

## PERMANENT GENETIC RESOURCES

# Twenty-three new microsatellite loci in the stable fly, *Stomoxys calcitrans* (L.) (Diptera: Muscidae)

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## Abstract

The stable fly, *Stomoxys calcitrans* (L.), is a significant pest of cattle. Twenty-three microsatellite markers were isolated from a repeat-enriched genomic library of *S. calcitrans*. We characterized variation at these markers and found that 17 loci were polymorphic in two fly populations from Florida. Two to nine alleles were observed among the variable microsatellite loci and expected heterozygosities ranged from 0.03704 to 0.85115. These markers will be useful for characterizing population genetic differentiation and for tracking the migration patterns of stable flies in the USA and worldwide.

**Keywords:** cosmopolitan pest, genetics, microsatellite, stable fly

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The stable fly *Stomoxys calcitrans* (L.) has a worldwide distribution and is a significant pest of man and animals (Zumpt 1973; Christensen 1982). Adult fly biting reduces weight gain and feed efficiency in cattle. Additionally, this fly is a vector for several diseases (Weiman *et al.* 1992). A genetic study of *S. calcitrans* in North America (samples from Canada, Nebraska and Texas) employing allozymes and mitochondrial markers reported low genetic diversity, supporting the hypothesis that this species experienced a bottleneck during colonization of North America (Szalanski *et al.* 1996). The reported  $F_{ST}$  values among geographical populations were low suggesting slight levels of genetic differentiation among them (Szalanski *et al.* 1996). However, given the low levels of polymorphism observed at these markers (see Szalanski *et al.* 1996), the development of highly polymorphic markers such as microsatellites are needed to better understand both the colonization history and gene flow among fly populations in the USA. To that end, we developed 23 new microsatellite loci for *S. calcitrans* and characterized their variation in two populations from Florida.

Total genomic DNA was isolated from 10 flies of *S. calcitrans* from a laboratory colony at the Agricultural Research Service Center for Medical, Agricultural and Veterinary Entomology (ARS-CMAVE) using the Puregene DNA

isolation kit (Gentra Systems). Extracted DNA was used to construct an enriched library for di-, tri- and tetranucleotide repeats using protocols modified from Kandpal *et al.* (1994) and Fleischer & Loew (1996). Genomic fragments enriched for nucleotide repeats were polymerase chain reaction (PCR) amplified using *Sau3AI* linker primers, ligated into a TOPO TA pCR2.1 vector (Invitrogen Corp.), and transformed into One Shot *Escherichia coli* cells (Invitrogen Corp.). Cloned fragments of 432 colonies were PCR amplified with M13 primers and resulting amplicons were purified and sequenced using M13 primers and standard fluorescent sequencing chemistry (ABI PRISM BigDye terminator chemistry, Applied Biosystems). Purified sequencing reactions were run on an ABI 3700 sequencer at the DNA Sequencing Core Facility at the University of Florida.

Out of the 432 analysed sequences, 60 contained some type of repeat motif. From these, we were able to successfully design 49 pairs of primers from flanking regions of the microsatellites using the Primer 3 program (Rozen & Skaletsky 2000). Twenty-three primer pairs successfully amplified a product of the expected size, and were used to genotype 51 wild-caught flies from two sampling sites in Florida separated by 50 km (Bell, 24 flies and Hague, 27 flies). DNA from the head + thorax was extracted as above. For comparisons, we also included three microsatellite loci previously developed for *S. calcitrans* and *Stomoxys niger niger* (Gilles *et al.* 2004, 2005).

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**Table 1** Characteristics of microsatellite loci isolated from *Stomoxys calcitrans* in two populations of stable flies in Florida (USA). Allelic diversity and observed and expected heterozygosities for the Bell and Hague populations are in top and bottom rows, respectively

Locus	Primer sequence (5'-3')	Repeat motif of clone	$T_a$ (°C)	Size of cloned allele (bp)	Allele size range (bp)	Total no. of alleles	No. of alleles per population	$H_O$	$H_E$	GenBank Accession no.
Stom B11	F: FAM-GCCACATTGGCATGTGTAC R: CCACAATCACTCACCCACTG	(AG) <sub>11</sub>	60	144	133-149	23	9	0.82609	0.81449	EU919517
Stom G03N	F: NED-TCAGACAAACACAGGAATTTGAA R: GGAGTACGTTTTAAGAGAAAAATAAACTG	(CA) <sub>6</sub>	60	119	111-125	20	8	0.80000	0.72436	EU919518
Stom CA172	F: PET-AGCAACAGCCGAACACCATA R: AGCATTGAATGACTGAGCGAAT	(GT) <sub>13</sub>	50	97	86-105	20	8	0.70000	0.82051	EU919519
Stom G06N	F: FAM-TCGATTTATTAAGGCAGCCATA R: TGATTTTTTATCCCTAAACATAGTGG	(GA) <sub>6</sub>	50	152	147-170	24	7	0.58333	0.68085	EU919520
Stom D12N	F: PET-TCTGGCTTTTATGTGTGTAATTTG R: TGCAGTATGGGTACAACCTGAGC	(CA) <sub>7</sub>	60	122	114-124	24	5	0.37500	0.65337†	EU919521
Stom E10	F: FAM-CAATGGCATTATGCAATCCAG R: GCGGAAGACTGAGTGTGCT	(CAA) <sub>5</sub>	60	192	179-195	23	4	0.39130	0.51304	EU919522
Stom CA165	F: PET-CGGTCCAGCTAAATTTACCATAAG R: CTTAAGCGGCAGAAAGTCTAT	(CT) <sub>11</sub>	60	154	142-156	19	4	0.05263	0.70697†	EU919523
Stom D02N	F: PET-AACCGGAACACCACAATA R: CCAAAATTTCCAATTTGC	(CAA) <sub>7</sub>	50	116	112-118	24	3	0.04167	0.04167	EU919524
Stom F9	F: PET-GGATCTAAAGGCTCACAGCATAA R: CGAATATTTCTCTGTGTTTCA	(CA) <sub>8</sub>	50	105	99-103	24	3	0.41667	0.35816	EU919525
Stom C10F2	F: FAM-CAAATAATGTTGGCATTAAAGAA R: TATATTTTATGCTGGGGCTTGC	(TTG) <sub>4</sub>	60	134	128-130	23	2	0.17391	0.16232	EU919526
Stom D4	F: FAM-GGACGTCGAAGAAAAAGACG R: GTGAAGCTCCTTCTGACTGCT	(TG) <sub>5</sub>	60	75	69-71	24	2	0.08333	0.08156	EU919527
Stom F5	F: PET-GCCCTCCACAGGCAAGTA R: TCTTTGTCGTTAACCATAGCTGA	(TG) <sub>5</sub>	60	64	61-63	24	2	0.37500	0.43883	EU919528
Stom G11N1	F: PET-TCCAAGTACCTTACCCTTGGT R: TGCTCTTGCTACAAAATTGGAC	(CAG) <sub>5</sub>	50	159	151-156	23	2	0.26087	0.34783	EU919529‡
Stom G11N2	F: VIC-GTCCAATTTTGTAGCAAGAGCA R: GCAGTACATCCATCCAACCATA	(CAA) <sub>4</sub>	50	168	166-169	24	2	0.45833	0.50975	EU919529‡
Stom CAG12	F: FAM-CGTTATAATTGCGCTGTCTGTG R: GAATTGAAACGCAACGCTAGT	(GT) <sub>5</sub> GC (GT) <sub>3</sub>	50	90	83-86	24	2	0.20833	0.19060	EU919530
Stom CA104	F: VIC-GGTTAAAGGTTTGGCTTCC R: CTTGGGATCTACTTCATTCTCTTG	(CCA) <sub>3</sub>	50	63	54-56	22	2	0.59091	0.42600	EU919531
Stom CA117	F: NED-ACTCCTTCCGACTGTTAATTCAG R: GCAGAAGTTGTGAGGAAATTGAA	(CA) <sub>5</sub>	50	79	75-80	24	2	0.45833	0.36082	EU919532
Sc A6	F: FAM-CTATCAACGTGGGAAAATTG R: AGTTGGCTGCTACGTTGTG	(CA) <sub>8</sub>	60	N/C	143-153	24	4	0.79167	0.71188	AY626555§
Sc E4	F: PET-CATCAGCGAAACATCAGAG R: CTGGTCCGGCTATTTAATG	(CA) <sub>12</sub>	60	N/C	114-130	24	9	0.70833	0.85816*	AY626558§
Sn F1	F: PET-GGGTAATGGCTTTGCTCTAAG R: TGCCCTCAATTATCATTGC	(GA) <sub>11</sub>	50	N/C	181-197	24	8	0.70833	0.80230*	AY734526¶
						27	7	0.70370	0.72886	

$T_a$ , annealing temperature; N/C, not cloned;  $N$ , number of genotypes successfully scored;  $H_O$ , observed heterozygosity;  $H_E$ , expected heterozygosity; significant deviations from Hardy-Weinberg equilibrium are denoted with \* and † (significant at  $\alpha = 0.05$  and  $\alpha = 0.01$ , respectively); ‡ loci Stom G11N1 and N2 were developed from different regions of same cloned sequence (Stom G11N); § Gilles *et al.* 2004, ¶ 2005.

The 23 loci were amplified in separate 15- $\mu$ L PCRs containing 7.5  $\mu$ L of 2 $\times$  *Taq-Pro COMPLETE* (2.0 mM MgCl<sub>2</sub>; Denville Scientific), 0.23  $\mu$ M of each primer, at least 10 ng template DNA and water. Thermal cycling profiles were: initial denaturation at 94 °C (1 min), followed by 35 cycles at 94 °C for 30 s, primer-specific annealing temperature for 45 s (Table 1), and 72 °C for 1 min; a final extension of 72 °C for 10 min. PCR amplicons were diluted 100:1 and run on an ABI 3730xl 96-capillary sequencer. GeneScan 600 LIZ was included in every well as an internal size standard (Applied

Biosystems). Microsatellite genotypes were scored using GeneMarker version 1.60 software (SoftGenetics, LLC). Expected heterozygosity, allelic diversity and Hardy-Weinberg disequilibrium for each locus in each population were calculated using Arlequin version 3.1 (Excoffier *et al.* 2005) (Table 1). Genotypic disequilibrium among loci was estimated (325 pairwise locus comparisons based on 6500 permutations) using FSTAT version 2.9.3 (Goudet 2001).

Seventeen out of the 23 new loci were polymorphic (Table 1) and six were monomorphic (GenBank Accession

nos EU919533–EU919538) in the surveyed populations. Among the variable loci, the number of alleles per locus ranged from two to nine, and the observed and expected heterozygosities ranged from 0.03704 to 0.82609 and from 0.03704 to 0.85115, respectively (Table 1). Four out of the 17 polymorphic loci significantly deviated from Hardy–Weinberg expectations in at least one population (Table 1). In every case, genotype proportions for these loci showed a significant deficiency of heterozygotes probably caused by the presence of null alleles at these loci. No significant linkage disequilibrium was detected after Bonferroni correction (adjusted  $P = 0.000154$ ). All three previously developed microsatellite loci (Sc A6, Sc E4 and Sn F1; Gilles *et al.* 2004, 2005) amplified successfully and showed similar results to those for the 17 newly developed variable loci (Table 1).

In summary, we developed and characterized 23 new microsatellite markers for *S. calcitrans*, 17 of which were polymorphic in two Florida populations. These microsatellite loci will be useful for investigating genetic structure and gene flow of *S. calcitrans* in the USA, for reconstructing the colonization history, as well as for monitoring for secondary introductions into the USA.

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### References

- Christensen C (1982) External parasites of dairy cattle. *Journal of Dairy Science*, **65**, 2189–2193.
- Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–50.
- Fleischer RC, Loew SS (1996) Construction and screening of microsatellite-enriched genomic libraries. In: *Molecular Zoology: Advances, Strategies, and Protocols* (eds Ferraris JD, Palumbi SR), pp. 461–468. Wiley-Liss, Inc, New York.
- Gilles J, Litrico I, Sourrouille P, Duvallet G (2004) Microsatellite DNA markers for the stable fly, *Stomoxys calcitrans* (Diptera: Muscidae). *Molecular Ecology Notes*, **4**, 635–637.
- Gilles J, Litrico I, Duvallet G (2005) Microsatellite loci in the stable fly, *Stomoxys niger niger* (Diptera: Muscidae) on La Réunion Island. *Molecular Ecology Notes*, **5**, 93–95.
- Goudet J (2001) *FSTAT version 2.9.3: A Program to Estimate and Test Gene Diversities and Fixation Indices*. Available from URL: <http://www.unil.ch/izea/software/fstat.html>
- Kandpal RP, Kandpal G, Weissman SM (1994) Construction of libraries enriched for sequence repeats and jumping clones, and hybridization selection for region-specific markers. *Proceedings of the National Academy of Sciences, USA*, **91**, 88–92.
- 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* (eds Krawetz S, Misener S), pp. 365–386. Humana Press, Totowa, New Jersey.
- Szalanski AL, Taylor DB, Peterson RD (1996) Population genetics and gene variation of the stable fly populations (Diptera: Muscidae) in Nebraska. *Journal of Medical Entomology*, **33**, 413–420.
- Weiman GA, Campbell JB, Deshazer JA, Berry IL (1992) Effects of stable flies (Diptera: Muscidae) and heat stress on weight gain and feed efficiency of feeder cattle. *Journal of Economic Entomology*, **85**, 1835–1842.
- Zumpt F (1973) *The Stomoxysine Biting Flies of the World. Taxonomy, Biology, Economic Importance and Control Measures*. Gustav Fischer Verlag, Stuttgart, Germany.