On the Topography of the Genetic Fine Structure

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ON THE TOPOGRAPHY OF THE GENETIC FINE STRUCTURE

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In an earlier paper,[1] a detailed examination was made of the structure of a small portion of the genetic map of phage T4, the *r*II region. This region, which controls the ability of the phage to grow in *Escherichia coli* strain K, consists of two adjacent cistrons, or functional units. Various *r*II mutants, unable to grow in strain K, have mutations affecting various parts of either or both of these cistrons. The topology of the region; i.e., the manner in which its parts are interconnected, was intensively tested and it was found that the active structure can be described as a string of subelements, a mutation constituting an alteration of a point or segment of the linear array.

This paper is a sequel in which inquiry is made into the topography of the structure, i.e., local differences in the properties of its parts. Specifically, are all the subelements equally mutable? If so, mutations should occur at random throughout the structure and the topography would be trivial. On the other hand, sites or regions of unusually high or low mutability would be interesting topographic features.

The preceding investigation of topology was done by choosing mutants showing no detectable tendency to revert. This avoided any possible confusion between recombination and reverse mutation, so that a qualitative (yes-or-no) test for re-

combination was possible. The class of non-reverting mutants automatically included those marked by relatively large alterations, which will be referred to as "deletions." Such a mutant is defined for the present purposes as one which inter-

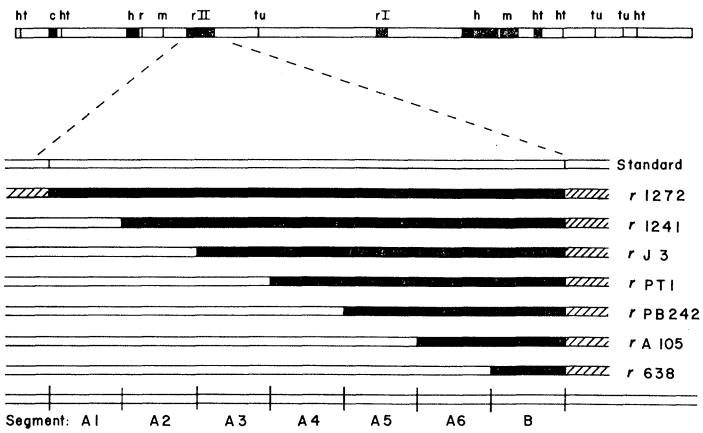


FIG. 1*a*.—At the top, the *r*II region is shown compared with the entire genetic map of the phage. This map is a composite[15] of markers mapped in T4 and the related phage T2. Seven segments of the *r*II region are defined by a set of "deletions" beginning at different points and extending to the right-hand end (and possibly beyond, as indicated by shading).

sects (fails to give recombination with) two or more mutants that do recombine with each other. Deletions provided overlaps of the sort needed to test the topology and to divide the map into segments.

The present investigation of topography, however, is concerned with differentiation of the various points in the structure. For this purpose mutants which do revert are of the greater interest, since they are most likely to contain small alterations. As a rule (there are exceptions) an *r*II mutant that reverts behaves as if its alteration were localized to a point. That is to say, mutants that intersect with the same mutant also intersect with each other. In a cross, recombination can be scored only if it is clearly detectable above the spontaneous reversion noise of the mutants involved. Therefore, the precision with which a mutation can be mapped is limited by its reversion rate. The detailed analysis of topography can best be done with mutants having low, non-zero reversion rates.

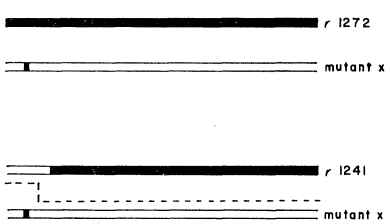


FIG. 1*b*.—Mapping a mutation by use of the reference deletions. If mutant x has a mutation in segment 1, it is overlapped by *r*1272, but not by *r*1241. Therefore, standard-type recombinants (as indicated by the dotted line) can only arise when x is crossed with *r*1241.

Some thousands of such *r*II mutants, both spontaneous and induced, have been analyzed and the resultant topographic map is presented here.

Assignment of Mutations to Segments.—To test thousands of mutants against one another for recombination in all possible pairs would require millions of crosses. This task may be greatly reduced by making use of deletions. Each mutant is first tested against a few key deletions. The recombination test gives a negative result

if a deletion overlaps the mutation in question and a positive result if it does not overlap. These results quickly locate a mutation within a particular segment of the map. It is then necessary to test against each other only the group of mutants having mutations within each segment, so that the number of tests needed is much smaller. In addition, if the order of the segments is known, the entire set of point mutations becomes ordered to a high degree, making use of only qualitative tests.

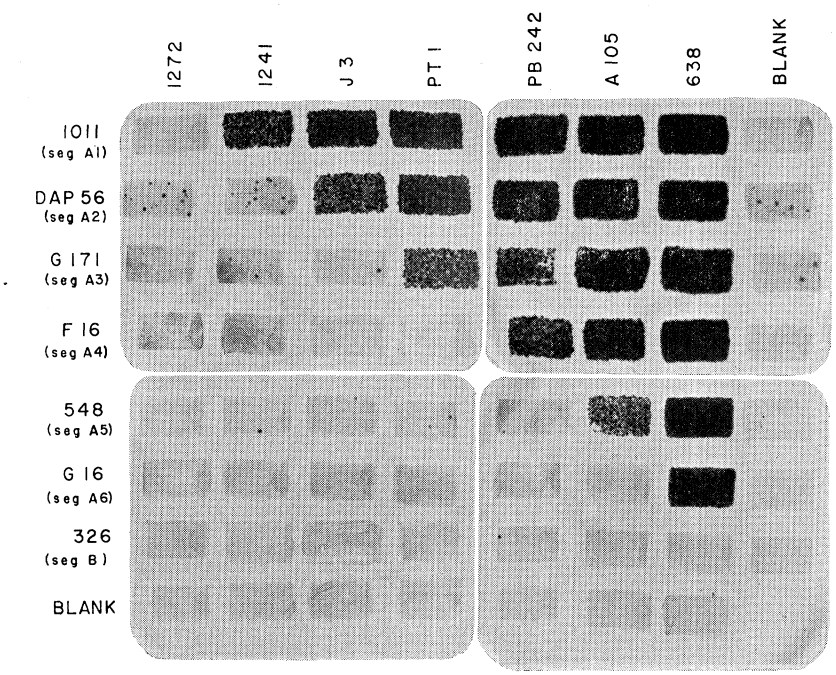


FIG. 2.—Crosses for mapping *r*II mutations. The photograph is a composite of four plates. Each row shows a given mutant tested against the reference deletions of Figure 1*a*. Plaques appearing in the blanks are due to revertants present in the mutant stock. The results show each of these mutations to be located in a different segment.

Procedure for crosses—The broth medium is 1% Difco bacto-tryptone plus 0.5% NaCl. For plating, broth is solidified with 1.2% agar for the bottom layer and 0.7% for the top layer. Stocks are grown in broth using *E. coli* BB which does not discriminate between *r*II mutants and the standard type. To cross two mutants, one drop of each at a titer of about 10^9 phage particles/ml is placed in a tube and cells of *E. coli* B are added (roughly 0.5 ml of a 1-hour broth culture containing about 2×10^8 cells/ml). The *r*II mutants are all able to grow on strain B and have an opportunity to undergo genetic recombination. After allowing a few minutes for adsorption, a droplet of the mixture is spotted (using a sterile paper strip) on a plate previously seeded with *E. coli* K. If the mutants recombine to produce standard type progeny, plaques appear on K. A negative result signifies that the proportion of recombinants is less than about 10^{-3}% of the progeny.

Within any one segment, however, the order of the various sites remains undetermined. This order can still be determined, if desired, by quantitative measurements of recombination frequencies.

In order to facilitate this project many more deletions have been mapped than were described in the previous paper. These suffice to carve up the structure into 47 distinct segments. By virtue of the proper overlaps, the order of almost all of

these segments is established. Observe first the seven large mutations in Figure 1*a*. These are of a kind which begin at a particular point and extend all the way to one end. Thus, they serve to divide the structure into the seven major segments shown.

Consider a small mutation located in the segment Al, as indicated in Figure 1*b*. It is overlapped by *r*1272 and therefore when crossed with it cannot give rise to standard type recombinants. It will, however, give a positive result with *r*1241 or any of the others, since, with them, recombinants can form as indicated by the

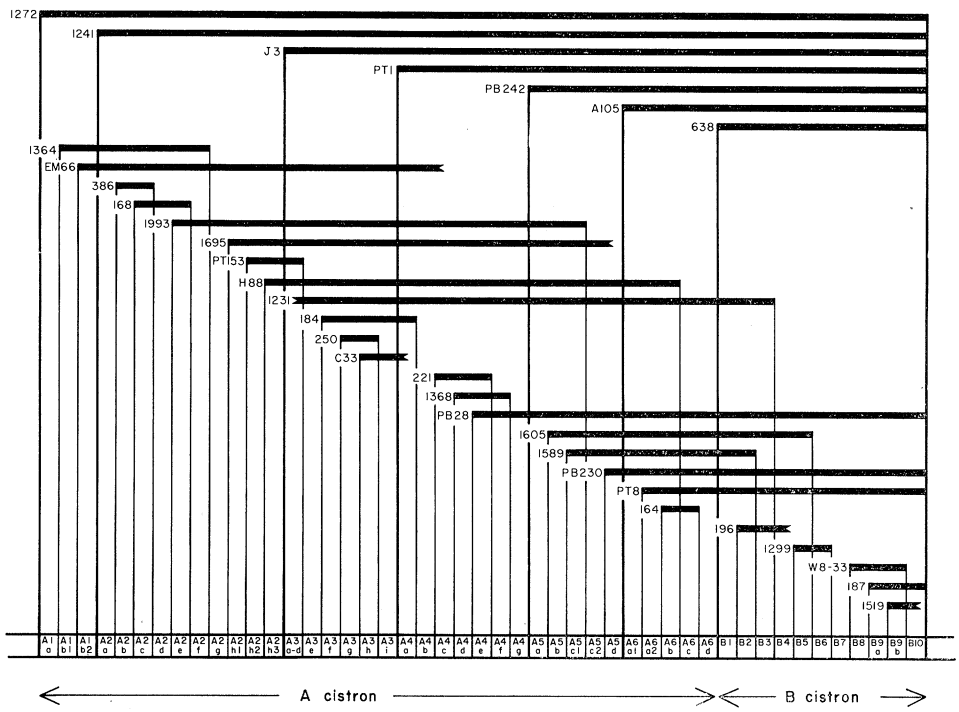


FIG. 3.—Deletions used to divide the main segments of Figure 1 into 47 smaller segments. (Some ends have not been used to define a segment, and are drawn fluted.) The A and B cistrons, which are defined by an independent functional test, coincide with the indicated portions of the recombination map. Most of the mutants are of spontaneous origin. Possible exceptions are EM66, which was found in a stock treated with ethyl methane sulfonate, and the PT and PB mutants, which were obtained from stocks treated with heat at low pH. The PT mutants were contributed by Dr. E. Freese.

dotted line. A point mutation located in the second segment will give zero with mutants *r*1272 and *r*1241 but not with the rest, and so on. Thus, if any point mutant is tested against the set of seven reference mutants in order, the segment in which its mutation belongs is established simply by counting the number of zeros. Figure 2 shows photographs of the test plates for seven mutants, each having its mutation located in a different segment.

Only these seven patterns, with an uninterrupted row of zeros beginning from the left, have ever been observed for thousands of mutants tested against these seven deletions. The complete exclusion of the other 121 possible patterns confirms the linear order of the segments.

Now a given segment can be further subdivided by means of other mutations having suitable starting or ending points. Figure 3 shows the set used in this study and the designation of each segment. Each mutant is first tested against the seven which have been chosen to define main segments. Once the main segment is known,

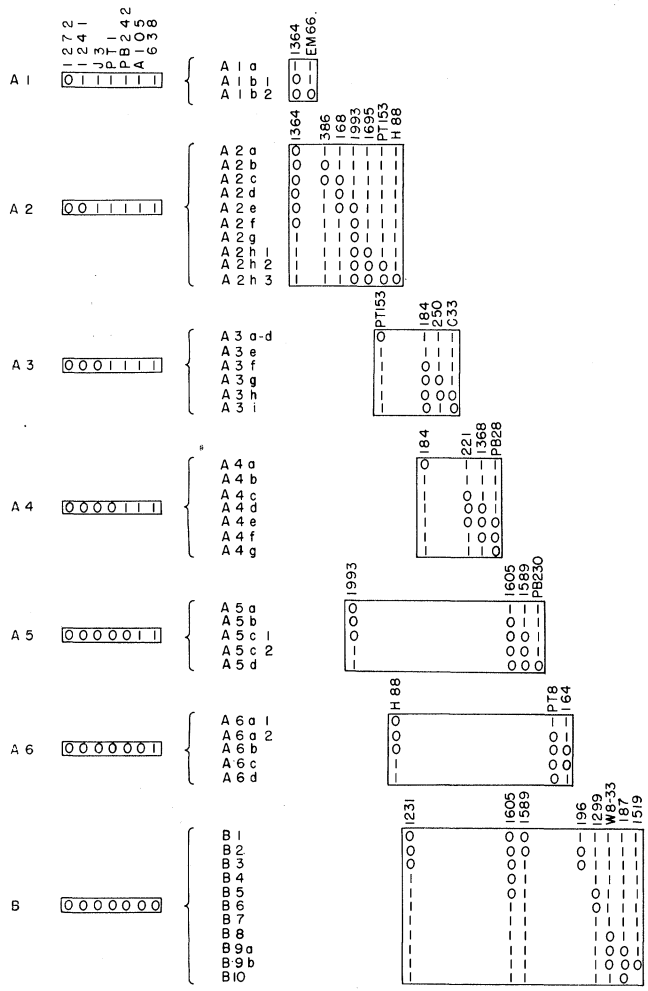


FIG. 4.—The test pattern which identifies the location of a point mutation in each of the segments of Figure 3. The test is done in two stages. An unknown mutant is first crossed with the "big seven" of Figure 1 in order. *Zero* signifies no detectable recombination and *one* signifies some, and the number of zeros defines the major segment. Once this is known, the mutant is crossed with the pertinent selected group of deletions to determine the small segment to which it belongs.

the mutant is tested against the appropriate secondary set. Figure 4 shows the pattern which identifies the location of a point mutation within each of the small segments. Thus, in two steps, a point mutation is mapped into one of the 47 segments.

The order of the first 42 segments, Ala through B6, is uniquely defined. Unfortunately, there remains a gap between $r1299$ and rW8-33. Therefore the order of segments B8 through B10, although fixed among themselves, could possibly be the reverse of that shown.[17] Also if there exists space to the right of segment B10, a mutation in that segment might map as if it were in segment B7, so that the latter segment must be tentatively regarded as a composite.

In the previous topology paper, the possibility that the structure contains branches was not eliminated. As pointed out by Delbrück, the existence of a branch would not lead to any contradiction with a linear topology if loss of a segment containing the branch point automatically led to loss of the entire branch.

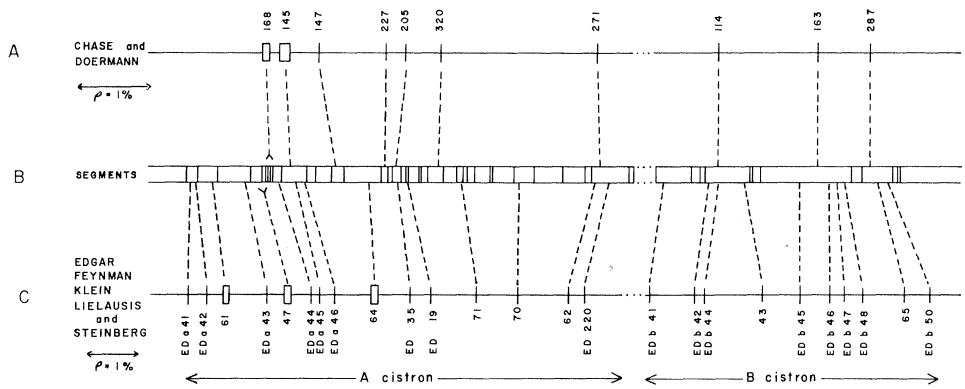


FIG. 5.—Correlation of the results of deletion mapping with the conventional method. *A:* The map constructed by Chase and Doermann[2] for ten rII mutants of phage T4B, using quantitative measurements of recombination frequency. The interval between adjacent mutations is drawn proportional to the frequency of recombination in a cross between the two. *C:* The map constructed in similar fashion by Edgar *et al.* (personal communication) with rII mutants of the very closely related phage T4D. The procedure used by Edgar *et al.* gives higher recombination frequencies. Therefore, the scales of the two maps are adjusted in the figure to produce a good over-all fit. Some of the mutations cover several sites and are drawn as having a corresponding length. A gap is left between the two cistrons because crosses between mutations in different cistrons give abnormally high frequencies due to the role of heterozygotes[16].

All of these mutations have also been mapped by the deletion method, and dotted lines indicate their locations in the various segments (*B*). The length of each segment is drawn in proportion to the number of distinct sites that have been found within it.

To show that a given segment is *not* a branch, it is required to find a mutation which penetrates it partially. From the mutations shown in Figure 3, it can be concluded that no branch exists that contains more than one of the 47 segments.

Comparison of Deletion Mapping by Recombination Frequencies.—The conventional method of genetic mapping makes use of recombination frequency as a measure of the distance between two mutations and requires careful quantitative measurements of the percentage of recombinant type progeny in each cross. By the method of overlapping deletions the order of mutations can be determined entirely by qualitative yes-or-no spot tests. Maps obtained independently by the two methods are compared in Figure 5. The upper part of the figure (*A*) shows the order obtained by Chase and Doermann[2] for a set of ten mutants, the distance between adjacent mutations being drawn proportional to the percentage of standard-type recombinants occurring among the progeny of a cross between the two. The central part of the figure (*B*) shows the rII region divided into the segments of

Figure 3, with the size of each segment drawn in proportion to the number of distinct sites which have been discovered within it (see below). As indicated by the dotted lines, there is perfect correlation in the order. In the lower part of the figure, a similar comparison is made for a set of *r*II mutations in the closely related phage strain T4D, which have been mapped, using recombination frequencies, by Edgar, Feynman, Klein, Lielausis, and Steinberg. Again the order agrees perfectly with that obtained by the use of deletions.

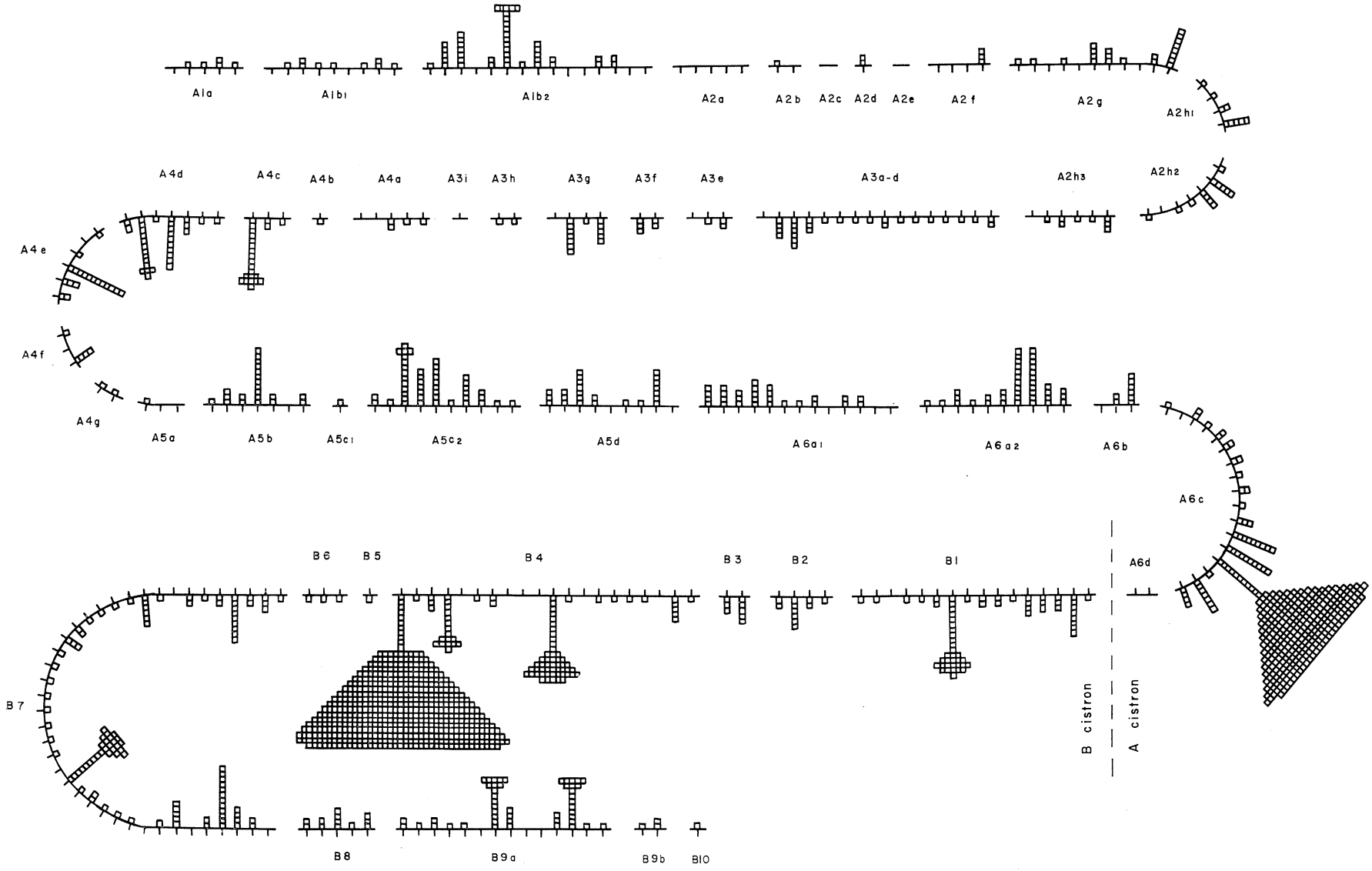
Topography for Spontaneous Mutations.—We now proceed to map reverting mutants of T4B which have arisen independently and spontaneously. The procedure is exactly as in Figure 4: first localizing into main segments, then into smaller segments. Finally mutants of the same small segment are tested against each other. Any which show recombination are said to define different sites. If two or more reverting mutants are found to show no detectable recombination with each other, they are considered to be repeats and one of them is chosen to represent the site in further tests. A set of distinct sites is thus obtained, each with its own group of repeats.

This procedure is based on the assumption that revertibility implies a point mutation. While this is a good working rule for *r*II mutants, a few exceptions have been found which appear to revert (i.e., give rise to some progeny which can produce detectable plaques on stain K) yet fail to give recombination with two or more mutants that do recombine with each other. If a mutant chosen to represent a "site" happens to be of this kind, mutations it overlaps will appear to be at the same site. Therefore, a group of "repeats" remains subject to splitting into different groups when they are tested against each other. This has not yet been done for all of the sites described here. It is, of course, in the nature of the recombination test that it is meaningful to say that two mutations are at different sites, while the converse conclusion is always tentative.

Figure 6 shows the map obtained for spontaneous mutants, with each occurrence of a mutation at a site indicated by a square. Within each segment the sites are drawn in arbitrary order. Other known sites are also indicated even though no occurrences were observed among this set of spontaneous mutants.

That the distribution is non-random leaps to the eye. More than 500 mutations have been observed at the most prominent "hotspot," while, at the other extreme, there are many sites at which only a single occurrence, or none, has so far been found.

To decide whether a given number of recurrences is significantly greater than random, the data may be compared with the expectation from a Poisson distribution. Figure 7 shows a distribution calculated to fit the least hot of the observed spontaneous sites, i.e., those at which one or two mutations have occurred, on the assumption that these sites belong to a uniform class of sites of low mutability. Comparing the observations with this curve, it would seem that if a site has four occurrences, there is a two-thirds probability that it is truly hotter than the class of sites of low mutability. Those having five or more are almost certainly hot. It can be concluded that at least sixty sites belong in a more mutable class than the coolest spots. Whether the hot sites can be divided into smaller homogeneous groups, assuming a Poisson distribution within each class, is difficult to say. Each of the two hottest sites is obviously unique.


A1a
A1b1
A1b2
A2a
A2b
A2c
A2d
A2e
A2f
A2g
A2h1
A4d
A4c
A4b
A4a
A3i
A3h
A3g
A3f
A3e
A3a-d
A2h3
A2h2
A4e
A4f
A4g
A5a
A5b
A5c1
A5c2
A5d
A6a1
A6a2
A6b
A6c
A6d
B6
B5
B4
B3
B2
B1
B7
B8
B9a
B9b
B10
B cistron
A cistron

From the distribution it can be predicted that there must exist at least 129 spontaneous sites not observed in this set of mutants. This is a minimum estimate since it is calculated on the assumption that the 2-occurrence sites are no more mutable than the 1-occurrence sites. If this is not correct, the predicted number of

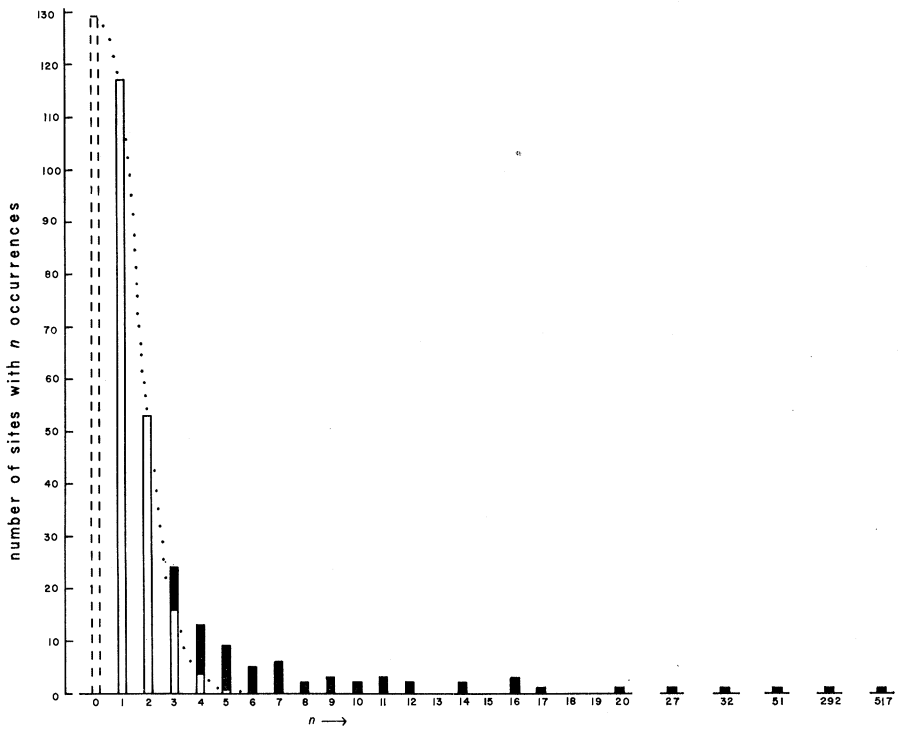


FIG. 7.—Distribution of occurrences of spontaneous mutations at various sites. The dotted line indicates a Poisson distribution fitted to the numbers of sites having one and two occurrences. This predicts a minimum estimate for the number of sites of comparable mutability that have zero occurrences due to chance (dashed column at n = 0). Solid bars indicate the minimum numbers of sites which have mutation rates significantly higher than the one- and two-occurrence class.

0-occurrence sites will be larger. Also, of course, there could exist a vast class of sites of much lower mutability. With 251 spontaneous sites identified and at least 129 more to be found, the degree of saturation of the map achieved with this set of 1,612 spontaneous mutants can be no greater than 66 per cent.

←

FIG. 6.—Topographic map of the *r*II region for spontaneous mutations. Each square represents one occurrence observed at the indicated site. Sites with no occurrences indicated are known to exist from induced mutations and from a few other selected spontaneous ones. The order of the segments is known for A1a through B7, but is only tentative for B7 through B10.[17] The arrangement of sites within each segment is arbitrary.

Each mutant arose independently in a plaque of either standard-type T4B or, in somewhat less than half of the cases, revertants of various *r*II mutants. All revertants (except F) gave results very similar to T4B. The pattern for *r*II mutants isolated from revertant F differs noticeably only in a reduced rate at the hotspot 117 (the site of its original *r*II mutation) and therefore does not significantly alter the topography. All the data for mutants isolated from standard type and from revertants are pooled in this figure.

Topography for Induced Mutations.—By the use of specific mutagens, new topographic features are revealed. This has been shown for *r*II mutants induced during reproduction of the phage inside the bacterial host cell with 5-bromouracil (Benzer and Freese[3]), proflavine (Brenner, Barnett, and Benzer[4]), and 2-aminopurine (Freese[5]). Other effective mutagens are 2,6-diaminopurine (Freese[5]) and 5-bromodeoxycytidine (Gregory, personal communication). Mutations may also be induced *in vitro*, i.e., in extracellular phase particles, by ethyl methane sulfonate (Loveless[6]) and nitrous acid (Vielmetter and Wieder;[7] Freese;[8] Tessman[9]).

*r*II mutants induced by all of these mutagens have now been mapped with respect to each other and spontaneous ones, and the results are given in Figure 8 (facing page 416) which shows the locations of over 2,400 induced and spontaneous mutations. Only *r*II mutants that have low reversion rates and are not too "leaky" on K have been included.

Each "spectrum" differs obviously from the spontaneous one. While the specificities of the various mutagens overlap in many respects, each differs significantly from the others at specific points. In making the comparison it must be borne in mind that the total number of mutants mapped is not the same for each mutagen and also that each induced set inevitably includes some proportion of spontaneous mutants. (An upper limit to this background can be set from the number of occurrences at the hottest spontaneous sites.) Also, none of the spectra are "saturated." Therefore, even if two mutagens act similarly upon a given site, it is possible, due to chance, that a few occurrences would be observed in one spectrum and not the other. Within these limitations, the map shows the comparative response at each site to each mutagen as well as the locations of various kinds of hotspots in various segments of the *r*II region.

The study of the induced mutations has added 53 new sites to the 251 identified by the spontaneous set alone, bringing the total to 304. (Four sites more are shown in Figure 8, but they come from a selected group of mutants outside this study.) Thus, a closer approach toward saturation of all the possible sites must have been made. By lumping together all the data, both spontaneous and induced, one can again make an estimate of the number of sites which must be detectable if one were to continue mapping mutants in the same proportion for the same mutagens. The result is that there must exist still a minimum of 120 sites not yet discovered. This appears discouragingly similar to the estimate based on spontaneous mutations alone. However, it need not be surprising if the use of mutagens brings into view some sites which have extremely low spontaneous mutability. With 308 sites identified and at least 120 yet to be found, the maximum degree of saturation of the map is 72 per cent.

Discussion.—One topographic feature, non-random mutability at the various sites, is obvious. Another question is whether mutable sites are distributed at random, or whether there exist portions of the map that are unusually crowded with or devoid of sites. The mapping technique used here defines only the order of sites from one segment to another (but not within a given segment). The distance between sites remains unspecified. However, all mutations in a segment more distal to a given point must be farther away than those in a more proximal segment. If the number of sites in a segment is used as a measure of its length, as in Figure 5, it can be seen that there is no major discrepancy between these distances and those

defined in terms of another measure of distance, recombination frequency. On a gross scale, therefore, there is no evidence for any large portion of the *r*II region that is unusually crowded or roomy with respect to sites. This does not necessarily mean that some other measure of distance would not reveal such regions, since it is at least conceivable that mutable sites coincide with points highly susceptible to recombination. The distribution of sites on a finer scale, within a small segment, remains to be investigated.

The number of points at which mutations can wreck the activity of a cistron is very large. This would be expected if a cistron dictates the formation of a poly peptide chain and "nonsense" mutations[10] are possible which interrupt the completion of the chain. Such mutations would be effective at any point of the structure, whereas ones which lead to "missense," i.e., the substitution of one amino acid for another, might be effective at relatively special points or regions which are crucial in affecting the active site or folding.

It would be of interest to compare the number of genetic sites to the material embodiment of the *r*II region in terms of nucleotides. Unfortunately, the size of the latter is not well known. Estimates based upon its length, in units of recombination frequency compared to the length of the entire genetic structure, are uncertain. A more direct attempt has been made using equilibrium sedimentation in a cesium chloride gradient and looking for a change in density of mutants known by genetic evidence to have portions of the *r*II region deleted (Nomura, Champe, and Benzer, unpublished). This technique has been successful in characterizing defective mutants of phage λ (Weigle, Meselson, and Paigen[11]) and is sufficiently sensitive to detect a decrease of 1 per cent in the amount of DNA per phage particle, but has so far failed with *r*II mutants. Although other explanations are possible, this result may suggest that the physical structure corresponding to the *r*II region represents less than 1 per cent of the total DNA of the phage particle, or less than 2,000 nucleotide pairs. If this is so, the number of possible sites would be of the order of at least one-fifth of the number of nucleotide pairs.

The data show that, if each site is characterized by its spontaneous mutability and response to various mutagens, the sites are of many different kinds. Some response patterns are represented only once in the entire structure. According to the Watson-Crick model[12] for DNA, the structure consists of only two types of elements, adenine-thymine (AT) pairs and guanine-hydroxymethylcytosine (GC) pairs. This does not mean, however, that there can only be two kinds of mutable sites, even if a site corresponds to a single base pair. Considering only base pair substitutions, a given AT pair can undergo three kinds of change: AT can be replaced by GC, CG, or TA. Certain of these changes may lead to a mutant phenotype, but some may not. The frequency of observable mutations at a particular AT pair will be determined by the sum of the probabilities for each type of change, each multiplied by a coefficient (either one or zero) according to whether that specific alteration at that particular pair does or does not represent a mutant type. Thus, if the probability that a base pair will be substituted is independent of its neighbors, the various AT sites may have seven different mutation rates. Similarly, there are seven rates possible for the various GC sites, so that it would be possible to account for fourteen classes by this mechanism. Some of these may have (total) spontaneous mutation rates that are similar. If a mutagen induces only certain substitu-

tions, it will facilitate further discriminations between sites but there should still be no more than fourteen classes.

If one allows for interactions between neighbors, the number of possible classes increases enormously. Such interactions are to be expected. As an example, consider the fact that AT pairs are held together much less strongly than are GC pairs.[13] If several AT pairs occur in succession, this segment of the DNA chain will be relatively loose, making it easier to consummate an illicit base pairing during replication. Thus, guanine and adenine, which make a very satisfactory pair of hydrogen bonds but require a larger than normal separation between the backbones, could be more readily accommodated. This would lead, in the next replication, to a replica in which one of the AT pairs has been substituted by a CG pair, with the orientation of purine and pyrimidine reversed. Thus, a region rich in AT pairs will tend to be more subject to substitution. If the same (standard-type) phenotype can be achieved by alternative sequences, the ones containing long stretches of AT pairs would tend to be lost because of their high mutability. In other words, cistrons ought to have evolved in such a way as to eliminate hotspots. The spontaneous hotspots that are observed would be remnants of an incomplete ironing-out process. In fact, a map of the *r*II region of the related phage T6 (Benzer, unpublished) also shows hotspots at locations corresponding to *r*131 and *r*117. However, while the first of these has a mutability similar to that in T4, the second is lower by a factor of four.

This point is emphasized by the data on reverse mutations. It is not uncommon for an *r*II mutant to have a reverse mutation rate that is greater than the total forward rate observed for the composite of at least 400 sites. That some of these high-rate reverse mutations represent true reversion (and not "suppressor" mutations) has been established in several cases by the most stringent criteria, including the demonstration that the revertant has exactly the same forward mutation rate at the same site as did the original standard type (Benzer, unpublished). It would therefore appear that certain kinds of highly mutable configurations are systematically excluded from the standard form of the *r*II genetic structure, and a mutation may recreate one of these banned sequences.

In the attempt to translate the genetic map into a nucleotide sequence, the detection of the various sites by forward mutation is necessarily the first step. By studies on the specificity of induction of reverse mutations,[14] one site at a time can be analyzed in the hope of identifying the specific bases involved.

Summary.—A small portion of the genetic map of phage T4, the two cistrons of the *rII* region, has been dissected by overlapping "deletions" into 47 segments. If any branch exists, it cannot be larger than one of these segments. The overlapping deletions are used to map point mutations and the map order established by this method is consistent with the order established by the conventional method that makes use of recombination frequencies. Further dissection has led to the identification of 308 distinct sites of widely varied spontaneous and induced mutability. The distributions throughout the region for spontaneous mutations and those induced by various chemical mutagens are compared. Data are included for nitrous acid and ethyl methane sulfonate acting *in vitro*, and 2-aminopurine, 2,6-diaminopurine, 5-bromouracil, 5-bromodeoxycytidine, and proflavine acting *in vivo*. The characteristic hotspots reveal a striking topography.

It is a pleasure to thank Mrs. Karen Sue Supple, Mrs. Joan Reynolds, and Mrs. Lynne Bryant for their indefatigable assistance in mapping mutants and bookkeeping. I am indebted to Dr. Robert S. Edgar and his associates for permission to make use of their unpublished data in Figure 5 and to Dr. Ernst Freese for several deletions as well as mutants induced with 2-aminopurine and 5-bromodeoxyuridine. This research was supported by grants from the National Science Foundation and the National Institutes of Health.

* Given by invitation of the Committee on Arrangements for the Annual Meeting as part of a Symposium on Genetic Determination of Protein Structure, Robley C. Williams, Chairman.

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[3] Benzer, S., and E. Freese, these PROCEEDINGS, **44,** 112 (1958).

[4] Brenner, S., L. Barnett, and S. Benzer, *Nature*, **182,** 983 (1958).

[5] Freese, E., *J. Molec. Biol.*, **1,** 87 (1959).

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[10] Crick, F. H. C., J. S. Griffith, and L. E. Orgel, these PROCEEDINGS, **43,** 416 (1957).

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[14] Freese, E., these PROCEEDINGS, **45,** 622 (1959).

[15] Brenner, S., in *Advances in Virus Research* (New York: Academic Press, 1959), pp. 137–158.

[16] Edgar, R. S., *Genetics*, **43,** 235 (1958).

[17] The terms topology and topography are used here in the following senses (Webster's New Collegiate Dictionary, 1959)—*topology:* the doctrine of those properties of a figure unaffected by any deformation without tearing or joining; *topography:* the art or practice of graphic and exact delineation in minute detail, usually on maps or charts, of the physical features of any place or region.

[18] *Note added in proof.* Recent data have established that the orientation shown for segments B8 through B10 is the correct one.

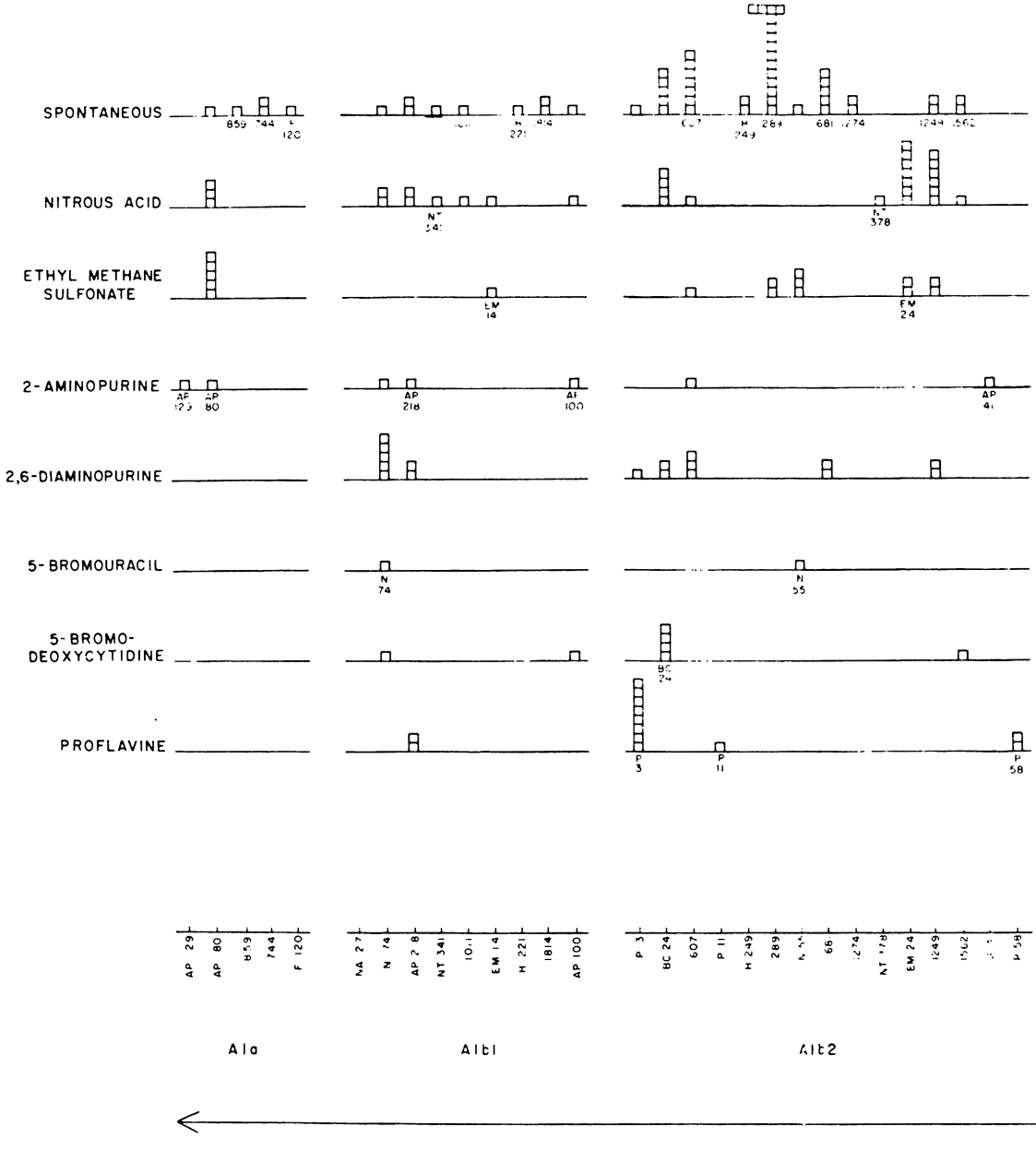
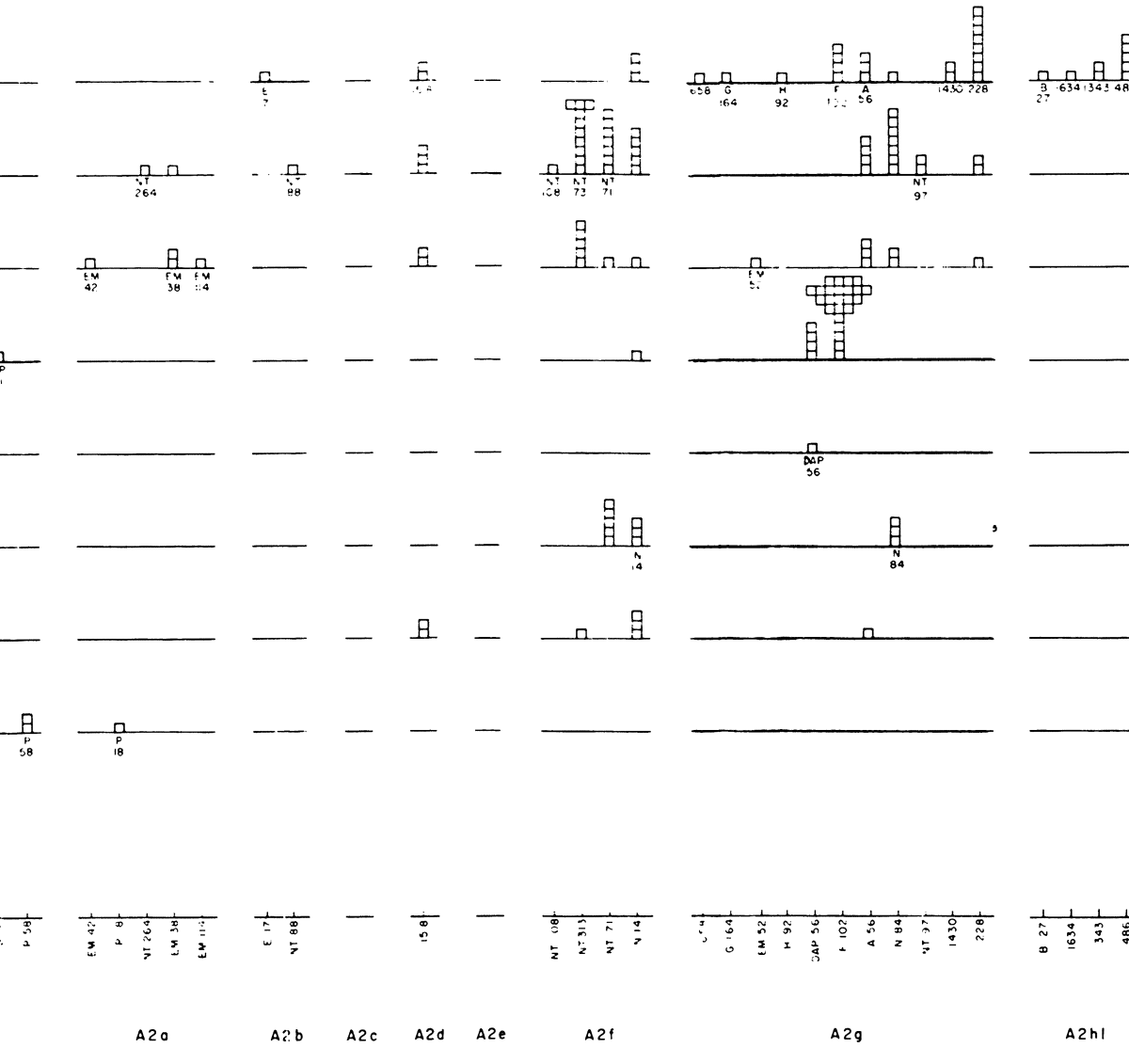


FIG. 8.—To
various mutag
and are not ve

Spontaneous

Nitrous acid
pH around 6.5
was 2×10^{-2}
isolated from t
minutes to giv
picked from b
also been map
large clones pr

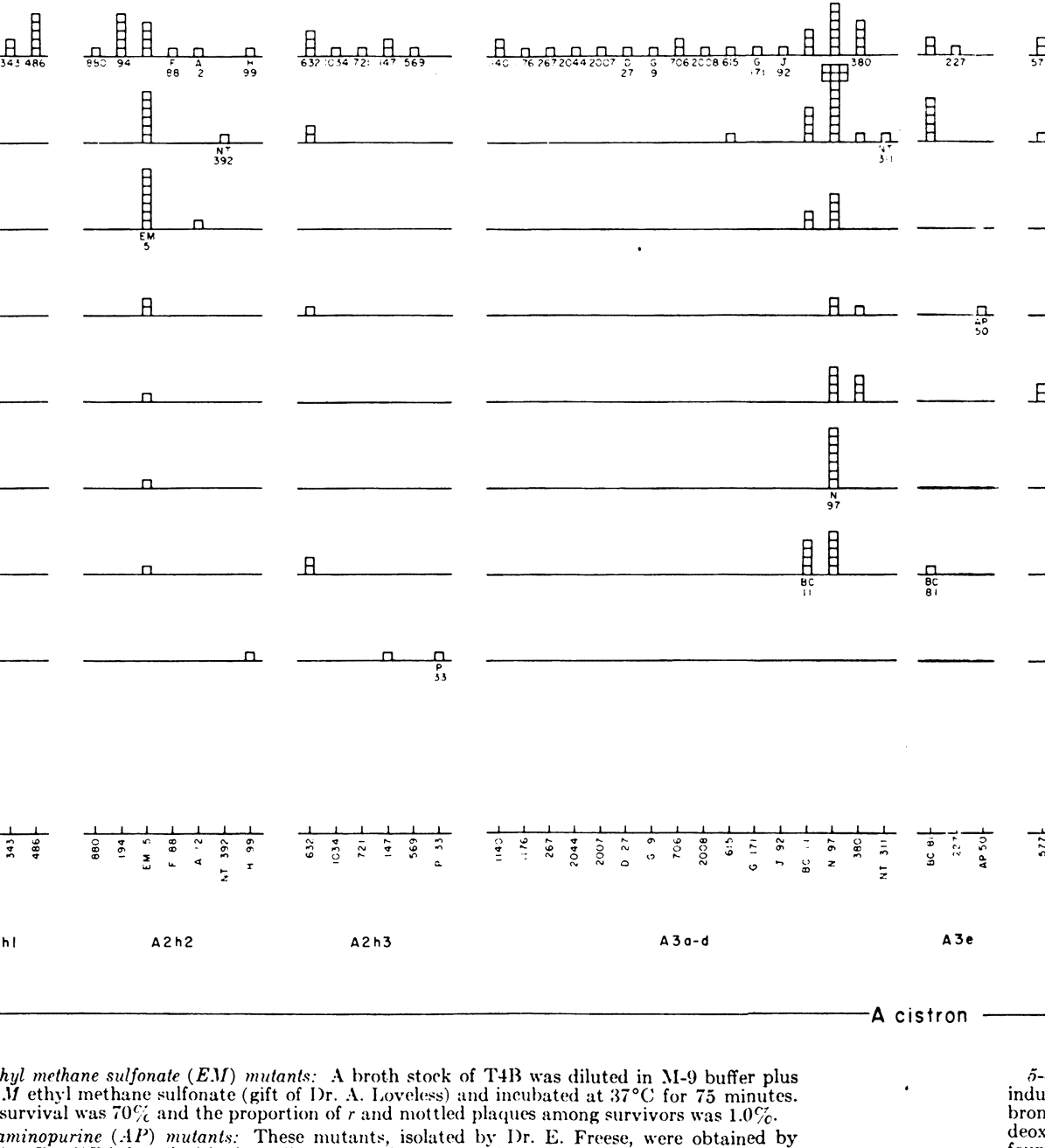


—Topographic map of the *r*II region for mutations arising spontaneously and induced by utagens. In each case, only *r*II mutants have been used that have low reversion rates ot very leaky.

neous mutants: See legend to Figure 6.

acid (NT) mutants: Standard type T4B was diluted in M-9 buffer plus 1.8 *M* $NaNO_2$, d 6.5, and incubated at 37°C for 80 minutes. The fraction of phage particles surviving 10^{-2} and included 0.4% of *r* type and mottled plaques. Four fifths of the mutants were rom this stock and the rest from a second stock exposed under similar conditions for 20 o give 0.3% mutants. For nitrous acid, as for the other mutagens below, mutants were om both *r* type and mottled plaques. Spontaneous mutants in the untreated stock have mapped (not shown here) and confirm that most of the NT hotspots cannot be due to es previously present.

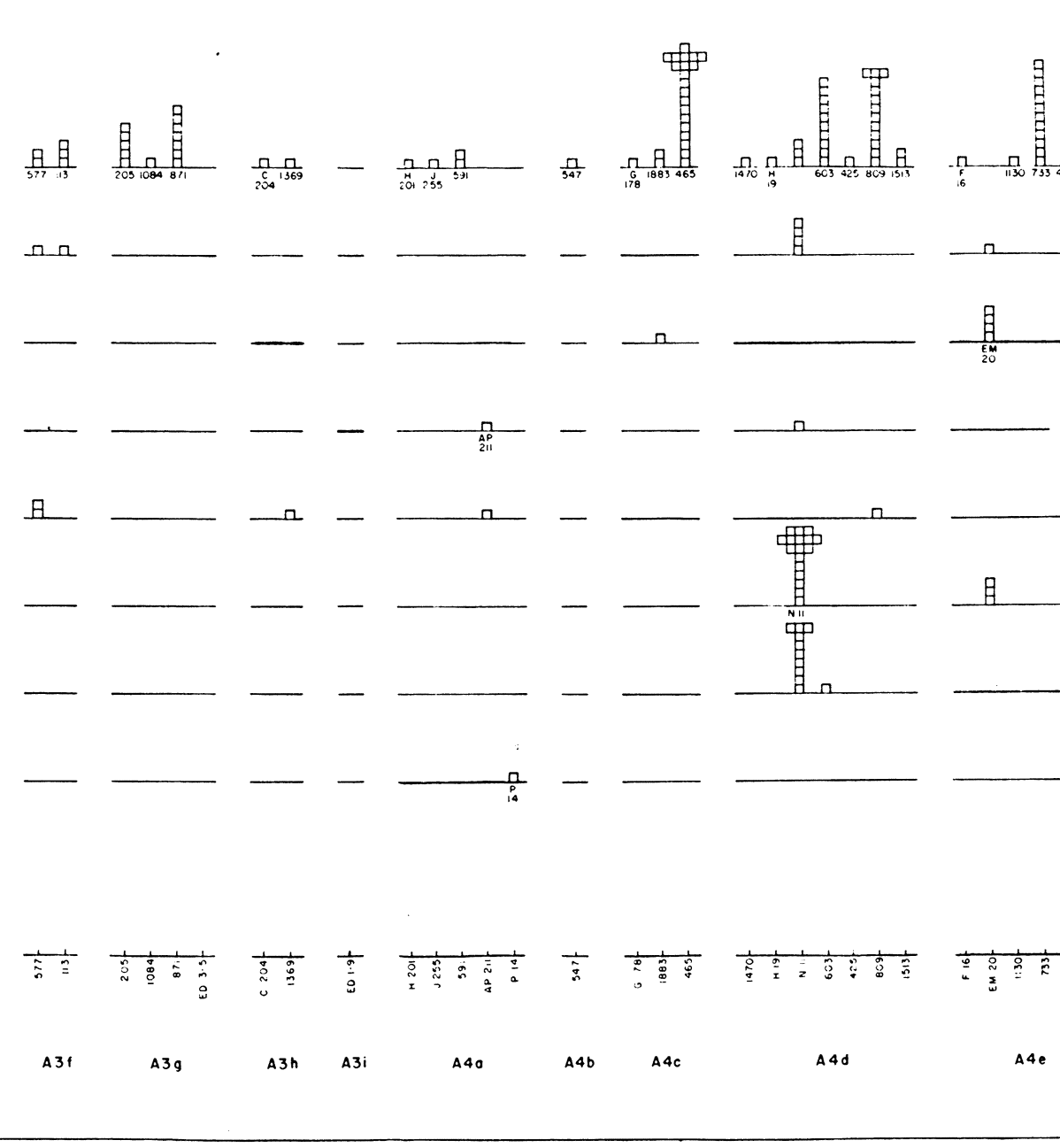
Ethyl
0.12 *M*
The surv
2-amin
growing
2,6-dia
into brot
minutes
infected
mutants
pendently
that the



hyl methane sulfonate (EM) mutants: A broth stock of T4B was diluted in M-9 buffer plus *M* ethyl methane sulfonate (gift of Dr. A. Loveless) and incubated at 37°C for 75 minutes. survival was 70% and the proportion of *r* and mottled plaques among survivors was 1.0%.

aminopurine (AP) mutants: These mutants, isolated by Dr. E. Freese, were obtained by ing *E. coli* B infected with phage T4B in a medium containing 2-aminopurine. See Freese.[5]

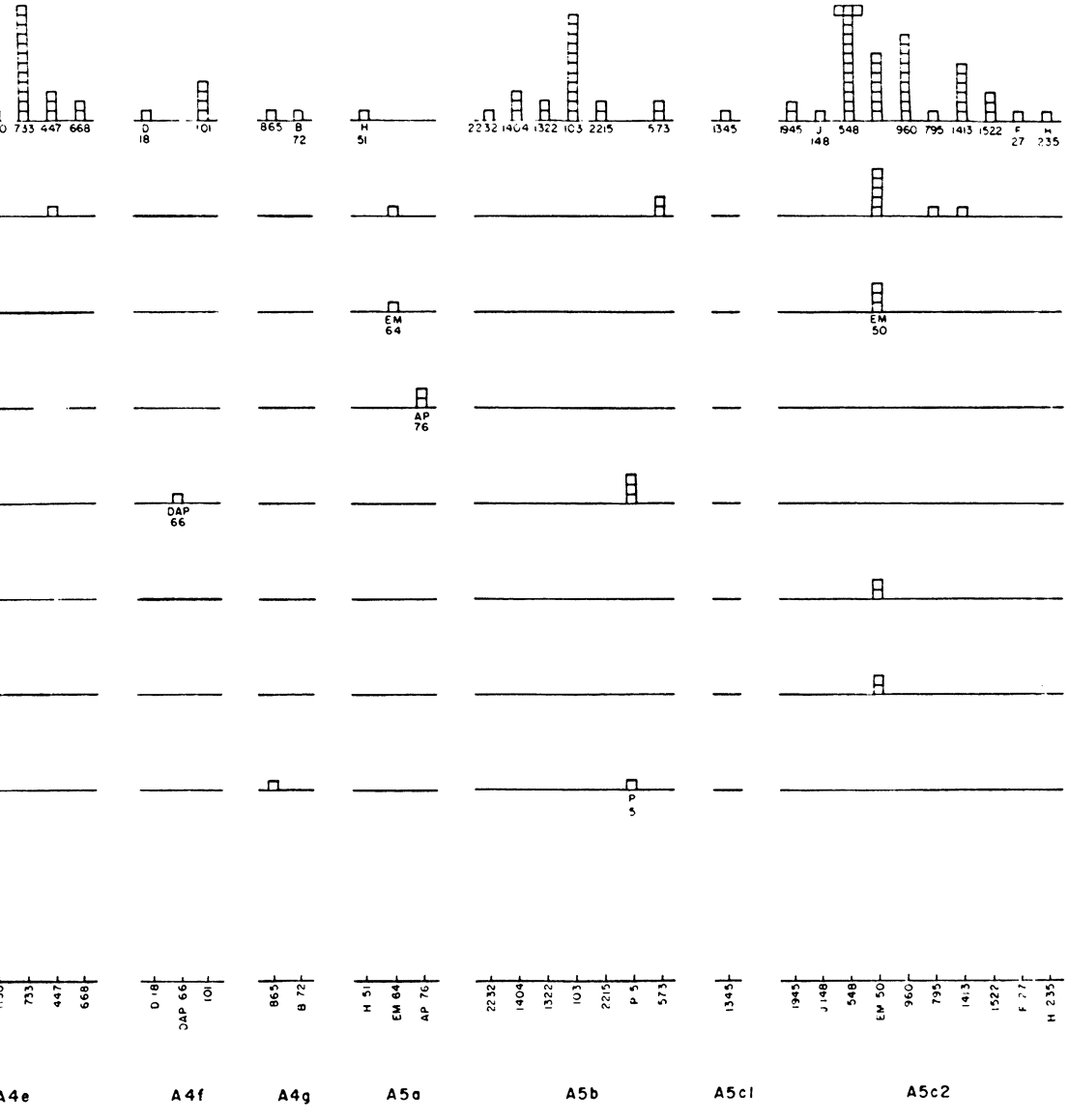
6-diaminopurine (DAP) mutants: *E. coli* B infected with T4B at low multiplicity were diluted broth containing 2.5 mg/ml. of 2,6-diaminopurine (Sigma Chemical Co.) at 37°C. After 60 ites the culture was treated with chloroform. The average yield of phage particles per ted cell was 90 and the proportion of *r* and mottled plaques was about 1.9%. The DAP ants were isolated from platings of a single stock, so that they did not necessarily arise inde- ently. However, only a small fraction of the mutants present in the stock was used, so the probability that two were derived from the same burst was small.



5-bromouracil (*N*) *mutants:* These include the mutants of Benzer and Freese[3], which were induced by growth of T4B on *E. coli* B in synthetic medium containing sulfanilamide plus 5-bromouracil. Added to these are the data for mutants isolated in the presence of 5-bromodeoxyuridine and thymidine (Freese[5]). Dr. Freese contributed mutants representing sites not found in the first set.

5-bromodeoxycytidine (*BC*)*mutants:* The effectiveness of this mutagen on phage was discovered by Dr. J. Gregory, who kindly supplied a sample synthesized by Dr. D. W. Visser. The procedure used was the same as for the DAP mutants except that the mutagen was 5-bromodeoxycytidine at a concentration of 5×10^{-4} *M*. The average yield was 80 and the proportion of *r* and mottled plaques was 0.8%.

Proflavine (*P*) *mutants:* These are the mutants, described by Brenner, Barnett, and Benzer[4], induced by proflavine during the growth of T4B on *E. coli* B. Each mutant was isolated from an independent burst.



TOPOGRAPHY OF THE GENETIC FINE STRUCTURE

FIG. 8.—*For detailed legend, unfold chart.*

PULL OUT CHART

(continued on reverse)

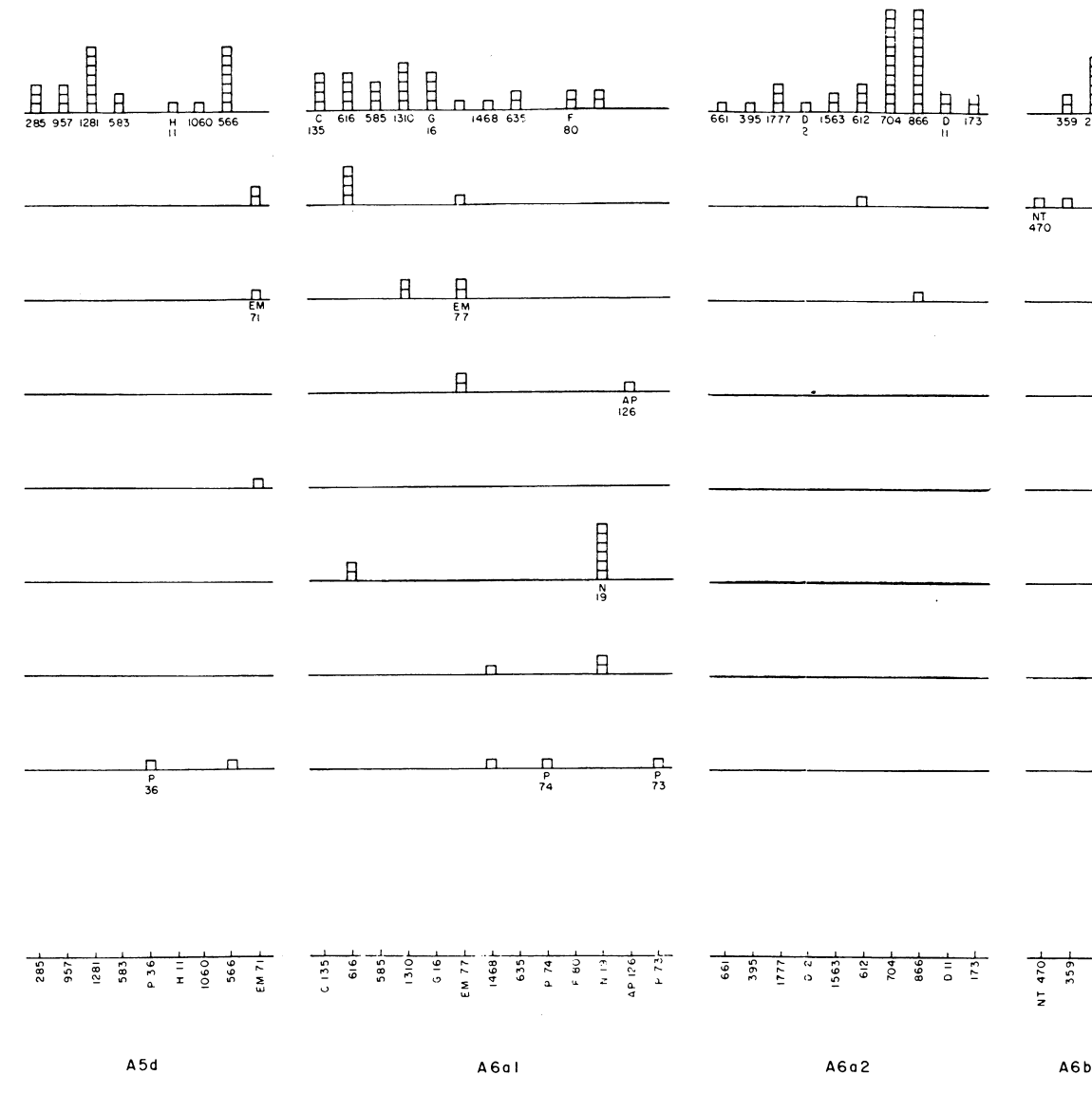
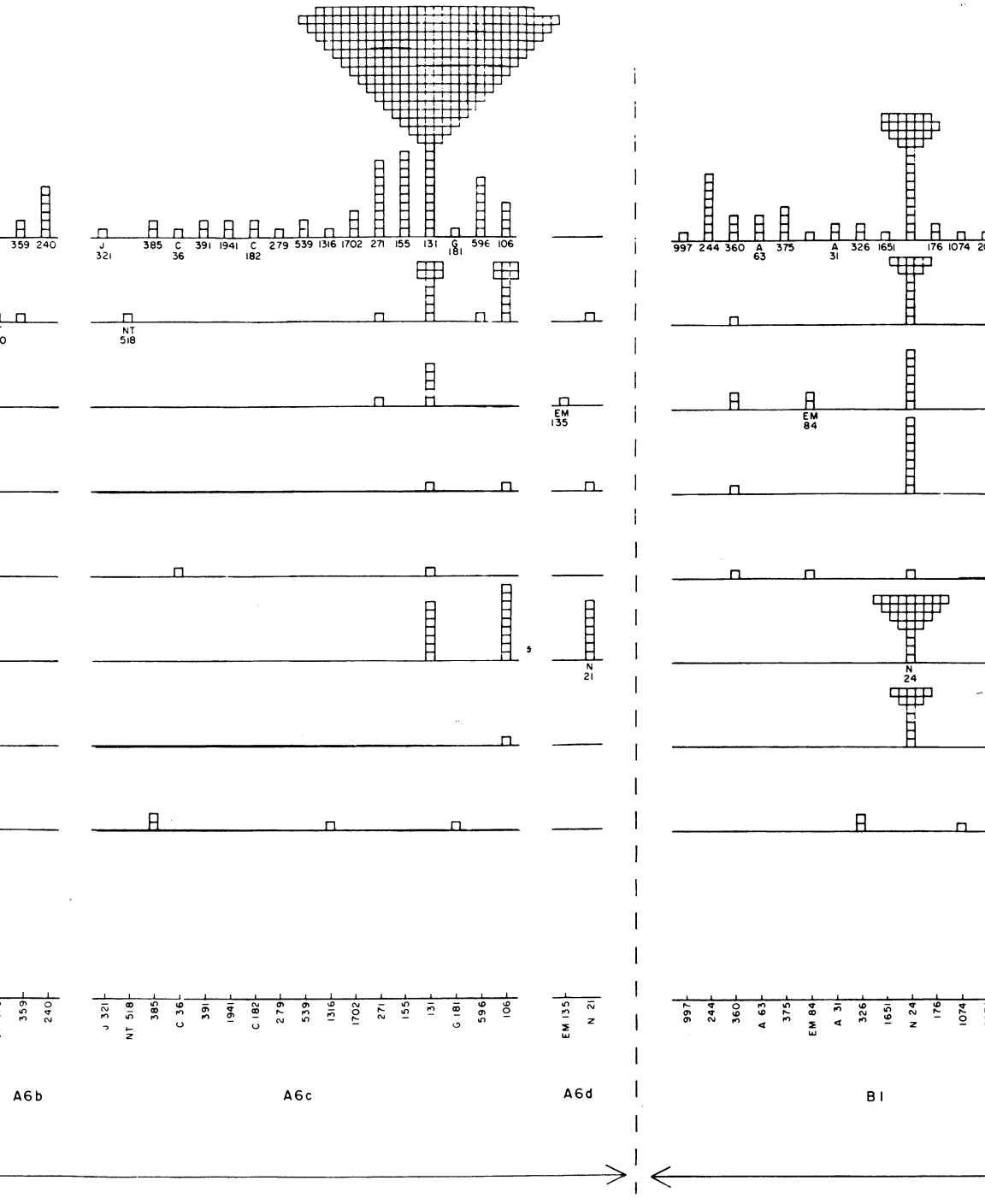
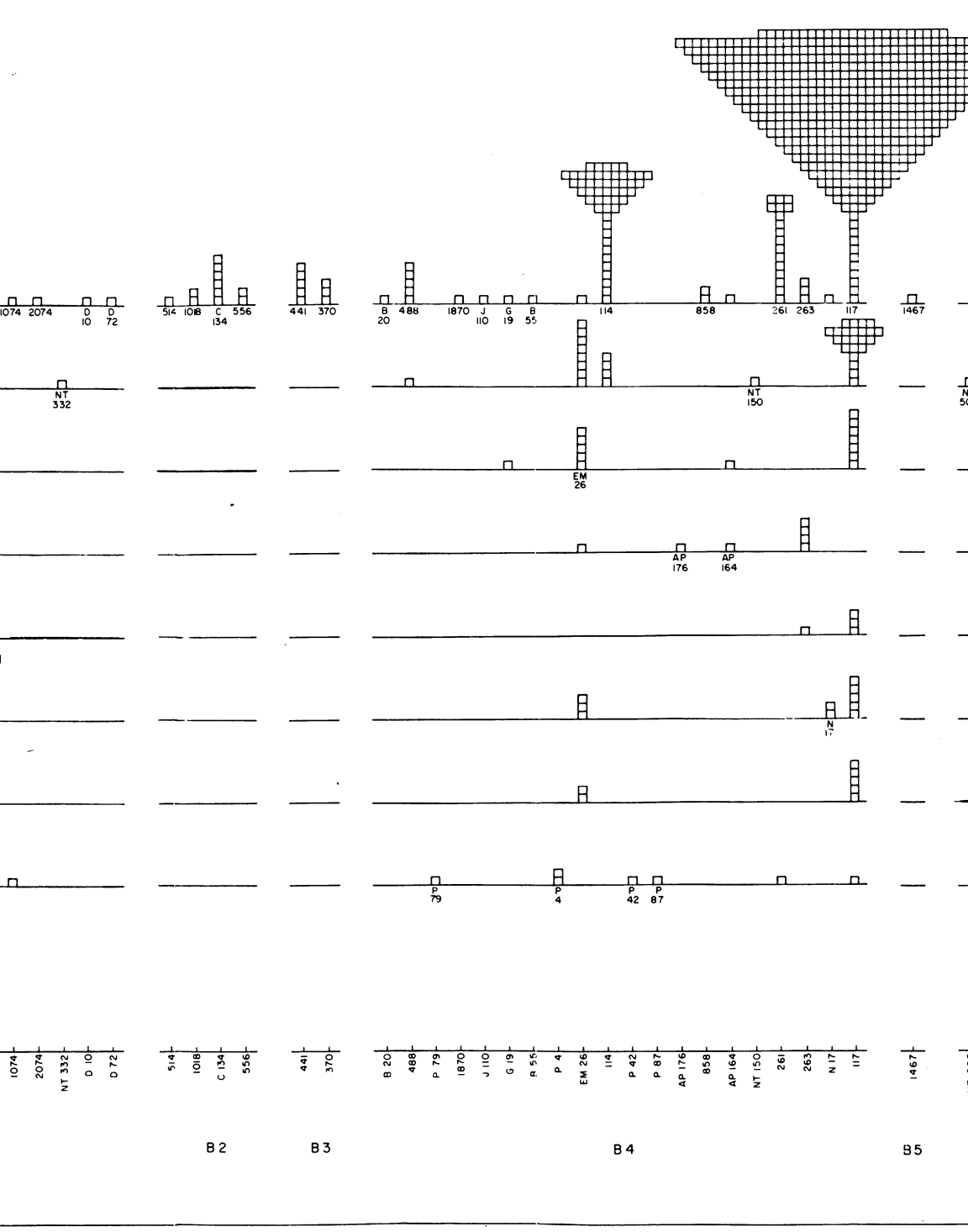
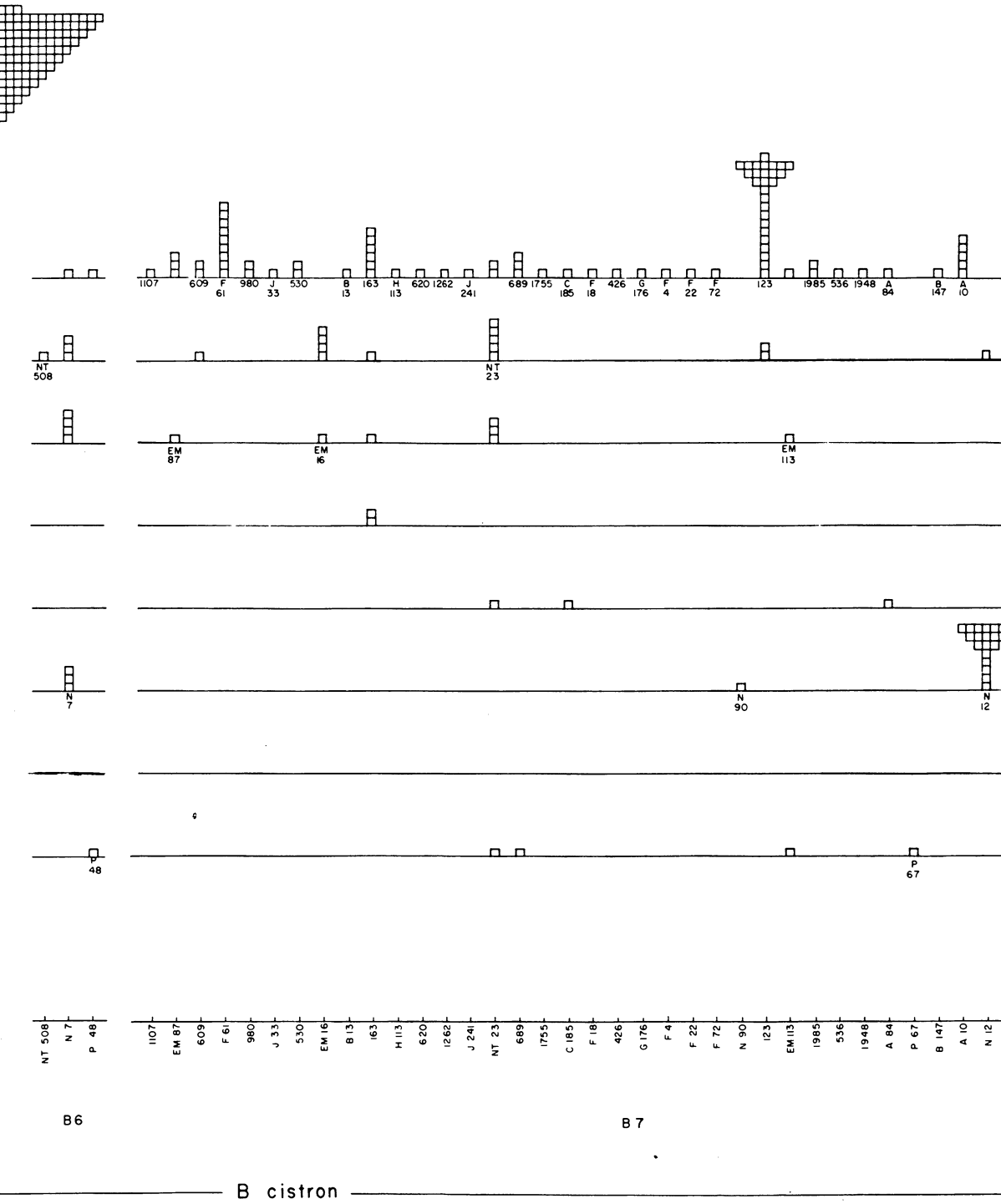


FIG. 8.—*continued. Full legend is given on front of chart.*


359 240
J 321
385 C 36 391 1941 C 182 279 539 1316 1702 271 155 131 G 181 596 106
NT 518
EM 135
N 21
997 244 360 A 63 375 A 31 326 1651 176 1074
EM 84
N 24
J 321 NT 518 385 C 36 391 1941 C 182 279 539 1316 1702 271 155 131 G 181 596 106
EM 135 N 21
997 244 360 A 63 375 EM 84 A 31 326 1651 N 24 176 1074
A6b
A6c
A6d
B1


1074 2074 D 10 D 72
514 1018 C 134 556
441 370
B 20 488 1870 J 110 G 19 B 55 114 858 261 263 117 1467
NT 332
NT 150
EM 26
AP 176 AP 164
N 17
P 79 P 4 P 42 P 87
1074 2074 NT 332 D 10 D 72
514 1018 C 134 556
441 370
B 20 488 P 79 1870 J 110 G 19 B 55 P 4 EM 26 114 P 42 P 87 AP 176 858 AP 164 NT 150 261 263 N 17 117
1467
B2 B3 B4 B5


1107
609
F 61
980
J 33
530
B 13
163
H 113
620
1262
J 241
689
1755
C 185
F 18
426
G 176
F 4
F 22
F 72
123
1985
536
1948
A 84
B 147
A 10
NT 508
NT 23
EM 87
EM 16
EM 113
N 7
N 90
N 12
P 48
P 67
NT 508
N 7
P 48
1107
EM 87
609
F 61
980
J 33
530
EM 16
B 13
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H 113
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J 241
NT 23
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F 18
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G 176
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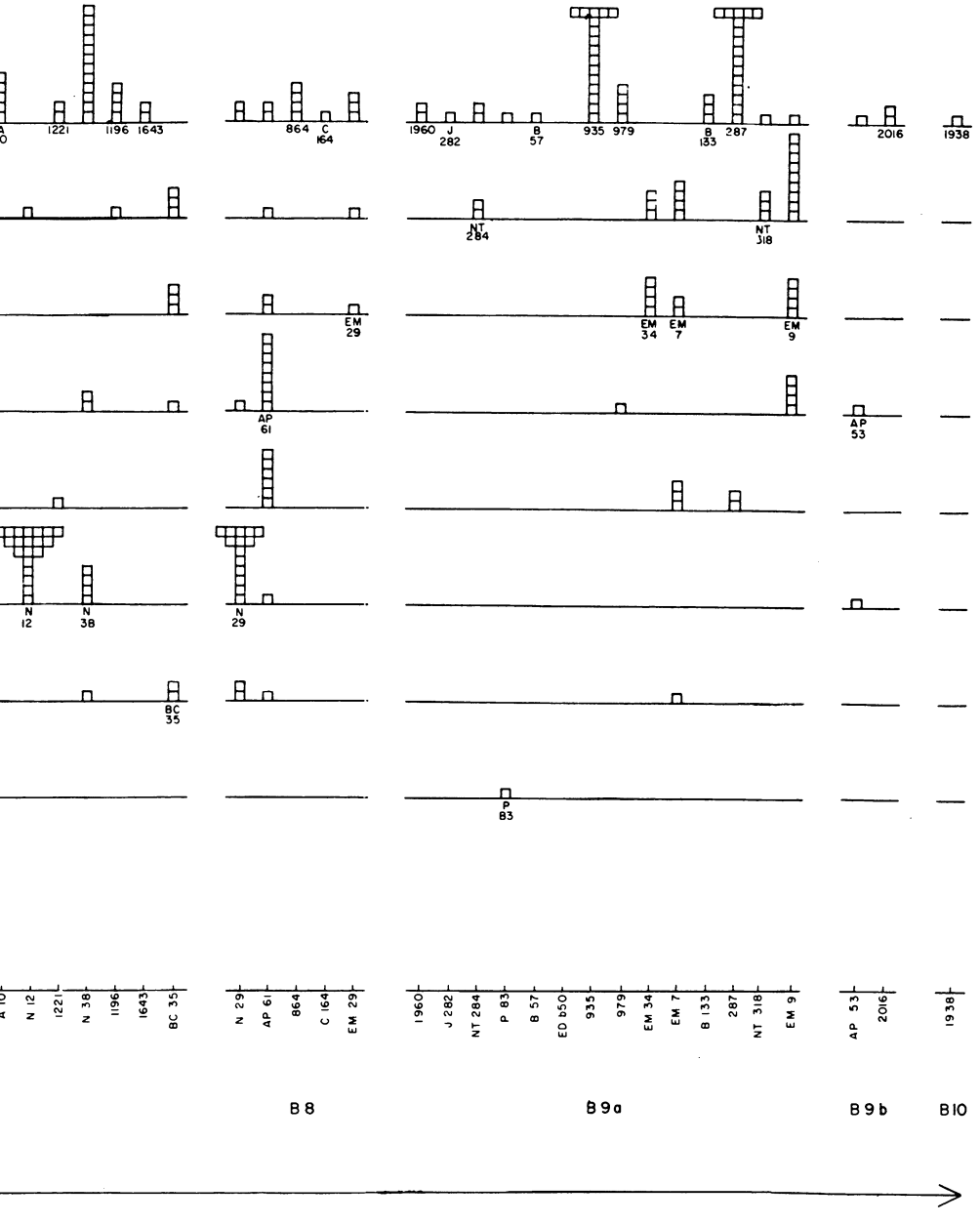
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1221
1196
1643
864
C 164
1960
J 282
B 57
935
979
B 133
287
2016
1938
NT 284
NT 318
EM 29
EM 34
EM 7
EM 9
AP 61
AP 53
N 12
N 38
N 29
BC 35
P 83
1960
J 282
NT 284
P 83
B 57
ED 650
935
979
EM 34
EM 7
B 133
287
NT 318
EM 9
AP 53
2016
1938
B8
B9a
B9b
B10