Unusually High Recombination Rate Detected in the Sex Locus Region of the Honey Bee (Apis mellifera)

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ABSTRACT

Sex determination in Hymenoptera is controlled by haplo-diploidy in which unfertilized eggs develop into fertile haploid males. A single sex determination locus with several complementary alleles was proposed for Hymenoptera [so-called complementary sex determination (CSD)]. Heterozygotes at the sex determination locus are normal, fertile females, whereas diploid zygotes that are homozygous develop into sterile males. This results in a strong heterozygote advantage, and the sex locus exhibits extreme polymorphism maintained by overdominant selection. We characterized the sex-determining region by genetic linkage and physical mapping analyses. Detailed linkage and physical mapping studies showed that the recombination rate is < 44 kb / C M in the sex-determining region. Comparing genetic map distance along the linkage group III in three crosses revealed a large marker gap in the sex-determining region, suggesting that the recombination rate is high. We suggest that a "hotspot" for recombination has resulted here because of selection for combining favorable genotypes, and perhaps as a result of selection against deleterious mutations. The mapping data, based on long-range restriction mapping, suggest that the Q DNA-marker is within 20,000 bp of the sex locus, which should accelerate molecular analyses.

GENETIC recombination resulting from sexual reproduction is ubiquitous in the animal kingdom. Most commonly, both males and females are diploid and are derived from the fusion of male and female gametes. However, in many species only the females are diploid while males are haploid and are derived directly from unfertilized eggs. Arrhenotoky, in which males are haploid, occurs in ~20% of animals, such as ticks and mites (Acarina), white flies (Aleyroidea), scale insects (Coccoidea, Margarodidae), thrips (Thysanoptera), bark beetles (Scolytidae), and rotifer (Monogononta) (Bell 1982) and characterizes the entire insect order Hymenoptera, which contains >200,000 species (Bell 1983). In 1845, Dzierzon discovered that female honey bees (Apis mellifera) arise from fertilized eggs and males arise from unfertilized eggs. This was the first rigorous report of a biological mechanism of sex determination (Bell 1983). Later studies showed that male eggs are unfertilized (Petrunkevitsch 1901) and haploid (Nachtshem 1913), and that there are no heteromorphic sex chromosomes as compared to other genetic systems (Sander son and Hall 1948).

The genetic basis of haplo-diploid sex determination remained a puzzle until complementary sex determination was found in the wasp Bracon hebetor (Whiting 1933, 1939, 1943). The developmental trajectory of male or female is determined by the sex locus. Females are heterozygous while normal males are haploid, and have just 1 allele. Individuals that are diploid and homozygous develop into diploid males that have degenerate testes with reduced quantities of diploid sperm (Hung et al. 1974), or, in the case of honey bees, are eaten by their nestmate workers. This locus exhibits strong symmetrical overdominant selection that can maintain large numbers of alleles within populations (Whiting 1943, 1945; Laidlaw et al. 1956; Wright 1965; Adams et al. 1977; Yokoyama and Nei 1979). Consequently, 9 (Bracon hebetor, Whiting 1943), 19 (Apismellifera, Adams et al. 1977), 24 (Bombus terrestris, Duchat eau et al. 1994), and up to 66–88 (Solenopsis invicta, Ross et al. 1993) sex alleles have been found in studied populations. One of the most interesting problems to be solved is how large numbers of sex alleles were generated and how the homozygous and heterozygous states are distinguished using up to 66 functionally equivalent sex alleles. Because heterozygosity at the sex locus results in females, and inbreeding results in a higher proportion of diploid males, this mechanism by itself contributes to a maintenance of genetic variation in a population, which may confer a selective advantage during evolution in haplo-diploid systems (Bell 1983).

The complementary nature of the sex-determining system is widespread in the order Hymenoptera and has been demonstrated in more than 12 species belonging to the bee family (Bark beetles (Scolytidae), and rotifera (Monogononta), thrips (Thysanoptera), mites (Acarina), white flies (Aleyroidea), scale insects (Coccoidea, Margarodidae), thrips (Thysanoptera), bark beetles (Scolytidae), and rotifer (Monogononta)).
to different superfamilies (Cook 1993), although there are exceptions for habitually inbreeding species, including the large superfamily Chalcidoidea (Crozier 1971, 1977). In Nasonia vitripennis recent findings suggest a strong support for a genomic imprinting model of sex determination (Dobson and Tanouye 1998). There sex depends on the correctly imprinted chromosome of paternal origin.

The honey bee, Apis mellifera, provides a model system for studying the mechanism of sex determination and the population genetics of sex alleles. In the past few years several DNA markers have been mapped near the sex locus (Beye et al. 1994; Hunt and Page 1994, 1995). However, these markers were derived from independent mapping studies and, as a consequence, their map relationships to each other were unknown.

In this study, we determine the map order of the sex locus and three flanking markers, analyze linkage groups of three mapping populations comprising the sex locus, and physically map linked markers to compare physical and genetic distances around the sex locus. This is a first attempt to identify and characterize a sex factor in a complementary sex-determining system showing strong overdominant selection.

MATERIALS AND METHODS

Source of bees: Honey bees used for linkage analysis were derived from instrumentally inseminating a virgin queen with a haploid male derived from her mother (this is referred to as a “mother-daughter” mating; see Laidlaw and Page 1997). In this case, a queen had a 50% chance of being inseminated with a male having a sex allele identical by descent with the one she inherited from her mother (see Figure 1), resulting in 50% of the offspring derived from this cross being homozygous at the sex locus and, therefore, diploid males. Of 57 mother-daughter matings performed, 32 demonstrated the “shot brood” phenotype indicative of diploid male production.

Sixteen queens producing shot brood were further screened to identify those that were heterozygous for two markers, Z (Beye et al. 1994) and Q (Hunt and Page 1994), that were suspected to flank the sex locus. Workers of a queen that had a high frequency of double heterozygous workers for Q and Z were used for the analysis. The diploid males were more likely to be homozygous for Z and Q marker alleles because they were linked to a sex allele common to both parents. One queen showing highly skewed worker genotypes was selected for further analysis. More than 300 newly emerged worker offspring were sampled from the colony. DNA was extracted using standard procedures (Hunt and Page 1995).

Sex-linked sequence tagged sites (STS) and microsatellite markers: From the cloned and sequenced Z marker fragment (Beye et al. 1994, 1996), primers were designed up and downstream of the microsatellite motif (TTTC), (Beye et al. 1998a): (5’ to 3’), Z1 AGCCGCTAAATATAATTC, Z2 GGAAAGAGG GTATTATAC. Amplification was carried out as described previously (Beye et al. 1998a). PCR products were resolved in gels containing 0.6% agarose and 1% Synergel (Diversified Biotech, Boston, MA). Random amplified polymorphic DNA (RAPD) markers linked to the X locus were cloned and sequenced as described (Hunt and Page 1994) and specific primers were designed. The sequences are the following:

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Q1:  AGCCGCTAAATATAATTC
Q2:  GGAAAGAGG GTATTATAC
X1:  AGCCGCTAAATATAATTC
X2:  GGAAAGAGG GTATTATAC
Z1:  AGCCGCTAAATATAATTC
Z2:  GGAAAGAGG GTATTATAC
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Figure 1.—Breeding scheme and the inferred genotypes of the sex locus and sex-linked markers. A virgin queen was mated by instrumental insemination to a drone derived from her mother. The drone is expected to share a sex allele along with linked marker alleles in common with the queen. The figure shows the genotype of the sex locus and the linked markers in their presumed orientation to the telomere and centromere. Individuals homozygous for the sex locus develop into diploid males. Except for recombinants, the diploid males are expected to be homozygous for the marker alleles.

Pulsed-field gel electrophoresis and Southern analysis: High molecular weight DNA was prepared from different hives of A. m. carnica bees (Berlin), following the protocol of Beye et al. (1999). Briefly, the mature pupae (colored eye stage) were taken from a hive. Nuclei were obtained by differential sucrose density gradient centrifugation, and then embedded in low-melting-point agarose inserts. The bulk of the DNA had a size >1.6 Mbp as determined by pulsed-field gel electrophoresis (PFGE). One-fourth to one-third of a plug (~4 μg of DNA) was digested overnight with 30–50 units of rare cutting restriction enzymes (AscI, NotI, FseI, SfiI, SmaI, MluI, Pmd, RsrlI, and Nael) in the standard reaction buffer (Promega, Madison, WI, and New England, Beverly, MA) supplemented with acetylated bovine serum albumin (Promega) to a final concentration of 0.1 mg/ml. The DNA was fractionated by PFGE in a CHEF-DRII gel apparatus (Bio-Rad) (1% agarose-gel, 0.5 × TBE (50 mm TrisHCl, 50 mm boric acid, 1 mm EDTA), 6 V/cm, 16”) under the following conditions: pulse times 60–100 sec for 24 hr for fragments >600 kb, pulse times 4–24 sec for 24 hr for
fragments between 600 and 100 kb, and pulse times 0.5–24 sec for 17 hr for fragments between 200 and 20 kb. To resolve fragments between 50 and 2 kb, a nonpulsed 0.6% agarose gel was run with 3 V/cm for 24 hr.

After electrophoresis the DNA was briefly depurinated by incubating the gel in 0.2 M HCl for 15 min. Capillary transfer of DNA onto a Hybond N+ membrane (Amerham, Buckinghamshire, UK) was carried out under alkaline conditions as described by Sambrook et al. (1989), and the membrane was baked for 120 min at 80°.

**Probes and probe hybridization:** An identified cosm Id clone, MPM Gc72E11247Q2, of the gridded honey bee library was used as Z marker probe (Beye et al. 1998b, 1999). The Q and sts335–33 marker probes were obtained by eluting the PCR product from a 2% agarose gel using standard techniques.

Labelled probes were either generated by random labeling (Z clone, MPM Gc72E11247Q2) or primer-specific labeling (Q and sts335–33 marker). Hybridization was carried out overnight following standard conditions (Beye et al. 1996). Washes were performed under stringent conditions, and membranes were exposed to Hyperfilm-MP (Amerham) with intensifying screens for 3 days to 2 wk at −70°. The same membranes were used for the hybridization of different probes.

The previous probe was stripped by incubation with 10 mm Tris-HCl pH 7.5 and 50% formamide at 70° for 30 min following two washes of 2× SSC (20× SSC: 3.0 M NaCl, 300 mm NaCitrate, pH 7.5) 0.1% SDS at 88° for 30 min. Stripped membranes were exposed to films for at least 3 days to assure that the previous signal had been completely removed.

**Independent linkage maps of linkage group III comprising the sex locus:** Genomic linkage maps were constructed from three different crosses resulting in three independent mapping populations. Two crosses were between bees of European origins (intraspin cross) (Hunt and Page 1995; R. E. Page, M. K. Fondrck, G. J. Hunt, E. Guzmán-Novoa, M. A. Humphries, K. Nguyen and A. S. Greene, unpublished results) and one cross was between bees of European (A. m. ligustica), and African (A. m. subulata) origins, the “wide” cross (Hunt and Page 1995; Hunt et al. 1998; R. E. Page, M. K. Fondrk, G. J. Hunt, E. Guzmán-Novoa, M. A. Humphries, K. Nguyen and A. S. Greene, unpublished results). Maps were based on male progeny (94 and 153 for the European cross, 179 for the African/European cross) of single queens. Because of male haploidy each male represented a single meiotic event and allowed direct determination of linkage relationships based on the segregation of RAPD markers. Detailed methods for generating RAPD markers and construction of honeybee linkage maps have been previously reported (Hunt and Page 1995; Hunt et al. 1998). The linkage group designations refer to the previously published map (Hunt and Page 1995). Linkage identity of linkage group III (comprising the sex locus) across different mapping populations was confirmed by STSs and segregating RAPD markers that were shared by two or three mapping populations. Shared RAPD markers were identified by the same 10-nucleotide primer in separate mapping populations that were of the same approximate size and map position relative to other markers in two or three mapping populations. RAPD markers were generated by polymerase chain reactions with 10-nucleotide DNA primers of arbitrary sequence and separated on agarose-Synergel gels (Diversified Biotech). Mapmaker software was used for linkage mapping with the Kosambi mapping function (Kosambi 1944) to convert recombination frequency into mapping distance (Hunt and Page 1995; Hunt et al. 1998).

Relative gap distances between markers along linkage group III were calculated as proportions of the total map distances (map distance between markers/total map size of linkage group III). The distribution of map distances of linkage group III was skewed toward smaller values (Kolmogoroff-Smirnov test for normality according to Lilliefors), probably because smaller map distances were easier to detect. However, we were able to eliminate the skew using an arc sine root transformation of the proportion data (P > 0.1, Kolmogoroff-Smirnov test for normality according to Lilliefors).

**RESULTS**

**Linkage analysis of Z and Q:** Linkage analysis was performed on workers, in which the recombination fraction between the markers and the sex locus was determined directly as the proportion of homzygotes for any marker. Individuals that were homozygous for a marker resulted from a crossover event between the marker and the sex locus during meiosis in the queen. Noncrossover marker homozygotes were not recovered in the progeny because they became diploid males, which are removed from the hive. Altogether, 271 worker offspring were heterozygous for Z and 23 showed a homzygous recombinant genotype. The recombination fraction of 7.8% corresponds to a map distance of 7.9 cm between the sex locus (X) and the Z marker (Kosambi 1944). In 287 individuals that could be genotyped for Q, only one recombinant homzygous genotype was found (sample no. 165), while the remainder were heterozygous for the marker, yielding a map distance of 0.3 cm. Individual no. 165, which was homzygous for Q, was heterozygous for Z, suggesting that Z and Q flank the sex locus. On the basis of our data, the likelihood of detecting a crossover event between X and Q is ~0.003; between X and Z, ~0.078. If Q and Z are flanking the X locus (the flanking model) there would have been only one crossover between Q and X and none between Z and X. So the likelihood of getting such a recombinational event is 0.00276. If Q and Z did not flank the X locus (the same side model) there would have been crossovers in both intervals. The likelihood of getting a recombinational event between both Q/X and Z/X is ~0.0002. The likelihood ratio then is 0.00276/0.0002 or 13.8. The flanking model is significantly better than the same side model using a likelihood ratio test statistic (G-test; P < 0.05; Sokal and Rohlf 1995).

Linkage data were combined with previous results to generate a combined linkage map of the sex locus region (Figure 2). The map is oriented relative to the telomere and centromere based on the ordering of markers relative to the end of linkage group III of the honey bee linkage map (Hunt and Page 1995) and the demonstration that the Z marker is located near the end of the chromosome based on fluorescence in situ hybridization (Beye et al. 1996). Distance and ordering of the Q and sts335–33 markers relative to the sex locus is based on previous linkage map data (Hunt and Page 1994, 1995; Hunt et al. 1998).

**Long-range restriction map of the sex locus region:** A detailed restriction map was generated using the closely
linked Q, Z, and sts335-.33 markers. High-molecular-weight DNA was isolated from honey bee pupae and digested with 10 rare-cutting restriction enzymes. Restriction fragments were fractionated by pulsed-field gel electrophoresis (CHEF) using different size ranges, blotted and hybridized consecutively with the probes sts335-.33, Q, and the cosmid containing the Z marker on the same membrane (Figure 3). The analysis revealed two DNA fragments that hybridize to Q and Z. These include a Ascl fragment of 780 kb and a NotI fragment of 400 kb. Most notably, the sts335-.33 marker hybridized to fragments of the same size. Coincidental sizes and comigration of fragments were excluded on the basis of analysis of double-restriction digests. For example, in double restrictions of NotI/SgfI the Q-marker probe hybridized to a 370-kb fragment while the Z cosmid detected a fragment of 35 kb. The sum of the two bands is equivalent to the 400-kb band observed in single NotI restrictions. Similar results were obtained for the NotI/SfiI double restrictions. Further analysis with several rare-cutting restriction enzymes led to the construction of a refined map around the sex locus region (Figure 4). Restriction sites of FseI, MluI, PmeI, RsrII, and NaeI were not included in the map because more than one site was found between Q and Z. SmaI sites in the vicinity of the Q/335-.33 region were mapped, because one SmaI site was located between the closely linked markers Q and sts335-.33. The SmaI fragment containing the sts335-.33 marker was mapped close to the NotI/SfiI restriction sites on the telomeric side of the map, while the SmaI/Q fragment was mapped by double-restriction analyses. Because sts335-.33 has a larger genetic distance from the sex locus than Q, the only possible ordering places Z and Q flanking the sex locus. Taking the distance of the SmaI site between Q and sts335-.33 into account, the physical distance between Z and Q is <360 kb. Considering the 8.2-cM map distance of Q and Z, the recombination rate is 44 kb/cM in the proximaty of X. Because only restriction sites close to the genetic markers Q and Z were mapped, the average recombination rate is even higher than 44 kb/cM in the sex locus region. The sex locus should be as close as 10,000–20,000 bp to Q, assuming that recombination is evenly distributed in this region.

**Distribution of recombination rate along the sex locus linkage group III:** Independent linkage maps of the sex locus region (Figure 5A) and of linkage group III (Figure 5, B, C, and D) were generated. A, C, and D are new maps of the X linkage group III while the B map has been published previously (Hunt and Page 1995). In two linkage groups designated as B and C ("intrastrain" cross and "wide" cross, respectively) the Q (stsQ16-58h) and the sts335-.33 markers precisely map the sex locus region. In map D X is localized by the Z marker (stsZ) mapping data. RAPD markers derived from the same primer and of approximately the same fragment size were considered as potentially homologous. Another indication of homology was con-
served orders of markers between the three maps. Potential homology is indicated (dashed and connecting lines in Figure 5) between markers on linkage group III. However, the independent linkage groups could not be joined together because a large gap of distance (up to 39.7 cM) is found in all three maps in the region of the sex locus. This is the largest map distance found independently in all three maps and the arcsine transformed map distances showed that there is low probability that we would generate such large map distance by chance. We used the gap distance (for Figure 5D, we assume a distance of 35 cM, which was our maximum detection threshold with this mapping population) and looked for the probability of obtaining a map distance by chance as great or greater than that observed (t-test of arcsine transformed data: Figure 5B: $P < 0.001$, $n = 26$; Figure 5C: $P < 0.001$, $n = 22$; Figure 5D: $P < 0.001$, $n = 22$). Moreover, considering the fact that a gap is found independently in all three maps, the probability is even lower. Another point of view is that average spacing of >1000 independent RAPD markers is $\sim 3.5$ cM for the honeybee genome (Hunt and Page 1995). These findings are evidence that genetic recombination.

Figure 4.—Physical map combined with genetic mapping data (Figure 2) that contains the sex locus of the honey bee. Long-range restriction map encompassing the sex locus and the markers Z, Q, and sts335-33, which was constructed using DNA restricted with combinations of restriction enzymes Acl (A), NotI (N), SgfI (Sg), SfiI (Sf), and SmaI (Sm). Note that SmaI restriction sites could only be determined in the vicinity of the Q and sts335-33 markers, because more than one site occurs in the Z/Q region. The mapping data showed that Z and Q are flanking the sex locus and that the distance is $< 360$ kb, resulting in a recombination rate of 44 kb/cM in the vicinity of the sex locus.

Figure 5.—Independent linkage maps of the sex locus linkage group III. The linkage group designation refers to the previously published map B (Hunt and Page 1995). The identity of group III (comprising the sex locus) across different mapping populations was confirmed by STSs and segregating RAPD markers that were shared by two or three mapping populations (dashed and connecting lines). Shared RAPD markers were identified by the same 10-nucleotide primer in separate mapping populations that were of the same approximate size and map position relative to other markers in two or three mapping populations. Mapmaker software was used for linkage mapping and Kosambi mapping function (Kosambi 1944) was used to convert recombination frequency into mapping distance (Hunt and Page 1995; Hunt et al. 1998). Presumed location of the sex locus ($X$) is based on location of sequence-specific sex-linked markers Z (stsZ) and Q (stsQ16-58) (Hunt and Page 1994; Beye et al. 1998a).
is not equally distributed along the linkage groups. There are other regions in the honey bee genome with high recombination rates, as evidenced by other gaps in the published maps. The genetic recombination rate is higher in the sex locus region than in the remainder of linkage group III, resulting in large map distances, assuming markers are randomly distributed.

**DISCUSSION**

**Relationship between recombination and physical distance:** The ordering of the sex-linked markers Z and Q allowed the first precise physical restriction mapping of a CSD factor. The small physical distance within the 360-kb Q/Z restriction fragment containing the sex locus favors the idea of a single sex factor “gene” (or genes that are very closely linked) in complementary sex determination. It has been postulated (Nöthiger and Steinmann-Zwicky 1985) that the seemingly different mechanisms of sex determination in insects may be based on a common strategy whereby an early acting primary signal irreversibly sets the state of a downstream sex-specific key gene. In Drosophila melanogaster the primary signal is given by the X:A ratio that regulates the sex-specific splicing of the key gene Sxl (Sex-lethal; Parkhurst and Meneely 1994). According to the proposed principle, a “sex locus gene” in the honey bee is the primary signal and should regulate, in the hemi- or and in the heterozygous state, a downstream, sex-specific key gene(s).

The physical mapping results support those of Hunt and Page (1995) for the extremely high recombinational rate of honey bees compared to other higher eukaryotes (e.g., Drosophila, which has nearly the same physical genome size as the honey bee but has roughly a physical-to-genetic distance of 575 kb/cm; Merriam et al. 1991). Using random amplified polymorphic DNA (RAPD) markers, Hunt and Page estimated an average recombinational rate of 52 kb/cm throughout the estimated 3500 cm of the honey bee genome. Other studies have shown that the relationships between recombinational and physical distances can vary up to 100-fold across the genome (e.g., in tomato: Ganal et al. 1989; Segal et al. 1992). Our estimate based on the first physical mapping suggests that linkage maps based on RAPD analyses are not necessarily inflated by errors in genotyping.

**High recombination rate in the sex-determining region:** The striking feature of the physical and linkage mapping results is that a high recombination rate (< 44 kb/cm) is found within a 360-kb fragment containing the sex factor. Chromosomal exchange is not suppressed as proposed by Whiting (1943), but instead recombination rate is unusually high in the CSD region. The high recombinational rate is further supported by large map distances (gaps) of linkage group III (up to 39.7 cm distance) found in all three maps around the sex-determining region (Figure 5). Although ~1000 independent RAPD markers were generated (Hunt and Page 1995; Hunt et al. 1998; R. E. Page, M. K. Fondrk, G. J. Hunt, E. Guzmán-Novoa, M. A. Humphries, K. Nguyen and A. S. Greene, unpublished results) and ~150 multilocus fingerprint markers were screened for sex linkage (Beye et al. 1994), the gap of linkage group III persists. The best sex-linked markers, Q and Z, have a map distance to X of 0.3 and 7.9 cm, respectively. Assuming the 3500 cm for the honey bee genome (Hunt and Page 1995) 1150 random markers should result in a nearest random marker of 1.5 cm to X with the upper 95% confidence interval for the distance of 4.5 cm (Martin et al. 1991). The large gap and the large distance of Z from X suggest that recombination is frequent in the sex-determining region, resulting in large map distances between physically close markers.

The finding of such elevated rates of recombination in the vicinity of the sex locus suggests an adaptive explanation. Allele matching (homozygosity) at the sex locus results in a diploid male with zero fitness. This results in strong selection favoring allelic diversity at the sex locus. As a consequence, the sex locus in honey bees exhibits extreme levels of polymorphism maintained by symmetrical overdominant selection (Wright 1965; Adams et al. 1977; Yokoyama and Nei 1979). In general, the number of sex alleles is thought to be maintained in a population by the balance between mutation and selection, both of which lead to increases in allele numbers and allelic diversity, and the loss of alleles due to genetic drift (Wright 1965; Yokoyama and Nei 1979).

Recombination in and around the sex locus may be selected as a consequence of different mechanisms. Strobeck et al. (1976) proposed that recombination will be favored near genes showing a heterozygous advantage because favorable alleles of genes neighboring such a locus can recombine with different sex alleles. Without recombination, favorable linked genes could not increase in frequency because strong overdominant selection at the linked sex locus would prevent it. A favorable allele could not be more frequent than the sex allele to which it is linked. This could explain the apparent high recombination rate between the sex locus and the centromere.

High recombination may also be expected within the sex locus if detrimental mutations with small effects accumulate faster than they can be eliminated by selection (Kondrashov 1984, 1988; Charlesworth 1990). As detrimental mutations increase, the sex allele loses its complementary function. In complementary sex determination a loss of function of an allele may have severe fitness effects by resulting in the production of a diploid male. Recombination within the gene could rescue the gene function in some gametes and reduce the deleterious effects.

**Further molecular analysis of the sex locus:** Detailed understanding of the function, genome organization,
and the population biology of sex locus haplotypes requires the isolation and molecular characterization of the sex locus. Since we do not know the biochemical function or the product of the sex locus, we are currently attempting to clone this gene using the flanking markers described here. The successful outcome of this approach depends on the relationship between physical and genetic distance, which may vary up to 100-fold. The high recombination rate in the vicinity of the sex locus favors the idea of a marker-based cloning attempt.

On the basis of the mapping data, we suggest that the Q marker is as close as 10,000–20,000, which would enable us to isolate the sex factor by a simple chromosomal walking or landing strategy, without tedious mutational analysis.

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LITERATURE CITED


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