ORGANIZATION OF THE CHORION GENES OF *BOMBYX MORI*, A MULTIGENE FAMILY. II. PARTIAL LOCALIZATION OF THREE GENE CLUSTERS

MARIAN R. GOLDSMITH AND EILEEN CLERMONT-RATTNER

Department of Developmental and Cell Biology, University of California, Irvine, Irvine, California 92717

Manuscript received October 26, 1978

ABSTRACT

Chorion genes of the inbred stock Ascoli have been localized to three linked clusters by analysis of testcross progeny. Electrophoretic variants screened by isoelectric focusing served as markers. The clusters are designated *Ch 1*, *Ch 2*, and *Ch 3*. The gene order is *Ch 1–Ch 2–Ch 3–Y*, with relative map distances of approximately 0.4 m.u. for *Ch 1–2*, 3.3 ± 0.9 m.u. for *Ch 2–3*, and 21 m.u. for *Ch 3-Y*. In a separate testcross using different markers, two chorion regions were localized 2.3 m.u. and 3.1 m.u. from *p*. These markers could not be assigned to *Ch 1*, *2*, or *3* because there is at present no test for allelism in this system.

THE silkmoth chorion consists of 50 to 100 proteins of similar molecular weight, pKi, and amino acid composition (KAFATOS *et al.* 1977). Amino acid sequence analysis of purified chorion proteins from the saturniid *Antheraea polyphemus* suggests that they are encoded by two or more multigene families that evolved by a process of gene duplication and subsequent mutation (REGIER *et al.* 1978; RODAKIS 1978). Sequencing of cloned cDNA synthesized from *A. polyphemus* chorion mRNA further reveals significant homology between members of two of these gene families (KAFATOS *et al.* 1978).

The chromosomal organization of known multigene families may be in tandem clusters or dispersed at many loci. Numerous examples of multigene clusters are known, such as the ribosomal RNA (TARTOF 1975), histone (KEDES 1976; LIFTON *et al.* 1978) and antibody genes (HOOD, CAMPBELL and ELGIN 1975). Repeated transcribed DNA sequences that show a nonclustered arrangement were recognized with the help of molecular cloning technology, and include the copia genes of *Drosophila melanogaster* (FINNEGAN *et al.* 1978). The evolutionary significance of these two gene arrangements is not known. Of particular interest is whether there are any regulatory or functional properties that are unique to clustered or dispersed multigene families, and thus reflect the role of natural selection in their evolution and arrangement.

The detailed chromosomal organization of the chorion genes is presently under investigation. By straightforward Mendelian analysis using electrophoretic variants as genetic markers, we have demonstrated that a large number of chorion

genes are linked to chromosome *2 in Bombyx mori* ($n = 28$; GOLDSMITH and BASEHOAR 1978). In this paper we report the results of a subsequent investigation designed to test the intensity of linkage among a group of chorion markers. We also report the partial localization of these chorion genes near the origin of chromosome *2*.

MATERIALS AND METHODS

Silkworm stocks and rearing: Silkworm stocks C108, Ascoli, Hiko and 703B were obtained from Y. TAZIMA and A. MURAKAMI at the National Institute of Genetics, Misima, Japan. Stocks P22 and 751 (dO3 × P22) were obtained from B. SAKAGUCHI and H. DOIRA at the Department of Sericulture, Faculty of Agriculture, Kyushu University, Fukuoka, Japan. The hybrid stock P22/703B was generated from stocks 751 and 703B by crossing p^s *Y* adults from the initial mating of a 751 female ($p^s +^G +^Y$ [P22]/*p Gr*^{B} *Y* [d03]) with a 703B male (*p Gr*^{B} *Y* [703B]/*p Gr*^{B} *Y* [703B]). The hybrid has been maintained for three generations by mating p^s *Y* females with $p +^Y$ males. Rearing was carried out as previously described (GOLDSMITH and BASEHOAR 1978).

Sample preparation and screening: Solubilization and isoelectric focusing of chorion samples on polyacrylamide gels were as previously reported (GOLDSMITH and BASEHOAR 1978), except that the voltage was increased to 900V during the last hour of electrophoresis to improve protein separations. Samples were screened from one to five times, depending on their overall resolution compared to parental chorion standards included in each gel. All reported markers were resolved in a minimum of two sample runs, except where indicated.

Chorion nomenclature: Chorion markers are designated as previously described (GOLDSMITH and BASEHOAR 1978). Markers are given a letter prefix indicating the stock of origin and then numbered consecutively from the basic end of the isoelectric focusing pattern. Hybrid bands, which are composites of comigrating markers, are designated with both sets of markers separated by a slash, such as P9/10, Figure 5.

RESULTS

Genetic rationale and summary of the results: To determine the degree of linkage among chorion genes, F_1 hybrids were produced by crossing two strains differing in a large number of chorion markers. The F_1 males, in which intrachromosomal crossing over can take place, were subsequently testcrossed to the homozygous females of one parental strain, and the progeny were scored for chorion phenotype on isoelectric focusing gels. Since the chorion genes are codominant and many are linked, all or most of the markers present in the homozygous parent will be expressed in the progeny, providing a phenotypic background in the gel patterns. To facilitate mass screening, we usually selected for the testcross female the parental strain against which recombinants could most easily be scored by isoelectric focusing. Thus, we were able to score recombinants only among markers that originated in one of the two stocks used for the F_1 cross (the nontestcross female; Figure 1).

We examined two strain combinations for the intensity of linkage between chorion genes, Ascoli *vs.* C108 and Ascoli *vs.* Hiko. These results are summarized in Table 1. Progeny fell into three phenotypic classes: Class P, identical to the homozygous testcross parent; Class F, identical to the F_1 parent, and various Class R recombinants, which carried some but not all of the Ascoli markers. The

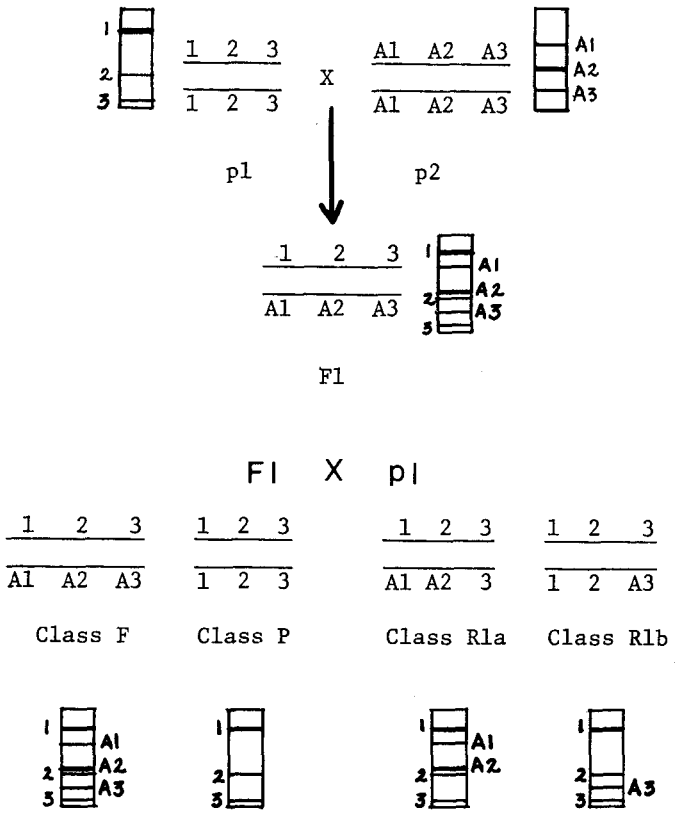

FIGURE 1.—Mating scheme to test for intensity of chorion gene linkage. Each number corresponds to a structural gene whose product yields a protein at the indicated gel position; chromosome map positions are arbitrary. p1 and p2 are different homozygous inbred stocks. Depicted are theoretical chorion protein patterns produced by parental, F_1 and testcross individuals.

relative yields of Class P and Class F progeny approximated the 1:1 ratio expected for linked genes. The frequency of recombinants ranged from 0.4% to 4.2%, and were calculated independently for paired reciprocal classes (see below).

We also examined a third strain combination, P22 *vs.* 703B, to localize chorion

TABLE 1

Summary of progeny in testcrosses involving Ascoli markers

Cross	Parentals Class P	Parentals Class F	Recombinants Class R*	Total	Recombinant frequency*
C108 × Ascoli/C108	119	110	10,1	240	0.42,004
Hiko × Ascoli/Hiko	46	37	2	85	0.024

* Reciprocal recombinants are combined; each value represents a crossover in a single chromosomal region relative to chorion markers; recombination frequencies are calculated for each class of recombinants (see RESULTS).

TABLE 2

Segregation of chorion markers in Ascoli vs. *C108 testcrosses*

Progeny class	Markers	No.
F	**A1a**, **A1**, A4, A6, A7, **A12**, **A18**, A24, A26	110
P	**4**, **5**, **6**, [17/18]	119
R1a	**A1a**, **A1**, A4, A6, A7, **A12**, [17/18]	4
R1b	A18, A24, A26, **4**, **5**, **6**	6
R2	A6, A7, **A18**, A24, A26	1
Total		240

The data was pooled from three pair matings. Markers listed in boldface are qualitative, in plain type are quantitative, and in brackets are distinguishable by morphology.
Frequency of R1a + R1b = 10/211 = 0.042; Frequency of R2 = 1/211 = 0.004.

genes with respect to other chromosome *2* markers. In this case, the frequency of chorion recombinants was estimated at 0.8%. The leftmost chorion marker was approximately 2.3 m.u. from the *p* locus at the origin.

Testcross of Ascoli vs. *C108 markers*: In order to examine the linkage of Ascoli markers, Ascoli/C108 males from a mating of C108 × Ascoli were testcrossed to homozygous C108 females, and 240 progeny were scored on isoelectric focusing gels (Table 2). We observed a total of ten Ascoli markers in 110 Class F progeny, including four qualitative markers (bands A1a, A1, A12, and A18) and six quantitative markers (bands A4, A6, A7, A16, A24, and A26; Figure 2). Four of these were key protein bands routinely used to identify Class F (bands A1, A6, A12, and A18), and scored on all screening gels. The remainder were distinguishable in 85% of the samples. A total of 119 Class P progeny were identified by the absence of these markers, revealing the presence of four underlying C108 bands (bands 4,5,6, and 17/18).

In addition to the parental classes, we observed three classes of chorion recombinants, R1a, (four cases), R1b (six cases), and R2 (one case). Classes R1a and R1b were reciprocals. Class R1a expressed only seven of the Ascoli markers (bands A1a, A1, A4, A6, A7, A12 and A16), whereas Class R1b expressed the remaining three bands (A18, A24, and A26). The third recombinant class, R2, expressed a new combination of five markers (bands A6, A7, A18, A24, and A26). In this case, the reciprocal was not recovered, despite a detailed examination of the screened samples for the predicted phenotype (expression of bands A1a, A1, A4, and A7).

The occurrence of these recombinants allowed us to begin constructing a linear map for chorion genes (Figure 3). As reciprocals, Classes R1a and R1b define two sets of tightly linked genes, designated *Ch 1–2*, and *Ch 3*, that were separated by crossing over within a single chromosomal region (Figure 3, crossover 1). Their combined frequency represents the genetic distance between the nearest chorion markers in each set, or 4.2 m.u. Since Class R2 expresses all the markers of R1b, but only two markers from R1a, it defines a second site of crossing over within the R1a group (Figure 3, crossover 2). This divides the R1a group into two tightly linked clusters, designated *Ch 1* and *Ch 2*. The frequency

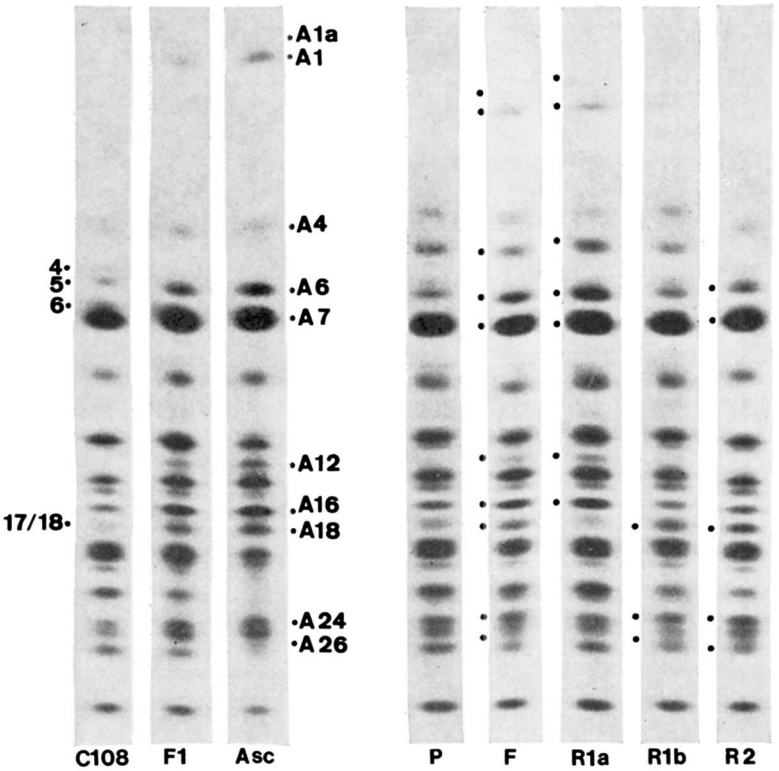

FIGURE 2.—Isoelectric focusing patterns of chorion proteins from progeny of C108 × Ascoli/C108 testcrosses. Numbered bands were scored as discussed in the text (Table 2). Left and right groups of samples were screened on different gels, leading to apparent differences in mobilities of some bands (*e.g.*, A1a, A1, A4).

of Class R2 establishes an approximate distance of 0.4 m.u. between the nearest neighbors of *Ch 1* and *Ch 2*.

We were also able to estimate the map position of the chorion loci from this experiment. The Ascoli markers were originally linked to the dominant gene *Y* (locus 25.6), and the C108 markers to its wild-type allele. We obtained a crossover frequency of 21% between *Y* and the rightmost chorion genes, as defined by Class F and Class P phenotypes (Table 3a). The rightmost known marker on

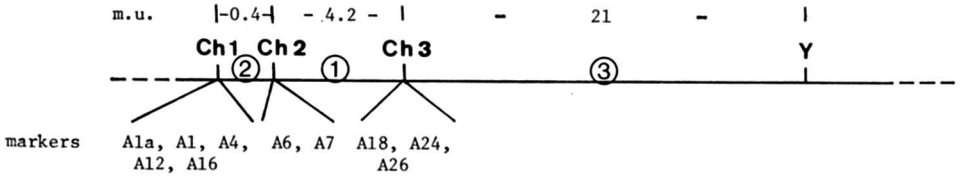

FIGURE 3.—Diagram showing the relative map positions of three chorion complexes, derived from the data in Tables 2 and 3. The circled numbers give relative locations of crossovers described in the text.

TABLE 3

Recombination between Y *and chorion markers*

a. Parental chorion classes

Larval phenotype	Number of progeny Class F	Class P	Total
$+^Y$	34	92	211
Y	74	11	

Chorion-*Y* recombination frequency = (34+11)/211 = 0.21

b. Recombinant chorion classes

Larval phenotype	Number of progeny R1a	R1b	R2	Total
$+^Y$	4	2	1	9
Y	0	3	0	

chromosome *2* is *Rc* at 31.8 m.u. (CHIKUSHI 1972). This suggests that the chorion genes are near the origin of the second chromosome. The relative linkage of Class R1a to $+^Y$ and R1b to *Y* after undergoing crossing over allowed us to predict the chorion gene order from the origin as *Ch 1–Ch 2–Ch 3*. We infer that two of the Class R1b recombinants and the Class R2 recombinant underwent additional crossovers between *Ch 3* and *Y* (Figure 3, crossover 3), since they expressed all the markers of *Ch 3* and were phenotypically $+^Y$. This is consistent with the expected number of double crossovers (21% of single crossover progeny, or two).

Testcross of Ascoli vs. *Hiko markers*: These results were confirmed in a second series of crosses made between Ascoli/Hiko males and Hiko females. In all we recovered a total of 37 Class F, 46 Class P, and two Class R offspring, yielding a recombination frequency of 2.4 m.u. (Table 4). Class F was characterized by the presence of six of the Ascoli markers observed in the previous crosses (bands A1, A4, A6, A18, A24 and A26, Figure 4). Class P was either lacking these bands or expressed them at low intensity. The single recombinant class produced only three of the Ascoli markers (bands A18, A24, and A26); we did not observe the reciprocal recombinant in this experiment.

The phenotype and relatively high frequency of the recombinant class suggest that it corresponds to Class R1b of the testcross of Ascoli/C108 with C108.

TABLE 4

Segregation of chorion markers in Ascoli vs. *Hiko testcrosses*

Progeny class	Markers	No.
F	**A1**, A4, A6, A18, **A24**, **A26**	37
P	all class F markers missing or in low intensity	46
R	A18, **A24**, **A26**	2
Total		85

Frequency of R = 2/85 = 0.024.

The data was obtained from a single pair mating. Markers listed in boldface are qualitative and in plain type are quantitative.

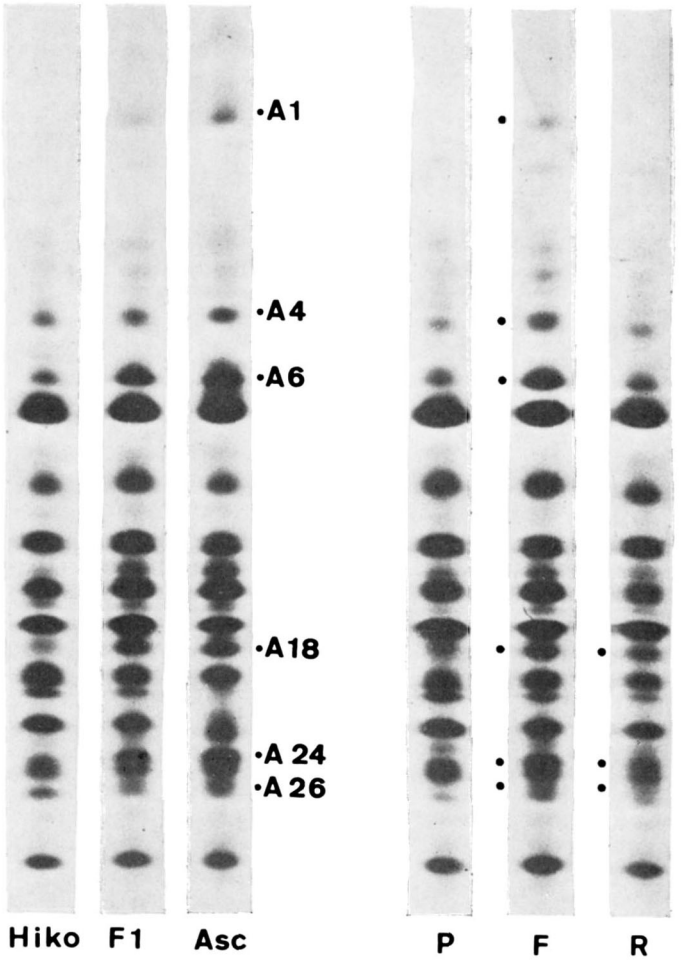

FIGURE 4.—Isoelectric focusing patterns of chorion proteins from progeny of Hiko × Ascoli/Hiko testcrosses. Numbered bands were scored as discussed in the text (Table 4).

Averaging the data from the two crosses sets the distance between the closest markers of *Ch 2* and *Ch 3* at 3.3 ± 0.9 m.u. It was not possible to establish the linkage to *Y* in these crosses because both Hiko and Ascoli stocks carry the dominant allele.

Localization near the origin: The proximity of chorion loci *Ch 1*, *Ch 2*, and *Ch 3* to one another allowed us to confirm their localization on the left end of chromosome *2*. Essentially, we constructed a three-point cross, treating alternate sets of chorion variants as single genetic loci, and mapping them in relation to outside markers at the origin (*p* vs. p^3 larval skin pattern) and at *Y*. We attempted to include a fourth locus, *Gr*(6.9), which affects chorion morphology and protein composition (NADEL 1977; unpublished observations). However, the chosen allele, Gr^B, showed approximately 50% penetrance in controls and

thus could not be scored conclusively in the progeny. The strategy of the cross was similar to that of previous experiments, except that we used a third homozygous stock for the testcross female instead of one of the parents used to construct the F_1. Thus P22/703B males (p^s $+^{Gr}$ $+^{Y}$[P22]/p Gr^B Y[703B], $p^s > p$) were testcrossed to homozygous recessive C108 females (p $+^{Gr}$ $+^{Y}$[C108]/p $+^{Gr}$ $+^{Y}$[C108]). This allowed us to score recombinants in the distal markers in late larval instars and select primarily p-Y recombinant females for rearing to adulthood. Such an approach significantly reduced the labor of growing the larvae and screening chorion patterns.

In the absence of recombination between chorion loci, we expected to find two chorion phenotypes in the progeny, one corresponding to P22/C108 (Class P/C) and the other to 703B/C108 (Class B/C; Figure 5). The intensity of linkage of P22 and 703B markers to their respective p and Y alleles would reflect their relative location on the left or right end of chromosome *2*.

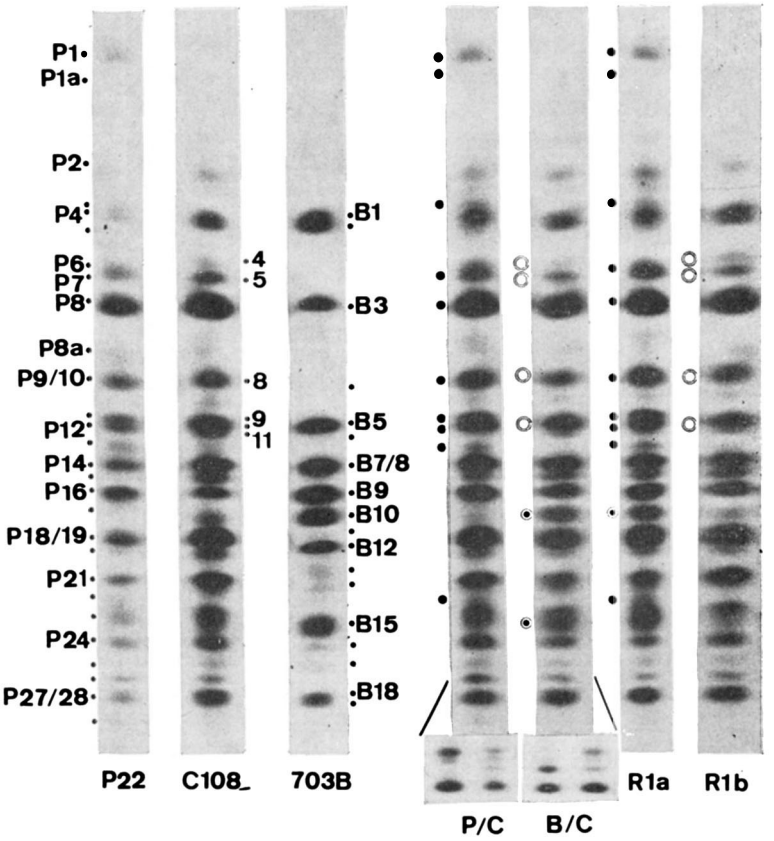

FIGURE 5.—Isoelectric focusing patterns of chorion proteins from progeny of C108 × P22/703B testcrosses. Standard band positions are given for homozygous stocks P22 and 703B. All other patterns are labeled with markers scored as discussed in the text (Table 5). Inset: resolution of heterogeneity in acidic proteins in a separate gel run. Symbols: closed circles, P22 markers; enclosed dots, 703B markers; open circles, C108 markers.

To establish nonrecombinant or parental chorion phenotypes, we screened ten $p^s +^Y$ and 21 p Y progeny from two matings. The $p^s +^Y$ offspring yielded the predicted Class P/C phenotype, characterized by ten markers originating in stock P22 (bands P1, P1a, P4, P7, P8, P9/10, P11, P12, P13 and P22), of which four were qualitatively unique (bands P1, P1a, P11, and P13; Figure 5, Table 5). The pY offspring produced the class B/C phenotype, recognized by the absence of the P22 markers. This uncovered four C108 bands (4,5,8, and 9/11), which was expected because the homozygous Gr^B phenotype has a markedly reduced spectrum of chorion proteins. All of the p Y progeny screened from cross one (12 samples) also expressed two 703B markers, bands B10 and B15. However, none of the p Y progeny from cross two produced these proteins (nine tested; data not shown). The simplest explanation for this result is that the genes for B10 and B15 had been heterozygous in the original 703B parent and were inherited only by the F_1 male used in cross one. Nevertheless, we are unable to rule out a second possibility: that there is a modifier present in the C108 stock that either suppresses the expression of B10 and B15 or alters their electrophoretic mobility so that they are no longer observable at their usual pI, and thus comigrate with other major bands on the gel. In the latter case, we must postulate that only the C108 female from cross two carried the suppressor or modifier gene.

We also observed heterogeneity in a number of acidic proteins, each parental class segregating into two phenotypic subclasses (Figure 5, inset). We believe that this was caused by heterozygosity previously observed in C108 markers that migrate in this region of the gel (GOLDSMITH and BASEHOAR 1978). We grouped both phenotypes in scoring parental classes.

The results are summarized in Table 6. We recovered 49 p-Y crossover progeny (18.6%), of which 45 expressed either P/C or B/C phenotypes in approximately equal frequency. This indicated that none of these crossovers had occurred between chorion markers. Further, the chorion genes remained more tightly linked to the p than to the Y locus, with six individuals (12.2%) undergoing recombination between p and the complete set of chorion markers (Figure 6, cross 1). This places the leftmost chorion genes tested in this experiment at a minimum of 2.3 m.u. from the origin.

TABLE 5

Segregation of chorion markers in C108 × P22/703B testcross progeny

Progeny class	Markers
P/C	**P1**, **P1a**, P4, P7, P8, P9/10, **P11**, [P12], **P13**, P22
B/C*	B10, [B15], **4**, [5], 8, [9/11]
R1a	**P1**, **P1a**, P4, P7, P8, P9/10, **P11**, [P12], **P13**, P22, B10, [B15]
R1b*	**4**, [5], 8, [9/11]

The data was pooled from two pair matings. Markers listed in boldface are qualitative, in plain type are quantitative, and in brackets are distinguishable by morphology.

* Markers B10 and B15 were missing from the B/C progeny of one cross; consequently, none of the progeny from this cross were scored as class R1b recombinants.

TABLE 6

Recombination between p *and chorion genes*

Larval phenotype	B/C	P/C	Number of progeny R1a	R1b	Total
$p+^{Y}$	21	2	0	3	49
$p^{3}\,Y$	4	18	1	0	

Frequency of recombination: *p—Y** = 90/484 = 0.186; *p—ch*† = 6/49 × 0.186 = 0.023; *p—B10*‡ 4/24 × 0.186 = 0.031.

Data are pooled from two crosses except where indicated.
* Data not shown.
† *ch* refers to the chorion marker nearest to *p*.
‡ Data taken only from cross 1 (see text).

Among the 24 *p-Y* crossover progeny of cross one, we identified four chorion recombinants that fell into reciprocal classes. Class R1a resembled the P/C phenotype, but expressed the additional 703B marker, B10 (one case; Figure 5); Class R1b resembled the B/C phenotype but lacked B10 and B15 (three cases). We were unable to score marker B15 in the R1a sample with certainty. The occurrence of the $p^{3}\,Y$ (P22–B10) recombinant implies that none of the P22 markers are allelic to B10 and, further, that B10 must be to the right of the P22 genes (Figure 6, crossover 2). The minimum genetic distance of the B10 locus from the origin is equal to the total frequency of the Class R1a and R1b recombinants, or 3.1 m.u. This places the B10 locus approximately 0.8 m.u. to the right of the other chorion genes tested in this cross.

DISCUSSION

We have shown that there is a minimum of three chorion clusters near the left end of chromosome *2*. Whether the genes are tandemly linked within each cluster, analogous to the histone genes (KEDES 1976; LIFTON *et al.* 1978), or

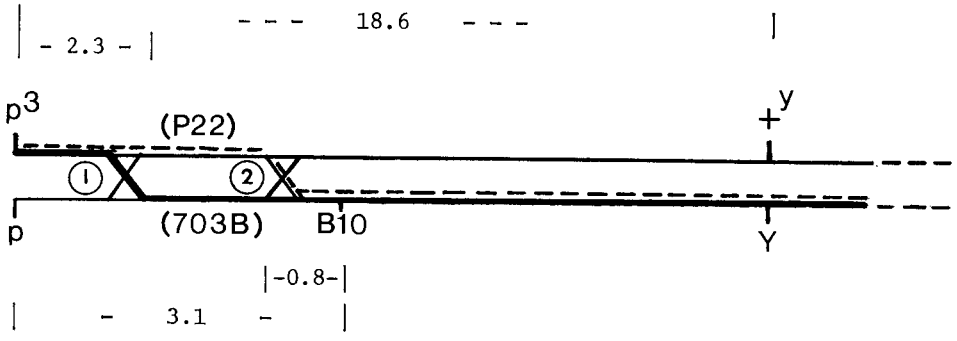

FIGURE 6.—Diagram showing the relative map positions of chorion markers, derived from the data in Tables 5 and 6. Parentheses are used to indicate the boundaries of the chorion markers found in stocks P22 and 703B. The circled numbers give relative locations of crossovers described in the text. Other numbers refer to approximate map distances in m.u.

separated by long stretches of DNA coding for nonchorion proteins cannot be determined from our experiments, which are limited to a sensitivity in the range of one map unit. Fine-structure analysis at a level that would resolve this question is now possible with recombinant-DNA techniques. We are presently carrying out such experiments.

The exact number and localization of chorion complexes in relation to the outside markers *p* and *Y* are still under investigation. In the absence of functional or structural tests for allelism, we cannot be certain that the P22 and 703B markers used for mapping chorion genes relative to the origin correspond to any of the Ascoli markers used to define *Ch 1*, *2* and *3*. Although the recombination frequencies are suggestive, the mapping error is large enough to prevent a comparison of the exact values obtained from different kinds of crosses. Thus, it is possible that some of the markers from stocks P22 and 703B are situated in new chorion clusters. We are answering this question directly by examining the linkage of Ascoli, P22 and 703B genes with respect to one another.

We used the C108-Ascoli strain combination to specify chorion clusters because it produced a high yield of recombinants. This is probably not caused by a high level of recombination, but rather due to the sensitivity of the isoelectric focusing gel assay for this particular combination of chorion markers. On the other hand, the yield of recombinants in the cross C108 × P22/703B may have been reduced by the presence of the Gr^B gene. Gr^B is *cis*-dominant, and the homozygote produces a chorion lacking approximately 60% of the normal number of chorion components (NADEL 1977). It is not known whether the Gr^B phenotype is caused by a deletion of several chorion structural genes or by the mutation of a regulatory gene. Our finding of a low *p-Y* recombination frequency of 18.6% relative to the standard value of 25.6% (CHIKUSHI 1972) may reflect the presence of a large deletion in this region. However, if Gr^B is a mutant regulatory site preventing the expression of adjacent chorion structural genes, then recombinants that exchanged P22 markers for their 703B alleles might be indistinguishable from the 703B phenotype. Either mechanism could account for our failure to recover any recombinants among the ten P22 markers visible in the P/C chorions. Nevertheless, we have not eliminated other explanations such as the possibility that the P22 markers belong to a single tightly linked cluster within which crossing over is rare.

In the light of the pleiotropic effect of Gr^B and other *Gr* mutations on chorion gene expression, (NADEL 1977; SCHMIDT and GOLDSMITH, in preparation), it is of great interest to establish the exact chromosomal relationship between *Gr* and the chorion loci. Given the *Ch 3-Y* crossover value of 21 m.u. and the standard map position of *Y* as 25.6 m.u. (CHIKUSHI 1972), we estimate that the *Ch 3* locus is at approximately 4.6 m.u. B10 maps at a similar location with respect to *p*, namely 4.1 m.u. Both are somewhat distal to *Gr*, whose reported position is 6.9 m.u. (CHIKUSHI 1972). Nevertheless, we feel that only a direct test of the localization of *Gr vs.* specific chorion markers will give an unequivocal answer to this question. We are presently carrying out these experiments.

An assumption of these studies is that the recombinant phenotypes reflect

true genetic crossing over. The similarity of recombination frequencies in three different crosses, the detection of two classes of reciprocal recombinants, the increase in the yield of recombinants in the progeny selected for crossing over between the outside markers *p* and *Y*, and the ability to construct a linear gene map reinforce the likelihood of this assumption. A final proof would be provided by the demonstration that the recombinants breed true. We were unable to carry out this test because most of the progeny were not bred for further study. However, we have observed transmission of a recombinant chorion pattern in crosses not reported here. We are presently repeating the C108-Ascoli experiments to examine the inheritance of the recombinant phenotypes. By using appropriate stocks for progeny testing, we will also be able to investigate the linkage of the untested C108 markers.

Our finding that groups of co-segregating chorion markers can be separated by genetic recombination lends strong support to the idea that the observed electrophoretic differences in chorion proteins originating in different inbred strains are encoded at the structural gene level, not caused by post-translational modification. Thus, it would be necessary to postulate a minimum of two or three linked modifiers to account for the results reported here. This seems unlikely, particularly in view of our observation that all chorion electrophoretic variants thus far examined in true-breeding strains show co-dominant inheritance in F_1 hybrids (GOLDSMITH and BASEHOAR 1978; GOLDSMITH and CLERMONT-RATTNER, in preparation). This is consistent with other evidence for a multiplicity of distinct chorion structural genes, including amino acid composition (KAFATOS *et al.* 1977) and protein sequencing (REGIER *et al.* 1978; RODAKIS 1978) studies, and sequencing of cloned cDNA's derived from chorion mRNA (KAFATOS *et al.* 1978). Nevertheless, we still cannot rule out the possibility that some apparent co-segregation of chorion markers is due to secondary processing of common precursor molecules.

The chorion system appears to have features of both dispersed and clustered multigene families. Although the extent of dispersion found thus far is an order of magnitude less than the 30 to 35 chromosomal sites occupied by the *412* and copia genes (FINNEGAN *et al.* 1978), the evolutionary basis for failing to maintain a single cluster may be the same. Further comparisons are difficult to make until more is known about the copia and *412* gene families and until we have localized a greater number of chorion genes. The segregation of chorion genes into separate complexes also resembles the organization of the mammalian κ, λ and H antibody genes into three clusters. The latter are unlinked, which may reflect relatively longer evolutionary time since their separation (HOOD 1976). In the next paper of this series we will report the gene composition of the chorion clusters, each of which contains representatives of two or more families (GOLDSMITH and CLERMONT-RATTNER, in preparation). This suggests that the evolutionary sequence of the divergence and physical separation of the chorion genes may have also differed from that of the antibody genes.

We acknowledge the expert secretarial assistance of P. DUNCAN. This work was supported by National Science Foundation Grant PCM 76–82450 to M. R. GOLDSMITH.

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Corresponding editor: G. LEFEVRE