Linkage Analysis of Sex Determination in *Bracon* sp. Near *hebetor* (Hymenoptera: Braconidae)

Alisha K. Holloway,* Michael R. Strand,† William C. Black IV‡ and Michael F. Antolin*

**Department of Biology, Colorado State University, Fort Collins, Colorado 80523, †Department of Entomology, University of Wisconsin, Madison, Wisconsin 53706 and ‡Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523*

Manuscript received November 19, 1998
Accepted for publication September 7, 1999

ABSTRACT

To test whether sex determination in the parasitic wasp *Bracon* sp. near *hebetor* (Hymenoptera: Braconidae) is based upon a single locus or multiple loci, a linkage map was constructed using random amplified polymorphic DNA (RAPD) markers. The map includes 71 RAPD markers and one phenotypic marker, *blonde*. Sex was scored in a manner consistent with segregation of a single "sex locus" under complementary sex determination (CSD), which is common in haplodiploid Hymenoptera. Under haplodiploidy, males arise from unfertilized haploid eggs and females develop from fertilized diploid eggs. With CSD, females are heterozygous at the sex locus; diploids that are homozygous at the sex locus become diploid males, which are usually inviable or sterile. Ten linkage groups were formed at a minimum LOD of 3.0, with one small linkage group that included the sex locus. To locate other putative quantitative trait loci (QTL) for sex determination, sex was also treated as a binary threshold character. Several QTL were found after conducting permutation tests on the data, including one on linkage group I that corresponds to the major sex locus. One other QTL of smaller effect had a segregation pattern opposite to that expected under CSD, while another putative QTL showed a female-specific pattern consistent with either a sex-differentiating gene or a sex-specific deleterious mutation. Comparisons are made between this study and the in-depth studies on sex determination and sex differentiation in the closely related *B. hebetor*.

SEX determination in most Hymenoptera is via haplodiploidy: males develop parthenogenetically from unfertilized haploid eggs while females develop from fertilized diploid eggs. In some (chalcidid) Hymenoptera, haplodiploid sex determination likely depends upon a mechanism of genomic imprinting (Dobson and Tanouye 1998). On the other hand, most Hymenoptera display a genetic mechanism of sex determination called complementary sex determination (CSD). Under CSD, sex is determined by homozygous or heterozygous genotypes of a single "sex locus" with a large number of alleles (Whiting 1943; Heimpel *et al.* 1999). Under CSD, diploid individuals that are heterozygous at the sex locus develop as females, while individuals that are hemizygous (haploid) or homozygous (diploid) at the sex locus develop as males. In Hymenoptera, diploid males are inviable, sterile, or produce sterile (triploid) daughters, and as a consequence the deleterious effects of inbreeding under CSD are severe (see reviews by Stouthamer *et al.* 1992; Cook 1993; Cook and Crozier 1995). CSD has been identified in >30 species within four superfamilies of Hymenoptera including the primitive sawflies, indicating that CSD is likely the ancestral means of sex determination in this group.

The molecular genetic mechanism that triggers CSD has yet to be identified (Stouthamer *et al.* 1992; Cook 1993; Beukeboom 1995; Cook and Crozier 1995). Different sex alleles of the sex locus have no gender tendencies; homozygotes for any sex allele develop as males (Cook and Crozier 1995). It has been hypothesized that the products of two different sex alleles may form an active heterodimer that serves as an unambiguous signal for sex determination (Crozier 1971; Hunt and Page 1994; Beukeboom 1995). Evidence from a number of species points to CSD being controlled by a single sex locus or at least a group of tightly linked genes found in one region of the genome (Cook 1993; Cook and Crozier 1995). Multilocus CSD, where a number of widely dispersed genes would have to be homozygous to produce diploid males, has similar effects to single-locus CSD, but it is difficult to distinguish from single-locus CSD without comprehensive genetic analyses. Two studies of CSD in which sex was treated as a single Mendelian locus [*Bracon hebetor* (Whiting 1961) and the honeybee (Hunt and Page 1994)] mapped the sex locus to a single region in the genome. However, two lines of evidence point to there being other genes involved in sex determination or somatic sex differentiation. First is the existence of heritable variation for the development of intersexes (*i.e.*, individuals with a "male"

Corresponding author: Michael F. Antolin, Department of Biology, Colorado State University, Fort Collins, CO 80523.
E-mail: antolin@lamar.colostate.edu

genotype but "female" characteristics) in species like *B. hebetor* (von Borstel and Smith 1960). Second, survival of diploid males differs among species that have been otherwise shown to have CSD (see Stouthamer *et al.* 1992; Cook 1993; Holloway *et al.* 1999).

CSD was first characterized in the parasitoid wasp *B. hebetor* (Whiting 1943). Previously, we mapped the genome of *B. hebetor* using random amplified polymorphic DNA-single-strand conformation polymorphism (RAPD-SSCP) markers (Antolin *et al.* 1996). However, we have been unable to carry out a more detailed genetic analysis of CSD in *B. hebetor* because diploid males of that species have low survival, making it difficult to correctly estimate the recombination frequencies of markers closely linked to the sex locus. In contrast, we recently described a closely related species to *B. hebetor*, currently identified as *Bracon* sp. near *hebetor* (Heimpel *et al.* 1997), which produces viable diploid males (Holloway *et al.* 1999). The existence of viable diploid males allows for detection of both sex-determining genes and other somatic sex-determining genes (*e.g.*, genes that specify pathways of sexual differentiation; see Marin and Baker 1998). In this study, we constructed a linkage map for *B.* sp. near *hebetor* from backcross populations using markers derived from RAPD fragments subjected to SSCP analysis (Black and Duteau 1996). We examined sex in two ways. First, we examined the placement of the sex locus as a single locus with two alleles under CSD, with homozygous diploid males and heterozygous diploid females. Second, we treated sex as a binary morphological trait and searched for quantitative trait loci (QTL) that influence sex.

MATERIALS and METHODS

Source of insects: *Bracon* sp. near *hebetor* (Hymenoptera: Braconidae) is a parasitoid wasp that attacks moth larvae in a number of lepidopteran families. This wasp was originally identified as *B. hebetor*, but the two species have been shown to be reproductively isolated and genetically distinct (Heimpel *et al.* 1997). The population used in this study originated from Barbados and has been maintained in the laboratory since 1992, with most rearing on *Plodia interpunctella* larvae as described by Heimpel *et al.* (1997).

During general colony maintenance, a spontaneous recessive light body color mutation arose in our *B.* sp. near *hebetor* culture that was described as *blonde* (*bl*; Holloway *et al.* 1999). Wild-type wasps (+) have black eyes, black sclerites on the thorax and abdomen, dark wing veins, and a tan body color. Wasps with the *blonde* mutation have black eyes, light brown sclerites on the thorax and abdomen, light wing veins, and a creamy-yellow body color.

The marker was used to help map the position of the sex locus. Several backcross families that included both females and diploid males were generated by reciprocal crosses between laboratory populations with the two body colors. Mated females were allowed to oviposit for 5 days on *P. interpunctella* fifth instar larvae while their male mates were fed honey and kept in an incubator at 22° to prolong life span. F_1 females were then backcrossed to these males and provided *P. interpunctella* hosts for 10–14 days of oviposition. Both male and female backcross progeny were collected and frozen at −80° for molecular analysis. Wasps were housed in environmental chambers at 28°, 16L:8D, and 70% humidity [see Strand and Godfray (1989) for laboratory rearing methods of *P. interpunctella* and Bracon].

Two families were selected for analysis based on the size of the backcross generation, one heterozygous (+/*bl* female) × blonde (*bl* male) backcross family (12A) and one heterozygous (+/*bl* female) × wild-type (+ male) backcross family (16W). From family 12A, 39 diploid males and 40 females were used, and 40 diploid males and 40 females were used from family 16W. The body color mutation was mapped in family 12A.

Initially, four RAPD primers were used to screen for possible diploid males by identifying males with heterozygous genotypes at codominant loci (all primers from Operon Technologies, Alameda, CA). Diploid males are necessary for determining the placement of the sex locus because of cosegregation of linked markers. Markers closely linked to the sex locus will have low recombination rates and segregate with sex alleles in the cross. If diploid males are homozygous at the sex locus, they should also be homozygous for closely linked RAPD markers. In contrast, females that are heterozygous at the sex locus should be heterozygous for linked RAPD markers.

DNA isolation: DNA was isolated by salt extraction following the protocol of Coen *et al.* (1982). DNA was resuspended in 100 μl TE (10 mm Tris-HCl, 1 mm EDTA, pH 8.0). DNA was stored in a −80° freezer; DNA used for PCR reactions was stored at 4°.

PCR amplification: RAPD-PCR protocols were as described in Black *et al.* (1992), with 1 μl of DNA template used in each 50-μl reaction. A negative control (all reagents except template DNA) was used to check each set of PCR reactions for contamination. PCR products were stored at 4° until electrophoresis.

Electrophoresis to detect SSCP: SSCP analysis and silver staining protocols were as described in Hiss *et al.* (1994), Antolin *et al.* (1996), and Black and Duteau (1996). Samples were electrophoresed on large (35 × 50 cm), thin (0.4 mm) glycerol (5%) polyacrylamide (5%, 2% cross-linking) gels. Shark tooth combs (6 mm) were used to create lanes for loading samples (4 μl of PCR product mixed with 1.5 μl of loading buffer). Electrophoresis proceeded at 350 V at room temperature for 15 hr, and gels were stained with silver nitrate to detect the mobility of the different DNA conformations.

Gel scoring: Amplified fragments were scored directly from dried gels by measuring band mobility relative to a 1-kb size marker (BRL Laboratories). To estimate sizes of the amplified DNA fragments, size standards were fitted to an inverse function that relates fragment size and mobility (Schaffer and Sederoff 1981). RAPD markers were named by the primer designation followed by a period and the estimated size of the fragment. Only repeatable bands that segregated in a Mendelian fashion were scored and used in analyses. Banding patterns for each primer were similar between families.

Data analysis: The data include RAPD markers with both codominant and dominant alleles. Codominant markers are informative in a backcross as long as the F_1 mother inherits different alleles from the P_1 mother and father. For typical RAPD markers, where the presence of a band is dominant, a marker is informative only if the dominant-band-present allele is inherited from the P_1 mother. Therefore, the genotypes of backcross progeny are scored as either A (homozygous for alleles from the P_1 father) or H (heterozygous). All markers were informative in all individuals because exact genotypes for progeny could be determined and because the phase of all markers was known.

Offspring genotypes were entered into JOINMAP v2.0 and coded as a backcross (Stam and van Ooijen 1995). JOINMAP

allowed data from both families to be combined into one linkage map, and converted distances between markers from recombination fractions to map units (cM) by the Kosambi mapping function (Kosambi 1944). A threshold logarithm of odds (LOD) score of 3.0 was used to group markers. DRAWMAP v1.1 (van Ooijen 1994) was used to plot a linkage map.

The trait sex was examined in two different ways. First, sex was treated as a single locus as expected under CSD, with males coded as A (homozygotes) and females as H (heterozygotes). Second, sex was treated as a quantitative trait scored as 0 (male) or 1 (female). Mapping QTL for binary traits using linear regression has been proven effective, especially for backcross populations (Visscher *et al.* 1996; Xu and Atchley 1996; Xu *et al.* 1998). The order of markers obtained from JOINMAP was used as the framework map for QTL analysis of each family via MapQTL (van Ooijen and Maliepaard 1996). Putative QTL were identified when LOD scores from MapQTL exceeded 2.0. Segregation of markers flanking putative QTL was tested for deviation from expected frequency by *G*-tests in contingency tables, with probabilities corrected for experiment-wise error (Sokal and Rohlf 1995).

In addition, to determine threshold LOD scores for accepting the presence of a QTL and estimate the relative effects of the QTL, the data from each family were analyzed using a maximum-likelihood mixture model, which uses a probit analysis of binary traits (Xu *et al.* 1998), using a FORTRAN program (BINARYQTL, available from Shizhong Xu, University of California, Riverside, E-mail: xu@genetics.ucr.edu). In addition to providing LOD scores and genetic variances associated with each QTL, the program was modified to carry out a permutation test to set the threshold level for accepting a QTL, following the "shuffling" permutation procedure of Churchill and Doerge (1994). Threshold LOD values were determined from the 95th percentile of maximum LOD of 1000 permutations of the data in each family.

RESULTS

A linkage map of *B.* sp. near *hebetor*: A total of 24 RAPD primers resulted in a large number of repeatable amplified fragments (Table 1). There were, on average, 14.8 (±1.3 SE) fragments per primer in family 12A and 17.2 (±1.9 SE) in family 16W. Polymorphic fragments used in the analysis ranged in size from 235 to 2855 bp. In family 12A there were 3.7 (±0.4 SE) polymorphic bands per primer and 2.4 (±0.4 SE) in family 16W. Family 12A had 88 polymorphic RAPD markers; 72 of these were informative for mapping. Only 9 of the 24 primers were used in family 16W because the grandparents shared many of the same RAPD alleles resulting in fewer polymorphic loci in the backcross. The 9 primers used in family 16W resulted in 29 polymorphic RAPD markers and 22 of these were informative for mapping. Only 10 of the markers shared between the two families were polymorphic in both families, giving a total of 84 RAPD markers used in the analysis. Of these, 62% were codominant. According to χ^2 goodness-of-fit tests, segregation ratios of 4 of the 84 markers deviated significantly from expected ratios (*i.e.*, 1:1) after correction for multiple comparisons ($\chi^2 \geq 10.83$, d.f. = 1, $P < 0.05$; Sokal and Rohlf 1995). Two of these were from family 12A and were unlinked (C4.270, D10.1185); the others mapped to linkage groups V and VII (Figure 1).

TABLE 1

Number of RAPD-SSCP fragments amplified and markers analyzed for backcross families 12A and 16W

Primer	No. of fragments	No. of polymorphic loci	Dominant markers	Codominant markers
		Family 12A		
A01	23	5	3 (1)	1
A02	27	3	2	1
A05	17	8	4 (2)	2
A10	15	4	1	3
A13	19	4	1 (2)	1
A16	10	2	0	2
A19	6	1	0	1
A20	17	1	0	1
AM07	14	5	1 (1)	3
B01	16	5	1	4
B04	16	2	1	1
B07	16	2	0	2
B10	14	4	1	3
C02	15	5	2	3
C04	24	7	3 (3)	1
C05	14	2	1	1
C08	10	2	0	2
C14	12	4	0 (2)	2
C17	9	4	2 (2)	0
C20	28	6	2 (3)	1
D04	14	5	1	4
D09	5	2	0	2
D10	9	3	1	2
D15	5	2	0	2
Total	355	88	27 (16)	45
		Family 16W		
A01	12	2	1	(1) 0
A02	26	4	0 (2)	2
A10	21	2	1	1
A13	18	2	0	2
A16	20	3	0	3
AM07	16	3	1 (1)	1
B1	8	3	0	3
C14	13	5	0 (3)	2
C20	21	5	4	1
Total	154	29	7 (7)	15

Numbers in parentheses indicate markers that were uninformative for mapping. For backcrosses, only dominant markers where the band-present allele originates in the grandmother are informative for mapping.

The final map, with grouping LOD of 3.0, included 71 RAPD markers, the phenotypic marker *blonde*, and the sex locus. Bracon species are known to have 10 chromosomes (Whiting 1961), and in this study, 10 linkage groups were formed with a total map length of 536.1 cM (Figure 1). Lowering the LOD threshold for linkage to 2.5 did not change the number or size of

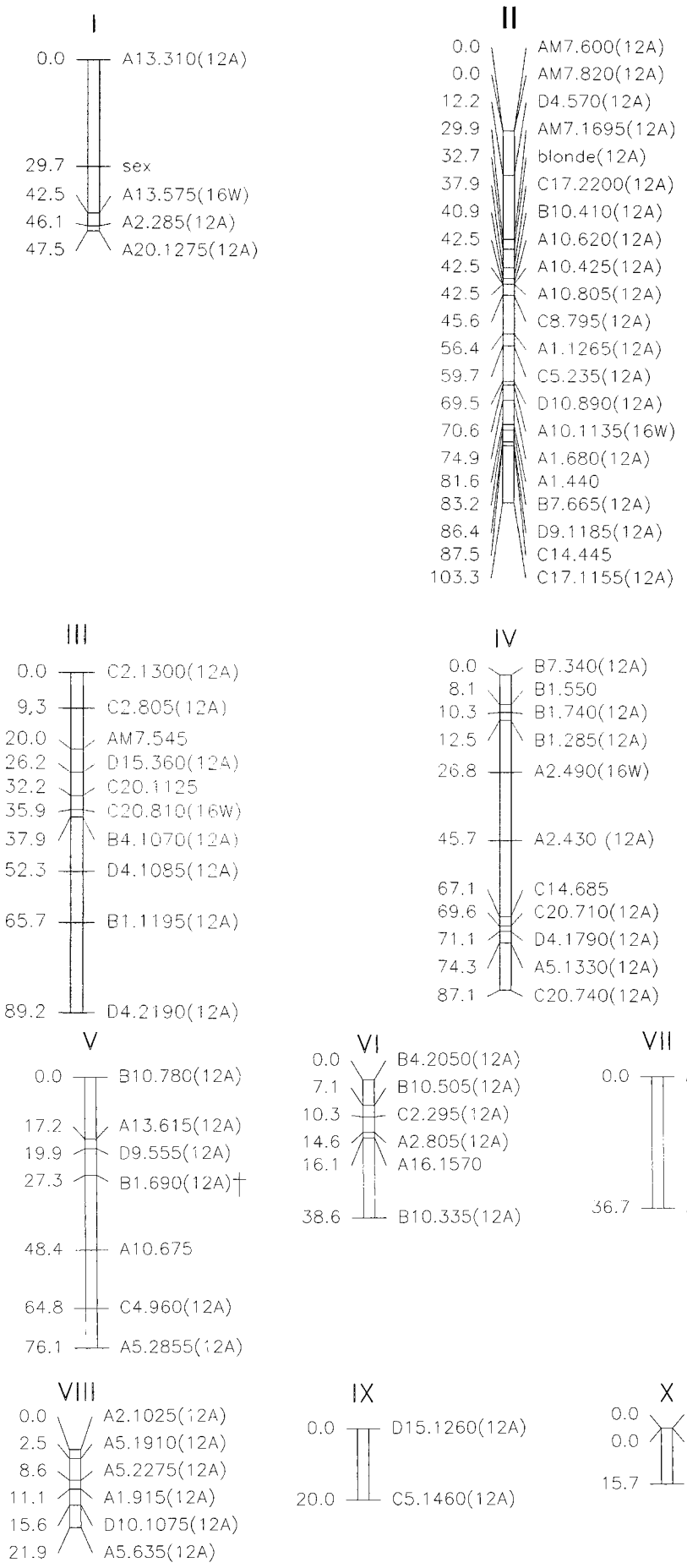

Figure 1.—A linkage map of *B.* sp. near *hebetor* based on RAPD markers. Loci are listed at right and the total centimorgan distance at left. Markers that were only found in one family are noted with the family name in parentheses. All of the markers were mapped with a minimum LOD score for linkage of 3.0. Markers that deviated from expected 1:1 segregation ($P < 0.05$) appear with a † to the right of the marker name.

TABLE 2

Segregation patterns of markers where MapQTL identified putative QTL at LOD > 2

Linkage group marker (position)	Family	LOD (probability[a])	Males		Females	
			A (homozygote)	H (heterozygote)	A (homozygote)	H (heterozygote)
Linkage group I						
A13.310 (0.0)	12A	4.26 (0.002)	27	12	9	31
QTL (29.0)	12A	11.59 (<0.001)				
A13.575 (42.5)	16W	14.59 (<0.001)	34	6	3	37
A02.285 (46.1)	12A	10.47 (<0.001)	31	8	4	35
A20.1275 (47.5)	12A	9.36 (<0.001)	30	9	5	35
Average			30.5	8.8	5.3	34.5
Linkage group III						
AM7.545 (20.0)	16W	1.97 (0.04)	9	31	22	18
C20.1125 (32.2)	16W	2.24 (0.02)	11	29	25	14
QTL (35.9)		3.32 (0.002)				
C20.810 (35.9)	16W	3.32 (0.002)	11	29	28	11
Average			10.3	29.7	25	14.3
AM7.545 (20.0)	12A	0.14 (>0.99)	17	22	21	19
D15.360 (26.2)	12A	0.02 (>0.99)	19	20	21	19
C20.1125 (32.2)	12A	0.13 (>0.99)	19	20	23	17
B4.1070 (37.9)	12A	0.82 (>0.99)	14	25	23	17
D4.1085 (52.3)	12A	0.23 (>0.99)	16	23	20	19
Average			17.0	22.0	21.6	18.2
Linkage group V						
A13.615 (17.2)	12A	0.03 (>0.999)	17	22	25	15
D9.555 (19.9)	12A	0.33 (0.99)	19	20	24	14
B1.690 (27.3)	12A	2.09 (0.123)	21	18	34	6
QTL (46.0)		2.06 (0.137)				
A10.675 (48.4)	12A	0.00 (0.137)	20	19	21	19
A10.675 (48.4)	16W	0.01 (>0.999)	20	20	21	19
C4.960 (64.8)	12A	0.33 (>0.999)	19	20	25	15
Average			19.2	19.8	25.8	13.8

These patterns were subsequently reanalyzed using a residual variance model (Xu *et al.* 1998). Reported LOD values are from MapQTL; probabilities are from permutation tests (Churchill and Doerge 1994).

[a] Probabilities determined by genome-wide permutations of the data to provide experiment-wise error rates using the methods of Churchill and Doerge (1994).

linkage groups, and all linkage groups except group VII remained intact even when the threshold LOD was raised to 5.0. Below LOD 2.5, 8 markers from family 12A and 5 from family 16W remained unlinked. All 10 markers that were polymorphic in both of the families were included on linkage groups. Of the 10 linkage groups, 4 were large (>60 cM) and 6 were small (15–50 cM). The average distance between markers was 7.45 cM.

QTL analysis of sex: When sex was treated as a single locus and included in linkage analysis, a single locus identified on linkage group I included the sex locus with two flanking markers (A13.310 and A13.575) 29.7 and 12.8 cM away (Figure 1).

When sex was treated as a quantitative trait, several putative QTL were found at LOD > 2.0 on linkage groups I, III, and V (Table 2). To further examine markers surrounding the putative QTL, two flanking markers on each side were tested for segregation patterns that differed between the sexes (Table 2). Linkage group I had a QTL located between the same markers that flanked the sex locus when sex was treated as a single locus. Segregation of all markers on this group was consistent with complementary sex determination, with significantly more heterozygous females and homozygous males than expected by chance (see Table 2; $G = 144.49$, d.f. $= 1$, $P < 0.001$, all probabilities adjusted for experiment-wise error). Markers on linkage group III had a pattern opposite to that expected from CSD, with more heterozygous males and homozygous females than that expected by chance (overall $G = 28.37$, $P < 0.05$). This pattern was found in both family 16W ($G = 35.18$, $P < 0.05$), where a statistically significant QTL was found (see below), and in family 12A ($G = 6.33$, $P < 0.10$).

The third putative QTL on linkage group V showed a sex-specific segregation pattern; markers surrounding B1.690 had significantly too few heterozygous females ($G = 9.0$, $P < 0.004$). This pattern is consistent with segregation of either a sex-specific deleterious allele (low survival of heterozygous females) or a somatic sex-differentiating gene (homozygous individuals develop female characteristics).

Analysis of the data via probit analysis in the mixture model, using BINARYQTL (Xu *et al.* 1998), showed that LOD values from BINARYQTL and MapQTL were always within 10% of each other. Permutation of the data using the BINARYQTL approach yielded experiment-wise 95% LOD thresholds of 2.58 and 1.90 for the presence of a QTL in families 12A and 16W, respectively. These values show that the QTL identified on linkage group I are statistically supported in both families, that the QTL on linkage group III are statistically supported in family 16W but not in family 12A, and that the putative QTL on linkage group V has some but less than significant statistical support (Table 2).

Finally, BINARYQTL provides estimates of the genetic variance associated with each QTL, and this analysis showed that the QTL on linkage group I must correspond to the major sex locus underlying complementary sex determination. The genetic variance associated with the sex locus QTL on linkage group 1 was 420.25, compared to genetic variance of 0.27 for linkage group III and 0.28 for linkage group V. In family 16W the genetic variance associated with the sex locus QTL was 1.42, compared to a variance of 0.32 for the QTL on linkage group III, and a variance of 0.05 for the marker on linkage group V. Even without converting these values to the correct scale underlying sex, it is clear that >75% of the genetic variance in sex arose from the sex locus QTL on linkage group I.

DISCUSSION

Sex determination, linkage analysis, and QTL: When sex was treated as a Mendelian locus under CSD and scored as homozygotes (male) or heterozygotes (female), it unambiguously mapped to the small linkage group I. The QTL analysis of both families (12A and 16W) adds support to the hypothesis of a major sex-determining gene on linkage group I. Whiting (1943) hypothesized that the sex locus in *B. hebetor* is a polygenic chromosomal segment and that all primary and secondary sex-determining genes must lie in the same segment, with genes for male traits being recessive. Our data for *B.* sp. near *hebetor* do not support this hypothesis. Another QTL on linkage group III showed a sex-specific pattern of segregation, even though it was opposite to that expected under CSD, with more heterozygous males and more homozygous females than expected by chance.

These data are consistent with a single gene being responsible for sex determination, but with other sex-differentiating and sex-specific genes found elsewhere in the genome. The data are inconsistent with purely single-locus CSD. In many sexually reproducing organisms, other genes work in concert with the sex-determining genes or act as modifiers of sex determination and regulators of somatic sexual differentiation (Wachtel 1994; Marin and Baker 1998). This appears to be the case in *B.* sp. near *hebetor*. Both the QTL on linkage group III (homozygous females and heterozygous males) and the putative QTL on linkage group V (a deficiency of heterozygous females) showed sex-specific segregation. On linkage group V, marker B1.690 and flanking markers had more homozygous than heterozygous females, but equal numbers of both genotypes in males. This indicates a sex-limited gene for female development in this region.

This result also compares favorably with results from the closely related *B. hebetor*. In that species, not only was the sex locus described (Whiting 1943), but subsequent genetic analyses demonstrated that one form of intersex is controlled by another gene in the region near the *orange* locus (von Borstel and Smith 1960; Whiting 1961). Intersexes are individuals with a genetic predisposition to be one sex, yet they have tissues with characteristics of the other sex (*e.g.*, male head, female abdomen). It is possible that the QTL found in our study of *B.* sp. near *hebetor* correspond to a homologue of same sex-differentiating gene or a gene with similar effects.

Polymorphisms and linkage patterns: This genomic analysis of *B.* sp. near *hebetor* again demonstrates the high resolution of RAPD markers when analyzed as SSCP on large-format polyacrylamide, as was seen in *B. hebetor* and the mosquito *Aedes aegypti* (Antolin *et al.* 1996). Codominant markers comprised 62% of all markers used in the analysis, with alleles that differ in mobility by as little as 1 mm.

As has been reported in other linkage studies using RAPDs (Hunt and Page 1995; Kazmer *et al.* 1995; Antolin *et al.* 1996), markers amplified by the same RAPD primer had a tendency to group together. This clumping could result from repetitive regions of the genome (Williams *et al.* 1990) or from different conformations of the same loci (SSCPs). An example of amplifying of repetitive regions is the three closely linked markers from primer B01 on linkage group VIII (Figure 2). Markers that are likely SSCPs of the same locus are seen as pairs of markers with no recombination between them (AM07.600 and AM07.820; A10.425 and A10.620; and C02.615 and C02.600). The female parent was heterozygous for all of these loci. However, the male parent always had the slow allele for one marker and the fast allele for the other marker in each pair. The father having opposite alleles for the marker pairs could indicate that these loci are in repetitive regions instead of SSCPs.

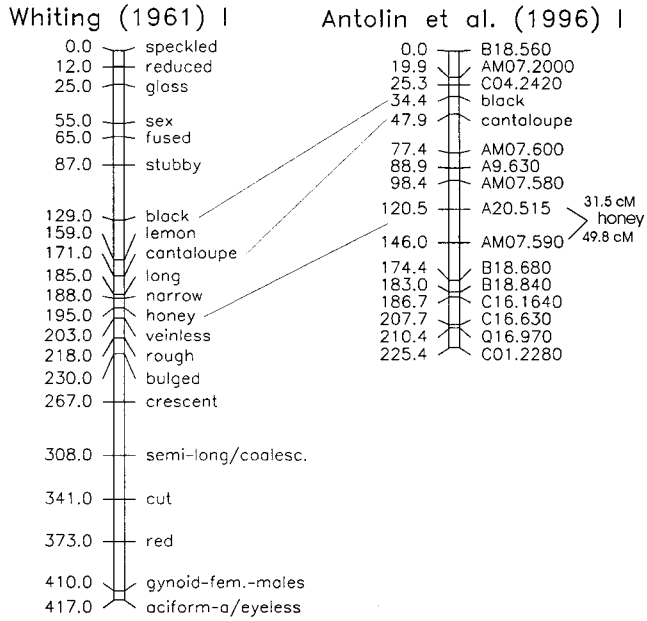

Figure 2.—Representation of *B. hebetor* linkage group I from Whiting (1961) showing the position of the sex locus and linkage group I from Antolin *et al.* (1996) showing comparative positions of body color mutations.

Comparison of *B.* sp. near *hebetor* and *B. hebetor*: Because *B.* sp. near *hebetor* and *B. hebetor* are closely related, we may expect them to have similar-sized genomes. The total map lengths of two previous studies of *B. hebetor* are 625 cM for a morphological map (Whiting 1961) and 1156 cM for a combined RAPD and morphological map (Antolin *et al.* 1996), compared to a total of 536.1 cM for *B.* sp. near *hebetor*. MAPMAKER/EXP 3.0b (Lander *et al.* 1987) was used to create the *B. hebetor* map in Antolin *et al.* (1996). When the data from Antolin *et al.* (1996) were reanalyzed with JOINMAP v2.0, using the same linkage groups and order of markers as in the previous map, the total map length was only 882.3 cM, a reduction of 274 cM. Using JOINMAP to completely reanalyze the data resulted in a map with 11 linkage groups and a total length of 759.7 cM.

Part of the difference in the map lengths from the two programs arises from different algorithms for estimating distances between markers. The JOINMAP algorithm uses local weighting of the two flanking markers on either side of the interval to be estimated, with weights based on LOD (Stam and van Ooijen 1995). On the other hand, MAPMAKER estimates distance between markers from the recombination fractions of those markers without weighting (Lander *et al.* 1987). For instance, when data from family 12A for markers on linkage group II of *B.* sp. near *hebetor* were entered into MAPMAKER, the map for linkage group II expanded from 103.3 to 204.9 cM. Small linkage groups with markers that are more evenly spaced are not affected by weighting.

Even when differences between mapping algorithms are taken into consideration, differences in map lengths between *B. hebetor* and *B.* sp. near *hebetor* remain. Several possibilities exist for the discrepancy in map lengths. First, the addition of four markers that grouped at LOD < 3.0 in the previous studies (Antolin *et al.* 1996) added 130 cM to the total map length. Second, because of inbreeding of *B.* sp. near *hebetor* in the laboratory, we may be seeing clustering of polymorphic regions of chromosomes interspersed with invariable regions. This would significantly underestimate map length of *B.* sp. near *hebetor* because the lack of genetic variation would reduce the number of recombinational events and the number of linkages that could be detected. Third, given that 10 linkage groups were found in *B.* sp. near *hebetor*, compared to 13 in *B. hebetor*, there may be chromosomal rearrangements causing the genomes of these two closely related species to differ in size.

The placement of the major sex locus seems to differ in *B. hebetor* and *B.* sp. near *hebetor*. While we found that the major sex locus is on a small linkage group in *B.* sp. near *hebetor*, the sex locus in *B. hebetor* was reported to be on a large linkage group (Whiting 1961; see Figure 2). However, examination of this linkage group reveals that the sex locus is tightly linked (10 cM) to one mutant locus, *fused*, which causes fused antennal segments. There is then a 42-cM gap between the next locus, *stubby* (stubby antennae), and the rest of the linkage group, beginning with the *black* locus (black body color). Bracon species are known to have 10 chromosomes (Crozier 1975), but only eight linkage groups were reported by Whiting (1961). This may indicate spurious linkages in the Whiting map, possibly between the stubby antennae and black body color mutations (Figure 2). Unfortunately, a more careful reanalysis of the Whiting data is not possible because the original data set cannot be obtained. By comparison, linkage group I from Antolin *et al.* (1996) also included the body color loci, *black*, *cantaloupe* (eye color), and *honey* (body color). The placement of these loci corresponds to locations on the Whiting map, but this linkage group is much smaller, with no large gaps above the *black body* locus. Determining whether the placement of the major sex locus corresponds between these two species or whether there have been significant chromosomal rearrangements will depend upon careful comparative genomic analyses, including physical mapping of markers and identification of the sex locus itself.

An important difference between *B. hebetor* and *B.* sp. near *hebetor* is that diploid males in *B. hebetor* are usually inviable, while they have high survival in *B.* sp. near *hebetor*. The viability of diploid males varies among Hymenoptera, which may indicate that if sex is determined by CSD in most bees, ants, and wasps, the pathways of sexual differentiation may differ among them (Stouthamer *et al.* 1992; Cook 1993; Holloway *et al.* 1999). Studies comparing sex determination in other groups

have shown that pathways of sex determination and sex differentiation can evolve rapidly (Bull 1983; Marin and Baker 1998). For example, in Diptera sex is primarily determined by the ratio of autosomes to sex chromosomes (genic balance). Homologues of *sex-lethal* (*sxl*), the major sex-determining gene that responds to ratios of autosomes to sex chromosomes in Drosophila, have been found in both the phorid fly, *Megaselia scalaris*, and the blowfly, *Chrysomya rufifacies*. However, *sxl* does not have a sex-determining function in either of these species (Muller-Holtkamp 1995; Sievert *et al.* 1997). Linkage mapping is an initial step for understanding the pathways that determine sex in organisms that exhibit CSD. Markers that are tightly linked with the sex-determining gene in honey bees have also been identified (Beye *et al.* 1994; Hunt and Page 1994). These studies provide a basis for future research on the genetics of sex determination and comparisons of the pathways of sexual differentiation in Hymenoptera that exhibit CSD.

We thank J. Herbers, T. T. Vaughn, and S. Xu for technical assistance and helpful discussions. This work was supported by United States Department of Agriculture-National Research Initiative competitive grant 94-37312-0062 to M.F.A. and M.R.S.

LITERATURE CITED

Antolin, M. F., C. F. Bosio, J. Cotton, W. Sweeney, M. R. Strand *et al.*, 1996 Intensive linkage mapping in a wasp (*Bracon hebetor*) and a mosquito (*Aedes aegypti*) with single-strand conformation polymorphism analysis of random amplified polymorphic DNA markers. Genetics **143:** 1727–1738.

Beukeboom, L. W., 1995 Sex determination in Hymenoptera: a need for genetic and molecular studies. BioEssays **17:** 813–817.

Beye, M., R. F. A. Moritz and C. Epplen, 1994 Sex linkage in the honeybee Apis mellifera detected by multilocus DNA fingerprinting. Naturwissenschaften **81:** 460–462.

Black, W. C., IV, and N. M. Duteau, 1996 RAPD-PCR and SSCP analysis for insect population genetic studies, pp. 514–531 in *The Molecular Biology of Insect Disease Vectors: A Methods Manual*, edited by J. Crampton. Chapman and Hall, New York.

Black, W. C., IV, N. M. Duteau, G. J. Puterka, J. R. Nechols and J. M. Pettorini, 1992 Use of the random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) to detect DNA polymorphisms in aphids. Bull. Entomol. Res. **82:** 151–159.

Bull, J. J., 1983 *Evolution of Sex Determining Mechanisms*. Benjamin/Cummings, Menlo Park, CA.

Churchill, G. A., and R. W. Doerge, 1994 Empirical threshold values for quantitative trait mapping. Genetics **138:** 963–971.

Coen, E. S., T. Strachan and G. Dover, 1982 Dynamics of concerted evolution of ribosomal DNA and histone gene families in the *melanogaster* species subgroup of *Drosophila*. J. Mol. Biol. **158:** 17–35.

Cook, J. M., 1993 Sex determination in the Hymenoptera, a review of models and evidence. Heredity **71:** 421–435.

Cook, J. M., and R. H. Crozier, 1995 Sex determination and population biology in the Hymenoptera. Trends Ecol. Evol. **10:** 281–286.

Crozier, R. H., 1971 Heterozygosity and sex determination in haplodiploidy. Am. Nat. **105:** 399–412.

Crozier, R. H., 1975 *Animal Cytogenetics, Vol. 3. Insecta 7: Hymenoptera*. Gebruder Borntraeger, Berlin.

Dobson, S. L., and M. A. Tanouye, 1998 Evidence for a genomic imprinting sex determination mechanism in *Nasonia vitripennis* (Hymenoptera: Chalcidoidea). Genetics **149:** 233–242.

Heimpel, G. E., M. F. Antolin, R. A. Franqui and M. R. Strand, 1997 Reproductive isolation and genetic divergence between two "strains" of *Bracon hebetor* (Hymenoptera: Braconidae). Biol. Control **9:** 149–156.

Heimpel, G. E., M. F. Antolin and M. R. Strand, 1999 Diversity of sex-determining alleles in *Bracon hebetor*. Heredity **82:** 282–291.

Hiss, R. H., D. E. Norris, C. Dietrich, R. F. Whitcomb, D. F. West *et al.*, 1994 Molecular taxonomy using single-strand conformation polymorphism (SSCP) analysis of mitochondrial ribosomal genes. Insect Mol. Biol. **3:** 171–182.

Holloway, A. K., G. E. Heimpel, M. R. Strand and M. F. Antolin, 1999 Survival of diploid males in *Bracon* sp. near *hebetor*. Ann. Entomol. Soc. Am. **92:** 110–116.

Hunt, G. J., and R. E. Page, 1994 Linkage analysis of sex determination in the honey bee (*Apis mellifera*). Mol. Gen. Genet. **244:** 512–518.

Hunt, G. J., and R. E. Page, 1995 Linkage map of the honey bee, *Apis mellifera*, based on RAPD markers. Genetics **139:** 1371–1382.

Kazmer, D. J., K. R. Hopper, D. M. Coutinot and D. G. Heckel, 1995 Suitability of random amplified polymorphic DNA for genetic markers in the aphid parasitoid, *Aphelinus asychis* Walker. Biol. Control **5:** 503–512.

Kosambi, D. D., 1944 The estimation of map distances from recombination values. Ann. Eugen. **12:** 172–175.

Lander, E. S., P. Green, J. Abrahamson, A. Barlow, M. Daly *et al.*, 1987 MAPMAKER, an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics **1:** 174–181.

Marin, I., and B. S. Baker, 1998 The evolutionary dynamics of sex ratio. Science **281:** 1990–1994.

Muller-Holtkamp, F., 1995 The sex-lethal gene homologue in *Chrysomya rufifacies* is highly conserved in sequence and exon-intron organization. J. Mol. Evol. **41:** 467–477.

Schaffer, H. E., and R. R. Sederoff, 1981 Improved estimation of DNA fragment lengths from agarose gels. Anal. Biochem. **115:** 113–122.

Sievert, V., S. Kuhn and W. Traut, 1997 Expression of the sex determining cascade genes *Sex-lethal* and *doublesex* in the phorid fly *Megaselia scalaris*. Genome **40:** 211–214.

Sokal, R. R., and F. J. Rohlf, 1995 *Biometry*, Ed. 3. W. H. Freeman and Co., New York.

Stam, P., and J. W. van Ooijen, 1995 Joinmap version 2.0: software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen, The Netherlands.

Stouthamer, R., R. F. Luck and J. H. Werren, 1992 Genetics of sex determination and the improvement of biological control using parasitoids. Environ. Entomol. **21:** 427–435.

Strand, M. R., and H. C. J. Godfray, 1989 Superparasitism and ovicide in parasitic Hymenoptera, theory and a case study of the ectoparasitoid Bracon hebetor. Behav. Ecol. Sociobiol. **24:** 421–432.

van Ooijen, J. W., 1994 DrawMap, a computer program for drawing genetic linkage maps. J. Hered. **85:** 66.

van Ooijen, J. W., and C. Maliepaard, 1996 MapQTL version 3.0: Software for the calculation of QTL position on genetic maps. CPRO-DLO, Wageningen, The Netherlands.

Visscher, P. M., C. S. Haley and S. A. Knott, 1996 Mapping QTLs for binary traits in backcross and F_2 populations. Genet. Res. **68:** 55–63.

von Borstel, R. C., and P. A. Smith, 1960 Haploid intersexes in the wasp Habrobracon. Heredity **15:** 29–34.

Wachtel, S. S., 1994 *Molecular Genetics of Sex Determination*. Academic Press, San Diego.

Whiting, A. R., 1961 Genetics of *Habrobracon*. Adv. Genet. **10:** 295–348.

Whiting, P. W., 1943 Multiple alleles in complementary sex determination of *Habrobracon*. Genetics **28:** 365–382.

Williams, J. G., A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V. Tingey, 1990 DNA polymorphisms amplified by arbitrary primers are useful genetic markers. Nucleic Acids Res. **18:** 6531–6535.

Xu, S., and W. R. Atchley, 1996 Mapping quantitative trait loci for complex binary diseases using line crosses. Genetics **143:** 1417–1424.

Xu, S., N. Yonash, R. L. Vallejo and H. H. Cheng, 1998 Mapping quantitative trait loci for complex binary traits using a heterogeneous residual variance model: an application to Marek's disease susceptibility in chickens. Genetica **104:** 171–178.

Communicating editor: Z-B. Zeng