AN ABSTRACT OF THE THESIS OF

Carole Mahaney Kurahara (Name of student) for the Master of Science (Degree)

in Microbiology (Major) presented on May 4, 1967 (Date)

Title: ANALYSIS OF SEGREGANTS OF F'GAL HETEROGENOTES IN ESCHERICHIA COLI

Abstract approved: Redacted for Privacy

Dr. D. K. Fraser

The purpose of this study was to isolate and characterize any segregants of the heterogenotes formed from sexduction by an F' donor. Following the mating of F' W4520, carrying an F' $\underline{gal}_1^+$ $\underline{gal}_2^+$ factor, with W3350, a $\underline{gal}_1^-$ $\underline{gal}_2^-$ recipient, a number of recombinant clones were picked and examined for segregants. From 5 such clones, 50 segregants of the gal^+ phenotype and 50 of the gal^- phenotype were isolated. The genotypes of these segregants were determined as follows. The gal^- segregants were examined to determine the genotype of any F factors carried, by crosses against three gal^- recipient types, which were streptomycin resistant mutants isolated early in the study. The failure of these segregants to produce recombinants in these crosses was taken as evidence that no F factors of the gal^+ type were carried by these segregants. The gal^- segregants were also analyzed for their chromosomal genotype

by crosses against two gal^- F' homogenotes formed earlier in the study. Again, failure of these crosses to produce gal^+ recombinants was noted and taken as evidence that these gal^- segregants carried chromosomal genotypes of the $\underline{gal}_1^- \underline{gal}_2^-$ type.

By a similar procedure, the 50 gal^+ segregants were examined to determine the genotype of any F factors carried, by crosses against the same 3 gal^- recipient types, which in this case, were azide resistant mutants isolated early in the study. The formation of gal^+ recombinants from these crosses was taken as evidence that these 50 gal^+ segregants carried F' factors of the type F $\underline{gal}_1^+ \underline{gal}_2^+$. The segregants were cured of these F factors with acridine orange and their chromosomal genotypes determined by crosses against the same two gal^- F' homogenotes used earlier. The absence of gal^+ recombinant formation from these crosses confirmed that these segregants carried chromosomes of the $\underline{gal}_1^- \underline{gal}_2^-$ type.

It was found that no variation of genotype existed among the 50 segregants of each type. The 50 gal^- strains were concluded to be either haploid parental types, with the genotype $\underline{gal}_1^- \underline{gal}_2^-$, resulting from loss of the F factor in the sexductional heterogenotes, or possibly homogenotes of the type $\underline{gal}_1^- \underline{gal}_2^- / F\ \underline{gal}_1^- \underline{gal}_2^-$, which were undetectable in this system. The 50 gal^+ segregants, carrying the genotype $\underline{gal}_1^- \underline{gal}_2^- / F\ \underline{gal}_1^+ \underline{gal}_2^+$, were of the same type as the heterogenotes from which they were segregated. Since no

recombinant types were found, it was concluded that the F' factors, in this system, undergo recombination with their host chromosome, during sexduction, at lower frequencies than expected from analogy with transduction by bacteriophage.

Analysis of Segregants of F'gal Heterogenotes in *Escherichia coli*

by

Carole Mahaney Kurahara

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1967

APPROVED:

Redacted for Privacy

Associate Professor of Microbiology
in charge of major

Redacted for Privacy

Chairman of Department of Microbiology

Redacted for Privacy

Dean of Graduate School

Date thesis is presented May 4, 1967

Typed by Donna Olson for Carole Mahaney Kurahara

ACKNOWLEDGMENTS

What we easily learn, we quickly forget; what we learn through suffering remains.

c. g. k.

The author wishes to express sincere gratitude to the following persons.

To Dr. D. K. Fraser in appreciation of her ability to encourage independent thought.

To the staff and graduate students of the Department of Microbiology. Special thanks go to Dr. W. E. Sandine for his encouragement and example.

To Willie C. Brown and Patti Jo Laudermilk for the support and understanding only the best friends can provide.

Special appreciation goes to my parents, Gene and Dorothy Mahaney, for giving me the courage to begin and the fortitude to complete this endeavor. To my husband, Jan, for his numerous sacrifices and constant encouragement, a special thank you.

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INTRODUCTION

Of the three mechanisms of genetic exchange known in bacteria: transformation, transduction and conjugation, the latter in particular, has been of great importance in linking the concepts of bacterial genetics to those of the classical genetics of higher organisms. The ability of Escherichia coli to conjugate and transfer genetic material to recipient cells resides in a discrete genetic determinant termed fertility or "F" factor, found only in donor cells.

The sex factors of E. coli K12 are members of a group of genetic determinants designated "episomes" by Jacob and Wollman (1961). Episomes are characterized by the fact that they are non-essential to the bacterial cell and are able to exist in two distinct states: the autonomous, independently replicating, cytoplasmic state, and the integrated or chromosomally attached state. In addition to F or sex factors, four other classes of episomic elements in bacteria have been investigated: temperate phage, colicinogenic factors, resistance transfer factors, and a transmission element of lactose determination in Salmonella typhosa, F_o-lac. Of these, temperate bacteriophage provide the clearest and best-defined example of the episome concept, and probably show the greatest similarity to

the F factor.

Differentiation of E. coli strains into donor (male) cells and recipient (female) cells is determined by the F factor, which is present in donors but absent in recipients. Donors which contain the F factor in the autonomous state are designated F^+, while donors harboring the F factor in the integrated state are designated Hfr. Such Hfr donors are isolated from F^+ populations and transfer chromosomal material from host cells to recipient cells at high frequency. In addition to the above two donor types, a third intermediate type, designated F prime (F'), has been identified. Such donors are thought to arise through genetic exchange between the sex factor and chromosome during the integrated state, followed by a return of the sex factor and its incorporated segment of chromosome to the cytoplasmic state. The process of genetic transfer by means of F' sex factors is termed F-duction or sexduction, and is quite analogous to transduction by bacteriophage.

In the present study, segregants of heterogenotes formed from sexduction by an F' donor were isolated and analyzed. By characterization of the genotypes of such segregants, information about the recombinational events occurring during sexduction could be obtained.

HISTORICAL REVIEW

Determination of Mating Types

Sexual differentiation in Escherichia coli K12 is determined by the presence or absence of a sex or fertility factor (F). Cells possessing this factor behave as males, or donors, while females, or recipients, lack this factor. In the case of F^+ donors, the sex factor is structurally independent of the bacterial chromosome, is capable of autonomous replication, and is transferred to recipients at a high frequency during conjugation (Cavalli-Sforza, Lederberg, and Lederberg, 1953; Hayes, 1953a; Lederberg, Cavalli, and Lederberg, 1952). A second type of donor, termed Hfr (high frequency of recombination), originates from F^+ populations. Such Hfr donors generate about one thousand times more recombinants when mated to F^- recipients than do equivalent F^+ donors. The F factor has become integrated into the cell chromosome in Hfr donors, and determines the linear oriented transfer of the chromosome beginning with the leading end, termed the origin. The sex factor itself, at least part of which is located at the terminal end of the chromosome during transfer, is transferred rarely due to spontaneous breakage of the chromosome. Only those recombinants which have received the terminal markers become Hfr donors, the remainder become F^- recipients of the recombinant type (Hayes, 1953b; Jacob and Wollman,

1961). It has been suggested that as a consequence of the integration of the F factor into the chromosome, the sex factor may be divided into two sections during transfer, one at each end of the chromosome. Recombinants which receive one section of F and not the other are defective donors which may become normal Hfr donors if allowed to acquire the other section of F by a second mating with an F^+ donor (Hayes, 1965, p. 660). The recombination event proposed as the mechanism by which integration of the sex factor and the chromosome occurs in Hfr donors will be described in detail later. A number of Hfr types have been isolated which differ in the location of the integration site of the sex factor and the chromosome. Such donors may differ both in the order of markers transferred and in the direction of transfer itself. In addition to these conventional types, two variant Hfr strains have been reported which differ in the number of sites of integration of a particular F factor and the host chromosome (Clark, 1963; Low, 1967). Matney et al. (1964) have prepared a map showing 17 attachment sites of the F factor of 44 known Hfr strains.

In addition to the above two donor types, F^+ and Hfr, a third type, termed F-prime (F'), has been isolated which possesses properties intermediate between F^+ and Hfr types. F' donors arise from Hfr populations and appear to carry a sex factor which has incorporated a segment of the bacterial chromosome. Such donors

therefore possess a region of homology between the sex factor and the chromosome. Integration of the sex factor at that point results in a high frequency chromosome transfer with the same orientation as in the Hfr parent. F' donors are therefore able to transfer their sex factor, with its incorporated chromosomal segment, at the same high frequency as F^+ donors and are also able to transfer other chromosomal markers at a fairly high frequency as in Hfr donors (Adelberg and Burns, 1959, 1960). The properties of such F' donors will be discussed below.

Nature of the F' State

An F' donor was first isolated from an Hfr strain by Adelberg and Burns (1959, 1960) and differed from the parental donor type in several ways. It transferred its chromosome with the same oriented sequence but only at about one-tenth the efficiency of the Hfr. The sex factor itself was transferred to recipients with the same high efficiency as in F^+ donor strains, but the recipients became intermediate donors and not F^+ cells; that is, the sex factor had acquired a memory of its Hfr location as a result of integration of chromosomal genes into the episome. Another difference noted between F' and Hfr strains, was the fact that treatment of F' donors with acridine orange resulted in a loss of the sex factor with its associated chromosomal genes, and conversion of the cells to

recipients. However, re-infection of these resulting F^- cells with a normal F^+ factor resulted in a return to the intermediate donor state. The original locus on the Hfr chromosome had preserved its affinity for the sex factor, possibly because of the incorporation of some genetic material from the sex factor into the chromosome at this site. This chromosomal site was termed sfa or sex factor affinity locus (Adelberg and Burns, 1960). Richter (1957) had also described a chromosomal locus which possessed a high affinity for a wild type F; however, the sex factor was lost in this case, making further observation of it impossible.

A short time after the original observation of the F' donor type, Hirota (1959, 1960) isolated a series of five similar F' variants, carrying different chromosomal genes. These F' episomes were also removable by acridine treatment (Hirota and Iijima, 1957). Jacob and his associates also reported another series of such variant clones isolated by screening stock Hfr cultures (Jacob and Adelberg, 1959; Jacob, Schaeffer, and Wollman, 1960).

Relatively little work has been reported describing the actual physical and chemical nature of F; however, the size and homology of F' DNA in relation to E. coli K12 chromosomal DNA was measured by Falkow and Citarella, using agar gel columns (1965). An F' genote consisting of F and the lac gene cluster was found to be equal to 2.5 percent of the E. coli genome. The wild type F alone was

shown to be 1.9 percent of the bacterial chromosome in size, or equal to about 1×10^5 nucleotide pairs. This F factor alone was shown to consist of three regions: 1) a region of about 40 percent of the genome which is homologous with the E. coli genome and is presumably active in host-episome relationships (DNA of 50 percent G + C content), 2) a region equal to about 50 percent of the F genome, non-homologous with the host chromosome, and concerned purely with sex factor activities (DNA of 50 percent G + C content), and 3) a region of 10 percent of the genome, also non-homologous and probably concerned with a specific function of the sex factor (DNA of 44 percent G + C content).

The number of sex factors per cell has been estimated from several types of experiments. First, the amount of β-galactosidase produced by heterogenotes carrying an F-lac$^+$ factor was slightly more than twice that yielded by haploid lac$^+$ bacteria (Jacob and Wollman, 1961). Secondly, Driskell and Adelberg (1961) have shown that when uninucleate F^+ cells are mixed with F^- recipients, the F^+ lose their sex factors to become F^- at the same rate as the recipients become F^+. This suggests that uninucleate cells have one sex factor (Hayes, 1965). Thirdly, F^+ cells which have accepted an autonomous F' lac factor appear to lose their resident factor, as if one excludes the presence of others (Scaife and Gross, 1963). The above results indicate that the average number of F per cell is

small, possibly one per nucleus. Stouthamer, de Haan, and Bulten (1963), using acridine orange curing, calculated that the average number of copies of F was two at cell division, and that only one F was transferred per donor with rapid multiplication after transfer resulting in not more than three to four F factors per cell.

Formation of F' Donors

The episomic elements which are known to be able to undergo attachment to the bacterial chromosome include λ bacteriophage and F factors. Campbell (1962) proposed a model for the integration of these structures with the cell chromosome, based on pairing followed by crossing-over (Figure 1). Campbell's model was developed as an explanation of λ phage interaction with the host chromosome. Vegetative phage becomes integrated with the host chromosome at a specific region of homology possessed by both structures, resulting in the prophage state. Normally, this integration may be reversed by prophage induction, and the phage is again released as an autonomous element. If, however, improper crossing-over occurs, the gal locus of the chromosome is replaced by a segment of the phage genome and results in the release of λ dg, that is, defective phage units carrying the chromosomal gal locus. The formation of the F' state may occur by a similar mechanism. Proper "relooping" and crossing-over of an Hfr chromosome with its integrated

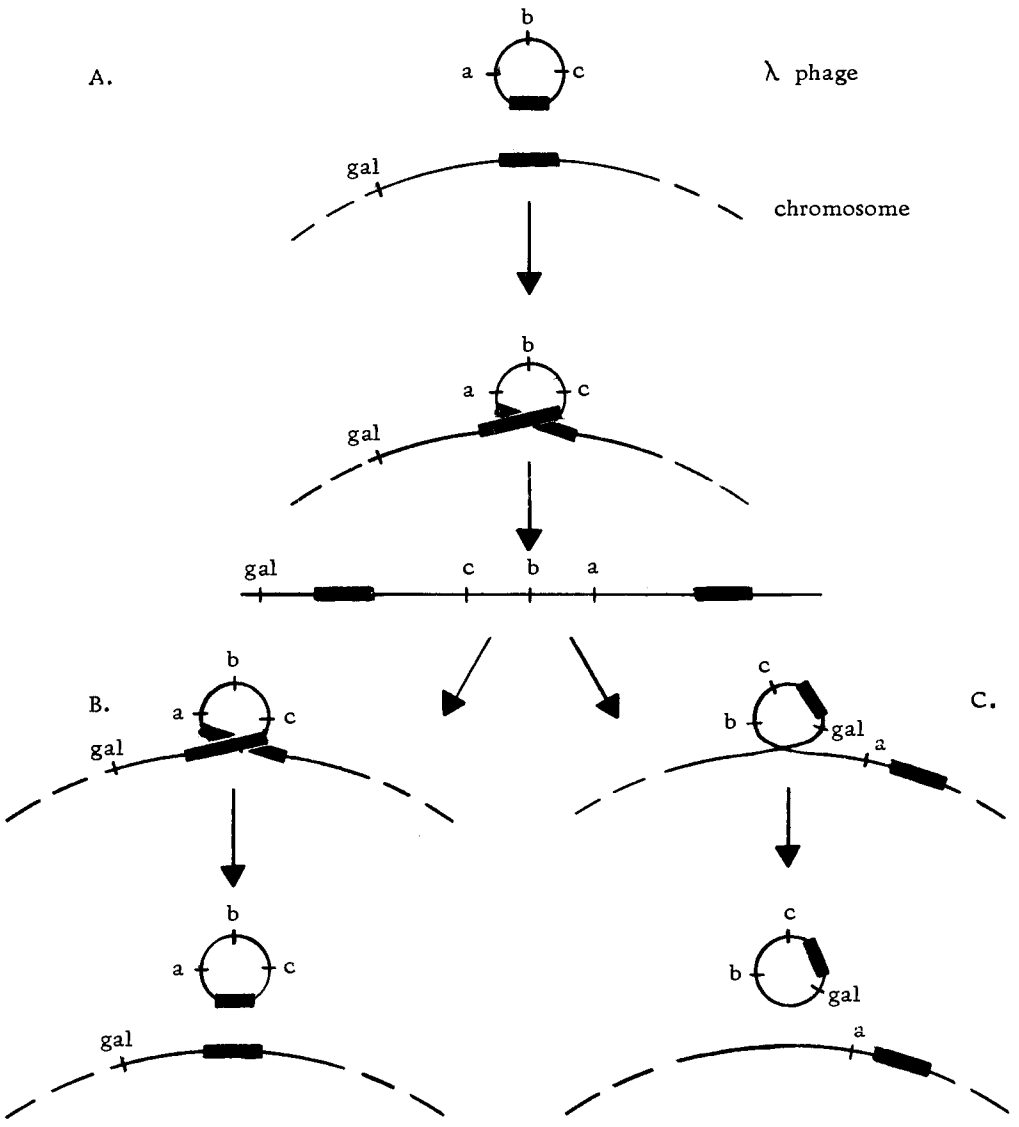

Figure 1. Interaction of λ phage and host chromosome.

A. Pairing between homologous regions (-▬-) of bacterial chromosome and phage, followed by crossing over, resulting in prophage state.

B. Reversal of integration process, leading to release of normal phage.

C. Improper "re-looping", followed by crossing over, resulting in formation of λ dg (Campbell, 1962).

F would lead to the F^+ state. However, if the Hfr chromosome were to re-loop imperfectly, a sex factor carrying a piece of chromosomal DNA would result, as well as a chromosome in which a section of variable length had been deleted and replaced by F DNA. This model would thus explain the sex factor affinity locus, described earlier, as consisting of a fragment of F DNA incorporated into the chromosome.

Broda, Beckwith, and Scaife (1964) presented a model for the formation of an F' factor carrying both proximal and distal regions of the parental Hfr chromosome (Figure 2). It was proposed that F' sex factors are formed by a reciprocal genetic exchange between two sites on the bacterial chromosome, in the two regions on either side of the inserted sex factor. Such an exchange resulted in two circular structures: the sex factor with its incorporated chromosomal loci, and the chromosome itself, which would show a deletion of these loci. Scaife and Pekhov (1964) and Scaife (1966) demonstrated the existence of a deletion in the strain from which this particular F' had arisen.

Alternation of States in F' Donors

F' sex factors are characterized by the fact that, unlike the F factor in the parental Hfr types, they are able to exist autonomously in the cell and undergo transfer to recipient cells independently of

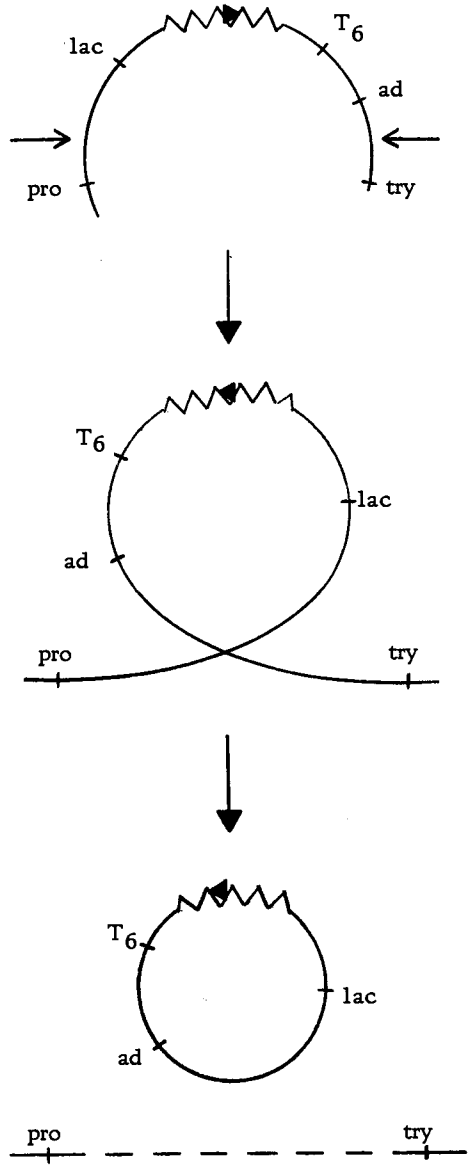

Figure 2. F' factor formation. Crossing over between two sites of homology (represented by arrows) on the Hfr chromosome with its inserted F, resulting in the formation of an F' factor with its incorporated chromosomal loci, and a deletion (represented by the broken line) in the chromosome (Broda, Beckwith and Scaife, 1964).

the host chromosome. In addition to this independent state, F' sex factors also possess the ability to become integrated into the host chromosome and promote its transfer, at a high frequency, to recipients.

In the autonomous state, F' sex factors behave as "replicons" or genetic units which are capable of independent replication (Jacob, Brenner, and Cuzin, 1963). In the Hfr state, on the other hand, the F replication system has become non-functional and the F factor is replicated as part of the chromosomal replicon. This model was substantiated in part on the basis of isolation of thermosensitive mutants carrying an F' lac factor. The F factor of such mutants was capable of independent replication at low temperatures but lacked this ability at high temperatures. It was demonstrated that at high temperatures the F' factor was usually lost, resulting in lac^- cells. Occasionally, lac^+ cells were found in which the F' factor had become integrated into the host chromosome and was able to promote chromosomal transfer to recipients.

The chromosome transfer by F' donors, which occurs as a consequence of integration of the F' sex factor into the host chromosome, was studied by Scaife and Gross (1963). It was concluded that such a transfer occurred as a result of a crossover in the F' donor between the F-merogenote and the homologous chromosomal region. A linear structure, with its incorporated sex factor and possessing

the lac segment in duplicate, was thus transferred to recipient cells, where further recombination could occur with the corresponding region of the recipient chromosome. This model predicts that transfer of chromosomal markers in F' strains is delayed compared with transfer of the same markers in parental Hfr strains. Such a delay was reported by de Haan and Gross (1962), de Haan and Stouthamer (1963), and by Matney and Achenbach (1962), using different F' systems. It was concluded by Pittard, Loutit, and Adelberg (1963) that the delay observed in the F' transfer of the chromosome corresponds to the time required to transfer the "merogenote" or segment of genetic material incorporated into the F factor. After extensive analysis of zygotes formed from recipients which had received the F' donor chromosome, Pittard and Adelberg (1964) concluded that F' strains transfer their F-merogenote with 100 percent efficiency, and that chromosomal transfer occurs only as a consequence of recombination between the chromosome and F-genote. If this is so, then in F' strains in which no region of homology between chromosome and F-genote exists, no chromosome transfer should occur. Pittard and Ramakrishnan (1964) demonstrated that in F'_{14}, which carries a chromosomal deletion equal to the entire F-genote, chromosome transfer occurs only at barely detectable frequencies.

The crossing over event in F' cells which leads to chromosome

transfer is analogous to the crossing over event which produces an Hfr strain from an F^+ strain. However, the latter transition appears to be quite stable, while in the F' case the integration of F into the chromosome is thought to be highly reversible (Adelberg and Pittard, 1965). These authors assigned possible rate constants to the transition between autonomous and integrated states in F' cells. Their interpretation was that in the F' case, the F-merogenote exists autonomously about 90 percent of the time and is integrated and capable of recombination only about 10 percent; the two states are thus in rapid equilibrium. However, evidence in support of the fact that recombination occurs between the F factor and the chromosome in F' cells rests only on the fact that chromosomal material is transferred from F' donors to recipient cells by the integrated sex factor. The possibility for transfer to occur is thus restricted to the 10 percent of the time during which the F factor is integrated into the chromosome of the F' cell.

Association of Chromosome and Episome

To explain the association of λ prophage to the host chromosome, Jacob and Wollman (1961) proposed a hypothesis based upon attachment of the prophage to the chromosome, either along the entire length of the phage or at a specific region. It was suggested that a similar attachment of episome to chromosome might exist in

the case of the F factor.

Campbell (1962) has proposed a model, which is now generally accepted as the case, predicting that the prophage or episome is actually inserted into the host chromosome and not merely attached. Pairing and recombination between the circular episome and chromosome occurs at a site of homology, and the episome is inserted linearly into the chromosome. In the case of prophage, induction would result from a re-looping of the circular chromosome, bringing the homologous regions together and allowing recombination which again returns the phage to an autonomous state. A defective re-looping may explain λ dg formation, just as such a defective re-looping in the case of an inserted F in an Hfr cell might cause the formation of an F' or an intermediate donor (Broda, Beckwith, and Scaife, 1964).

Evidence in support of this insertion model was further supplied by Campbell (1963) in studies of the segregation patterns of lysogenic heterogenotes. Observations which substantiate Campbell's model were also reported in 1965 by Franklin et al. who showed that deletions, in particular lysogenic bacteria, involved the simultaneous elimination of bacterial genes and segments of prophage genome.

Transduction by λ dg, which may be formed according to Campbell's model (Figure 1), results in the formation of

heterogenotes of the type $\underline{gal}^- / \lambda \underline{gal}^+$. Such partial diploids, possessing both the gal^- allele of the recipient and the gal^+ allele of the donor, continue to yield haploid gal^- segregants of the parental type, through loss of the phage or exogenote. In addition, recombination occurs between the gal regions of the endogenote and exogenote, resulting in the segregation of homogenotes of the type $\underline{gal}^- / \lambda \underline{gal}^-$. In highly efficient or HFT transduction (Morse, Lederberg, and Lederberg, 1956) involving phage of the type $\underline{gal_1}^- \underline{gal_2}^+$ and recipients of the type $\underline{gal_1}^+ \underline{gal_2}^-$, both cis and trans heterogenotes are segregated. Complementation tests involving the trans heterogenotes have been performed to determine whether gal^- mutations belong to the same or different functional units, or cistrons. Extensive analysis of the segregants from heterogenotes formed by transduction with λ phage has been done by Lederberg (1960).

The process of genetic transfer of chromosomal loci by means of F factors, termed F-duction or sexduction, is analogous to transduction in bacteriophage (Jacob and Adelberg, 1959). Heterogenotes of the type $\underline{z}^-/F\underline{z}^+$ result from matings of a donor F' carrying the wild type allele and recipients carrying the recessive allele. Such heterogenotes segregate homogenotes of the type $\underline{z}^-/F^-\underline{z}^-$ as a result of recombination between exogenote and endogenote. Also segregated, but less frequently, are haploid types possessing the genotype of the recipient parent. Complementation tests may be

performed involving sexduction by F' factors carrying a pair of mutational sites, for example, $\underline{z}_1$ and $\underline{z}_2$.

Hirota and Sneath (1961) noted the similarity between F' heterogenotes and transductional heterogenotes such as produced by λ*gal* and P1*lac*. However, in contrast to the case of transduction by phage, no extensive analysis of segregants from heterogenotes formed by F' sexduction has been reported to date.

METHODS AND MATERIALS

Bacterial Strains

Four strains of Escherichia coli K12, obtained from the stock collection of Dr. D. K. Fraser at Oregon State University, were used throughout this work (Table 1). These strains consisted of one F' donor and three F^- recipients, all prototrophs.

Table 1. Description of bacterial strains.

Strain Number	Galactose Utilization[a]	Response to Streptomycin[b]	Response to Azide[b]	Genotype
W4520	$\underline{Gal}^+$	S^s	A^s	F' $\underline{Gal}_1^+$ $\underline{Gal}_2^+$
W3101	$\underline{Gal}^-$	S^s	A^s	F^- $\underline{Gal}_1^-$ $\underline{Gal}_2^+$
W3102	$\underline{Gal}^-$	S^s	A^s	F^- $\underline{Gal}_1^+$ $\underline{Gal}_2^-$
W3350	$\underline{Gal}^-$	S^r	A^s	F^- $\underline{Gal}_1^-$ $\underline{Gal}_2^-$

[a] + indicates ability to ferment galactose.
- indicates lack of ability to ferment galactose.

[b] s indicates ability to grow in presence of agent.
r indicates lack of ability to grow in presence of agent.

Strains W3101 ($\underline{gal}_1^-$) and W3102 ($\underline{gal}_2^-$) are non-galactose fermenting mutants lacking in transferase and kinase activity respectively; W3350 is a double mutant (Lederberg, 1960). The strains with gal^+ phenotype appear as dark green shiny colonies while the gal^- strains appear as pink mucoid colonies, on appropriate media.

Culture Propagation and Preservation

Cultures were maintained by storage at 4°C on sterile nutrient agar slants. Log phase cultures were prepared by adding a one percent inoculum of the cells to sterile Luria (L) Broth (Table 2) followed by incubation at 37°C with shaking for 3 hours, resulting in broth cultures of approximately 2 x 10^8 cells/ml.

Table 2. Composition of luria (L) broth medium.

Ingredient	Grams per liter
Tryptone	10.0
Yeast Extract	5.0
NaCl	5.0
Agar[a]	15.0
Distilled Water	1 liter
pH is adjusted to 7.4	

[a]Added when Luria agar was desired.

Before use in conjugation experiments, carbohydrate utilization and response of the strains to streptomycin and azide was confirmed by exposure to eosin methylene blue (EMB) agar containing appropriate concentrations of the carbohydrate and antibacterial agents (Table 3).

Table 3. Composition of eosin methylene blue (EMB) agar.

Ingredient	Grams per liter
Difco EMB agar base[a]	27.5
Distilled water	1 liter
Galactose	10
Streptomycin sulfate[b]	
Sodium azide[c]	

[a]Base contains peptone, dipotassium phosphate, agar, eosin Y and methylene blue indicators.

[b]Sterilized separately and added, when desired, to final concentration of 1000 μg/ml.

[c]Sterilized separately and added, when desired, to final concentration of 125 μg/ml.

Conjugation Technique

Conjugation of the F' donor cells and F^- recipients was carried out according to a modification of the methods of Adelberg and Burns (1960) and de Haan and Gross (1962). Log phase cultures of donors and recipients were prepared by adding a one percent inoculum of an overnight culture to L broth, followed by incubation with shaking for three hours at 37°C. Counting in a Petroff-Hauser cell counter at this time showed approximately 2×10^8 cells/ml. The donor cells were then diluted to 10^4 per ml and recipients to approximately 10^7

per ml. A mating mixture of donors to recipients in a 1 to 10 ratio was prepared and incubated statically at 37°C for 1 hour to allow mating. One tenth of one ml of the mixture was then spread on EMB agar to which one percent galactose had been added, thus giving a concentration of recipients of about 10^6 per plate and, more important, a concentration of donors of about 10^2 per plate which gave a maximum number of recombinants of about 10^2 per plate. Recombinant selection was done by adding either streptomycin or azide to the agar to prevent growth of the gal^+ donors. Gal^+ recombinants appeared as small raised colonies or papillae which outgrew the gal^- recipient background after incubation of the plates at 37°C for two days. Papillae were then picked with a sterile toothpick or wire needle, resuspended in L broth, and streaked on the same media to give isolated colonies. Controls for the conjugation experiments consisted of agar plates of donors or recipients only, diluted to the same extent as on the recombinant plates. No growth of the donor strains occurred, since the cells were sensitive to the particular agent used. The recipient strain was able to grow, but formed no gal^+ papillae.

In cases where conjugation of a large number of strains was necessary, two drops of a log phase culture of recipient cells were added to two drops of a log phase culture of donor cells previously diluted 1:10. The mixture was incubated 1 hour at 37°C statically

and a loopful spread on EMB agar to which 1 percent galactose had been added as well as streptomycin or azide to prevent donor development on the plates. The spots, 10 per plate, of the conjugation mixtures, were examined following incubation of the plates for 2 days at 37°C. Gal^+ recombinants appeared as papillae in the gal^- background of the spots. A spot of the particular recipient used in the crosses on a given plate was also spread on the plate as a control.

Isolation of Streptomycin Resistant and Azide Resistant Mutants

Since an antibacterial agent was added in certain crosses to the recombinant selection plates to prevent the development of donors, it was necessary in these cases that the recipient cells be resistant to the agent used, to allow development of recombinants on the plates. In the case of the streptomycin resistant mutants, the single step method of W. C. Brown (1962) was used. Log phase cultures were concentrated by centrifugation and resuspended in sterile saline, to give at least 10^9 cells/ml. One ml of cells was spread on EMB agar containing 1 percent galactose and 1000 μg/ml streptomycin sulfate. The plates were incubated overnight at 37°C with porcelain covers to allow drying and then for 2 days more at the same temperature. Any streptomycin resistant colonies were picked from the plates with a sterile wire needle and restreaked on EMB agar containing 1000 μg/ml streptomycin sulfate to obtain stable

streptomycin resistant mutants. Mutants were designated S^r.

Similarly, recipients resistant to 125 μg/ml sodium azide were obtained by exposure of a large number of cells to agar plates containing 125 μg/ml sodium azide. Such mutants were designated A^r.

Acridine Orange Curing

The method of Hirota (1959) was used to inactivate or eliminate the F factor in the F' donors. Log phase cultures of the F' donors were diluted to a final concentration of 10^4 cells/ml in L broth at pH 7.6, containing 50 μg/ml of acridine orange. The mixture was incubated overnight, followed by dilution and plating to yield isolated colonies. Curing of the strains was confirmed by crosses against appropriate recipient strains. Untreated donor strains were crossed against the same recipients, as controls.

Isolation of Gal^- F' Strains

Two donor strains were isolated as a result of recombination between the W4520 donor and the W3101 and W3012 recipients. In one case, the F' donor, W4520, was mated to the $\underline{gal}_1^-$ strain, W3101. Several possible recombinant types could result including: 1) $\underline{gal}_1^- \underline{gal}_2^+$ / F $\underline{gal}_1^+ \underline{gal}_2^+$ strains, which give a gal^+ phenotype on EMB-gal agar; 2) $\underline{gal}_1^- \underline{gal}_2^+$ types, which are strains of the

same type as in the first case, that have become haploid through loss of F; and 3) $\underline{gal}_1^- \underline{gal}_2^+$ / F $\underline{gal}_1^- \underline{gal}_2^+$ strains, which are gal^- recombinants of the homogenote type. In the case of the same donor, W4520, crossed to the $\underline{gal}_2^-$ recipient, W3102, similar results could be obtained. It was the third type in each case which was the desired gal^- F' homogenote, and hence was isolated as follows. After mating of the W4520 F' donor with W3101 ($\underline{gal}_1^-$) and W3102 ($\underline{gal}_2^-$), a large number of recombinant papillae were picked and streaked on the same media to reveal the galactose phenotype of the colonies. Any gal^- colonies were picked and tested for their donor ability, since those from the second type which had lost the F would obviously carry no donor ability while those of the third type would exhibit such ability. The gal^- colonies which were picked were isolated and mated to each of the three gal^- recipients, W3101, W3102, and W3350. The $\underline{gal}_1^- \underline{gal}_2^+$ / F $\underline{gal}_1^- \underline{gal}_2^+$ recombinant donors would give gal^+ papillae on crosses with the $\underline{gal}_1^+ \underline{gal}_2^-$ recipient (W3102) only. The other donor, $\underline{gal}_1^+ \underline{gal}_2^-$ / F $\underline{gal}_1^+ \underline{gal}_2^-$ would give gal^+ papillae on the $\underline{gal}_1^- \underline{gal}_2^+$ recipient (W3101) only. Thus, two gal^- F' donor strains were isolated with $\underline{gal}_1^- \underline{gal}_2^+$ / F $\underline{gal}_1^- \underline{gal}_2^+$ and $\underline{gal}_1^+ \underline{gal}_2^-$ / F $\underline{gal}_1^+ \underline{gal}_2^-$ genotypes. Somewhat contrary to expectation, the gal^- F' donors occurred with fairly high frequency, while the haploid parental gal^- types were found only rarely. These two donors were particularly

useful in conjugation experiments in that the addition of streptomycin or azide to recombinant selection media was unnecessary. Even though these strains carried the F and hence possessed donor ability, they lacked the gal^+ phenotype which would interfere with the observation of gal^+ recombinant papillae in mating experiments.

Isolation and Characterization of Segregants

Sexduction by the F' donor, W4520, into the double mutant recipient, W3350, yielded sexductional heterogenotes similar to the transductional heterogenotes of λ phage. The main part of this study was concerned with the isolation and analysis of segregants from heterogenotes resulting from the F' sexduction. Throughout this paper, the word "segregants" refers to this group of strains isolated from the original sexduced cells, whether the strains are altered or unaltered from the original type. The procedure followed in this isolation and analysis is outlined in Figure 3.

As outlined in Figure 3, 30 recombinant papillae resulting from sexduction by the F' donor were streaked to give isolated colonies. From each of 5 of these 30 streaks, 10 gal^+ and 10 gal^- segregants were picked for further analysis. These 50 gal^+ and 50 gal^- strains were checked for stability by streaking to isolate any further segregants.

Both types of segregants were then analyzed for the genotype

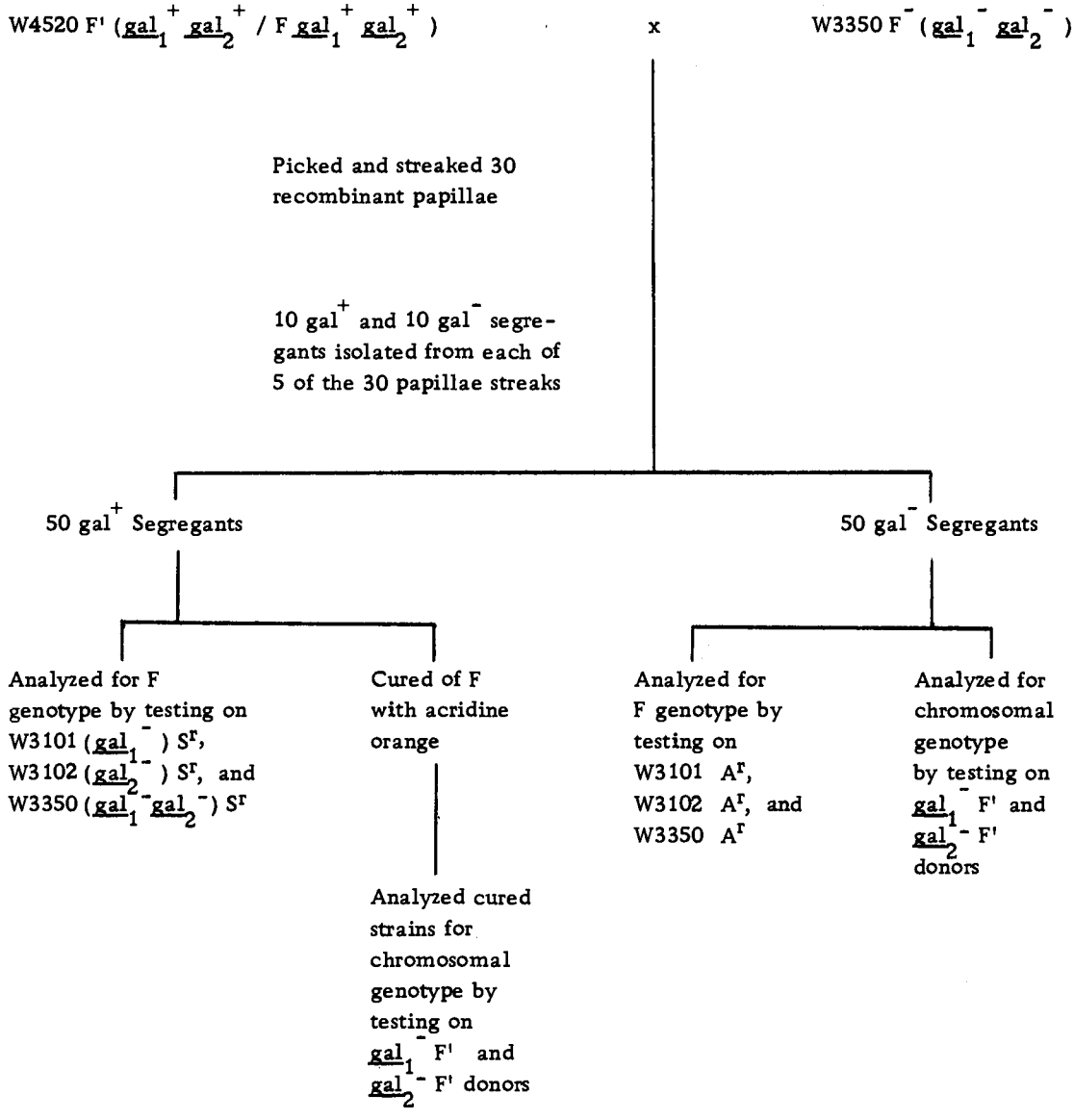

Figure 3. Isolation and analysis of segregants from sexduction by F' gal_1^+ gal_2^+ donors.

of any F factor still present and the genotype of their chromosomes, by crosses on appropriate gal^- strains. First, the 50 gal^- segregants were crossed on the 3 gal^- recipients, W3101, W3102, and W3350, to determine whether these segregants still carried the F factor, that is, were still diploid types. The formation of gal^+ recombinants from these crosses would indicate that these gal^- segregants had transferred F of the type F $\underline{gal}_1^+ \underline{gal}_2^-$ or F $\underline{gal}_1^- \underline{gal}_2^+$. The lack of recombinant formation would indicate that the F segment in the heterogenote had been lost, or possibly that an F factor of the type F $\underline{gal}_1^- \underline{gal}_2^-$ was present in these gal^- strains, which would not be detected by these crosses.

The chromosomal genotype of these gal^- strains was determined by testing for the formation of gal^+ recombinants in crosses on the two gal^- F' donors. In the case of the $\underline{gal}_1^- \underline{gal}_2^+ / F \underline{gal}_1^- \underline{gal}_2^+$ homogenote, gal^+ recombinants would be formed with recipient chromosomes of the $\underline{gal}_1^+$ type. The other donor, $\underline{gal}_1^+ \underline{gal}_2^- / F \underline{gal}_1^+ \underline{gal}_2^-$, would yield gal^+ recombinants in crosses with recipient chromosomes of the $\underline{gal}_2^+$ type. The absence of gal^+ recombinant formation in both cases would indicate that the chromosomes of the gal^- segregants were $\underline{gal}_1^- \underline{gal}_2^-$.

The 50 gal^+ segregants were tested for the genotype of the F factor, if still carried, by crosses on the 3 gal^- recipients, W3101, W3102, and W3350. In this case, azide was used as a selective

agent against the gal^+ donors. The use of azide was necessary since the 50 gal^+ strains used as donors in this case were S^r. The formation of gal^+ recombinants from crosses with these gal^+ segregants as donors and the $\underline{gal}_1^-$ recipients only would indicate that these gal^+ segregants were of the F $\underline{gal}_1^+$ genotype. A similar result with the $\underline{gal}_2^-$ recipient only would indicate that these gal^+ segregants were F $\underline{gal}_2^+$. The double mutant recipient, W3350, would be expected to form gal^+ recombinants with the 50 segregants as donors only if both $\underline{gal}_1^+$ and $\underline{gal}_2^+$ were transferred. Again it must be noted that the crosses performed here to test the genotype of these 50 gal^+ segregants would not select for an F of the type F $\underline{gal}_1^-$ $\underline{gal}_2^-$.

In order to determine the chromosomal genotype of these 50 gal^+ segregants, it was first necessary to cure the strains of any F factors present. This was done by exposure of the strains to acridine orange following the method described earlier. The strains were exposed to the agent and the resulting gal^- colonies tested for the loss of F by crosses against the three A^r recipients. If no gal^- colonies were obtained after acridine orange exposure it would indicate that the strains still carried F and were noncurable by this method. The cured strains were tested for the genotype of the chromosome by crosses with the two gal^- F' donors, just as was described earlier in the determination of the chromosomal type of the 50 gal^- segregants. The two donor homogenotes would form

recombinants possessing the ability to ferment galactose only if the complementary genotype was supplied by the cured segregants used as recipients. In this way, it was possible to determine the genotype of the chromosome of the 50 gal^+ segregants.

Confirmation of Genotype of W4520 F' Donor

The gal^+ phenotype exhibited by the F' donor used in this study could result from several possible genotypes. Conjugation experiments were done to establish the genotype of the F carried by this donor followed by curing of the strain with acridine orange and subsequent crosses with suitable donors to determine the genotype of the chromosome. Crosses were done with W4520 as donor and W3101 ($\underline{gal}_1^- \underline{gal}_2^+$) and W3102 ($\underline{gal}_1^+ \underline{gal}_2^-$) as recipients, following the method described earlier. The formation of gal^+ recombinants with both recipient types would confirm that the F carried by this donor was $\underline{gal}_1^+ \underline{gal}_2^+$. The donor F' strain was subjected to acridine orange exposure, following the method described earlier. To confirm that the strain had been cured of its donor ability, crosses were done using colonies from cells exposed to acridine orange as donors and the three gal^- recipient strains, W3101, W3102, and W3350. The crosses and controls were the same as described earlier in the acridine orange curing of the 50 gal^+ strains. The cured W4520 strains, no longer donors, were then examined for

chromosome genotype by crosses on the two gal$^-$ F' donor strains isolated earlier. Recombinant formation in both donor cases would confirm that the chromosome of the W4520 strain was $\underline{gal}_1^+ \underline{gal}_2^+$. It was thus possible to confirm the genotype of both the F and its host chromosome in this W4520 F' donor.

RESULTS

Isolation and Characterization of Segregants

The gal^+ and gal^- segregants from the sexductional heterogenotes were isolated and analyzed according to the procedure in Figure 3. The two types of segregants were isolated directly from streaks of the heterogenotes resulting from the F' sexduction. Because of the large number of crosses involved in the analysis of segregants, the number isolated was limited to 50 of each type, i.e., 10 gal^+ and 10 gal^- segregants from each of 5 recombinant papillae. The stability of these segregants was confirmed, since streaking of the 50 gal^+ and gal^- types resulted in development of gal^- segregants only in the case of 2 gal^+ strains. Analysis of these gal^- segregants from the two gal^+ strains revealed that they were either haploid gal^- strains of the recipient parental type, $\underline{gal}_1^- \underline{gal}_2^-$ or homogenotes of the $\underline{gal}_1^- \underline{gal}_2^- / F \underline{gal}_1^- \underline{gal}_2^-$ type, which were not detectable in this system. The 50 gal^+ and 50 gal^- segregants were analyzed for the genotype of F, if carried, and chromosome, as outlined in Figure 3.

The results of analysis of the 50 gal^- segregants for the presence of the F factor are shown in Table 4. It may be seen that in no case were gal^+ recombinants formed with the 50 gal^- strains as donors, a fact which confirms that no F of the type $F \underline{gal}_1^+ \underline{gal}_2^-$ or

Table 4. Results of crosses of the gal$^-$ segregants in characterization of donor ability.[a]

Gal$^-$ Strain	Recipient Strains		
	W3101	W3102	W3350
1-1	-	-	-
1-2	-	-	-
1-3	-	-	-
1-4	-	-	-
1-5	-	-	-
1-6	-	-	-
1-7	-	-	-
1-8	-	-	-
1-9	-	-	-
1-10	-	-	-
2-1	-	-	-
2-2	-	-	-
2-3	-	-	-
2-4	-	-	-
2-5	-	-	-
2-6	-	-	-
2-7	-	-	-
2-8	-	-	-
2-9	-	-	-
2-10	-	-	-
3-1	-	-	-
3-2	-	-	-
3-3	-	-	-
3-4	-	-	-
3-5	-	-	-
3-6	-	-	-
3-7	-	-	-
3-8	-	-	-
3-9	-	-	-
3-10	-	-	-
4-1	-	-	-
4-2	-	-	-
4-3	-	-	-
4-4	-	-	-
4-5	-	-	-
4-6	-	-	-
4-7	-	-	-
4-8	-	-	-
4-9	-	-	-
4-10	-	-	-
5-1	-	-	-
5-2	-	-	-
5-3	-	-	-
5-4	-	-	-
5-5	-	-	-
5-6	-	-	-
5-7	-	-	-
5-8	-	-	-
5-9	-	-	-
5-10	-	-	-

[a] + indicates presence of gal$^+$ recombinants after crossing.
- indicates absence of gal$^+$ recombinants after crossing.

F $\underline{gal}_1^-$ $\underline{gal}_2^+$ was carried by the gal^- segregants. However, any F factors of the type F $\underline{gal}_1^-$ $\underline{gal}_2^-$ would not have been detected by this method.

The results of crosses to determine the chromosomal genotype of the 50 gal^- segregants are shown in Table 5. It may be seen that in no case did gal^+ recombinants result from crosses of these segregants with the two gal^- F' donors. These results ruled out the possibility that any of these 50 gal^- segregants carried chromosomes of the type $\underline{gal}_1^+$ $\underline{gal}_2^-$ or $\underline{gal}_1^-$ $\underline{gal}_2^+$. It was thus concluded that the 50 segregants, lacking F, were either haploid strains, of the same type as the original recipient, $\underline{gal}_1^-$ $\underline{gal}_2^-$, or were $\underline{gal}_1^-$ $\underline{gal}_2^-$ / F $\underline{gal}_1^-$ $\underline{gal}_2^-$ strains.

Table 6 shows the results of analysis of the 50 gal^+ segregants for the genotype of any F factors carried. In all 50 cases, the gal^+ segregants behaved as donors in crosses with the 3 recipients, as evidenced by the formation of gal^+ recombinants in all crosses. It was therefore concluded that all 50 segregants carried an F factor of the type F $\underline{gal}_1^+$ $\underline{gal}_2^+$, the same type as carried by the original F' donor.

It was necessary to cure the 50 gal^+ segregants of the F factor, in order to analyze the chromosomal genotypes of these strains. Treatment of the 50 gal^+ segregants with acridine orange resulted entirely in gal^- progeny from 42 of the 50 strains, and entirely in

Table 5. Results of crosses of the gal$^-$ segregants in determination of chromosomal genotype.[a]

Gal$^-$ Strain	Donor Strains	
	$\underline{gal}_1^-\ \underline{gal}_2^+$ / F $\underline{gal}_1^-\ \underline{gal}_2^+$	$\underline{gal}_1^+\ \underline{gal}_2^-$ / F $\underline{gal}_1^+\ \underline{gal}_2^-$
1-1	-	-
1-2	-	-
1-3	-	-
1-4	-	-
1-5	-	-
1-6	-	-
1-7	-	-
1-8	-	-
1-9	-	-
1-10	-	-
2-1	-	-
2-2	-	-
2-3	-	-
2-4	-	-
2-5	-	-
2-6	-	-
2-7	-	-
2-8	-	-
2-9	-	-
2-10	-	-
3-1	-	-
3-2	-	-
3-3	-	-
3-4	-	-
3-5	-	-
3-6	-	-
3-7	-	-
3-8	-	-
3-9	-	-
3-10	-	-
4-1	-	-
4-2	-	-
4-3	-	-
4-4	-	-
4-5	-	-
4-6	-	-
4-7	-	-
4-8	-	-
4-9	-	-
4-10	-	-
5-1	-	-
5-2	-	-
5-3	-	-
5-4	-	-
5-5	-	-
5-6	-	-
5-7	-	-
5-8	-	-
5-9	-	-
5-10	-	-

[a] + indicates presence of gal$^+$ recombinants after crossing.
- indicates absence of gal$^+$ recombinants after crossing.

Table 6. Results of crosses of the gal^+ segregants in characterization of donor ability.[a]

Gal^+ Strain	Recipient Strains		
	W3101 A^r	W3102 A^r	W3350 A^r
1-1	+	+	+
1-2	+	+	+
1-3	+	+	+
1-4	+	+	+
1-5	+	+	+
1-6	+	+	+
1-7	+	+	+
1-8	+	+	+
1-9	+	+	+
1-10	+	+	+
2-1	+	+	+
2-2	+	+	+
2-3	+	+	+
2-4	+	+	+
2-5	+	+	+
2-6	+	+	+
2-7	+	+	+
2-8	+	+	+
2-9	+	+	+
2-10	+	+	+
3-1	+	+	+
3-2	+	+	+
3-3	+	+	+
3-4	+	+	+
3-5	+	+	+
3-6	+	+	+
3-7	+	+	+
3-8	+	+	+
3-9	+	+	+
3-10	+	+	+
4-1	+	+	+
4-2	+	+	+
4-3	+	+	+
4-4	+	+	+
4-5	+	+	+
4-6	+	+	+
4-7	+	+	+
4-8	+	+	+
4-9	+	+	+
4-10	+	+	+
5-1	+	+	+
5-2	+	+	+
5-3	+	+	+
5-4	+	+	+
5-5	+	+	+
5-6	+	+	+
5-7	+	+	+
5-8	+	+	+
5-9	+	+	+
5-10	+	+	+

[a] + indicates presence of gal^+ recombinants after crossing.
- indicates absence of gal^+ recombinants after crossing.

gal^+ progeny from 8 of the 50 strains. Results of crosses of the 50 strains after acridine orange exposure, to confirm curing, are shown in Table 7. It was evident that the segregants had been cured of the F factor in 42 cases. Eight of the fifty segregants were apparently non-curable by this method since they continued to behave as donors even after exposure to acridine orange.

The phenotype of the cured strains was gal^-. However, to distinguish between the possible types, $\underline{gal}_1^- \underline{gal}_2^+$, $\underline{gal}_1^+ \underline{gal}_2^-$, and $\underline{gal}_1^- \underline{gal}_2^-$, it was necessary to examine the chromosomes of these strains further. Subsequently, after curing of those 42 strains in which it was possible to do so, the chromosomal type was confirmed by crosses of these strains, now recipients, with the two gal^- F' donors. These results are tabulated in Table 8. It may be seen that none of the 42 strains tested produced gal^+ recombinants with either donor type. It was concluded that the 42 strains possessed chromosomes of the $\underline{gal}_1^- \underline{gal}_2^-$ genotype, in spite of the presence of the F' $\underline{gal}_1^+ \underline{gal}_2^+$ factor in these segregants, prior to curing.

Confirmation of Genotype of W4520 F' Donor

As may be seen from Table 9, crosses of this donor on the two recipients, W3101 and W3102, resulted in recombinant formation in each case. Hence, this donor transferred an F of the type,

Table 7. Results of crosses with acridine orange treated gal^+ segregants in confirmation of curing.[a]

	Recipient Strains		
Strain	W3101 A^r	W3102 A^r	W3350 A^r
1-1	+	+	+
1-2	-	-	-
1-3	-	-	-
1-4	-	-	-
1-5	-	-	-
1-6	-	-	-
1-7	-	-	-
1-8	-	-	-
1-9	-	-	-
1-10	-	-	-
2-1	-	-	-
2-2	-	-	-
2-3	-	-	-
2-4	-	-	-
2-5	-	-	-
2-6	-	-	-
2-7	+	+	+
2-8	-	-	-
2-9	-	-	-
2-10	-	-	-
3-1	-	-	-
3-2	-	-	-
3-3	-	-	-
3-4	-	-	-
3-5	-	-	-
3-6	-	-	-
3-7	-	-	-
3-8	+	+	+
3-9	+	+	+
3-10	-	-	-
4-1	-	-	-
4-2	-	-	-
4-3	+	+	+
4-4	-	-	-
4-5	-	-	-
4-6	-	-	-
4-7	+	+	+
4-8	-	-	-
4-9	+	+	+
4-10	-	-	-
5-1	-	-	-
5-2	+	+	+
5-3	-	-	-
5-4	-	-	-
5-5	-	-	-
5-6	-	-	-
5-7	-	-	-
5-8	-	-	-
5-9	-	-	-
5-10	-	-	-

[a] + indicates presence of gal^+ recombinants after crossing.
- indicates absence of gal^+ recombinants after crossing.

Table 8. Results of crosses of acridine orange cured gal^+ segregants in determination of chromosomal genotype.[a]

Acridine orange cured strains	Donor Strains	
	$\underline{gal}_1^- \underline{gal}_2^+$ / F $\underline{gal}_1^- \underline{gal}_2^+$	$\underline{gal}_1^+ \underline{gal}_2^-$ / F $\underline{gal}_1^+ \underline{gal}_2^-$
1-2	-	-
1-3	-	-
1-4	-	-
1-5	-	-
1-6	-	-
1-7	-	-
1-8	-	-
1-9	-	-
1-10	-	-
2-1	-	-
2-2	-	-
2-3	-	-
2-4	-	-
2-5	-	-
2-6	-	-
2-8	-	-
2-9	-	-
2-10	-	-
3-1	-	-
3-2	-	-
3-3	-	-
3-4	-	-
3-5	-	-
3-6	-	-
3-7	-	-
3-10	-	-
4-1	-	-
4-2	-	-
4-4	-	-
4-5	-	-
4-6	-	-
4-8	-	-
4-10	-	-
5-1	-	-
5-3	-	-
5-4	-	-
5-5	-	-
5-6	-	-
5-7	-	-
5-8	-	-
5-9	-	-
5-10	-	-

[a] + indicates presence of gal^+ recombinants after crossing.
- indicates absence of gal^+ recombinants after crossing.

F $\underline{gal}_1^+$ $\underline{gal}_2^+$. Curing of the donor of the F factor was evident from the absence of recombinant formation with the three recipient types. The cured strain was then crossed to the two gal^- F' donors. Results of these crosses confirmed that the chromosomal genotype of this strain was $\underline{gal}_1^+$ $\underline{gal}_2^+$. The complete genotype of the F' donor, W4520, was thus established as $\underline{gal}_1^+$ $\underline{gal}_2^+$ / F $\underline{gal}_1^+$ $\underline{gal}_2^+$.

Table 9. Results of crosses of acridine orange treated and untreated W4520 F' to confirm genotype.[a]

Untreated W4520		Acridine orange treated W4520				
Recipients		Recipients			Donors	
W3101	W3102	W3101	W3102	W3350	$\underline{gal}_1^-\underline{gal}_2^+$ / F$\underline{gal}_1^-\underline{gal}_2^+$	$\underline{gal}_1^+\underline{gal}_2^-$ / F$\underline{gal}_1^+\underline{gal}_2^-$
+	+	-	-	-	+	+

[a] + indicates presence of gal^+ recombinants after crossing.
- indicates absence of gal^+ recombinants after crossing.

DISCUSSION

Just as in the case of transduction with lambda phage, sexduction by the F' $\underline{gal}_1^+$ $\underline{gal}_2^+$ factors resulted in the formation of heterogenotes which were diploid for the gal region. The fact that these strains, which were isolated from sexduction of the $\underline{gal}_1^-$ $\underline{gal}_2^-$ recipients by the F' factor, were heterogenotes, was deduced from analysis of the gal^+ and gal^- strains segregated by these sexductants. It would be expected, from analogy with transductional heterogenotes, that such sexductional heterogenotes would segregate recombinant types, such as homogenotes, as well as haploid parental types. The possible genotypes of the 50 gal^+ and 50 gal^- segregants which were analyzed are listed in Table 10. It was noteworthy that in both segregational types, all 50 strains proved to be of the same genotype. As far as could be determined using this system of analysis, the gal^+ segregants were of the genotype $\underline{gal}_1^-$ $\underline{gal}_2^-$ / F $\underline{gal}_1^+$ $\underline{gal}_2^+$, while the gal^- segregants were of the genotype $\underline{gal}_1^-$ $\underline{gal}_2^-$. It was therefore concluded that the gal^- segregants were probably haploid parental types resulting from loss of the F from the heterogenotes. The genotype of the 50 gal^+ segregants indicated that in the heterogenote, no recombination had occurred between the F' $\underline{gal}_1^+$ $\underline{gal}_2^+$ exogenote and the $\underline{gal}_1^-$ $\underline{gal}_2^-$ endogenote of the recipient. Within the scope of these studies then, no recombinant types were segregated

Table 10. Possible genotypes of segregants from sexduction of $\underline{gal}_1^-\ \underline{gal}_2^-$ recipients by F' $\underline{gal}_1^+\ \underline{gal}_2^+$ donors.[a]

Gal^+ Segregants	Gal^- Segregants
$\underline{gal}_1^+\ \underline{gal}_2^+ / F\ \underline{gal}_1^+\ \underline{gal}_2^-$	$\underline{gal}_1^-\ \underline{gal}_2^- / F\ \underline{gal}_1^+\ \underline{gal}_2^-$
$\underline{gal}_1^+\ \underline{gal}_2^+ / F\ \underline{gal}_1^-\ \underline{gal}_2^+$	$\underline{gal}_1^-\ \underline{gal}_2^- / F\ \underline{gal}_1^-\ \underline{gal}_2^+$
$\underline{gal}_1^-\ \underline{gal}_2^+ / F\ \underline{gal}_1^+\ \underline{gal}_2^-$	$\underline{gal}_1^+\ \underline{gal}_2^- / F\ \underline{gal}_1^+\ \underline{gal}_2^-$
$\underline{gal}_1^+\ \underline{gal}_2^- / F\ \underline{gal}_1^-\ \underline{gal}_2^+$	$\underline{gal}_1^-\ \underline{gal}_2^+ / F\ \underline{gal}_1^-\ \underline{gal}_2^+$
$\underline{gal}_1^+\ \underline{gal}_2^+ / F\ \underline{gal}_1^-\ \underline{gal}_2^-$	$\underline{gal}_1^+\ \underline{gal}_2^- / F\ \underline{gal}_1^-\ \underline{gal}_2^-$
$\underline{gal}_1^+\ \underline{gal}_2^+ / F\ \underline{gal}_1^+\ \underline{gal}_2^+$	$\underline{gal}_1^-\ \underline{gal}_2^+ / F\ \underline{gal}_1^-\ \underline{gal}_2^-$
$\underline{gal}_1^+\ \underline{gal}_2^- / F\ \underline{gal}_1^+\ \underline{gal}_2^+$	$\underline{gal}_1^-\ \underline{gal}_2^- / F\ \underline{gal}_1^-\ \underline{gal}_2^-$
$\underline{gal}_1^-\ \underline{gal}_2^+ / F\ \underline{gal}_1^+\ \underline{gal}_2^+$	
$\underline{gal}_1^-\ \underline{gal}_2^- / F\ \underline{gal}_1^+\ \underline{gal}_2^+$	

[a]Not including any possible haploid segregant types.

from the sexductional heterogenotes. Transductional heterogenotes of λ gal vary extremely widely in stability, and in general show much more recombination between prophage and chromosomal genes (Fraser, 1962). It was possible to conclude from analysis of the segregants that recombinants, i.e., heterogenotes, formed by the introduction of an F' $\underline{gal}_1^+ \underline{gal}_2^+$ factor into a $\underline{gal}_1^- \underline{gal}_2^-$ recipient, were quite stable diploids of the cis heterogenote configuration, $\underline{gal}_1^- \underline{gal}_2^- / F \underline{gal}_1^+ \underline{gal}_2^+$. It may be noted that the trans heterogenotes of the genotype $\underline{gal}_1^+ \underline{gal}_2^- / \lambda \underline{gal}_1^- \underline{gal}_2^+$, which were analyzed in transduction studies, were not formed and analyzed in this case.

It was of interest that eight of the fifty gal^+ strains were noncurable with the acridine orange technique used. A possible explanation is that these particular F factors were inaccessible to the agent, i.e., they might have been inserted in the host chromosome in all cells of the subcultures. The eight strains were not isolated from the same one of the five original papillae from the cross of W4520 F' with W3350, and were randomly distributed among the fifty gal^+ segregants.

It is a characteristic of the F' system in general, that upon mating with a suitable recipient, the F-merogenote itself is transferred at frequencies of almost 100 percent. However, F' donors also characteristically transfer chromosomal markers but at a lower frequency than transfer of the F-genote occurs. Such

chromosomal transfer has been shown to occur only following integration of the F into the chromosome, possibly by a crossover event between the F-merogenote and a homologous region of the chromosome (Adelberg and Pittard, 1965). From this work, crossover leading to integration of the F into the chromosome in F' cells has been predicted by these authors to occur during vegetative growth so that the F is inserted into the chromosome in 10 percent of the cells, and exists 90 percent of the time in an independent state. Application of these principles to the present study would predict that, at least some of the time, insertion of the F into the chromosome might be expected to lead to recombination. The lack of recombination evident in these studies may thus be an indication of low frequency of insertion of the F factor into the chromosome, that is, insertion occurred at a somewhat lower frequency than the proposed 10 percent of the time. If the frequency of insertion is as high as predicted by Adelberg and his associates from the transfer data, then the markers carried by the inserted episome are evidently much less accessible to recombination with chromosomal markers than in the apparently similar case of λ gal.

It has been suggested by Jacob, Brenner, and Cuzin (1963), as well as by Lark (1966), that replicons, including episomes and chromosomes, are independently attached to the bacterial membrane and begin replication following activation by some surface reaction or signal. In support of this model is the fact that episomes multiply

rapidly immediately after transfer to recipients but subsequently settle down to multiplication at the same rate as the host chromosome. In keeping with the results of the present study then, the opportunity for interaction between F and chromosome, leading to transfer of chromosomal markers, may be limited only to the transfer period itself, with independent replication being the rule in non-conjugating F' cells.

Several possible mechanisms have been proposed which could account for the recombination between F factor and chromosome in F' cells. In the first place, recombination may occur by classical double crossing over as in the case of higher organisms. However, the reciprocal recombination usually evident in such crossing over has been reported in only one case in F' bacteria. Herman (1965) detected reciprocal recombination between an F-merogenote and host chromosome under conditions where both recombinant F factor and recombinant chromosome could be detected. In the case of the transducing phage, λ, the recombinational event between prophage and chromosome appears to be of the non-reciprocal type (Driskell-Zamenhof, p. 169).

Recombination by Campbell's model presents a second possibility. Insertion of the F factor into the chromosome by pairing and single crossing over at a site of homology, with the subsequent possible release of defective phage or F' factors, was outlined in

Figure 1. It should be noted that the recombination predicted by Campbell's model is also reciprocal. No such recombinants were observed in this study.

The isolation of the two gal$^-$ F' homogenotes was a proof that episome-chromosome recombination was possible in this system. Recombination between F' donors which were F $\underline{gal}_1^+$ $\underline{gal}_2^+$ and recipients which were $\underline{gal}_1^-$ $\underline{gal}_2^+$, in one case, and $\underline{gal}_1^+$ $\underline{gal}_2^-$ in the other case, led to formation of homogenotes of the type $\underline{gal}_1^-$ $\underline{gal}_2^+$ / F $\underline{gal}_1^-$ $\underline{gal}_2^+$ and $\underline{gal}_1^+$ $\underline{gal}_2^-$ / F $\underline{gal}_1^+$ $\underline{gal}_2^-$. Since these donors were isolated for use in test crosses and not for analysis in themselves, the exact frequency of occurrance was not determined. However, such homogenotes were found with relatively high frequency.

Thus, it appears that by far the most common recombinational event observed in these studies consisted of the formation of homogenote strains by a process which copies the entire chromosomal region into the episome. The mechanism of formation of these homogenotes is unknown, but the recombination involved is obviously of a non-reciprocal type.

SUMMARY

The purpose of this study was to isolate and characterize any segregants of the heterogenotes formed from sexduction by an F' donor. From the mating of F' W4520, carrying an F' $\underline{gal}_1^+$ $\underline{gal}_2^+$ factor, and W3350, a $\underline{gal}_1^-$ $\underline{gal}_2^-$ recipient, recombinant clones were picked and streaked to isolate any segregant types. From 5 such recombinant clones, 50 gal^+ and 50 gal^- segregants were isolated.

The genotypes of both the gal^- and gal^+ segregants were characterized by crosses against appropriate gal^- strains. In the case of the gal^- segregants, it was determined that either the F factors had been lost, yielding haploid strains of the $\underline{gal}_1^-$ $\underline{gal}_2^-$ genotype, similar to the recipient parental type or the gal^- segregants were homogenotes of the undetectable type $\underline{gal}_1^-$ $\underline{gal}_2^-$ / F $\underline{gal}_1^-$ $\underline{gal}_2^-$. It was demonstrated that the gal^+ segregants were carrying F' factors of the type F $\underline{gal}_1^+$ $\underline{gal}_2^+$. When cured of the F by acridine orange, the gal^+ segregants were shown to be carrying chromosomes of the genotype $\underline{gal}_1^-$ $\underline{gal}_2^-$. Hence, these gal^+ segregants were of the same genotype as the heterogenotes from which they were isolated, $\underline{gal}_1^-$ $\underline{gal}_2^-$ / F $\underline{gal}_1^+$ $\underline{gal}_2^+$.

The lack of segregation of recombinant types from the sexductional heterogenotes was taken as evidence that, in this F'

system, the F factors fail to undergo recombination with the host chromosome to the extent that analogy with studies of transductional heterogenotes would predict. The isolation of the two gal$^-$ F' homogenotes was taken as evidence that recombination, or some form of copying of the chromosomal genes into the episome, was possible in this system; however, this process was of a non-reciprocal type.

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