name	location	genetic map position	primers	PCR conditions	size relation	used in	source
X chromosome							
period (*per*)	3B1-2	4.5	CCC GTC CAC GAG GGC AGC GGG GGC TTC TCC ATC TCG TCG TTG TG	55°C 1min	sim:380 se:350	sim1	(Gleason and Ritchie 2004)
sevenless (*sev*)	10A4	24.4	ATT AAA GTG CAA TTA ACT AT AAG CGA CAA GTT TCA ATT AAC	52°C 1min	sim:275 se:230	se	(Colson *et al.* 1999; Gleason and Ritchie 2004)
no-on-or-off-transient A (*nonA*)	14B18-C1	36.1	GAG TAT GCA CGC TAT GAG CA GTT GTC AAC TTC GCG TTT TC	52°C 1min	sim:430 se:580	se	(Gleason and Ritchie 2004)
forked (*f*)	15F4-7	38.9	morphological marker	-	-	sim1, sim2	-
Second chromosome							
net (*nt*)	21B1	0.0	morphological marker	-	-	sim1, sim2	-
anterior open (*yan*)	22D1	4.1	TAA TGG GGA ATG GGT GAA TG GCC GTG CTC TTT TCT CTT ACG	52°C 1min	sim:98 se:90	se	(Gleason and Ritchie 2004)
spalt (*sal*)	32E4-F1	41.0	GCC ACG ATG AAA CTA CTG GTG C ACT CCT CCC TGG CCA ATT C	55°C 1min	sim:290 se:230	se, sim1	(Gleason and Ritchie 2004)
Galpha47A	47A7-9	72.8	GAA ATA GGA ATC ATT TTG AAT GGC AAT TAA AAA CAA AAA ACC TGA GCG	52°C 1min	sim:180 se:155	se, sim1	(Gleason and Ritchie 2004; Schug *et al.* 1998)
mastermind (*mam*)	50C23-D3	83.1	GGC GGC CTA CCA GTT TTC CA CCT GTT GCT CCC AGG TTT GC	52°C 30s	sim:300	sim1, sim2	(Civetta *et al.* 2002)
SRPK	51F11-12	87.3	CAG AGG GAG GAG CGC AAG AGT TGT GGC GGC TGT TGC CC	55°C 30s	sim: 350 se:358	sim1, sim2	this paper
acc004516	52D12	89.1	TCG TCG CCC GTT AAT ATA ACC GTT CGT GGG TCA AAT AG	55°C 1min	sim:295 se:250	se, sim1	(Colson *et al.* 1999)
Amylase distal (A*my-d*)	53F12	92.4	TAC GTG GAT GTG ATC TTC AA GAT GAC CTC CTG GAC GAT	48°C 30s	sim:403 se:380	sim1, sim2	(Civetta *et al.* 2002)
grainy head (*grh*)	54E10-F1	94.2	TCC ACG ACA ACG ATC TCG CA CTA ACA ATG TCG CCG GGA TG	55°C 1min	sim:115 se:100	sim1, sim2	(Gleason and Ritchie 2004)
plum (*pm*)	57C7-8	102.0	morphological marker	-	-	sim1, sim2	-
twist (*twi*)	59C2	113.8	GTG TTC CTG CCC CTC TAC AA CGA GCT GGA GTA AGC ACT GC	55°C 1min	TaqI cuts sim	se	(Gleason and Ritchie 2004)
Third chromosome							
temperature-induced-paralytic-E (*tipE*)	64A10	12.8	TCA CCA GCT GAA AGT CCA GA ACT CGT CGT CAT CGT CTT GC	55°C 1min	sim:490 se:475	se, sim1	(Gleason and Ritchie 2004)
Esterase 6 (*Est-6*)	69A1	44.4	CAC AGA GGA TGG TGG CTA CA CCG GAC GCA TTT CCA CTT CT	58°C 1min	sim:510 se:470	se	(Gleason and Ritchie 2004)
scarlet (*st*)	73A3	55.2	morphological marker	-	-	sim1, sim2	-
Accessory gland-specific peptide 76A (*Acp76A*)	75F5	60.9	CGT CGC TCC TCT TTC AAA ATA CAA TAC AAC TCG GCA GGA GGA TCA ACA TAC CC	55°C 1min	BanI cuts sim	se, sim1	C. Jones, personal communication
ripped pocket (*rpk*)	82C5	63.6	AGC CGG CGA TGA CTT GGA CTA CTT T AGC GCT TTT ACT CTT TTG AAT TCC CTG ACT	55°C 1min	PVUII cuts sec	se, sim1	C. Jones, personal communication
rotund (*rn*)	84D3	69.8	CGC CCC ACG ATG CAG AAC AAA C ACC CGC TGC CAA GTG ACG ACA TT	60°C 1min	AluI cuts sim 2x, se 3x	sim2	adapted from C. Jones, personal communication
E2F transcription factor (*E2F*)	93E9-F1	72.0	CGC TCT TCA ACA ATA TCG ATG C GCA AGA ACT CAG ACT GTG ATG	55°C 1min30	HincII cuts se	sim2	this paper
insulin receptor (*InR*)	93E4	72.7	CCT TAT AGC TTG CAA ATC CAT GG GAC CAA GGC ATA TCC GTC GA	52°C 1min	HindIII cuts sim	sim2	this paper
Heat shock RNA omega (*hsr*)	93D4	72.8	GGG CAA GGG CCC ACG TAG T CGA TAT CAA CTT TCA ACG CCC A	52°C 1min	DdeI cuts sim 3x, se 2x	sim2	this paper
ebony (*e*)	93C7-D1	72.9	morphological marker	-	-	sim1, sim2	-
nanos (*nos*)	91F7	77.8	CAA GCG AAC AAC AGC AAG GC CCC TAT CGG CCA CGA CGA TT	52°C 1min	XmnI cuts se	sim1	this paper
couch potato (*cpo*)	90D1-E1	82.4	TCG CAC GAG TCC AAC TCC ACG GAG TCC ATG CTC TGC	50°C 1min	sim:280 se:250	se, sim1	(Civetta *et al.* 2002)
Relish (*Rel*)	85C3	112.5	GAC CAT GGC CAT CGG GCT C TAA GTC AGG AGT TCG GAC GC	55°C 1min	BseAI cuts se	sim1	adapted from C. Jones, personal communication
slowpoke (*slo*)	96A14-17	121.3	GAT GCT GCC AAC ATC ATG AG CGA CGT CTT GAA CGA TCT CA	52°C 1min	sim:320 se:340	se, sim1	(Gleason and Ritchie 2004)
Fourth chromosome							
cubitus interruptus (C*i*)	102A1-3	-	GCG AGT ATC CGG GAT GTA GC CCG TTG CCT AGC CAA AAC AG	55°C 1min	SacI cuts se	se, sim1	this paper

Supplemental Table 1. Markers used and their relative map order. Cytological locations were obtained from Flybase (http://flybase.bio.indiana.edu/) and are for *D. melanogaster*. Fragments were PCR amplified using ABgene Thermo-Start PCR Master Mix 2.0mM $MgCl_2$ (#AB-0938/20). All PCR reactions followed this format: 95°C for 15 min, 94°C for 30 sec, annealing temperature as indicated for 30 sec, 72°C for an elongation time as indicated, 35 cycles, 72°C for 8 min. PCR fragment sizes are approximate. sim1: first *D. simulans* backcross, sim2: second *D. simulans* backcross, se: *D. sechellia* backcross.

LITERATURE CITED

CIVETTA, A., H. M. WALDRIP-DAIL and A. G. CLARK, 2002 An introgression approach to mapping differences in mating success and sperm competitive ability in Drosophila simulans and D. sechellia. Genet Res **79:** 65-74.

COLSON, I., S. J. MACDONALD and D. B. GOLDSTEIN, 1999 Microsatellite markers for interspecific mapping of Drosophila simulans and D. sechellia. Mol Ecol **8:** 1951-1955.

GLEASON, J. M., and M. G. RITCHIE, 2004 Do quantitative trait loci (QTL) for a courtship song difference between Drosophila simulans and D. sechellia coincide with candidate genes and intraspecific QTL? Genetics **166:** 1303-1311.

SCHUG, M. D., K. A. WETTERSTRAND, M. S. GAUDETTE, R. H. LIM, C. M. HUTTER *et al.*, 1998 The distribution and frequency of microsatellite loci in Drosophila melanogaster. Mol Ecol **7:** 57-70.