Studies on a mutator gene in Escherichia coli
by Guylyn Rea Warren

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY in Genetics
Montana State University
© Copyright by Guylyn Rea Warren (1967)

Abstract:
The precise location of mut-1, one of three separately isolated mutator genes which have been studied
extensively in bacterial systems, has been determined by the use of transduction and Hfr x F- timed
mating methods. It has been found to map very close to the locus for sodium azide resistance (azi),
between azi and the locus for T1,T5 resistance (ton).

Mut-1 is thus not allelic to either of the other two mutator genes (ast in E. coli, mut-3 in S.
typhimurium). The existence of three mutator loci indicates that the mutant DHA polymerase model is
inadequate as a general explanation for all instances of mutability.

Some mutagens have been found to produce effects on mapping data, such as "loosening of linkage" by
UV irradiation; therefore, the possibility of such an effect produced by mut-1 was investigated. Paired
transduction experiments using isogenic mutable and non-mutable phage donor strains showed no
measurable effects of mut-1 on linkage distances. Paired conjugations using isogenic mutable and
non-mutable derivatives of HfrC as donors suggested a slight increase in numbers of quadruple
crossover types caused by mut-1 although their effect on timed mating data used for location of mut-1
was negligible.

Since the effects of mut-1 on linkage data are negligible, the mutator genes mut-1, ast and mut-3 may
be ordered as follows with respect to other well-known loci in the region.

met mut-3 purA thi thr leu val azi mut-1 ton ast pro lac
STUDIES ON A MUTATOR GENE
IN ESCHERICHIA COLI

by

GUYLYN REA WARREN

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree
of
DOCTOR OF PHILOSOPHY
in
Genetics

Approved:

Head, Major Department

Chairman, Examining Committee

Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

August, 1967
ACKNOWLEDGMENTS

The author wishes to thank Dr. P. D. Skaar for his guidance and constructive criticism throughout the course of this study and especially in the preparation of this manuscript. The assistance of Dr. S. R. Chapman and Dr. G. R. Julian in manuscript preparation, and the earlier support of Dr. R. H. McBee and Dr. W. G. Walter, without which an advanced degree would not have been pursued, are also gratefully acknowledged.

This work was supported, in part, by a National Defense Education Act Fellowship, and, in part, by a grant (C-3902) to Dr. P. D. Skaar from the National Cancer Institute, Public Health Service.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>VITA.</td>
<td>11</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>iii</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>viii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>9</td>
</tr>
<tr>
<td>Bacterial and bacteriophage strains</td>
<td>9</td>
</tr>
<tr>
<td>Media and supplements</td>
<td>10</td>
</tr>
<tr>
<td>Nutrient Agar (NA)</td>
<td>10</td>
</tr>
<tr>
<td>L Broth (LB)</td>
<td>10</td>
</tr>
<tr>
<td>L Agar (LA)</td>
<td>11</td>
</tr>
<tr>
<td>Eosin Methylene Blue Agar (EMB)</td>
<td>11</td>
</tr>
<tr>
<td>Pennassay Broth (PB)</td>
<td>11</td>
</tr>
<tr>
<td>Davis Minimal Agar</td>
<td>11</td>
</tr>
<tr>
<td>Tris Glucose Minimal Agar (TGA)</td>
<td>11</td>
</tr>
<tr>
<td>Phage Dilution Fluid (PDF)</td>
<td>11</td>
</tr>
<tr>
<td>Top Layer Agar (TL)</td>
<td>11</td>
</tr>
<tr>
<td>Saline</td>
<td>11</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>12</td>
</tr>
<tr>
<td>Sodium Azide</td>
<td>12</td>
</tr>
<tr>
<td>Valine</td>
<td>12</td>
</tr>
<tr>
<td>Growth Factors</td>
<td>12</td>
</tr>
<tr>
<td>Storage of bacterial and bacteriophage strains and preparation of working cultures and lysates</td>
<td>12</td>
</tr>
<tr>
<td>Bacterial strains</td>
<td>12</td>
</tr>
<tr>
<td>Bacteriophage techniques</td>
<td>12</td>
</tr>
<tr>
<td>Transduction procedures</td>
<td>13</td>
</tr>
<tr>
<td>Tests of transductants for marker alleles</td>
<td>13</td>
</tr>
<tr>
<td>Mating Procedures</td>
<td>14</td>
</tr>
<tr>
<td>Gene terminology</td>
<td>15</td>
</tr>
<tr>
<td>EXPERIMENTAL RESULTS</td>
<td>17</td>
</tr>
<tr>
<td>The position of the mut-1 gene as inferred from transductions</td>
<td>17</td>
</tr>
<tr>
<td>Linkage of mut-1 to leu</td>
<td>17</td>
</tr>
<tr>
<td>Positions of val and mut-1 in relation to azi and leu</td>
<td>19</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>--------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>The position of mut-l as inferred from timed matings</td>
<td>22</td>
</tr>
</tbody>
</table>
| Effect of mut-l on transductional and conjugational recombina-
| tion frequencies                                           | 25   |
| Effect of mut-l on transductional linkage                    | 25   |
| Effect of mut-l on conjugational recombination frequency    | 26   |
| The precise location of mut-l                               | 28   |
| DISCUSSION                                                  | 30   |
| SUMMARY                                                     | 36   |
| LITERATURE CITED                                            | 37   |
# LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>PERCENT CO-TRANSDUCTION OF UNSELECTED MARKERS WHEN LEU&lt;sup&gt;+&lt;/sup&gt; OR THR&lt;sup&gt;+&lt;/sup&gt; WERE SELECTED</td>
<td>18</td>
</tr>
<tr>
<td>II.</td>
<td>FREQUENCIES OF LEU&lt;sup&gt;+&lt;/sup&gt; TRANSDUCTANTS CARRYING THE EIGHT DIFFERENT ARRAYS OF THREE UNSELECTED MARKERS</td>
<td>21</td>
</tr>
<tr>
<td>III.</td>
<td>FREQUENCIES OF CROSSING OVER IN COMPARABLE REGIONS OF ISOGENIC MUT&lt;sup&gt;+&lt;/sup&gt; AND MUT&lt;sup&gt;-&lt;/sup&gt; STRAINS</td>
<td>26</td>
</tr>
<tr>
<td>IV.</td>
<td>FREQUENCIES OF UNSELECTED MARKERS AMONG LAC&lt;sup&gt;+&lt;/sup&gt; PROGENY OF MATINGS. A COMPARISON BETWEEN MATINGS WHERE THE DONOR IS MUT&lt;sup&gt;+&lt;/sup&gt; AND WHERE THE DONOR IS MUT&lt;sup&gt;-&lt;/sup&gt;</td>
<td>28</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

1. Pedigree of K12 strains used.............................. 10

2. Kinetics of transfer of unselected donor characteristics among recombinants selected for Lac\textsuperscript{+} inheritance as a function of time of interruption after mating. All points are averages of four separate experiments using EMB-lac agar as the selective medium. Mating was interrupted every 2.5 minutes after dilution of GW6 into W1177 as described in the text............. 24

3. Mapping of mutator region expressed in percent crossing over. The a. and b. figures represent isogenic Mut\textsuperscript{+} and Mut\textsuperscript{-} donors respectively with a. the result of 318 colonies tested and b. the result of 270........................................... 27

4. A composite map of the mutator region. The figure represents the pooled results of 968 Plkc transductants using Mut\textsuperscript{+} donors GW1 and GW2, a composite map of the region......................... 29
ABSTRACT

The precise location of mut-1, one of three separately isolated mutator genes which have been studied extensively in bacterial systems, has been determined by the use of transduction and Hfr x F\textsuperscript{−} timed mating methods. It has been found to map very close to the locus for sodium azide resistance (azi), between azi and the locus for T1,T5 resistance (ton).

Mut-1 is thus not allelic to either of the other two mutator genes (ast in E. coli, mut-3 in S. typhimurium). The existence of three mutator loci indicates that the mutant DNA polymerase model is inadequate as a general explanation for all instances of mutability.

Some mutagens have been found to produce effects on mapping data, such as "loosening of linkage" by UV irradiation; therefore, the possibility of such an effect produced by mut-1 was investigated. Paired transduction experiments using isogenic mutable and non-mutable phage donor strains showed no measurable effects of mut-1 on linkage distances. Paired conjugations using isogenic mutable and non-mutable derivatives of HfrC as donors suggested a slight increase in numbers of quadruple crossover types caused by mut-1 although their effect on timed mating data used for location of mut-1 was negligible.

Since the effects of mut-1 on linkage data are negligible, the mutator genes mut-1, ast and mut-3 may be ordered as follows with respect to other well-known loci in the region.

met mut-3 purA thi thr leu val azi mut-1 ton ast pro lac
INTRODUCTION

Studies by R. A. Emerson (1914), in which he proposed the cause of mosaicism in plants to be mutation, cleared the way for studies of increased mutation rates at various loci in genetically suitable material. In nearly every type of organism which has been the focus of intensive genetic study, instances of mutational instability have been reported.

Mutable loci at which forward mutations are unstable and revert to wild type or some other state with high frequency have been described in plant, animal, and bacterial systems. Such loci as rose-alpha and lavender-alpha investigated by Demerec (1931), and the color locus p* reported by Dawson (1955) in Delphinium, the dwarf locus in Portulaca (Blakeslee, 1920; Faberge and Beale, 1942), and the c locus controlling anthocyanin pigmentation of the kernel in maize (Schwartz, 1960) have been studied in higher plants. In Drosophila, the miniature-gamma locus (Demerec, 1929a, 1929b) and mt-3a (Zamenhof, 1945) have been investigated. Lederberg (1952), Zamenhof et al. (1958) and Barnett and deSerres (1963) have found such mutants in microorganisms.

"Controlling elements" of mutation, probably episomal, have been studied extensively in maize in such systems as a1-Dt (dotted) by Rhoades (1938, 1941) and Nuffer (1961), and activator-dissociator by McClintock (1951, 1956). In Drosophila, controlling elements, possibly viral, have been found by Mampell (1943, 1945, 1946), by Levitan (1962), Levitan and Williamson (1965) and Ehrman and Williamson (1965). Systems of episomal control of mutation in bacteria have been described by Gunderson et al. (1961, 1963) in Escherichia coli and by Dawson and Smith-Keary (1963) in
Salmonella typhimurium. A system of viral effect on mutation has been described in E. coli by Taylor (1964) caused by the temperate bacteriophage mu-1, and one in maize caused by an RNA virus, barley stripe mosaic (BSMV), by Sprague et al. (1963). In all of these systems the controlling element is heritable and often infectious, but it cannot be mapped at any specific and unique locus in the genome.

The system of particular interest here is that of a true mutator gene, which has a unique chromosomal locus and is inherited in a Mendelian fashion, and which affects rates of mutation at other loci not closely linked with it. Several such genes have been studied in Drosophila by Demerec (1937), Plough and Holthausen (1937), Goldschmidt (1939, 1945), Neel (1942) and Ives (1943, 1950). Most of these gene affect the yellow locus as well as causing chromosomal aberrations, with the exception of hi, studied by Ives (1950), which affects the forked locus. Demerec (1937) proposed that the presence of mutator genes in wild populations of Drosophila would allow increased rates of evolution by maintaining greater adaptability to changes than that allowed by normal mutation rates.

Speyer (1965) has recently discovered a series of mutator alleles in bacteriophage T4. Gene 43, which has been identified as the structural gene for T4 DNA polymerase (Edgar, 1964; deWaard, 1965), is the locus of these alleles. Tests of the rII system of T4 indicated that temperature sensitive (ts) mutant L-56 caused a 2000-fold increase in the reversion frequency of spontaneous forward mutations in the rII region and also affected the back mutation frequency of some mutations caused by base
The mutagenic ts alleles do not affect reversion of deletions or of frame shift rII mutations. It was proposed that mutant DNA polymerases may permit mistakes in base pairing, thereby increasing mutation frequency.

Four independent mutable strains of bacteria have been described, three of Escherichia coli and one of Salmonella typhimurium. The first was a substrain of E. coli K12, 58-278M*, discovered by Treffers et al. (1954) by virtue of its enhanced (ca. 100-fold) mutation rate from streptomycin sensitivity to resistance. Subsequently, by means of appropriate F\(^+\) x F\(^-\) crosses, Skaar (1956) showed that a localized region of the E. coli linkage map was responsible for the enhanced mutability. He concluded that a single mutator gene was involved. Since more than one of the mutator loci to be discussed have been given the designation mut, for the sake of clarity in this presentation the mutant allele of 58-278M* will be referred to as mut-1. Skaar (1956) also concluded that the mut-1 locus was very close to the leucine (leu) locus, which marks the Y-10 line (Lederberg, 1947). Further, although the pertinent recombinants were not numerous enough to allow an unambiguous answer, it appeared that the mut-1 locus was to the "right" of leu, between leu and the locus for Tl,T5 resistance (ton), rather than between the leu and threonine (thr) loci. Later HfrC x F\(^-\) crosses performed by Gunderson et al. (1961) were interpreted by these workers to indicate that the mut-1 locus was not close to leu, but was rather to the "left" of thr, between that locus and the methionine (met) locus.
There are a number of clues as to the mode of action of mut-1.

(1) The multiplicity of effects of mut-l observed by Treffers et al. (1954) suggested a generalized effect, and hence a mutagenic gene product. They reported enhanced mutability for such diverse traits as resistance to chloramphenicol, sodium fluoride and bacteriophages T1, T4, T5, and T3h, and fermentation of sugars, but no effect on the mutation rate from T2 sensitivity to resistance. Skaar's demonstration (1956) that the streptomycin locus affected was only remotely linked to mut-1, and that the near-by loci, thr and thi (thiamine), were also affected, strengthened this inference. Auxotrophic mutants are significantly more frequent in mut-l cultures (Skaar, personal communication) and these constitute the same general array of types observed in non-mutable strains, mapping throughout the genome. Of especial significance is the finding (Pierce, 1966b) that mut-l is capable of increasing the mutation rate in the virulent bacteriophage T4. This argues that the "mutagen" is not a defective polymerase since this phage makes its own DNA polymerase.

(2) Bullas and Skaar (personal communication), (upon one occasion), isolated appreciable amounts of a fifth UV-absorbing component from the DNA of a mut-l strain. Several of its properties, including its unique spectral profile and its ability to satisfy growth requirements of a thymine-requiring bacterium, suggest that it was not an artifact, but rather a mut-l specific substance (possibly the mutagen) produced only under certain conditions of growth.

(3) Treffers et al. (1954) pointed out the marked discrepancy
between the array of streptomycin resistant mutants evoked by mut-1 and those which arise in other K12 strains. In the latter, 25% are streptomycin dependent; whereas, less than 1% of those arising in mut-1 strains are dependent. Since dependence and resistance without dependence were thought to be allelic (Newcombe and Nyhom, 1950) they suggested an allele-specific action of mut-1. Though these types of streptomycin resistance are not allelic (Hashimoto, 1960), this suggestion was supported by the work of Bacon and Treffers (1961a, 1961b), who found that mut-1 selectively raises the rate of one specific mutation type (the S48 type) at the ornithine locus.

Finally, Yanofsky (1966) showed that amino acid replacements, resulting from action by mut-1 on the tryptophane synthetase A protein, are consistent with a base transversion from A-T to C-G in the A cistron.

The second mutable strain was a substrain of MSFE and was designated the Harvard strain (ATCC 11887) of E. coli by Goldstein and Smoot (1955) and is also referred to as DZ-1 by Zamenhof (1966). Populations of this organism grown on nutrient medium normally contain a variety of auxotrophic mutants, at a mean frequency of about 4%. Goldstein and Smoot (1955) found that many of these auxotrophs are unstable and revert to wild type with high frequency. The mutability of this strain was found to be a stable hereditary characteristic and was proposed to be the result of a mutator gene. This gene was recently mapped by Zamenhof (1966) and was given the designation ast, for the Greek astasia (instability, mutability). Ast was found to lie between the locus for T1,T5 resistance (ton) and that
for proline (pro). The percentage of auxotrophic mutants in DZ-1 ranged from 5 to 12, while that of its closest known ancestor was only 0.005, and 50% of these mutants were highly unstable. The mapping of this gene was done with KL2 derivatives of DZ-1 which were F-lac since establishment of F in DZ-1 strains was impossible (possibly due to lack of homology with the F genome or to restriction) precluding selection of DZ-1 Hfr. Possible host restriction or modification of DZ-1 DNA by KL2 strains was noticed, causing "unlinking" of markers in crosses. It was observed that ast does not appreciably increase the mutation rate to streptomycin resistance in its host strain (DZ-1) but does so in K12 strains, suggesting a difference between strains in the mechanism of this resistance. Further, ast causes an increase in rate of mutation from T2 sensitivity to resistance in K12 strains. However ast affects loci for amino acid, purine and pyrimidine requirements, phage resistance other than T2, resistance to antibiotics and sugar fermentation as does mut-1. Also like mut-1, ast has recently been found to be capable of affecting virulent phage (T4) mutation rates (Zamenhof, 1967).

The third mutable strain was isolated and studied extensively by Miyake and Demerec (1959) and Miyake (1960) from S. typhimurium and has been designated LT-7. The mutability of this strain was shown to be caused by a single mutator gene, which will be referred to hereafter as mut-3 in order that it may be distinguished from the first isolated mutator gene. This gene exhibits allele specific activity and affects the rate of mutation at the streptomycin locus, as well as at all others
tested, with 87.7% of the auxotrophs produced exhibiting high reversion frequencies. This gene was mapped by Hfr x F" crosses and was reported to be located to the "left" of thr, near the purine A (purA) locus (Demerec et al., 1965; Miyake, 1960) as inferred from timed matings, although extensive data are not available. Mut-3 also has the peculiar property of increasing the efficiency of its host as a receptor for transduction by the transducing phage PLT-22 H1 (Miyake and Demerec, 1959). Kirchner is said to have found an unusual base present in mut-3 strains, but he has not been able to introduce this substance into cells to prove that it is mutagenic (in Pierce, 1966a), in agreement with his earlier mutagen hypothesis (Kirchner, 1960). He also has evidence of other possible mutator genes and other mutagenic bases in Salmonella.

The fourth mutable strain was isolated by Zamenhof (1958a, 1958b) by selection of a 5 bromouracil (5 BU) resistant mutant of E. coli I (ATC 11117) which requires thymine. The strain was designated I/Br and yields pinpoint colonies at high rates on nutrient medium as does the Harvard strain, DZ-1, which harbors ast. This mutability must be a property of resistance to 5 BU since all resistant mutants were unstable and back mutants to sensitivity were stable. This type of mutant cannot be isolated without the presence of 5 BU but the presence of 5 BU was not required for the expression of mutability of the resistant strains. 5 BU resistant mutants of other strains did not exhibit this mutability.

The three mutator genes which have been mapped are located within a segment constituting only about 10% of the E. coli genome. The map of Salmonella (Demerec et al., 1965) indicates close correlation of the
area containing mut-3 with the same area of the E. coli map (Taylor and Thoman, 1964). Despite the fact that each of the mutator genes seems to have its unique pattern of action, the possibility that they are allelic requires re-examination. On the basis of published positionings, ast and mut-3 are the loci further apart. Hence, they are least likely to be misplaced in the genome. The mut-l locus, however, is between the others (according to Skaar) and the allelism of mut-l with both mut-3 (Demerec, 1959) and ast (Zamenhof, 1966) has been suggested. In the present investigation, the precise position of mut-l is examined, using the more powerful tools of transduction and timed Hfr matings.

The precise positioning of mut-l, in addition to answering questions about the multigenic basis of mutability, is a necessary prerequisite to any further study of a possible gradient of mutability due to proximity to the gene.

Certain mutagens (e.g. ultraviolet light) affect recombination frequencies (Jacob and Wollman, 1955, 1959) and therefore representation of linkage distances in mapping. The possibility of a similar effect of mut-l was investigated. This possibility is interesting for its own sake, and is also a necessary part of an investigation attempting to position the gene.
MATERIALS AND METHODS

Bacterial and bacteriophage strains

The bacterial strains used were derivatives of *Escherichia coli* Kl2.

CS19 (Skaar, 1956) requires phenylalanine and is mutable and is therefore 58-278M* (Treffers et al., 1954) renamed after loss of its biotin requirement. GW1 is a derivative of CS19 which is resistant to sodium azide and to valine.

W677 is a multiply marked derivative of Y10 (Lederberg, 1947) which requires threonine, leucine and thiamine, cannot ferment lactose, maltose, mannitol or xylose, and is resistant to coliphages T1 and T5. W1177 is a streptomycin resistant derivative of W677.

W1895 is a derivative of 58-161 which is HfrC, the Cavalli strain (Cavalli, 1950) and requires methionine.

GW6 was constructed by transduction of W1895 by Plkc grown on GW1 and is HfrC, requires methionine, is mutable, and is resistant to sodium azide. GW6 was used as the donor in timed mating experiments.

GW2 was constructed by transduction of W677 with Plkc grown on GW1 and requires threonine and thiamine, does not ferment lactose, maltose, mannitol or xylose, is resistant to coliphages T1 and T5, is mutable and resistant to sodium azide and valine. GW3 is isogenic with GW2 with the exception that it is not mutable. These strains were used as isogenic donors in paired transduction experiments.

The phage strains used were Plkc, a mutant of Pl with an increased efficiency of plating on Kl2 strains, which was used as the generalized transducing agent; and T5, a virulent coliphage used to test the resistance
Only new characteristics are indicated, at all other loci the strains are identical with their immediate predecessors.

Figure 1. Pedigree of KL2 strains used.

of transductants and recombinants to T1,T5.

Media and supplements

Final concentrations are given in grams/liter of distilled water unless otherwise indicated. Media were sterilized in an autoclave at 18 pounds pressure for 20-25 minutes.

Nutrient Agar (NA): Difco Nutrient Broth 8, NaCl 5, agar 15,

**L Agar (LA):** LB plus 10 grams agar, for phage add 1 ml/l 2.5 M CaCl₂ (Lennox, 1955).

**Eosin Methylene Blue Agar (EMB):** Bacto-Casitone 8.0, Bacto-Yeast Extract 1, NaCl 5, K₂HPO₄ 2.0, eosin Y 0.4, methylene blue 0.065, sugar 10.0, agar 15.

**Pennassay Broth (PB):** Bacto-Beef Extract 1.5, Bacto-Yeast Extract 1.5, Bacto Peptone 5.0, Bacto-Dextrose 1.0, NaCl 3.5, K₂HPO₄ 3.6, KH₂PO₄ 1.3.

**Davis Minimal Agar (DMA):** KH₂PO₄ 2.0, K₂HPO₄ 7.0, crystalline sodium citrate 0.5, crystalline MgSO₄ 0.1, crystalline (NH₄)₂SO₄ 1.0, Bacto-Dextrose 1.0, agar 15. The salts were added to 500 ml of water and sterilized. The glucose and agar were added to 500 ml water and mixed with the salt solution after sterilization.

**Tris Glucose Minimal Agar (TGA):** NaCl 5.4, KCl 3.0, NH₄Cl 1.1, CaCl₂ 0.011, MgCl₂ 0.095, FeCl₃ 0.00162, KH₂PO₄ 0.0872, Na₂SO₄ 0.0227. Tris (hydroxymethyl) aminomethane 12.1. sugar 2.0, agar 10.0. The salts were added to 500 ml water and sterilized, the sugar and agar were added to 500 ml water and mixed with the salts after sterilization just prior to pouring into petri plates. In some cases lactose was substituted for glucose as the sugar and was added by diluting in 10 mls sterile water and adding to the agar after sterilization.

**Phage Dilution Fluid (PDF):** NaCl 3, Bacto-Peptone 1, MgSO₄ 2.46.

**Top Layer Agar (TL):** Bacto-Nutrient Broth 8.0, NaCl 5.0, agar 6.5.

**Saline:** NaCl 8.6.
Streptomycin: (dihydrostreptomycin sulphate) 250 mg/ml.

Sodium Azide: 5 ml/l of 0.4 M solution - used in LA.

Valine: 5 ml/l of 20 mg/ml solution - used in TGA.

Growth Factors: The following were added to TGA or DMA when required: methionine 0.02, threonine 0.04, leucine 0.02, thiamine 0.001, phenylalanine 0.02.

Storage of bacterial and bacteriophage strains and preparation of working cultures and lysates.

Bacterial strains. Bacterial strains were stored on nutrient agar slants at 4°C and transferred every 4-6 months. Transfers always included a single colony isolation and tests for relevant growth requirements. All inoculations were made directly from the stock slants and stocks in prolonged use were reisolated and tested periodically.

All cultures were grown in an incubator or water bath at 37°C. Penassay and L Broth cultures were grown overnight (12-16 hours) and log phase cultures in these media were obtained by diluting 1/10 from an overnight culture and incubating 90-120 minutes, depending upon the medium.

Bacteriophage techniques. Phage lysates were obtained by the confluent lysis plate technique described by Adams (1959). After collection, phage suspensions were spun in a Servall angle head centrifuge to remove cells and the supernate decanted and treated with chloroform. The resulting lysates were stored at 4°C. A concentration of $2.5 \times 10^{-3}$ M CaCl$_2$ was added to both L Agar and top layer agar when working with phage Plkc.

Lysates were assayed by the agar-layer method at appropriate
dilutions (in PDF) on L agar plus calcium. Lysates were aerated 20 minutes at 37°C to remove chloroform prior to transduction procedures.

Transduction procedures

High-titer lysates (at least 10^9 phage/ml) of F1kc were harvested on appropriate donor bacteria as above. Incubation of a 1/10 dilution of an overnight culture of W677, the recipient, for 150 minutes in LB produced a log phase culture of approximately 10^8 cells/ml. Five mls of this culture were centrifuged for five minutes and resuspended in 0.5 ml LB plus calcium, which allowed concentration to 10^9 cells/ml. 0.5 ml of the phage suspension, with chloroform removed, was added allowing multiplicities of infection of approximately 1:1. This phage-cell mixture was incubated 30 minutes to allow maximum adsorption and was then diluted, a step found to be critical for frequency of transduction, by a factor of five in saline and allowed to stand at room temperature for 15 minutes. The cells were then washed twice by centrifugation in saline, resuspended in 1 ml saline, and 0.1 ml samples of this suspension spread on appropriate selective media. For example, if leucine were the selective requirement, the medium was supplemented with threonine and thiamine. The freshly spread plates were incubated at room temperature for two hours as suggested by Luria et al. (1960), and then incubated 48 hours at 37°C. Control plates were spread with the recipient strain alone to check for spontaneous revertants and the phage lysate was tested for sterility in each experiment.

Tests of transductants for marker alleles. Recombinant clones were
picked and streaked on nutrient agar, single colony isolations were made from these streaks and L broth cultures (5 ml) inoculated from each. These cultures were incubated 12-22 hours and tested for unselected markers and checked for the selected marker as well. Growth requirements such as those for threonine and leucine were tested by patching on appropriately supplemented TGA and noting absence (-) or presence (+) of growth after 48 hours of incubation; resistance to valine by patching on TGA plus valine and all necessary supplements and noting absence (s) or presence (r) of growth after 24 hours; resistance to sodium azide by patching on L agar plus sodium azide and reading at 24 hours; and resistance to T5 by cross brushing the cultures with T5 on nutrient agar and scoring presence (s) or absence (r) of plaques or lysis of the streaks. Fermentation markers were tested on EMB plus the appropriate sugar and noting color reaction. Tests for mutability were performed by spreading 0.1 ml of each culture on nutrient agar plus streptomycin as described by Skaar (1956). Any clones not giving a positive test were diluted, re-grown, and tested again. After scoring each broth culture was stored at 4°C for future reference.

Mating procedures

Matings following the kinetics of transfer of the mutator gene (mut-l) and other pertinent loci were performed by the method developed by de Haan and Gross (1962) as modified by Wendt (1965). Log phase Hfr and F' cultures were mixed to concentrations of 1-2 x 10^7 Hfr/2-4 x 10^8 F' per ml., usually by diluting the Hfr 1/10 into the F' when grown in penassay
broth. Mating mixtures were incubated for five minutes to allow pairing and then diluted into $10^{-2}$ PB (PB diluted in saline) to halt further pairing. 0.1 ml. samples were removed from the diluted mixture at either 2.5 or 5 minute intervals and spread on appropriately supplemented TGA (usually with lactose) or on EMB-lactose to assay recombinants. In all cases resistance to streptomycin was the contra-selective marker, and streptomycin was added to all media to kill the donor cells. Each recombinant was picked and purified on the selective medium and tested for markers as in the transductions, with the exception that a new test for mutability was devised, since the recombinants are streptomycin resistant. The mutable allele causes a 100-fold increase in mutation rate from mannitol minus to mannitol fermentation (Skaar, personal communication), therefore, the cultures were patched on EMB-mannitol and the presence or absence of mutability scored by observing increase in papillation.

**Gene terminology**

As nearly as possible the suggestions of Demerec et al. (1966) were followed as regards genetic terminology. When the genotype of the strains is discussed small italicized letters are used, and when phenotype is discussed the first letter of the designation is capitalized, not italicized and such characteristics are resistance, sensitivity etc., as conferred by alleles of the same gene may be discussed. The abbreviations most commonly used are as follows:
<table>
<thead>
<tr>
<th>locus</th>
<th>genotype</th>
<th>allelic phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>threonine</td>
<td>thr</td>
<td>Thr(^-) or Thr(^+)</td>
</tr>
<tr>
<td>leucine</td>
<td>leu</td>
<td>Leu(^-) Leu(^+)</td>
</tr>
<tr>
<td>valine</td>
<td>val</td>
<td>Val(^s) Val(^r)</td>
</tr>
<tr>
<td>sodium azide</td>
<td>azi</td>
<td>Azi(^s) Azi(^r)</td>
</tr>
<tr>
<td>mutability</td>
<td>mut(^-)</td>
<td>Mut(^-) Mut(^+)</td>
</tr>
<tr>
<td>Tl,T5 receptors</td>
<td>ton</td>
<td>Ton(^s) Ton(^r)</td>
</tr>
</tbody>
</table>

The plus sign used in phenotypic designations normally indicates the wild type ability to perform a function and does so here with the exception of its use with Mut\(^+\) where it is the designation for mutability while Mut\(^-\) is the wild type.
EXPERIMENTAL RESULTS

The position of the mut-1 gene as inferred from transductions

Linkage of mut-1 to leu. Due to the disparity of the conjugal mapping data of Skaar (1956) and that of Gunderson et al. (1961), it was first necessary to investigate the relative proximity of mut-1 to leu and thr. This problem was approached by transductional techniques, which allow detection of linkage only between loci that are situated close enough together on the chromosome to be included in one fragment carried by an infecting phage particle. The generalized transducing phage Plkc, a strain of the largest of the temperate phages, which is competent on Kl2 strains of E. coli and known to co-transduce leu and thr (Lennox, 1955), was chosen as the agent of transfer. Transduction experiments were performed as described in Materials and Methods. Since mut-1 cannot be selected directly, selection for another locus (leu or thr) was imposed and the frequency of co-transduction of unselected markers was scored. Proximity of mut-1 to leu was examined by imposing selection of those transductants manifesting the donor (GW1) phenotype for leucine (Leu+) and scoring their respective phenotypes for the val, thr, mut-1 and ton (T1,T5 resistance) loci. A common recipient strain, W677, was used in all transduction experiments, thus minimizing the variability of transducing ability from strain to strain shown by Pl (Lennox, 1955). The results of this experiment are reported in Table I, a. As is to be expected among Plkc transductants, the donor thr allele was found among very few (less than 1%) and the donor ton allele was never found (Lennox, 1955). Among these transductants 82.5% carried the donor val allele and
TABLE I. PERCENT CO-TRANSDUCTION OF UNSELECTED MARKERS WHEN LEU⁺ OR THR⁺ WERE SELECTED.

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient</th>
<th>Selected marker</th>
<th>No.</th>
<th>Unselected marker inheritance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. GW1 W677</td>
<td>Leu⁺</td>
<td>200</td>
<td>1</td>
<td>82.5 * 20.5 0</td>
</tr>
<tr>
<td>b. GW1 W677</td>
<td>Leu⁺</td>
<td>458</td>
<td>0.6</td>
<td>76.4 19.4 14.6 0.5</td>
</tr>
<tr>
<td>c. GW1 W677</td>
<td>Leu⁺</td>
<td>192</td>
<td>0</td>
<td>83.9 19.3 15.6 0</td>
</tr>
<tr>
<td>d. GW2 W677</td>
<td>Leu⁺</td>
<td>111</td>
<td>0.9</td>
<td>72.0 23.4 20.7 0</td>
</tr>
<tr>
<td>e. GW2 W677</td>
<td>Leu⁺</td>
<td>207</td>
<td>0</td>
<td>76.5 24.2 23.2 0</td>
</tr>
<tr>
<td>f. GW3 W677</td>
<td>Leu⁺</td>
<td>159</td>
<td>0</td>
<td>71.7 24.6 ** 0</td>
</tr>
<tr>
<td>g. GW3 W677</td>
<td>Leu⁺</td>
<td>111</td>
<td>0</td>
<td>79.3 20.7 ** 0</td>
</tr>
<tr>
<td>h. GW1 W677</td>
<td>Thr⁺</td>
<td>96</td>
<td>4.2</td>
<td>1.0 0 0 0</td>
</tr>
</tbody>
</table>

Each transductant clone was picked, streaked, and purified by single colony isolation on NA (nutrient agar). Transductions a., b and h. were selected on DMA (Davis Minimal) plus appropriate supplements, while the rest were selected on TGA (Tris Glucose Minimal).

* No test of these transductants was made for azide resistance.
** No test for mutability was possible, since the donors were Mut⁻.

20.5% carried the donor mut⁻1 allele. This indication of the closeness of mut⁻1 to leu was further tested by the performance of a transduction identical to the first but imposing selection for donor phenotype at the thr locus. The results of this experiment are reported in Table I, h. Among 96 transductants tested, 4% carried the donor leu allele and 1% carried the donor val allele. None of them carried the donor mut⁻1 allele; in other words, mut⁻1 was not observably co-transduced with thr.
The results of these experiments indicate a much closer linkage of mut-1 to leu than to thr, but that mut-1 is not so close to leu as is val. On control platings, spontaneous revertants of the recipient strain to either Leu+ or Thr+ were never present in concentrations greater than 1/10^8 cells; corrections for background mutation were therefore unnecessary.

Positions of val and mut-1 in relation to azi and leu.

Unambiguous positioning of mut-1 or val to the "left" or "right" of leu cannot be inferred from the data of the first two experiments due to the small number of Thr+ transductants tested, although location to the "right" is suggested. A locus controlling resistance or sensitivity to sodium azide (azi) has been positioned by transduction (Lennox, 1955) and timed mating (Maccacaro and Hayes, 1961) techniques to the "right" of leu and between it and ton. Since this is the only azide locus which has been described in E. coli and it is co-transducible with leu, azi was assumed as the "right" reference locus for the following transduction experiments. This assumption will be examined later. In order to establish the positions of val and mut-1 unambiguously in relation to leu and azi, two separate transductions were performed imposing selection for Leu+ and a total of 650 transductants were purified and scored for unselected markers. The results are reported in Table I, b. and c. Among 650 Leu+ transductants, approximately 80% were Val+, 19% were Azi+ and 15% were Mut+ (or mutable). Since frequency of transfer is directly proportional to distance from the selected marker (Lennox, 1955), these data indicate that leu is
closer to val than to azi and closer to azi than to mut-
; but frequencies alone have no bearing on the "right" or "left" positioning of the un-
selected markers. The ordering of these loci can be accomplished by looking at the actual recombinant types resulting from integration of the trans-
duced fragment of donor genome. The eight possible recombinant phenotypes resulting from a four factor cross such as this are represented in Table II and also the pooled numbers of each type scored in the experiments. According to the above results val can only be located in one of two alternative positions, either (1) between the leu and azi loci, or (2) on the opposite side of leu from azi but closer to leu than is azi. If the first is correct, the number of Azi\textsuperscript{r} transductants (receiving the donor azi allele) should be dependent on the number of those which are also Val\textsuperscript{r} (carry the donor allele for val), while if the second is correct the number of Azi\textsuperscript{r} types should be independent of the number also Val\textsuperscript{r}.

Actually, among 508 Val\textsuperscript{r} transductants, 390 were also Azi\textsuperscript{s} while 118 were Azi\textsuperscript{r}; whereas among 142 Val\textsuperscript{s} transductants, 132 were also Azi\textsuperscript{s} and only 10 were Azi\textsuperscript{r}. In other words, the ratio of donor to recipient azi alleles among those transductants carrying donor val alleles is 1:3, while this ratio among transductants with recipient val alleles is 1:10. This coupling of val and azi alleles argues that val is between leu and azi.

The location of mut-
 in relation to azi and leu can be ascertained in the same way. If mut-
were on the side opposite leu from azi the donor alleles of mut- and azi should occur more or less independently in the transductants; but if they are on the same side, with mut- only
TABLE II. FREQUENCIES OF LEU\(^+\) TRANSDUCTANTS CARRYING THE EIGHT DIFFERENT ARRAYS OF THREE UNSELECTED MARKERS.

<table>
<thead>
<tr>
<th>Recombinant Phenotypes</th>
<th>No.</th>
<th>Inferred cross-over type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leu(^+)Val(^s)Azi(^s)Mut(^-)</td>
<td>132 *</td>
<td>1-2</td>
</tr>
<tr>
<td>Leu(^+)Val(^r)Azi(^s)Mut(^-)</td>
<td>386</td>
<td>1-3</td>
</tr>
<tr>
<td>Leu(^+)Val(^r)Azi(^r)Mut(^-)</td>
<td>30**</td>
<td>1-4</td>
</tr>
<tr>
<td>Leu(^+)Val(^r)Azi(^r)Mut(^+)</td>
<td>88</td>
<td>1-5</td>
</tr>
<tr>
<td>Leu(^+)Val(^s)Azi(^r)Mut(^+)</td>
<td>6</td>
<td>1-2-3-5</td>
</tr>
<tr>
<td>Leu(^+)Val(^r)Azi(^s)Mut(^+)</td>
<td>4</td>
<td>1-3-4-5</td>
</tr>
<tr>
<td>Leu(^+)Val(^s)Azi(^r)Mut(^-)</td>
<td>4</td>
<td>1-2-3-4</td>
</tr>
<tr>
<td>Leu(^+)Val(^s)Azi(^s)Mut(^+)</td>
<td>0</td>
<td>1-2-4-5</td>
</tr>
</tbody>
</table>

A total of 650 Leu\(^+\) transductants of W677 (Thr\(^-\) Leu\(^-\) Val\(^s\) Azi\(^s\) Mut\(^-\) Ton\(^r\)) by Plk grown on GW1 (Thr\(^+\) Leu\(^+\) Val\(^r\) Azi\(^r\) Mut\(^+\) Ton\(^s\)) were purified and characterized as to the five unselected markers. Only three were Thr\(^+\) (all in the group marked with an asterisk). Only one was Ton\(^s\) (in the group marked with a double asterisk). The inferred gene order was: end of fragment-1-leu-2-val-3-azi-4-mut-5-end of fragment, where numbers refer to cross-over regions. Thr is within region 1, ton is within region 5.

slightly farther away than azi, there should be a preponderance of parental combinations. There were 528 Azi\(^s\)Mut\(^-\) and 94 Azi\(^r\)Mut\(^+\) transductants (parental types); 34 Azi\(^r\)Mut\(^-\) and 4 Azi\(^s\)Mut\(^+\) (recombinant types). This greater frequency of parental combinations is consistent with the location of mut\(^-\) on the same side of leu as azi, between the latter and and ton.
The positions of azi and val inferred here agree with those presented by Hayes (1964). If regions of crossing-over are assigned for the ordering of loci inferred above, (end of fragment-1-leu-2-val-3 azi-4-mut-1-5-end of fragment), numbers of crossovers necessary for the formation of each transductant type can be scored. This has been done and the results are presented in Table II. The quadruple crossover types are clearly the most infrequent as is to be expected if the gene order is correct.

**The position of mut-1 as inferred from timed matings.**

The transduction experiments just described locate mut-1 as first proposed by Skaar (1956) and are in disagreement with the location proposed by Gunderson et al. (1961). Since the latter based their conclusions upon Hfr x F’ matings an investigation of its location using this technique as well was thought necessary. Since the azi locus used as a "right" reference marker was mapped by use of a newly isolated azide resistant mutant never mapped before, its assumed location would be confirmed as well by these matings.

An HfrC (high frequency donor) derivative which carried the same alleles at mut-1 and azi as GW1 was obtained by transduction of W1895 with Plkc grown on GW1, with selection for azide resistance imposed. Although the mutation rate from Azis to Azir in the W1895 recipient was high, it was felt that the probability of the Mut+ transductant picked being a spontaneous mutant rather than a carrier of the azi allele of GW1 was virtually nil. This Mut+AzirHfrC (Cavalli, 1950) strain will be referred to as GW6, and was used as the donor strain in timed matings.
As in transduction, it was necessary to test mut-1 as an unselected marker. The recipient strain, Wl177, was a streptomycin resistant mutant of W677.

Since gradient of transmission in bacterial matings is predominantly a function of transfer rather than crossing over, decisive results will only be obtained if the marker to be located lies distal to the selective marker (Hayes et al., 1963). HfrC transfers markers beginning with lac (lactose fermentation) and continuing to the left, transferring leu at about 20 minutes; therefore lac was chosen as the selective marker.

Matings were performed as described by Wendt (1966) with 0.1 ml. samples of the diluted mating mixture spread on selective medium plus streptomycin every 2.5 minutes following a five-minute allowance for pairing. The pooled results of four separate timed matings are represented in Figure 2. The lac donor allele entered at approximately 10 minutes after mixing as is expected of HfrC (Hayes et al., 1963) and the times of entry of the donor azi, leu and thr alleles agree with those of Taylor and Thoman (1964), although ton (T1,T5 rec on their map) enters approximately two minutes later than expected. Maccacaro and Hayes (1961) reported 1.5 minutes separating thr and azi in contrast to the one minute difference seen here. They also reported two minutes between ton and azi while a difference of one minute is found here. The method of mating interruption used does not allow presentation of two phase kinetics. (Taylor and Thoman, 1964).

Since joint transduction of loci separated by more than 1.5 minutes is extremely rare, the close entry times of the jointly transducible loci investigated, within one minute from thr to azi, is to be expected
Figure 2. Kinetics of transfer of unselected donor characteristics among recombinants selected for Lac⁺ inheritance as a function of time of interruption after mating. All points are averages of four separate experiments using EMB-lac agar as the selective medium. Mating was interrupted every 2.5 minutes after dilution of GW6 into W1177 as described in the text.
(Taylor and Thomas, 1964). The times of entry support the assumption that the azide resistance locus is the one previously mapped, and that the loci mapped here do fall to the "right" of leu, between it and ton. It may then be stated that the gene in question, mut-1, is not allelic to either ast or mut-3, but has its own unique position in the same general region.

**Effect of mut-1 on transductional and conjugational recombination frequencies**

In the course of the transduction experiments strains were developed to detect a possible effect of the mutable allele of mut-1 on recombination frequency similar to the "loosening of linkage" noticed following ultraviolet exposure of donor bacteria and of phage lambda (Jacob and Wollman, 1955, 1959). Since mutator genes may produce mutagenic substances (Kirchner, 1960), this consequence of mut-1 is possible.

**Effect of mut-1 on transductional linkage.** As a test, isogenic Mut+ (GW2) and Mut− (GW3) derivatives of W677, available as previously isolated transductants, were used as donors in paired Plkc transduction experiments, with W677 as the common recipient. The results of two separate paired experiments of this type are presented in Table I, e.,f.,g. and h. The order of genes was assumed as described in previous sections, results of the two experiments were pooled and frequency of crossovers and crossover percentages was scored for each region as given in Table III, a. and b. and linkage maps of the Mut+ donor and Mut− donor genomes were constructed in units of % crossing-over. These maps are presented in Figure 3. The transductants were selected for inheritance of the donor leu allele as
TABLE III. FREQUENCIES OF CROSSING OVER IN COMPARABLE REGIONS OF ISOGENIC MUT+ AND MUT- STRAINS.

<table>
<thead>
<tr>
<th>Donor Type</th>
<th>No. Tested</th>
<th>Number of crossovers in region:</th>
<th>Total to right of leu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mut+ (GW2)</td>
<td>318</td>
<td>80 166 77 323</td>
<td>(25.2%) (52.5%) (24.2%) (101.6%)</td>
</tr>
<tr>
<td>Mut- (GW3)</td>
<td>270</td>
<td>68 143 63 274</td>
<td>(25.2%) (53.0%) (23.3%) (101.5%)</td>
</tr>
<tr>
<td>Mut+ (GW1)</td>
<td>650</td>
<td>140 396 142 678</td>
<td>(21.5%) (60.9%) (21.8%) (104.3%)</td>
</tr>
</tbody>
</table>

Leu+ transductants of W677 (Leu- Val8 Azi8 Mut-) were obtained using Plkc grown upon GW2 (Leu+ Val8 Azi8 Mut+) and upon the isogenic strain GW3 (Leu+ Val8 Azi8 Mut-). Crossovers in region 2 (between leu and val), region 3 (between val and azi), and in regions 4 and 5 (between azi and end of fragment) enumerated. Equivalent data for the transductions of W677 by GW1 (Leu+ Val8 Azi8 Mut+) are included for comparison.

Before. Since crossovers occurring in regions 4 and 5 cannot be scored in the Mut- donor transductions these regions have been pooled. It can be seen that the linkage maps constructed from these data are nearly identical. Hence it is concluded that mut-1 has no measurable effects on linkage maps obtained by transduction procedures, and the ordering of loci as previously presented is correct.

Effect of mut-1 on conjugational recombination frequency.

This same phenomenon was then investigated in conjugation experiments,
leu  val  azi  end of fragment  Total Units

a.  25.2  52.2  24.2  101.6

b.  25.2  53  23.3  101.5

Figure 3. Mapping of mutator region expressed in percent crossing over. The a. and b. figures represent isogenic Mut+ and Mut- donors respectively with a. the result of 318 colonies tested and b. the result of 270.

since a possible effect of the mutable allele of mut-1 on conjugational recombination frequencies had been noticed earlier in this laboratory (Skaar, personal communication). GW6 and Wl895 were used as donor strains differing only at mut-1 and azi loci, and each was allowed to mate with Wl177 for 45 minutes, at which time the plateau of marker entry should be reached and the affect of depression by streptomycin contra-selection should not be great. Samples were spread on TGA-lactose plus methionine, threonine, thiamine and leucine to select for transfer of the donor allele of lac. Recombinant classes resulting from such an experiment and and their frequencies are reported in Table IV. Again, the linkage maps inferrable for the two crosses are nearly identical. A more subtle effect may be reflected in the data, however, since 11 of 202 recombinants (5.5%) of GW6 matings are quadruple crossover types while only 2 of 93 (2%) are of this type using Wl895. Since the number of Wl895 recombinants tested was small, no firm conclusions can be drawn.
TABLE IV. FREQUENCIES OF DESELECTED MARKERS AMONG LAC+ PROGENY OF MATINGS. A COMPARISON BETWEEN MATINGS WHERE THE DONOR IS MUT+ AND WHERE THE DONOR IS MUT-.

<table>
<thead>
<tr>
<th>Recombinant Phenotypes</th>
<th>Crossover type</th>
<th>No. if donor is Mut+</th>
<th>Mut-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ton^rAzi^SLeu^-</td>
<td>d</td>
<td>107</td>
<td>54</td>
</tr>
<tr>
<td>Ton^S Azi^SLeu^-</td>
<td>d</td>
<td>19</td>
<td>8</td>
</tr>
<tr>
<td>Ton^S Azir^rLeu^-</td>
<td>d</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Ton^S Azir^rLeu^+</td>
<td>d</td>
<td>59</td>
<td>28</td>
</tr>
<tr>
<td>Ton^r Azir^SLeu^+</td>
<td>q</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Ton^r Azir^Leu^+</td>
<td>q</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ton^r Azir^rLeu^-</td>
<td>q</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ton^r Azir^rLeu^+</td>
<td>q</td>
<td>11</td>
<td>2</td>
</tr>
</tbody>
</table>

W1895 and GW6 were each allowed to mate with W1177 for 45 minutes at which time 0.1 ml samples of the mating mixtures were spread on TGA-lactose agar for selection of Lac+ recombinants with addition of streptomycin for contra-selection of donors. Recombinants were isolated and scored as in text. The d represents the double crossover types and the q quadruple crossover types necessary if the gene order is lac - ton - azi - leu. All Azir recombinants of the GW6 cross were also Mut+.

The precise location of mut-1

Since there are no obvious changes in mapping data produced by the mutable allele of mut-1 the results of all GW1 and GW2 transductions (all Mut+ donors) have been pooled to present a composite map of the region. This map, with linkage distances expressed as % crossing over, is presented in Figure 4.
Figure 4. A composite map of the mutator region. The figure represents the pooled results of 968 Plkc transductants using mut+ donors GW1 and GW2, a composite map of the region.
DISCUSSION

It was noticed that the results presented in Table I, the percent co-transduction of unselected markers with leu, are variable from experiment to experiment. For example, the percent co-transduction of val varies from 73% to 84% or by some 11 percentage points. The values of azi co-transduction vary by 5 percentage points and those for mut-1 by 9. This variability is probably due to experimental error, although there may have been some effect by changing the type of minimal medium used to select and test the transductants (DMA to TGA). The minimal medium was changed since the transductants were unstable when tested for requirements (or appeared to be) on Davis Minimal Agar. Each transductant culture seemed to contain a mixture of + and - cells for each requirement, and if such + and - clones were picked, streaked and purified, each seemed also to contain a mixture again. The transductants tested and recorded in Table I, b. were first selected on DMA and tested on DMA and on TGA. The TGA tests for markers showed no variable colony size but tested as they had on DMA. This media effect was pursued no farther. Even though there is variability between experiments, the % co-transduction values may always be arranged in the same sequence by order of magnitude within experiments and therefore the ordering of the loci in question is considered accurate.

Linkage maps constructed from data obtained by transduction using isogenic Mut+G2 and Mut"G3 donors indicate no effect of the presence of the mutator allele of mut-1, as measurable by these methods. This is not to say that mut-1 has no effect on recombination frequency in
transductional systems. Recombination frequency could be increased along the whole fragment such that numbers of all recombinant types would be increased without altering the relative frequency of the major types. The only unambiguous data bearing on the possible recombination effects of mut-l would be a comparison of frequencies of quadruple crossover types in the mutable and non-mutable systems. The relative frequency of these should increase with an overall increase in the probability of crossing over. Among 318 transductants by Plkc grown on a mutable donor, 3 (0.8%) were of quadruple crossover type, while of 270 transductants obtained by use of non-mutable donor, 2 (0.9%) were of quadruple crossover type. Equivalent frequencies are expected if mut-l exerts no effect. Although no mut-l effect is indicated here, the data are not numerous enough to pose as proof. The data do seem to argue however, against one specific effect, namely the "weakening" of donor DNA in the presence of mut-l, causing fragmentation and thereby distorting the linkage map.

The rather preliminary experiments designed to detect possible effects of mut-l on conjugational recombination frequencies served the purpose for which they were intended in this study. That is, they confirmed that the location of the azi locus to the "right" of leu was not an effect produced by linkage distortion due to mut-l. Since of 202 recombinants from GW6 x W1177 crosses 12 were of quadruple crossover type while only 2 of 93 from W1895 x W1177 crosses were of quadruple type, or a ratio of 6%:2%, an effect of mut-l in conjugational recombination
frequency cannot be dismissed. A tripling of the frequencies of quadruple crossovers in the mutable system is seen although the non-mutable sample size is too small to warrant any conclusion at this time. This effect is under further investigation.

The positioning of mut-l reported here agrees with that proposed by Skaar (1956) but opposes that by Gunderson et al. (1961). It is necessary to examine the data upon which this discrepancy is based. Gunderson et al. (1961) crossed an HfrC Try~ with an F"Met"Thr"Mut++. Selection was imposed for transfer of met in one experiment, for transfer of thr in another. The frequency of transfer of the wild-type allele of mut-l (Mut~) was slightly higher (61%) where selection was for met than where selection was for thr (54%). A difference of this kind would be expected if the mut-l locus is to the left of threonine; no difference would be expected if the mut-l locus were to the right of thr. A total of 96 recombinants were examined in each experiment cited. A simple chi-square calculation shows that the probability of the observed difference being due to chance alone is 0.46. In view of the present investigation, it appears that the difference was indeed fortuitous.

In the presentation of a composite map of the mutator region in Figure 5 the data from GW1 and GW2 transductions was pooled. Although there are slight differences between the frequencies of co-transduction of unselected markers and in frequencies of crossing over, as reported in Table III, of GW1 and GW2, the data are not different in the way expected, if loosening of linkage occurred. Since the data of GW2 and GW3 agree and
both are W677 derivatives, it is possible that the strain difference from
GW1, a CS19 derivative, causing difference in transducing ability by Plkc
accounts for the differences in data. The ends of the composite map may
be foreshortened due to irregularities in the size of fragments of
bacterial genome carried by Plkc, although Ikeda and Tomizawa (1965a.)
find that nearly all particles of bacterial genome carried by Pl are of
the same size, the size of the normal phage genome.

The positioning of the mut-1 gene bears on hypotheses concerning the
mode of action of mutator genes. Speyer's hypothesis (1965) is that
mutator genes in bacteria could be DNA polymerase mutants allowing produc-
tion of an enzyme permitting mistakes in base pairing as caused by the
mutant ts alleles of gene 43 in T4. Although the allelism of both ast
(Zamenhof, 1966) and mut-3 (Demerec and Miyake, 1959) with mut-1 had been
proposed the positioning of mut-1 by this study clearly shows that this
gene is not located in the same place as either of the other mutator genes.
The area encompassing the mutator genes and other previously mapped loci
may be represented as follows:

met mut-3 purA thi thr leu val azi mut-1 ton ast pro lac

During the course of mapping each mutator gene, test markers previously
mapped in non-mutator strains were found in their normal positions,
seemingly precluding any major chromosomal rearrangements between strains
as explanation of differing mutator gene locations. The fact that these
separately isolated and mapped mutator genes are not allelic seems to
contradict the polymerase hypothesis, unless it is supposed that there is
more than one polymerase locus, as do the findings that both mut-l (Pierce, 1966b) and ast (Zamenhof, 1967) increase rates of mutation in the virulent phage T4, which specifies its own DNA polymerase.

In the light of this evidence the hypothesis of Kirchner (1960) seems more plausible. He proposed that the action of mut-3 involves production of a mutagenic substance, possibly an aberrant base, causing pairing mistakes during DNA replication. Such a substance would affect T4 mutation rates.

Since DNA repair enzymes which are able to repair mutations caused by several different agents (Boyce and Howard-Flanders, 1964; Kohn et al., 1965) have been described and it has been suggested that they may recognize sequences along DNA molecules thereby influencing the net mutability of a segment (Zamenhof, 1967), it is possible that the mutator genes described could be sites of production of such enzymes. These enzymes might (in mutant form) influence mutation rates. At this time no sites of repair control have been mapped in the exact positions as the mutator genes. Phage T4 carries its own gene for a UV repair enzyme (u+) (Stahl et al., 1961) just as it does its own DNA polymerase locus; therefore, the effects of mutator genes on T4 may also argue against this hypothesis.

The result of the action of mut-l, as inferred from amino acid substitutions in mutant proteins of the A cistron of tryptophane synthetase (Yanofsky, 1966), is a unidirectional A-T to C-G base transversion in DNA. This finding explains the allele specificity of mut-l and suggests a definite role of transversions in the evolution of higher G-C
content in DNA, a supposition made before by Freese (1963) but this is the first demonstration of the existence of transversions in vivo.

In the presence of some mutagenic agents such as nitrosoguanidine (NTG), resistant mutants have a lower spontaneous rate of mutation than normal, and they revert to normal when the mutagenic agent is removed (Woody-Karrer and Greenberg, 1964). The system, therefore, moves to correct any disruption of equilibrium (mutability compensation) in accord with the principle of Le Chatelier (Zamenhof, 1967). This would imply that mutator strains should always be selected against but this is not always true. It has been noticed that ast strains are at a selective advantage when grown in mixed populations with wild type strain B of E. coli (Zamenhof and deGiovani, 1958) while mut-1 strains are at a definite disadvantage when grown with E. coli B, although they have not been tested against isogenic non-mutable K12 strains (Mecklenburg, personal communication). In the case of NTG resistance, UV resistance was also indicated in the mutants, implicating enzymic repair mechanisms.

It has been estimated that 20% of all mutations are undetectable due to the degeneracy of the genetic code (Goldberg and Wittes, 1966) and mutations involving essential genes and causing death, those involving other regions than essential ones in proteins or those in regulatory regions may never be noticed. If the hypothesis of Kirchner (1960) is true it is then possible that a mutagenic substance such as an aberrant base may be present in relatively high concentrations in mutable cells of mut-1 strains. Experiments designed to detect such a substance are now in progress.
The precise location of mut-1, one of three separately isolated mutator genes which have been studied extensively in bacterial systems, has been determined by the use of transduction and Hfr x F" timed mating methods. It has been found to map very close to the locus for sodium azide resistance (azi), between azi and the locus for T1,T5 resistance (ton).

Mut-1 is thus not allelic to either of the other two mutator genes (ast in E. coli, mut-3 in S. typhimurium). The existence of three mutator loci indicates that the mutant DNA polymerase model is inadequate as a general explanation for all instances of mutability.

Some mutagens have been found to produce effects on mapping data, such as "loosening of linkage" by UV irradiation, therefore, the possibility of such an effect produced by mut-1 was investigated. Paired transduction experiments using isogenic mutable and non-mutable phage donor strains showed no measurable effects of mut-1 on linkage distances. Paired conjugations using isogenic mutable and non-mutable derivatives of HfrC as donors suggested a slight increase in numbers of quadruple crossover types caused by mut-1 although their effect on timed mating data used for location of mut-1 was negligible.

Since the effects of mut-1 on linkage data are negligible, the mutator genes mut-1, ast and mut-3 may be ordered as follows with respect to other well-known loci in the region.
LITERATURE CITED


Warren, C.I.
Studies on a mutator gene in Escherichia coli.