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Quantitative Trait Loci for Sexual Isolation Between *Drosophila simulans* and *D. mauritiana*

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ABSTRACT

Sexual isolating mechanisms that act before fertilization are often considered the most important genetic barriers leading to speciation in animals. While recent progress has been made toward understanding the genetic basis of the postzygotic isolating mechanisms of hybrid sterility and inviability, little is known about the genetic basis of prezygotic sexual isolation. Here, we map quantitative trait loci (QTL) contributing to prezygotic reproductive isolation between the sibling species *Drosophila simulans* and *D. mauritiana*. We mapped at least seven QTL affecting discrimination of *D. mauritiana* females against *D. simulans* males, three QTL affecting *D. simulans* male traits against which *D. mauritiana* females discriminate, and six QTL affecting *D. mauritiana* male traits against which *D. simulans* females discriminate. QTL affecting sexual isolation act additively, are largely different in males and females, and are not disproportionately concentrated on the *X* chromosome: The QTL of greatest effect are located on chromosome *3*. Unlike the genetic components of postzygotic isolation, the loci for prezygotic isolation do not interact epistatically. The observation of a few QTL with moderate to large effects will facilitate positional cloning of genes underlying sexual isolation.

THE early stage of speciation in animals is often characterized by the appearance of prezygotic isolation (ethological barriers to interspecific mating) and postzygotic isolation (reduced viability and fertility of interspecific hybrids). Although recent progress has been made toward understanding the genetic basis of postzygotic isolation (Wittbrodt *et al.* 1989; Ting *et al.* 1998; Barbash *et al.* 2003; Presgraves *et al.* 2003), relatively little is known of the genetic architecture of sexual isolation—arguably the most important form of reproductive isolation in animals (Mayr 1963; Coyne and Orr 1997, 1998). In Drosophila, for instance, sister species that occur in the same location show much higher levels of sexual isolation than of postzygotic isolation (Coyne and Orr 1997). Previous studies of prezygotic isolation in Drosophila have localized genes affecting sexual isolation to whole chromosomes or chromosome arms (Zouros 1981; Coyne 1989, 1993, 1996a,b; Wu *et al.* 1995; Noor 1997; Ting *et al.* 2001), but so far there has been no refined mapping to chromosome regions small enough to facilitate positional cloning of candidate loci.

Drosophila simulans and *D. mauritiana* are sibling species in the *D. melanogaster* subgroup. *D. simulans* is a cosmopolitan species largely commensal with humans, while *D. mauritiana* is restricted to the island of Mauritius. *D. mauritiana* probably arose after colonization of the island by a *D. simulans*-like ancestor ~250,000 years ago (Kliman *et al.* 2000). This species pair has several reproductive barriers, including gametic isolation, conspecific sperm precedence (Price 1997; Price *et al.* 2000, 2001), male-limited hybrid sterility, and behavioral (sexual) isolation. The sexual isolation is asymmetrical—in the laboratory, *D. mauritiana* females rarely mate with courting *D. simulans* males, but the reciprocal cross occurs readily. The sexual isolation is thus based on a species difference in female mating preference (*D. mauritiana* females discriminate against *D. simulans* males, but *D. simulans* females do not discriminate against *D. mauritiana* males) as well as a difference in an unknown male trait (or traits) against which the females discriminate (Coyne 1989). Moreover, although *D. simulans* females readily copulate with a courting *D. mauritiana* male, the copulations are often abnormally short, with many terminating prior to the time needed for adequate sperm transfer (Coyne 1993). The shortened copulations are thus a form of postmating, prezygotic isolation. Thus, at least three traits are involved in prezygotic isolation, and there may be others, such as ecological differences, that have not been studied.

F_1 females are fertile and can be readily crossed to *D. mauritiana* males to produce backcross (BC) progeny

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segregating for genes from both species. Thus, we can map the genes contributing to species divergence in female mate choice, the male traits that are discriminated against, and the attenuated period of copulation. Such data enable us to address longstanding questions regarding the genetic basis of prezygotic isolation: Do few or many genes contribute to reproductive isolating mechanisms? What are the relative magnitudes of their effects? How are these loci distributed throughout the genome? Do they interact epistatically? Do the same loci contribute to reproductive isolation in males and females and in the two species? If genes can be mapped to fairly restricted genomic regions, it may also be possible to identify the specific loci involved in sexual isolation.

MATERIALS AND METHODS

Drosophila strains: All flies were maintained in 8-dram vials containing standard cornmeal-agar-Karo media on a 12 hr:12 hr light:dark cycle at 24°. *D. simulans* Florida City (FC) is an isofemale line collected in Florida City, Florida, in 1985 (Coyne 1989). *D. mauritiana* synthetic (SYN) is derived from six isofemale lines collected in Mauritius in 1981 and combined in 1983 (Coyne 1989). Backcross hybrids were produced by crossing 4-day-old virgin *D. simulans* FC females to virgin *D. mauritiana* SYN males and then backcrossing virgin F_1 females to virgin *D. mauritiana* SYN males. Female backcross hybrids are either homozygous *D. mauritiana* or heterozygous *D. mauritiana/simulans* and have mitochondrial DNA from *D. simulans*. Male backcross hybrids have the same autosomal and mitochondrial genotypes as the females, but the *X*-linked loci are either pure *D. simulans* or *D. mauritiana*, and the *Y* chromosome is from *D. mauritiana*. Three separate groups of backcross hybrids were produced: (A) 1005 BC females, (B) 1002 BC males, and (C) 1002 BC males.

Courtship behavior: Three sets of "no-choice" mating assays were conducted, in which single BC individuals were paired with single pure-species individuals: (A) BC females and *D. simulans* males, (B) *D. mauritiana* females and BC males, and (C) *D. simulans* females and BC males. Experiment A reveals the quantitative trait loci (QTL) in BC females that lead to lack of mating with *D. simulans* males, experiment B the QTL in BC males that lead to lack of mating with *D. mauritiana* females (most probably through rejection by those females, since all males court *D. mauritiana* females persistently), and experiment C reveals the QTL in BC males that lead to shortened copulation and reduced sperm transfer after mating with *D. simulans* females.

In all three crosses, BC flies were collected as virgins and sorted by sex using brief exposure to CO_2 and kept in uncrowded vials for 4 days prior to use in experiments. Four-day-old virgin BC and pure-species flies were transferred by aspiration to vials containing standard cornmeal-agar-Karo media within 1.5 hr of "lights on." Experiments were conducted at room temperature, which varied from 21° to 23°. For experiments A and B we recorded whether or not copulation occurred within 45 min and, for those flies that did copulate, copulation latency (time to copulation) and copulation duration. In experiment C, we recorded data (copulation latency and duration) for only those flies that mated.

Molecular markers: We tested 10 *D. simulans* FC and 10 *D. mauritiana* SYN individuals for polymorphism at 53 microsatellite markers using *D. melanogaster* primers (Schug *et al.* 1998; Gockel *et al.* 2001; J. Gockel, personal communication) and 45 single-nucleotide polymorphisms (SNPs) previously developed for inbred lines of these species (Liu *et al.* 1996; Zeng *et al.* 2000). The parental lines were fixed for different alleles of 13 microsatellite and 6 SNP variants. An additional 12 informative markers were developed using PCR primers described in Zeng *et al.* (2000) and digesting PCR products with a battery of restriction enzymes. One marker [at *Su(z)*] was developed *de novo* utilizing *D. melanogaster* sequence to design primers to amplify the homologous sequence from *D. simulans* and *D. mauritiana* and identifying an informative insertion/deletion variant by direct sequencing. Table 1 lists the 32 markers, their cytological locations, and conditions for genotyping.

All BC flies from the mating behavior assays were stored at −80° in 0.5-ml eppendorf tubes. DNA was extracted from each BC individual using the Puregene (Gentra Systems, Research Triangle Park, NC) single-fly DNA extraction protocol, with minor revisions involving increased centrifugation times and pipette transfer of supernatant rather than pouring. The genotypes of the ~3000 backcross hybrids were determined for all 32 markers (*i.e.*, ~96,000 genotypes). The marker map was constructed using MapMaker.

QTL mapping: QTL for copulation latency and copulation duration were mapped in each backcross population using composite interval mapping (CIM; Zeng 1994) and implemented using QTL Cartographer software (Basten *et al.* 1999). CIM tests whether an interval between two markers contains a QTL affecting the trait while simultaneously controlling for the effect of QTL located outside the interval using multiple regression on marker cofactors. Marker cofactors were chosen by forward selection-backward elimination stepwise regression. The likelihood-ratio (LR) test statistic is $-2\ln(L_0/L_1)$, where L_0/L_1 is the ratio of the likelihood under the null hypothesis (*i.e.*, there is no QTL in the test interval) to the alternative hypothesis (there is a QTL in the test interval). LR test statistics were computed every 2 cM with marker cofactors 10 cM or more from the test location. We used permutation analysis to determine appropriate significance thresholds that take into account the multiple tests performed and correlations among markers. We permuted trait and marker data 1000 times and recorded the maximum LR statistic across all intervals for each permutation. LR statistics calculated from the original data that exceed the fiftieth greatest LR statistic from the permuted data are significant at the experimentwise 5% level under the null hypothesis (Churchill and Doerge 1994; Doerge and Churchill 1996). The approximate boundaries of regions containing QTL were determined by taking 2-LOD intervals (9.22 LR) surrounding the point of greatest significance and interpolating the cytological location of the interval on the basis of the observed amount of recombination between flanking markers.

Two methods were used to map QTL for copulation occurrence in experiments A and B. First, CIM as described above was used for the binary data, where individuals that mated were assigned a value of 1 and those that did not mate were assigned a value of 0. Second, we used an extension of CIM based on logistic regression (Xu and Atchley 1996), which assumes that the binary trait is connected to its continuous underlying liability by a threshold model (Falconer and Mackay 1996). The same window size (10 cM) and marker cofactors used for CIM were also used in the logistic model.

We estimated the effects of each QTL as the difference between heterozygous *simulans/mauritiana* genotypes and homozygous *mauritiana* genotypes at the peak LR, scaled by the phenotypic standard deviation.

We evaluated pairwise epistatic interactions between all significant QTL within each experiment using either the marker positioned at the highest LR of each QTL peak or the haplotype of the two markers flanking the QTL peak. Tests for

TABLE 1

Molecular polymorphisms discriminating *D. simulans* FC and *D. mauritiana* SYN

Marker name	Cytological location	Type	Primers	PCR T_A	Restriction endonuclease
ewg	1A	s	ATAACAGCAACCAGCGGCGG GGGCATCCATCCTCACATTGG	64°	*Acc*I
DMU56661	4F	m	TATTTCGCTAACAAACCGGC AACGCGATCACAAACATCAA	NA	NA
DELTEX	6B	m	ACGCAATAAGTTGGCGTA AATCAGGATAATGCCTAAT	NA	NA
AC004114	8E	m	TTTTATTCCAGCCATCAGGC TGCGGTCCTTTACCATAAGC	NA	NA
v	9F	s	TGTCCCTATGCAGGAAACGG TGAACAGATGCTCATCGTGC	52°	*Taq*I
DROEXO2	13F	m	TGCAGGGCACCTTCTCTCCA GAACGCTTGATTTAGATTTGGG	NA	NA
DMARIADNE	16F	m	AACACTGTCCCCATCCACAT TCTGTTCAACTCCTTCGGCT	NA	NA
run	19E	s	AGTGCATACCGAGAATCCGC ATTGATGGCGATTGCGGAGG	53°	*Bsi*EI
DROEXPAND	21C	m	GTGATCGATCCCGCTGTC TCCGGTTTCCAATTAGCTTG	NA	NA
Gpdh	26A	s	CCCCTGTTCACGGCTATTC CTGGTGATTTGATCTATGCGG	60°	*Hin*fI
AC005889	30A	m	GCGTGGCTGGCATATAG TAAGCCCCCTCGTGTAATTG	NA	NA
prd	33C	s	GATGCAAGGTGAGTGTCTATC GCCATGGGATACACGTAGCT	52°	*Tsp*509I
AC002474	38D	m	GATGCTGTCCTTCGGACTTC AACAACAAAGCCCATTCTGC	NA	NA
DucC	42C	s	AAGAGGCCACAGAGCAGC TTACCCGAGAAGATGATGGC	65°	*Alu*I
eve	46C	s	TTGTGGACCTCTTGGCCACC AACTCCTTCTCCAAGCGACC	63°	*Dra*I
Su(z)	49E	id	GTTACAACTGGAGCCGGGTA CACAATTGGATTGGGTTTCC	62°	NA
sli	52D	s	TTACCAGCTTTAAGGGCTGC CATTTGTTCTCCAGGCAAGG	50°	*Aci*I
AC004365	58A	m	GCTTTATCAATGCAGCCTCC GGCCCCAATATGTCCTCGCC	NA	NA
twi	59C	s	TCCCTGCAGCAGATCATCCC ATCACTCGAGCTGAGCATGC	63°	*Hin*fI
ve	62A	s	GAGAACCCAACGCAGAATGT ATATCCTCCGACTCCGGAAG	52°	*Pst*I
h	66D	s	ACTCAAGACTCTGATTCTGG TGTCTTCTCCAGAATGTCGG	55°	*Dra*I
CycA	68E	s	ATTTCGCCGTGCTCAATG ACGTCATGGTTCTCTTTGTCG	57°	*Hin*fI
Eip71CD	71D	s	CCTGTATGGAGCCACCCG GGGGCTGAGATTTAGCGATG	55°	*Bsm*AI
rdgC	77B	id	CAAAGACATCGACTCAGCTACG CGAACTCTCCACGATGCC	62°	NA
5-HT2	82C	s	TGACGATTCCCCTCCTCC CGCCCACTGATAGGAATTTG	52°	*Hin*fI
Antp	84B	s	ACGGACGTTGGAGTTCCCGA ACATGCCCATGTTGTGATGG	60°	*Mse*I
DROHOXNK4	93D	m	CTGAAGTTGAAGTCCGAGCC TACATGTGCTGCATCTGTTGC	NA	NA
DROTRXIII3	88B	m	GACCGTTTGTTTGCCTTGAT TGCCTGTACAAGTCTGACCG	NA	NA

(*continued*)

TABLE 1

(Continued)

Marker name	Cytological location	Type	Primers	PCR T_A	Restriction endonuclease
DMTF125	95C	m	CTCGAGCGGGCCATACAAGA TGATTGAAGAGGCCACTCAA	NA	NA
Ald	97A	s	ATGGGCCCTCACCTTCTC GTGGTCATCCACATGCAAAG	52°	*Xmn*I
DROROUGH	97D	m	AAGCAATGCCACACAATGAG CGGTTATTTTTTTTCCTTGGC	NA	NA
Efld2	100E	s	GACTGGTCTCCTCAAGCCAG AGCCTCGTGGTGCATCTC	62°	*Sfc*I

Cytological locations are given on the basis of *D. melanogaster* cytology (FlyBase Consortium 2003). The marker type is m, microsatellite; s, SNP; id, insertion/deletion. The PCR protocol for all microsatellites is 1 cycle 95°, 5 min; 5 cycles 95°, 45 sec, 68°, 5 min, 72°, 1 min, decreasing the T_A each cycle by 2°; 4 cycles 95°, 45 sec, 58°, 2 min, 72°, 1 min, decreasing the T_A each cycle by 2°; 27 cycles 95°, 45 sec, 50°, 2 min, 72°, 1 min; and 1 cycle 72° 5 min. The PCR protocol for all other markers is 1 cycle 94°, 5 min; 30 cycles 94°, 1 min, T_A°, 45 sec, 72° 1 min; 1 cycle 72°, 5 min; where T_A is listed. Primers are listed from the 5′ end. Microsatellite markers were run on a 6% polyacrylamide gel and imaged with a LICOR Gene Readir 4200 DNA analyzer. PCR fragments containing SNPs were digested with a restriction endonuclease and then run on a 3% agarose gel and manually genotyped. T_A, annealing temperature; NA, not applicable.

epistasis were calculated for the binary data with a log-linear model using PROC CATMOD and for copulation duration with an ANOVA using PROC GLM, using SAS 8.2 software. Significance thresholds were determined via a Bonferroni correction.

RESULTS AND DISCUSSION

We generated three groups of ~1000 BC individuals between *D. mauritiana* and *D. simulans* and measured components of mating behavior in tests of single BC individuals with pure-species individuals. For each BC individual we recorded whether or not copulation occurred and copulation latency (the time until copulation occurred) and copulation duration for those flies that did copulate. We genotyped all BC individuals for 32 evenly spaced molecular markers fixed for alternate alleles in the two pure species stocks and constructed a recombination map on the basis of the 3000 BC hybrids (Table 2). We performed genome scans for QTL affecting reproductive isolation in each of the BC populations, using CIM (Zeng 1994). Since copulation occurrence is a binary trait (mated or not mated), we analyzed the binary data using standard CIM and also using a logistic regression model (Falconer and Mackay 1996; Xu and Atchley 1996; Tao *et al.* 2003).

Cross A: QTL in *D. mauritiana*/*D. simulans* BC females affecting discrimination against *D. simulans* males: QTL affecting the discrimination of *D. mauritiana* females against pure-species *D. simulans* males were mapped by pairing BC females with *D. simulans* males. A total of 239 of the 1005 BC females tested mated (23.8%). At least seven QTL, all with large effects, affect female mate choice: two on the *X*, two on the second, and three on the third chromosome (Figure 1A; Table 3). An additional QTL on the third chromosome was identified using the logistic model. The magnitudes of these effects are roughly consistent with a previous study that mapped at least one factor affecting female sexual isolation to each major chromosome, with the effects of the autosomes much greater than that of the *X* chromosome (Coyne 1989). We detected no QTL for copulation latency or duration. This is expected for copulation duration since attenuated copulation is seen only in matings between *D. simulans* females and *D. mauritiana* males.

Cross B: QTL in *D. mauritiana*/*D. simulans* BC males that *D. mauritiana* females discriminate against: We mapped QTL associated with traits of male *D. simulans* against which female *D. mauritiana* discriminate by pairing *D. mauritiana* females with BC males. A total of 459 of the 1002 flies tested copulated (45.8%). At least three QTL with large effects, all on chromosome *3*, contribute to differences between males causing sexual isolation (Figure 1B; Table 3). Two additional QTL with smaller but significant effects were detected with the logistic model, one each on the *X* and third chromosomes. These data are consistent with a previous study in which at least one gene on each of the *X* and third chromosomes affected sexual isolation of male BC hybrids (Coyne 1996b). Again, no QTL for copulation latency or duration were detected. The lack of genes reducing copulation duration is expected, given the nature of the cross. However, the lack of genes for copulation latency in either this cross or cross A shows that sexual isolation occurs primarily through refusing rather than delaying copulation.

TABLE 2

Molecular markers and map positions

Marker no.	Marker names	Cytological location	*r*	Genetic distance (cM)
		X chromosome		
1	*ewg*	1A	0.0000	0.00
2	*Dmu56661*	4F	0.1663	17.29
3	*Deltex*	6B	0.0851	25.88
4	*AC004114*	8E	0.1437	40.67
5	*v*	9F	0.0691	47.62
6	*DroExo2*	13F	0.2563	75.93
7	*DroMariadne*	16F	0.1893	95.85
8	*run*	19E	0.1311	109.27
		Chromosome *2*		
9	*DroExpand*	21C	0.0000	0.00
10	*Gpdh*	26A	0.2537	27.96
11	*AC005889*	30A	0.1561	44.11
12	*prd*	33C	0.1660	61.36
13	*AC002474*	38D	0.3451	103.78
14	*DucC*	42C	0.2828	135.83
15	*eve*	46C	0.0854	144.45
16	*Su(z)*	49E	0.1219	156.89
17	*sli*	52D	0.1341	170.64
18	*AC004365*	58A	0.2571	199.06
19	*twi*	59C	0.1030	209.51
		Chromosome *3*		
20	*ve*	62A	0.0000	0.00
21	*h*	66D	0.2148	22.97
22	*CycA*	68E	0.1379	37.13
23	*Eip71CD*	71D	0.1143	48.77
24	*rdgC*	77B	0.1485	64.08
25	*5-HT2*	82C	0.0714	71.27
26	*Antp*	84B	0.0575	77.05
27	*DroHoxNK4*	93D	0.0490	81.97
28	*DroTrxIII3*	88B	0.2840	114.20
29	*Dmtf125*	95C	0.3667	161.00
30	*Ald*	97A	0.1523	176.73
31	*DroRough*	97D	0.0793	184.73
32	*Efld2*	100E	0.2060	206.63

r is the recombination rate between two adjacent markers. The genetic distance *d* was inferred from *r* using the Kosambi map function, $d = \frac{1}{4}\ln[(1 + 2r)/(1 - 2r)]$.

Cross C: QTL in *D. mauritiana*/*D. simulans* BC males that *D. simulans* females discriminate against: The genetic basis of shortened copulation between *D. simulans* females and *D. mauritiana* males was studied by pairing *D. simulans* females with BC males. The duration of copulation ranged from 0.58 to 49.35 min (SD = 7.30 min). At least six autosomal QTL (one on the second and five on the third chromosome) with moderate effects are associated with the traits in *D. mauritiana* males that cause this form of reproductive isolation (Figure 1C; Table 3). A single QTL for copulation latency mapped to the tip of chromosome *3* (Figure 1C; Table 3). Again, these results are consistent with a previous study of shortened copulation in this interspecific cross, which showed at least one gene with large effects on both the second and third chromosomes and a marginally significant contribution from the *X* chromosome (Coyne 1993).

The only diagnostic morphological difference between these species is the posterior lobe of the male genital arch, which is long and thin in *D. mauritiana* and broad and helmet shaped in *D. simulans* (Coyne 1993). It is possible that the *D. simulans* females sense the aberrant shape of the smaller *D. mauritiana* arch and use this as a cue to terminate copulation prematurely (Coyne 1993). Under this hypothesis, we expect QTL affecting copulation duration and those affecting the size and shape of the genital arch in the same interspecific backcross to colocalize. A minimum of eight QTL affect the difference in morphology of the male genital

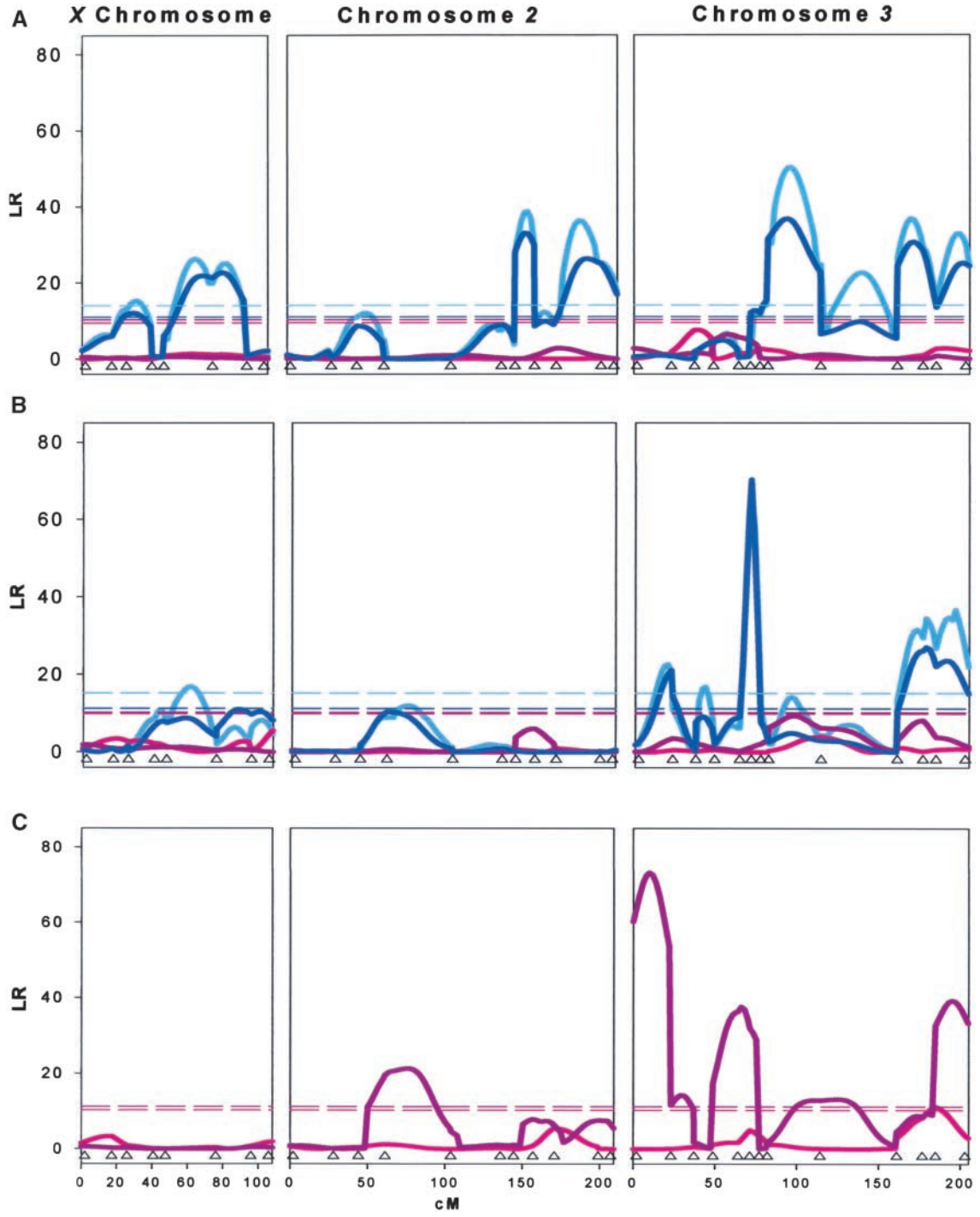

Figure 1.—QTL affecting prezygotic reproductive isolation between *D. simulans* and *D. mauritiana*. (A) BC females paired with *D. simulans* males. This group identifies the QTL in *D. mauritiana* females that affect their discrimination against *D. simulans* males. (B) BC males paired with *D. mauritiana* females. This group identifies the QTL in *D. simulans* males that *D. mauritiana* females discriminate against. (C) BC males paired with *D. simulans* females. This group identifies the QTL in *D. mauritiana* males that *D. simulans* females discriminate against. Molecular markers are indicated as open triangles on the *x*-axis. Plots are LR test statistics for copulation occurrence (dark blue, copulation occurrence, CIM; light blue, copulation occurrence, logistic model), copulation latency (pink), and copulation duration (purple) as determined by composite interval mapping. Significance thresholds for each trait were determined by permutation and are denoted by dashed lines with the same color code as the traits.

arch in backcrosses of F_1 females to *D. mauritiana* males: two on the *X* chromosome, two on the second chromosome, and four on the third chromosome (Zeng *et al.* 2000). The third chromosome QTL affecting genital morphology at 64BC–65E and 97AB–100E do indeed overlap two QTL that we detected for copulation dura-

TABLE 3

QTL affecting prezygotic reproductive isolation

		CIM				Logistic CIM			
Cross	Trait	Region	Peak	LR	Effect	Region	Peak	LR	Effect
BC females × *D. sim.* males	Copulation occurrence	5E–7F	6E	12.11	6.13	5E–8C	7B	15.32	0.709
		10F–16F	15A	22.71	8.59	9D–16F	12D	26.31	0.948
		46C–49E	47F	32.99	10.90	46C–49E	48C	38.64	1.188
		52F–59C	56B	26.25	10.88	53B–59C	55B	36.26	1.242
		88B–93F	91C	36.79	13.39	88F–93D	91B	50.26	1.382
		—	—	—	—	87B–94E	85E	22.56	0.978
		95C–97D	96D	30.67	9.70	95C–97D	96B	36.80	1.077
		97D–100E	99F	25.12	8.83	97D–100E	99E	32.92	0.977
BC males × *D. mau.* females	Copulation occurrence	—	—	—	—	10D–12F	11D	16.84	−0.725
		64D–67A	66C	21.19	−12.19	64A–66D	65F	22.67	−0.983
		—	—	—	—	69A–71B	70C	16.87	0.958
		81B–83E	82C	70.31	−17.55	79E–83E	82C	66.07	−1.349
		95D–100E	97B	27.06	−9.51	95C–100E	99C	36.70	−0.870
BC males × *D. sim.* females	Copulation duration	30A–36F	34D	21.33	0.333				
		62A–66D	64A	73.29	0.583				
		66D–68E	67D	14.28	0.306		NA		
		71D–84B	79B	37.73	0.398				
		85F–90E	87A	13.19	0.277				
		97D–100E	99A	39.37	0.401				
	Copulation latency	97C–97F	97D	11.19	0.213				

QTL regions are estimated from 2-LOD support intervals ($P \leq 0.05$). Note that there is a large inversion from 84F to 93F in relation to *D. melanogaster*. The peak is the cytological location with the highest LR. QTL effects are in phenotypic standard deviation units. NA, not applicable.

tion, at 62A–65E and 97D–100E, respectively (Table 3), but the remaining six QTL affecting genital morphology and three QTL affecting behavior map to different genomic locations. Thus, while differences in the size and shape between the genital arches of *D. mauritiana* and *D. simulans* males may provide some cue for *D. simulans* females to prematurely terminate copulation with *D. mauritiana* males, this cannot be the major cue.

Overall QTL number, effect, and location: Our results indicate that relatively few QTL (from three to seven or five to eight, depending on the model used) with moderate to large effects contribute to behavioral isolation between these species. This statement must be tempered by the usual caveat that these are minimum numbers, as larger samples and a more dense marker map would have greater power to detect QTL with smaller effects and to separate any linked genes located within significant QTL regions. However, our sample sizes of ~1000 BC individuals in each experiment are uncommonly large, and the statistical support for the mapped QTL is generally very high. It is therefore possible that a few genes with large effects account for the behavioral isolation between this species pair. The effects of all QTL were in the same direction as the difference in behavior between the pure species, but we detected too few QTL in each experiment to apply a formal statistical test for positive selection (Orr 1998).

Some models of sexual isolation via sexual selection predict that genes involved in sexual isolation should accumulate preferentially on the *X* chromosome. Sex chromosomes tend to accumulate genes creating sexually antagonistic phenotypes: those traits that that are selectively favored in one sex but disadvantageous in the other (Rice 1984), and also advantageous recessive alleles (Charlesworth *et al.* 1987). In contrast to this prediction, we found that autosomal loci had the greatest effects for all traits involved in prezygotic isolation of this species pair. A similar result was noted in previous studies of sexual isolation between these two species (Coyne 1989, 1993, 1996a,b), between *D. simulans* and *D. sechellia* (Coyne 1992), and between *D. pseudoobscura* and *D. persimilis* (Noor 1997). These results imply that sexual antagonism is not the driving force for the evolution of whatever traits yield sexual isolation.

Epistatic interactions: We tested for epistatic interactions for each significant QTL within each mapping population. The only significant interaction observed was in cross A (BC females × *D. simulans* males) between

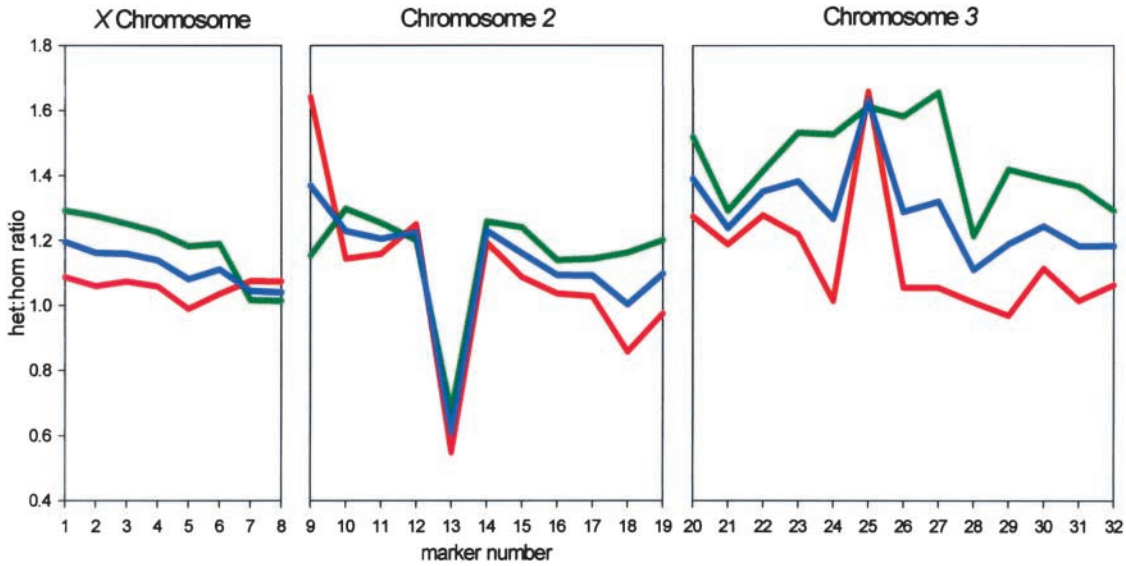

FIGURE 2.—The ratio of heterozygotes to homozygotes for BC females crossed to *D. simulans* males (red), BC males crossed to *D. mauritiana* females (green), and BC males crossed to *D. simulans* females (blue).

the markers at the tip of the third chromosome (*Ald* and haplotype *DROROUGH/Efld2*, $P < 0.0007$), but we attribute this to linkage rather than epistasis. This is in sharp contrast to QTL affecting postzygotic isolation (DAVIS *et al.* 1994; COYNE 1996a; HOLLOCHER *et al.* 1997; PRESGRAVES 2003; TAO *et al.* 2003), among which substantial epistasis occurs.

Candidate loci: The significant regions containing QTL affecting prezygotic reproductive isolation do not contain candidate genes affecting mating behavior identified by mutagenesis in *D. melanogaster* (SOKOLOWSKI 2001; FLYBASE CONSORTIUM 2003). However, we note that a polymorphism in 5-hydroxytryptamine 2 (5-HT2) was exceptionally strongly associated with the occurrence of mating when BC males were paired with *D. mauritiana* females (Figure 1B), yet the flanking markers were not significant. It is thus possible that polymorphisms at 5-HT2 cause the difference in interspecific mating behavior attributable to this QTL. The 5-HT2 gene codes for a serotonin receptor that is expressed in the central nervous system and is part of the large family of receptors that interact with G proteins. The product of 5-HT2 acts on phospholipase C (SAUDOU and HEN 1994), encoded by *no receptor potential A* (*norpA*), which been shown to affect visual and circadian behaviors as well as neurophysiology (INOUE *et al.* 1985; KANEKO *et al.* 2000). This gene is of particular interest since several other genes involved in serotonin metabolism, such as *Dopa decarboxylase* (TEMPEL *et al.* 1984) and *pale* (BUCHNER 1991), have been shown to affect mating behavior in *D. melanogaster*. In addition, we have shown that an *eagle* (*eg*) mutation in which the number of serotinergic neurons is dramatically reduced (DITTRICH *et al.* 1997; LUNDELL and HIRSH 1998) fails to complement QTL affecting variation in mating behavior between two strains of *D. melanogaster* (MOEHRING and MACKAY 2004, accompanying article, this issue).

Hybrid viability loci: We also assessed whether the markers exhibited segregation distortion in the backcross hybrids, as would be expected if they were associated with differences in viability. The ratio of heterozygotes to homozygotes is shown for each marker in Figure 2. We expected a decrease in the number of heterozygous individuals at loci that are linked to *D. simulans* genes that decrease viability when present in a *D. mauritiana* genetic background. This was observed for marker *AC002474* at cytological location 38D, implicating factors affecting viability near this locus. Remarkably, however, there were a consistently greater number of heterozygotes than homozygotes for all other genotypes. This implies heterosis for viability in interspecific hybrids, which seems counterintuitive. The most parsimonious explanation is that inbreeding depression for viability occurred independently in the *D. simulans* and *D. mauritiana* stocks during their long-term laboratory culture and that the observed heterosis in the interspecific crosses is not related to speciation.

Female discrimination *vs.* male traits being discriminated against: Comparison of the map locations of QTL for the discrimination of BC females against *D. simulans* males and QTL affecting traits in male BC hybrids against which female *D. mauritiana* discriminate allows us to address the question of whether female discrimination has the same genetic basis as the male traits that are discriminated against. The answer is clearly "no," as only one QTL region, at the tip of the third chromosome (95D–100E), appears in both analyses (Figure 1, A and B; Table 1). (The region from 10D to 12F is also implicated by the logistic analysis.) Further, this single overlapping region might not represent the same genes

for reproductive isolation in males and females, as there may be linked genes separately affecting the sexes within this region. Independent genetic architectures of female preference and preferred male traits have also been demonstrated in previous analyses of these species pairs (Coyne 1989, 1993, 1996a,b), between *D. arizonensis* and *D. mojavensis* (Zouros 1981) and between two "races" (Wu *et al.* 1995) of *D. melanogaster* (Ting *et al.* 2001). Genes causing behavioral isolation in males and females usually map to different chromosomes (as in *Ostrina nubialis*) or to different regions of the same chromosomes (Butlin and Ritchie 1989; Ritchie and Phillips 1998). This result is not unexpected; it would be surprising if interspecific differences in traits such as morphology, song, behavior, or chemistry were based on the same genes used to perceive and evaluate those traits. However, there are some cases of apparent genetic coupling between male traits and female preferences for those traits (Hoy *et al.* 1977; Ritchie 1992).

QTL in male *D. simulans vs.* male *D. mauritiana*: We mapped three QTL affecting traits in male BC hybrids against which *D. mauritiana* females discriminate prior to copulation and six QTL affecting traits in male BC hybrids against which *D. simulans* females discriminate after copulation. QTL mapped to the third chromosome in both experiments, with regions of overlap at 64D–67A, 81B–83E, and 97D–100E (Figure 1, B and C; Table 3). Additional regions on the second (34C–35F) and third (90C–90E) chromosomes showed overlap with the logistic analysis. To assess whether this overlap between the two experiments was greater than expected by chance, we computed the fractions of the third chromosome encompassed by the QTL mapped in each experiment, assuming for simplicity that each of the 240 cytological subdivisions on the third chromosome is the same physical size. The four QTL affecting *D. simulans* male traits together cover 25.4% of chromosome *3* and the four QTL affecting *D. mauritiana* male traits together cover 68.8% of chromosome *3*. We therefore expect $25.4 \times 68.8 = 17.5\%$ overlap in QTL locations between the two experiments by chance. Since the observed regions of overlap uncovered 20.4% of the third chromosome, we conclude that it is unlikely that these two isolating barriers involve the same genes.

Civetta and Cantor (2003) mapped a single QTL affecting differences in copulation duration between *D. simulans* and *D. sechellia* to 84A–86B on chromosome *3*, which overlaps the QTL affecting copulation duration that we detected between *D. simulans* and *D. mauritiana* from 85F–86B. Similar calculations to those described above indicate that the amount of QTL overlap is less than expected by chance. Thus, it does not appear that mutations at a common set of genes affect behavioral isolation between *D. simulans* and these two sibling species. Our observation that relatively few QTL with moderate to large effects underlie prezygotic reproductive isolation between *D. simulans* and *D. mauritiana* is a favorable scenario for future positional cloning of genes responsible for behavioral reproductive isolation.

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