

## TECHNICAL NOTE

### Microsatellite markers for interspecific mapping of *Drosophila simulans* and *D. sechellia*

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**Keywords:** *Drosophila sechellia*, *Drosophila simulans*, mapping, microsatellite

Received 24 April 1999; revision accepted 17 May 1999

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The sister species *Drosophila simulans* and *D. sechellia* provide an excellent system for investigating the genetics of speciation and adaptation. The two species have different ecologies and differ markedly in a number of reproductive traits (R'Kha *et al.* 1991; Coyne *et al.* 1991). Attempts have been made to localize genes responsible for these differences. However, the number of markers employed was limited (Joly *et al.* 1997; Jones 1998). The development of a relatively dense genetic map for *D. simulans* and *D. sechellia* will allow more powerful analyses to be undertaken.

Most published *Drosophila* sequences were characterized from *D. melanogaster*. The close relationship between *D. melanogaster*, and the *D. simulans* complex (*D. simulans*, *D. sechellia* and *D. mauritiana*) implies that a reasonable fraction of these microsatellites should be informative in all four species. We screened microsatellites selected from *D. melanogaster* in order to develop genetic markers for mapping studies in the *D. simulans* group. Some microsatellites were selected from the literature (see references in Table 1), others were found in GenBank (using the program 'FINDPATTERNS' from the GCG package, Genetics Computer Group Inc. 1994) and in the Berkeley *Drosophila* Genome project website ([HTTP://fruitfly.berkeley.edu/seq\\_tools/patternSearch.html/](http://fruitfly.berkeley.edu/seq_tools/patternSearch.html/)). Finally, primer sequences for one marker (DS01340) was provided by C. Schlötterer (Institut fuer Tierzucht und Genetik, Veterinaermedizinische Universitaet Wien).

One hundred and seven microsatellite loci were selected and primers designed when necessary. Single-fly DNA was extracted in Tris-EDTA-NaCl buffer with 200 µg/mL proteinase K (Gloor *et al.* 1993). PCR reactions were conducted in prealiquoted 96-well microplates (Advanced Biotechnologies) with 0.625 U *Taq* DNA polymerase, 75 mM Tris-HCl, 20 mM (NH<sub>4</sub>)<sub>2</sub> SO<sub>4</sub>, 2.5 mM MgCl<sub>2</sub>, 0.01% Tween 20 and 0.2 mM of each dNTP to a total volume of 12.5 µL. One µL of DNA and 7 pmol of each primer were added to the PCR mix. Amplification was performed using a GeneAmp PCR 9700 system (Perkin-Elmer) with the following conditions: 94° for 4 min (94° for 1 min, 55° for 1 min, 72° for 1 min), 35–72° for 8 min. Out of 107 loci, 86 amplified in *D. simulans* and *D. sechellia*, and were tested for variability using five isofemale lines each

of *D. sechellia* and *D. simulans*. The *D. simulans* lines include two African (sim133, sim148, from UMEA *Drosophila* stock centre), one Israeli (kindly provided by Prof. Eviator Nevo, University of Haifa, Israel), one European (sim132, from UMEA *Drosophila* stock centre) and one American line. *D. sechellia* lines were obtained from the Mid-America *Drosophila* Stock centre, Bowling Green, Ohio. Variability was assessed by calculating the heterozygosity ( $H = 1 - \sum x_i^2$ , where  $x_i$  are the allele frequencies). We identified 71 loci which discriminate with various effectiveness between the two species, although limited sequencing of some loci indicated that the allele sizes are sometimes caused by mutations outside the microsatellite (Colson & Goldstein 1999). The discriminating power of the markers was defined as the probability of obtaining two different alleles when one homozygous line of each species was chosen at random. The discriminating power of the markers varies from 0.2 to 1, but is higher than 0.75 in over 50% of them (Table 2). This indicates that it is possible to reach a resolution of 10–20 centimorgans (cM) between selected lines of *D. simulans* and *D. sechellia*. It is likely that the same kind of power can be achieved between *D. simulans* and *D. mauritiana*. The genetic location of a subset of 38 markers was determined from the progeny of F1 females (our unpublished data). For this set, the intervals between markers do not exceed 18.3 cM, with an average of 8.8 cM.

#### Acknowledgements

We thank Dr C. Schlötterer for providing information on primer sequences, and Prof. E. Nevo for providing the Israeli *D. simulans* line. This work was funded by a BBSRC grant to Dr D. Goldstein.

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**Table 1** Cytological and genetic locations (genetic locations were determined from *D. simulans*/*D. sechellia* hybrid females), variability and discriminating power of 71 microsatellite loci in *D. simulans* (*D. sim*) and *D. sechellia* (*D. sech*). When the microsatellites were taken from the literature, the reference has been included (when the locus name is different, the original locus name is indicated after the reference)

Locus name	Acc. no	Cytological location	Genetic location	Repeat (in <i>D. mel</i> )	Size range (bp)	No. of alleles		Heterozygosity		Discr.	Reference
						<i>D. sim</i>	<i>D. sech</i>	<i>D. sim</i>	<i>D. sech</i>		
AF047180	AF047180	01B1-B14	1-0	(AT) <sub>16</sub>	248-250	1	1	0	0	1	
dmu566661	U566661	04F1-F2	1-9.0	(AC) <sub>15</sub>	230-250	2	3	0.48	0.56	1	
drootua	M30885	07F1		(AT) <sub>9</sub>	139-144	1	1	0	0	1	Goldstein & Clark (1995)
ds09021	G684034	08B5-B8	1-17.7	(GT) <sub>12</sub>	122-124	1	1	0	0	1	
drosev2	J03158	10A1-A2	1-24.4	(TTG) <sub>9</sub> ,(TA) <sub>7</sub>	236-274	1	1	0	0	1	Goldstein & Clark (1995)
dmtena	X68794	11A6-A9	1-28.4	(AT) <sub>14</sub>	90-98	3	1	0.56	0.48	0.8	Goldstein & Clark (1995)
dmu18774	U18774	13D	1-33.9	(AC) <sub>12</sub>	167-200	3	2	0.64	0.56	0.88	
dmariadne	X98309	15F		(AC) <sub>14</sub>	102-110	2	3	0.32	0	0.36	
dmtropni	X58188	16F3-F6	1-41.4	(AC) <sub>11</sub>	110-114	3	1	0.56	0.48	0.8	Goldstein & Clark (1995)
DROPASSOV	L13306	19E	1-50.7	(AT) <sub>14</sub>	253-263	1	2	0	0	1	
af017777	AF017777	19F3-F6		(AT) <sub>14</sub>	111-119	2	1	0.32	0	0.2	
ac006472	AC004115	21B7-C2		(AG) <sub>17</sub>	169-176	2	1	0.48	0	1	
droexpand	L14768	21C4	2-0	(CAG) <sub>8</sub>	80-83	2	1	0.48	0	0.6	Goldstein & Clark (1995)
droyanetsb	M97694	22D1-2	2-4.1	(GT) <sub>19</sub>	80-88	2	1	0.48	0	1	Goldstein & Clark (1995)
ac006472	AC006472	45E1-46A2		(AG) <sub>16</sub>	268-270	2	1	0.32	0	0.2	
ds01340		24A1-A2		(AG) <sub>9</sub>	160-162	2 (1null)	1	nd	0	1	
ac005732	AC005732	24C3-D2	2-9.5	(GT) <sub>13</sub>	208-254	4	1	0.72	0	1	
ac004373	AC004373	24F1-F2		(AT) <sub>15</sub>	174-178	2	1	0.32	0	0.2	
drogpdha	J04567	25F5	2-21.6	(CT) <sub>7</sub>	176-179	2	1	0.32	0	0.2	
ac004758	AC004758	26A1-C1		(AG) <sub>16</sub>	191-203	2	1	0.48	0	1	
ac003052	AC003052	27A1-B2		(AT) <sub>23</sub>	239-286	2	1	0.32	0	0.2	
AC005555	AC005555	29A1-C1	2-31.4	(GT) <sub>12</sub>	166-178	4	1	0.72	0	0.8	
dmbigene	X53275	30F		(CAG) <sub>5</sub>	131-137	2	1	0.32	0	0.2	Michalakis & Veuille (1996) (bib)
drodans	J03148	31E1-E7		(CAG) <sub>5</sub>	273-281	1	2	0	0.32	0.8	
g410		33E9-E10	2-44.5	(CT) <sub>11</sub>	125-133	1	2	0	0.32	1	Harr <i>et al.</i> (1998)
ac006302	AC006302	34C4-D2		(AAT) <sub>11</sub>	218-250	3	2	0.64	0.32	1	
ac004118	AC004118	35B2-B3		(CT) <sub>15</sub>	267-270	2	1	0.32	0	0.2	
dromhc	M61229	36B1		(AC) <sub>13</sub>	90-98	4	1	0.72	0	0.8	Goldstein & Clark (1995)
drodorsal	M23702	36C	2-54.3	(CAG) <sub>9</sub>	210-228	3	2	0.44	0.32	1	Michalakis & Veuille (1996) (dl)
ac002474	AC002474	38D1-D2		(CA) <sub>13</sub>	255-258	1	2	0	0.48	0.6	
ac004759	AC004759	38E1-E9	2-64.0	(CCT) <sub>11</sub>	231-237	2	1	0.32	0	1	
DM1433	Y12573	46E2-E4		(AG) <sub>11</sub>	101-162	2	2	0.48	0.32	0.88	
drogpad	M31129	47A	2-72.8	(GT) <sub>9</sub>	150-166	2	1	0.32	0	1	Schug <i>et al.</i> (1997)

dmp20	Y00795	49F9-F13	2–80.8	(AC) <sub>10</sub>	76–86	2	1	0.32	0	0.8	Goldstein & Clark (1995)
dmu19731	U19731	51A1-A2		(GT) <sub>9</sub>	172–178	3	1	0.44	0	0.6	
AC004306	AC004306*	51E9-F2		(GT) <sub>9</sub>	142–146	2	1	0.48	0	0.4	
ac004516	AC004516	52D2-D15		(AC) <sub>18</sub>	238–276	3	1	0.44	0	1	
AC004248	AC004248	52D9-D15	2–89.3	(AC) <sub>21</sub>	133–159	3	5 (1null)	0.56	0.8	1	
aco04641	AC004641	53D1-E2		(AC) <sub>22</sub>	234–236	2	1	0.48	0	0.4	
ds00361		54B1-B2	2–93.1	(AC) <sub>8</sub>	142–156	4	1	0.68	0	0.8	Schlötterer <i>et al.</i> (1997)
dmelf1	X15657	55A1		(CAG) <sub>6</sub>	165–186	3	2	0.56	0.48	0.76	Goldstein & Clark (1995) (elf)
ac004640	AC004640	54F1–55A1		(AT) <sub>13</sub>	242–246	3	1	0.56	0	0.8	
ds08687a		57C5-D1	2–102.4	(GT) <sub>11</sub>	173–189	3	1	0.44	0	1	Schlötterer <i>et al.</i> (1997)
ac004564	AC004564	57E1-E2		(GT) <sub>12</sub>	106–113	1	1	0	0	1	
ac004365	AC004365	58A4-B1	2–107.6	(AT) <sub>17</sub>	204–210	2	1	0.48	0	1	
ds08011		59A1-B2	2–111.9	(GT) <sub>8</sub>	101–111	3	1	0.48	0	1	Harr <i>et al.</i> (1998)
dm92d10s	Z50696	59A1-D8		(AT) <sub>19</sub>	104–122	2	2	0.48	0.48	0.36	
dmrhob	X52454	62A	3–0	(AC) <sub>10</sub>	198–206	3	3	0.56	0.64	0.72	Schug <i>et al.</i> (1997)
ac004343	AC004343	62A1-A2		(CT) <sub>11</sub>	202–212	4 (1null)	1	0.72	0	0.8	
ac004658	AC004658	63D2-E1	3–6.5	(AC) <sub>12</sub>	112–126	3	2	0.56	0.48	0.88	
dmu36477	U36477	64A		(AT) <sub>14</sub>	103–107	2	2	0.48	0.32	0.88	
drodsrsc	K01043	64B	3–16.5	(AT) <sub>7</sub>	312–317	1	3	0	0.56	0.8	Schug <i>et al.</i> (1998) (dmsrsc)
ac004352	AC004352	65C5-D1		(AG) <sub>14</sub>	267–283	3	1	0.44	0	0.6	
dmu14395	U14395	65D1-D3	3–28.3	(CT) <sub>12</sub>	263–281	5	2	0.8	0.32	0.84	
drolamb2a	M58417	67C	3–37.9	(ATT) <sub>5</sub>	150–207	4	1	0.72	0	0.8	Schug <i>et al.</i> (1998)
drogtpaap	M86655	67D2-D3		(CAG) <sub>5</sub>	220–226	2	1	0.48	0	0.4	
AC006414	AC006414*	89A1-A5	3–94.7	(GT) <sub>9</sub>	192–198	4	1	0.72	0	0.8	
dmz60mex	X58286	71C-D		(TTC) <sub>8</sub>	85	1	null	0	nd	1	Goldstein & Clark (1995)
dm22f11t	Z83456	73A1-B7	3–55.2	(AT) <sub>22</sub>	185–203	4	4	0.72	0.72	1	
dmcathpo	X52286	75D-E1	3–60.4	(ACC) <sub>6</sub>	108–117	4	1	0.72	0	0.8	Schug <i>et al.</i> (1997)
AC001665	AC001665	84C1-C4	3–69.4	(AC) <sub>9</sub>	134–140	3	2	0.56	0.32	0.96	Schug <i>et al.</i> (1998) (dro17dc4z)
drohoxnk4	M27292	91E1-E3		(CAG) <sub>5</sub>	136–139	2	1	0.32	0	0.2	
dronanos	M72421	91F13	3–77.8	(AT) <sub>18</sub>	104–110	4	1	0.72	0	0.8	Goldstein & Clark (1995)
dmehab	X72303	90B1-B2	3–84.4	(AGCC) <sub>5</sub>	353–361	3 (1null)	2	0.64	0.32	0.6	Schug <i>et al.</i> (1997)
drotropi2	K03277	88F5		(AT) <sub>11</sub>	84	2 (1null)	1	0.48	0	0.6	Schug <i>et al.</i> (1997)
dmtrxiii	Z50152	88B3	3–100.3	(AC) <sub>9</sub>	211–214	1	2	0	0.32	0.2	
dmprosper	Z11743	86E	3–107.1	(AC) <sub>6</sub>	110–114	3	1	0.56	0	0.4	Schug <i>et al.</i> (1997) (prospero)
				(AG) <sub>12</sub>							
dmu25686	U25686	93F		(AT) <sub>5</sub>	153–155	1	1	0	0	1	
dmtf125	X98235	95C6-C8	3–119.7	(CAG) <sub>6</sub>	289–297	4	1	0.72	0	0.8	
dmsidna	X79340	96E1-E4		(GC) <sub>6</sub>	126–128	2	1	0.32	0	0.2	Schug <i>et al.</i> (1998)
DMU43090	U43090	99D6-D9	3–132.8	(CAG) <sub>8</sub>	180–186	3	1	0.56	0	0.4	

\*GenBank sequence reversed compared to our sequence.

Name	Forward primer	Reverse primer	Remarks
AF047180	tac ctt agg aaa ccc gac cc	tct tgt tgc gaa ttt tgt tca	
dmu56661	tat ttc gct aac aaa ccg gc	aac gcg atc aca aac atc aa	
ds09021	gat ctt ttc atg tgt tat tt	ccg ttt tgt ttg gca act tt	
drosev2	att aaa gtg caa tta act at		
dmu18774	gat cct tgg cgg cag ggg agc gaa	tat gca act cct tgc aca a	
dmariadne	aac act gtc ccc atc cac at	tct gtt caa ctc ctt cgg ct	
dmtena		tca aga gtc gct caa tgg c.	
dropassov	gtg gaa atg gca gag gag ag	gtt gtt cat ttg ttt agc gg	
af017777	att agc taa ctc caa gaa cg	aat cct ctc agc tca gcg ta	
ac004115	ttc tgc ccg ctt aac ctc ta	aag cta agt cag cat ccc c.	
ac006472	tcc tcc atg taa aga taa acg ct	aac tcg caa att gcc taa cg	
ds01340	gga gcg caa tgc tgt tta agt	gga gta gtg cct gtc tcg gac	
ac005732	taa ttt ggc aca aac cac c.	gcc gca taa tgg tca aaa gt	
ac004373	aat gcg tgt gtt tgg atg aa	gtc cca gtc tcc cag tga aa	
drogpdha	cat tgg aaa agt gag cgg at	ttg gtt tgc act cca cac at	
ac004758	tgc ttt cgc ttt cgg tat ct	aac gga gtg cct atg cat t	
ac003052	atc gtc gaa cga gac cgt a	tcg att taa ttg cgg tgt ga	
AC005555	ggt tgc tgg gag aaa gac	gcc aca cat tcg cat ctc	
dmtrxiii3	gac cgt ttg ttt gcc ttg at	tgc ctg tac aag tct gac cg	
ac006302	tgt ttt cca tgc cag cta gt	gcc cgg aaa att ctt gtt ta	
ac004118	cca act tgg gcg aga gaa tt	gct taa ttg cct cac tgt gc	
dromhc		aca tta ccg ata ttg gat gca	
ac002474	gat gct gtc ctt cgg act tc	aac aac aaa gcc cat tct gc	
ac004759	aca gac gga aag cca aaa tg	cac tcc gcc tcg ttt ctt ac	
DM1433	gct tga aga atc cct gct tg	tga ttt ttg cca gtt cag c.	
u19731	acc ttt ttc tcg cag tgc at	att gtg gct ggc tgt tta cc	
AC004306	gag aca ccc ctt gac gag tg	ctc aaa aca aac cca gtc tc	
ac004516	tcg tcg ccc gtt aat ata	acc gtt cgt ggg tca aat ag	
AC004248	caa ttt ccc tcg cac tga cac	cgg aaa cga acg ggc gat aag	
ac004641	tca agt agg ggg tgt cgt tc	aac caa caa cta att gcg gc	
dmelf1	tgt aag caa agg ccc aga gag	gag tct gga gct gta act gaa	
ac004640	ccg taa gcc cat aag cgt aa	ggc tac ggc tag agt tcg tg	
ac004564	gaa ttt caa aat ggg cga aa	att cac gtg cta tgt gca gc	
ac004365	gct tta tca atg cag cct cc	ggc ccc aat atg tcc tcg cc	
dm92d10s	ccg aat cga tgt agt tcc ttg	aag gct ccg gtc ctt gtt ag	
ac004343	acg gta att gcg gat gag ac	acg atg gca aca agg atc tc	
ac004658	att tgg tcc acg aga gat tt	tgg gaa aac tgt gcc aca ta	
dmu36477	cgg cga gcc aaa ctc tta t	att att tgt ggc aaa agc gg	
ac004352	tcc tcg gtg aga ccg taa tc	ggg cag agg aaa agc act ca	
dmu14395	ggg cag agg aaa agc act c.	tcg gtg aga ccg taa tct gc	
drogpaap	ctg aaa tac ggc agc aga c.	tag gcg ttc atg ttg ctc gt	
dm22f11t	gga tgc tcg gat acc aaa aa	tcg cct gtg act tag att gc	
dmprosper	caa taa cca cac gca ttc c.	aac cac ttc ctg ttt ggc c.	
drodans	tgc cca gca tca cat gat ac	ggt ttt tat gga aga gag gg	
drotropi2	gta cat ccc gaa tcc cac ac	aat aca ctg aaa ctg ttg ggg g	ann.t: 60c
dmehab	att tat tag ttt ttt gct aat act tgc	aga gtt ctt gtt gta ttt ata c.	
dronanos	gcg aag tat tca ttt caa cac a	tgc tgg cgg ttg ttt cat	
drohoxnk4	ctg aag ttg aag tcc gag cc	tac atg tgc tgc atc tgt tgc	
dmu25686	cga taa ttt act ctg tgc tcc	cag ctc aca caa aag gca aa	
dmtf125	ctc gag cgg gcc ata caa ga	tga ttg aag agg cca ctc aa	
DMU1043090	tgc acc cag caa tac cag ta	gct gtt gtc gtg gtg ctg	
AC006414	gaa aga gct cca agg caa tca gg	tgt ttc cca gga cag gat aag cg	

**Table 2** Primer sequences of new markers and modified primer sequences of published markers

Michalakakis Y, Veuille M (1996) Length variation of CAG/CAA trinucleotide repeats in natural populations of *Drosophila melanogaster* and its relation to the recombination rate. *Genetics*, **143**, 1713–1725.

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