PROPERTIES AND FUNCTIONS OF THE SEX FACTOR OF

ESCHERICHIA COLI

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The thesis is concerned with the sex factor of *Escherichia coli* K12. The sex factor, which confers upon its host cell the ability to transfer genetic material, can exist either as an autonomous particle (in *F*+ cells) or integrated with the chromosome (in Hfr cells). In the introduction an account is given of present knowledge on its structure, functioning and relation to its host cell. Special attention is paid to ideas and experimental results concerned with the mechanism by which the sex factor becomes integrated with the chromosome.

In the first experimental section an account is given of studies with a series of newly isolated Hfr strains. The results obtained are consistent with a scheme for integration involving genetic recombination between the sex factor and the chromosome. In the second section the isolation of a new type of F-prime factor (a variant sex factor carrying chromosomal genes) is described. A mechanism for its origin is proposed and evidence supporting this model is presented. This particular type of F-prime factor was found to differ from other types previously described in that it gives rise to Hfr strains of a single type. This effect was analysed and an explanation is proposed.
The third section is concerned with the genetic analysis of the property of some strains of *E. coli* of allowing phage T3 to form plaques only with low efficiency. It is shown that this heritable property is carried on the sex factor. In this context a structure formed by recombination between an F-prime factor and a mutant sex factor is described.
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INTRODUCTION

A. Genetic systems in bacteria.

The heritable information of living matter is carried by genetic determinants (genes). Their function is to specify the type of proteins which a given cell produces; these proteins in turn mediate the chemical reactions of the cell. Genes are composed of deoxyribonucleic acid (DNA) except in some viruses, in which they are composed of ribonucleic acid (RNA). RNA can also act as an unstable carrier of information in all organisms. The nucleic acids are macromolecules with a repeating sequence of alternate phosphate and sugar residues. To each of the sugar residues is attached one of four bases. These are adenine, cytosine, guanine and thymine (in DNA) or uracil (in RNA). The order in which they are arranged in a gene, like the letters in an alphabet, constitutes the message (that is, the information) which specifies the order of amino acids in the corresponding protein.

We can study the properties of genetic material and its role in the organization of living matter by the use of mutations and the recombination of genes from different individuals. Microorganisms are excellent material for this type of investigation. This is partly because of the relative simplicity of these organisms, and partly through the development of selective techniques for detecting rare events, such as mutations, within the large populations which can easily be handled.
In microorganisms there are three known processes by which genetic material can be transferred. In all, the fact of transfer is recognised by the acquisition by a recipient strain of properties characteristic of a donor strain. In transformation, purified DNA from the donor strain enters the recipient strain. In transduction, a virus leaving its bacterial host can carry with it host genetic information which may be expressed in the second host. Conjugation involves a complex series of events in which two bacteria come into physical contact, and genetic material is transferred through a bridge from one to the other. In contrast to higher organisms, in bacteria it is rare for a complete complement of genetic material to be transferred. In *Escherichia coli* K12 conjugation is mediated by a small extrachromosomal particle, the sex factor F. This sex factor is the subject of the present thesis.
B. **Conjugation in E. coli K12**

(i) **The discovery of conjugation**

In 1946 Tatum and Lederberg demonstrated genetic recombination in *E. coli* K12. If a mixture of cultures of two auxotrophic strains were plated on unsupplemented media some colonies (about $10^{-7}$ of the input) were able to grow. The possibility that these colonies were growing syntrophically was excluded by the demonstration that their prototrophic character was preserved through repeated purification and isolation of single colonies. Transformation was also excluded as a mechanism since on centrifugation the prototroph-forming ability sedimented with the same kinetics as the cells, and nothing less than the presence of whole cells of both parental types gave recombinants. Such recombination required physical contact between cells (Davis, 1950). In contrast to transformation, numbers of markers were frequently transferred together.

Lederberg (1947) attempted to construct a genetic map by examining the inheritance of unselected markers among prototrophic recombinants. As more markers were studied, it appeared that they could not be fitted into an internally consistent linear map (Lederberg *et al.*, 1951). It was also noted that, in general, recombinants inherited more markers from one parent than from the other. Apparently, therefore, the mating system was radically different from those in higher organisms.
In retrospect, we know that these difficulties derive from Lederberg's initially stated assumptions. These were that mating involves complete genetic complements from the two parents, that segregation is regular, and that the contribution of unselected markers is determined exclusively by their linkage relationships to the selected markers. These assumptions turn out to be invalid because of the peculiar nature of the mating system in *E. coli*, in which there is differentiation of the mating strains into donor and recipient, and transfer of only part of the male genetic complement to the female.

(ii) The sex factor

In 1952 Hayes demonstrated (Hayes, 1952a) that in a cross between two strains, which we shall call A and B, if A but not B was sensitive to streptomycin, the addition of streptomycin inhibited the formation of recombinants. However, in a cross between a streptomycin-sensitive A strain and a resistant B strain in the presence of a streptomycin recombinant arisen. Hayes concluded that mating was between a donor (A) and a recipient (B) and that the donor's viability was not essential for recombinant formation.

From independent investigations, Lederberg and his collaborators also inferred that in *E. coli* there were two mating types, which they called *F*<sup>+</sup> and *F*<sup>−</sup>, and which corresponded respectively to types A and B; *F*<sup>+</sup> x *F*<sup>−</sup> and *F*<sup>+</sup> x *F*<sup>+</sup> crosses were fertile, but *F*<sup>−</sup> x *F*<sup>−</sup> crosses were sterile (Lederberg et al., 1952).
Two other discoveries about this time were transduction (Zinder and Lederberg, 1952), in which genetic transfer is mediated by an infective particle, and the induction by ultraviolet light (UV) of the lytic cycle of the lysogenic phage \( \lambda \), which had recently been discovered in \( E. coli \) K12 (Weigle and Delbrück, 1951; Lederberg and Lederberg, 1953). It had been observed (Haas et al., 1948) that UV light increased the fertility of genetic crosses. Hayes (1952b) was able to show that this effect was upon the \( F^+ \) parent, and suggested, by analogy with transduction and the induction effect, that fertility was mediated by an infective and inducible virus-like particle.

This hypothesis was tested by using a strain which although derived from an \( F^+ \) strain, was now \( F^- \). After mixed overnight culture with an \( F^+ \) strain about half the reisolates of this strain had regained the \( F^+ \) property. This compared with a frequency for recombinant formation of about \( 10^{-6} \) (Hayes 1953a,b). This infective, but non-lethal, particle was called the sex factor, \( F \).

(iii) Chromosomal transfer by Hfr cells

The mechanism of conjugation in \( E. coli \) was further clarified by the isolation by Cavalli-Sforza (1950) and by Hayes (1953b) of two donor strains termed Hfr (for high frequency of recombinants) which yielded about a thousand times more recombinants than the original donor strain from which they were
derived. These Hfr strains transferred only certain markers with this high frequency, unlike the approximately equal frequency for all markers by F\(^+\) strains. In these strains the sex factor was no longer infective, though it was still present, since occasional derivatives arose with properties similar to those of the original F\(^+\) strain.

Wollman and Jacob (1955) discovered that conjugation was interrupted by violent agitation, which separated mating pairs. They showed that for a given Hfr strain each gene had a characteristic time of entry so that the Hfr population is homogeneous with respect to the sequence in which it transfers its genes. It was inferred that the chromosome is transferred linearly. They also found that the more frequently a gene was transferred, the earlier it is carried over to the female; from this they concluded that in the population of conjugating cells mating is interrupted in a random fashion, so that only a very few recipients receive the whole of the male chromosome.

This conclusion was supported by their zygotic induction experiments. Phage \(\lambda\) can exist stably in some strains of \textit{E. coli} K12 without killing the host cell. In this "lysogenic" state (so called because the potentiality for independent multiplication of the phage and lysis of the cell remain) the phage genome ("\(\lambda\) prophage") has a chromosomal location close to the gal cluster of genes. In crosses between Hfr strains lysogenic for phage \(\lambda\), and non-lysogenic
recipient cells when \(\lambda\) prophage enters the female cell it is induced and begins the lytic cycle (Jacob and Wellman, 1956a). In such crosses only recombinants expressing genes transferred before the prophage are found. In confirmation of the inference that breaks occur, it was found that the number of each type of recombinant bears an inverse relation to the distance of the gene from the \(\lambda\) locus; the chance of a break occurring between the gene and the \(\lambda\) locus is greater the greater the distance between the two points (Jacob and Wellman, 1956a).

These experiments proved that only a part of the donor chromosome need be transferred to yield recombinants in Hfr x F\(^-\) crosses. If we adopt the hypothesis, which will be justified later, that fertility of F\(^+\) strains is in principle similar to that of Hfr strains, they invalidate Lederberg's primary assumption that recombinants arise from complete zygotes.

When a number of different Hfr strains had been isolated it was found that, in each, different chromosomal segments were transferred first. It was also found that two genes, one transferred very early and the other very late by a given Hfr strain, would be linked in transfer by other Hfr strains. To explain this, Jacob and Wellman proposed that the chromosome of the F\(^+\) cell is circular and that Hfr strains arise by integration of the sex factor at various points in this circle. All the available evidence is consistent with this model and recently Cairns (1963) has shown by autoradiographic techniques that the E. coli chromosome is indeed
circular. This model, of course, also explains the disappearance of the autonomous sex factor from Hfr cells. The problem of the mechanism of integration of the sex factor in the formation of Hfr strains will be discussed in detail later.

(iv) The basis for the fertility of $F^+$ cultures

The simplest way to explain the fertility of $F^+$ populations is that it is due to the existence of a minority of cells which have become Hfr in type; that there will be different types of Hfr strain each transferring a different part of the $F^+$ strain's chromosome with high frequency, and that the proportion of such cells remains low because of reversion to the $F^+$ state.

An experiment by Jacob and Wollman (1956b) provides strong evidence that a large part at least of the fertility can be accounted for in this way. Their experiment is an adaptation of Luria and Delbrück's test (1943) to demonstrate the random and clonal nature of bacterial mutation. Jacob and Wollman were able to show that if a series of small populations of an $F^+$ culture were grown up in parallel, the variance in fertility between these cultures was far greater than could be ascribed to sampling errors, but was consistent with the assumption that the fertility was due to Hfr clones arising as rare mutation-like events. They then proceeded to isolate Hfr strains from the tubes containing the most fertile cultures.
Whereas in crosses between Hfr and F^- strains the recombinants arising at high frequency are almost always female, those from F^+ x F^- crosses are usually F^+ males. This might be caused by secondary infection with F particles; on the other hand, in reconstruction experiments using F^+ cells to which a minority of Hfr cells had been added the number of recombinants remaining F^- significantly exceeded that found in control F^+ matings (Reeves, 1960). A possible explanation is that in F^+ populations most of the Hfr cells will have arisen in the last generation or two and will retain autonomous sex factors as well as integrated ones, so that F infection is superimposed on chromosome transfer. It is also possible that there is a second component to F^+ fertility. In the first section of this thesis some experimental evidence on this question will be presented.
C. The properties of the sex factor

(i) Composition

The sex factor is composed of DNA. Herman and Ferro (1962) have shown that when F+ cells are labelled with tritiated thymine (which is incorporated into DNA but not into RNA) in F+ x F- crosses, when only the sex factor appears to be transferred, the label is found in the recipient cells in the predicted amounts. Another approach has been through the use of mitomycin C, an antibiotic which under certain conditions inhibits the synthesis of DNA but not RNA. Driskell and Adelberg (1961) showed that under such conditions mitomycin C inhibits the incorporation of radioactive phosphorus (32P) into the F-prime factors (sex factors carrying in addition chromosomal genes) and into the chromosome to the same extent. Similar results were obtained with ordinary F+ cells. They concluded that the sex factor and F-prime factors, like the chromosome, contain DNA but not RNA.

Various estimates for the size of the sex factor using the rate of its inactivation by 32P decay as a measure (Herman and Ferro, 1962; Lavallé and Jacob, 1961; Driskell and Adelberg, 1961) give values in the order of 10^5 phosphorus atoms. This corresponds to 5 x 10^4 nucleotide pairs, and to 1-2% of the size of a chromosome of E. coli. If we assume a triplet code, and about three hundred amino-acids per protein, this could code for about 50 (\frac{5 \times 10^4}{3 \times 10^2 \times 3}) genes. Like other genetic material,
the sex factor can undergo genetic recombination (Adelberg and Burns, 1960); this point will be discussed in detail in sections I and III.

(ii) Functions

Fertility probably involves many functions, of which very few have been identified. In male cells a surface antigen not found in female cells is recognised (Orskov and Orskov, 1960) and it is likely that this antigen is responsible for contact formation. Periodate phenotypically devirilises male cells, possibly by modifying this antigen; it is known that it breaks C-C bonds if the carbon atoms carry -OH groups in the cis position, as are for instance found in the sugar residues of carbohydrates. Subsequent growth in periodate-free medium restores the ability to form contacts (Sneath and Lederberg, 1961).

Recently a number of RNA phages which are specific to male cells have been isolated (Loeb, 1960; Dettori et al., 1961). It appears that they absorb to the male contact sites, since cells which have become resistant to these phages cannot form contacts with female cells; periodate-treated cells are also temporarily resistant to these phages (Dettori et al., 1961).

Fimbriae are projections growing from the surface of gram-negative bacteria. Those of E. coli are about 70Å in diameter, and many times longer. They are absent from some strains. Since both male and female strains may carry up to
several hundred fimbriae per cell, there were no grounds for believing that they were involved in conjugation. However, it has recently been observed by Crawford and Gesteland (1964) that in fimbriated male strains the male-specific phage R17 attaches with high efficiency to two to four of the morphologically indistinguishable fimbriae. When such a strain is subcultured in the presence of acridine orange, which eliminates the autonomous sex factor (Hirota, 1960) cells of the clones which become female (as determined in matings) lose the ability to plate the male-specific phage M12 (Hofschneider, 1963) and have no fimbriae able to absorb the phage (Brinton et al., 1964). The latter authors also found that when the sex factor of a fimbriated strain was infected into a fimbriated female strain, the clones which had become fertile now had a few phage-absorbing fimbriae, and gave plaques with phage M12. They concluded that the contact sites are located on special F-fimbriae, and suggested that these fimbriae provide the conjugation bridge between mating cells.

It has been observed that normal fimbriae have an axial hole 25\(\text{\AA}\) in diameter, wide enough to carry a DNA double helix.

Crosses in which male cells are used as recipients have about 1\% - 10\% of the efficiency of the corresponding cross with a female recipient. This efficiency can be increased by converting male recipients into phenotypic females (F-phenocopies), unable to act as donors, by growing to saturation under vigorously aerated conditions.
We may ask what prevents normal males from acting as efficient females. If the contact sites are indeed very localised, this question becomes especially pertinent. Possibly when cells become males there are additional changes over the whole cell surface.

A property of the sex factor found in some strains of \textit{E. coli} K12 is that of lowering the efficiency of plating (e.o.p.) of phage T3 on these strains. An analysis and discussion of this phenomenon, of which the relation to sexuality is unclear, will be presented in the third section of this thesis.
D. The relation of the sex factor to the cell

(i) The sex factor as a representative of a class of autonomous particles

There are two principal aspects to this problem. The first is the manner in which the sex factor's duplication and functioning is controlled when it is in each of its alternative states. The second is the nature of the sex factor's association with the chromosome in the integrated (Rfr) state, and the mechanism involved in this integration. These problems are of general interest since they also apply to a number of different types of factor observed in E. coli. Apart from the sex factor, the best known of these are the temperate phage $\lambda$, the resistance transfer factors (RTFs) and the colicin determinants (see, for instance, Campbell, 1962). They are similar in that for the cell they are dispensable and that they can exist physically apart from the chromosome. Phage $\lambda$ and the sex factor conform to the original definition by Jacob and Wollman (1958b) of "episomes," in that they can also have an alternative chromosomal location; this has not been demonstrated for any of the various RTFs or colicin determinants which have been studied.

In the instance of phage $\lambda$ the chromosomal or integrated state is the usual one; the phage genome is only autonomous when it is undergoing vegetative growth, which leads to the killing and lysis of the host cell. However, in the case of the sex factor, the RTFs and the colicin determinants the autonomous state is not lethal to the host cell and the number of particles per cell remains low.
(ii) The number of sex factors per nucleus

Scaife and Gross (1962) showed that replication of
F-prime factors (variant sex factors carrying chromosomal genes)
involves an exclusion system. If two F-prime factors (Flac\textsuperscript{+} and
Fgal\textsuperscript{+}) were both introduced into a female cell, one or other type
is eliminated. It was also found that both F\textsuperscript{+} and, more efficiently,
Hfr cells can exclude superinfecting sex factors or F-prime factors.
However, when a sex factor is introduced into a female cell, all
products of division of that cell carry a copy of the sex factor
(Lederberg, 1958). Since the host cell is multinucleate, these
observations point to the multiplication of the sex factor at a
faster rate than that of the cell until there is just one sex
factor per nucleus. In Hfr strains the resident sex factor is
apparently more entrenched, so that it is always the introduced,
autonomous factor which is excluded.

The replication of the autonomous sex factor can be
inhibited while that of the chromosome proceeds, either by growth
of the strain in the presence of acridine orange, which
specifically inhibits the replication of the autonomous sex factor
(Hirota, 1960), or by growing at 42\textdegree a strain with a mutant sex
factor unable to replicate at this temperature (Cuzin, 1962).
It is then found that although the population increases, the
absolute number of cells carrying the autonomous sex factor remains
constant, indicating that if there is more than one sex factor per
nucleus on cell division they must all enter the same daughter
cell (Jacob et al., 1963; Stouthamer et al., 1963). Also, the amount of β-galactosidase produced by cells with the lac + gene carried on an Flac + factor is only 2-3 times greater than that than that given by the corresponding haploid lac + strain (Jacob et al., 1960).

As daughter cells inherit the sex factor with very high efficiency, it has been argued that the division process must involve a non-random distribution of the daughter sex factors so that each of the daughter cells will receive just one, neither more nor less.

(iii) The replicon model

Whether autonomous or integrated, these particles can replicate in the cell. This contrasts with genetic material introduced for instance by conjugation, which can only persist in the host cell if it is recombined into the chromosome. Jacob and Brenner (1965) have proposed that structures, such as the chromosome and the autonomous sex factor, which are able to replicate as a unit should be termed "replicons," and that they are characterized by having a site at which replication may begin. Since the number of autonomous sex factors per chromosome, like the number of integrated sex factors, remains constant, this replication must somehow be related to that of the chromosome and the cell as a whole.
The replication of the *E. coli* chromosome at a given moment is only taking place at a single point, which proceeds along the length of the chromosome (Cairns, 1963). Yoshikawa and Sueoka (1963) have demonstrated that in a strain of *Bacillus subtilis* the point at which this replication cycle is initiated is always the same, and Nagata (1963) has produced evidence that in cells of Hfr strains of *E. coli* replication proceeds from the point at which the sex factor is integrated. In contrast, in a female strain there was no unique initiation point. He concluded that the sex factor in the integrated state influences chromosome duplication. However, Cuzin (1962) has observed that at 42°C temperature-sensitive F-prime factors, unable to replicate autonomously at this temperature, can replicate as part of the chromosome replicon. It appears, therefore, that the sex factor's control over chromosome replication must be limited to defining the point at which it starts.

Jacob et al. (1963) have presented a model which relates the transfer of the sex factor and of the chromosome to both their location and their replication. They suggest that the chromosome and the sex factor are both attached to the cell membrane and that division of these attachment sites initiates, as a step in the growth of the cell, the division of these replicons. The presence of a limited number of such sites (for instance one per nucleolus) might explain the localisation of the
contact-forming sites (Pirtton et al., 1964) and why two types of sex factor cannot stably coexist in a cell.

It is proposed that the formation of effective contacts and conjugation bridges activates the F system of replication, and that replication begins. This proceeds so that one of the daughter DNA duplexes is transferred to the female cell, and one remains in the donor cell. If the sex factor is integrated into the chromosome it is the chromosome which is transferred; if not, simply the sex factor. However, it is not yet proven that transfer and replication are indeed related.
E. The integration of phage $\lambda$ into the chromosome

(i) Formal possibilities

Both phage $\lambda$ and the sex factor can become associated with the chromosome at a particular region or regions. Phage $\lambda$ always integrates at a point close to the cluster of genes concerned with galactose utilization (gal loci), whereas independently isolated Hfr strains have the sex factor integrated at a variety of positions. The mechanism of this integration has been the subject of much recent work in which concepts originally developed for phage $\lambda$ have been extended to the sex factor system.

Formally an episome may become physically associated with the chromosome either by insertion into its continuity or by lateral attachment (Bertani, 1958; see Figure 1). Evidence against insertion models was put forward by Jacob and Wollman (1958c) from experiments with the lysogenic phage $\phi$18, which in transduction experiments with phage $\lambda$63 appeared to integrate at a point between two linked methionine markers, met1 and met2. They found that the distance between the two met genes was no greater in strains lysogenic for $\phi$18 than in the analogous non-lysogenic strains. Further, in interrupted mating experiments using Hfr strains they found that when the chromosome was transferred in one direction the prophage could again be mapped between the two met loci. However, in transfer in the other direction, the lysogenic property apparently only entered
1. Before integration

\[\text{bacteriophage 18} \quad - \quad \text{the bacterial chromosome} \quad \text{met 1 met 2.}\]

2. After integration

\[\text{Model 1.} \quad \quad \quad \text{Model 2.}\]

\[\text{met 1. met 2.} \quad \quad \quad \text{met 1. met 2.}\]

Figure 1.
Figure 1. Formal models for the association of an episome such as \( \Phi 18 \) (see text) with the chromosome. Model 1 assumes a lateral attachment of the episome to the chromosome; model 2 involves insertion of the episome into the continuity of the chromosome.
after both the *met* genes. They concluded that the $\phi$18 prophage was not collinear with but parallel to the chromosome, and that it overlapped the *met1* gene; that is, it was not inserted (Figure 1).

(ii) **The Campbell insertion model**

More recently evidence has been obtained that at least $\lambda$ phage and the sex factor integrated by an insertion mechanism. In 1960 Calef and Licciardello had observed in mapping experiments that the genes on the chromosome of phage $\lambda$ appeared to be arranged in a different order, depending upon whether the phage was in the integrated or the vegetative state. Campbell (1962) proposed that their results could be explained if it was assumed that the chromosome of vegetative $\lambda$ is circular, and that on integration it becomes linear. In this way genes at different ends of the linear structure and unlinked would become genetically linked in the circular structure. He pointed out that a single reciprocal crossover between such a circular phage chromosome and the bacterial chromosome would linearise the phage chromosome and simultaneously insert it into the bacterial chromosome (Figure 2).

(iii) **The mapping of $\lambda$**

More $\lambda$ mutants have now been obtained, and with these it has been possible to confirm Calef and Licciardello's original
Figure 2.
Figure 2. Campbell's model for the formation of $\lambda$ prophage by circularization and recombination (after Rothman, 1965). ABCD is a hypothetical region of homology between the chromosome and
conclusion that there are two different orders of genes. However, in vegetative λ as in the prophage these markers are in a linear rather than a circular structure, although the latter may exist as a minority class, as there seems to be weak linkage between terminal markers. These results argue against a simple Campbell model, though the λ phage structure which is actually integrated into the chromosome might be circular although vegetative λ is linear (Figure 2).

Physicochemical evidence does exist for a circular form of the phage chromosome. Hershey, Burgi and Ingraham (1963) have shown that the DNA of λ phage can exist either as a linear structure or, probably by cohesion of the ends, as a circular structure. These two forms have now been observed using electron microscopy. Further evidence suggests that these "sticky ends" are regions of single stranded DNA (Strack and Kaiser, 1965).

On the Campbell model, to account for the two distinct orders of the genes in the λ phage we must assume that the crossover occurs in a specific region of the phage λ genome, and that in the linear vegetative phage this region is non-terminal. If it was terminal, the arrangement of genes in the vegetative phage and the prophage would not be different. It is a prediction of this model that the two orders of genes would be permutations of the type 1 2 3 4 5 6 7, 3 4 5 6 7 1 2.
Support for this model comes from two sets of experiments in which \( \lambda \) prophage markers were mapped with respect to each other and to the chromosome. In transduction experiments using phage \( \Phi^+ \) as transducing agent, Rothman (1964) selected for \( \text{gal}^+ \) recombinants in a cross between strains lysogenic for genetically marked \( \lambda \) phages. She found that among these recombinants different \( \lambda \) markers from the donor were inherited at different frequencies, and that a linkage map of the prophage markers could be constructed. In other experiments Franklin et al. (1965) used a hybrid prophage constructed by crossing phages \( \lambda \) and \( \Phi^80 \) (Matsushiro, 1963). This hybrid phage had inherited the immunity and chromosomal location of \( \Phi^80 \) but most other genes, including those determining its host range properties, from phage \( \lambda \). The chromosomal location of \( \Phi^80 \) is close to the loci for tryptophan synthesis and for sensitivity to phage \( \text{T}1 \). Some \( \text{T}1 \)-resistant mutants carry more or less extensive deletions in this region. When \( \text{T}1 \)-resistant mutants were isolated from a culture lysogenised with the \( \lambda / \Phi^80 \) hybrid it was found that in some of those with deletions a variable amount of the prophage genome was also deleted. They were able to produce an unambiguous map in which the prophage markers could be ordered linearly with respect to each other and to the chromosome.

The orders obtained by these two methods are consistent with each other, and differ from the order of genes in vegetative \( \lambda \) phage. The simplest, though not the only, explanation for this type of relation between genetic events within
and outside the prophage region is that the λ prophage is truly inserted by a crossover, of the type suggested by Campbell, between the chromosome and the vegetative λ phage.

Campbell (1963) has examined the segregation pattern of single lysogens from doubly lysogenic strains of the type $\text{gal}^{-}/(\lambda)(\lambda_{\text{gal}})$ (see below). By looking for $\text{gal}^{-}$ segregants and determining the frequency with which different λ markers were also lost, he was able to show non-random loss of λ characters and from these to deduce linkage relationships. This genetic map is also consistent with his model, but as with work of Rothman and of Franklin et al. does not completely exclude other models.

(iv) The formation of defective, $\text{gal}^{+}$ transducing (λdg) particles.

A great merit of Campbell's model of recombination-mediated insertion is that it helps to explain the formation of defective, $\text{gal}^{-}$-transducing λ (λdg) particles. It had been found that λ can transduce the closely linked $\text{gal}$ locus. Lysogenic cultures are inducible by ultraviolet light; such induced lysates, but not spontaneous ones, transduce the $\text{gal}$ region with a low frequency (low frequency transduction or LFT). If a $\text{gal}^{+}$ transductant is itself used as donor for transduction to a new $\text{gal}^{-}$ recipient, $\text{gal}^{+}$ recombinants arise with very high frequency (high frequency transduction or HFT) (Morse et al., 1956). However, if HFT lysates are used at low multiplicities of infection, the $\text{gal}^{+}$
transductants obtained, although immune to superinfection with phage $\lambda$, are found not to liberate phage $\lambda$ on induction. In gaining the gal cluster of genes these $gal^+$ transducing $\lambda$ particles have lost a variable part of their own genome, and are defective. These particles can only produce a lysate if an intact $\lambda$ phage is also present in the cell to provide one or more enzyme functions which are missing from the transducing phage. It turns out that a particular region of the $\lambda$ genome is lost (Arber, 1958; Campbell and Balbinder, 1958). This is interstitial in vegetative $\lambda$; one end of this region is similar in different $\lambda_{dg}$ species while the other is variable and may start in the middle of a cistron (Arber, 1958; Campbell, 1959). The amount of chromosomal material gained is not correlated with the size of the deletion, since the resulting $\lambda_{dg}$ isolated may vary in density by as much as +8% to -14% from the normal (Weigle et al., 1959). Of known chromosome markers only gal and the closely linked bio (Rollman, 1963) are transduced by $\lambda$.

On Campbell's model, the insertion of $\lambda$ is by recombination, while induction occurs by a simple reversal of this process. Recombination is imagined to occur normally in the hypothetical region ABCD (Figure 3). If, however, it occurs instead between points 1 and 2 in the figure, the region lost by the chromosome will not be a normal $\lambda$ but one lacking part of the genome and carrying chromosomal genes. It is found that the region deleted in the various $\lambda_{dg}$ isolates is indeed as predicted on the basis of the determined order of genes in the $\lambda$ prophage.
1. The "fragment" released

2. The release of $\lambda bg$ by a genetic exchange

Figure 3.
Campbell's model for the formation of $\Delta g$ particles (see text). It is assumed that the $\lambda$ prophage is colinear with the chromosome. The upper diagram shows the release of a fragment carrying the gal gene and part only of the $\lambda$ genome. In the lower diagram the same event is represented as a genetic exchange between the two points marked with arrows, giving a circular $\Delta g$ structure.
Thus the fixed point (in the diagram, the right hand ABCD region) coincides with the terminal cistron in the prophage further from the gal locus. However, since separate λdg isolates differ in size and genetic content (see Figure 4), any recombinational model must admit that there cannot be a unique pair of points 1 and 2. This will be discussed in section II in connexion with the origin of F-prime factors.
Figure 4.
Figure 4. The formation of $\lambda_{dg}$ particles; the $\lambda$ genome (line 1) is shown as colinear with the chromosome. Line 2 shows the portion of $\lambda$ which is always (full line) or sometimes (broken line) absent from $\lambda_{dg}$ particles. The third line represents the material always (full line) or sometimes (broken line) present in defective $\lambda$ particles. The distance to which the left hand side of line 3 may extend is unknown.
F. **F-prime factors**

(i) **The discovery of F-prime factors**

Among the experimental difficulties in studying the relationship of the sex factor to the chromosome is the absence, at present, of a genetic map for F analogous to that of \( \lambda \). However, a useful experimental substitute has been found in genetically marked F-prime factors.

It was known that Hfr bacteria can yield derivatives in which the sex factor has returned to the autonomous state. Furthermore, Adelberg and Burns (1960) showed that one such sex factor was variant, since on introduction into a F\(^-\) cell it was found to have an affinity for the chromosomal region at which the sex factor had been integrated in the parent Hfr strain. When this variant sex factor was removed by the action of acridine orange from cells of the type in which it arose, and a normal sex factor was introduced, this second sex factor now had an affinity for this same region. On the assumption (which will be discussed later) that this type of affinity is due to genetic homology, it was, therefore, concluded that recombination could occur between the chromosome and an integrated sex factor.

By analogy with the formation of \( \lambda \) \( \delta g \) particles from lysogenic bacteria, Jacob and Adelberg (1959) predicted and showed that some autonomous sex factors derived from Hfr bacteria carried known chromosomal genes. Such variant sex factors, termed F-prime factors, can mediate chromosome transfer with high frequency and with
(i) F-prime factor-mediated chromosome transfer

When pro\(^+\) recombinants formed in chromosome transfer by a lac\(^-\)/Flac\(^+\) donor were examined, it was found that in some of them the lac\(^+\) gene had preceded pro\(^+\) on the transferred chromosomal structure (Scaife and Gross, 1963). It was suggested that F-prime factor-mediated chromosome transfer depends upon a recombination event (Figure 5) between the substituted sex factor and the chromosome, giving rise to a transient Hfr structure in which the chromosome is diploid for the region carried by the F-prime factor (Scaife and Gross, 1963; Cuzin and Jacob, 1963). In such a strain one of the lac alleles would be transferred early and the other late; which is transferred first depends on whether the crossover event occurs to the left or to the right of the lac locus (Figure 5). Cuzin and Jacob found that some of the persistently lac\(^+\) clones isolated after growth at high temperature of a lac\(^-\) strain carrying a temperature-sensitive Flac\(^+\) factor were clones of these types. It is assumed that these partially diploid structures are normally unstable because of the reversibility of the recombination process.

Further evidence that a recombination event is involved comes from the finding that if the region borne by the sex factor is deleted from the chromosome the ability to mediate oriented chromosome
Figure 5. DONOR STRUCTURES

Type 1 Donor

Type 2 Donor
Figure 5. The partial diploid Hfr structures formed by a reciprocal recombination event between the F-prime factor Plac* and the chromosome (Scaife and Gross, 1964). Which structure is formed depends upon which side of the lac locus the cross-over occurs. The genetic material of the sex factor is represented by the wavy line, and that of the chromosome by the straight line. The arrow represents the leading extremity of the structure transferred during mating.
transfer at high frequency is lost (Cuzin and Jacob, 1964).

Clark and Margulies (1965) have confirmed that a recombination event is involved. They showed that an F\textsuperscript{lac} factor carried in a strain lacking the ability to recombine is able to mediate its own transfer but not that of the chromosome.

It appears that transfer of the sex factor, F-prime factor and the chromosome are fundamentally similar. In each the sex factor, which is a DNA structure, mediates its own transfer and that of any DNA to which it is attached, to female cells in an oriented fashion. Thus the Hfr chromosome is formally analogous to the sex factor, differing from it in having "the bacterial chromosome integrated in the sex factor" (Jacob et al., 1963).

In chromosomal transfer, however, for the sex factor to be transferred the whole chromosome must first be transferred (Figure 6). The transfer of an F-prime factor of itself and of the chromosome both fit into this scheme. Which type of transfer occurs depends upon whether or not the F-prime factor has recombined with the chromosome.

This scheme assumes that the different directions of chromosome transfer by Hfr strains are determined by a polarity inherent in the sex factor itself. If, as we imagine by analogy with \(\lambda\), T4 phages and the bacterial chromosome, the sex factor is a circular structure, it must break at a point for transfer to take place. The nature of the mechanism which might be involved in the specification of polarity or the point of breakage is unknown.
Figure 6.
Figure 6. The relationship between the structures transferred by $F^+$, $F'$ and Hfr donor strains (Scaife and Gross, 1964). In each case, it is the sex factor (represented by the wavy line) which mediates transfer and specifies its polarity. The sex factor is shown as colinear with the chromosomal genes of the $F$-prime factor and the chromosome.
6. The mechanism of the \( F' \rightarrow \text{Hfr} \) transition

Both phage \( \lambda \) and the sex factor are DNA structures which can integrate into the chromosome; when integrated they can both recombine with the chromosome, to give rise respectively to \( \lambda \) particles and F-prime factors. This parallel prompts us to see whether insertion by recombination can explain the integration of the sex factor into the chromosome.

Integration by recombination would depend upon the existence of regions of genetic homology between the sex factor and the chromosome. We should, therefore, predict that the sex factor will not integrate into the chromosome completely at random. If we assume that the direction of chromosome transfer by an Hfr strain is imposed by a polarity inherent in the sex factor, we should expect that at each site of integration only Hfr strains transferring the chromosome in one direction would arise. This is because pairing between DNA double helices with homologous base sequences can only occur with one polarity. For instance the base sequence

\[
\text{ATGCGAGC}
\]

can pair with

\[
\text{TACGCTCC}
\]

but it cannot pair with this sequence in the reverse order

\[
\text{CCGTGCAT}
\]

These predictions, which are common to all models involving pairing as the basis for Hfr formation, are tested and confirmed in the work detailed in the first section of this thesis.
H. The formation of F-prime factors

The analogy has been drawn between the relationship of bacteria lysogenic for λ and λdg particles on the one hand, and of Hfr bacteria and F-prime factors on the other. Predictions made on the basis of this comparison can be tested using F-prime factors carrying several mappable markers. One such structure has been isolated, analysed and compared with respect to the relative positions of these markers on the F-prime factor and on the ancestral Hfr chromosome. The results obtained are consistent with a simple type of deletion model for the origin of such F-prime factors analogous to Campbell's model for the formation of λdg particles. An account of this work is given in the second section of this thesis.
MATERIALS AND METHODS

A. Bacteria and bacteriophages

1. Hfr donor strains

\[
\text{Hfr Cavalli } \text{met}^- \text{str}^F T_4^S T_6^S \text{sugars}^+ (\lambda)^+ \quad \text{(Cavalli-Sforza, 1950)}
\]

\[
\text{Hfr P4x } \text{met}^- \text{str}^S \text{sugars}^+ \quad \text{(Adelberg and Burns, 1960)}
\]

\[
\text{Hfr 13 } \text{met}^- \text{str}^S (\lambda)^- \quad \text{(Hirota and Sneath, 1961)}
\]

2. F\(^+\) donor strains

\[
\text{58-161 } \text{met}^- \text{str}^S \quad \text{(Lederberg, 1947)}
\]

\[
\text{W1655 } \text{met}^- \text{str}^S T_4^S T_6^S \text{sugars}^+ (\lambda)^- \lambda^F \quad \text{(Lederberg and Lederberg, 1953).}
\]

3. F-prime donor strain

\[
\text{W1655 } (\text{Flac}^+) \quad \text{(Scaife, 1964)}. \quad \text{Isolated by}
\]

\[
\text{infecting strain W1655 } F^- \quad \text{(see below) with}
\]

\[
\text{a lac}^+ \text{ revertant of an Flac}^- \text{ factor carried}
\]

\[
\text{by strain 23.10.S. The Flac factor arose}
\]

\[
\text{in the Hfr strain P4x (see above).}
\]

4. Recipient (F\(^-\)) strains

\[
\text{58-161 } \text{met}^- \text{str}^S \quad \text{(Hayes, 1955)}
\]

\[
\text{W1655 } \text{met}^- \text{str}^S \quad \text{(Scaife, 1964)}
\]

\[
\text{W677 } \text{thr}^- \text{leu}^- \text{pro}^- \text{str}^S \text{asi}^S. \quad \text{A pro}^- \text{ derivative of strain W677 (Hayes, 1953).}
\]

\[
\text{The pro}^- \text{ gene was introduced by transduction}
\]

\[
\text{from strain 510 (Reeves, pers. comm.)}
\]
\( \text{W677 thr}^- \text{leu}^+ \text{pro}^- \text{str}^+ \). A \text{str}^\text{F} derivative, kindly provided by Dr. R.C. Clowes, of the above strain. The \text{leu}^+ marker from strain \( \text{W1655} \) was introduced into it, using phage \text{Piko} as the transducing agent.

\( \text{W945 thr}^- \text{leu}^- \text{pro}^- \text{str}^+ \text{lac}^- \text{met}^- \text{gal}^- \) (Cavalli-Sforza and Jinks, 1956).

\( \text{C600 thr}^- \text{leu}^- \text{pro}^- \text{str}^+ \text{lac}^- \) (Appleyard, 1954)

\( \text{P678 thr}^- \text{leu}^- \text{pro}^- \text{str}^+ \text{lac}^- \) (Jacob and Wollman, 1961)

\( \text{J62 pro}^- \text{try}^- \text{his}^- \text{str}^+ \text{lac}^- \) (Clowes and Rowley, 1954)

\( \text{J677 pro}^- \text{try}^- \text{his}^- \text{str}^+ \text{lac}^- \) (supplied by Dr. P.C. de Haan)

(ii) Other \text{E. coli} strains

Strain D (Demerec and Pano, 1945)

Strain C (Bertani and Weigle, 1953)

(iii) \text{Bacteriophages}

Phages T3 and T6 (Delbruck, 1946)

Phage \text{Piko} (Lennox, 1955)

Phage \mu (Dettori et al., 1961)
Figure 7.
The genetic map of *Escherichia coli* K12, showing the markers used or mentioned in this thesis. The arrows represent the origins and direction of transfer of Hfr strains mentioned in the text. The abbreviations used are the following.

- **ade**: adenine
- **asi^r/asi^s**: resistance/sensitivity to azide
- **B1**: vitamin B1
- **gal^+/gal^-**: ability/inability to ferment galactose
- **his**: histidine
- **lac^+/lac^-**: ability/inability to ferment lactose
- **leu**: leucine
- **mann**: mannitol
- **met**: methionine
- **pro**: proline
- **str^r/str^s**: streptomycin resistance/sensitivity
- **T1^r/T1^s**: resistance/sensitivity to phage T1
- **T6^r/T6^s**: resistance/sensitivity to phage T6
- **thr**: threonine
- **try**: tryptophan
- **λ**: prophage λ
- **18**: prophage 18
- **C**: origin of Hfr strain Cavalli
- **F**: origin of Hfr strain Hayes
- **F4x**: origin of Hfr strain F4x
- **13**: origin of Hfr strain 13
- **B1-12**: origins of Hfr strains B1-12 (see text)
B. Media

Nutrient broth. 2.5% Oxoid Nutrient Broth No. 2.

Minimal media as described by Lederberg and Tatum (1947), but without asparagine.

Nutrient and minimal agar were made by solidifying the appropriate liquid media with 1.25% and 1.5% Davis New Zealand Agar respectively.

Buffer. An aqueous solution (pH approx. 7.2) with 0.7% anhydrous NaH₂PO₄, 0.4% NaCl, 0.3% KH₂PO₄ and 0.02% MgSO₄·7H₂O.

Amino acids, vitamin B₁₂ and streptomycin were added to give final concentrations of 20, 10 and 250 μg/ml. respectively.

Eosin-methylene blue medium as described by Lederberg (1950)

C. Methods

Strains were maintained at 4°C on nutrient agar slopes. All experiments were performed at 37°C. Stationary-phase cultures were obtained by inoculating into nutrient broth and incubating without aeration. After overnight growth such cultures contain about 10⁹ cells per ml. To obtain cultures in the logarithmic phase of growth, stationary phase cultures were diluted tenfold into broth. In crosses such cultures were generally incubated for 1/₂ hr. (giving about 2 x 10⁶ cells per ml.) on a rotor (to give some aeration), before mating. Matings were interrupted by violent agitation with a microrid flask shaker running at full speed.
A. Introduction

The object of these experiments was to establish whether in a typical $F^+$ strain of *E. coli* K12 stable Hfr strains arise with their origins (that is, the point at which the sex factor is integrated) distributed quite randomly around the chromosome, or whether there are specific regions at which the sex factor can integrate stably. An $F^+$ strain was used as a source of independently and spontaneously arising Hfr strains, which were then characterised, and with these it was shown that such specific regions do exist.

B. The isolation of Hfr strains

1. Principle

Jacob and Wellman (1956b) had isolated Hfr strains using an adaptation, described in the introduction (p. 14) of Luria and Delbrück's experiments (1943) which had demonstrated the clonal nature of bacterial mutation. In crosses against a female strain Jacob and Wellman determined from the recombinant yield, assuming that fertility of these cultures was due to Hfr cells, which of a series of $F^+$ cultures had most Hfr cells. They then isolated Hfr strains from the most fertile cultures by plating out dilutions and detecting fertile colonies by replica
plating onto a lawn of $F^-$ cells on selective medium on which only recombinants will grow.

In the present study the Luria and Delbruck fluctuation method was also used for obtaining cultures which in crosses give rise to many recombinants, but Hfr strains were then isolated from these by Cavalli and Lederberg's sub-selection enrichment method (1955). This involves estimating how many Hfr cells there are in a culture from the recombinant count, and then diluting the culture so that when aliquots are dispensed into a series of tubes only a few tubes will receive an Hfr cell. In this minority of cultures the Hfr population will, therefore, be enriched with respect to the background of $F^+$ cells because the ratio of $F^+$ cells to Hfr cells in these few tubes will be smaller than in the original undiluted culture. After incubation it can be found which cultures are fertile; after several such cycles stable Hfr clones may be isolated. This method has been used independently by Makela (1963) for the isolation of Hfr strains in Salmonella

We cannot accurately estimate the number of Hfr cells in an $F^+$ culture from the number of a given class of recombinants which it gives. The relation between these two numbers depends upon the mating efficiency of the Hfr strain in question as well as on the distance of the point at which the sex factor integrates on the chromosome from the selective marker used. In practice empirical coefficients were used for the dilutions. Growth was
limited as much as possible because at least some Hfr strains grow more slowly than their F+ ancestor (p. 58).

The present method differs from Jacob and Wollman's in two further respects. No ultraviolet irradiation was used, and the cells were kept in broth at all stages of the isolation procedure, so that mutations to auxotrophy which might accompany the transition from the F+ to the Hfr state would not necessarily be lethal.

The strain used in this study was W1655 F+, a methionineless, non-lysogenic derivative of the original Lederberg (1947) strain 58-161. It is sensitive to streptomycin and to all the phages of the T series and it ferments the same sugars as the wild type K12 strain. In matings it gives approximately equal numbers of recombinants for markers distributed around the chromosome, so that there is no special affinity of the sex factor for any particular region of the chromosome analogous to that described by Richter (1961). Each Hfr strain isolated was derived from a culture started from a different F+ colony; there was therefore no possibility of the repeated isolation of the same Hfr clone.

(ii) Techniques

An overnight broth culture of the F+ strain, derived from a single colony, was diluted into broth to give a suspension with about 160 cells per ml. Forty-four 1 ml. aliquots were
dispensed into tubes, which were then incubated at 37° for 5½ hr., to give populations of about $5 \times 10^6$ cells per ml. 0.15 ml. samples were withdrawn and mated for one hour at 37° with 0.1 ml. of a culture of an F^− strain (about $2 \times 10^7$ cells) suspended in buffer. A drop of this mixture was then plated onto selective medium. The cultures which gave rise to many recombinants were inferred to contain a large clone of Hfr cells with an origin relatively close to the selected marker. The F^+ cultures were stored at 4°C until it was known which of them would be used for the next cycle of the enrichment procedure.

Cultures were only enriched if they gave more than four times the average number of recombinants. Such a culture was then diluted into fresh broth for the next cycle, so that only a minority of 1 ml. aliquots would contain an Hfr cell, using the number of recombinants obtained as the approximate dilution factor. One ml. volumes of this diluted culture were then dispensed into a fresh series of tubes, incubated for 4½ hr., mated with the female and plated as before. After between one and three cycles of dilution, incubation and mating, the culture was sufficiently enriched to allow plating on nutrient agar for the picking of single Hfr colonies. This process was repeated to obtain each Hfr isolate.

(iii) Results

In the first series of isolations undertaken, selection was for Hfr strains which transfer thr^+ leu^+ early. The recipient
strain used was W245 thr^- leu^- str^- R^+. Six independently arising Hfr strains were obtained; these were characterized by one-hour crosses and by interrupted matings (Figure 8). The data presented in Table I allow us to construct a consistent map of the sequence of gene transfer of these Hfr strains. It will be seen that all these Hfr strains (strains B1-6) transfer pro^+ and thr^+ leu^+ with high efficiency. In addition, it was shown that in crosses with strain P678 thr^- leu^- str^+ R^+, Hfr strains B1-6 did not transfer the lac^+ character, although this character was transferred efficiently by strains Hfr Hayes and Hfr Cavalli. It was, therefore, concluded that the origins of all these strains are in the small region between pro and lac. In interrupted mating experiments each of these Hfr strains transferred the pro^+ and thr^+ leu^+ loci after about 5½ and 13 min. mating respectively.

To determine whether another class of Hfr strains exists in which the sex factor is also integrated between the pro and lac loci, but which transfer the chromosome in the opposite direction, a second series of isolations was undertaken. The recipient strain used was J62 pro^- try^- his^- str^-, and the isolation of Hfr strains transferring the try^+ gene (which is about the same distance as the thr leu loci from the pro lac region) was attempted. Six further Hfr strains (B7-12) were isolated. They fell into three groups: whereas strain B12 transferred his^+ very early, the origins of strains B8 and B11 fell between the T6 and lac loci. These two strains, which have very similar transfer kinetics, transfer ade^+...
Figure 8.

Recombinants per plate

Time at which mating is interrupted

- $\times$ pro$^+$ str$^+$ (Hfr B3 donor)
- $\triangle$ thr$^+$ leu$^+$ str$^+$ (Hfr B3 donor)
- $\bullet$ pro$^+$ str$^+$ (Hfr B4 donor)
- $\circ$ thr$^+$ leu$^+$ str$^+$ (Hfr B4 donor)
The selection of pro+ and thr+ leu+ recombinants in interrupted matings between Hfr strains B3 and B4 and the F- strain W677. 0.05 ml. of an overnight culture of strain B3 was inoculated into 5 ml. of prewarmed broth. After 1.5 hr. incubation on the rotator at 37°C the culture was mixed with 5 ml. of an overnight culture of strain W677 (thr leu B+ pro str+). Samples were withdrawn at intervals, diluted tenfold into buffer, and violently agitated for thirty seconds to separate mating pairs. After a further five-fold dilution in buffer, 0.2 ml. samples were spread on minimal medium, supplemented with glucose, B1, threonine, leucine and streptomycin for the selection of pro+ recombinants, and with glucose, B1, proline and streptomycin for the selection of thr+ leu+ recombinants. An identical procedure was followed for strain B4.
The mapping of the origins of Hfr strains B1-12. Young broth cultures of the Hfr and the F- strains, each at about $4 \times 10^8$ cells per ml, were mated for one hour. 0.1 ml. samples of $10^{-6}$ dilutions were plated onto selective media containing streptomycin. In the crosses with strains B4-6, the female strain used was W677 thr+ leu+ pro+ strF and that used with strains B7-12 was J62 pro+ try+ his+ strF. The sequences of gene transfer by Hfr strains B1-3 were inferred directly from interrupted mating experiments.

<table>
<thead>
<tr>
<th>Cross between strains</th>
<th>pro+</th>
<th>thr+ leu+</th>
<th>B+</th>
</tr>
</thead>
<tbody>
<tr>
<td>B4 x W677</td>
<td>66</td>
<td>44</td>
<td>4</td>
</tr>
<tr>
<td>B5 x W677</td>
<td>146</td>
<td>55</td>
<td>12</td>
</tr>
<tr>
<td>B6 x W677</td>
<td>670</td>
<td>326</td>
<td>65</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>pro+</th>
<th>try+</th>
<th>his+</th>
</tr>
</thead>
<tbody>
<tr>
<td>B7 x J62</td>
<td>73</td>
<td>263</td>
</tr>
<tr>
<td>B9 x J62</td>
<td>62</td>
<td>209</td>
</tr>
<tr>
<td>B10 x J62</td>
<td>112</td>
<td>517</td>
</tr>
<tr>
<td>B8 x J62</td>
<td>0</td>
<td>200</td>
</tr>
<tr>
<td>B11 x J62</td>
<td>0</td>
<td>186</td>
</tr>
<tr>
<td>B12 x J62</td>
<td>10</td>
<td>64</td>
</tr>
</tbody>
</table>
early (Figure 9) and lac+ late. Hfr strains B7, 9 and 10 form the third class. All three transfer try+ after 7-10 minutes and his+ late.

(iv) Conclusion

These findings strongly suggest that there are specific regions where the sex factor can stably integrate to give Hfr strains. The segment between the pro and lac loci is one such region; a total of twelve Hfr strains transferring pro early and lac late have been isolated. As well as the six Hfr strains B1-6, and three more strains isolated by the present writer using a replica plating technique, Hfr strains B2 and R4 (Reeves, 1959) and P4x (see Figure 7) also have the sex factor integrated in this region. The small segment of the chromosome between the F6 and lac loci appears to be another such region, since three Hfr strains (B6, B11 and 15) (Hirot a and Sneath, 1961); see also p. 82 have their origins here.

Since Hfr strains with the sex factor integrated between pro and lac which transfer lac first were looked for but not found, another conclusion is that at least in this region the sex factor can only integrate to give Hfr strains transferring the chromosome with one polarity.
Figure 9.

Recombinants per plate

Time at which mating is interrupted

○ ade<sup>+</sup>str<sup>r</sup>(Hfr B8 donor)

● ade<sup>+</sup>str<sup>r</sup>(Hfr B11 donor)
The selection of ade+ recombinants in interrupted matings between F+ strains B6 and B14 and the F- strain 1777. 0.05 ml. of an overnight culture of strain B6 was inoculated into 5 ml. of prewarmed broth. After 1.5 hours incubation on the rotor at 57°C, the culture was mixed with 5 ml. of an overnight culture of strain 1777 (2 ade+ str-). Samples with withdrawn at intervals, diluted tenfold into buffer, and violent agitated for 30 seconds to separate mating pairs. After a further five-fold dilution in buffer, 0.2 ml. samples were spread on minimal medium supplemented with glucose, B1 and streptomycin, for the selection of ade+ str+ recombinants. An identical procedure was followed with strain B14.
To distinguish between apparently similar Hfr strains

(1) Transduction using phase P1

An obvious approach was to compare the linkage between the sex factor and the closest known chromosomal markers, using transduction with phase P1ko (Lennox, 1955). In such an experiment Hfr strain B1 was used as a donor and a pro⁻ lac⁻ strain (a pro⁻ leu⁺ derivative of strain W677 thr⁻ leu⁻ B⁺ lac⁻ strB) was used as the recipient. Although both pro⁺ and lac⁺ transductants were obtained, of several hundred recombinants tested none had inherited either the unselected marker (that is, lac⁺, pro⁺, respectively) or the sex factor.

Since de Witt and Adelberg (1962) had reported joint transduction of the lac⁺ character and the sex factor in an experiment in which Hfr strain P4x was used as the donor and a recipient strain analogous to strain W677 as the recipient, this Hfr strain was then used in a control experiment. In contrast with their findings, none of the lac⁺ transductants obtained carried the sex factor. Because of the absence of any positive results, this approach was not pursued further.

(ii) The rate of reversion from the Hfr to the F⁺ state

It was thought that each species of Hfr strain might have a characteristic stability. The proportion of F⁺ cells among the descendents, in a known number of generations, of single cells of the different Hfr strains were, therefore, determined and compared.
Overnight broth cultures of each of these strains were diluted $\times 5 \times 10^9$ into broth and dispensed as 1 ml aliquots so that the initial number of cells in any one tube was unlikely to be more than one. After overnight incubation those tubes which yielded growth were checked for fertility in crosses with appropriate recipient strains and dilutions were plated for the recovery of isolated colonies. One hundred colonies derived from each Hfr strain were resuspended in broth and scored for fertility of the Hfr or the $F^+$ type by mixing with an equal volume of a culture of an $F^-$ strain and then plating the mixture for recombinant colonies on selective media. This technique gives better discrimination than the use of replica plating. In general, each clone gave a clear Hfr or $F^+$ level of fertility. The few clones which were doubtful, and all apparent $F^+$ clones, were retested to ensure that low fertility was not an artifact.

It was found that with none of the Hfr strains tested (B1-41) had the proportion become greater than 3% after the thirty generations of growth from one cell to about $10^9$ cells. To obtain greater resolution, therefore, clones which had been serially subcultured through a total of about eighty generations were examined.

It was important to show that the results obtained by this method from a given Hfr strain are reproducible. All determinations were, therefore, made on two or three parallel cultures, each starting from a different single cell of the Hfr
strain. It was confirmed that within an Hfr strain the data from parallel experiments are very similar. For example, in the experiment given on Table III, the three Hfr B7 clones subcultured in parallel gave rise to 2/33, 3/33 and 4/34 revertants.

The results are presented in this table. Hfr strains with similar origins tended to give similar numbers of F+ cells, but different Hfr strains gave rise to very different numbers of F+ cells. Thus Hfr strains B8 and B11 resembled each other, as did strains B7, 9 and 10. An exception was strain B1, which differed significantly from strains B2-6. No revertants were found among the descendants of strains B2-6, so that these strains were indistinguishable within the limits of the experiment.

A very interesting point emerges from a comparison of the thirty and eighty generation samples from strains B7, 9 and 10. It appears that during growth of these strains the proportion of F+ cells increases exponentially. The simplest explanation, assuming a constant rate for the Hfr → F+ transition, is that the Hfr cells grow more slowly than F+ cells. To account for the observed rate of increase of the F+ population in these cultures the Hfr cells would have to grow at only 0.076 of the rate (1.0) for F+ cells. The experimental result obtained in growth rate experiments using strains Hfr M10 and M1655 F+ was 0.79. We may, therefore, conclude that there is a difference in growth rate, sufficient to explain this effect, between Hfr strains of the B7 type and the F+ strain from which they arose. However, slower growth is not a general property
<table>
<thead>
<tr>
<th>Hfr strain</th>
<th>Approximate number of generations of growth</th>
<th>Number of colonies tested</th>
<th>Number of revertant clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>80</td>
<td>200</td>
<td>3</td>
</tr>
<tr>
<td>B2</td>
<td>&quot;</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>B3</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>B4</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>B5</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>B6</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>B7</td>
<td>30</td>
<td>&quot;</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>&quot;</td>
<td>91</td>
</tr>
<tr>
<td>B9</td>
<td>30</td>
<td>&quot;</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>&quot;</td>
<td>96</td>
</tr>
<tr>
<td>B10</td>
<td>30</td>
<td>&quot;</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>&quot;</td>
<td>99</td>
</tr>
<tr>
<td>B8</td>
<td>80</td>
<td>&quot;</td>
<td>1</td>
</tr>
<tr>
<td>B11</td>
<td>&quot;</td>
<td>&quot;</td>
<td>3</td>
</tr>
</tbody>
</table>

**Table III.** The formation of $F^+$ derivatives during subculture of Hfr strains B1-11. Overnight broth cultures of Hfr strains B1-11 were diluted $2 \times 10^{-10}$ into broth and dispensed as 1 ml aliquots. After overnight incubation dilutions and platings were made for the recovery of 30 generation colonies. Cultures from each Hfr strain were also diluted $2 \times 10^{-6}$ into broth; these cultures were in turn incubated overnight. After three cycles of dilution and overnight incubation, dilutions and platings were made for the recovery of 80 generation clones. Colonies were tested for the Hfr character by suspending in broth, incubating, crossing with appropriate $F^-$ strains ($\Delta 677$ thr leu $B^+$ pro $str^+$ for strains B1-6 and J62 pro $try^+$ his $str^+$ for strains B7-11). Standard loopfuls of the mating mixtures were plated onto selective media for the recovery of $thr^+ leu^+$ (strains B1-6) and $try^+$ (B7-11) recombinants. In each case the results given are the sum of two or three parallel experiments.
of Hfr strains, since in reconstruction experiments strains W1655
F+ and Hfr B1 appeared to grow at the same rate (Table IV).

(iii) The transfer-race technique

The object of this refinement of the interrupted mating experiment is to exclude some of the factors which cause fluctuations between the results of different experiments. Comparisons between pairs of Hfr strains are made by using them in a mixed mating with the same F- strain, so that all sampling errors and environmental effects cancel out. The contribution which each of the two donor strains makes to the recombinant population can be assessed by labelling one of them with a non-selective marker which is inherited with high frequency by the selected recombinants. In the present study the entry times of thr+ leu+ for Hfr strains B1 and B3 were compared, since although they were indistinguishable in simple interrupted mating experiments in the reversion experiment (see p. 58) only strain B1 gave F+ derivatives at a measurable rate. Aside resistance (argF) was used as the marker labelling one of the Hfr strains; since both strains transfer their arg allele just before thr+ leu+, all the selected recombinants will have received the arg locus, irrespective of the time at which mating is interrupted. If one of the strains transfers the thr+ leu+ loci earlier than the other strain does, in mixed matings the "winning" strain will contribute a higher proportion of the first-formed recombinants; these can be recognised by the arg allele which they carry.
Table IV. Growth rates of strains Hfr B1 and W1655 F⁺ compared. W1655 F⁺ asi⁺, Hfr B1 asi⁻ and Hfr B1 asi⁺ were the strains used. Overnight broth cultures were diluted 10⁻⁴ and 0.1 ml of each were added to 5 ml of broth to give a mixed culture. These mixed cultures were grown at 37°C without shaking. After incubation for two hours and for six hours, forty minutes dilutions were plated for single colonies, which were tested for their asi character by resuspending in buffer and streaking onto nutrient agar supplemented with Na/500 (final concentration) sodium azide.
An azide-resistant derivative of strain B3 was isolated and the following pairs of strains were together mated with the female strain W677 thr^- leu^- B^ pro^- str^ asi^ s 

B3 asi^ s + B3 asi^ f 

B3 asi^ s + B1 asi^ f 

The former cross (Table V) showed that the asi character itself did not affect the kinetics of mating. The results of the second cross show that within the limits of the experiment, Hfr strains B1 and B3 are indistinguishable with respect to the time of transfer of the thr^ + and leu^ + loci.

D. The analysis of recombinants formed in F^+ crosses.

We have confirmed Jacob and Wollman's demonstration that at least part of the fertility of F^+ populations is due to the generation of Hfr clones. In the present study, selection at each stage in the isolation procedure of the tube giving rise to most recombinants resulted in the enrichment and isolation of Hfr strains, so that we may conclude that these strains are indeed derived from the observed fertile clones. However, as was pointed out in the introduction, there is no proof that such clones are the sole basis for the fertility of F^+ cultures. We may now return to this question, and ask whether Hfr strains such as those isolated (B1-12) in the present study are sufficient to explain the fertility of strain W1655 F^+.

Strains B1-12 divide into three groups with respect to
### Table V. The transfer-race experiment.

Overnight broth cultures of the Hfr strains were diluted $10^{-2}$ into fresh broth. After 18 hours incubation at 37°C on a rotor the two Hfr cultures were mixed and a 5 ml. volume of the mixture was added to an equal volume of an F⁻ strain (W677 thr⁻ leu⁻ B₁ pro⁻ str⁻ asi⁻). At appropriate times samples were withdrawn, diluted tenfold into buffer, violently agitated for one minute, and further diluted fivefold. 0.2 ml. samples were plated on minimal media supplemented with glucose, proline, vitamin B₁, and streptomycin, for thr⁺ leu⁺ recombinants. Recombinant colonies were resuspended in buffer and streaked on plates of the selective media supplemented with $\frac{M}{1600}$ (final concentration) sodium aside. Under these conditions an unambiguous resistance/sensitivity result was obtained for the asi character.

<table>
<thead>
<tr>
<th>Hfr strains in cross</th>
<th>Time at which mating is interrupted</th>
<th>Total number of thr⁺ leu⁺ recombinants obtained per 0.2 ml. sample</th>
<th>Number of recombinants tested</th>
<th>asi⁻ %</th>
<th>asi⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>B₃ asi⁺ + B₃ asi⁻</td>
<td>12</td>
<td>96</td>
<td>108</td>
<td>71</td>
<td>66</td>
</tr>
<tr>
<td>B₃ asi⁺ + B₃ asi⁻</td>
<td>25</td>
<td>712</td>
<td>108</td>
<td>66</td>
<td>61</td>
</tr>
<tr>
<td>B₃ asi⁺ + B₁ asi⁻</td>
<td>12</td>
<td>150</td>
<td>72</td>
<td>47</td>
<td>65</td>
</tr>
<tr>
<td>B₃ asi⁺ + B₁ asi⁻</td>
<td>25</td>
<td>&gt;1000</td>
<td>72</td>
<td>47</td>
<td>65</td>
</tr>
</tbody>
</table>
the transfer of the pro lac try region of the chromosome. While strains B1-6 transfer pro+ but not lac+ or try+ with high frequency, and strains B8 and B11 transfer only try+, strains B7, 9 and 10 and also strain B12 transfer all three loci with high frequency. Thus, whereas pro+ and try+ are transferred by two of the three groups of strains, lac+ is only transferred by the B7 group. Therefore, if F+ fertility is due to Hfr clones (and assuming that strains B1-12 are a representative sample of these) we should expect that in F+ x F- crosses lac+ will be transferred less frequently than either pro+ or try+ and also that the proportion of the lac+ recombinants which are pro+ will be greater than the proportion of pro+ recombinants which are lac+.

Strain 21655 F+ (from which strains B1-12 had been isolated) was mated with strain J62 F- pro- try- his- lac- strF in a one hour cross, selecting for pro+ and for try+ recombinants. A peculiarity of strain J62 prevents direct selection for lac+ recombinants, but the frequency of inheritance of the lac+ character can be examined among the pro+ recombinants, which were also tested for the try character.

A control cross was made with Hfr strain B10, which transfers genes in the order O. try lac pro. It was found that of the pro+ recombinants 86% were lac+ and 69% were try+ (Table VI).

In the F+ cross 65% of the pro+ recombinants were also lac+. Since linkage between these genes (as determined in the Hfr cross) is 86%, 76% (that is, 65 x \(\frac{100}{86}\)) must have received the lac+
1. Approximate number of recombinants per ml. of mating mixture

<table>
<thead>
<tr>
<th>Cross</th>
<th>Recombinant class</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pro⁺</td>
</tr>
<tr>
<td>F⁺ x F⁻</td>
<td>1.8 x 10⁹</td>
</tr>
<tr>
<td>Hfr B10 x F⁻</td>
<td>9.4 x 10⁶</td>
</tr>
</tbody>
</table>

2. Analysis of pro⁺ recombinants

<table>
<thead>
<tr>
<th>Donor strain</th>
<th>Number of recombinants tested</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pro⁺ try⁺ lac⁺ try⁻ lac⁻ try⁺ lac⁻ try⁻</td>
<td></td>
</tr>
<tr>
<td>Hfr</td>
<td>63</td>
<td>64</td>
</tr>
<tr>
<td>F⁺</td>
<td>80</td>
<td>17</td>
</tr>
</tbody>
</table>

Table VI. Linkage between pro⁺, lac and try among pro⁺ recombinants in crosses of strains Hfr B10 and W1655 F⁺ against strain J62 pro⁺ try⁺ his⁺ lac⁺ str⁺. Logarithmically growing broth cultures were mated for one hour before dilution and plating on minimal selective medium for pro⁺ and try⁺ recombinants. Purified pro⁺ recombinant colonies were suspended in buffer and streaked onto selective media to score for the try and lac characters.

Hfr strain B10 transfers its markers in the order try⁺ lac⁺ pro⁺.
genes. Since Hfr strains B1-6 do not transfer lac⁺, according to these results only 100-76 = 24% of the pre⁺ colonies can possibly have come from this type of strain.

It might be argued that Hfr clones similar to strains B7, 9 and 10 would account for the pre⁺ recombinants which are lac⁺. However, only 19% of the pre⁺ recombinants from the P⁺ cross were try⁺. Since Hfr P10 showed 69% linkages between the pre⁺ and try⁺ markers, the maximum proportion of the pre⁺ recombinants which can have been derived from this type of donor is 19 \times \frac{100}{69} = 28\%.

We must, therefore, conclude that at least about half of the pre⁺ recombinants in P⁺ crosses do not derive from Hfr cells of either the B1 or B10 type. However, because of the relatively small samples of recombinants examined, this can only be a tentative conclusion.

E. The transfer of the T₆ locus by Hfr strains B8 and B11

In crosses with F⁻ strain 1774 (lac⁻ T₆⁺ ade⁻) Hfr strains B8 and B11, which are lac⁺ T₆⁺ ade⁺, transfer ade⁺ but not lac⁺ with high frequency: in such crosses the ade⁺ gene begins to enter the recipient cell after about eight minutes (Figure 9). When ade⁺ recombinants from one-hour crosses were tested for the inheritance of the T₆ gene, which is located between the lac and the ade loci, it was found that about 12% of these were T₆⁺ (Table VII). It was inferred that the T₆ locus is transferred

*It is assumed that the sizes of the DNA fragments transferred in the P⁺ and Hfr matings are similar. If this assumption is false, it follows that P⁺ fertility cannot be due to Hfr clones.
### Table VII

The inheritance of the donor T₆ alleles among ade⁺ recombinants in crosses of Hfr strains B8, B10 and B11 with strain 1177. Logarithmically growing cultures of strain 1177 (loc, B, ade, str⁻) and of the mat⁻ T₆ ade⁺ str⁺ strains were mixed. After one hour, one thousand-fold dilutions were plated onto minimal medium supplemented with glucose, vitamin B₁, and streptomycin for ade⁺ str⁺ recombinants, which were purified by restreaking on the same medium. Colonies were picked and resuspended in buffer, and these suspensions were cross-streaked against a phage T₆ suspension on nutrient agar.

<table>
<thead>
<tr>
<th>Donor strain</th>
<th>Number tested</th>
<th>Number which were T₆⁺</th>
<th>% T₆⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hfr B8</td>
<td>53</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Hfr B10</td>
<td>54</td>
<td>25</td>
<td>46</td>
</tr>
<tr>
<td>Hfr B11</td>
<td>52</td>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>

### Ada⁺ recombinants
early rather than late by strains B8 and B11, and that the origins of these strains lie between the $T_6$ and the $lac$ loci. However, the value of $12\%$ is strikingly low, and compares with a value of about $45\%$ among the $ade^+$ recombinants formed in a parallel cross with Hfr strain B10, which transfers both these markers rather late. It is proposed that this low efficiency of integration of the $T_6$ gene from strains B8 and B11 is a function of the gene's proximity to the origin of these strains.

It was shown that strain B11 transfers the $T_6$ gene, which is dominant, as efficiently as does strain B10, and that the $T_6$ phenotype is then expressed in the zygotes. In parallel crosses strains E10 and B11 were mated with strain 1177; after one hour the mating mixtures were diluted and challenged with $T_6$ phage for twenty minutes before plating, with controls, for $ade^+$ recombinants. In each case the addition of phage $T_6$ reduced the recombinant yield by about half (Table VIII). It, therefore, appears that the $T_6$ locus is transferred as efficiently by Hfr strain B11 as by strain B10, and that it can then be expressed, so that the low proportion of $ade^+$ recombinants from the B11 cross which are $T_6$ is due to a failure in integration rather than in transfer.

On the assumption that integration requires recombination we should expect this result if the distance between the origin of the transferred chromosome and the $T_6$ locus is small compared with the distance between the $T_6$ and $ade$ loci.
Table VIII. The expression of the $T_6^*$ phenotype in zygotes.

Hfr strains B10 and B11 were mated with strain 1177 $B_2 T_6^*ade^-str^+$, as described in Table VII. After thirty minutes the cultures were diluted tenfold into fresh prewarmed broth. After a further thirty minutes 0.1 ml. samples of appropriate dilutions were mixed with 0.1 ml. aliquots of a $T_6^*$ suspension (c. $3 \times 10^{11}$ particles/ml.); twenty minutes later these mixtures were plated directly onto minimal medium supplemented with glucose, streptomycin, and vitamin B$_2$ for the selection of ade$^+$ str$^+$ recombinants. Results are expressed in recombinants per ml. of the original mating mixture.
F. Discussion

When Hfr strains transferring the \textit{thr}^+ and \textit{leu}^+ loci as proximal markers were selected for, all six isolates (strains B1-6) were found to have their origins in a small chromosomal region between \textit{pro} and \textit{lac}, about ten minutes of transfer time away from the \textit{thr} \textit{leu} region. The length of this \textit{pro-lac} segment is about 2\% of that of the whole chromosome (Jacob and Wollman, 1961). A firm conclusion from this result is, therefore, that Hfr strains isolated by this method arise by non-random integration of the sex factor into the chromosome.

In simple interrupted mating experiments the origins of these six Hfr strains were indistinguishable. In more refined transfer experiments Hfr strains B1 and B3 could still not be distinguished, although these strains differed in that \textit{F}^+ derivatives were found in subcultures of strain B1 but not in those of strain B3. Attempts were also made to compare the linkage of the sex factors of Hfr strains B1-6 to neighbouring chromosomal genes by transduction, using phage \textit{P1} as the transducing phage. As mentioned earlier (p.56) no joint transduction of the sex factor with either \textit{pro} or \textit{lac} was obtained with strain Hfr B1 or with strain Hfr \textit{Pl}, with which co-
transduction was reported by de Witt and Adelberg (1962).

Attempts were made to isolate Hfr strains of the reciprocal type; that is, strains with the sex factor integrated in the same region as in strains B1-6, but transferring the
chromosome in the opposite direction. These were not found; if we assume, from the isolation of strains B1-6, and the existence of similar strains isolated by other workers (see p. 54) that specific integration sites do exist, this is good evidence that these sites have polarity. The isolation of other Hfr strains (B7-12) is also described; the fact that five out of six fall into two groups is strong support for the proposition that there are specific small regions where the sex factor can integrate to give rise to stable Hfr strains.

The repeated isolation of similar types of Hfr strain, and the absence of the reciprocal type to strains B1-6, would both be expected if the formation of Hfr strains is dependent on pairing between homologous regions of the DNA of the sex factor and of the chromosome. Models postulating that the sex factor can integrate with equal ease at all points on the chromosome are excluded, as are those which propose that the sex factor associates with non-DNA regions of the chromosome, unless assumptions on the nature of this attachment are made, to account for the observed polarity and the differences in stability between Hfr strains of the different types which were isolated. These results are, therefore, consistent with Campbell's model for insertion by recombination, although they do not exclude pairing without recombination as a mechanism for integration.

If Hfr strains are indeed formed by recombination
between the sex factor and the chromosome, the stability of different Hfr strains and the frequency with which these different types arise should both be functions of the length of the homologous region. This is because the possibility of effective pairing between the sex factor and the chromosome and the frequency of recombination between them should both be related to the length of this region. If return to the autonomous state is also by recombination, the rate at which this occurs should also be a function of the length of this region of homology. Short regions should, therefore, give rise rarely to very stable Hfr strains, and less stable Hfr types should arise more frequently by integration at other, longer regions of homology. We should expect that those types of Hfr strains which are actually isolated would be in the middle of this range of stabilities; the others would either arise too rarely, or else they would be too unstable to be isolated.

The stabilities of the strains B1-11 were, therefore, measured in growth experiments in which cultures were grown for a similar number of generations from a single fertile cell. Striking differences were observed in the numbers of F' cells in the subcultures of the different Hfr strains, although in general the strains within a group gave comparable results; thus, for instance, strains B7, 9 and 10 gave values respectively of 91%, 96% and 99%. An exception was strain B1, which gave revertants, and appeared to differ significantly in this respect from strains B2-6.
However, further investigation showed that the high proportion of $P^+$ cells in the cultures of Hfr strains B7, 9 and 10 was in each case mainly due to the fact that these Hfr strains grow much more slowly than the $P^+$ cells arising from them, rather than to an inherently high degree of instability. This result is of great interest, as it suggests that the growth rate of an Hfr strain is a function of the region at which the sex factor is integrated.

If these values are corrected for the hypothetical case where the Hfr and $P^+$ cells grow at equal rates, the reversion figure for Hfr strains B7, 9 and 10 after eighty generations growth is only about 3%. Thus when this correction is made, there is rather little difference in the stabilities of the Hfr strains in which it is measureable.

Jacob and Hollman's observation (see introduction) that persistent fertile clones arise in $P^+$ populations has been confirmed in the present study by the isolation of Hfr strains B1-12 following enrichment from $P^+$ cultures chosen for their fertility. However, the examination of recombinants obtained in an $F^+ \times F^-$ cross, using the same $F^+$ strain from which the Hfr isolates were obtained, gave very different frequencies for the inheritance of unselected markers from those predicted on the assumption that $P^+$ fertility is due to Hfr strains of the types isolated in the present study. In particular, this analysis shows that Hfr clones of the B1 and B7 types together cannot
contribute more than half the \( \text{pro}^+ \) recombinants obtained in the \( F^+ \) cross. It is, therefore, inferred that the types of \( \text{Hfr} \) strain actually isolated can only contribute a component to \( F^+ \) fertility. However, we must treat this conclusion with some caution, first because the samples tested were not very large, and secondly because the formation of a large number of \( \text{Hfr} \) clones similar in transfer properties to the \( \text{Hfr} \) Cavalli strain (that is, transferring both \( \text{lac}^+ \) and \( \text{pro}^+ \)) would explain this result. Yet, if these arise frequently in the present \( F^+ \) strain, it is surprising that strains of this type were not isolated.

A recombination model for the origin of \( \text{Hfr} \) strains would predict this difference, since in \( F^+ \) cultures the unstable and, therefore, non-isolable types of \( \text{Hfr} \) clones would make a large contribution. In contrast, if integration involves pairing but no recombination, those \( \text{Hfr} \) strains actually isolated should represent the types which are both the most stable and the most frequently arising in \( F^+ \) cultures. Another inference which might be drawn from this experiment is that the total number of sites at which the sex factor can integrate is indeed not large, since in \( F^+ \times F^- \) crosses different genes are transferred at significantly unequal frequencies. If there were a very large number of origins these frequencies would all be equal.

The Campbell model also allows us to explain the observation that some \( \text{Hfr} \) species grow more slowly than the strain from which they arise. If recombination occurs at regions of
limited homology distributed randomly around the chromosome, insertion will interrupt the genes at which the pairing and recombination between the chromosome and the sex factor takes place. This could lead to impairment of function and the consequent slow growth rate or even death of the cell. It should, however, be noted that since the procedure employed in the isolation of strains B1-12 involved competitive growth of the Hfr strains with strain W1655 F⁺ (although growth was limited as far as possible) we should not expect to isolate very defective Hfr strains. But one class of Hfr types which might still have been isolated were not observed; although at all stages in their isolation strains B1-12 were kept in broth, none had any nutritional requirements additional to those of the ancestral strain. Also like strain W1655 F⁺, they were able to utilise lactose.

A possible alternative explanation for the experimental result that strains B7, 9 and 10 grow more slowly than strain W1655 F⁺ is that growth rate is determined by the length of the chromosome, the DNA of which is replicated at a constant rate, and that Hfr strains, with the sex factor inserted into the chromosome, grow more slowly than the F⁺ strains because they have a longer chromosome. However, this is excluded on two grounds. In the first place, it predicts that all Hfr strains should grow more slowly than their F⁺ ancestors; this was not observed with strain B1. Secondly, the predicted slowing in
growth rate would be in the order of 1-2% (the size of the sex factor relative to that of the chromosome, and a difference immeasurable by the methods used in these experiments). The observed slowing in growth rate for strains B7, 9 and 10 was about 21%.

The existence of a class of Hfr clones unable to grow indefinitely, because of impaired functions, as well as slow growing strains, would in growing F+ cultures make for an increased proportion of Hfr cells with autonomous sex factors. If such cells could act as donors both of the sex factor and the chromosome, we may reconcile Reeves' observation (p. 15) that recombinants from an F+ x F- cross are generally F+ males with the view that the fertility of F+ cultures is due to Hfr clones. It would also be an additional reason, perhaps more important than that given on p. 74, why the stable Hfr strains isolated in the present study appear not to be sufficient to explain the pattern of inheritance of unselected markers observed in the analysis of recombinants obtained in an F+ x F- cross.
SECTION II

A NEW TYPE OF F-PRIME FACTOR

A. Introduction

In the introduction to this thesis the properties of F-prime factors were discussed, and it was mentioned that their mechanism of origin was unknown. The experiments which will now be described bear upon this question. An F-prime factor carrying several markers was isolated and the arrangement of genes on it was compared with their location on the chromosome of the Hfr strain from which the F-prime factor was derived; this comparison has allowed the proposal of a mechanism for the formation of F-prime factors. The factor isolated here gives rise to a single class of Hfr strains; it will be suggested that this instability is a function of the structure of this particular F-prime factor.

B. Isolation

(i) Method

The Hfr strain chosen for the isolation of F-prime factors was B11 (page 51) which transfers chromosomal genes in the order O. T6 ade+ tyr+ ... pro+ lac+. F. The lac marker could, therefore, be used selectively for the isolation of F-prime factors by Jacob and Adelberg's technique (see p. 36). Equal volumes of logarithmically growing cultures with about 4 x 10^8
cells per millilitre of the Hfr strain (met− lac+ strF) and of a lac− female strain (c600 thr− leu− B4 lac− strF) were mixed and aerated on a rotor at 37°C. After an hour the mixture was shaken violently to separate mating pairs and diluted one thousand-fold into broth containing 250 µg. of streptomycin per millilitre, which kills the donor strain cells. This culture was grown overnight at 37°C to allow autonomous sex factors which had arisen in the Hfr population to spread epidemically through the streptomycin-resistant population of strain C600 cells. It was expected that of these a small minority would be variants in which the lac+ region of the chromosome was associated with the autonomous sex factor, and that these would give rise to lac+ strF recombinants in the cells to which they were transferred. Dilutions of the culture were spread onto EMB lactose medium containing streptomycin; after two days incubation lac+ strF recombinants appeared as papillae on a background of recipient lac− strF cells.

Recombinants were picked, purified and tested for their ability to transfer the lac+ property to a lac− recipient strain. In test crosses against the female strain 1177 (B4 lac− T6 ade−) four of the five recombinant clones tested transferred lac+ with a high efficiency and were, therefore, tentatively assumed to harbour F-prime factors carrying the lac region. A very high proportion of the lac+ recombinants had also received the donor T6 and ade+ characters, so that the F-prime factors isolated apparently carried the segment of the chromosome bearing these genes as well as the lac region.
(ii) Characterisation; demonstration of F-prime ness

One of the F-prime factors, referred to as BB1, was studied further. As well as to strain 1177, it was transferred in mixed culture from the C600 strain to a female derivative of W1655 (ade leu), the F+ strain from which Hfr strain B11 had been isolated. The object of this operation was to obtain an intermediate male strain in which strict homology existed between the bacterial chromosome and the region carried by the F-prime factor. The experiments to be described were performed with either strain 1177 or W1655, each harbouring the F-prime factor BB1.

There are normally three operational criteria for recognising F-prime factors. The first is the ability to mediate chromosome transfer with high efficiency and with the polarity of the Hfr strain from which it was derived. The frequencies of transfer of the chromosomal genes pro* and try + by strain W1655/F'BB1 were compared (Table IX) with those of the ancestral strain Hfr B11. In parallel crosses, mating young broth cultures of the two donors with the recipient strain J62 (try pro F-) for one hour, it was found that in each case try + was transferred over one hundred times more frequently than pro*, indicating that in the two strains the chromosome is transferred with the same orientation.

The other two tests distinguish strains carrying F-prime factors from Hfr strains. First, F-prime factors mediate
<table>
<thead>
<tr>
<th>Donor strain</th>
<th>try⁺ str⁻</th>
<th>pro⁻ str⁺</th>
<th>ade⁻ str⁺</th>
<th>lac⁺ str⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1655/F'R11</td>
<td>3.1 x 10⁶</td>
<td>2.1 x 10⁴</td>
<td>8.0 x 10⁷</td>
<td>7.3 x 10⁷</td>
</tr>
<tr>
<td>W1655/Hfr B11</td>
<td>6.2 x 10⁶</td>
<td>2.1 x 10⁴</td>
<td>1.2 x 10⁷</td>
<td>2.0 x 10⁴</td>
</tr>
</tbody>
</table>

Table IX. The formation of different recombinant types by strains Hfr B11 and W1655/F'R11. Overnight cultures from single cells of these two strains were diluted tenfold into broth. After two hours incubation at 37°C they were mixed, in two pairs of crosses, with equal volumes of female strains J62 pro⁻ try⁻ his⁻ str⁻ and 1177 B⁻ lac⁻ ade⁻ str⁻. The mixtures were incubated for one hour at 37°C before mating was interrupted by violent agitation. Standard loopfuls (c. 0.015 g.) of appropriate dilutions were plated onto selective media for the recombinant types shown above. Results are expressed as the number of recombinants per millilitre of the original undiluted mixture.
their own transfer with high efficiency; this can easily be recognised if the F-prime factor carries a known genetic marker. Second, F-prime factors, like normal autonomous sex factors, can be eliminated from cells subcultured in the presence of acridine orange.

We know that the lac gene is transferred terminally by Hfr strain B11 (section I, p. 51, section II, p. 80); in contrast we find that the lac⁺ character we have equated with "BM" is transferred with high efficiency. The most likely hypothesis is that it is carried on an F-prime factor. The possibility that the origin of the Hfr strain has been translocated and that lac is now transferred proximally on the chromosome is excluded because the lac⁺ character can be serially transferred through a series of recipients. Thus it has been transferred from the original Hfr strain through strain G600 to the W1655 female, and from this strain to strain 1177; this strain in turn has then been shown to have the donor property (p. 94).

When strain W1655/BB1 was subcultured in the presence of acridine orange, it was found that some of the clones which were tested had lost the ability to transfer ade⁺ and lac⁺ with high efficiency (Table XI). This is a further demonstration of the autonomy of the sex factor.

There are three reasons for believing that the F-prime factor BB1 carries the T₆⁺ and ade⁺ genes as well as the lac locus, rather than merely mediating their chromosomal transfer with high
efficiency, as would be expected from a simple F-prime factor derived from strain Hfr B11 (see introduction, p. 36). First, in parallel crosses (Table IX) the absolute number of ade$^+$ recombinants, and the ratio of ade$^+$ recombinants to try$^+$ recombinants was much higher in the F-prime factor cross than in the Hfr strain B11 cross. Secondly, on Scaife and Gross’s (1963; p. 37) model for F-prime factor-mediated chromosome transfer, lac$^+$ would precede ade$^+$ if lac$^+$ is episomal and ade$^+$ is chromosomal. However, interrupted mating experiments (p. 84) appear to show that ade$^+$ precedes lac$^+$ in transfer (see however p. 86). Thirdly, in crosses between strains 11655/BB1 and 1177 B$^-$ lac$^{-}$ T$^R$ ade$^{-}$ the lac$^+$ T$^R$ ade$^+$ recombinants are found to be diploid and heterogenetic for these three characters; that is, they segregate clones with the lac$^-$ T$^R$ and/or ade$^-$ phenotypes.

The evidence thus suggests that "BB1" is an F-prime factor carrying the lac$^+$, T$^R$ and ade$^+$ genes; it is thus strikingly different from other F-prime factors, which apparently only carry genes transferred last by their ancestral Hfr strains.

(iii) A comparison of Hfr strains 13 and B11

The properties of F-prime factor BB1 are very similar to those of F-prime factor 13, isolated by Hirota and Sneath (1961). Each carries the lac, T$^R$ and ade loci and in each these genes are transferred with the same kinetics (Figure ) and linkages. It was of interest to compare the sites at which the sex factor is
integrated in Hfr strain B11, and in Hfr strain 13, from which F-prime factor 13 was derived. Unfortunately the original Hfr strain 13 was no longer extant, but a similar strain was used. This Hfr strain was derived by Dr. S. Brenner from a strain into which F-prime factor 13 had been introduced. Evidence will be presented (p. 90) which argues that the origin of this sex factor is at exactly the same point on the chromosome as in Hfr strain 13. Like Hfr strain B11 it transfers $T_6$ and ade early and lac late. Two very similar F-prime factors have therefore been derived from similar Hfr strains.

(iv) The ordering of markers on F-prime factors B11 and F13

An obvious means for the ordering of markers on F-prime structures, like those on the chromosome, is the interrupted mating technique. This was used by Hirota and Sneath (1961) who selected for ade$^+$ recombinants in a cross in which a strain carrying F-prime factor 13 was used as the donor. The recombinants from the earliest samples were neither $T_6^6$ nor lac$^+$, and they were female. At later times this class persisted as a diminishing proportion, but most recombinants were now lac$^+$, $T_6^6$ and male. These authors concluded that ade$^+$ is the first marker to be transferred, and, from slight differences, that $T_6^6$ precedes lac$^+$, but that both of these are closely linked to the sex factor, which was transferred last of all, analogous to the sex factor in Hfr crosses. The postulated order of transfer was
therefore o. ade^+ T_g^5 lac^+ F.

In their experiments two main classes of ade^+ recombinants can be distinguished; there are those which have only received the ade^+ character and those which have received the whole F-prime factor (that is, the ade^+, T_g^5, lac^+ and F determinants). It is presumed that the former class derive from matings in which transfer has been interrupted and that genes from the transferred fragment must then be integrated into the chromosome, since unlike a complete F-prime factor this fragment could not replicate autonomously.

In interrupted mating experiments in which strain W1655/BB1 was crossed against the female strain 1177 B_4^− lac^- T_g^5 ade^- we obtained similar results, although the inheritance of the sex factor was not studied. Almost all the purified lac^+ recombinants, from all times of sampling, were ade^+ (Figure 10). In contrast, as with F-prime factor 13, the proportion of the ade^+ recombinants which were lac^- increased, reaching a plateau. This is as expected if ade^+ precedes lac^- during transfer.

However, these results, which appear to show that both T_g^5 and lac^- are preceded by ade^+ in transfer by F-prime factor BB1, are open to a different interpretation. In transfer by Hfr strain B11 (and Hfr strain 13), only about 10% of the ade^+ recombinants obtained from one hour crosses between Hfr strain B11 (T_g^5 ade^+) and a T_g^5 ade^- female are T_g^5. On the other hand, when Hfr strain B10, which transfers the T_g^5 and ade^+ loci much later,
was used as donor, about half the \( \text{ade}^+ \) recombinants were \( T_6^S \).

From this it was suggested that the observed low frequency of integration of the \( T_6 \) locus is a function of its extreme proximity to the origin of strain B11. In the first section of this thesis a possible explanation for this effect is discussed.

In a model which will be presented later, we propose that the relative order of genes on the \( \text{F-prime} \) factor BB1 and on the chromosome of \( \text{Hfr} \) strain B11 are formally similar, so that the \( T_6 \) gene on the \( \text{F-prime} \) factor would be transferred before the \( \text{ade}^+ \) gene, and, from fragments of the \( \text{F-prime} \) factor, integrated with low frequency, as in the \( \text{Hfr} \) B11 cross. Therefore in interrupted mating experiments in which the \( \text{F-prime} \) factor is transferred there would be very few \( \text{ade}^+ \) recombinants which are \( T_6^S \) and have not received the whole \( \text{F-prime} \) factor, so that in transfer the \( T_6 \) gene will appear to be closely linked to the fertility property.

According to this argument, interrupted mating experiments on \( \text{F-prime} \) factors will only indicate whether a given marker is in the middle of the transferred \( \text{F-prime} \) structure (eg \( \text{ade}^+ \) on \( \text{F-prime} \) factor BM) or at one of the two extremities. If it is in the middle of the structure a fairly high proportion of the recombinants will not have received the fertility property; if it is at either of the two extremities virtually all the recombinants (like the \( T_6^S \) and \( \text{lac}^+ \) recombinants in this cross) will also have received the donor character. Consequently, since the inheritance of the \( T_6^S \) and \( \text{lac}^+ \) characters among the \( \text{ade}^+ \) recombinants in the
Figure 10.
Figure 10. The inheritance of ade<sup>+</sup> and lac<sup>+</sup> from F-prime factor BD1 in interrupted matings. A young broth culture of strain 1655 met<sup>+</sup> lac<sup>+</sup> ade<sup>+</sup> str<sup>S</sup> F<sup>+</sup>BD1 lac<sup>+</sup> ade<sup>+</sup> was mixed with an equal volume of strain 177 by lac<sup>−</sup> ade<sup>−</sup> str<sup>R</sup>. At intervals samples were withdrawn, agitated to separate mating pairs and plated after dilution on to minimal agar plates, appropriately supplemented for the selection of ade<sup>+</sup> str<sup>R</sup> and lac<sup>+</sup> str<sup>R</sup> recombinants. The resulting recombinant colonies were then picked, purified and tested for the lac and ade characters.

o = percentage of ade<sup>+</sup> recombinants which are lac<sup>+</sup>

• = percentage of lac<sup>+</sup> recombinants which are ade<sup>+</sup>
interrupted matings are so similar, we can be no more certain that 
$\text{lao}^+$, which the model to be presented in the discussion predicts 
is transferred after $\text{ade}^+$, is transferred at the tail end by 
F-prime factor BB1 than that the $T_6$ locus is.

There are other grounds for believing that an 
unambiguous order of markers on F-prime factor 13 cannot always be 
inferrred from the numbers and types of recombinants obtained in 
interrupted mating experiments. Sugino (1965) selected for $\text{ade}^+$ 
and $\text{mb}^-$ (methylene blue resistant) recombinants in such a cross, 
and then examined recombinants of each type for the inheritance of 
the unselected markers (that is, $\text{mb}^-$ and $\text{ade}^+$ respectively). He 
found that in each case the proportion of recombinants which had 
inherited the unselected marker rose with time (of Figure 10) so 
that each marker appeared to precede the other.

C. The Instability of F-prime factor BB1

(i) The nature of this effect

Two observations suggested that this factor was 
unstable. Cultures of strains carrying it gave rise to 
derivatives able to transfer $\text{ade}^+$ but not $\text{lao}^+$ with high 
efficiency. Also, if the F-prime factor was introduced into 
the strain 1177 ($E^-\text{lao}^-\text{ade}^-$) to form a diploid strain ($\text{lao}^- 
\text{ade}^-/\text{lao}^+\text{ade}^+$) after subculture many clones with the $\text{ade}^+ \text{lao}^-$, 
$\text{ade}^- \text{lao}^+$, and $\text{ade}^- \text{lao}^-$ phenotypes were obtained.

The rate at which the ability to transfer $\text{lao}^+$ and $\text{ade}^+$
with high frequency was lost was determined by the method used for measuring the rates of reversion of different Hfr strains to the \(F^+\) state (p. 56). \(10^{-10}\) dilutions into broth were made from an overnight culture of a known F-prime factor-carrying colony of strain \(\#1655\). One ml. aliquots were dispensed into a series of tubes so that no more than one cell was inoculated into each tube. After overnight growth platings were made for single colonies, which were tested for ability to transfer \(\text{ade}^+\) and \(\text{lac}^+\). All clones examined were found to have one of three types of fertility. As well as those clones able to transfer both \(\text{ade}^+\) and \(\text{lac}^+\) with high efficiency, there were some which transferred \(\text{ade}^+\) at high frequency but \(\text{lac}^+\) only at low frequency. This type, termed "class B" by J.G. Scaife (1964) had been observed by him among derivatives of a strain into which F-prime factor 13 had been infected. The reciprocal class (that is, cells transferring \(\text{lac}^+\) much more frequently than \(\text{ade}^+\)) was not found. Since in subcultures of strain \(\#1655/BB\) the fertility with respect to \(\text{ade}^+\) is roughly maintained while that with respect to \(\text{lac}^+\) drops by a factor of a thousand, such a class, if it exists, must be much rarer than "class B." A third class of clones transferred neither marker with high frequency and were therefore either \(F^+\) or \(F^-\) (Table X).

Subcultures were made of two F-prime factor-carrying clones and two "class B" clones; colonies obtained after one and after four cycles of growth starting from single cells of each type were tested for their fertility. The results of this experiment is
<table>
<thead>
<tr>
<th>Strain</th>
<th>Number of growth cycles</th>
<th>Number of clones tested</th>
<th>Markers transferred with high frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>lac⁺   ade⁺   ade⁺  none</td>
</tr>
<tr>
<td>W1655/F'BM</td>
<td>1</td>
<td>50</td>
<td>49        1     0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>50</td>
<td>35        4     11</td>
</tr>
<tr>
<td>W1655 (class B)</td>
<td>1</td>
<td>50</td>
<td>0         50    0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>50</td>
<td>0         32    18</td>
</tr>
</tbody>
</table>

Table X. The kinetics of the formation of derivatives by strains W1655/F'BM and W1655 (class B). Overnight broth cultures of two clones each of strains W1655/F'BM lac⁺ ade⁺ and W1655 (class B) lac⁺ ade⁺ were diluted x 10⁴ into broth. 1 ml. portions of each were dispensed into a series of tubes. After overnight culture, those clones obtained were checked for their fertility and one fertile culture from each original clone was diluted (a) x 10⁶; platings were then made on nutrient agar plates for the recovery of single colonies, and, (b) x 10⁴; 0.1 ml. of this dilution were inoculated into broth (5 ml.) for the first of three similar serial subcultures. After the fourth cycle of growth single colonies were again obtained. Colonies were picked and tested in broth in crosses with strain 1177 B lac⁻ ade⁻ for the ability to transfer the lac⁺ and ade⁺ characters with high frequency. Data from the parallel experiments are summed.
presented in table X. It was concluded that within the limits of
the experiment the W1655/BB1  \( \rightarrow \) class B transition is irreversible
and that there is a sequence which can be represented as
W1655/BB1  \( \rightarrow \) class B  \( \rightarrow \) F'. However, to account for the number
of F' clones obtained in the subculture of strain W1655/BB1
compared with the number obtained from strains of type B, we must
assume that strain W1655/BB1 can also give rise directly to F' cells.

(ii) The nature of class B cells

The following lines of evidence indicate that class B
clones derived from strain W1655/BB1 are Hfr donors similar to
each other and to strain Hfr B11.

1. As with Hfr strains, and unlike cells carrying an F-prime
factor, their fertility is unaffected by subculture in acridine
orange (Table XI).

2. Ade' recombinants obtained from crosses using these clones
as donors do not segregate ade'- clones when subcultured with
acridine orange.

3. Cells of this type transfer the chromosome in the same way
as Hfr strain B11. First, in crosses more try' recombinants
are obtained than his' recombinants; very few pro' recombinants
are obtained. The yield of each class of recombinants is
strikingly similar to that from Hfr strain B11, and the number of
try' recombinants is greater than that obtained in parallel crosses
using strain W1655/BB1 clones as donors. Secondly, in interrupted
<table>
<thead>
<tr>
<th>Strain</th>
<th>Presence of aeridine orange during culture</th>
<th>Number of colonies tested after subculture</th>
<th>Number of colonies transferring with high frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>lac⁺, ade⁺, ade⁺</td>
<td>none</td>
</tr>
<tr>
<td>W1655/BB1</td>
<td>+</td>
<td>20  2  5  13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>40  19  21  0</td>
<td></td>
</tr>
<tr>
<td>&quot;class B&quot;</td>
<td>+</td>
<td>30  0  30  0</td>
<td></td>
</tr>
<tr>
<td>(three clones)</td>
<td>-</td>
<td>30  0  30  0</td>
<td></td>
</tr>
<tr>
<td>Hfr B11 (control)</td>
<td>+</td>
<td>10  0  10  0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>10  0  10  0</td>
<td></td>
</tr>
</tbody>
</table>

Table XI. The effect of subculture in the presence of aeridine orange upon the fertility of strain W1655/BB1 and upon three "class B" clones. Single colonies of strain W1655/BB1, of three "class B" strains, and of strain Hfr B11 were inoculated into broth. After overnight incubation these were diluted 10⁻⁴ and 0.1 ml. aliquots of this dilution were inoculated into broth cultures with and without 50 μg/ml. aeridine orange. After 48 hours incubation single colonies were obtained by plating dilutions onto nutrient agar plates. These clones were picked and tested for the ability to transfer the lac⁺ and ade⁺ properties in crosses with strain 1177 B⁻ lac⁻ ade⁻ str⁻.
mating experiments \textit{try}^+ \textit{begins to be transferred at the same time as in transfer by strain Hfr B11. Third, as with strain Hfr B11 only about 10\% of the \textit{ade}^+ recombinants obtained in crosses of class B strains with the \textit{ade}^- \textit{le}a^- \textit{T}_{6}^F strain 1177 have also become \textit{T}_{6}^S, suggesting that in each the \textit{T}_{6} locus is very close to the origins.

It was therefore concluded that F-prime factor BB1 can only exist moderately stably in the cell, and that it gives rise to Hfr clones of a single type, similar to Hfr strain B11.

(iii) Phenotypic evidence for the instability of F-prime factor BB1.

The donor strain W1655/BB1 \textit{lao}^+ \textit{ade}^+ \textit{str}^S was mated with strain 1177 \textit{lao}^- \textit{ade}^- \textit{str}^F; \textit{ade}^+ \textit{str}^F recombinants were purified and tested for the lao character. Some of the \textit{ade}^+ \textit{lao}^+ clones, which were assumed to have received the whole F-prime factor, were subcultured. Single colonies were then isolated from these cultures and examined for the \textit{ade} and lao phenotypes. Although the results (table XIII) were not fully understood, several points emerged. The original \textit{ade}^+ \textit{lao}^+ clones segregate the recessive phenotypes (\textit{ade}^-, \textit{lao}^-) and are therefore diploid with respect to these characters. The \textit{ade}^+ and \textit{lao}^+ characters can be lost independently of each other, since the \textit{ade}^+ \textit{lao}^- and \textit{ade}^- \textit{lao}^+ classes are also found. Among the derivatives of the different clones which were subcultured the four possible phenotypes were found in significantly different proportions. It was also observed
Table XII. The distribution of phenotypes among derivatives of strain 1177 B− lac ade− F′R6K lac+ ade+ Ade+ recombinants were obtained by mating strains W1655/F′R6K lac+ ade+ str+ and 1177 B− lac− ade− str−. Recombinants which were also lac+ were recognized by replication onto EMB lactose plates. From each, a purified lac+ colony was inoculated into broth. After incubation single colonies were isolated by plating onto EMB lactose plates supplemented with adenine. These colonies were tested for the ade character by replicating onto plates with minimal medium supplemented with glucose and vitamin B₁.
that acridine orange had no obvious effect upon the distribution of the phenotypes.

Three ade- lac+ clones were tested for fertility in crosses with the F- strain J62 (pro- try- his-). One transferred lac+ and try+, but not pro+, with high efficiency. Since it also segregated lac- colonies it was apparently a partial diploid strain carrying an F-prime factor. Both the other clones gave try+ recombinants, but, as expected if they were Hfr cells like Hfr strain B11, did not transfer or segregate the lac+ character.

Other clones transferred neither lac+ nor try+ with high frequency, and were therefore neither Hfr nor F-prime-factor carrying males. Therefore the same three types of fertility are found among derivatives of strain 1177/BB1 (ade+ lac+) as among those of strain W1655/BB1 (ade+ lac+).

D. Discussion

1) A model for the formation of F-prime factors

The isolation and characterisation of a new F-prime factor, BB1, has been described; it is regarded as an F-prime factor carrying the lac+ T6 and ade+ characters because it mediates its own serial transfer and because it can persist in lac- T6 ade- recipient cells, giving partially diploid lac+ T6 ade+ derivatives. In addition, subculture of strains carrying it, such as W1655/BB1, in the presence of acridine orange, results in the loss of fertility.
Its novel feature is that it carries the $T_{6}$ and ade$^{+}$ characters, which the Hfr strain B11, from which it was derived, transfers early, in addition to the terminal lac$^{+}$ character.

Figures 11, A-D, represent a formal model for the origin of this F-prime factor. It is assumed that the F-prime factor is formed from the whole region between a pair of points $x$ and $y$; this includes the lac, $T_{6}$ and ade loci and the integrated sex factor. The observation (Hirota and Sneath, 1961; Sugino, 1965) that an analogous structure, F-prime factor 13, carries all the known genetic markers of this region of the chromosome (lac, pho, $T_{6}$, mb, ade) supports this assumption.

There are two steps in this formal scheme. Firstly, breaks occur at the points $x$ and $y$. Secondly, rejoining of the ends occurs in such a way as to (a) restore the circularity of the bacterial chromosome, and (b) give a closed structure, the F-prime factor, in which the arrangement of markers relative to each other is analogous to their arrangement on the Hfr chromosome.

In Figure 11D these two steps are incorporated into a single event. It is proposed that $x$ and $y$ represent regions of limited genetic homology and that pairing between them is followed by a reciprocal genetic exchange, analogous to that postulated (p. 71) as the mechanism by which the sex factor integrates into the chromosome to give rise to Hfr strains. This gives the two structures shown in Figure 11C.
Figure II.
The mechanism of F-prime factor formation in *E. coli*. A, B and C represent formally the sequence of events necessary for the formation of F-prime factor E61 (see text). A → B shows the release of the sex factor associated with a piece of the bacterial chromosome. In B → C there is union of the open ends of the resulting structures. By assuming a genetic exchange between sites x and y (see text) the above sequence can be expressed as a single event (D).

The sex factor, represented by a wavy line, is shown inserted in the chromosome (Campbell, 1962). The direction of transfer during conjugation is indicated by the arrow.
Several predictions follow from this model. First, in conjugation the chromosomal genes incorporated into the F-prime factor should be transferred with the same orientation as in their transfer by Hfr strain B11; that is, in the order $T_6\text{ ade lae F}$. However, because of the difficulties, described on p. 85, involved in mapping F-prime markers by interrupted mating experiments, it has not been possible to distinguish this order from that proposed by Hirota and Sneath (ade $T_6\text{ lae F}$), or even to prove that lae$^+$ is transferred after ade$^+$. Secondly, if the recombination event is reciprocal the chromosome from which the F-prime factor arose should have a deletion covering exactly the region contained in the F-prime factor. Consequently a strain carrying the F-prime factor and the chromosome from which it arose should be entirely haploid and not have any region of true homology between the F-prime factor and the chromosome. Experiments with strain W3747, in which F-prime factor 13 arose, indicate that this strain carries a deletion of the postulated type (Scuhe and Pohl, 1964). When transferred into recipient cells F-prime factor 13 then mediates its own transfer from these cells, and also that of the chromosome at high frequency and with the polarity characteristic of Hfr strain 13. However, only the F-prime factor itself is transferred with high frequency from the parent strain W3747. This distinction between the behaviour of F-prime factor 13 in the cell from which it arose, and in cells into which it is introduced, is
predicted on our model, since in a strain not carrying a region of homology between the chromosome and the F-prime factor recombination at high frequency between the two structures is not possible.

If strain W3747 is entirely haploid the removal of the F-prime factor with its many genes should be lethal, and only those cells not susceptible to the action of acridine orange will grow normally. One way in which a cell can become immune to the action of acridine orange is by integrating the F-prime factor into the chromosome, and indeed it was found that the survivors of such treatment of strain W3747, but not of other strains carrying F-prime factor 13, were stable Hfr clones able to transfer ade* with high frequency. However, the sex factor, together with the chromosomal genes carried on the F-prime factor, is not now integrated between pro and try, as in Hfr strains 13 and B11, but at various other points on the chromosome. This is to be expected if there is no region for which the cell is diploid, and the lac T6 ade region between pro and try is absent.

A third prediction of the model is that any Hfr strain with the sex factor integrated between points x and y could, like Hfr strain B11, give rise to F-prime factors carrying the lac, T6, and ade loci. Since our results show a close similarity between Hfr strains 13 and B11, Hirota and Sneath's observation that F-prime factor 13 carries these markers is consistent with the model.
This model can also be used to explain the formation of F-prime factors not carrying the proximally transferred part of their parental Hfr strain's chromosome if it is assumed that point z (Figure 11) may lie within the sex factor itself. A genetic exchange between a pair of points z' and y' would then result in the retention by the chromosome of part of the sex factor. This region would correspond to the sex factor affinity loci observed by Adelberg and Burns (1960) and Richter (1959).

The type of recombination between non-allelic regions of homology proposed here may be a universal property of all DNA, and a biologically important mechanism for the relocation of genes, so that the origin of F-prime factors is analogous to the formation of separate chromosomes in higher organisms. It is also a specific mechanism by which deletions might arise for instance in bacteria and phages.

(ii) An explanation for the instability of F-prime factor BB1

It was found that F-prime factor BB1, like F-prime factor 13, integrates into the chromosome to give stable, haploid Hfr strains similar to Hfr strain BB1, from which it was derived. We must explain why all these strains are of one type and how the cell becomes haploid. We must also explain why strains carrying other F-prime factors have not, so far, been found to yield such derivatives.

The formation of these Hfr cells can be related to
the fact that F-prime factors 13 and BB1 are apparently unique among F-prime factors so far examined in carrying genes located on both sides of the sex factor in their ancestral strains.

Scaife and Gross (1963) and Cuzin and Jacob (1963) inferred that unstable, partially diploid Hfr structures can arise by a reciprocal recombination event between the chromosomal genes on the F-prime factor and the homologous part of the chromosome. It is proposed that the stable haploid Hfr strains observed in the present study arise by two such cross-over events (Figure 12). If in a strain carrying F-prime factor BB1 or 13, one cross-over occurs in each of the regions of homology (the lac region and the T6 ada region) one on either side of the sex factor, a region of the chromosome will be exchanged for a portion of the F-prime factor which includes the sex factor itself. The sex factor will therefore be incorporated into the chromosome, so that a haploid Hfr chromosome will be formed.

A prediction of this model is that on examination other F-prime factors would divide into two groups; those which gave haploid, stable Hfr derivatives and those that did not. The former, like F-prime factor BB1, would be found to carry genes transferred early as well as late by the ancestral Hfr strain.

If recombination is really reciprocal, the material not integrated into the chromosome in the formation of Hfr derivatives of F-prime factor BB1 should contain one of the two
Figure 12.
Formal possibilities for reciprocal recombination between the chromosome of E. coli K12 and the F-prime factor BB1. According to the model for F-prime factor formation (see Figure 11) there are two regions of homology between these structures. Region 1 is the ade region, transferred early by the Hfr strain BB1; region 2 is the lac region, which is transferred late by this Hfr strain. The sex factor, represented by a wavy line, is shown inserted in the episome. The direction of transfer during conjugation is indicated by the arrow in the sex factor.

Reading the sequence of markers from the right-hand extremity of the sex factor,

A cross-over in region 1 gives the partial diploid

\[ T_6 \text{ ade try} \ldots \text{pro lac} \ T_6 \text{ ade lac} \ F \]

A cross-over in region 2 gives the partial diploid

\[ T_6 \text{ ade lac} \ T_6 \text{ ade try} \ldots \text{pro lac} \ F \]

A cross-over in each region gives a haploid Hfr chromosome

\[ T_6 \text{ ade try} \ldots \text{pro lac} \ F \]

and a structure \( T_6 \text{ ade lac} \).
copies present of each of the chromosomal genes carried by the
F-prime factor. Since the autonomous replication of these factors
appears to depend upon the sex factor itself, such structures in
newly formed Hfr cells could not replicate and would therefore be
lost. The finding that haploid Hfr derivatives of strain 1177/EB1
can show independent loss of the ada$^+$ and lac$^+$ characters (that is,
that these Hfr strains can carry either ada or lac allele) is
therefore consistent with the double cross-over model.

Since strains carrying F-prime factor EB1 mediate
chromosome transfer we can infer that these strains can give rise
to partial diploid Hfr strains of the type described by Cuzin and
Jacob and by Scaife and Gross. However, an interesting
prediction is that recombination in the T6 ada region and in the
lac region should give two different types of partial diploid.
If the order of genes on the F-prime factor and the chromosome
are similar, these diploids should transfer their chromosomes in
the following orders (Figure 12).

\[
\begin{align*}
0. & \ T6 \ \text{ada} \ \text{...} \ \text{lac} \ T6 \ \text{ade} \ \text{lac} \ F \text{ (arises by recombination in the T6 ada region)} \\
\text{and} \ 0. & \ T6 \ \text{ade} \ \text{lac} \ T6 \ \text{ade} \ \text{...} \ \text{lac} \ F \text{ (arises by recombination in the lac region)}
\end{align*}
\]

We should expect that as with the partial diploids
described earlier, these should be both unstable. A second cross-
over occurring in the same region as the original insertion
amounts to a simple reversal, giving back the F-prime factor and
and the haploid chromosome. However, a cross-over in the other region is formally similar to the mechanism proposed above for the formation of stable haploid Hfr strains, differing from it only in the existence of an intermediate stage. It might be possible to recognise such unstable partial diploids, and to establish whether they give rise to stable haploid Hfr cells, and if so, whether they can account for the observed rate of transition in strains carrying F-prime factor 2341 to the Hfr state.
SECTION III

THE RELATION OF THE PHAGE T3 RESTRICTION PROPERTY TO THE SEX FACTOR OF E. COLI K12.

A. Introduction

The efficiency of plating (e.o.p.) of phage T3 is similar on E. coli strains B and C, and on many K12 strains. However, on other K12 strains the e.o.p. is reduced by a factor of approximately $10^5$. We shall refer to this strain-specific property of giving a low e.o.p. as "restriction." It is not known whether restriction is due to a failure on the part of the phage to absorb, inject or multiply in the restricting bacterial strain. Although this question is of considerable interest, it does not affect the conclusions drawn from the genetic analysis described in this section. Restriction and non-restriction are designated $T_3^+$ and $T_3^-$ respectively.

In general the strains which restrict T3 are male, although both a non-restricting male and a restricting female were found among the many strains tested (Glover, S.G., and Schell, J., pers. comm.). This section of the thesis is concerned with the demonstration that the sex factor normally carries the determinant for the T3 restriction property, $T_3^+$, and with an analysis of the known exceptions to this rule.

B. Methods

Individual clones of bacteria were tested simultaneously...
for the $T^r_3$ property and for maleness; the criterion for maleness was the ability of the male-specific phage $\mu$ (Dettori et al., 1961) to infect and lyse the bacteria. Single colonies were suspended in broth and after two hours incubation at 37°C, these cultures were plated as lawns on Difco agar plates. A series of dilutions of a phage $T_3$ suspension and a high titre suspension of $\mu$ phage were each spotted onto these plates, which were then incubated overnight at 37°C. With restricting bacterial clones high concentrations of the $T_3$ phage gave small plaques (c. 1 mm. diameter). At these concentrations non-restricting clones gave zones of confluent clearing; higher dilutions gave plaques with diameters of up to 5 mm. The single concentration used of the male-specific phage $\mu$ either gave a zone of clearing or no zone. Clones giving a doubtful result were retested.

C. Results

(1) The inseparability of the phage $T_3$, restriction property from the sex factor of strain 58-161.

The phage $T_3$-restricting strain of E. coli K12 which was mainly used was 58-161 $F^+$. On subculture in the presence of acridine orange this strain loses the restriction and male properties together (Table XIII). In a control experiment with a fertile clone of an Hfr strain (Hfr Cavalli), originally derived from strain 58-161, neither property was lost. The simplest interpretation is that the $T^r_3$ character is carried on the sex
Table XIII. The concomitant loss of the properties of maleness and $T_1$ restriction on acridine orange treatment of restricting male $E. coli$ K12 strains. Overnight cultures of strains 58-161 $F^+T^+$ and Hfr Cavalli $T^+$ were diluted into fresh broth (pH 7.6) with and without 50 $\mu$g/ml. acridine orange, to a final concentration of about $10^8$ cells per ml. After 36 hours incubation, single colonies were isolated and tested.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Acridine orange treatment</th>
<th>Number of clones tested</th>
<th>Number of clones displaying the phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\mu^sT^+$</td>
</tr>
<tr>
<td>58-161 $F^+$</td>
<td>+</td>
<td>22</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Hfr Cavalli</td>
<td>+</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>
factor so that it can only be removed by acridine orange treatment when the sex factor itself is susceptible.

This hypothesis was tested by attempting a separation of the phage T₃ restriction and male properties in genetic crosses. In each of these crosses approximately equal numbers of donor and recipient bacteria were mixed and the mixture was incubated with gentle aeration at 37°C for one hour. Aliquots of dilutions were then plated on media selective for the recipient cell type.

In the first cross between two K12 strains, one $P^{+}_{2,3}$ and the other $P^{-}_{2,3}$, the sex factor and the $T^{+}_{2,3}$ property are transferred together (Table XIV). Of the recipient clones tested all those which had become $P^{+}$, and only those, had also become $T^{+}_{2,3}$. This, like the acridine orange experiment, appears to exclude a chromosomal location for $T^{+}_{2,3}$, since the sex factor and the $T^{+}_{2,3}$ property are transferred with a far higher frequency than any chromosomal marker. The transfer of the $T^{+}_{2,3}$ property is also mediated by an F-prime factor, $F\text{lac}^{+}$, in the same way as by the sex factor itself (cross 2).

The restriction property was also linked to the sex factor in chromosomal transfer by Hfr strain Cavalli. In a cross with the female strain W945 ($\text{thr}^{-} \text{leu}^{-} \text{B}^{-} \text{mann}^{-} \text{gal}^{+}$) recombinants were obtained at the frequencies expected from an Hfr cross. Only among the $\text{gal}^{+}$ recombinants ($\text{gal}^{+}$ being transferred near the tail end of the chromosome) were any recombinants male (Table XV). All these, and only these, restricted phage T₃.
The transfer of the $T_{3}^{+}$ property together with maleness. A mixture of the parental cultures was incubated at 37°C for one hour before dilution and plating for the recovery of single colonies of the recipient type. In the second cross C600 exconjugants which had become $lac^{+}$ were selected.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Type of cross</th>
<th>Donor strain</th>
<th>Recipient strain</th>
<th>Number of recipient exconjugant clones tested</th>
<th>$% F^{+}$ recipients which now restrict $T_{3}$ $F^{+}$ (or Flac+)</th>
<th>$F^{-}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$F^{+}T_{3}^{+} T_{3}^{+} \times F^{-}T_{3}^{-} T_{3}^{-}$</td>
<td>58-161 $F^{+}$</td>
<td>$W245 F^{-}$</td>
<td>60</td>
<td>87</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>Flac$^{+}T_{3}^{+} T_{3}^{+} \times F^{-}T_{3}^{-} T_{3}^{-}$</td>
<td>$W1655/Flac^{+}$</td>
<td>C600 $F^{-}$</td>
<td>60</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>$F^{+}T_{3}^{+} T_{3}^{+} \times F^{-}T_{3}^{-} T_{3}^{-}$</td>
<td>58-161 $F^{+}$</td>
<td>$W1655 F^{-}$</td>
<td>20</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>$F^{+}T_{3}^{+} T_{3}^{+} \times F^{-}T_{3}^{-} T_{3}^{-}$</td>
<td>$W1655 F^{+}$</td>
<td>C600 $F^{-}$</td>
<td>20</td>
<td>40</td>
<td>0</td>
</tr>
</tbody>
</table>
Table XV. The $T_3$ restriction and $\mu$-plating properties of recombinants obtained from a cross between strains Hfr Cavalli and W945 F'. Equal volumes of logarithmically growing cultures of strains Hfr Cavalli (met) and W945 (F$^-$ thr$^-$ leu$^-$ F$^-$ man$^-$ gal$^-$) were mixed. After 30', 50', and 120' respectively, plaques at different dilutions were made for thr$^+$ leu$^+$, man$^+$ and gal$^+$ recombinants. Purified recombinants were tested for their ability to plate $\mu$ and $T_3$ phages.

<table>
<thead>
<tr>
<th>Recombinant type</th>
<th>Number tested</th>
<th>$\mu^S_{T_3}$</th>
<th>$\mu^F_{T_3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>thr$^+$ leu$^+$</td>
<td>33</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td>man$^+$</td>
<td>33</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td>gal$^+$</td>
<td>33</td>
<td>28</td>
<td>5</td>
</tr>
</tbody>
</table>

The T$3$ restriction and $\mu$-plating properties of recombinants obtained from a cross between strains Hfr Cavalli and W945 F'. Equal volumes of logarithmically growing cultures of strains Hfr Cavalli (met) and W945 (F$^-$ thr$^-$ leu$^-$ F$^-$ man$^-$ gal$^-$) were mixed. After 30', 50', and 120' respectively, plaques at different dilutions were made for thr$^+$ leu$^+$, man$^+$ and gal$^+$ recombinants. Purified recombinants were tested for their ability to plate $\mu$ and $T_3$ phages.
The inability to separate phase $T_3$ restriction from the sex factor in the acridine orange experiments or in the transfer experiments with either Hfr or F$^+$ donors renders very unlikely the hypothesis that the $T_3^{F+}$ property, instead of being carried on the sex factor, is carried on a separate autonomous particle.

(ii) The analysis of two atypical strains

During the screening of a large number of strains, both an $F^{+}_{T_3}$ strain (W1655 $F^+$) and an $F^{-}_{T_3}$ strain (a 58-161 derivative) were found. To decide whether it is ever possible for W1655 cells to restrict phage $T_3$, the sex factor of strain 58-161 $F^{+}_{T_3}$ was introduced into a W1655 strain cured of its sex factor (Table XIV, cross 3). The W1655 exconjugants which had become male all restricted phage $T_3$; no clones were isolated in which the $T_3$ and male properties were separated. It was also observed that when an $F_{T_3}$ factor from a $T_3^{F+}$ strain is introduced into strain W1655 $F^-$, the recipient also becomes $T_3^{F+}$. The $T_3^{F+}$ property can therefore be expressed in strain W1655 in the presence of an introduced sex factor or F-prime factor.

The view that the sex factor native to strain W1655 is a mutant form of the sex factor receives support from the further observation that when it is transferred into a C600 female strain the male exconjugants do not restrict phage $T_3$ (Table XIV, cross 4).

A strain like 58-161 $F^{+}_{T_3}$, able to restrict phage $T_3$ but insensitive to phage $\mu$ might carry the $T_3^{F+}$ property either
chromosomally or autonomously, possibly on a defective sex factor. If this female strain, which does not give recombinants in crosses with female strains, still carries the $T_3^{+}$ property autonomously, we should predict that on subculture with acridine orange these cells would lose this property; this has been substantiated (Table XVI). If the autonomous particle carrying the $T_3^{+}$ property is a defective sex factor, although unable to mediate conjugation it should share an immunity pattern with the sex factor, and the two should be unable to exist stably in the same cell (Scaife and Gross, 1962). Further, it might be possible to obtain recombinants with the $F_3^{+}$ or $F_3^{-}$ types of defective sex factor. These predictions have both been fulfilled.

Dilutions of overnight cultures of strains 58-161 ($met^{-} F_3^{+}$) and C600 ($thr^{-} leu^{-} B^{-} F_3^{+} T_3^{-}$) were inoculated together into broth, and this mixed culture was then incubated for 24 hours. Finally, dilutions were plated onto minimal agar plates, supplemented with methionine, for the recovery of 58-161 $met^{-}$ clones. These clones were tested for their ability to plate phages $\mu$ and $T_3$. It was found (see Table XVII) that the great majority of clones had the sensitivity of either one parent or the other of the two phages; that is, they were either $\mu T_3^{-}$ or $\mu T_3^{+}$. No clones were $\mu T_3^{+}$, so that apparently the two properties were carried on two structures unable to cohabit stably in the same cell. Neither were there any $\mu T_3^{-}$ clones. This conclusion was reinforced by the analysis of a clone which was intermediate with respect both
<table>
<thead>
<tr>
<th>Acridine orange</th>
<th>Number of clones tested</th>
<th>$\mu_T^{+}$</th>
<th>$\mu_T^{-}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>36</td>
<td>27</td>
<td>9</td>
</tr>
<tr>
<td>-</td>
<td>36</td>
<td>36</td>
<td>0</td>
</tr>
</tbody>
</table>

Table XVI. The effect of subculture in the presence of acridine orange upon the $T_2^{+}$ property of an $F_T^{+}$ strain. Strain 5846 F $T_2^{+}$ was grown overnight in broth. Inoculations were made into fresh broth at pH 7.0 with and without 50 g/ml. (final concentration) acridine orange, to give an initial population of about $10^6$ cells/ml. After overnight growth dilutions and platings were made for single colonies, which were then tested.

*Because by an oversight the pH used here was 7.0 these results are not strictly comparable with those in table XIII.*
to the plating of phages \( \mu \) and \( T_3 \). Single isolated colonies were obtained by streaking this clone. Of twenty such colonies, none was still intermediate; 15 were \( \mu \frac{T_3}{\mu} \) and the other five were \( \mu \frac{F^+}{\mu} \).

If the \( T_3^{F+} \) property is indeed carried on the sex factor \( \mu \frac{T_3^{F+}}{T_3^{F-}} \) (or \( \mu \frac{T_3^{F-}}{T_3^{F+}} \)) recombinants might have been expected from the \( F^{T_3^{F+}} \times F^{T_3^{F-}} \) cross. Perhaps because of the non-selective nature of the system, they were not found. However recombination involving the \( T_3^{F+} \) property was obtained in a cross between strains carrying an \( \text{Flac}^+ \left( T_3^{F+} \right) \) F-prime factor and the \( T_3^{F-} \) sex factor discovered in strain \( \text{W1655} \). Strain \( \text{W1655 met}^+ \text{ str}^3 / \text{F lac}^+ \frac{T_3^{F+}}{T_3^{F-}} \) (the strain used in cross 2, table XIV) was mated for one hour with strain \( \text{C600 thr}^- \text{ leu}^- \text{ lac}^- \text{ str}^F \frac{T_3^{F-}}{T_3^{F+}} \). Dilutions were plated for \( \text{lac}^+ \text{ str}^F \) recombinants on EMB-lactose medium supplemented with streptomycin. Most of the purified \( \text{lac}^+ \) recombinants which were tested transferred the \( \text{pro}^+ \) property to strain \( \text{J62 pro}^- \text{ try}^- \text{ his}^+ \) with high efficiency. A few of these fertile \( \text{lac}^+ \) clones did not restrict phage \( T_3 \), and were presumed to carry recombinant \( \text{F lac}^+ \frac{T_3^{F-}}{T_3^{F+}} \) factors in which the \( T_3^{F-} \) allele had been acquired from the \( T_3^{F+} \) sex factor.

The atypical \( F^{T_3^{F+}} \) strains were also used to test whether the \( T_3^{F+} \) property can be transferred from \( F^{T_3^{F+}} \) strains. Cross 2 (Table XVIII), that is, \( F^{T_3^{F+}} \times F^{T_3^{F-}} \) suggests that in the absence of a sex factor able to allow the plating of \( \mu \) phage, the \( T_3^{F+} \) property is not transferred. It was also found that even
<table>
<thead>
<tr>
<th>Number of clones tested</th>
<th>Number of clones having the different phenotypes</th>
<th>Intermediate for both μ and T₃</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu ^T_{2J}$</td>
<td>$\mu ^R_{2J}$</td>
</tr>
<tr>
<td>96</td>
<td>0</td>
<td>36</td>
</tr>
</tbody>
</table>

The unstable cohabitation of the $T_{2J}^T$ and $\mu ^S$ characters among exconjugants from a cross between an $F^{T_{2J}T}$ strain and an $F^{T_{2J}R}$ strain. A tenfold dilution of an overnight broth culture of strain C600 $F^{T_{2J}}_R$ (thr leu $E'$) was mixed with an equal volume of a hundredfold dilution of a similar culture of strain 58-161 $F^{T_{2J}T}$ (met'). After 24 hours incubation at 37°C dilutions were plated onto minimal agar plates supplemented with methionine for the recovery of met' clones. These were then tested for their $\mu$ and $T_3$ plating properties.
when mating contacts were formed \((P^{+}\Delta_3^{x} x P^{-}\Delta_3^{x})\) the \(\Delta_3^{F^+}\) property is not transferred from \(P^{-}\Delta_3^{F^+}\) cells (cross 6; Table XVIII).

(iii) **The transfer of the phage \(T_3\) restriction property into \(E.\ coli\) strains C and B**

The \(\Delta_3^{F^+}\) property can also be transferred into \(E.\ coli\) strains C and B (crosses 7 and 8; Table XIX). The \(E.\ coli\) strain C cells which have received the sex factor can also restrict phage \(T_3\) although the e.o.p. is reduced only tenfold, instead of by a factor of \(10^5\) as in restricting K12 strains. The result of cross 9 (Table XIX) supports the view that this effect is host-specific; when the sex factor introduced into the C strain is transferred back into female K12 cells the e.o.p. of phage \(T_3\) on those clones which have become male is again \(10^{-5}\) compared with its e.o.p. on non-restricting strains. However, after a mixed incubation between an \(F^+\) K12 strain (58-161 \(F^+\Delta_3^{F^+}\)) and strain B (cross 8; Table XIX) the strain B clones which now restrict phage \(T_3\) do not plate \(\mu\). Also, after a cross between one such \(\Delta_3^{F^+}\) strain and a K12 strain (cross 10, Table XIX) none of the K12 clones tested were found to restrict phage \(T_3\), so that the \(\Delta_3^{F^+}\) and male properties have apparently been separated. A possible explanation is that the determinant for the \(\Delta_3^{F^+}\) property is indeed separate from the sex factor, but a more likely explanation will be advanced in the discussion.
<table>
<thead>
<tr>
<th>Cross</th>
<th>Type of cross</th>
<th>$\mathbf{T_3^{+}}$ strain</th>
<th>$\mathbf{T_3^{-}}$ strain</th>
<th>Number of clones tested or originally $\mathbf{T_3^{-}}$ strain</th>
<th>$%$ $\mathbf{F^+}$</th>
<th>$%$ $\mathbf{F^-}$</th>
<th>recipient clones which now restrict $\mathbf{T_3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>$\mathbf{F_T3^{+}} \times \mathbf{F_T3^{-}}$</td>
<td>$\mathbf{58-161 F^-}$</td>
<td>$\mathbf{G600 F^-}$</td>
<td>52</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>$\mathbf{F_T3^{+}} \times \mathbf{F_T3^-}$</td>
<td>$\mathbf{58-161 F^-}$</td>
<td>$\mathbf{W1655 F^+}$</td>
<td>56</td>
<td>100</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

Table XVIII. The absence of transfer of the $\mathbf{T_3^{+}}$ property from strain $\mathbf{58-161 F_T3^{+}}$. Incubation of the mixed cultures was for one hour. In cross 6 multiple matings were minimised by dilution of the mating mixture one-thousand fold five minutes after mixing.
<table>
<thead>
<tr>
<th>Cross</th>
<th>Type of cross</th>
<th>$T_{2}^{r+}$ strain</th>
<th>$T_{2}^{r-}$ strain</th>
<th>Number of clones tested of originally $T_{2}^{r-}$ strain</th>
<th>% $F^{+}$</th>
<th>2 recipient clones which now restrict $T_{2}^{r-}$</th>
<th>$F^{+}$</th>
<th>$F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>K12 $F^{+}T_{2}^{r+}$ x</td>
<td>58-161 $F^{+}$</td>
<td>C</td>
<td>60</td>
<td>100</td>
<td>100</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C $T_{2}^{r-}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>K12 $F^{r+}T_{2}^{r+}$ x</td>
<td>58-161 $F^{+}$</td>
<td>B</td>
<td>56</td>
<td>0</td>
<td>0</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B $T_{2}^{r-}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>C $F^{r+}T_{2}^{r+}$ x</td>
<td>C $T_{2}^{r-}$</td>
<td>C600 $F^{-}$</td>
<td>42</td>
<td>7</td>
<td>100</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>K12 $F^{r-}T_{2}^{r-}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>B $T_{2}^{r+}$ x</td>
<td>B $T_{2}^{r+}$</td>
<td>C600 $F^{-}$</td>
<td>50</td>
<td>0</td>
<td>-</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>K12 $F^{r-}T_{2}^{r-}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table XIX.** The $T_{2}^{r+}$ property in strains C and B. All matings were for one hour. As in the previous crosses the criterion for maleness was the ability to plate phage $\mu$. 
D. Discussion

In this section the hypothesis that phage T₃ restriction is carried on the sex factor of *E. coli* K12 strains was tested. The failure of transfer and acridine orange experiments to separate the properties of maleness and T₃ restriction in F⁺T₃⁺ and Hfr T₃⁺ strains was taken as verifying this hypothesis.

Two atypical strains were then examined. If it is assumed that T₃⁺ is in fact normally carried by the sex factor, then the inability of strain M1655 F⁺ to restrict phage T₃ is due to a deficiency in its sex factor. This view was confirmed by the isolation of F₃⁺ T₃⁻ strains from a cross between F₃⁺ T₃⁺ and F₃⁻T₃⁻ strains, as well as by the fact that when the sex factor of the non-restricting strain M1655 F⁺ was eliminated by acridine orange and substituted by a sex factor from a T₃⁺ strain, strain M1655 became restricting.

The other variant strain, 58-161 F₃⁻T₃⁺, was isolated by Spicer from an F⁺ culture (Hayes, 1953a). It can be regarded as carrying a defective sex factor. Like a normal sex factor, it is eliminated by subculture in the presence of acridine orange, and it is unable to cohabit stably in the same cell as an introduced sex factor. Additional evidence that the Spicer strain carries a defective sex factor is the observation (E. Meynell; unpublished) that in fluid cultures of this strain phage µ can undergo some multiplication. This increase in titre is far less than that observed in F⁺ cultures, but compares with a complete
absence of multiplication by phage $\mu$ on an aeridine orange-cured 58-161 strain.

In the present study the only separation of the $T_3^{F+}$ and phage $\mu$-plating properties originally present together in strain 58-161 $F_3^{T_3}F_3^{F+}$ was achieved in cross 8 (Table XIX), in which the $T_3^{F+}$ property but not the ability to plate phage $\mu$ was transferred to $E. coli$ strain B. The obvious inference was that the $T_3^{F+}$ property is not carried on the sex factor. However since all the other evidence is that the $T_3^{F+}$ determinant in this strain is located on the sex factor, we must consider alternative explanations for this result.

The criterion used here for deciding whether a clone carried the sex factor was the ability to plate phage $\mu$. This turns out to be an unreliable criterion since it is found that even among $E. coli$ K12 strains there are some which carry the sex factor or an $F_{plag}^+$ P-prime factor and on which phage $\mu$ plates badly or not at all. Two such strains are 1177 $lacr^-$ $B_1$ $ads^-$ and J62 $pro^-$ $trp^-$ $his^-$ (Breda; unpublished). It is possible that in such instances phage $\mu$ does not give lysis because the sex factor properties, such as male antigen production, are insufficiently expressed. Support for this hypothesis comes from the observation (Glover and Colson, 1965) that when an $F_{plag}^+$ P-prime factor is transferred from a K12 strain to a B strain of $E. coli$, the B clones which have become $lacr^+$ do not plate phage $\mu$ although they, like strains 1177 and J62, can act as donors of the F-prime factor.
In cross 10 (Table XIX) a $T_3^{+}$ derivative of strain B was mixed with a female K12 strain. It was found that none of the 50 K12 clones from this cross which were tested were $T_3^{+}$. However, it is possible that the sex factor, carrying the $T_3^{+}$ determinant, is in fact present in B($T_3^{+}$) cells, and that like the $lac^+$ mentioned above, it can mediate its own transfer from B strains to K strains, but that since this material carries the B modification it is subject to restriction when transferred back into *E. coli* K12 cells; a restriction system of this type in B to K crosses has been described by Glover and Colson *(loc. cit.*). A more critical test of fertility of such $T_3^{+}$ B strains would have been to attempt the transfer of the $T_3^{+}$ property into another B strain.
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