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A Dense Genetic Map of the Silkworm, *Bombyx mori*, Covering All Chromosomes Based on 1018 Molecular Markers

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ABSTRACT

A dense linkage map was constructed for the silkworm, *Bombyx mori*, containing 1018 genetic markers on all 27 autosomes and the *Z* chromosome. Most of the markers, covering ~2000 cM, were randomly amplified polymorphic DNAs amplified with primer-pairs in combinations of 140 commercially available decanucleotides. In addition, eight known genes and five visible mutations were mapped. Bombyx homologues of *engrailed* and *invected* genes were found to be closely linked, as in *Drosophila melanogaster*. The average interval between markers was ~2 cM, equal to ~500 kb. The correspondence of seven linkage groups to counterparts of the conventional linkage map was determined. This map is the first linkage map in insects having a large number of chromosomes ($n = 28$) that covers all chromosomes without any gaps.

GENOME research is being conducted on an increasing number of organisms, but insufficient attention has been paid to insects, which account for the majority of animal species. Only the fruit fly, *Drosophila melanogaster*, has been exhaustively studied genetically and is a subject of a genome research project as a model organism (FlyBase Consortium 1998). The malaria vector mosquitoes, *Anopheles gambiae* and *Aedes aegypti*, also have been extensively analyzed (Severson *et al.* 1993; Dimopoulos *et al.* 1996; Zheng *et al.* 1996). In addition, molecular linkage maps for a few Diptera and Hymenoptera species are available [*i.e.*, honeybee, *Apis mellifera* (Hunt and Page 1995); wasp, *Bracon hebetor* (Antolin *et al.* 1996); and mosquito, *A. albopictus* (Severson *et al.* 1995; Mutebi *et al.* 1997)]. However, the vast majority of insects, including economically and ecologically important pests, natural enemies, or producers, do not even have a complete genetic map that covers all chromosomes (for review, see Heckel 1993).

Bombyx mori, the domesticated silkworm, is one of the most genetically studied insects, apart from *D. melanogaster*. More than 200 mutations have been placed on linkage maps covering 900.2 cM and maintained as genetic resources (Doira 1992). *B. mori* itself has considerable importance as a producer of silk and, in recent years, recombinant proteins (Maeda 1989). Moreover, it belongs to the insect order Lepidoptera, which includes many serious agricultural pests. Therefore, advances in silkworm genomics will have a great impact not only on basic and applied research in the silkworm but also on comparative biology and applications such as pest control. I intend to establish a firm base that will enable map-based cloning in *B. mori*. Establishment of a high-density linkage map is the first milestone for this purpose.

Recent progress in polymerase chain reaction (PCR)-based markers such as randomly amplified polymorphic DNA (RAPD; Williams *et al.* 1990) and simple sequence repeats (SSR; Beckmann and Soller 1990) has greatly facilitated the construction of linkage maps for relatively small body-size organisms. Linkage maps have been constructed for the honey bee, *A. mellifera*, based on RAPDs (Hunt and Page 1995), the human malaria vector, *A. gambiae*, based on SSR (Zheng *et al.* 1996), and a wasp, *B. hebetor* (Antolin *et al.* 1996) and a mosquito, *A. albopictus* (Mutebi *et al.* 1997), based on RAPD-SSCP (single strand conformation polymorphism). Mapping with molecular markers has also been applied to the silkworm (Promboon *et al.* 1995; Shi *et al.* 1995), but the large number of chromosomes in the haploid genome ($n = 28$), typical of Lepidoptera, made it difficult to construct maps without missing any chromosomes. Cytogenetic mapping has also been difficult because of the small, numerous, and fairly indistinguishable chromosomes (Heckel 1993; Goldsmith 1995).

In this article, I present a dense linkage map of the silkworm mainly based on RAPDs using double primer pairs (Kurata *et al.* 1994). The map contains around 1018 genetic markers and covers ~2000 cM including all 27 autosomes and the *Z* chromosome. I also map a number of known genes and mutant loci and show the relationship between some of the newly established and conventional linkage groups.

MATERIALS AND METHODS

Silkworm strains and isolation of DNA: Silkworm strains C108 and p50 were identical to those used in the previous

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work (Promboon *et al.* 1995). An F_2 intercross was performed using F_1 sibs from a single pair mating of a C108 female by a p50 male. F_2 progeny were scored for sex and the *p* locus (plain; Doira 1992), and finally I used 169 male (*p*:39,+^{p}:130) and 186 female (*p*:44,+^{p}:142) larvae for DNA isolation. Genomic DNA was prepared from individual final instar larvae. A pair of whole silk glands was ground with a mechanical homogenizer within a microcentrifuge tube and suspended in DNA extraction buffer (50 mm Tris, pH 8.0, 100 mm NaCl, 20 mm EDTA) containing 150 μg/ml proteinase K. After digestion with proteinase K at 50° overnight, phenol extraction was carried out and DNA was recovered by isopropanol precipitation. DNA concentration was measured with a spectrophotometer (Beckman, Fullerton, CA), and 166 F_2 samples of higher DNA yield were selected for construction of a linkage map.

Additional matings were carried out to identify correspondence between molecular and established linkage groups (ELGs). Two recessive mutant strains, NISES 912 (*pe-re, ch*) and 920 (*lem, oc*), were used for this purpose (Sorita 1991). F_2 populations between a C108 mother and mutant father were reared and genomic DNA was isolated as described above.

RAPD analysis: Oligonucleotide primers were purchased from Operon Technologies (Alameda, CA; kits A, D, H, I, T, R, and Y). The reaction was performed in a total reaction mixture of 15 μl consisting of 25 ng template DNA, 0.5 μm primers, 1 unit of *Taq* polymerase (Takara, Kyoto, Japan), 0.1 mm for each of the dNTPs (Pharmacia, Piscataway, NJ), 50 mm KCl, 10 mm Tris-HCl (pH 9.0), 0.1% Triton X-100, and 2.5 mm $MgCl_2$. The amplification was carried out with a 3-min denaturation at 94°, followed by 45 cycles with a 1-min denaturation at 94°, a 2-min hybridization at 36°, and a 3-min elongation at 72°, ending with a 5-min final extension at 72°. The completed reaction (8 μl) was loaded on a binary gel [a mixture of 0.7% agarose (Takara) and 0.7% Synergel (Diversified Biotech, Boston) in 0.5× TBE buffer] and separated by electrophoresis. Gels were stained with ethidium bromide and photographed under UV light with Polaroid 667 film or a CCD-imaging processor (ATTO, Tokyo).

Establishment of expressed sequence tags (ESTs): Sequences of cloned silkworm genes were obtained from the GSDB, DDBJ, EMBL, and NCBI nucleotide sequence databases. Primers listed in Table 1 were designed with a program Oligo ver. 4.0 (National Biosciences, Plymouth, MN). PCR reactions were performed in the same buffer as described above except that the $MgCl_2$ concentration was 1.5 mm. The amplification was performed with a 3-min denaturation at 94°, followed by 45 cycles with a 1-min denaturation at 94°, a 2-min hybridization at 55°, and a 3-min elongation at 72°, followed by a 5-min final extension at 72°. Products were digested with eight restriction enzymes, *Afa*I, *Alu*I, *Dra*I, *Hae*III, *Hha*I, *Hin*fI, *Hpa*I, and *Mbo*I, to screen restriction fragment length polymorphisms (RFLPs) and to confirm whether expected fragments were amplified (Table 1).

Linkage analysis: The presence or absence of each polymorphic marker was scored for all 166 F_2 individuals. These data were processed to sort markers into linkage groups with the program MAPMAKER/Exp ver. 3.0 (Lander *et al.* 1987) with a statistical threshold LOD score of 3.0. Markers belonging to the same group were ordered by use of the "compare" command of MAPMAKER, and potentially false signals were checked by additional PCR amplifications. Because MAPMAKER/Exp ver. 3.0 cannot account for achiasmatic meioses, I could not use it to calculate genetic distances between loci. Instead, F_2 intercross data were converted to BC1 backcross data using the hypothesis described in results, and genetic distances between markers were then calculated using MAPMAKER.

Heteroduplex formation: Equal amounts of PCR products from both parental strains and loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol in H_2O) were mixed and denatured at 95° for 5 min. Then a 15-min hybridization was performed at 55° and cooled to 4° and the reaction was loaded onto an agarose gel [3% Metaphor XR (FMC, Rockland, ME) in 0.5× TBE, 1 m urea] in an ice-cold chamber.

RESULTS

Detection of RAPDs with double primer pairs: To obtain markers for linkage analysis, RAPD polymorphisms between two parental strains (C108 and p50) were screened using 140 10-mer commercially available primers. A total of 7757 primer combinations were tested and 1001 of them were selected for further analysis (Figure 1A). Small-scale linkage analysis was performed with 22 F_2 progeny, and I found 757 primer combinations generating segregating banding patterns (Figure 1B). More detailed mapping was carried out with an additional 144 F_2 progeny from the same single pair mating, and 1388 polymorphic bands generated from 719 primer pairs were finally used for map construction after eliminating unclear markers (data not shown; the list of RAPD markers used in this experiment will be available on http://ss.nises.affrc.go.jp/).

Mapping of known genes: I also tried to map cloned silkworm genes. ESTs were designed for more than 30 known sequences, and four of them showed clear polymorphisms between C108 and p50 (Table 1). Monomorphic PCR products were probed for RFLPs by digestion with a variety of restriction enzymes. As a result, I found RFLPs for three ESTs (Table 1). In addition, I used a previously reported intron length polymorphism of the prothoracicotropic hormone (PTTH) gene (Shimada *et al.* 1994). These eight markers were used to analyze 166 F_2 individuals from the cross between C108 and p50.

Identification of linkage groups: A total of 1388 RAPDs and eight ESTs were then grouped into linkage groups using MAPMAKER/Exp ver. 3.0. Sorting of markers revealed that there were 28 linkage groups that coincided with the haploid chromosome number of *B. mori*. However, there still remained the possibility that large gaps between markers might falsely assign markers on the same chromosomes to different linkage groups.

Achiasmatic oogenesis makes it possible to test whether linkage groups are really independent or not. As shown in Figure 2A, any F_2 individual cannot be homozygous for both maternal and paternal dominant markers on the same autosome (Promboon *et al.* 1995; Shi *et al.* 1995), since most of the markers used in this analysis were dominant (*i.e.*, RAPDs) and it was impossible to distinguish homozygotes from heterozygotes when markers were present. The only distinguishable homozygotes were scored as nulls. Consequently, the absence of a maternal marker in a certain F_2 progeny meant that the nonrecombinant autosome of the progeny was paternal, and vice versa (Figure 2A). This enabled us to type each F_2 individual for each linkage

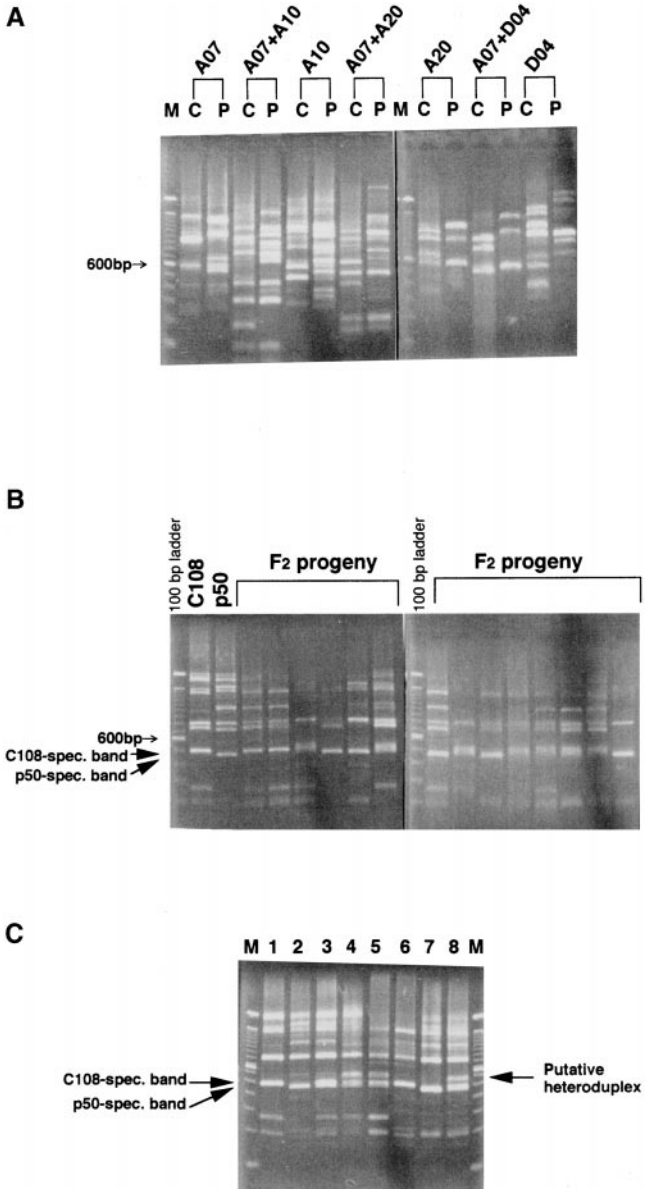


Figure 1.—(A) An example of RAPD analysis between parental strains C108 and p50, using single or double primers. (B) Typical RAPD banding patterns of linkage analysis using parental strains (C108 and p50) and 14 F_2 progeny. Primers OPA05 and OPD19 were used. C108- and p50-specific fragments (arrowheads) belonged to the same linkage group. (C) A putative heteroduplex was newly generated between C108- and p50-specific fragments. Electrophoresis of PCR products using the same primers as in B was carried out in a gel containing 1 m urea. M, 100-bp ladder (GIBCO BRL, Gaithersburg, MD); lane 1, C108; lane 2, p50; lanes 3 and 4, mixture of products from C108 and p50, not treated (lane 3) or heat denatured and annealed (lane 4); lanes 5–8, F_2 progeny. Note that the 600-bp "spike" band of the 100-bp ladder was split presumably because it was composed of two fragments whose sequences were different.

group as to whether its nonrecombinant chromosome was paternal or maternal, yielding a so-called *chromosome print* (Figure 3). To identify the *Z* chromosome, maternal dominant markers were expected to segregate in a 1:1 ratio because of haploidy and all paternal ones were expected to be present in F_2 males without fail (Figure 2B). One of the linkage groups fulfilled these requirements (Figure 3; Group 1).

Typing was determined when more than two null scores of C108- or p50-dominant markers were confirmed for each putative linkage group. If paternal and maternal markers of the same linkage group were found to coexist in the same F_2 individual, another PCR amplification was repeated until clear results were obtained. Even when linked markers on the same chromosome are separated by long gaps they cannot show inconsistent *chromosome prints*, and all 28 putative linkage groups were revealed to be independent from each other. Therefore, I concluded that the 28 linkage groups assembled by MAPMAKER covered all 27 autosomes and the *Z* chromosome.

Mapping of ESTs and visible markers: Segregation patterns of visible markers, sex, and the *p* locus, revealed that 2 of the 28 linkage groups corresponded to ELG 1 and 2. Early chorion gene A4, previously identified on ELG 2 (Goldsmith and Clermont-Rattner 1979), was found to be linked with the visible *p* locus, as expected. Analysis of sequences corresponding to egg specific protein and PTTH also showed the correspondences between 2 linkage groups and ELG 19 and 22 (Figure 4). The P25 and *invected* genes were newly identified to be located in ELG2 (Figure 4). In addition, the larval serum protein and vitellogenin genes belonged to linkage groups whose relationship between ELGs had not been previously identified (Figure 4).

Intriguingly, Bombyx homologues of *engrailed(en)* and *invected(inv)* genes (Hui *et al.* 1992) were found to be closely linked on ELG 2. Recombination occurred in only 1 of 166 F_2 progeny. The *en* and *inv* genes were previously reported to be closely located in *D. melanogaster* (Coleman *et al.* 1987). These results might suggest that the genome structure of this region is conserved, at least regionally, between the silkworm and the fruit fly.

Map construction: Achiasmatic oogenesis also facilitated evaluation of the reliability of the RAPD markers. False-negative signals could be detected, for example, when a maternal marker was absent in the progeny whose nonrecombinant chromosome was maternal. Exclusion of unreliable markers revealed by frequently generating false-negative signals greatly improved the accuracy of the linkage analysis. Out of 1388 RAPDs, 1010 were finally utilized for map construction.

C108-dominant and p50-dominant markers were separately ordered within each linkage group (Promboon *et al.* 1995). As a result, marker orders of 56 putative linkage groups were determined (Figure 4). Since MAPMAKER could not be adjusted for achiasmatic oogenesis, I could not calculate genetic distances between neighboring markers directly. Therefore, I converted

TABLE 1

ESTs used in this analysis

Name	Primer sequences	Polymorphism	
		C108	p50
Larval serum protein (D12523, D01179)	F TCAAAGACAAAGACATAGCC R CAGCAGAATCAGAGAAACAG	*ca.* 900 bp	*ca.* 1200 bp
Bm engrailed (M64335)	F TGGTCTCTTCTTTTTCTGTA R AGTTTATCTGTTTGGTTATG	*ca.* 630 bp	*ca.* 770 bp
Bm invected (M64336)	F TAGAGATGAAGGCGAGAGAA R CGACTGTTATGAAGACTGCT	*Alu*I susceptible	*Alu*I resistant
Silk protein P25 (D12521)	F TCATACCTTTTTCCCTGTCC R AATACTCGCTTCACCGTTCC	*Mbo*I resistant	*Mbo*I susceptible
Yolk protein (X04223)	F ATGCTATTGTTTCGCTTTTC R CTCTATTAGTGTCTGTTTGG	*Dra*I susceptible	*Dra*I resistant
Early chorion A4 (X58447)	F ACAAATACAAATGATGAAAA R AATACCGCACTATACTCTAA	*ca.* 700 bp	Not amplified
Vitellogenin (D30733)	F GAAACTAACTCAAAATGGTA R TAAGAACAAAGACAAAACAG	Not amplified	*ca.* 950 bp
PTTH[a] Prothoracicotropic hormone	F GACTCCTGCGATTTAGTTTC R CAAAGAAAGTTTATACAGTG	*ca.* 350 bp	*ca.* 490 bp

Accession numbers are indicated in parentheses.
[a] Synthesized according to Shimada *et al.* (1994).

F_2 intercross data to backcross (BC) 1 data with the criteria described below.

An individual having a C108-derived nonrecombinant chromosome is not informative for calculating recombination values between C108-dominant markers because it must show positive signals regardless of the crossing over in the other chromosome (Figure 2A). Therefore, I used only individuals confirmed as having a p50-derived nonrecombinant chromosome to calculate genetic distances between C108-dominant markers, and vice versa. The presence or absence of markers in such individuals could be interpreted as BC1 data (Figure 2A; for examples, see behavior of markers A, D, and E in type B progeny and markers F, G, and J in type A progeny). On average, 61.7 individuals were informative for C108-dominant markers and 62.0 individuals were informative for p50-dominant markers.

Connecting the C108- and p50-dominant linkage groups: Map construction as described above inevitably generated two linkage groups for each chromosome (Figure 4). Integration of the two groups required connecting markers between them. One candidate was codominant markers. The ESTs listed in Table 1 were useful for this purpose. In addition, I found a number of RAPDs that behaved like codominant markers. C108- and p50-dominant bands of such markers not only were of similar size but also generated putative heteroduplex products in heterozygotes (Figure 1B) as reported in previous work in the honeybee (Hunt and Page 1995). I tested whether these bands were codominant or not by heteroduplex formation (Figure 1C); bands that generated new heteroduplex bands were utilized as linking markers (Table 2).

Other candidates for map integration were markers showing a segregation ratio of 1:1. Many of them were on the *Z* chromosome of the maternal strain (Figure 2B). In this case, segregation behavior of paternal dominant markers in F_2 females could easily be integrated into those of maternal markers (Figure 2B).

On the other hand, heterozygosity in parental strains also leads to similar results. If a heterozygous dominant marker succeeds to an F_1 female, the segregation behavior in F_2 individuals would reveal the marker composition of the nonrecombinant chromosome, which would be reflected in *chromosome print* (Figure 2A; markers B and H). Only one marker, R121124, was classified in this category since its behavior completely agreed with *chromosome print* of Linkage Group O (Figure 3).

If an F_1 male inherits a locus that was heterozygous in the parental strains, its segregation pattern is informative for linkage analysis in both types of F_2 individuals, and is useful for connecting the two linkage groups (Figure 2A; markers C and I). I found several markers of this type (Table 2). Interestingly, some of these markers seemed to be clustered, suggesting that these chromosome regions of the parental strains remained heterozygous (Table 2). It may be a clue to the presence of recessive lethals.

The correspondence of molecular linkage groups to the conventional linkage groups: For utilization of the genetic resources of the silkworm mutants for molecular analysis, it is desirable to map conventional mutations

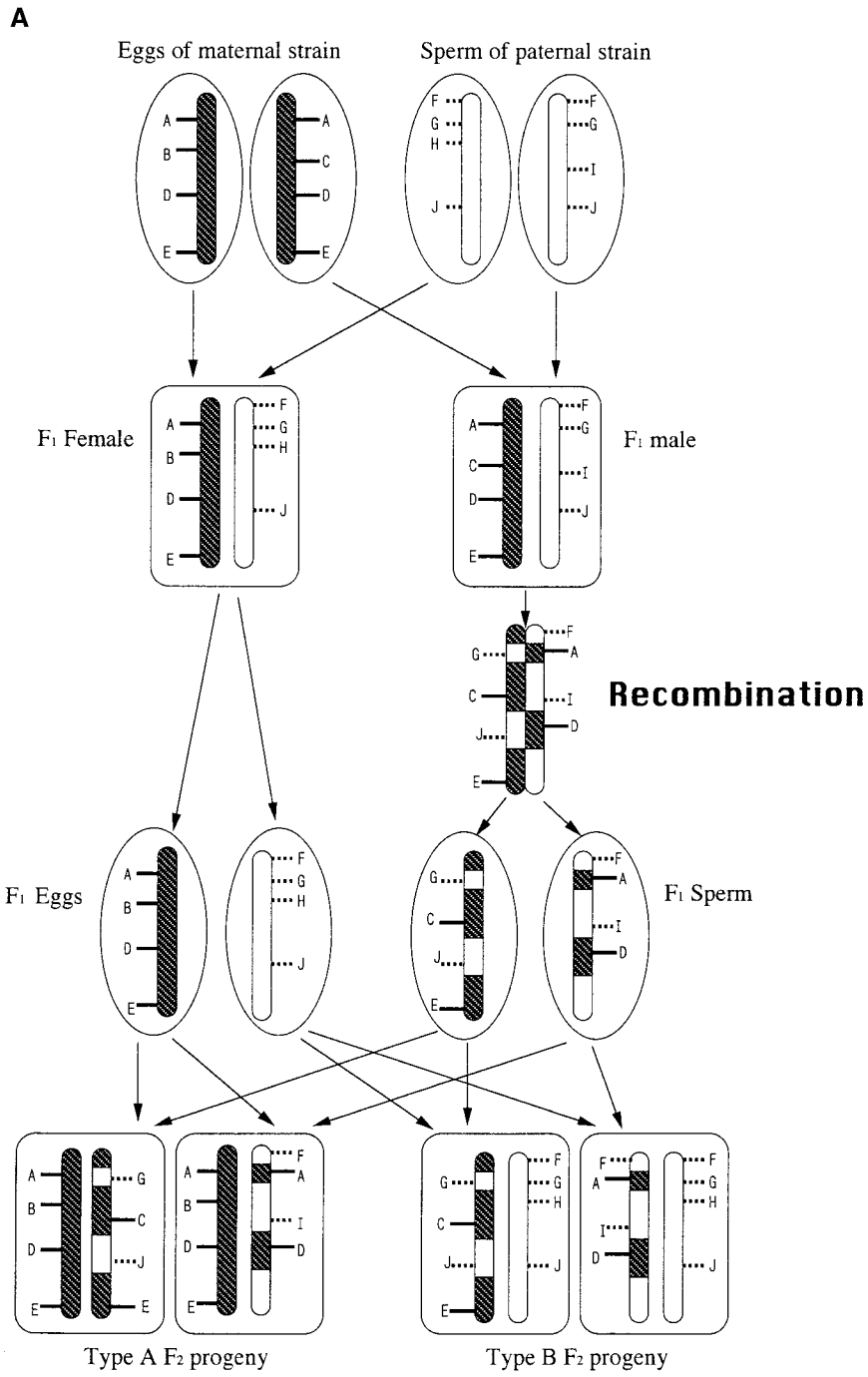


Figure 2.—Schematic representation of behavior of dominant markers on autosomes (A) and the *Z* chromosome (B) in an F_2 intercross. Note that type B progeny and F_2 males must be positive for paternal markers, and maternal markers are always heterozygous in these progeny. See the text for the case of heterozygous markers of parental strains (markers B, C, H, and I).

on the molecular linkage maps newly developed in this analysis. As a first step, I tried to find the correspondence of some of these molecular linkage groups with the conventional counterparts.

As described above, I could already find such relationships for ELG 1, 2, 19, and 22 (Figure 4). In addition, matings of C108 with two recessive mutant strains, NISES 912 (*pe-re, ch*) and 920 (*lem, oc*), were performed. *pe(pink eye)-re(red eye)* and *oc* (C translucent) loci belong to ELG 5, *lem* (lemon) to ELG 3, and *ch* (chocolate) to ELG 13, respectively (Doira 1992). F_2 linkage analysis revealed the molecular counterparts of ELG 3, 5, and 13 (Figure 5). Moreover, *pe*, *re*, and *oc* loci belonged to the same linkage group, as expected (Figure 5).

DISCUSSION

RAPD analysis is superior to other methods for linkage analysis in many respects. It requires a small amount of DNA and no special apparatus, expensive reagents, or complicated procedures. Therefore, it is suitable for large-scale analysis at low cost. Because it does not depend on knowing any specific sequences, such as SSR, restriction sites, repetitive elements, or expressed se-

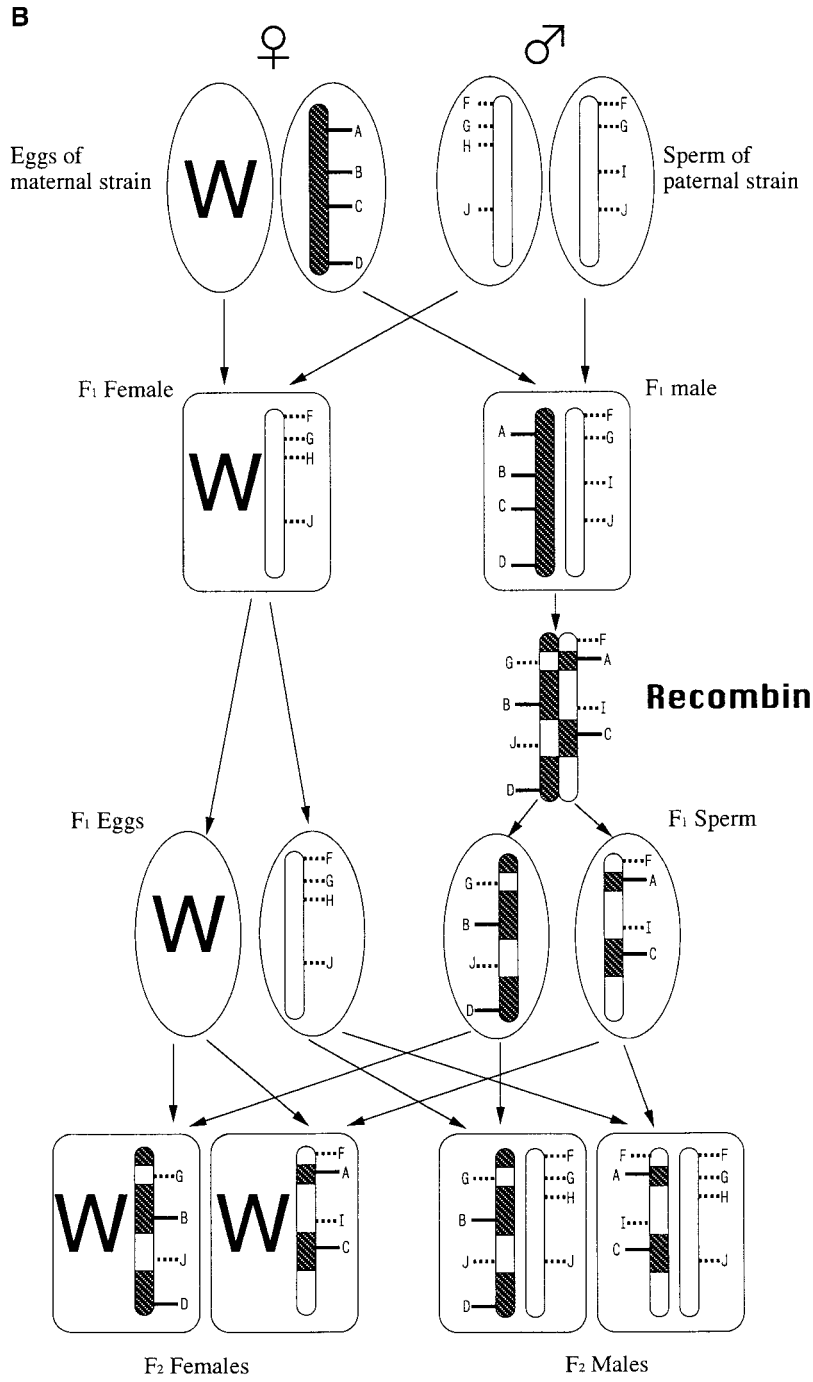
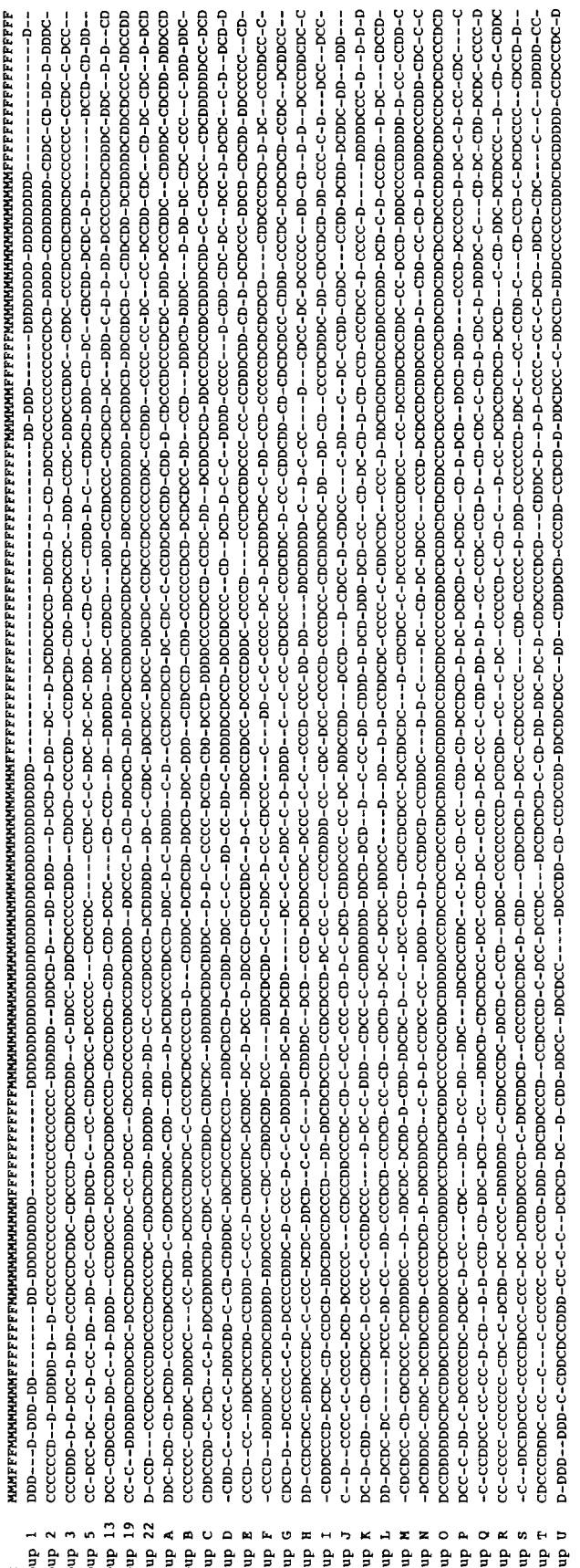


Figure 2.—(*continued*)

quences, there are no major limitations for improving map resolution.

In spite of these advantages, RAPD analysis has been treated as an uncertain method for mapping because of its so-called lower reproducibility. I improved reproducibility by screening a great number of primer pairs rather than single primers. It seems unusual that single primers are specifically utilized in RAPD analysis whereas double primers are commonly used in PCR for almost all other purposes. Using double primers has a great advantage, especially for constructing a dense map, due to the exponential increase of combinations of primer pairs using a limited number of primers (*e.g.*, Brickener *et al.* 1996). Screening of 10,000 single primers is far from feasible, if not impossible, but 10,000 primer pair combinations can be tested using only 143 primers.

The two main problems for utilization of double primers will be whether new information is obtained, and whether the banding patterns generated by this method are reproducible. As shown in Figure 1, the banding patterns generated with double primers were often quite different from those of each single primer, and many of them were amplified more consistently com-



pared to using a single primer. For the 719 primer pairs that I used a result of prescreening nearly 8000 pairs, there was no evidence that the use of double primers reduced reproducibility. I concluded that double primer RAPD analysis was a cost-effective tool for large-scale linkage analysis of small organisms. Anyone can easily utilize my map just by purchasing seven kits of commercially available primers.

Estimation of the fraction of the genome covered by the mapped region is difficult because autosomes have two parallel series of linkage groups in these experiments (Figure 4), and the extent of overlap should be confirmed by linking markers. Linkage groups of C108-dominant markers covered 2300.8 cM, whereas those of p50-dominant markers covered 2359.9 cM. However, my mapping strategy overestimated genetic distances when precise behavior of the nonrecombinant chromosome was not identified and untyped F_2 progeny (indicated as a dash in Figure 3) were excluded for calculation. Except for Linkage Group O, all autosomes retained this ambiguity (Figure 3). The sizes of Linkage Group O were 73.4 cM for C108-dominant markers and 116.4 cM for p50-dominant markers. If the calculation is performed just as for other groups, namely ignoring information obtained from R121124A, these sizes are 93.4 cM and 133.9 cM, respectively. In this case, the extent of overestimate varied from 15.0 to 27.2%. Using this value, the actual sizes of linkage groups were estimated as 1841.2–2020.5 cM for C108-dominant markers and 1894.2–2075.8 cM for p50-dominant markers. The mean intervals between markers were 3.54–3.89 cM for C108-dominant and 3.67–4.02 cM for p50-dominant markers. This resolution is sufficient for fine dissection of quantitative trait loci (QTL) or complex traits governed by multiple genes.

Considering potentially overlapping regions of the two series of linkage groups, the complete recombination length of *B. mori* was estimated to be ~2000 cM. Linkage maps previously reported cover 413 cM based on RFLPs (Shi *et al.* 1995; 60 markers), 897.4 cM based on RAPDs (Promboon *et al.* 1995; 169 markers), and 900.2 cM based on phenotypic markers (Doira 1992; 207 markers). My estimate is twofold greater in size than previous reports. However, the value seems not to be too large since the other maps have many gaps, unmapped loci, and linkage groups whose length is uncertain or very short in part due to the small number of markers. The physical genome size of *B. mori* is reported as 495 Mb (Gage 1974). Therefore, the average interval

Figure 3.—*Chromosome print* presents typing of nonrecombinant chromosomes for each F_2 individual (in column) and linkage group (in row). C, nonrecombinant chromosome derived from C108; D, nonrecombinant chromosome derived from p50 (Daizo); a dash indicates that origin of nonrecombinant chromosome was not identified.

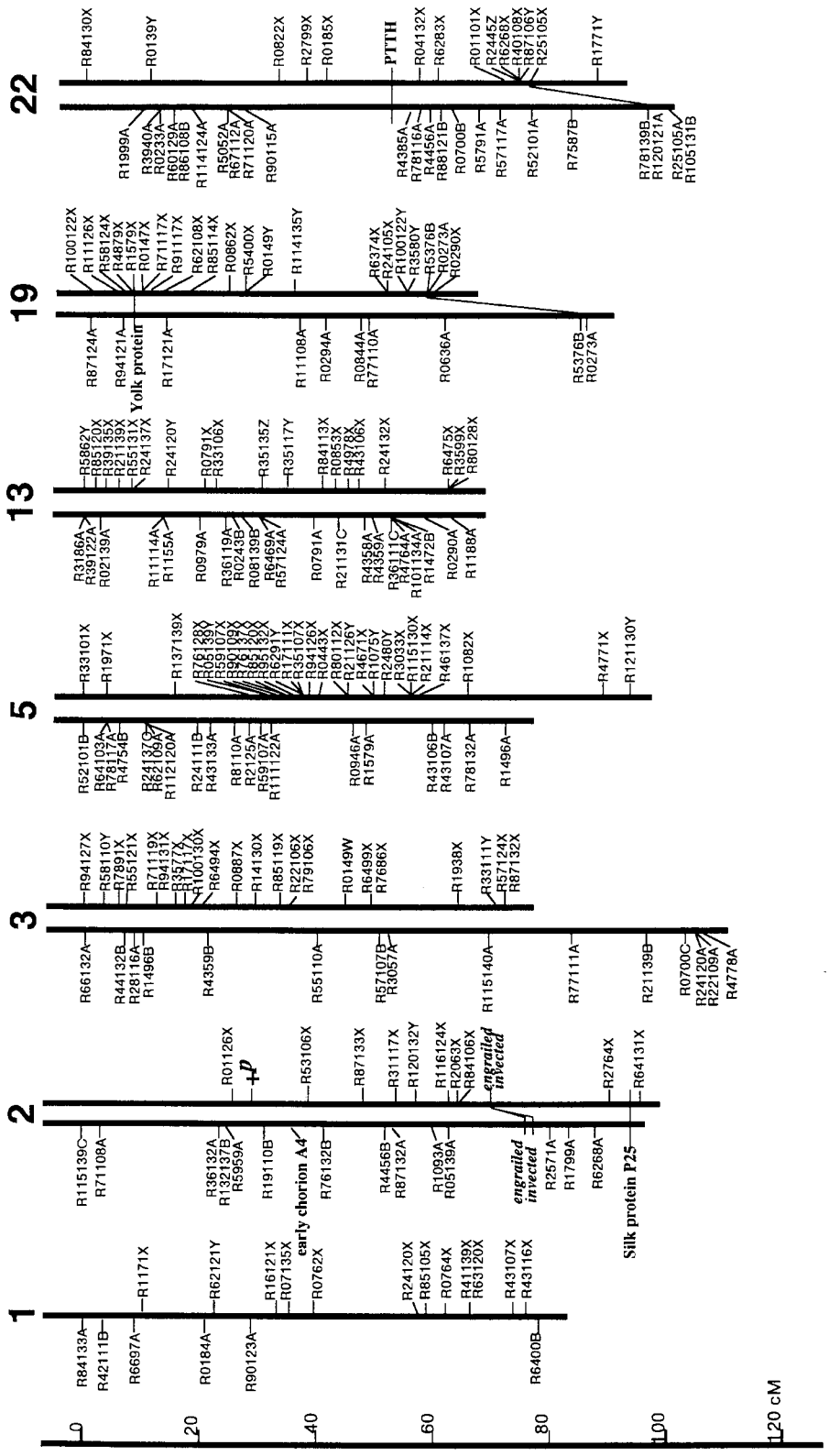
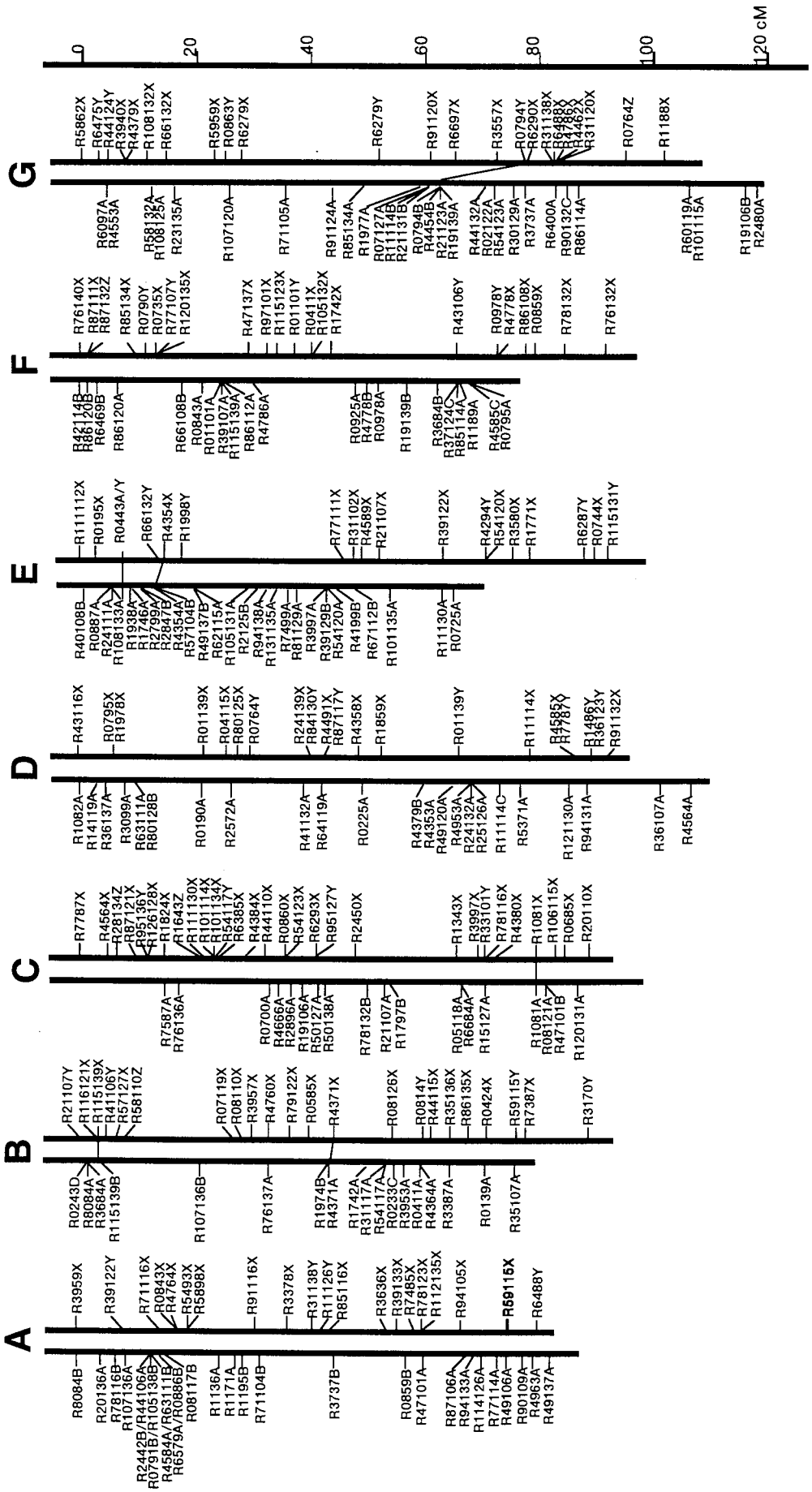


Figure 4.—A linkage map of *B. mori*. Twin bars represent linkage groups of C108-dominant markers (left) and p50-dominant markers (right). The complete list of markers will be available on http://ss.nises.affrc.go.jp/.

of markers is ~500 kb and the relationship between physical and genetic distances is ~250 kb/cM. This value is intermediate between the honey bee (52 kb/cM; Hunt and Page 1995) and the fruit fly (575 kb/cM; Merriam *et al.* 1991), agreeing well with the idea that crossing-over rates are inversely correlated with chromosome size, because average chromosome sizes are 11.1 Mb for the honey bee, 17.1 Mb for the silkworm, and 47 Mb for the fruit fly (Hunt and Page 1995).

Integration of linkage groups of C108- and p50-domi-



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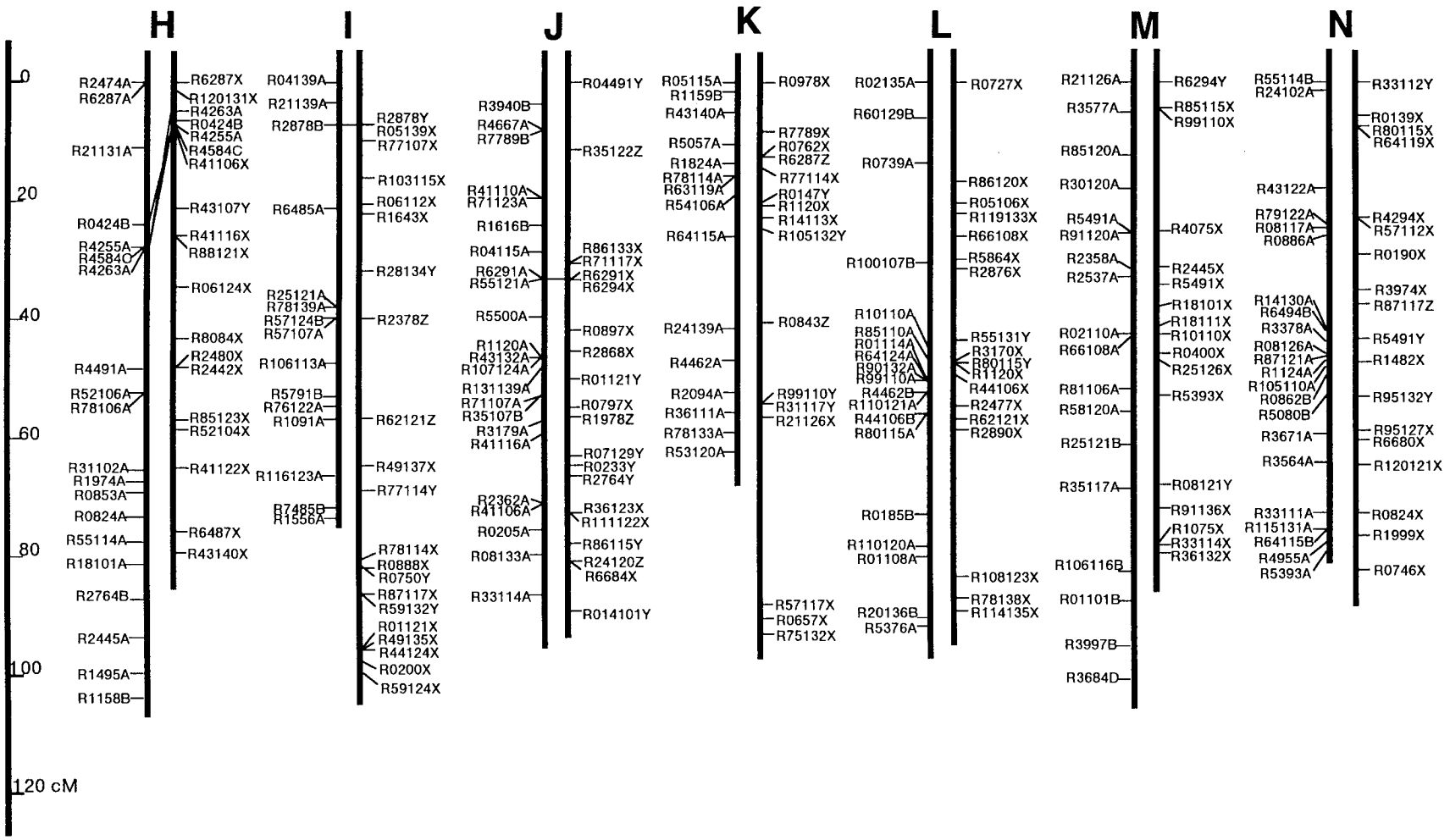

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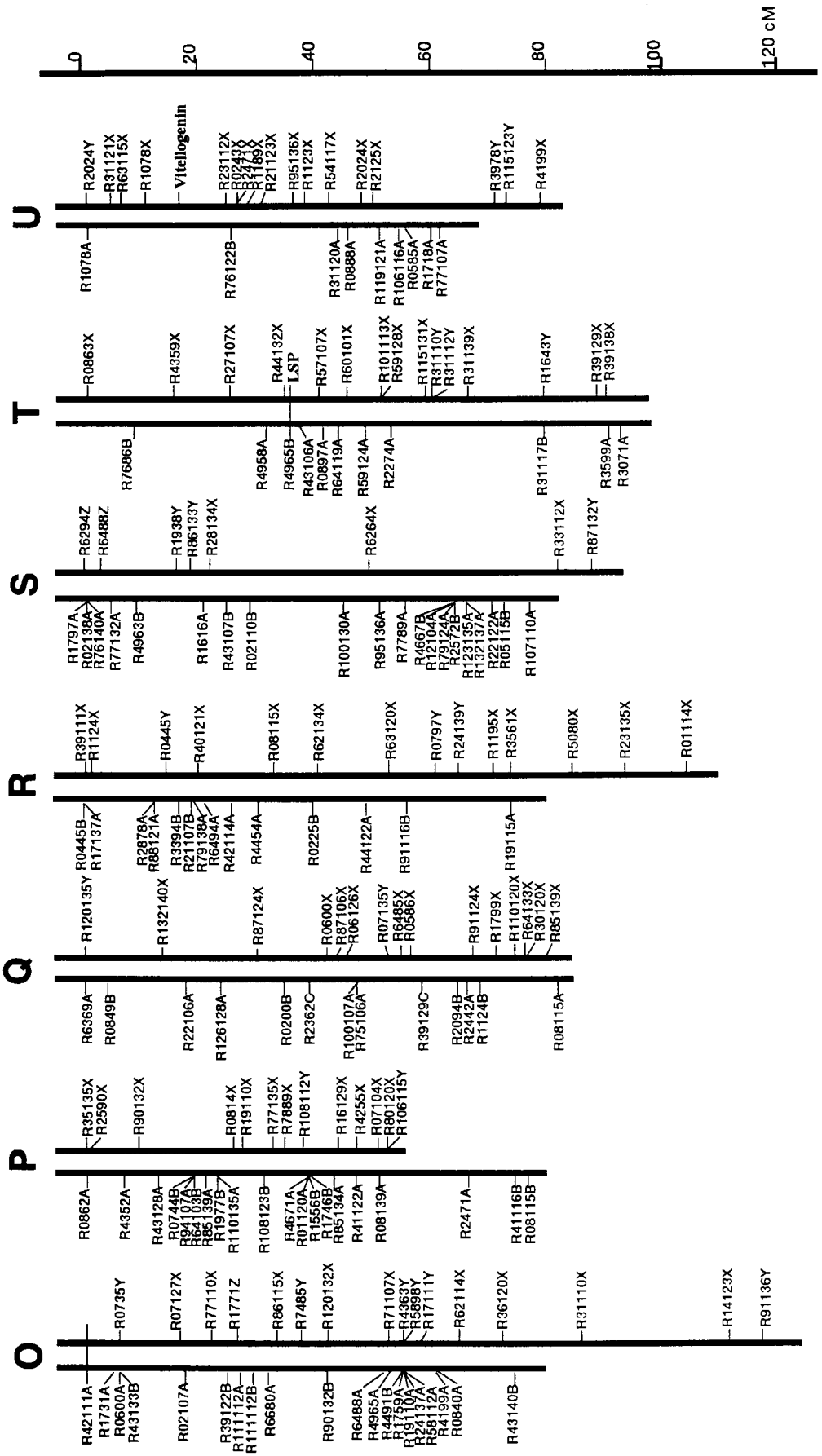

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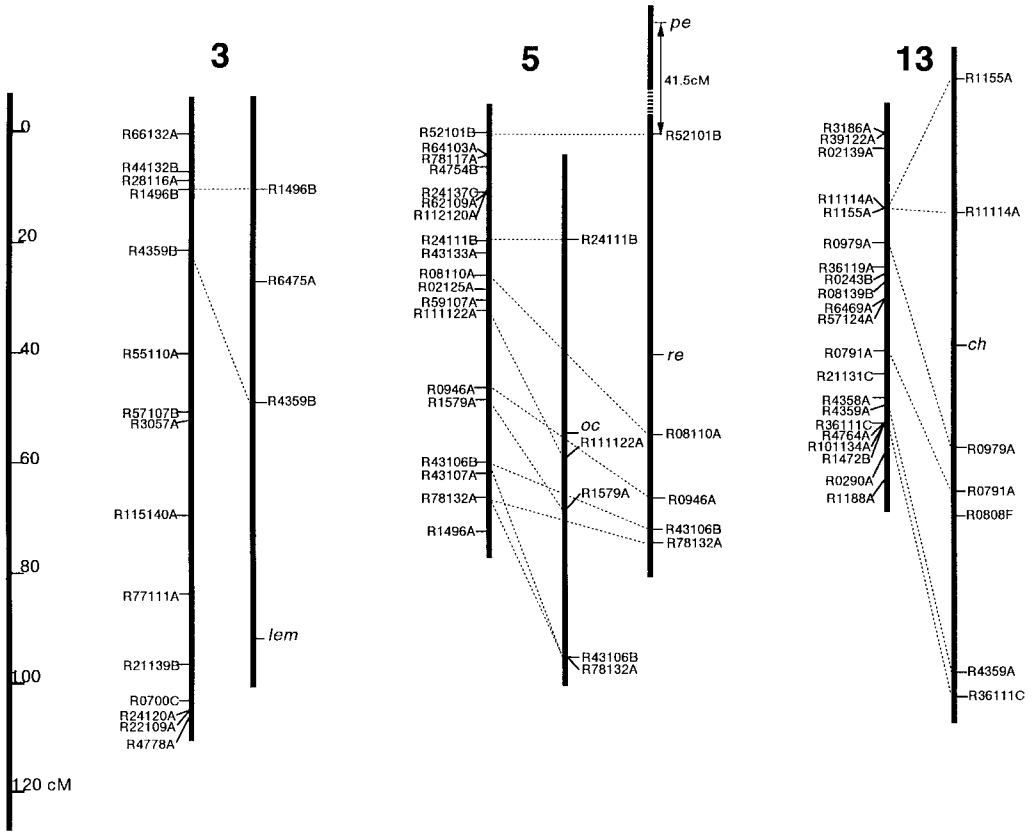

Figure 5.—The correspondence between molecular and phenotypic markers. The left bars show linkage groups of C108-dominant markers derived from mating with p50. The middle and right bars present those derived from matings with recessive mutants. Marker intervals seem to be larger in matings with mutants probably because the numbers of markers scored and F_2 individuals were much smaller.

nant markers is one of the remaining problems. It is critical to establish cost- and labor-saving methods to map codominant markers that connect both groups. PCR-based methods, such as SSCP (Hayashi 1991), may be useful for this purpose. SSCP analyses of RAPD markers were previously reported (Antolin *et al.* 1996; Mutebi *et al.* 1997). Alternatively, heteroduplex formation, used in this report for confirmation of codominant markers (Figure 1C), might also become an effective tool. I have succeeded in finding polymorphisms of several sequence tagged sites using this method (Y. Yasukochi, unpublished results).

TABLE 2

Linking markers between C108- and p50-dominant markers

Markers	C108 (bp)	p50 (bp)	Linkage groups
Codominant markers			
R115139B/X	500	480	B
R4371A/X	350	370	B
R1081A/X	1050	1150	C
R0443A/Y	780	950	E
R04354A/X	700	660	E
R0794B/Y	600	740	F
R2878B/Y	1100	1200	I
R6291A/X	360	290	J
R25105A/X	300	360	22
Markers segregated at a 1:1 ratio			
R0424B	800	—	H
R4255A	540	—	H
R4263A	300	—	H
R4584C	460	—	H
R42111A	280	—	O
R0273A	420	—	19
R5376B	580	—	19

The map reported here is the first molecular linkage map of insects having a large number of chromosomes (n = 28) with a one-to-one correspondence between linkage groups and chromosomes. The mapping strategy used in this experiment can be easily applied to other organisms having achiasmatic meioses found in heterozygous wild populations. The overall average distance of my map approaches nearly 2 cM/marker to date, and there are no fundamental limitations to improving it. We are now constructing a bacterial artificial chromosome (BAC; Shizuya *et al.* 1992) library. Combining the dense linkage map, the BAC library, and the mutant genetic resources of the silkworm, map-based cloning will be feasible within a few years, and light will be thrown on molecular mechanisms that cause various mutant phenotypes.

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