Linkage Map of the Honey Bee, *Apis mellifera*, Based on RAPD Markers

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ABSTRACT

A linkage map was constructed for the honey bee based on the segregation of 365 random amplified polymorphic DNA (RAPD) markers in haploid male progeny of a single female bee. The *X* locus for sex determination and genes for black body color and malate dehydrogenase were mapped to separate linkage groups. RAPD markers were very efficient for mapping, with an average of about 2.8 loci mapped for each 10-nucleotide primer that was used in polymerase chain reactions. The mean interval size between markers on the map was 9.1 cM. The map covered 3110 cM of linked markers on 26 linkage groups. We estimate the total genome size to be ~3450 cM. The size of the map indicated a very high recombination rate for the honey bee. The relationship of physical to genetic distance was estimated at 52 kb/cM, suggesting that map-based cloning of genes will be feasible for this species.

APIS mellifera L., the honey bee, is an important model organism for behavioral research because it is a colonial insect with complex social behavior. Many studies have characterized its fascinating behavioral repertoire, but little is known about the genetic bases of its behavior because of difficulties in maintaining selected stocks and the scarcity of genetic markers. However, it is now possible to construct detailed linkage maps from single crosses by following the segregation of molecular markers. In species such as the fruitfly *Drosophila melanogaster* and the house mouse *Mus musculus*, genetic mapping is a valuable tool for identifying chromosomal regions affecting behavioral traits (*e.g.*, DE BELLE *et al.* 1989; CARLIER *et al.* 1990). A saturated linkage map of the honey bee should facilitate the characterization of complex social behavioral traits in the same way that studies of crop plants have determined the number and locations of genes affecting agronomic traits (PATERSON *et al.* 1988, 1991; STUBER *et al.* 1992).

Although 27 linkage maps have been reported for insect species, very few are sufficiently complete to allow comparative studies (HECKEL 1993). By far, the best mapped insect genome is the fruit fly, *D. melanogaster*, with ~3800 mapped genes (KAFATOS *et al.* 1991; MERRIAM *et al.* 1991). The Drosophila map is based on morphological, behavioral and biochemical markers, plus *in situ* hybridizations of both cDNA and large genomic clones to polytene chromosomes. Maps currently are being developed for other dipteran species, such as the mosquitoes, *Aedes aegypti* (MUNSTERMAN and CRAIG 1979; SEVERSON *et al.* 1993) and *Anopheles gambiae* (ZHENG *et al.* 1991, 1993), and the Australian blow-fly, *Lucilia cuprina* (FOSTER *et al.* 1981).

Outside of Drosophila, the map of the silk moth, *Bombyx mori*, has the largest number of published markers (177 morphological and allozyme markers; DOIRA *et al.* 1992). Until now, no hymenopteran insect has been extensively mapped, but 38 morphological markers of the wasp, *Bracon hebetor*, have been included in a map that has one large linkage group of ~400 cM and eight minor groups with two to four markers each (WHITING 1961). A map of the chalcidoid wasp, *Nasonia vitripennis*, that contains 47 morphological markers also has been reported (SAUL 1993).

The honey bee genome has a haploid chromosome number of 16 (PETRUNKEWITSCH 1901) and a total size of ~180 megabase pairs (JORDAN and BROSEMER 1974). About 8–11% of the genome consists of moderate- to high-copy repetitive DNA that is arranged in the "long-period interspersion" pattern that is typical of Drosophila (CRAIN *et al.* 1976). Linkage studies have demonstrated three pairs of linked morphological markers (MACKENSEN 1958; LAIDLAW *et al.* 1965; WITHERELL and LAIDLAW 1977; reviewed by TUCKER 1986). Recently, fluorescence *in situ* hybridization has shown that ribosomal gene complexes are located on two separate honey bee chromosomes, but because of difficulty in karyotyping the honey bee, specific chromosomal assignments have not been made (BEYE and MORITZ 1993).

We present a map of the honey bee that is based on the segregation of RAPD markers that were scored in haploid males (drones). The map consists of 26 linkage groups and contains the malate dehydrognase (*Mdh-1*) gene, a gene for black body color (blk) and the major sex determination locus of the honey bee, the *X* locus. We also show that the recombinational size of the honey bee genome is very large relative to its physical size.

MATERIALS AND METHODS

Sources of bees: Two presumably unrelated bees, a haploid male (drone) and a diploid female (queen) were raised

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from stocks collected from commercial apiaries near Davis, California, and crossed by instrumental insemination (LAIDLAW 1977) to produce an F_1 daughter queen. The drone father of the F_1 was hemizygous for Mdh^M and the mother was Mdh^S/Mdh^S. The Mdh^F, Mdh^M and Mdh^S alleles observed in this study are believed to be the $Mdh\text{-}1^A$, -1^B and -1^C alleles, respectively, that were described by CONTEL *et al.* (1977). The drone father was black, whereas the queen mother of the F_1 was yellow and had drone progeny that were yellow with variable black markings. The colony into which the virgin F_1 queen was introduced was fitted with a queen excluder that allows the workers to pass but prevents the larger queens and drones from entering or exiting. Mature haploid drones, arising from unfertilized eggs of the virgin F_1 queen, were collected from inside the colony and were used as the mapping population. The haploid drone progeny of the F_1 queen will be referred to as H_1 individuals, to indicate haploid progeny of an F_1 individual. The *X* locus was mapped in a second population of diploid progeny resulting from a backcross of one H_1 male to a queen of a parental line (HUNT and PAGE 1994). Individuals that are heterozygous at this locus are female but homozygotes develop as diploid males. This backcross produced a 1:1 segregation of females and diploid males for analyses.

The linkage map was constructed from analyses of markers segregating in drone progeny of the F_1 queen (H_1 individuals). The use of a haploid population avoids loss of information caused by dominance of RAPD markers. After freezing the drones at −80°, the bodies were used for DNA extractions and the heads were used to determine the *Mdh* phenotype (HEBERT and BEATON 1989). Several drones had the Mdh^F allele, indicating that they were the result of eggs laid by unrelated worker bees, a phenomenon that occurs at a low level in honey bee colonies. These drones were excluded from further analysis. Ninety-four of the drone progeny of the F_1 queen were used for most of the linkage analyses but the mapping population was increased to 142 for some loci to confirm linkage when larger recombinant fractions resulted in lower LOD scores.

DNA extraction: Individual, frozen drones and queens were ground with pestles in microcentrifuge tubes that contained 350 μl of CTAB extraction buffer [1% hexadecyltrimethyl ammoniumbromide, 750 mM NaCl, 50 mM Tris-Cl (pH 8), 10 mM EDTA and 100 μg/ml of proteinase K]. After incubation at 60° for 2 hr, one-third volume of 1.5 M NaCl/50 mM Tris-Cl (pH 8) was added to the samples to prevent CTAB and polysacharide precipitation. Samples were extracted with phenol/chloroform and then with chloroform, and the DNA was precipitated with one-tenth volume of 3 M sodium acetate (pH 5) and two volumes of ethanol. Following centrifugation for 10 min at 4000 × *g*, the precipitate was washed with 70% ethanol and resuspended in 10 mM Tris (pH 7.6), 1 mM EDTA. The DNA was then quantified with a fluorometer (Hoeffer) and diluted to 3 ng/μl in 10 mM Tris, 0.3 mM EDTA.

Polymerase chain reactions: RAPD markers were generated in polymerase chain reactions using the method of WILLIAMS *et al.* (1990). Ten-nucleotide primers of arbitrary sequence were obtained from Operon Technologies (Alameda, CA) or the University of British Columbia Biotechnology Center (Vancouver, Canada). Amplifications in Perkin-Elmer 480 thermal cyclers were performed with these parameters: 45 cycles of 94°/1 min, 35°/1 min, 2-min ramp to 72° and 72°/2 min. Different cycling parameters were used with a small number of primers to increase output: five cycles as stated above were followed by 32 cycles of 94°/10 sec, 35°/30 sec, 72°/30 sec (D. LAVELLE and R. W. MICHELMORE, unpublished data). The faster cycling times did not significantly affect banding patterns but did reduce the intensity of some bands. Polymerase chain reactions (PCR) were performed in 12.5 μl reaction volumes that contained 0.5 μM primer, 100 μM each of dATP, dGTP, dCTP and dTTP (Pharmacia), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 0.25 U Taq polymerase and 3 ng of genomic DNA. Reactions were covered by one drop of mineral oil.

Primer screening: We screened 1000 primers against the two parents and the F_1 queen. Primers were selected that produced the maximum number of bright bands that were polymorphic between the parents and inherited in the F_1 queen. This screen allowed us in most cases to determine whether an allele was of maternal or paternal origin. In the mapping population of haploid drones, fragment-length polymorphisms occasionally were identified that could not be scored when primers were screened against the parental DNA. When the parental origin of a RAPD marker allele was uncertain, the allele was arbitrarily coded as if it had been inherited from one of the parents and if linkage was not detected at first, the data were then recoded to indicate inheritance from the other parent.

Gel electrophoresis and scoring of RAPD markers: Amplification products were resolved in 20 × 25 cm gels consisting of 1% Synergel (Diversified Biotech, Newton Centre, MA) and 0.6% agarose dissolved in 0.5× TBE (SAMBROOK *et al.* 1989). Gels had two sets of combs and were run at 3.6–4.6 V/cm of gel for ~600–700 V hr. Gels were stained with ethidium bromide for 25 min and destained from 10 min to 2 hr. The gels were photographed on a UV transilluminator with Polaroid 667 film. The gels were scored once just before data recording. Gels were scored a second time to check the completed data file after marker data had been organized into ordered linkage groups. This helped to reevaluate individual scores that caused unlikely double crossovers. Data for gel lanes that were difficult to score were listed as missing. Some markers were dropped from analyses because of difficulties in scoring.

Polymorphic RAPD markers were named by the primer designation, followed by a dash and the approximate size of the amplified fragment in kilobases. Presence/absence polymorphisms had no further designation, but the name was followed by the letter "f" if that marker appeared as a simple fragment-length polymorphism. An "h" rather than an "f" was assigned to fragment-length markers that generated an additional heteroduplex band from the two alternative fragments. Primers are listed as designated by the supplier; those obtained from Operon Technologies contain a letter first and then a number. Primers obtained from the University of British Columbia have a number designation. A few RAPD markers were converted to sequence-tagged-sites (STSs; OLSON *et al.* 1989) by cloning the polymorphic fragment and designing specific primers based on the terminal nucleotide sequence of the clone. STS markers are designated by the additional letters "sts" before the name of the original RAPD marker. The methods for producing STSs from RAPD markers were as reported (HUNT and PAGE 1994). Primer sequences were: stsQ16-.58, 5′-AGTGCAGCCAGCTACTGAGAG and 5′-AGTGCAGCCACGTGCCTGAAT; stsQ16-.59, 5′-GCCATTACTTTTGACAGAGATTAC and 5′-GCAGCCAGAATATAAGACGCTGTT.

Linkage analyses: Most of the analyses were performed with MAPMAKER software (LANDER *et al.* 1987; version 2.0 for the Macintosh obtained from S. V. TINGEY, E. I. duPont Demour Corp., Wilmington, DE). The data type was coded as "haploid." Putative linkage groups initially were determined by the results of pair-wise comparisons of markers using the "group" command with a minimum $LOD_{linkage}$ score of 3.0 for statistical acceptance of linkage, and minimum re-

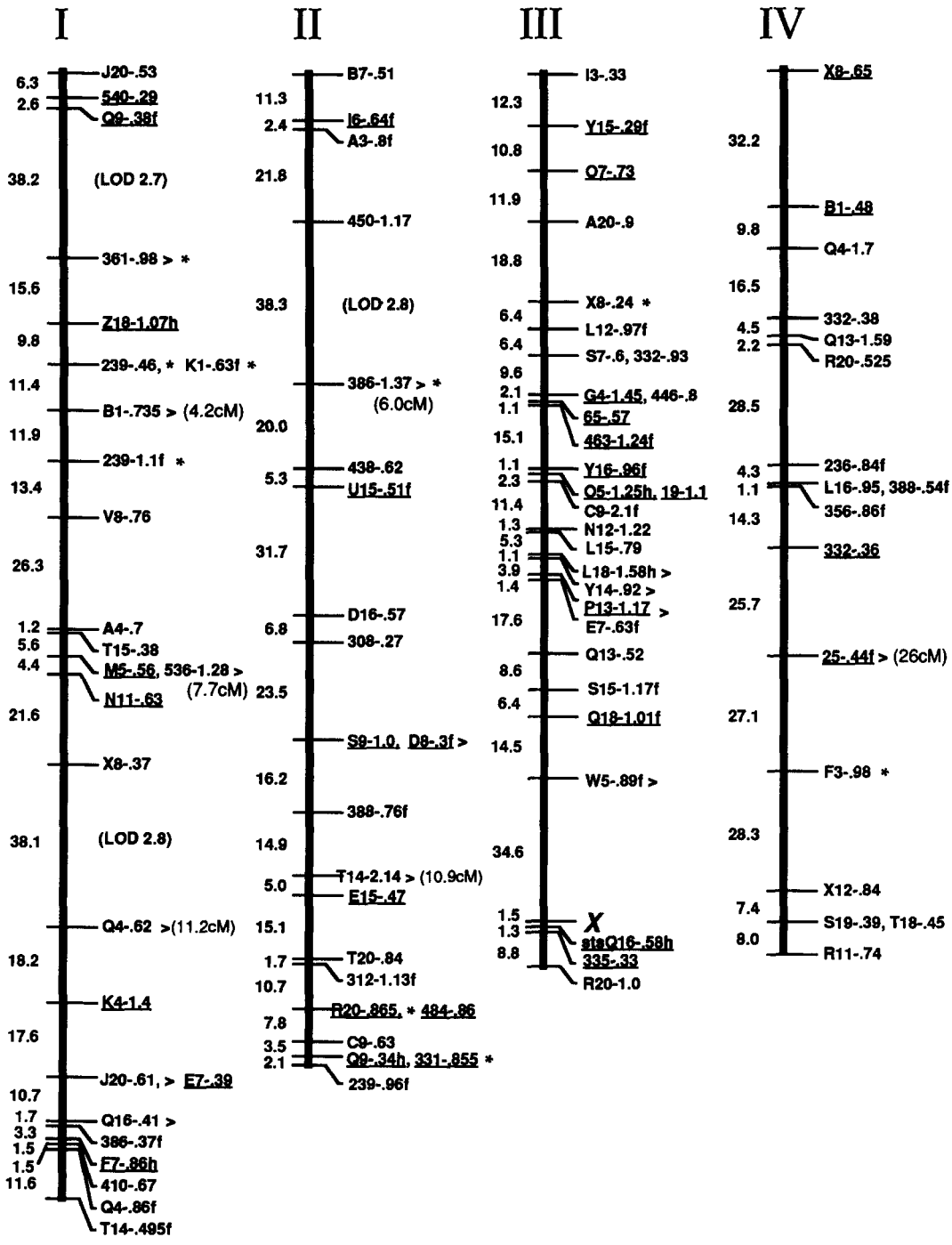

FIGURE 1.—A linkage map of the honey bee, based on RAPD markers. Data analyses were performed with MAPMAKER 2.0 for the MacIntosh using the Kosambi mapping function. Markers are named by the primer designation of the supplier (a letter followed by a number for primers from Operon Technologies, Alameda, CA, or a number for primers from University of British Columbia). The primer designation is followed by a dash and the approximate size of the amplified fragment in kilobases. Fragment-length polymorphisms are indicated by an "f" at the end of the marker name or an "h" if the fragments involved are known to form an additional heteroduplex band in reactions from heterozygous individuals. Presence/absence polymorphisms are not followed by a letter. Sequence-tagged-sites have the additional prefix "sts". Underlined markers were particularly easy to score and are considered to be the most likely markers to be reproducible in other labs (see Figure 2). All of the markers were mapped with a minimum LOD-score for linkage of 3.0, except for the four intervals that have lower LOD scores indicated. All of the markers for intervals >2 cM were ordered with an LOD 3.0 threshold except for markers that have a bar to the right of the marker name and the LOD score for that order is indicated to the right. Markers that deviated from the expected 1:1 segregation ($P < 0.05$) appear with an asterisk to the right of the marker name. Markers whose presence caused the map to expand by ≥2 cM when added to the analysis have a bracket (>) to the right of the name. The amount of map expansion (centiMorgans) is provided for those markers that cause ≥4 cM of expansion.

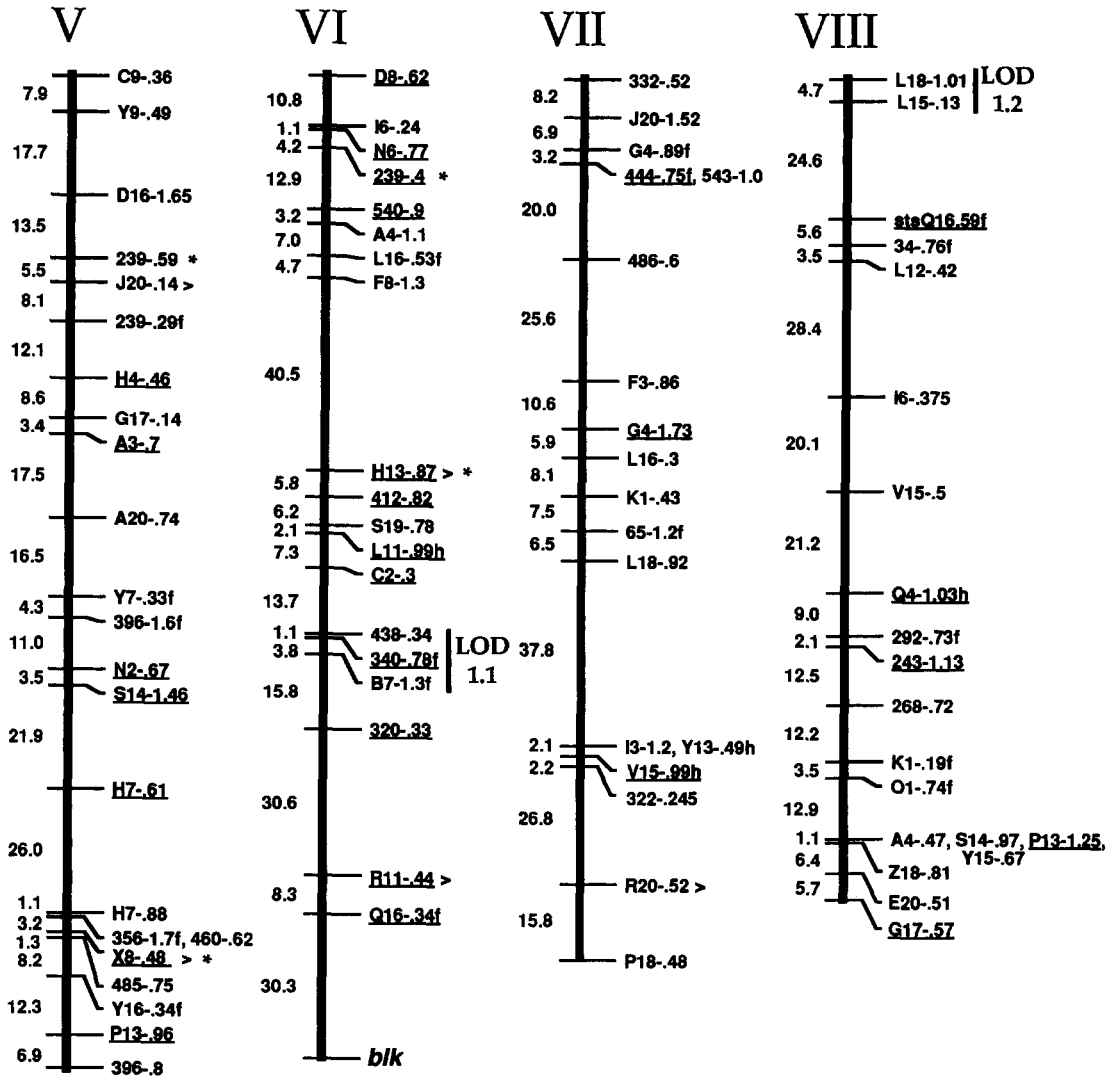

FIGURE 1.—*Continued*

combinant fraction of 0.34. A few loci that were not linked at LOD 3 with pair-wise estimates were still incorporated if the value for $LOD_{linkage}$ that is based on information from all the markers in the linkage group was ≥3.0 (the multipoint LOD value generated by the "linked?" command). Next, three-point analyses were performed on each linkage group, taking consecutive markers three at a time. A LOD value of 3 was used as the linkage criterium for triples and the three-point analysis generated the most likely order. The most likely order for the linkage group was tested with the "ripple" command. If any possible order for each triplet of markers did not decrease the log-likelihood of the map for that linkage group by ≥3.0 (indicating orders that are 1000 times less probable), the most likely order was shown and the markers with uncertain order were depicted with a bar next to them, along with the LOD value that represents the confidence statistic for that order (Figure 1). For markers linked at 2 cM or less, only the most likely order was depicted because these markers often could not be ordered at the more stringent LOD 3 threshold, because of an insufficient number of informative meioses.

The "Drop Marker" command was used to determine whether the presence of individual markers were causing an overestimate of map size because of experimental errors. Mapmaker 3.0 was used to detect putative errors in the data set with the default setting of 1% *a priori* probability of error (LINCOLN and LANDER 1992). The gels for data points with a $LOD_{error} \geq 1.0$ were rechecked a third time and data entries that were found to be incorrect were either corrected or entered as missing data. JoinMap software was used to merge the data from the two populations for mapping the region of the *X* locus (obtained from P. STAM, CPRO, Wageningen, The Netherlands; STAM 1993). The Kosambi mapping function was used to convert recombinant fractions to map distances (KOSAMBI 1944).

RESULTS

Polymorphism and segregation of RAPD markers: After screening 1000 primers, 132 were used for mapping with the haploid drone progeny of the F_1 queen (H_1 individuals). The primers generated 1018 scoreable marker-bands (an average of 7.7 per primer), of which 409 (40%) were polymorphic. Of the 409 markers used in linkage analysis, 375 (92%) initially were incorporated into 26 linkage groups. However, 10 mark-

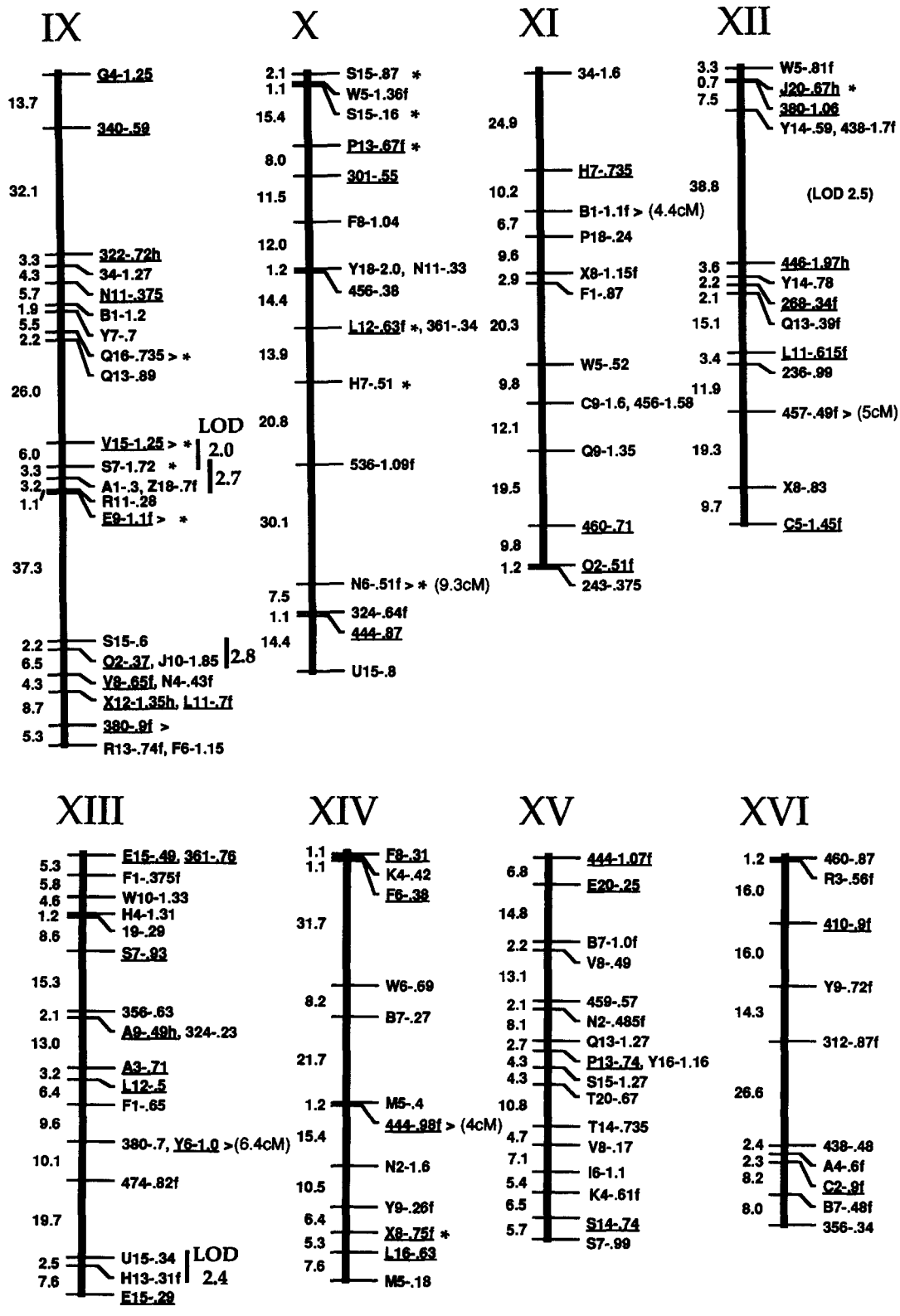

FIGURE 1.—*Continued*

ers were removed from analyses, as explained below. The final map contains 365 RAPD markers (Figure 1).

An average of 2.77 markers could be mapped for each primer that was used because the primers were prescreened carefully. Most of the primers produced presence/absence polymorphisms in the H_1 drones, but 114 (31%) of the markers that were mapped segregated as fragment-length polymorphisms (see Figure 2). For 16 of the fragment-length polymorphisms, an additional bright band was present in the reaction prod-

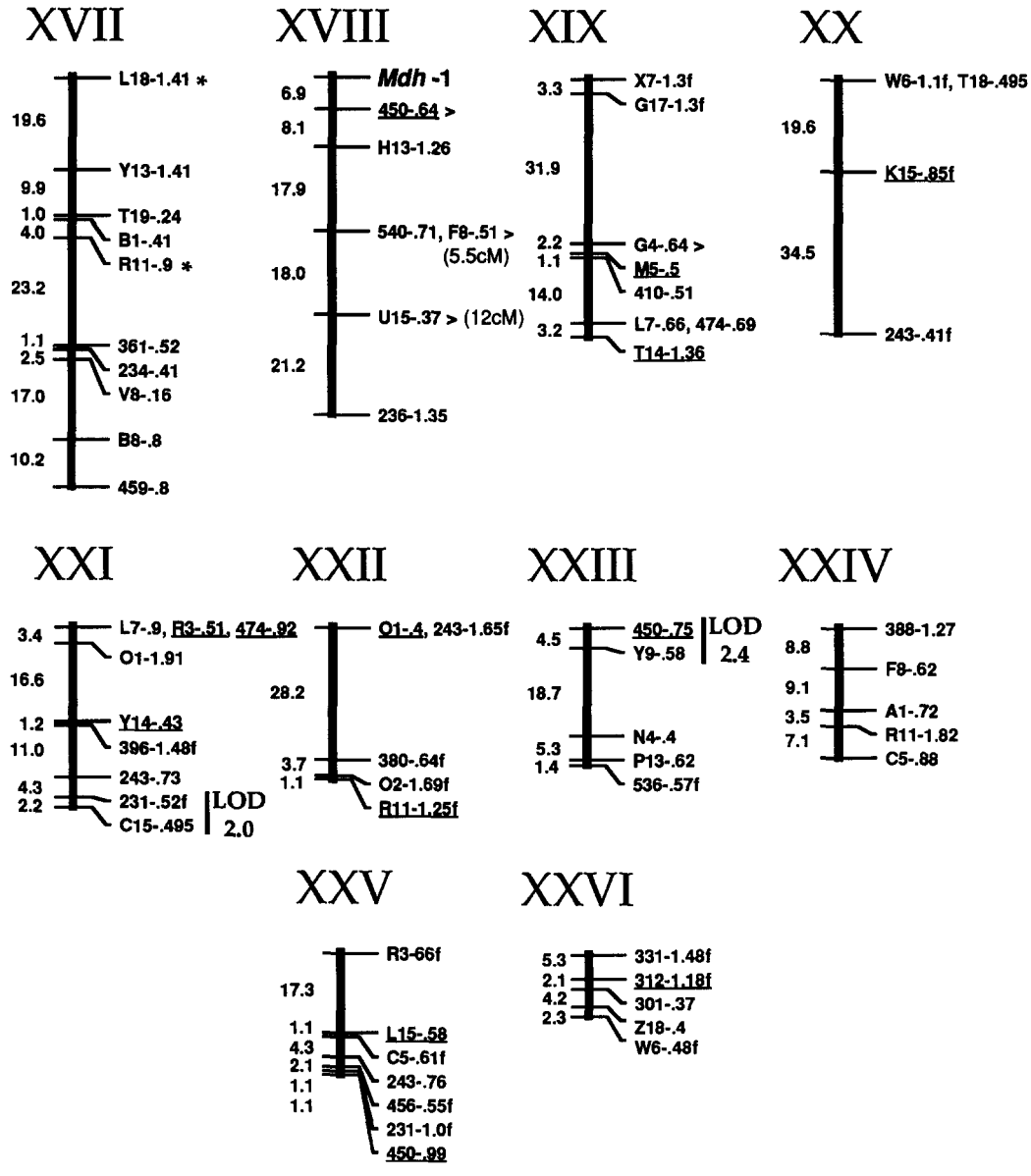

FIGURE 1.—*Continued*

ucts from the F_1 queen that was not present in either of her parents and was not inherited by her drone progeny. These bands migrated more slowly than the fragment-length polymorphism on the gel. We assume that these bands represent heteroduplexes that are generated from the two alternative fragments in a heterozygote (HUNT and PAGE 1992). In a few cases, heteroduplexes were confirmed by mixing amplification products from two drones that had different alleles, in the absence of polymerase activity, to reconstitute the heteroduplex band. In addition to the 16 heteroduplex markers that we were able to detect, there were probably additional fragment-length polymorphisms segregating in the H_1 drones that would produce heteroduplexes in heterozygotes. Our screen of the two parental bees and the F_1 queen only revealed heteroduplexes that appeared as a distinct band in the F_1 queen.

Segregation ratios that were skewed towards one of the parental alleles were not common, but in some instances segregation of RAPD markers deviated from the expected 1:1. Significant deviations ($\chi^2 > 3.84$; $P < 0.05$, 1 d.f.) were observed at 27 separate loci (indicated by asterisks in Figure 1). However, because of linkage, these only represent 16 independent loci. The skews may be caused by chance alone because we would expect 18 skews of this magnitude if all markers were independent of each other. However, tight linkage of markers in some clusters shows that the assumption of independent inheritance is not valid. Therefore, the number of loci that are expected to be skewed because of chance alone should be <18. Clusters of three or

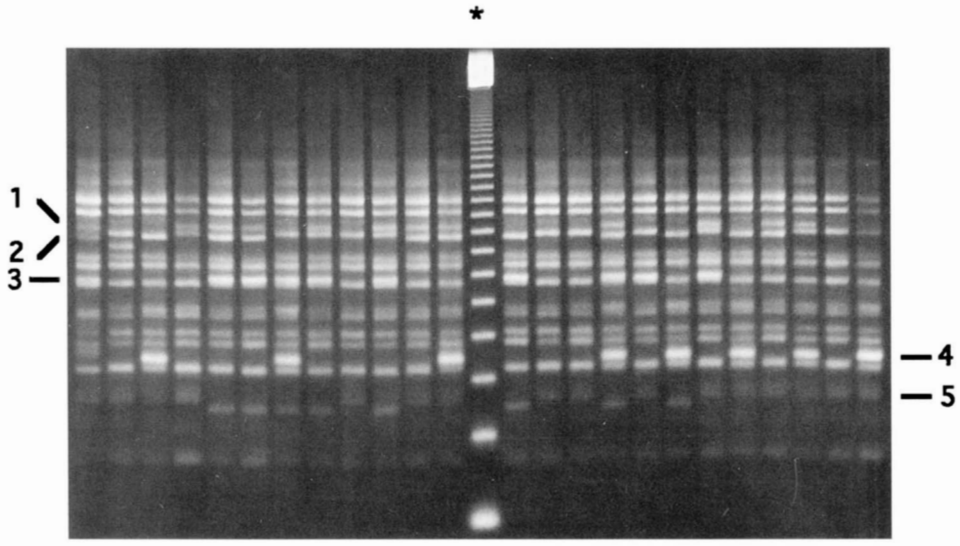

FIGURE 2.—Random amplified polymorphic DNA (RAPD markers) generated by primer UBC-239 demonstrate typical polymorphic markers. The first lane on the left represents the mother of the F1 queen and the other lanes contain DNA amplified from haploid drone progeny of the F1 queen, except for the molecular weight marker in the center lane (a 123-bp ladder, designated by *). The five markers that mapped to linkage groups were 1, 239–1.1f in group I; 2, 239-.96f in group II; 3, 239-.59 in group V; 4, 239-.4 in group VI; 5, 239-.29f in group V. An "f" at the end of the marker designation indicates a fragment-length polymorphism. An underlined marker is judged to be an exceptionally robust marker.

more markers showing skewed segregation appeared on linkage groups I, IX and X. Selection on gametes or haploid drones could have caused the skews on these three linkage groups. The skewed regions did not consistently favor alleles from either the paternal or maternal parent.

Genome size and error detection: The 26 linkage groups in Figure 1 span 3110 cM with an average marker spacing of 9.1 cM. Linkage was confirmed within a few of the groups (at LOD 3) by repeating specific markers with the genomic DNA from 48 additional haploid drones from the same queen. For example, this allowed us to confirm linkage of the *X* locus to the end of linkage group III and within linkage groups IV, VI, VII and IX. The recombinational size of the *A. mellifera* genome must be even >3110 cM, because of incomplete coverage by the 368 mapped loci. Honey bees have 16 chromosomes, therefore some of the 26 linkage groups must be physically linked.

The Drop Marker command was used to determine if the presence of individual markers was causing map expansion due to experimental errors. Overall, there was no tendency for expansion as markers were added to the analyses. We observed an insignificant average contraction of 0.01 ± 0.134 cM (means ± SD) as markers were added between their flanking markers. However, we found 40 internal markers that caused the map to expand ≥2.0 cM when added individually to the analysis [such markers are indicated (>) in Figure 1]. Eight markers that were found to cause expansion, or their adjacent markers, were omitted from the analyses because they were more difficult to score. Scoring errors could cause map expansion by adding spurious recombinants to the data set.

In some cases, the effect of a marker on map expansion could not be determined because there were no flanking markers (in the case of linkage group termini) or because dropping the marker resulted in fragmentation of the linkage group. For example in linkage group IV, marker 25-.44f, which caused the largest map expansion (26 cM), was retained because there were few markers in that region and the marker was very easy to score. Dropping the adjacent marker F3-.98 from the map resulted in fragmentation of the linkage group, therefore we could not assess the effect of F3-.98 on map expansion. Marker F3-.98 bridged a gap but was more difficult to score than 25-.44f. Alleles of F3-.98 also had the largest deviation from the expected 1:1 ratio of any internal marker ($\chi^2 = 8.19$; $P < 0.01$), suggesting that scoring errors could be inflating the map in this region. A similar problem in interpretation occurs for markers on the ends of linkage groups because errors could cause these markers to be incorrectly ordered, thus placing them distal to others. Two terminal markers were dropped from analyses due to difficulty in scoring and in one case, skewed segregation.

The error-checking algorithm of Mapmaker 3.0 was used to check for errors that were still undetected, even after the gels had been scored twice. The value of LOD_{error} is a measure of the likelihood that a given data entry is actually an error (LINCOLN and LANDER 1992). We scanned the 37,355 data entries from linked markers for high values of LOD_{error} and gels that resulted in data points with $LOD_{error} \geq 1.0$ were rescored. Values of $LOD_{error} \geq 1.0$ occurred for 280 (nearly 1%) of the 28,495 data entries for internal markers. Error detection is most powerful for internal markers where errors cause apparent double crossovers. One individual drone accounted for 6.8% of these high values. Marker-bands generated from this drone's DNA sometimes were amplified inconsistently. Therefore, data from this individual were omitted from further analyses, thus re-

ducing the sample size to 94 individuals for most loci. Sixty-six data points had LOD_{error} values of ≥2.0. One fragment-length marker that was difficult to score was responsible for nine of these assumed errors and data from this marker were omitted from analyses. Of the data entries with $LOD_{error} \geq 2.0$, 34 (51%) appeared to be incorrectly scored and were corrected. Corrections were made for 25% of all data entries with $LOD_{error} \geq 1.0$. Some of these corrections were actually for a flanking marker from the same individual that did not have a high LOD_{error} value. Gels for flanking markers were rescored when a change in the flanking marker data-point resolved the double crossover.

Genes on the map: The *X* locus for sex determination was mapped as previously described in a population consisting of 185 diploid backcross progeny from one haploid son of the F_1 queen (HUNT and PAGE 1994). JoinMap software was used to incorporate the *X* locus into the linkage map presented here by combining linkage data from the two populations with the default values for that software (STAM 1993). JoinMap combines data from various types of crosses by weighting the recombinant fraction from each data set by its standard deviation. The *X* locus appears on the distal portion of linkage group III, with an interval of ~34 cM separating it from the remainder of the group. Two RAPD marker loci linked to *X* segregated in both mapping populations (335-.33 and Q16-.58) and one marker fragment was cloned to produce an STS (stsQ16-.58) that is tightly linked to *X*.

A recessive allele for black body color (*blk*) mapped to the end of linkage group VI. This is probably the *bl* locus described by LAIDLAW and EL-BANBY (1962) that may be allelic to *abdome castanho* (*ac*) described by KERR (reviewed by TUCKER 1986). Crosses with several races of honey bee, including the African race, *A. mellifera scutellata*, indicated that *bl* was probably allelic to the major gene in other races and that three detectable alleles were involved (renamed *Y*, y^{bl} and y^{ac}; WOYKE 1977). Unfortunately, the stocks used to identify these alleles are no longer available to confirm the identity of this gene, so we will designate the allele that we mapped as *blk*. The *Mdh* locus was mapped to one of the smaller linkage groups (XVIII). This locus has been important for population studies in the honey bee because of the scarcity of other polymorphic allozymes in hymenopteran insects (METCALF 1975).

DISCUSSION

Variability of RAPD marker alleles: The F_1 queen was heterozygous for 40% of the RAPD markers that were generated by the primers used for mapping. The high rate of polymorphism of RAPD markers allowed us to map multiple loci with most primers. Some of the fragment-length polymorphisms had fragments of considerable size difference (20–150 bp) and a few fragment-length polymorphisms were characterized by multiple fragments for a single allele. Fragment-length polymorphisms that were amplified from the F_1 queen and generated visible heteroduplex bands tended to involve the brightest bands observed for that particular primer.

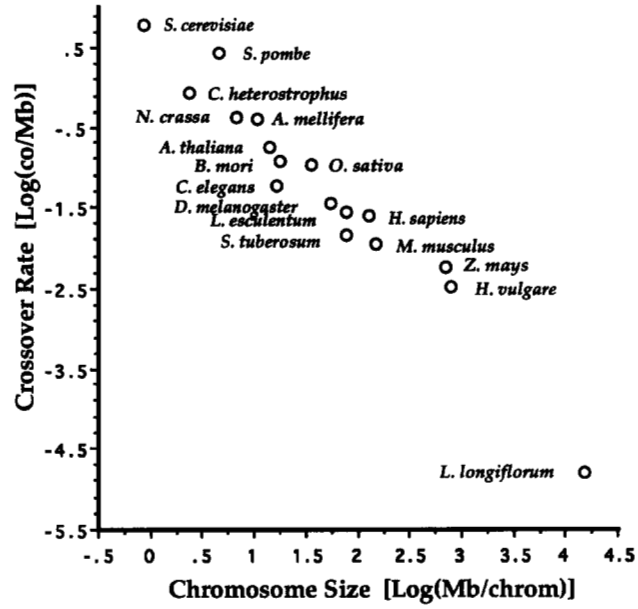

FIGURE 3.—A plot showing the relationship between recombination rate and chromosome size. The recombination rate, measured in log of crossovers per megabase pairs (crossovers/Mb) is plotted against chromosome size, measured in log of megabases per chromosome (Mb/chromosome) for 17 different species ($r^2 = 0.95$). The four species of fungi that appear above the honey bee (*Apis mellifera*) have an extended haploid phase in the life cycle. All of the species listed below the honey bee have a haploid phase that is reduced in duration and structural complexity. The species are, *Saccharomyces cerevisiae, S. pombe, Cochliobolus heterostrophus, Neurospora crassa, A. mellifera, Bombyx mori, Oryza sativa, Caenorhabditis elegans, Arabidopsis thaliana, Drosophila melanogaster, Lycopersicon esculentum, Homo sapiens, Mus musculus, Solanum tuberosum, Hordeum vulgare, Zea mays* and *Lillium longiflorum*. Estimates for the genetic size of incompletely mapped genomes were extrapolated from available linkage data. Data were obtained from KING and MORTIMER (1990), REITER *et al.* (1992), MORTON (1991), SHIELDS (1993), TANKSLEY *et al.* (1992) and TZENG *et al.* (1992).

Evidence for high recombination rates: The striking feature of the honey bee linkage map is its large size in recombination units. Scoring errors and PCR artifacts could have inflated our estimate of genome size by resulting in additional spurious crossovers. However, our analyses of map expansion that was caused by data from individual markers indicated that artificial map inflation was not excessive in this data set. The Drop Marker command did not detect a significant degree of map expansion as new markers were added to linkage groups. The phenomenon of map expansion can be the result of either experimental errors or an innapropriate estimate of interference by the mapping function. The

TABLE 1

List of chromosome sizes for some insect species

Order (Family)	Genus	No. species[a]	n[b]	Genome size (mb of DNA[c])	Chromosome size (mb/chromosome)	Reference[d]
Orthoptera (Crickets/Grasshoppers)						
(Gryllidae)	Gryllus	1	11	11,000	1,000	1
(Acrididae)	(6 genera)	9	9–12	5,629–15,278	469–1698	1, 2
(Eumastacidae)	Moraba	1	7	7,003	1,000	2
(Phasmatidae)	Bacillus	5	17–18	2,024–2,440	112–143	3
Diptera (Flies)						
(Sarcophagidae)	Sarcophaga	1	5	570	114	4
(Muscidae)	Musca	1	6	972	162	5
(Drosophilidae)	Drosphila	6	3–5	170–366	47–78	6
(Culicidae)	(4 genera)	31	3	219–1,737	73–580	7, 8, 5
(Dixidae)	Dixa	1	4	146	36.4	5
(Psychodidae)	Telmatoscopus	1	6	227	37.9	5
(Chironomidae)	Chironomus	1	4	187	47	9
(Cecidomiidae)	Mayetiola	1	4	93.5	23.4	10
Homoptera (True Bugs)						
(Aphididae)	Schizaphis	1	8	374	47	11
Coleoptera (Beetles)						
(Tenebrionidae)	(26 genera)	52	5–13	147–804	14.7–80	12, 14
(Dermestidae)	Dermestes	6	8	900–1,980	112–247	13
(Chrysomelidae)	(24 genera)	39	8–23	162–3,452	15.1–173	15
Lepidoptera (Butterflies/Moths)						
(Noctuidae)	Heliothis	1	31	408	13.2	16
(Bombycidae)	Bombyx	1	28	495	17.7	17
Hymenoptera (Bees/Ants/Wasps)						
(Pteromalidae)	Nasonia	1	5	312	62	18
(Megachilidae)	Megachile	1	16	283	17.7	19
(Braconidae)	Bracon	1	10	156	15.6	18
(Apidae)	Apis	2	16	178	11.1	19

[a] Number of species for which genome size was determined.
[b] n is the haploid chromosome number.
[c] Total haploid DNA content in megabases, assuming 1 bp = 642 D and 1 pg = 935 Mb.
[d] References for genome sizes are 1, SPARROW *et al.* 1972; 2, HEWITT 1979; 3, MARESCALCHI *et al.* 1990; 4, SAMOLS and SWIFT 1979; 5, JOST and MAMELI 1972; 6, LAIRD 1973; 7, RAO and RAI 1987; 8, BESANSKY and POWELL 1992; 9, WELLS *et al.* 1976; 10, SHUKLE and STUART 1994; 11, MA *et al.* 1992; 12, ALVAREZ-FUSTER *et al.* 1991; 13, REES *et al.* 1976; 14, JUAN and PETITPIERRE 1991; 15, PETITPIERRE *et al.* 1993; 16, TAYLOR *et al.* 1993; 17, GAGE 1974; 18, RASCH *et al.* 1977; 19, JORDAN and BROSEMER 1974.

correct mapping function should result in additivity of map distances for adjacent intervals by appropriate correction for chiasmata interference. The Drop Marker command is one way to test for the appropriate estimation of interference because it is the equivalent of performing three-point test crosses throughout the genome (see BAILEY 1961). Therefore, the fact that there was no significant expansion or contraction as markers were added to the map suggests that the Kosambi mapping function fit our data reasonably well.

Markers that were separated by >2 cM could be placed in orders that were 1000 times more probable (LOD 3) than alternative orders, with only a few exceptions. These results demonstrate that RAPD markers provide reliable data for mapping in haploids, if the primers are properly screened and selected. The few markers that could not be ordered at LOD 3 did not significantly inflate the map. When these markers were dropped from the seven largest linkage groups, the largest group contracted by 3.5%, but six of the seven groups actually expanded (by an average of 0.3%). Our ability to order the markers with high confidence was probably enhanced by the fact that the map relied almost entirely on molecular markers. Morphological and physiological markers are often difficult to score and may have lower penetrance due to environmental or developmental factors.

Our error rate appeared to be similar to that reported by DIETRICH *et al.* (1992) for a mouse linkage map based on simple sequence repeat markers (SSRs). In their data set consisting of SSR markers, 1.1% of data for markers at internal loci of linkage groups had values of $LOD_{error} > 1$, of which 46% were determined to be actual errors. In the present study, a little <1% of the data for our internal markers had values of LOD_{error} that were >1, and 25% of these were errors. The larger mean spacing between markers in the honey bee map (9 cM, *vs.* the 4 cM spacing reported for the mouse)

makes this type of error detection less efficient, but the similarity in error rates between the two studies is another indication that RAPD markers are reliable.

The current map covers 3110 cM. However, the map includes 10 gaps because there are 26 linkage groups and only 16 chromosomes in the honey bee. These gaps must cover ≥34 cM each because we had a high likelihood of detecting linkage between markers spaced at closer intervals. Therefore, we estimate the complete genome size to be 3450 cM, yielding an average chromosomal length of >200 cM. Although recombination rates will vary in different crosses, we can estimate the average relationship between physical distance and genetic distance. Given a physical genome size of 178,000 kilobases (using the data of JORDAN and BROSEMER 1974 and assuming 1 bp = 642 D) and a recombinational size of 3450 cM for the honey bee, the relationship between these two measures is an average of 52 kb/cM.

The honey bee should be a more practical subject for map-based cloning of genes than most model organisms because of the high rate of recombination (present study) and the low level of repetitive DNA (CRAIN *et al.* 1976). For example, the Drosophila genome has nearly the same physical size as the honey bee but has roughly 575 kb/cM (MERRIAM *et al.* 1991) and the ratio of physical to genetic distance for the crucifer plant, *Arabidopsis thaliana,* is at least twice that of the honey bee (CHANG *et al.* 1988; REITER *et al.* 1992). We counted crossovers in the five largest linkage groups of each haploid male and found an average of two crossovers/group. However, the actual number of crossovers per bivalent in these groups is estimated to be four because the haploid male inherits an egg genome containing only one of the two sister chromatids that could have participated in a crossover during meiosis. So for each crossover, there is a 50% chance that the drone would receive the nonrecombinant chromatid. Our estimate is conservative because double crossovers flanking a single marker-locus were not included in the estimate and the linkage groups may be incomplete. Another way to estimate the average number of crossovers per chromosome is to divide the total recombinational size of the genome by 50 cM and by the number of chromosomes. This calculation yields an estimate of 4.3 crossovers/chromosome for the honey bee.

Comparisons between diverse species have shown a significant correlation between the rate of meiotic recombination and the average physical size of chromosomes (MORTIMER *et al.* 1992). Figure 3 shows a log-log plot of the number of crossovers per megabase pairs of DNA *vs.* the number of megabases per chromosome for 17 different species with genomes that span a wide range of physical sizes. The plot shows that crossover rates are inversely correlated with chromosome size ($r^2 = 0.95$). The relationship between high crossover rates and chromosome size is primarily a result of the fact that there is a relatively constant number of crossovers per chromosome. Most species have an average of about two to three crossovers per bivalent, regardless of chromosome size. For this reason, species with small chromosomes have more crossovers per unit of physical distance. However, there are species in Figure 3 that have have four or more crossovers per bivalent and these include species with high crossover rates per unit of physical distance (*A. mellifera, S. cerevisiae* and *S. pombe*) as well as species with relatively low rates (*Z. mays* and *H. vulgare*). The fact that the three species with very small chromosomes are not constrained to two to three crossovers per bivalent suggests that there may have been selection for high recombination rates in these species (*S. pombe* has 10–12 crossovers per chromosome). The honey bee has the highest reported crossover rate for any species of higher eukaryote. Four species of fungi that have long haploid phases and have higher crossover rates than the honey bee are shown in Figure 3.

Small chromosomes and high rates of crossovers per megabase seem to be associated with organisms that have a long haploid stage in their life cycle (Figure 3). It is unclear how general this association is because of the scarcity of recombination maps and chiasma frequency data. However, we can make comparisons between haploid and diploid species based on physical sizes of chromosomes to see if there is a trend towards smaller chromosomes in species with prolonged haploid stages. The honey bee has chromosomes that are roughly 11 Mb in size. In a survey of 1619 diploid angiosperms, only two species have chromosomes that are smaller than the honey bee (*Cardamine amara* and *Ruta montana*, with chromosome sizes of 6 and 9 Mb, respectively, and possibly *Luzula pilosa*). Two species of Epilobium have chromosomes that are about the same size as honey bee and seven other species have chromosomes that are between 12 to 15 Mb (BENNETT and SMITH 1976, 1991; BENNET *et al.* 1982).

We were unable to find a diploid insect species that has smaller chromosomes than the honey bee, however, a few beetles in the families Chrysomelidae and Tenebrionidae have relatively small chromosomes, as does the Noctuid moth, *Heliothis zeae* (Table 1). Unfortunately, there are very few estimates of physical chromosome size for haplo-diploid insects. The chromosomes of *A. cerana* are the same size as the honey bee (11 Mb) and those of a leaf-cutting bee, *Megachile rotundata*, are 17 Mb (Table 1). The wasp, *B. hebetor,* has an average chromosome size that is just a little larger than the honey bee (13.5 Mb) and the large size of one linkage group (400 cM) is indicative of a high crossover rate for this species (WHITING 1961). However, the chalcidoid wasp, *Nasonia vitripennis,* has ~60 Mb per chromosome, so we would expect a recombination rate that was much lower than Bracon, on the basis of the correlation between chromosome size and crossover rate shown in

Figure 3. The preliminary map for Nasonia is evidence for a lower recombination rate than that of Bracon because the entire map spans only about half the distance of the Bracon map, even though it contains more markers (SAUL 1993).

Prospects for a saturated linkage map: Despite the high recombination rate, a saturated map of the honey bee should be achieved soon. Because of the efficiency of RAPD marker analysis, most of the results presented here were performed by one person in two years. It now should be possible to complete a similar project in less time and to incorporate more mapped genes and STS markers into the map. Useful fragment-length marker bands from this map will be cloned so that STS primers can be designed. Most of these STS markers probably will not have rates of polymorphism that are as high as SSRs, unless the amplified fragments actually contain such SSRs (ESTOUP *et al.* 1993). However, the STS markers should be useful because we will choose markers with large fragment-length polymorphisms, so that they can be easily resolved on agarose gels. The small amount of DNA needed for PCR-based markers allowed us to retain most of the DNA from the original mapping population. Thus, new markers that are mapped in other populations also can be integrated with the map reported here.

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