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The Genetic Basis of Prezygotic Reproductive Isolation Between *Drosophila santomea* and *D. yakuba* Due to Mating Preference

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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 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 santomea* and *D. yakuba*. We mapped at least three QTL affecting discrimination of *D. santomea* females against *D. yakuba* males: one *X*-linked and one autosomal QTL affected the likelihood of copulation, and a second *X* chromosome QTL affected copulation latency. Three autosomal QTL also affected mating success of *D. yakuba* males with *D. santomea*. No epistasis was detected between QTL affecting sexual isolation. The QTL do not overlap between males and females and are not disproportionately concentrated on the *X* chromosome. There was some overlap in map locations of QTL affecting sexual isolation between *D. santomea* and *D. yakuba* with QTL affecting sexual isolation between *D. simulans* and *D. mauritiana* and with QTL affecting differences in pigmentation between *D. santomea* and *D. yakuba*. Future high-resolution mapping and, ultimately, positional cloning, will reveal whether these traits do indeed have a common genetic basis.

DESPITE the probable importance of sexual isolation as a primary reproductive barrier during the process of speciation (Coyne and Orr 2004), we know relatively little about the genetic basis of interspecific mate discrimination. Yet such genetic studies can answer important questions about speciation. Is sexual isolation based on few genes or many? If many, do a few genes contribute to most of the sexual isolation? Do "mate discrimination" genes tend to occur in similar regions of chromosomes among different species in the same group, implying that sexual isolation may involve identical genes in different speciation events? Do the same chromosome regions (and possibly the same genes) contribute to mate preference in males and females? Finally, what is the normal function of genes involved in sexual isolation? This last question can be answered only by identifying those genes, an endeavor that must begin by their fine-structure localization.

Previous studies of prezygotic isolation in Drosophila have mapped genes affecting sexual isolation to whole chromosomes, chromosome arms, or large sections of chromosomes (Zouros 1981; Coyne 1989, 1993, 1996a,b; Wu *et al.* 1995; Noor 1997; Ting *et al.* 2001; Williams *et al.* 2001; Gleason and Ritchie 2004; Takahashi and Ting 2004), but so far there have been only a few studies localizing quantitative trait loci (QTL) affecting sexual isolation between species with high resolution by linkage to molecular markers (Civetta and Cantor 2003; Moehring *et al.* 2004)—the first step toward positional cloning of candidate loci. Here, we report the results of mapping QTL causing sexual isolation between two sister species, *Drosophila yakuba* and *D. santomea*, using 32 species-specific molecular markers to localize chromosome regions involved in mate discrimination. *D. yakuba* is widespread across sub-Saharan Africa and on islands near the continent, inhabiting open areas such as savannas, montane grassland, and, in human-colonized areas, disturbed habitats such as plantations and cut-over fields. *D. santomea*, discovered in 1998, is endemic to São Tomé, an 860-km^2 volcanic island 255 km off the coast of Gabon (Lachaise *et al.* 2000), where it inhabits only montane rain and mist forests. *D. yakuba* also inhabits São Tomé. On the mountain of Pico de São Tomé, *D. yakuba* lives at elevations

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below 1450 m, while *D. santomea* lives at elevations above 1150 m. Between these elevations, the species' ranges overlap at an ecotone between plantations and virgin rain forest, forming a hybrid zone in which one finds a low frequency (~1%) of hybrids (Lachaise *et al.* 2000; Llopart *et al.* 2005). Molecular evidence puts the divergence between *D. yakuba* and *D. santomea* at ~400,000 years ago (Cariou *et al.* 2001; Llopart *et al.* 2002).

The species show substantial sexual isolation in the laboratory (Coyne *et al.* 2002, 2005; Llopart *et al.* 2002): the two interspecific matings occur less frequently than intraspecific matings, with the mating between *D. santomea* females and *D. yakuba* males occurring very rarely. In this latter pairing, *D. yakuba* males court *D. santomea* females persistently, but are usually rejected. In the reciprocal pairing, weaker sexual isolation is evinced by *D. santomea* males courting *D. yakuba* females less ardently than conspecific females (Coyne *et al.* 2005). Thus most sexual isolation is due to discrimination against some traits of *D. yakuba* by *D. santomea* females. There is no enhanced sexual isolation ("reinforcement") between strains of these species taken from the area of sympatry (Coyne *et al.* 2002).

MATERIALS AND METHODS

Drosophila strains: All flies were maintained in 8-dram vials containing standard cornmeal-agar-Karo media on a 12:12 hr light:dark cycle at 24°. We used a strain of *D. yakuba* named Taï18, an isofemale line collected by D. Lachaise in 1983 in the Taï rainforest on the border between Liberia and the Ivory Coast. *D. yakuba* Taï18 contains a polymorphic inversion on the second chromosome (*2Rn*) that distinguishes the species from *D. santomea*. We therefore eliminated this inversion from the stock to make it homosequential to *D. santomea*, enhancing our mapping capabilities. We inbred 30 lines of Taï18 by full-sib mating for seven generations. We tested these inbred lines for the presence of the inversion by crossing the inbred *D. yakuba* Taï18 males to *D. santomea* females and observing whether or not the polytene chromosomes of F_1 larvae contained the loops diagnostic of inversion heterozygosity. We saved stocks in which all F_1's lacked the inversion and thus were homosequential to *D. santomea*. Four of these inbred lines were intercrossed in equal numbers to create the *D. yakuba* ST strain used in this study. *D. santomea* STO.4 is an isofemale line collected in March of 1998 in the Obo Natural Reserve on São Tomé at 1300 m altitude (Lachaise *et al.* 2000). Further description can be found in Llopart *et al.* (2002).

Cytology: The two species strains are homosequential, but their exact cytology in relation to *D. melanogaster* is not known, hindering our ability to accurately define QTL boundaries and candidate loci within QTL regions. Therefore, we determined the cytology of *D. yakuba* in relation to *D. melanogaster* by BLASTing (http://www.ncbi.nlm.nih.gov/BLAST/) 6-kb pieces of the *D. yakuba* genome (Release 1.0, April 2004; http://genome.ucsc.edu/cgi-bin/hgGateway) to *D. melanogaster* every 100 kb, for example, base pairs 100,000–106,000 of the right arm of chromosome *2*. Occasionally it was necessary to use larger stretches of sequence or sequence closely adjacent to the 100-kb interval due to transposon insertions, microsatellites, etc. We also visually inspected the projected base pair alignment (http://genome.ucsc.edu/cgi-bin/hgGateway) for any genomic segments ≥5 kb that were out of order.

TABLE 1

Sexual isolation between pure *D. yakuba* (ST strain) and *D. santomea* (STO.4 strain)

Pairings			
Female	Male	% mated	Mean CL (SE)
D. yakuba	*D. yakuba*	85.6	15.28 (1.07)
D. yakuba	*D. santomea*	47.8	22.23 (1.53)
D. santomea	*D. yakuba*	14.4	14.38 (2.26)
D. santomea	*D. santomea*	71.1	10.32 (0.93)
BC *santomea*	*D. yakuba*	NA	14.97 (0.49)
D. santomea	BC *yakuba*	NA	18.70 (0.50)

Ninety pairs of flies were watched for 45 min for each of the four pairings of pure species. Females from the backcross to *D. santomea* were observed until 50% had mated and males from the backcross to *D. yakuba* were observed for 45 min, when ~50% had mated. Each pair was observed individually. We recorded whether or not mating occurred during that period, and, if so, the mean copulation latency (CL) of the matings. Mean copulation latencies and their standard errors (SE) are given in minutes.

Crosses: The sexual isolation between these species has been described extensively (Coyne *et al.* 2002, 2005). Heterospecific matings occur less often than conspecific matings, with a particular dearth of matings between *D. santomea* females and *D. yakuba* males (this lack of mating occurs despite ardent courtship by the *D. yakuba* males and thus is due largely to discrimination by *D. santomea* females). Table 1 shows the extent of discrimination between pure lines, demonstrating the typical pattern of sexual isolation described above. For matings that do occur, the copulation latency (time from introduction of flies into observation vials until copulation takes place) is particularly long in the mating between *D. yakuba* females and *D. santomea* males.

The strong sexual isolation between *D. santomea* females and *D. yakuba* males suggested that the genetics of sexual isolation in females should be studied by pairing pure *D. yakuba* males with *D. santomea*/*D. yakuba* backcross (BC) females (F_1 hybrid males are sterile, so that individuals of mixed genotype must be generated in backcrosses) and the genetics of sexual isolation in males should be studied by pairing pure *D. santomea* females with *D. santomea*/*D. yakuba* BC males. Preliminary experiments and previous data (Coyne *et al.* 2002) showed that sexual isolation of mixed genotypes was strongest in males from the backcrosses of F_1 hybrid females to *D. yakuba* males and strongest in females from the backcross of F_1 hybrid females to *D. santomea* males.

To produce F_1 females, 4-day-old virgin *D. yakuba* ST females were crossed to *D. santomea* STO.4 males. Virgin F_1 females were then backcrossed in two ways: (A) to *D. santomea* STO.4 males, producing 535 BC females, or (B) to *D. yakuba* ST males, producing 539 BC males. BC females are either homozygous *D. santomea* or heterozygous *D. yakuba*/*D. santomea* and have mitochondrial DNA from *D. yakuba*. BC males are autosomally either homozygous *D. yakuba* or heterozygous *D. yakuba*/*D. santomea*, the *X*-linked loci are either pure *D. yakuba* or *D. santomea*, and the *Y* chromosome and mitochondrial DNA are from *D. yakuba*.

Mating behavior: Two 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. yakuba* ST males and (B) BC males and *D. santomea* STO.4 females. Experiment A reveals the QTL in BC females that lead to lack

of mating with *D. yakuba* males. Since *D. yakuba* males mate readily with conspecific females in the assay time period, we presume that heterospecific alleles at QTL (*i.e.*, those for *D. santomea* female mating behavior) are the likely cause of reduced mating success by *D. yakuba* males. Experiment B reveals QTL in BC males that lead to lack of mating with *D. santomea* females. Again, heterospecific alleles (*i.e.*, those for *D. yakuba* male mating behavior) are the likely cause of this reduced mating success.

The BC flies were collected as virgins and sorted by sex under brief CO_2 exposure and kept in uncrowded vials for 4 days before use in experiments. Four-day-old virgin BC and pure-species flies were transferred by aspiration to 8-dram 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°. Forty pairs of flies were watched during each observation session. For flies in experiment A, we watched flies for a variable period until about half of them had mated; this period varied from 20 to 57 min. We watched flies from experiment B for a constant period of 45 min, which also yielded an ~50% frequency of copulation. In both experiments, flies that did not mate were given a mating score of "0," while those that mated were given a score of "1." For those flies that did copulate, copulation latency (time from introduction of flies into vials to copulation) was recorded in minutes; individuals that did not mate were not used in the analysis of copulation latency.

Molecular markers: We used the same 32 strain-specific markers and conditions for genotyping described in Carbone *et al.* (2005), with two exceptions. We included *Ngp* between *Sara* and *Kr* and excluded *janB* between *ymp* and *krz*. The forward and reverse primers for *Ngp* were, respectively, 5′-AGAACAATTGGCCCAAAAGA-3′ and 5′-CCTCGGATCTAGCAT CTTCG-3′. The primer annealing temperature for this marker was 55°, and the nucleotide difference was detected as a restriction length polymorphism following digestion with *Bam*HI.

All BC flies from the mating behavior assays were stored at −80° in 0.5-ml Eppendorf tubes. Genomic 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. Genotyping was performed using restriction fragment length polymorphism analysis by PCR amplification from genomic DNA, using RedTaq DNA Polymerase (Sigma, St. Louis) followed by restriction enzyme digestion (see Carbone *et al.* 2005 for primers, restriction enzymes, and conditions). Digested products were run on a 3% agarose gel stained with ethidium bromide, imaged with the Bio-Rad ChemiDoc System PC RS-170 and Quantity One software (version 4.2.1), and manually genotyped. Individuals were scored as a 0 if homozygous and a 1 if heterozygous. The genotypes of the 1074 backcross hybrids were determined for all 32 markers (*i.e.*, 34,368 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), 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 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 50th 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). We estimated the effects of each QTL as the difference between heterozygous *yakuba/santomea* genotypes and homozygous pure-species genotypes at the peak LR, scaled by the phenotypic standard deviation. 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 by dividing the cytology within the region according to the observed amount of recombination between flanking markers.

Although the assumption of normality when calculating CIM is violated by the analysis of the binary trait of copulation occurrence, a previous study (Moehring *et al.* 2004) has shown that using 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), reveals the same QTL peaks as those found with CIM.

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 epistasis were calculated for the binary trait of copulation occurrence with a log-linear model using PROC CATMOD and SAS 8.2 software (SAS Institute, Cary, NC). Significance thresholds were determined via a Bonferroni correction.

RESULTS AND DISCUSSION

Cytology: The chromosome band order in *D. yakuba* differs from *D. melanogaster* by many inversions and translocations (Ashburner 1989). We used publicly available sequences of these species to define the cytological differences more precisely. The *D. yakuba* cytology relative to *D. melanogaster* is given at 100-kb intervals in supplemental Table 1 (http://www.genetics.org/supplemental/), the exact base pair breakpoints of each cytological segment are given in supplemental Table 2 (http://www.genetics.org/supplemental/), and an overall comparison between *D. yakuba* and *D. melanogaster* genomes is given in Figure 2.

The cytological order in *D. yakuba* in relation to *D. melanogaster* is as follows, where "*" denotes the centromere: *X* chromosome (also see Figure 2), |1A1–2B14|11A9–11A1|5D3–6D7|5C5–2B14|11A9–15A1|10C10–9F5|19B3–18B1|8D9–6D7|5C5–5D3|11A1–10C10|15A1–18B1|8D9–9F5|19B3–20E|*; chromosome *2*, |21A2–25B1|28D2–26B8|31E2–34E2|25C10–25B2|28D3–31E2|26B8–25C10|34E2–35B8|42B2–47A9|35F11–35B8|42A15–41F7|*|40F1–39E1|36A2–36D3|36A2–35F12|47A9–47F3|38F2–39D4|

36D2–38D2|50D4–47F3|38F1–38D2|50D3–51F7|58C1–54C1|58E1–59B2|53E1–51F8|58C1–58E1|54C1–53E1|59B2–60F5|; and chromosome *3*, |61A5–63D4|67C5–66B11|63B8–66B11|63B8–62D4|67C5–71B5|75E2–72F1|71B5–72D7|78F4–75E2|72F1–72D7|78F4–80C4|*|82A1–83B1|90A6–93F2|84E9–84A1|84E10–86E13|89F1–89D2|87A2–88A3|89D2–88A3|87A2–86E13|89F1–90A6|83B1–84A1|93F2–95C13|99D1–95C13|99D1–100D1|. Note that the molecular cytology differs substantially from that inferred from observations of banding patterns of salivary chromosomes (Ashburner 1989).

Molecular marker map: We genotyped 1074 *D. yakuba/D. santomea* backcross hybrids for 32 molecular markers. The cytological locations of the markers (relative to *D. melanogaster*), recombination rates, and map distances are given in Table 2. Note that the map is expanded relative to *D. melanogaster*—by 21, 37, and 60%, respectively, for the *X*, second, and third chromosomes. Increased recombination rates relative to *D. melanogaster* have also been previously observed for the sibling species *D. simulans* and *D. mauritiana* (Ashburner 1989; Liu *et al.* 1996; Zeng *et al.* 2000; Moehring *et al.* 2004) and *D. simulans* and *D. sechellia* (Liu *et al.* 1996; Macdonald and Goldstein 1999; Civetta and Cantor 2003; Gleason and Ritchie 2004).

We assessed whether the markers exhibited segregation distortion, as would be expected if they were associated with differences in hybrid viability (Table 2). Although the proportion of heterozygotes was significantly skewed, averaged over all loci ($\chi^2_{31} = 75.32$, $P \leq 0.001$), no particular marker was especially deviant. It is interesting to note that there was a tendency toward an increased number of homozygotes for the *X* chromosome (Table 2), while there was a tendency toward an increased number of heterozygotes for autosomes. The *X* chromosome data are consistent with multiple polygenic *X*-linked loci, each contributing to small reductions in hybrid viability. The excess of heterozygotes on the autosomes may be due to inbreeding depression for viability that occurred in the parental species strains during long-term laboratory maintenance, so that the observed heterosis in the interspecific crosses is not related to speciation. The same explanation was previously proposed to account for the genomewide excess of heterozygotes in *D. simulans/D. mauritiana* backcross hybrids (Moehring *et al.* 2004).

QTL for mating behavior: We mapped QTL affecting the discrimination of BC to *D. santomea* females against pure-species *D. yakuba* males. We detected two additive QTL affecting copulation occurrence, one on the *X* chromosome and one with large effect on chromosome *3* (Table 3, Figures 1A and 2). One QTL for copulation latency mapped to the *X* chromosome in this hybridization (Table 3, Figures 1A and 2), but it should be noted that our power to detect QTL for copulation latency is lower than that for copulation occurrence since only half of the individuals mated and had latency scores.

TABLE 2

Molecular markers and map positions

Chromosome	Marker	Cytological location	*r*	*d*	Prop. het.
X	*y*	1A5	0.0000	0.00	0.4802
	per	3B1–2	0.0255	2.62	0.4877
	sog	13E1	0.1316	17.89	0.4790
	v	9F11	0.0683	25.23	0.4742
	rux	5D2	0.2131	53.01	0.4788
	f	15F4–7	0.0643	59.89	0.4993
	bnb	17D6	0.0800	68.61	0.4998
	Hex-A	8E10	0.0366	72.41	0.4965
	AnnX	19C1	0.0453	77.16	0.4956
	su(f)	20E	0.0255	79.77	0.4986
2	*l(2)gl*	21A5	0.0000	0.00	0.5228
	Rad1	23A1	0.0623	6.65	0.5275
	RpL27A	24F3	0.0637	13.47	0.5285
	salr	32E4–F1	0.1371	29.49	0.5376
	Rep4	34B4	0.0722	37.29	0.5527
	His3	39D3–E1	0.1679	57.75	0.5558
	barr	38B1–2	0.0464	62.62	0.5546
	Sara	57E6	0.0994	73.70	0.5543
	Ngp	54C8	0.1409	90.25	0.5432
	Kr	60F5	0.3506	150.65	0.5134
3	*Lsp1γ*	61A6	0.0000	0.00	0.5129
	dib	64A5	0.1631	19.74	0.5199
	sfl	65B3–4	0.1168	33.04	0.5281
	Est-6	69A1	0.2273	63.36	0.5108
	Ssl1	80B2	0.2086	90.35	0.5346
	ry	87D9	0.1036	101.96	0.5277
	Rpn5	83C4	0.1612	121.42	0.5417
	AP-50	94A15–16	0.0803	130.17	0.5311
	Mlc1	98A14–15	0.1697	150.90	0.5288
	ymp	96E	0.0858	160.32	0.5261
	krz	100E3	0.1418	176.99	0.5019
4	*ci*	102A1–3	0.0000	0.00	0.4984

The order of the markers in the first column reflects their relative positions in *D. yakuba* and *D. santomea*, inferred from the *D. yakuba* genome project (Release 1.0, April 2004; http://www.genome.wustl.edu/projects/yakuba/). Cytological locations are given on the basis of *D. melanogaster* cytology (Lemeunier and Ashburner 1976). *r*, the recombination rate between two adjacent markers; *d*, the genetic distance inferred from *r* using the Haldane map function [$d = -\frac{1}{2}\ln(1 - 2r)$]; Prop. het, the proportion of genotypes that were heterozygous for that marker.

These results are consistent with those of Coyne *et al.* (2002), in which hybrid females actually have lower copulation latencies in tests to males of both species than do conspecific females.

We mapped QTL affecting the discrimination of BC to *D. yakuba* males against pure-species *D. santomea* females. We detected three autosomal QTL, one on the second chromosome and two on chromosome *3* affecting copulation occurrence (Table 3, Figures 1B and 2). These QTL displayed additive gene action, as

TABLE 3

QTL affecting copulation occurrence and latency between *D. yakuba* and *D. santomea*

BC cross	Trait	QTL	Peak	LR	Effect	Effect/σ_p	R^2
F_1 females × *D. santomea* males	BC female mating to *D. yakuba* male	1A–3B	4B	13.42	−0.21	−9.52	0.0225
		82A–88B	85E	48.21	−0.30	−14.06	0.0908
F_1 females × *D. santomea* males	Latency to BC female mating *D. yakuba* male	7D–16D	10E	11.81	4.91	0.42	0.0439
F_1 females × *D. yakuba* males	BC male mating to *D. santomea* female	48D–50D	48A	10.22	−0.16	−7.44	0.0188
		68A–73E	69A	12.73	−0.16	−7.60	0.0200
		97D–95C	96E	14.67	−0.16	−7.22	0.0231

QTL regions are estimated from 2 LOD support intervals ($P \leq 0.05$) and the cytological locations were extrapolated from recombination rate between markers in comparison to the *D. yakuba* cytological map. The peak is the cytological location with the highest likelihood ratio (LR). Effects were estimated from the least-squares means of the two genotype classes as [homozygous − heterozygous] and are also given scaled by the phenotypic standard deviation (σ_p). R^2 is the proportion of the variance accounted for the QTL and is estimated by $R^2 = (s_0^2 - s_1^2)/s^2$, where s^2 is the variance of the trait, s_0^2 is the sample variance of the residuals, and s_1^2 is the variance of the residuals (Basten *et al.* 1999).

there was no evidence for pairwise epistasis between significant QTL. We did not detect any QTL affecting variation in copulation latency in this mapping population. Coyne *et al.* (2002) evaluated the mating success of reciprocal F_1 *D. santomea/D. yakuba* male hybrids paired with both pure species. Hybrid males with a *D. yakuba X* chromosome mated as frequently to either *D. santomea* or *D. yakuba* females as did conspecific males, indicating a negligible effect of the *X* chromosome on mating occurrence, as observed here. However, Coyne *et al.* (2002) also observed that hybrid males with a *D. santomea X* chromosome had reduced mating success with both *D. yakuba* and *D. santomea* females. One interpretation of this unusual result is that QTL on the *D. santomea X* chromosome interact epistatically with QTL on the *D. yakuba* autosomes, causing behavioral sterility. While this does not appear to be the case—no significant epistatic interactions were detected between *X* chromosome markers with autosomal markers (after Bonferroni correction)—it is possible that increased sample size would increase the power to detect these interactions. Our observation that no QTL for copulation latency were detected in pairings of backcross males to *D. santomea* females is also consistent with the previous observations of Coyne *et al.* (2002).

Comparison with other studies: Previously, we mapped QTL affecting sexual isolation in the *D. simulans/D. mauritiana* hybridization and observed at least seven QTL affecting traits in *D. mauritiana* females leading to reduced mating success with *D. simulans* males and at least three QTL in *D. simulans* males leading to reduced mating success with *D. mauritiana* females (Moehring *et al.* 2004). We can thus compare numbers, effects, locations, and coincidence of QTL across both studies to gain insight regarding the genetic basis of interspecific sexual isolation. In both cases, relatively few QTL, with moderate to large effects, contribute to behavioral isolation between species. It is thus possible that a few genes with relatively large effects generally account for sexual isolation. This statement must be tempered with the usual caveat that more QTL could be detected with larger numbers of backcross individuals and a greater density of markers.

In contrast to predictions of some models of sexual isolation via sexual selection, which postulate preferential accumulation of genes affecting sexual isolation on the *X* chromosome (Rice 1984; Charlesworth *et al.* 1987), we observed that autosomal loci had the greatest effects on most traits involved in prezygotic isolation for both the *D. santomea/D. yakuba* and *D. simulans/D. mauritiana* hybridizations. Similar results were also obtained in studies of sexual isolation between *D. simulans* and *D. sechellia* (Coyne 1992) and between *D. pseudoobscura* and *D. persimilis* (Noor 1997).

Another emerging theme from several interspecific hybridizations is that genes affecting sexual isolation are not the same in males and females. None of the QTL detected for female and male mating success overlapped in the *D. santomea/D. yakuba* hybridizations reported here, a finding previously observed for sexual isolation between *D. simulans/D. mauritiana* (Coyne 1989, 1993, 1996a,b; Moehring *et al.* 2004), *D. arizonensis/D. mojavensis* (Zouros 1981), and two "races" of *D. melanogaster* (Ting *et al.* 2001). This is also often true for sexual isolation in other species (Butlin and Ritchie 1989; Ritchie and Phillips 1998) and indicates that the complex genetic architecture underpinning the many morphological, behavioral, and chemical signals used in courtship does not typically overlap the genetic architecture required to perceive and evaluate these signals. There are, however, some instances in which male traits and female preference for the traits are genetically coupled (Hoy *et al.* 1977; Ritchie 1992; Marcillac *et al.* 2005).

Is it possible that the same genetic mechanisms contribute to sexual isolation in independent speciation

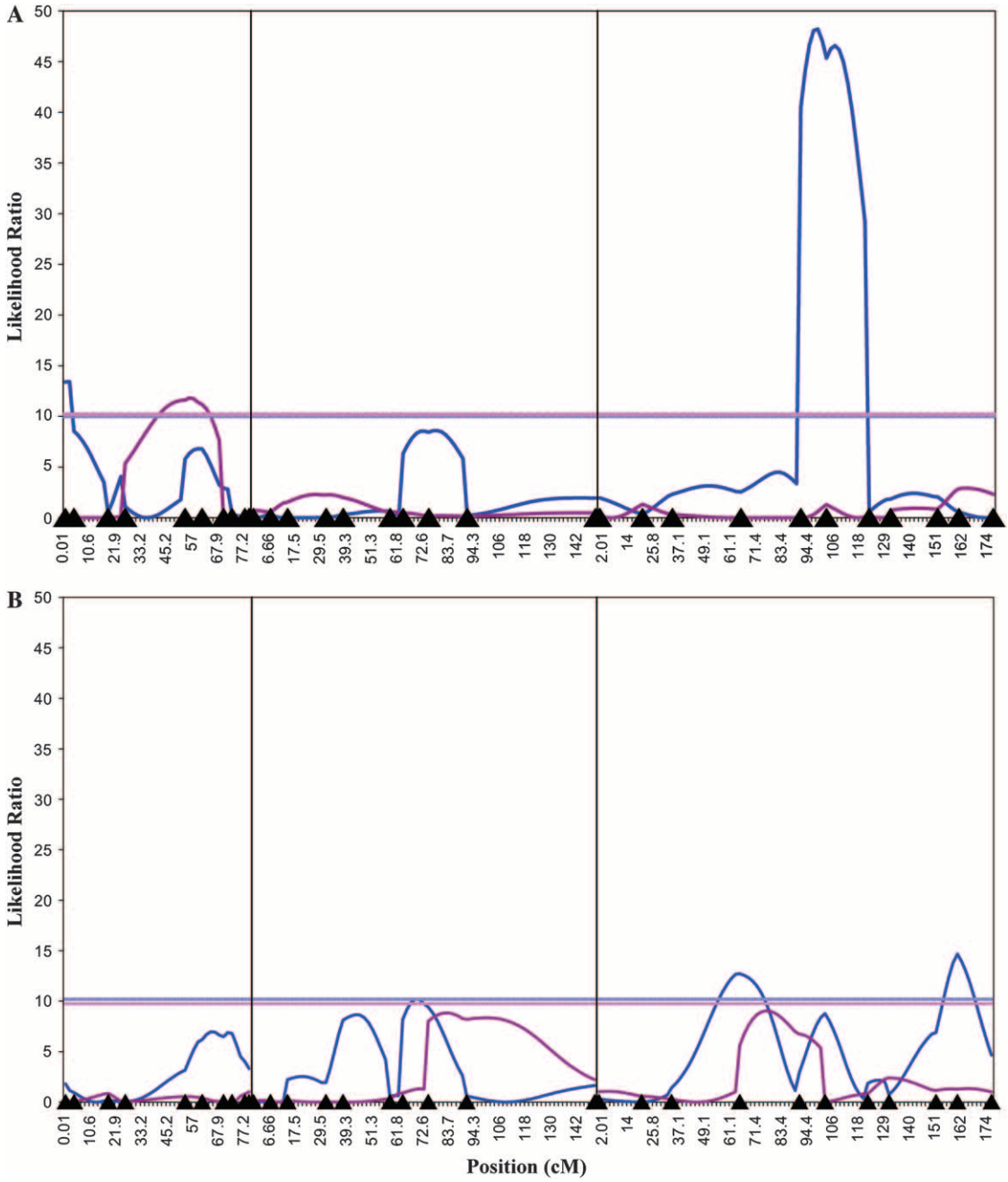

FIGURE 1.—QTL for the *X*, second, and third chromosomes affecting copulation latency (time to copulation) and occurrence (whether or not copulation occurred) in backcross hybrids between *D. yakuba* and *D. santomea*. There were no QTL for the small fourth chromosome. (A) F_1 females backcrossed to *D. santomea* males. Resulting females are tested against *D. yakuba* males. (B) F_1 females backcrossed to *D. yakuba* males. Resulting males are tested against *D. santomea* females. Plots are the likelihood-ratio (LR) test statistics for copulation latency (purple) and copulation occurrence (blue) as determined by composite interval mapping. The significance thresholds were determined by permutation testing, are represented by correspondingly colored dashed horizontal lines, and are all approximately LR = 10. Marker locations are represented by black triangles on the *x*-axis and are in the same order from left to right as the order listed in MATERIALS AND METHODS: *y*, *per*, *sog*, *v*, *rux*, *f*, *bnb*, *Hex-A*, *AnnX*, *su(f)*, *l(2)gl*, *Rad1*, *RpL27A*, *salr*, *Rep4*, *His3*, *barr*, *Sara*, *Ngp*, *Kr*, *Lsp1γ*, *dib*, *sfl*, *Est-6*, *Ssl1*, *ry*, *Rpn5*, *AP-50*, *Mlc1*, *ymp*, and *krz*. Note that markers are spaced according to recombination distance.

events? We can address this question by comparing the locations of QTL affecting female and male mating success in the *D. simulans*/*D. mauritiana* and *D. santomea*/*D. yakuba* hybridizations. If QTL affecting sexual isolation do not colocalize, then we can rule out a common genetic basis. On the other hand, colocalization does not necessarily correspond to common genetic mechanisms, which must be addressed by further high-resolution mapping in both species pairs. One of the two QTL affecting female sexual isolation in the *D. santomea*/*D. yakuba* hybridization

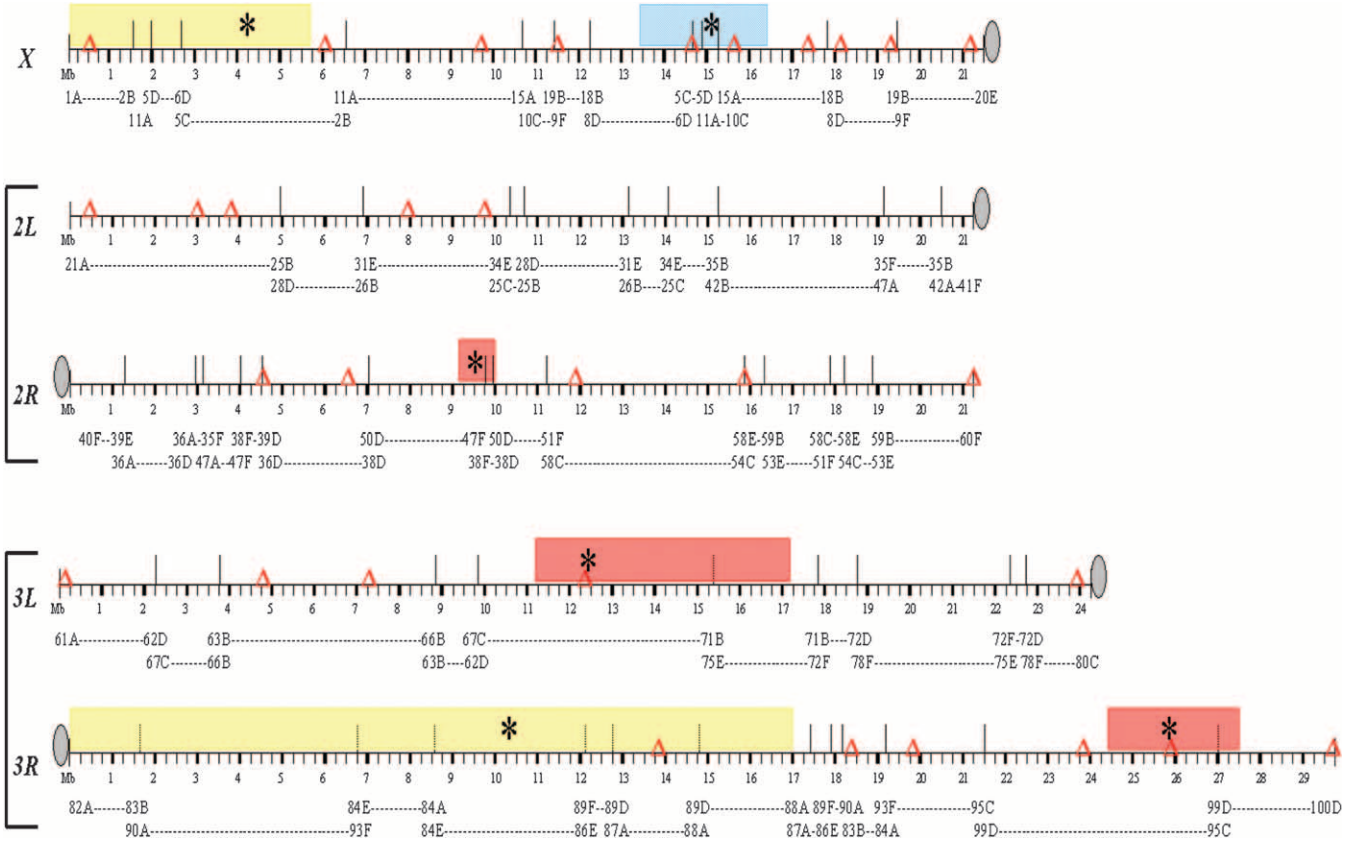

FIGURE 2.—The significant regions from QTL mapping when compared to *D. melanogaster* cytology. Short vertical lines below the horizontal are every 250 kb; the short thick lines are every 1 Mb. Tall vertical lines above the horizontal represent the inversion/translocation breakpoints. Colored boxes represent QTL regions: BC female copulation occurrence (yellow) and latency (blue) when mated to *D. yakuba* males and BC male copulation occurrence (red) when mated to *D. santomea* females. "*" indicates the peak of the QTL. Centromeres are represented by gray circles. Open red triangles represent markers, with the markers in the same order as listed in MATERIALS AND METHODS: *y*, *per*, *sog*, *v*, *rux*, *f*, *bnb*, *Hex-A*, *AnnX*, *su(f)*, *l(2)gl*, *Rad1*, *RpL27A*, *salr*, *Rep4*, *His3*, *barr*, *Sara*, *Ngp*, *Kr*, *Lsp1*γ, *dib*, *sfl*, *Est-6*, *Ssl1*, *ry*, *Rpn5*, *AP-50*, *Mlc1*, *ymp*, and *krz*. Note that markers are spaced according to base pair distance.

(82A–88B) does overlap with one of the seven QTL affecting female sexual isolation in the *D. simulans/D. mauritiana* hybridization (88B–93F), although the QTL peaks do not coincide (85E *vs.* 91C, respectively). In addition, two of the three QTL affecting male sexual isolation between *D. santomea* and *D. yakuba* (68A–73E, peak LR at 69A; and 95C–97D, peak LR at 96E) also affect male sexual isolation between *D. simulans* and *D. mauritiana* (69A–71B, peak LR at 70C; and 95D–100E, peak LR at 97B). However, the male traits are not identical in the two experiments. Here, we mapped QTL affecting reluctance of *D. yakuba* males to court *D. santomea* females; whereas MOEHRING *et al.* (2004) mapped QTL affecting male *D. simulans* traits against which female *D. mauritiana* discriminated. Nevertheless, we note that the 95–100 region on chromosome *3* has been repeatedly implicated in studies mapping QTL for sexual behavior in Drosophila (MOEHRING and MACKAY 2004; MOEHRING *et al.* 2004; this article). A positional candidate gene in this region, *E(Spl)*, both fails to complement QTL affecting variation in male mating behavior (MOEHRING and MACKAY 2004) and exhibits altered transcript abundance between lines selected for increased and decreased copulation latency (MACKAY *et al.* 2005).

Finally, we can ask to what extent QTL affecting sexual isolation overlap those affecting the large morphological difference in abdominal pigmentation between *D. santomea* and *D. yakuba* (LACHAISE *et al.* 2000; LLOPART *et al.* 2002; CARBONE *et al.* 2005). *D. santomea* is completely devoid of any pigmentation while *D. yakuba* shows the sexually dimorphic pattern typical of the *D. melanogaster* group: females' yellow abdomens are striped with black, while those of males have black tips. In addition to this being a major morphological difference between these two species, pigmentation has also been shown previously to have an impact on mating success (*e.g.*, STURTEVANT 1915; BASTOCK 1956). We find that the single QTL at 15F4–20E affecting pigmentation in (*D. yakuba/D. santomea* F_1 ♀ × *D. santomea* ♂) BC hybrid females (CARBONE *et al.* 2005) overlaps the single QTL at 7D–16E affecting copulation latency for these females paired with *D. yakuba* males mapped in

this study. In addition, the two autosomal QTL at 34B4–57E6 and 69A1–83C4 affecting pigmentation in (*D. yakuba*/*D. santomea* F_1 ♀ × *D. yakuba* ♂) BC hybrid males (Carbone *et al.* 2005) overlap two of the QTL at 48D–50D and 68A–73E, affecting copulation success of these males paired with *D. santomea* females mapped in this study. These observations raise the interesting hypothesis that common genes may underlie the evolution of the morphological and behavioral differences between these species. Llopart *et al.* (2002) have shown that any effect of pigmentation on sexual isolation in this species pair is not due to a visual cue, since there was no difference in measures of sexual isolation between these species determined under light and dark conditions. It remains possible, however, that genes affecting pigmentation may have pleiotropic effects on other behavioral traits, such as locomotion, that could be a component of mating behavior. Alternatively, it is possible that selective sweeps at pigmentation loci resulted in fixation of chromosomally linked regions containing genes affecting mating preference. These hypotheses can be distinguished by jointly mapping QTL affecting pigmentation and mating behavior in backcross hybrids.

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