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The Genetic Basis of Postzygotic Reproductive Isolation Between *Drosophila santomea* and *D. yakuba* Due to Hybrid Male Sterility

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ABSTRACT

A major unresolved challenge of evolutionary biology is to determine the nature of the allelic variants of "speciation genes": those alleles whose interaction produces inviable or infertile interspecific hybrids but does not reduce fitness in pure species. Here we map quantitative trait loci (QTL) affecting fertility of male hybrids between *D. yakuba* and its recently discovered sibling species, *D. santomea*. We mapped three to four *X* chromosome QTL and two autosomal QTL with large effects on the reduced fertility of *D. yakuba* and *D. santomea* backcross males. We observed epistasis between the *X*-linked QTL and also between the *X* and autosomal QTL. The *X* chromosome had a disproportionately large effect on hybrid sterility in both reciprocal backcross hybrids. However, the genetics of hybrid sterility differ between *D. yakuba* and *D. santomea* backcross males, both in terms of the magnitude of main effects and in the epistatic interactions. The QTL affecting hybrid fertility did not colocalize with QTL affecting sexual isolation in this species pair, but did colocalize with QTL affecting the marked difference in pigmentation between *D. yakuba* and *D. santomea*. These results provide the basis for future high-resolution mapping and ultimately, molecular cloning, of the interacting genes that contribute to hybrid sterility.

UNDERSTANDING the genetic basis of speciation—the splitting of a group of interbreeding populations into two reproductively isolated groups—is a major challenge of evolutionary biology. Yet the framework in which we must seek to determine the genetic basis of postzygotic reproductive isolation—the inviability or sterility of interspecific offspring—has been clear from the beginning of the last century. First, hybrid dysfunction must be caused by deleterious epistatic interactions ("incompatibilities") between genes that function perfectly well in pure-species backgrounds. DOBZHANSKY (1937) and MULLER (1940) proposed a simple two-locus model that explains how such incompatibilities could arise if different alleles at the two loci become fixed in the two species. In this model, the ancestral species has genotype $A_1A_1B_1B_1$, and its two descendant species have genotypes $A_1A_1B_2B_2$ and $A_2A_2B_1B_1$, respectively. There would be no selection against deleterious interactions between A_1 and A_2 or B_1 and B_2 in the pure species, but such interactions may exist between alleles A_1 and B_2 in the separate lineages, causing inviability or sterility of the $A_1A_2B_1B_2$ hybrids. Second, HALDANE (1922, p. 101) noted that: "When in the F_1 offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterozygous [heterogametic] sex." One of the causes of Haldane's rule thus is likely to be incompatibilities involving the sex chromosomes with each other or with autosomes (other factors probably also contribute to Haldane's rule; see COYNE and ORR 2004).

Thus, to understand the genetic architecture of postzygotic reproductive isolation, we must identify the interacting pairs (or more) of genes affecting inviability and fertility. Most progress to date has been made by studying hybridizations of the four Drosophila species, *Drosophila melanogaster*, *D. simulans*, *D. sechellia*, and *D. mauritiana* (COYNE and ORR 2004). *D. simulans*, *D. mauritiana*, and *D. sechellia* are particularly suitable for the genetic analysis of factors affecting male hybrid fertility, since crosses between *D. simulans* and either of the other two species yield sterile males but fertile females that can be backcrossed to either species. (Since these species are allopatric and fertile hybrids can be produced, one could argue that full speciation between them has not yet been completed.) Early work used mapping with visible morphological markers to show that genes affecting male fertility in interspecific backcross males mapped to all chromosomes except the fourth (COYNE 1984; COYNE *et al.* 1991; COYNE and BERRY 1994), including the *Y* chromosome (JOHNSON *et al.* 1993; JOLY *et al.* 1997; DERMITZAKIS *et al.* 2000), and

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that the *X* chromosome had a disproportionately large effect (for review see Coyne and Orr 2004). Further genetic dissection revealed at least three *X*-linked factors affecting sperm motility in hybrid *D. simulans/D. mauritiana* and *D. simulans/D. sechellia* backcross males (Coyne and Charlesworth 1986, 1989). One region with a large effect on hybrid male fertility, closely linked to *forked* (Coyne and Charlesworth 1986, 1989), later mapped to the *Odysseus* (*Ods*) locus (Perez *et al.* 1993), which was eventually localized to a 8.4-kb region containing a rapidly evolving homeobox gene (Ting *et al.* 1998). However, the genetic architecture of hybrid male sterility is typically highly polygenic. High-resolution analysis of the other two *X*-linked factors of large effect revealed at least seven genes with smaller effects on sperm mobility in *D. simulans/D. mauritiana* hybrid males (Cabot *et al.* 1994; Davis and Wu 1996). A second factor with small effect, closely linked to *Ods*, is required for full sterility when this region of *D. mauritiana* is introgressed into *D. simulans* (Perez and Wu 1995). Further complexity arises in identifying individual loci since the same genes do not affect hybrid male sterility in reciprocal introgressions of the same region of *D. simulans* into the *D. mauritiana* background (Palopoli and Wu 1994).

High-resolution mapping has also shed light on the genetic mechanisms underlying the large effect of the *X* chromosome on sterility of male hybrids between these species. Autosomal introgressions have largely recessive effects on hybrid male sterility (Hollocher and Wu 1996; True *et al.* 1996; Tao *et al.* 2003a), but there are also proportionately more genes affecting hybrid male sterility on the *X* chromosome than on the autosomes (Tao *et al.* 2003a). Thus, Haldane's rule for sterility of male hybrids between these species is attributable to both the recessivity of genes causing hybrid incompatibilities and a higher rate of evolution of genes affecting hybrid male sterility, perhaps through sexual selection (reviewed by Laurie 1997; Coyne and Orr 2004).

The extent to which these features of the genetic architecture of loss of fitness in interspecific hybrids are general can be addressed only by equally comprehensive studies of additional pairs of species. The recent discovery of *D. santomea*, a species endemic to the island of São Tomé (Lachaise *et al.* 2000), has greatly expanded the opportunities for genetic analysis. Molecular phylogenetic analysis shows that *D. yakuba* and *D. santomea* are sister species within the *D. melanogaster* subgroup (Lachaise *et al.* 2000; Cariou *et al.* 2001). This pair represents a speciation event independent of that separating the ancestor of *D. melanogaster* from that of the *D. simulans* triad, as well as the two speciation events in which a *D. simulans*-like ancestor produced its island descendants *D. sechellia* and *D. mauritiana* (Lachaise *et al.* 1988).

D. santomea and *D. yakuba* show substantial sexual isolation when tested in the laboratory and differ in male genital morphology and in pigmentation (*D. yakuba* has the black abdominal pigmentation typical of all other species in the *D. melanogaster* group, while *D. santomea* lacks any pigmentation (Lachaise *et al.* 2000; Coyne *et al.* 2005). In interspecific crosses, F_1 male hybrids are sterile but female hybrids are fertile; the latter can be backcrossed to either parental species (Lachaise *et al.* 2000; Cariou *et al.* 2001). In addition to behavioral isolation and intrinsic hybrid sterility, the species also show conspecific sperm precedence: when *D. yakuba* females are multiply inseminated by both conspecific and *D. santomea* males, they produce very few hybrid progeny, regardless of the order of mating (Chang 2004). Here, we use quantitative trait locus (QTL) mapping to identify chromosomal regions affecting fertility in *D. yakuba/D. santomea* male backcross hybrids. These data offer a comparison to previous work on other traits within this species pair, comparisons to loci for fertility in other species pairs, and a basis for future high-resolution genetic analysis.

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 the *D. yakuba* ST and *D. santomea* STO.4 strains described in the accompanying article (Moehring *et al.* 2006, this issue) as parental strains for QTL mapping. *D. yakuba* ST was derived from the isofemale strain *D. yakuba* Taï18 by eliminating the polymorphic inversion distinguishing it from *D. santomea*, thus creating a strain homosequential with *D. santomea* for improved mapping resolution (Moehring *et al.* 2006).

Crosses: Previous studies (Lachaise *et al.* 2000; Coyne *et al.* 2004) have shown that crosses between these species produce fertile F_1 females but sterile F_1 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 (BC) in two ways: (A) to *D. yakuba* ST males, producing 550 BC males, and (B) to *D. santomea* STO.4 males, producing 550 BC males. Male backcross hybrids have autosomal loci with one chromosome that is pure (A) *D. yakuba* or (B) *D. santomea* while the other chromosome is a mixture of the two parental genomes; *X*-linked loci and the *Y* chromosome are either pure (A) *D. yakuba* or (B) *D. santomea*.

Fertility assay: Four-day-old virgin males were scored for fertility by removing their testes and lightly crushing them in Ringer's solution under a coverslip (Coyne *et al.* 2004). To provide a quantitative measure of fertility, each male was scored for degree of fertility on an eight-point scale of increasing degree of motile sperm formation: 0, testes completely atrophied; 1, testes of normal size, no spermatids or sperm; 2, testes of normal size containing spermatids but no free sperm; 3, testes of normal size containing a few free sperm, but none of them motile; 4, testes of normal size, many free sperm but none of them motile; 5, testes of normal size, a few free sperm of which a few were motile; 6, testes of normal size, a few free sperm, most of which were motile; 7, testes of normal size, many free sperm of which a few were motile; and 8, testes of normal size, many free sperm of which many were motile. A few individuals had one atrophied testis and one larger testis; these contained no sperm or spermatids and thus were scored as "1." All scoring was done by a single observer.

Molecular markers and genotypes: Flies were immediately frozen at −80° after they were scored for fertility. The sample then underwent DNA extraction and marker-specific PCR amplification and restriction endonuclease digestion. Each of the 1100 samples was scored for 32 molecular markers that are roughly evenly spaced throughout the genome, as described in the accompanying article by Moehring *et al.* (2006, this issue). The markers and their cytological (relative to *D. melanogaster*, Drysdale *et al.* 2005) and recombination map positions are: *y*, 1A5, 0.00; *per*, 3B1–2, 2.62; *sog*, 13E1, 17.89; *v*, 9F11, 25.23; *rux*, 5D2, 53.01; *f*, 15F4–7, 59.89; *bnb*, 17D6, 68.61; *Hex-A*, 8E10, 72.41; *AnnX*, 19C1, 77.16; *su(f)*, 20E, 79.77; *l(2)gl*, 21A5, 0.00; *Rad1*, 23A1, 6.65; *RpL27A*, 24F3, 13.47; *salr*, 32E4–F1, 29.49; *Rep4*, 34B4, 37.29; *His3*, 39D3–E1, 57.75; *barr*, 38B1–2, 62.62; *Sara*, 57E6, 73.70; *Ngp*, 54C8, 90.25; *Kr*, 60F5, 150.65; *Lsp1*γ, 61A6, 0.00; *dib*, 64A5, 19.74; *sfl*, 65B3–4, 33.04; *Est-6*, 69A1, 63.36; *Ssl1*, 80B2, 90.35; *ry*, 87D9, 101.96; *Rpn5*, 83C4, 121.42; *AP-50*, 94A15–16, 130.17; *Mlc1*, 98A14–15, 150.90; *ymp*, 96E, 160.32; *krz*, 100E3, 176.99; and *ci*, 102A1–3, 0.00.

QTL mapping: QTL for fertility were mapped three different ways in each backcross population. First, fertility was treated as a continuous trait using the full range of scores (see above). Second, whether or not spermatids were present was considered as a binary trait since this is the stage to which spermatogenesis proceeds for one direction of the cross (*D. yakuba* mothers) but not the other (*D. santomea* mothers). Third, whether or not sperm were present was considered as a binary trait, for comparison to previous work. The assumption of normality in composite interval mapping (CIM) (Zeng 1994) is violated when fertility is considered as a binary trait. However, we showed previously (Moehring *et al.* 2004) that using an extension of CIM on the basis of 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), gives the same results as CIM performed on binary data for large sample sizes.

QTL were mapped using CIM, implemented using QTL Cartographer software (Basten *et al.* 1999), exactly as described in the accompanying article (Moehring *et al.* 2006, this issue). Empirical experimentwise 5% significance thresholds, which take account of the multiple tests performed and correlations among markers, were determined by permutation (Churchill and Doerge 1994; Doerge and Churchill 1996). The effects of each QTL were estimated as the difference between heterozygous *D. santomea/D. yakuba* genotypes and homozygous pure species genotypes at the peak likelihood ratio (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 estimating the cytological location of the interval by dividing the cytology within the region according to the observed amount of recombination between flanking markers (for the *D. yakuba* cytology relative to *D. melanogaster*, see Moehring *et al.* 2006, this issue). We evaluated pairwise epistatic interactions 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 data with a log linear model using PROC CATMOD and for overall fertility with an ANOVA using PROC GLM, using SAS 8.2 software (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Fertility of pure species and F_1 hybrids: Table 1 describes the fertility assay of the two pure species and the two reciprocal F_1 hybrids. As expected, the pure species were fertile and the hybrid males sterile. In the two pure species, >90% of males had some motile sperm (stages 5–8), with most falling into stage 8 (testes of normal size, many free sperm of which many are motile). In contrast, none of the F_1 hybrids had any motile sperm. However, there was a difference in the stage of spermatogenesis attained in the two reciprocal hybrids: nearly all males with a *D. santomea* mother had testes of normal size but no spermatids or free sperm (stage 1), while male offspring of the reciprocal cross had testes of normal size and spermatids whose differentiation had not progressed to sperm (stage 3). This difference in the degree of spermatogenesis between the two reciprocal hybrids, which is highly significant ($\chi^2_8 = 182.6$, $P < 0.001$), was also described by Coyne *et al.* (2004) (Table 3).

TABLE 1

Fertility of males of pure species and F_1 hybrids

	Stage of spermatogenesis								
Genotype	0	1	2	3	4	5	6	7	8
S	0	1	2	3	0	5	10	1	82
Y	0	0	2	0	0	6	12	5	75
F_1, $S \times Y$	6	93	1	0	0	0	0	0	0
F_1, $Y \times S$	0	4	94	2	0	0	0	0	0

At least 100 individuals were scored for each genotype. S = *D. santomea* STO.4 *strain*; Y = *D. yakuba* ST strain. For F_1 hybrids, the genotype of the female parent is given first. See materials and methods for definitions of spermatogenesis stages.

QTL for fertility in *D. yakuba* backcross males: We mapped three *X* chromosome QTL with large effects on the reduced fertility of *D. yakuba* backcross males, in the *per–sog* (3B–13A), *v–rux* (8D–5D), and *AnnX–su(f)* (19C–20E) intervals when fertility was considered as a continuous trait. We use the cytological locations when reporting the regions (rather than recombination distance) as the end goal is to locate the genes responsible for hybrid sterility, which will most likely be accomplished through comparisons to the genome of the model system of *D. melanogaster*, making the cytology, rather than the recombination distance, of relevance. The same three QTL were detected when fertility was considered as a binary trait of presence or absence of sperm, although these QTL tended to map to larger intervals, consistent with the lower power of mapping threshold traits (Xu and Atchley 1996) (Table 2, Figures 1A and 2). We identified six QTL when the presence or absence of spermatids was considered as a dichotomous trait: *per–sog* (11E–13A), *v–rux* (7D–7B), *f–bnb* (15F–18B), *su(f)* (9A–20E), *Rep4–Sara* (34B–57E), and *Ssl1–ry* (82A–83C). Particularly striking is the effect of the *v–rux* interval, which explains >47% of the variance for this trait.

Thus at least four genes are responsible for hybrid sterility when considered as a continuous trait or for

TABLE 2

QTL affecting fertility in offspring of crosses between *D. yakuba* and *D. santomea*

BC cross	Trait	QTL	Peak	LR	Effect	Effect/σ_p	R^2
F_1 females × *D. yakuba* males	Fertility (scored 0–8)	3B–13A	11E	91.08	1.48	0.72	0.1178
		8D–5D	5C	25.88	0.91	0.44	0.0294
		19C–20E	20E	85.05	1.69	0.82	0.0978
	Presence/absence of spermatid	11E–13A	12D	132.49	0.43	20.11	0.1561
		7D–7B	7C	1874.56	1.01	47.60	0.7298
		15F–18B	16F	26.28	0.29	13.47	0.0332
		9A–20E	20E	35.14	0.24	11.46	0.0339
		34B–57E	49A	19.52	−0.14	−6.83	0.0196
		82A–83C	84B	20.19	−0.15	−6.99	0.0215
	Presence/absence of sperm	1A–13A	11E	56.80	0.23	14.50	0.0865
		8A–15D	5D	14.35	0.13	8.11	0.0173
		9D–20E	20E	66.95	0.29	18.41	0.0897
F_1 females × *D. santomea* males	Fertility (scored 0–8)	3B–13E	12F	36.53	0.60	0.40	0.0592
		14A–18C	19B	11.95	0.44	0.29	0.0178
		5D–20E	17F	21.11	0.47	0.31	0.0317
		41F–51E	49A	11.84	0.33	0.21	0.0197
		65F–80B	70D	19.38	0.42	0.28	0.0324
	Presence/absence of spermatid	11B–13A	12B	107.91	0.40	21.24	0.1849
		13E–7A	8C	21.10	0.27	14.14	0.0504
		5C–16A	5D	19.26	0.18	9.46	0.0259
		16F–9B	17F	29.26	0.19	10.38	0.0387
	Presence/absence of sperm	19B–17D	7C	17.07	0.11	8.84	0.0342
		45F–57E	50A	16.92	0.10	8.09	0.0295

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 listed when scaled by dividing 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).

presence or absence of sperm, since the *D. santomea* *X*-linked loci must interact with at least one *D. yakuba* autosomal or *Y*-linked locus. In this backcross we mapped autosomal QTL only for the presence of spermatids. If *D. santomea* *X*-linked loci interact with *D. yakuba* *Y*-linked loci, this interaction could be at least partially responsible for reduced fertility of backcross males. We also note that the third chromosome region from *ry-AP-50* (87D9–94A16) approached formal significance (LR = 10.17) for presence of sperm and further that epistatic interactions may occur between *X*-linked QTL and autosomal QTL that do not themselves have a main effect on fertility.

We tested for epistatic interactions among the QTL (Table 3) in two ways. First, we considered only interactions between QTL having significant main effects on hybrid fertility and used a Bonferroni correction to assess significance. We found a significant interaction for fertility scored on the continuous scale between the QTL in the *per–sog* interval and the QTL in the *v–rux* interval. In addition, we observed an epistatic interaction between the QTL in the *per–sog* interval and the QTL at the base of the *X* chromosome [*HexA–su(f)*, 9D–20E] for fertility considered as the dichotomous trait of presence/absence of sperm. The backcross design affords us the opportunity to test for *X*–autosome interactions that may define pairs of loci contributing to hybrid incompatibilities. Therefore, we assessed interactions between the three *X* chromosome QTL and all autosomal markers, even though autosomal markers were formally significant only for presence of spermatids.

We found interactions between the QTL in the *per–sog* interval and the *ry* and *Rpn5* markers on chromosome *3* and the *RpL27A* marker on chromosome *2*, for the continuous fertility data. For the dichotomous trait presence of spermatids, interactions were observed between the QTL in the *per–sog* interval and the *l(2)gl*, *RpL27A*, and *Ngp* markers on chromosome *2* and *Est-6* on chromosome *3*. Epistatic interactions for this trait were also identified between the *v–rux* QTL interval and *His3*, *barr*, *ry*, *Rpn5*, *AP-50*, and *ymp*. It is interesting to note that the *v–rux* interval, which had the highest effect on this trait, also had the greatest number of epistatic interactions. Considering the presence of sperm, we found epistatic interactions between the *per–sog* interval QTL and the third chromosome markers *ry* and *AP-50* and the second chromosome markers *Rad1* and *Rpl27A*. All of the interactions were in the expected direction, with double heterozygotes (and hemi-heterozygotes for *X* markers) being the least fertile and double

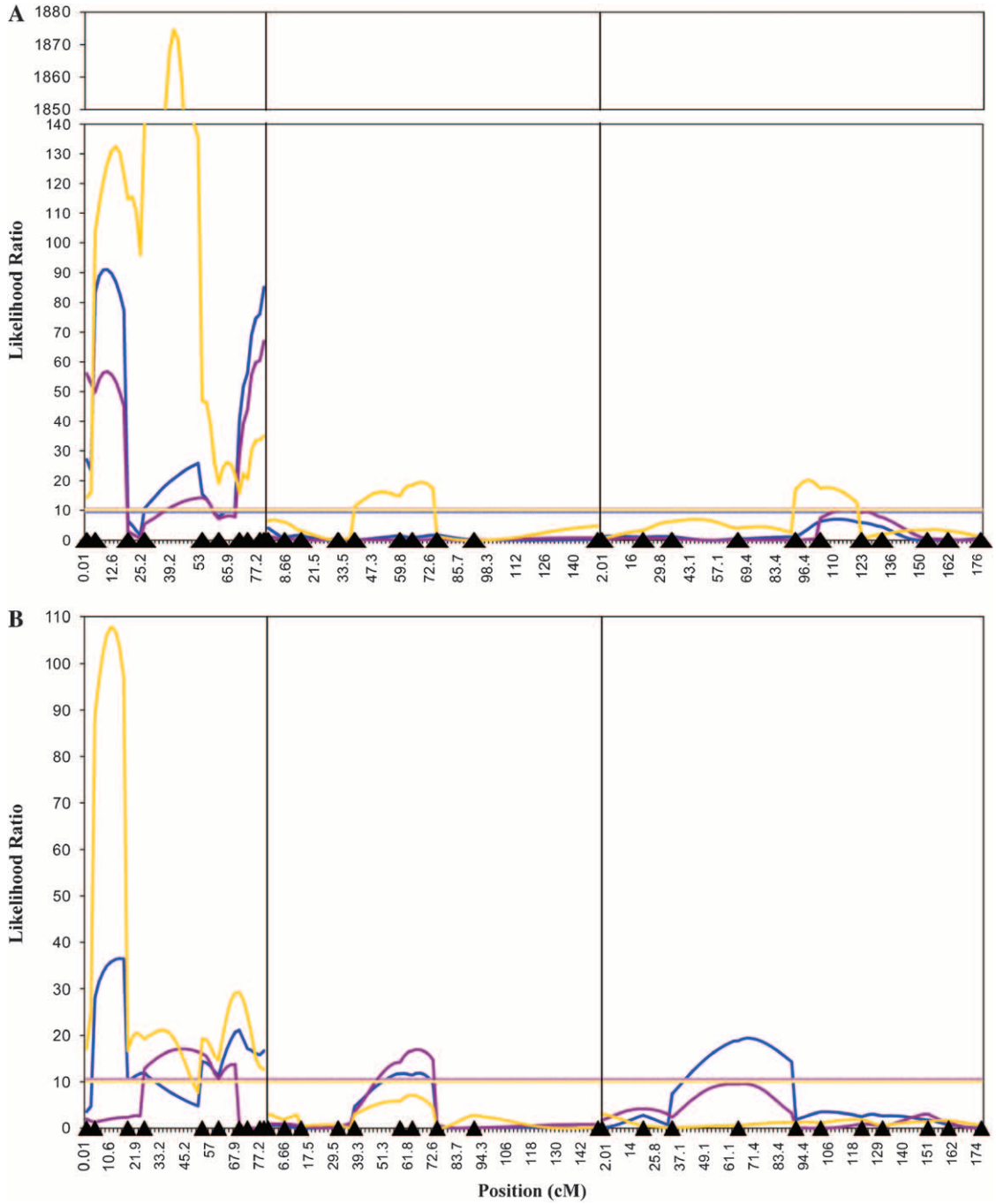

FIGURE 1.—QTL for the *X*, second, and third chromosomes affecting fertility in backcross hybrids between *D. yakuba* and *D. santomea*. There were no QTL for the small fourth chromosome. (A) F_1 females backcrossed to *D. yakuba* males. The *y*-axis from likelihood ratio (LR) 140–1850 is truncated. (B) F_1 females backcrossed to *D. santomea* males. Plots are the LR test statistics for overall fertility score (blue), presence of spermatids (yellow), and presence of sperm (purple) 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.

homozygotes (and hemi-homozygotes) the most fertile. Although these interactions would not be significant if corrected for multiple tests, it is possible that the hybrid incompatibilities in this backcross are at least partially attributable to interactions between *X* chromosome QTL and recessive or partially recessive autosomal QTL.

These results are consistent with those of COYNE *et al.* (2004), who used visible morphological markers to

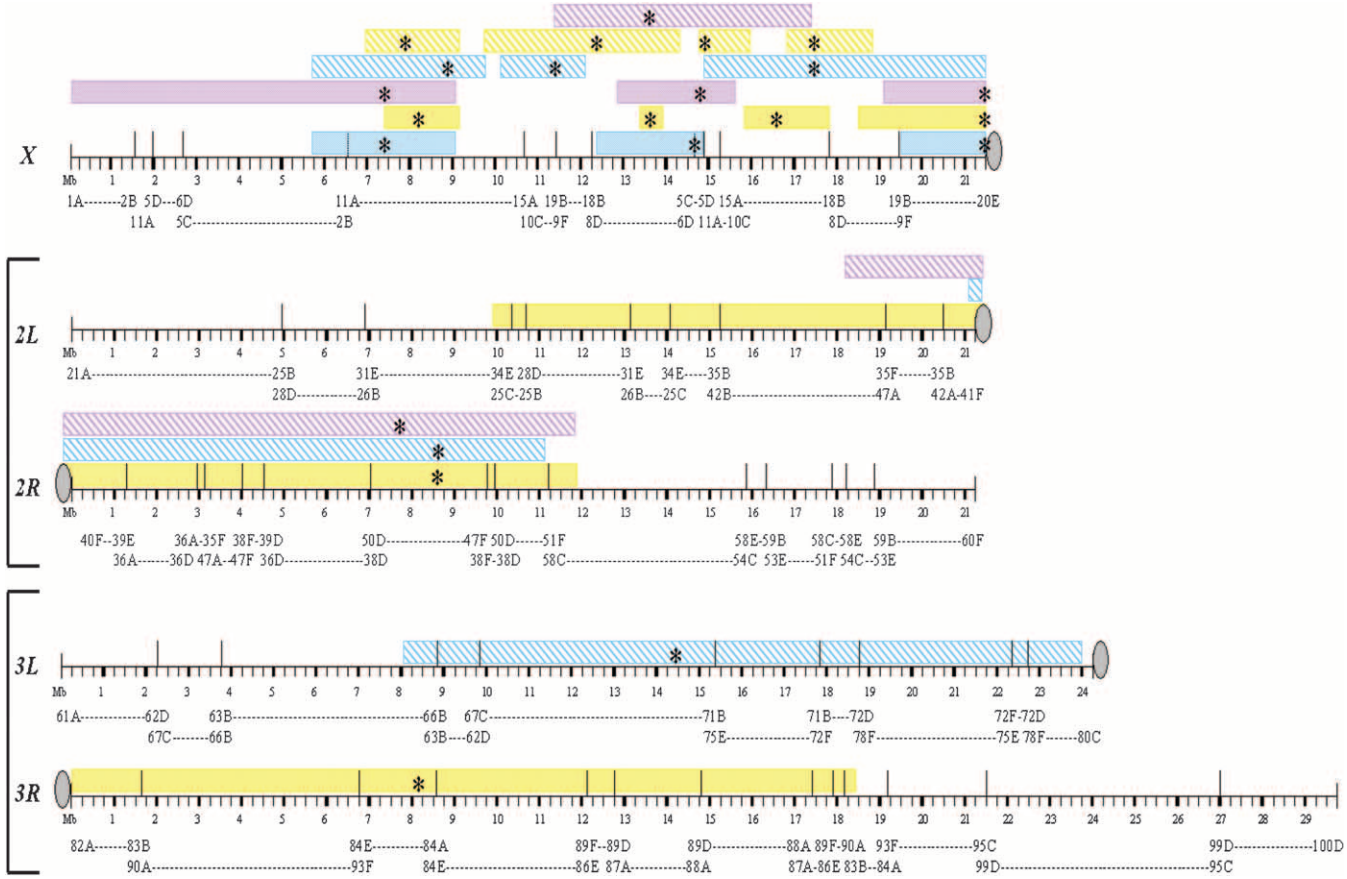

FIGURE 2.—The significant regions from QTL mapping when compared to *D. melanogaster* cytology. Short vertical lines below the horizontal are every 250 kb; the short thick lines are every 1 Mb. Tall vertical lines above the horizontal represent the inversion/translocation breakpoints. Colored boxes represent QTL peaks: overall fertility (blue), presence of spermatids (yellow), and presence of sperm (purple) scored for BC males from F_1 females × *D. yakuba* males (solid boxes) and BC males from F_1 females × *D. santomea* males (hatched boxes). * indicates the peak of the QTL. Centromeres are represented by a gray circle. 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, Lsp1γ, dib, sfl, Est-6, Ssl1, ry, Rpn5, AP-50, Mlc1, ymp*, and *krz*. Note that markers are spaced according to base pair distance.

estimate the relative contributions of the *X* chromosome and autosomes to the reduced fertility (scored as presence or absence of motile sperm) of *D. yakuba* backcross males. COYNE *et al.* (2004) observed a large effect of the *X* chromosome and showed that this effect was attributable to at least three genes, in the regions between *w* (at cytological location 3B) and *g* (12B), *g* and *sn* (7D), and *sn* to the base of the chromosome. These regions exactly match the QTL mapped here by linkage to molecular markers. COYNE *et al.* (2004) also detected a smaller but significant effect of the second chromosome marker *no* (*notch*). However, this effect was in the direction of increased sterility of the homozygous *no D. yakuba* genomic segment relative to the heterozygous *no*/+ *D. yakuba/D. santomea* genotype, possibly implying that homozygosity for *no* itself causes a reduction of fertility in a hybrid background. If this explanation is true, we would not expect to detect an effect of the *no* region when using molecular markers to map QTL affecting hybrid sterility, as indeed observed in this study. Finally, COYNE *et al.* (2004) noted a significant effect of the *D. yakuba Y* chromosome, consistent with a role of *X*–*Y* interactions in hybrid sterility.

QTL for fertility in *D. santomea* backcross males: We mapped three *X* chromosome QTL and two autosomal QTL with large effects on the reduced fertility of *D. santomea* backcross males, when fertility is considered as a continuous trait (Table 2, Figures 1B and 2). The *X* chromosome QTL mapped to the *per*–*sog* (3B–13E), *sog*–*rux* (14A–18C), and *rux*–*su(f)* (5D–20E) intervals, and the autosomal QTL mapped to the region from *His3*–*barr* (41F–51E) on chromosome *2* and the region from *sfl*–*Ssl1* (65F–80B) on chromosome *3*. Again, the *X* chromosome has a disproportionately large effect on hybrid sterility. Four QTL were detected on the *X* chromosome when fertility was measured according to whether or not spermatids were present, in the *per*–*sog* (11B–13A),

TABLE 3

Epistatic interactions between marker regions

Fertility QTL	Trait	Marker 1	Marker 2	*P*-value
D. yakuba BC males	Fertility	*per–sog*	*v–rux*	0.0070*
			ry	0.026
			Rpn5	0.05
	Spermatids	*per–sog*	*I(2)gl*	0.045
			RpL27A	0.04
			Ngp	0.038
			Est-6	0.02
		v–rux	*His3*	0.016
			barr	0.009
			ry	0.005
			Rpn5	0.006
			AP-50	0.017
			ymp	0.049
	Sperm	*per–sog*	*HexA–su(f)*	0.0057*
			ry	0.032
			AP-50	0.027
			Rad1	0.024
			RpL27A	0.014
D. santomea BC males	Fertility	*per–sog*	*Ssl1*	0.016
		v	*barr*	0.0013*
			Ssl1	0.007
		bnb	*barr*	0.033
			Ssl1	0.026
	Spermatids	*per–sog*	*rux–f*	0.02
			bnb	0.0029*
			His3	0.0002*
			barr	0.0002*
		v	*rux–f*	0.012*
			bnb	0.0005
			salr	0.0003*
			Rep4	0.0002*
			Ngp	0.0001*
		rux–f	*barr*	0.0004*
		bnb	*barr*	0.0002*

Pairwise epistatic interactions were calculated using the marker at the highest LR peak or the haplotype of the two markers flanking a peak. Trait is fertility scored as a continous trait (fertility), the presence/absence of spermatids (spermatids), or the presence/absence of sperm (sperm). * denotes formal significance after Bonferroni correction.

v (13E–7A), *rux–f* (5C–16A), and *bnb* (16F–9B) regions. We also detected two QTL for hybrid fertility when considering fertility as the threshold trait of presence of sperm, one on the *X* chromosome in the *v–rux* interval (19B–17D) and one on chromosome *2* in the *Rep4–Sara* interval (45F–57E). The results from the different measurements of fertility are in good agreement, since two of the *X* chromosome QTL overlap, the second chromosome QTL is detected in two of the analyses, and there is a strong suggestion of linkage on chromosome *3* in the threshold model in the *sfl–Ssl1* region (LR = 9.56) (Table 2, Figures 1B and 2).

When we evaluated epistatic interactions between QTL with significant main effects on fertility considered as a continuous trait in *D. santomea* backcross males, we observed an interaction between the *X*-linked QTL at 14A–18C (near *v*) and the second chromosome QTL. In addition, we observed multiple interactions between *X* chromosome QTL and the autosomal QTL when we tested for all possible *X*–autosome interactions. The QTL in the *per–sog* interval interacts with the third chromosome QTL, with a peak at *Ssl1*. The QTL near *v* interacts with both the second and third chromosome QTL, with peak associations at *barr* and *Ssl1*, as does the QTL near *bnb*, also with peak associations at *barr* and *Ssl1*. When fertility is measured as presence of spermatids, we observed that the *per–sog* QTL had epistatic interactions with both the *rux–f* and *bnb* QTL and that *v* also interacted with both the *rux–f* and *bnb* QTL. When testing for all possible *X*–autosome interactions, the *per–sog* interval interacted with most of the autosomal regions, with formally significant peaks at *His3* and *barr*; *v* significantly interacted with *salr*, *Rep4*, and *Ngp*; and both *rux-f* and *bnb* had significant interactions with *barr*. As with the epistatic interactions in *D. yakuba* backcross males, all of the interactions were in the expected direction, with double heterozygotes (and hemi-heterozygotes) being the least fertile and double homozygotes (and hemi-homozygotes) the most fertile.

Thus, the genetics of hybrid sterility are partially overlapping but not identical between *D. yakuba* and *D. santomea* backcross males, in terms of both main effects of and epistatic interactions between QTL (Table 2, Figures 1 and 2). Fine mapping these QTL to smaller intervals will resolve whether the basis of sterility in reciprocal hybridizations between *D. yakuba* and *D. santomea* is due to the same genetic loci.

QTL affecting sexual isolation and pigmentation differences between *D. yakuba* and *D. santomea*: In the accompanying article, we mapped QTL affecting the discrimination of *D. yakuba* males against pure-species *D. santomea* females by pairing *D. yakuba* backcross hybrid males with *D. santomea* females (Moehring *et al.* 2006, this issue). There is one scenario under which we might expect the same QTL to affect hybrid mating behavior and fertility, and that is if sexual selection is driving the rapid evolution of male hybrid sterility. However, this appears not to be the case. The QTL affecting sexual isolation in *D. yakuba* male backcross hybrids all mapped to the autosomes (Moehring *et al.* 2006), while the QTL affecting reduced fertility in *D. yakuba* male backcross hybrids mapped mainly to the *X* chromosome (this report).

We also mapped QTL affecting the marked difference in pigmentation between *D. yakuba* and *D. santomea* (Carbone *et al.* 2005). The same four QTL affected variation in pigmentation in *D. yakuba* and in *D. santomea* male backcross hybrids: 1A5–13E1 (*y–sog*), 15F4–20E [*f–su(f)*], 34B4–57E6 (*Rep4–Sara*), and 69A1–83C4 (*Est6–Rpn5*). Strikingly, these QTL overlap with the QTL affecting reduced male hybrid fertility at the tip and the base of the *X* chromosome and the chromosome *2*

locus in *D. yakuba* and *D. santomea* backcross males and with the chromosome *3* QTL affecting reduced male hybrid fertility in the *D. santomea* backcross males. Is it possible that the coincidence of QTL affecting postzygotic reproductive isolation and morphological variation is a byproduct of adaptive fixation of genes affecting loss of black pigmentation in *D. santomea*, as predicted from the Dobzhansky-Muller model of evolution of hybrid incompatibility (Dobzhansky 1937; Muller 1940)? If so, one expects that the same genes have pleiotropic effects on both traits, an hypothesis that can be tested by simultaneous high-resolution mapping of pigmentation and fertility in male interspecific hybrids.

QTL affecting hybrid fertility in other Drosophila species pairs: Our data fully recapitulate the most prominent feature of the genetic architecture of hybrid male sterility observed in other Drosophila species pairs, namely, a disproportionately large effect of the *X* chromosome (reviewed by Coyne and Orr 2004). In addition, the low amount of QTL overlap seen here when comparing reciprocal hybridizations has also been observed in reciprocal hybridizations of *D. simulans/D. mauritiana* (Perez *et al.* 1993; Palopoli and Wu 1994). The breadth of the QTL peaks observed in this study, spanning several markers, also suggests that high-resolution mapping will reveal multiple closely linked QTL affecting male fertility in *D. santomea/D. yakuba* hybrids, as observed for *D. simulans/D. mauritiana* hybrids (Cabot *et al.* 1994; Palopoli and Wu 1994; Perez and Wu 1995; Davis and Wu 1996; True *et al.* 1996; Tao *et al.* 2003b). While we must be reserved in speculating about candidate genes given the rather coarse resolution of our mapping, we note that *Ods* (at 16D) is in the *X*-linked QTL for all three fertility-scoring methods in males resulting from a backcross to *D. santomea*, but is only within the QTL for the presence of spermatids in males from the backcross to *D. yakuba*.

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