ABSTRACT

CHROMOSOME TRANSFER IN SALMONELLA PULLORUM MEDIATED BY F-PRIME FACTORS CARRYING SALMONELLA GENES

By

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When F-prime factors carrying *Salmonella* genes are introduced into *Salmonella pullorum* they are able to initiate transfer of the chromosome. A partial linkage map of *S. pullorum* has been derived using this genetic system. The linkage map of *S. pullorum* compared with that of *Salmonella typhimurium* appears to have an inverted *cysB trp* region, and a transposed *thr* locus. The FT59 (*pyrB*$^+$) factor was isolated from *Salmonella abony* and the FT71 (*trp*$^+$) and FT77 (*cysE*$^+$ *pyrE*$^+$ *rfa*$^+$) factors were isolated from *S. typhimurium*. Both the FT59 and FT71 factors in *S. pullorum* mobilize the chromosome in the opposite direction than when in *S. typhimurium*. The FT77 factor in *S. pullorum* appears to localize in an area between the *pro* and *ilv* loci and to transfer the chromosome in two directions. There appears to be good fine structure homology between *S. typhimurium* and *S. pullorum* since crosses between them mediated either by transduction or conjugation yield recombination frequencies which are analogous to intra-species crosses.

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This thesis is dedicated to Marie, Steven and George.

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INTRODUCTION

Jacob, Brenner and Cuzin (62) proposed a model for chromosome mobilization during conjugation which was an extension of their replicon model. They proposed that the Hfr chromosome results from the integration of the F factor into the bacterial chromosome. Once the F factor is integrated into the chromosome, its replication is controlled by the host cell chromosome.

Campbell proposed a specific insertion model with high predictive value (19). He suggested that the episome becomes associated with the bacterial chromosome by a single reciprocal crossover which integrates the episome linearly into the chromosome. The frequency at which the episome integrates into and detaches from the chromosome is a function of the degree of homology between the two replicons. The Campbell model predicts that an F-prime factor is formed if a crossover occurs between sites bracketing the integrated F^+, rather than at the original sites. Scaife and Pekhov (100) demonstrated that an Escherichia coli strain possessing an F-prime factor carried a deletion on its chromosome corresponding to the chromosome genes carried by the F-prime factor.

The chromosomal fragment carried by a F-prime factor confers a high affinity for the homologous region of the chromosome causing frequent integration at that site (98). The frequency of chromosome transfer is determined by the frequency of donor crossover between the F-prime factor and the chromosome (99). Consequently, in a culture of intermediate donors approximately 10% of the cells transfer the chromosome with the same orientation of the parental Hfr. Integration of a sex factor is usually studied by observing its ability to mobilize the bacterial chromosome during conjugation.

Bacterial conjugation occurs in several genera, but most experimentation has involved either E. Coli K-12 or Salmonella typhimurium. Though the guanine to cytosine ratio of S. typhimurium and E. coli K-12 are the same, 50%, (118) there is a considerable difference in genetic fine structure. Both low transduction frequencies (31) and poor in vito nucleic acid hybridization (81) between the two genera have been used to demonstrate the lack of fine structure homology. Divergence of the two species at the chromosomal level is evidenced by the inversion of the pyrF-cysB trp region (60,95,96,97). Otherwise, the sequence of genes in S. typhimurium is very similar, if not the same as that found in E. coli K-12 (95,105).

Transduction between Salmonella pullorum and S. typhimurium was reported by Schoenhard in 1963 (117) and has been demonstrated

many times since then. The transfer of an F-lac$^+$ episome from E. coli to S. pullorum and back again, as well as be-between S. pullorum was described by Robinson in 1964.(117). Since then, F-prime factors carrying S. typhimurium genes have been reported (95). It seemed clear that the time was right to develop a conjugation system in S. pullorum by use of F-prime factors isolated from and carrying S. typhimurium genes. If this conjugation was successful then it would be possible to determine the gross structure of the S. pullorum chromosome. To these ends this research was done.

S. pullorum unlike most Salmonella species is non-motile and does not produce H_2S. It is a slow growing species which has been classified as a group D Salmonella (somatic antigens 9 and 12) in the Kaufman White Schema (34). S. pullorum is the causative agent of fowl typhoid and is commonly isolated from chickens (body temperature 41-43C) while S. typhimurium is usually isolated from mice (body temperature 36-38C). However, there is a high degree of fine structure homology between S. pullorum and S. typhimurium as shown by the high transduction frequencies obtained from interspecies crosses (117).

LITERATURE REVIEW

Part I. Early History

In 1946 Joshua Lederberg, a Ph.D. student of Tatum, discovered conjugation in bacteria. He assumed that the two parental types of bacteria were equal partners, and the fusion between them led to the formation of fully diploid zygotes. The wild type of *E. coli* strain used in this work was labeled K-12 (71).

Employing streptomycin sensitive and resistant strains of *E. coli* K-12, Hayes (51) found that the viability of one of the strains was essential to the fertility of the cross. From this experiment, he concluded that conjugation was a heterothallic system in which recombination is mediated by a one-way transfer of genetic material from the donor to the recipient bacterium. This one-way transfer was further substantiated when Hayes (52) demonstrated that pre-treatment of donor cells with ultra violet light increased the yield of recombinants as much as 50-fold while pre-treatment of the recipient cells decreased the yield of recombinants.

In bacterial conjugation a donor is characterized by the presence of an autonomous, transmissible genetic element

which is designated F, for fertility (20). There are three types of unique genetic elements that confer donor ability on the bacterium within which they reside. Luria (76) termed these genetic elements "conjugans," they are the F (fertility) factors, the CF (colicinogenic) factors and the RTF (resistance transfer) factor.

Part II. Chromosome Mobilization

The F factor. The F factor is a small piece of deoxyribonucleic acid (DNA) with a molecular weight of 4.5×10^7 daltons, which is comparable to about 2% of the *E. coli* chromosome. The F factor exists as a circular piece of double stranded DNA (41,42). The DNA of the F factor can code for 40-80 genes, but only a few gene functions are presently known.

It would appear that the F factor contains few if any chromosomal genes, since there is no preferential origin and direction of F-mediated gene transfer as is the case with F-prime containing males (2). However, since 40% of the DNA of the F factor hybridizes with chromosomal DNA from *E. coli* (35), it would appear that the two replicons are phylogenetically related. The largest fraction of F DNA (9/10) has a GC ratio of 50% like the chromosome of *Escherichia*, *Shigella* and *Salmonella*. The other fraction of F DNA (1/10) has a GC ratio of 44% (35,93).

The autonomous male fertility factor may be eliminated from the cell by treatment with acridine dyes (57). However, the acridines do not inhibit the replication of the F-factor when it is integrated into the host cell chromosome (56). It is thought that the acridine dyes preferentially inhibit multiplication of the autonomous F-factor (107).

Only a few gene functions have been directly related to the presence of the F-factor in a bacterial cell. The most actively studied F-gene function has been the biosynthesis of a specialized fimbriae designated as "F" fimbriae (18).

"F" fimbriae. Fimbriae, first referred to as "pili" (17) are a widely distributed class of bacterial surface appendages. Two types of fimbriae have been reported (110). They are structural and "F" fimbriae. "F" fimbriae are rods $2\text{-}10\text{x}10^{3}$nm in length with a diameter of 8.5nm and an axial hole 2.0-2.5nm in diameter. They are generally longer and thicker than structural fimbriae and they often have a knob of variable shape on their distal end (82). "F" fimbriae are recognized by their selective adsorption of a male specific bacteriophage (111).

Male specific phage were first isolated by Loeb in 1960 (72) as a phage that would form plaques on lawns of E. coli K-12 donor strains but not on recipient strains (F^{-}).

However, Hourichia and Adelberg (58) were unable to demonstrate plaque formation in strains of Proteus mirabilis harboring the F^+-factor, but they could show an increase in phage titer when these cells were infected with the male specific phage.

Nature of the conjugal tube. The nature of the union between conjugation partners and the extent of the material transferred is not entirely clear. Bridges between cells consisting of cytoplasmic extensions 100 to 300 nm in width have been repeatedly observed by Anderson in electron microscope preparations of conjugating cells (3). Brinton, Gemski and Carnahan (18) argued that these cytoplasmic extensions were artifacts since these bridges were frequently seen joining male cells. They suggested that the true conjugal bridges were the "F" fimbriae.

If "F" fimbriae are involved in bacterial conjugation and phage penetration then conjugation should interfere with phage invasion and phage invasion should disrupt bacterial conjugation. Silverman et al. (104) have performed this type of experiment and their results indicated that conjugation and phage penetration were mutually exclusive.

If the large diameter bridges described previously by Anderson are the true conjugal bridges, then one might expect extensive transfer of protein and RNA through them

during conjugation. If "F" fimbriae of 2.0-2.5 nm in diameter are the conjugal bridges, then the passage of molecules having a Stokes radius greater than 2.5nm would be restricted. Rosner (92) did not find a significant transfer of B-galactosidase during mating between F^+ and F^- cells. Silver (103) found that the transfer of labeled ribonucleic acid (RNA) or protein from Hfr to F^- cells did not exceed the lower limit of detection of about 1%. Thus the transfer of material other than DNA, if it occurs, appears to be relatively minor in bacterial conjugation. Since chromosomal DNA has not been found in "F" fimbriae (113) the nature of the union between the two conjugation partners remains unanswered.

Model for chromosome mobilization. Jacob, Brenner and Cuzin in 1963 (62) proposed a model for chromosome mobilization during conjugation which was a simple extension of their replicon model and is currently the model in vogue. They proposed that the Hfr chromosome results from the fusion of the F factor and the bacterial chromosome. Once the F factor integrates into the chromosome, its replication is controlled by the host cell chromosome. This integrated F factor is thought to be in juxtaposition with both the plasma membrane and the "F" fimbriae. The event of conjugation triggers the start of a new round of replication beginning at the "F" replicator, such that the origin, which is the

lead point in transfer, is duplicated first. Genetic transfer is coupled necessarily with DNA replication. Consequently the transferred molecules are synthesized at the time of mating. One replica is driven into the female cells by the same forces which ensure DNA replication.

The Hfr donor transfers its genes in a polarized fashion beginning at the origin (location of F factor) and proceeding sequentially along the linkage group until the integrated sex factor is reached and is itself transferred. Random chromosome breakage during transfer terminates the transfer process and results in a higher number of early markers near the origin being transferred than late markers (64).

Jacob and Wollman (64) followed the appearance of a series of donor markers in recipient cells as a function of time by separating the mating pairs at various times with a Waring Blendor. By this procedure, called interrupted mating, they were able to translate the distance between genes into time units.

Transfer replication. The predominance of evidence seems to suggest that DNA synthesis in the male is necessary for chromosome transfer. Barbour (6) found that F'lac transfer was severely restricted in the presence of Nalidixic Acid, which is a known inhibitor of DNA replication. Gross and Caro (48) studied chromosome transfer in Hfr

males using quantitative autoradiography and concluded that the DNA is replicated prior to or during transfer. They also concluded from the intensity of beta tracks that it was double stranded DNA that was transferred during conjugation. Freifelder (40) also demonstrated that the DNA transferred during conjugation was replicated prior to or during transfer. He mated a thymine-requiring cell in the presence of 5-bromouracil and was able to show the presence of 5-bromouracil in the recombinant by virtue of its sensitivity to ultraviolet light. However, Cohen et al. (24) demonstrated the presence of single stranded DNA in "mini cells" when they were mated with F^+ donor cells of E. coli K-12.

Chromosome mobilization by rec^- donors. Implicit in the model of Jacob et al. (62) is the concept that chromosome transfer during conjugation is mediated by an integrated sex factor. Thus, chromosome transfer by F^+ cells is due to a small fraction of the cells being Hfrs. However, Clowes and Moody (23) did find chromosome transfer mediated by certain conjugans (F, F-prime, colV2 and col3) in recombinationless (rec^-) donor strains. In these strains the chromosome was transferred at a decreased level and there was no preferential origin of transfer even with F-prime strains. Furthermore, Curtiss and Stallions (28) found that only about 10% of the recombinants formed in

$F^+ x F^-$ matings were due to stable Hfr donors. Thus it appears that chromosome mobilization does not depend entirely on recombination or even transient association of the two replicons; conjugans and chromosome.

Role of female. Some 11 years ago, Fisher (38) published experiments which demonstrated that only the male cell required an available source of energy during mating; therefore, the female was assumed to play a passive role in conjugation. It is currently believed that this conclusion was wrong and that the female actively participates in DNA transfer during conjugation.

Freifelder (40) mated Hfr and F'lac male strains of E. coli K-12 with purine requiring recipients. He found that mating in the absence of purine markedly reduced the yield of recombinants and concluded that DNA transfer required some, as yet unknown, function of the female. Bonhoeffer et al. (13) found that the donor genome is not transferred to a temperature sensitive recipient at the restrictive temperature. He concluded that the recipient strain contained a component which was necessary for DNA synthesis in the recipient and for transfer of DNA during conjugation. Spelina (106) mated synchronously dividing Hfr cells with randomly dividing F^- cells and found that recombinant formation was not influenced by the time in the division cycle at which the male cells were taken. When

synchronously dividing female cells were used with randomly dividing Hfr cells, the yield of recombinants was greatly influenced by the time in the division cycle of the female. The highest yield of recombinants occurred with females which were in the middle of the division cycle. This corresponded nicely with Helmstetter's data (54) which indicated that DNA replication began when bacteria were in the middle of cellular division.

Gene pseudoinversion. Pittard and Walker (89) employing both proximal and distal unselected markers found that an obligatory interaction occurred between the donor and recipient DNA molecules in the region immediately adjacent to the lead end of the transferred DNA. It has been shown also that markers transferred early during conjugation are integrated at a lower frequency than more distal markers (74,45). Thus, the true order of genes located near the origin has to be deduced by an obligatory analysis of the genetic constitution of several classes of recombinants. The explanation for the "pseudoinversion" of genes located near the origin is thought to be due to the presence of F-DNA on the lead region of the transferred DNA (27). The F portion of lead segment has little homology with the recipient chromosome thus exerting an anti-pairing effect and reducing effective synapsis of the respective alleles. This would necessitate that the obligatory

recombination event must occur at a more distal locus, thus reducing the recombination frequency of genes located 1-2 min from the origin as compared to genes located 5 min from the origin (74,23). After the lead region of the donor DNA has recombined with the recipient chromosome it is assumed that the female winds in the DNA. This would in part assure effective synapsis of the respective alleles.

Part III. Recombination

The central problem of bacterial conjugation seems to be the very process of recombination itself. One might reasonably ask how two DNA molecules get close enough to enable switching of exact and identical nucleotide sequences. The phosphate groups on the DNA are all negatively charged under the normal physiological conditions of the cell. If the DNA strands did pair closely enough to exchange bonds, strong forces of repulsion would have to be overcome. Further, the phosphate ester bonds are relatively stable bonds, and considerable energy would be required to break and reform them.

Recombinationless mutants. Since it seemed likely that enzymes participate in the events leading to the formation of the completed recombinant DNA structure, Clark and Margulies (22) undertook the isolation of mutants in which one or more of the hypothetical recombinant enzymes

would be defective. Two mutants were isolated that were unable to form recombinants with suitable Hfrs. These mutants, designated rec$^-$, were found to be much more sensitive than the parental strain to the effects of ultraviolet light. Flanders (59) found a definite correlation between the rec$^-$ mutation and the inability of rec$^-$ strains to excise pyrimidine dimers and repair other DNA lesions. Oppenheim and Riley (85) also demonstrated that enzymes participate in recombinant formation. They showed that gene integration in bacterial conjugation involved a physical association mediated by covalent bonds of parental DNA molecules.

Segregation. The measurement of segregation frequency in merozygotes gives information about the fate of the donor genome from the moment of its insertion until the moment of pure recombinant formation. Bresler (16) was not able to demonstrate essentially haploid clones until at least three to nine generations after termination of mating. He assumed that the exogenote was replicated with the endogenote and gradually integrated. However, Wood (115) found widely different segregation patterns depending on the Hfr strain employed. Thus, during conjugation there is a time period during which a transient merozygote exists.

In 1951, Lederberg et al. (70) discovered the presence of stable merozygotes in an F$^-$ strain of E. coli K-12. This strain multiplied mainly as a partial diploid cell,

segregating a few haploid recombinants at a low frequency. This strain was thought to possess a defective recombinase. Low (75) studied the inheritance patterns of cells from a Hfr x F^- rec^- mating and found that the rec^- zygotes and their progeny retained the alleles of both parents. The behavior of these rec^- zygotes bears a remarkable similarity to the stable merozygotes previously described and it is becoming clear that usually the rec^- recombinants are not normal.

Part IV. Episome Chromosome Interaction

Campbell's Model. In 1962, Campbell proposed a specific insertion model with high predictive value (19). Since then, the model has been extensively tested in several systems and extensive experimental evidence has been obtained in support of it. Campbell suggested that the episome becomes associated with the bacterial chromosome by a single reciprocal crossover at which time the episome integrates linearly into the chromosome. The mechanism that detaches an episome is exactly the reverse of that causing insertion, and seems to be a function of loop formation which allows synapsis followed by crossing over. The frequency at which the episome integrates into or detaches from the chromosome is a function of the degree of homology between the two replicons. This model accounts

for the rare alteration between the F^+ and Hfr states of the F factor (64).

Primary F-prime strains. The Campbell model predicts that an F-prime factor is formed if a crossover occurs between sites bracketing the integrated F^+, rather than at the original sites. Such an event would yield a circular F-prime factor and a chromosome with a deletion corresponding to the chromosome fragment incorporated by the F-prime factor. Scaife and Pekhov (100) demonstrated that an E. coli strain possessing an F-prime factor carried a deletion on its chromosome corresponding to the chromosomal genes carried by the F-prime factor. This parental strain possessing the F-prime factor is termed a primary F-prime strain; it is an F-prime strain descended directly from an Hfr. Primary F-prime strains would be expected to lack a region of homology between the replicons and thus chromosome transfer would be randomly oriented and the recombinant yield would be of the order found with the F^+ factor. Scaife and Pekhov (100) demonstrated this with the primary F-prime strain that they originally isolated.

Secondary F-prime strains. F-prime factors are transferred autonomously to the recipient during conjugation, and they are converted to intermediate donors which are designated secondary F-prime strains. The chromosomal fragment carried by an F-prime factor confers a high

affinity for the homologous region of the chromosome in the intermediate donor, and this affinity results in frequent integration at that site. Consequently, a culture of intermediate donors contains approximately 10% of cells which are able to transfer the chromosome with the same orientation as the parental Hfr. The other cells continue to transfer the F-prime factor in the autonomous state (29,99). A secondary F-prime strain when compared to the parental Hfr will show a delay in time of transfer of a marker equal to that needed for transfer of the F-prime factor (98,100). This delay is defined as the lead time, whereas dead time is defined as the time interval from contact formation until gene transfer.

Consequence of episome integration. It is implicit in Campbell's model that the episome is linearly integrated into the chromosome. Pittard (87) found that when the sex factor is integrated at a site between two cotransducible genes, their linkage is markedly reduced. Mapping studies also indicated that the F-prime factor is integrated by insertion into the chromosome. This conclusion was drawn from the analysis of chromosomal markers transferred by an F-prime strain (98,100).

If an episome integrates by insertion into the chromosome, then the insertion within a gene should inactivate that gene. Beckwith et al. (9) employed this concept to

direct the transposition of an Hfr derivative. They employed the E. coli strain that was sensitive to T6 phage and possessed a temperature sensitive F'lac replicase. They grew this strain in the presence of T6 phage at the elevated temperature with lactose as the only energy source. Employing this procedure, they were able to isolate Hfr derivatives with the episome integrated in the region coding for T6 phage receptor sites. The rationale of this experiment was two-fold: (1) once integrated the replication of episomal genes would be accomplished by the host chromosome replicase system and (2) integration within a gene coding for a phage receptor site would inactivate that gene and the cell would then be phenotypically resistant to the phage. In other experiments Beckwith et al. (9), employing the temperature sensitive sex factor isolated by Cuzin and Jacob (29), were able to isolate both clockwise and counterclockwise transposition Hfrs. These experiments demonstrated that a gene inversion and/or transposition was not necessarily a lethal event.

Part V. Conjugal Systems in Other Genera

The predominance of work done with bacterial conjugation has employed fertile strains of E. coli K-12. Conjugal systems have been established in a number of other strains and genera of bacteria by application of the basic concepts gleaned from the K-12 system.

Chromosomal markers have been transferred by mating E. coli K-12 Hfr donors with S. typhimurium (7), Salmonella typhosa (8), and Shigella (76,102) recipients. Such matings result in an apparent lower frequency of chromosomal transfer than with E. coli Hfr x E. coli F^- matings. Usually the former type matings result in the formation of unstable partial diploids (7,44).

Genetic compatibility by sexduction does not require that the interacting organisms display a similarity in DNA base composition, since the transferred material does not need to exchange with the resident chromosome (35). A number of conjugans have been transferred from E. coli K-12 to strains of Shigella, Salmonella, Serratia and Proteus at a high frequency (2). In Serratia and Proteus strains infected with the E. coli F-factor, the DNA band profile obtained by density-gradient centrifugation shows a minor band identical to E. coli DNA. If the F-exogenote is lost from these strains either spontaneously or by curing, the satellite DNA band disappears (35). This allows a unique way of isolation and study of E. coli conjugans.

Shigella x Shigella matings do not yield recombinants, even when Shigella cells are used which had received the F-factor from E. coli. This might possibly be due to the lack of homology between the F-factor and the Shigella chromosome (76).

The F-factor from E. coli was first introduced into Salmonella abony, which subsequently served as a donor of F to other Salmonella strains. S. abony strains possessing the F^+ factor were mated with S. abony recipients and yielded recombinants at a frequency similar to that found in E. coli matings. Since chromosome transfer could be demonstrated Mäkela (78) attempted to isolate Hfr Salmonella donors from F^+ strains which had been subjected to ultra violet light and replicating onto a recipient. All attempts were futile using this procedure. Hfrs were isolated by Sib selection, and in many cases a high percentage of the apparent Hfrs transferred the F-factor at a high frequency. From an S. abony Hfr (SW1444) an F-prime factor FT59 was isolated. This factor when introduced into other Salmonella strains transferred the chromosome in the same direction and with the same orientation as the Hfr from which it was isolated (79,80).

Schneider and Falkow (102) developed another method of Hfr selection. They obtained an Hfr by terminal marker selection in a cross between an Hfr E. coli and a Shigella flexneri recipient. The resultant Shigella Hfr had the same gene sequence as E. coli.

Conjugation and recombination have been reported to occur in Pseudomonas aeruginosa (73). The conjugan responsible for these events (FP) behaves very much like the F-factor of E. coli K12. A mating system also has been

discovered in Pasteurella pseudotuberculosis (68). A strain of Vibrio cholera which produces a bacteriocin that kills vibrios has been found to yield recombinants when mixed with certain Vibrio cholera strains which lack the determinant for bacteriocin production (12). In 1956 Belser and Bunting (10) studied what they thought was a conjugation system in Serratia marcescens. However, in 1963, Dushman (33) proved that their results were mainly the consequence of syntrophism.

Part VI. Phylogenetic Relation Between Escherichia coli and Salmonella typhimurium

Even though the guanine to cytosine ratio of S. typhimurium and E. coli are the same, 50%, and they have approximately the same gene sequence (96,95,109), a considerable divergence in fine structure homology has been detected by fine structure analysis as indicated by the inability to transduce genes between the two genera (31). These genetic results ~~:~~ results earlier in vitro nucleic acid hybridization studies (81), which demonstrated poor homology between the two genera. The corresponding genes are not truly allelic, although enough chromosomal homology exists to allow genetic exchange when larger units are transferred by conjugation (109,95).

In both S. typhimurium and E. coli the trp-cysB-pyrF genes are localized within a short segment of the chromosome

but are, comparatively speaking, inverted (95). If evolution proceeds by the integration of new genetic information into simpler clustered sequences, then a diffusion of the clusters must ensue. Since no known genes other than cysB are located between trp and pyrF in both S. typhimurium and E. coli then strong selective pressure must have existed during evolution for maintenance of the trp-cysB-pyrF cluster in these species (105).

Ino and Demerec (60) suggest that E. coli and S. typhimurium have diverged in two distinct fashions that are of potential evolutionary significance. Divergence at the intra-genic level is indicated by low transduction frequencies between the two genera and divergence at the chromosomal level is demonstrated by the inversion of the trp-cysB-pyrF region (97). This knowledge of gene orientation is important because of its bearing on models of gene transcription and regulation. It is also important to realize that the environment would be expected to play a large role in diffusion, inversion and/or transposition of gene clusters.

Part VII. *Salmonella pullorum*

S. pullorum is commonly isolated from chickens and is the causative agent of fowl typhoid. It is slow growing in comparison to other *Salmonella* species. It is classified as a group D *Salmonella* strain by the Kauffman White Schema and has an antigenic structure of 9, 12. The somatic factor 12 is the presumed receptor for PhageP22.

Schoenhard (117) was able to transduce genes from *S. typhimurium* to *S. pullorum* employing the PLT-22 phage, thus indicating fine structure homology between the species. Robinson and Schoenhard (91) demonstrated that *S. pullorum* could accept and transfer conjugans at a high frequency with *E. coli* K-12 donor and recipient strains.

MATERIALS AND METHODS

Chemicals. N'-methyl-N'-nitro-N-nitrosoquanidine (NTG) was obtained from Aldrich Chemical Company, Milwaukee, Wisconsin. Sodium azide was obtained from Distillation Products Industries, Rochester, New York. All other chemicals employed were reagent grade.

Bacteria. S. pullorum, strain MS35, was selected as the prototype organism from the stock collection of Dr. D. E. Schoenhard; it was designated wild type. S. pullorum, strain MS35, and auxotrophic mutants derived from it are described in Table 1. S. typhimurium and E. coli K12 strains used in this experiment are described in Tables 2 and 3 respectively. The donor strains of S. pullorum which were isolated during this investigation are listed in Table 4. The linkage map of S. typhimurium is shown in Figure 1. The chromosomal distribution of relevant markers and the point of origin and direction of transfer of the various male strains used in this investigation are located on the linkage map.

Media. The E minimal medium was that described by Vogel and Bonner (112). Sterile D-glucose was added to a final concentration of 0.5%. The A minimal medium (119)

Table 1. Characteristics of Salmonella pullorum recipient strains.

Strain No.	Mating Type	Relevant Genetic Markers[a]	Origin or Ref.
MS35W[b]	F⁻	cysA1 cysJ1 leu-1	(66)
MS18	F⁻	leu-1	MS35
MS350	F⁻	strA1	MS18
MS351	F⁻	strA1 tyr-1	MS350
MS352	F⁻	strA1 ilv-2	MS350
MS353	F⁻	strA1 ser-1	MS350
MS354	F⁻	strA1 met-1	MS350
MS355	F⁻	strA1 trp-1	MS350
MS356	F⁻	strA1 leu-2	MS350
MS357	F⁻	strA1 thy-1	MS350
MS358	F⁻	strA1 pro-2	MS350
MS359	F⁻	strA1 thr-2	MS350
MS360	F⁻	strA1 ara-1	MS350
MS361	F⁻	strA1 xyl-2	MS350
MS362	F⁻	strA1 gal-2	MS350
MS363	F⁻	strA1 glyA1	MS350
MS364	F⁻	strA1 his-1	MS350
MS365	F⁻	strA1 his-1 pro-1	MS364
MS366	F⁻	strA1 his-1 pro-1 thr-1	MS365
MS367	F⁻	strA1 his-1 pro-1 thr-1 ilv-1	MS366
MS368	F⁻	strA1 his-1 pro-1 thr-1 ilv-1 azi-1	MS367
MS369	F⁻	strA1 his-1 pro-1 thr-1 ilv-1 gal-1	MS367
MS370	F⁻	strA1 his-1 pro-1 thr-1 ilv-1 gal-1 xyl-1	MS369
MS374	F⁻	strA1 pro-1 thr-1 ilv-1 gal-1	MS369
MS36	F⁻	cysA1 cysJ1 leu-1 trp-2	MS35
MS37	F⁻	cysA1 cysJ1 leu-1 his-2	MS35
MS81	F⁻	leu-1 cysE1	MS18
MS82	F⁻	leu-1 cysE1 glyA2	MS81
MS90	F⁻	leu-1 cysE1 ilv-3 strA2	MS81
MS100	F⁻	leu-1 cysB1	MS18
MS103	F⁻	leu-1 cysB1 trp-3	MS100
MS104	F⁻	leu-1 cysB1 trp-3 his-3	MS103
MS105	F⁻	leu-1 cysB1 trp-3 his-3 gal-3 strA3	MS104

[a] Where possible, we have used the same symbols as Sanderson (95) and have followed the conventions suggested by Demerec et al. (30) for genotypic and phenotypic symbols.

[b] W = wild type (streptomycin sensitive).

[c] MS81, 82, 90, 100-105 isolated by B. Klooster.

Table 2. Characteristics of *Salmonella* strains.[a]

Strain No.	Mating Type	Relevant Genetic Markers	Source	Reference
SU219	Hfr	hisD23 gal-50	Hartman	
SB172	Hfr	purC7 hisR1272	Hartman	
SA534	Hfr	serA13	Sanderson	
SA535	Hfr	serA13	Sanderson	
SA536	Hfr	serA13	Sanderson	
SB394	F'	trp-109/F_{ts} lac^+	Hartman	(4)
SH59	F'	/FT59($pyrB^+$)	Hartman	(79)
SB890	F'	his-712 ser-821 arg-501/FT80 (his^+)	Hartman	(37)
SU694	F'	trpA52 cysB12 pyrF146 ilv-178/FT71 (trp^+)	Sanderson	(94, 97)
SU695	F'	trpA52 cysB12 pyrF146 ilv-178/FT73 (trp^+ $cysB^+$ $pyrF^+$)	Sanderson	(94, 97)
SA523	F'	met-483 cysE396/FT76 (xyl^+ $cysE^+$ $pyrE^+$ rfa^+)	Sanderson	
SA532	F'	met-483 cysE396/FT77 ($cysE^+$ $pyrE^+$ rfa^+)	Sanderson	
SH634	F^+		Hartman	(78)
MST90	F^-			
MST100	F^-	trp-109	SB394	
MST119	F^-	trp-109 his-1 thr-1 pro-1	MS100	
MST120	F^-	cysA20	Hartman	
MST121	F^-	cysC1021	Hartman	
MST123	F^-	pro-107	Hartman	
MST124	F^-	cysH75	Hartman	
MST125	F^-	cysEb	Hartman	
MST172	F^-	purC7 hisR1272	SB172	

[a]All strains described are *S. typhimurium* with the exception of SH59 and SH634 which are *S. abony* strains.

Table 3. Characteristics of Escherichia coli strains.

Strain No.	Mating Type	Relevant Genetic Markers
AB257	Hfr	met
AB311	Hfr	thr leu
AB312	Hfr	thr leu
AB785	F'	met/F-lac$^+$
3349	F'	his ile/F-his
W6	F$^+$	met
AB113	F$^-$	his leu thr
MSE311[a]	F$^-$	thr leu

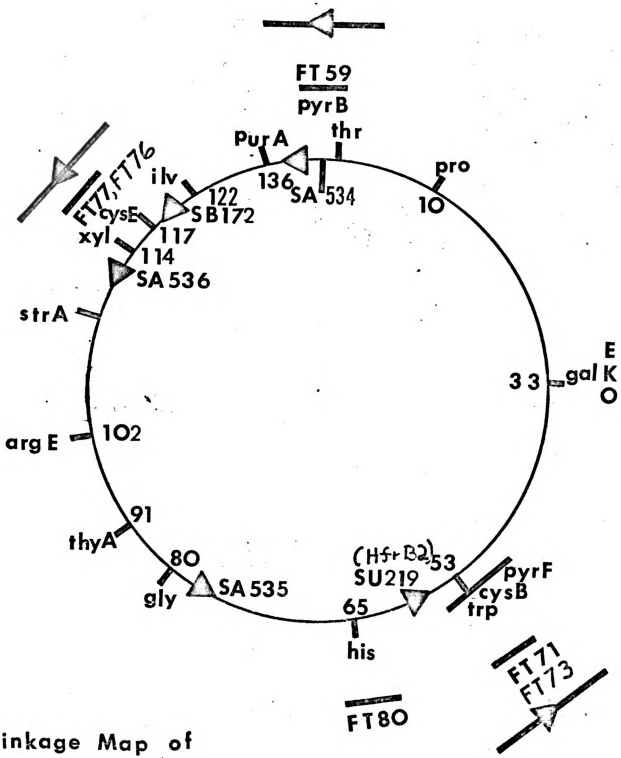
[a]MSE311 isolated as an MS2 insensitive mutant of AB311.

Table 4. Donor strains of Salmonella pullorum.

Strain No.	Mating Type	Relevant Genetic Markers	Derived from This Mating
MS800	F^+	cysA1 cysJ1 leu-1	W6 x MS35
MS801	F^+	cysA1 cysJ1 leu-1 lac^+	SH634 x MS35
MS802	F'	cysA1 cysJ1 leu-1/F lac^+	AB785 x MS35
MS803	F'	cysA1 cysJ1 leu-1/F_{ts} lac^+	SB394 x MS35
MS804	F'	cysA1 cysJ1 leu-1 his-2/F-his^+	3349 x MS37
MS805	F'	cysA1 cysJ1 leu-1 his-2/FT80$_+$(his^+)	SB890 x MS37
MS806	F'	cysA1 cysJ1 leu-1/FT59 ($pyrB^+$)	SH59 x MS35
MS807	F'	cysA1 cysJ1 leu-1 trp-2/FT71 (trp^+_+)	SU694 x MS36
MS808	F'	cysE1 glyA2 leu-1/FT76 (xyl^+_+ $cysE^+$ $pyrE^+_+$ rfa^+)	SA523 x MS82
MS809	F'	cysE1 glyA2 leu-1/FT77 ($cysE^+$ $pyrE^+$ rfa^+_+)	SA532 x MS82
MS810	F'	cysA1 cysJ1 leu-1 his-4 trp-2/FT71 (trp^+)[a]	---

[a]Derived from MS807.

Figure 1. Linkage map of the S. typhimurium chromosome showing the points of origin and direction of transfer of the various male strains used. The Hfr strains are indicated on the circle and the F-prime factors in the expended portion.



Linkage Map of

Salmonella typhimurium

lacking citrate was used for the detection of carbohydrate utilization. Filter sterilized carbohydrates were added to a final concentration of 0.2%. Where necessary, amino acids were added to a final concentration of 25 µg/ml. L broth and L agar containing 10g of tryptone (Difco), 5g of yeast extract, and 10g of NaCl per liter of distilled water were employed for routine cultivation. Nutrient broth consisting of 8g nutrient broth (Difco) and 5g NaCl per liter of distilled water was employed in dilution and plating of mating mixtures. Difco agar was used at 1.5% final concentration unless otherwise stated. MacConkey Agar Base (Difco) and Levine Eosin Methylene Blue Agar without lactose (BBL) were routinely employed for the detection of carbohydrate fermentation. The pH was adjusted to 7.1 and after autoclaving, a filter sterilized carbohydrate solution was added to a final concentration of 1%. The glucose concentration of the various carbohydrate sources was determined by the Glucostat (Worthington). Bacto SIM Medium (Difco) was employed for the detection of sulfide and indole production. The medium described by Ball (5) was employed for the detection of motility. Dihydrostreptomycin Sulfate was added to a final concentration of 1200 µg/ml in minimal media and 250 µg/ml in complete media.

Mutagenic treatment. Mutants isolated during the course of this work were induced by NTG following the method recommended by Adelberg, Mandel and Chen (1). Five ml of logarithmic phase cells (2 x 10^8 cells/ml) growing in E minimal broth were washed by filtration and then resuspended in 10 ml of TM buffer pH 6.0 containing 100 µg of NTG/ml. The suspension was incubated for 20 min at 37C with shaking and then 1 ml was filtered to remove the excess NTG. These treated cells were resuspended in 10 ml of E minimal broth properly supplemented to permit growth of the desired mutant type and incubated with aeration for at least five generations.

Also penicillin treatment as described by Gorini and Kaufman (47) was employed to enrich for the desired mutants. Ten ml of the NTG treated suspension (5 x 10^8 cells/ml) were centrifuged and the pellet resuspended in 1 ml of E minimal broth. A 0.1 ml aliquot of the resuspended pellet was placed in 10 ml of E minimal broth supplemented with 10% sucrose, 0.5% glucose, 0.01 M $MgSO_4$ and other growth requirements of the parental cell type. The culture was grown with aeration for two generations or 3 hr and Penicillin G then added to a final concentration of 2,000 units/ml. The suspension was incubated at 37C without shaking. After 4 hr incubation, when about 50% of the cells had become spheroplasts, the action of penicillin was stopped by chilling and the

culture centrifuged. The pellet was resuspended in 10 ml of E minimal broth properly supplemented to permit growth of the mutant type. After two to three cyles of penicillin enrichment the cells were plated on L agar and the mutants isolated by the replica plating technique.

Determination of UV sensitivity. Logarithmic phase cells (1 x 10^8 cells/ml) growing in L broth were centrifuged and then resuspended in an equal volume of A minimal broth. A 3 ml sample of cells was placed in an open glass 60 cm^2 petri dish at a distance of 48 cm from a horizontal, 30-watt General Electric G30T8 germicidal lamp emitting primarily at a wavelength of 253.7 nm. The suspension was shaken during exposure to UV light and then immediately diluted and plated on L agar. The plates were incubated at 37C for 24 hr. Extreme care was taken to avoid photoreactivation.

Bacteriophage sensitivity. Sensitivity or insensitivity to the bacteriophage MS2, a donor-specific RNA bacteriophage isolated by A. J. Clark, was used to indicate the presence or absence, respectively, of the F factor in the tested culture. The following procedure was employed to detect sensitive donor strains. It is based on the observation that S pullorum donor strains infected with MS2 will allow an increase in titer of the phage to a maximum of 2 x 10^8 phage/ml even though MS2

does not form plaques on donor strains of S. pullorum. A loopful of an overnight culture was inoculated into a tube containing 2 ml of L broth supplemented with 200 µg of $CaCl_2$/ml and previously inoculated with 10^3 MS2 phage/ml. This suspension was incubated overnight at 37C without aeration. After incubation two drops of chloroform were added to each tube and then each tube was swirled. After incubation at 37C for 15 min a loopful from each tube was placed onto a fresh lawn of E. coli AB312 growing on an L soft agar overlay on L agar plate. Approximately 10 tubes could be tested per plate. The plates were scored after 3hr incubation at 37C. Sensitive strains carrying the MS2 phage gave a clear zone of lysis 1 cm in diameter; whereas, strains without MS2 phage produced no clearing. Extreme care was taken to avoid transferring any excess chloroform to the lawn of E. coli AB312.

Technique of bacterial mating. The techniques employed were essentially those previously described by Anton et al. (4). Overnight aerated L broth cultures of the donor and the recipient were diluted 1:20 in L broth followed by incubation for 3 hr at 37C without aeration. Five ml of the donor (1×10^8 cells/ml) were mixed with 5 ml of the recipient (1×10^8 cells/ml) and the mixed suspension filtered on a pre-wet membrane filter, Millipore HA 0.45µ, 25mm. The filter was immediately

placed on a prewarmed L soft agar, plate, 0.75% agar and incubated for 3 hr at 37C. After incubation the millipore filter was removed from the agar and placed into the tube containing 2 ml of nutrient broth, and agitated with a Vortex Jr. Mixer for 60 sec to resuspend the cells. Further dilutions were made in nutrient broth. One tenth ml aliquots were pipetted from the various dilutions of the mating mixture into tubes containing 3 ml of E minimal soft agar, 0.75%, kept at 45C. The tubes were mixed and then poured onto E minimal agar plates which were supplemented when necessary. The plates were then incubated for 96 hr at 37C. Growth of the donor strains and plate-mating were prevented by omitting from the minimal medium a supplement required by the auxotrophic donor strain, preferably a requirement determined by the distal region of the donor genome, and by adding 1200 µg/ml of dihydrostreptomycin to eliminate the streptomycin sensitive donor strain.

Interrupted matings were performed as above except that several 3ml portions of donor and recipient were used and the millipore filters were removed from the agar at appropriate time intervals. After the cells were resuspended in nutrient broth the cell suspension was transferred to a fluted screw cap test tube to further agitate for 2 min to disrupt the mating pairs. Time zero

was taken as the time at which the cells were drawn onto the Millipore Filter.

Centrifuge matings were conducted in a manner analogous to the millipore matings. Ten ml of the donor (1×10^8 cells/ml) was mixed with 10 ml of the recipient (1×10^8 cells/ml) and the mixed suspension poured into a pre-warmed centrifuge tube. The suspension was then centrifuged for 4 min at 10,800 x g and then incubated at 37C for 30-60 min. After the prescribed time interval, the mating suspension was agitated with a Vortex Jr. Mixer for 2 min to resuspend the cells.

Interrupted centrifuge matings were performed as above except that four to eight tubes containing 10 ml of the donor (1×10^8 cells/ml) were poured into the pre-warmed centrifuge tubes and then 10 ml of the recipient cells (1×10^8 cells/ml) were added to each tube. The mating suspensions were swirled and immediately centrifuged. The cells were centrifuged for 4 min at 10,800 x g at 37C. Time zero was taken as 1 min after the start of centrifugation. The centrifuge tubes were removed and incubated as described previously. At appropriate time intervals the mating cells were resuspended and 2 ml of the suspension was transferred to a fluted screw cap test tube to further agitate for 2 min to disrupt the mating pairs.

Scoring unselected markers. Recombinant clones were purified by streaking on minimal medium of the same composition as that used for initial selection and incubating for 4 days at 37C. Single colonies were then spread into patches on minimal medium of the same composition as that used for initial selection and incubated 24-48 hr. These patches of colonies were tested for their inheritance of unselected markers by replicating them onto plates of medium appropriately supplemented. In instances where streptomycin was not used for counter-selection it was necessary to rule out cross feeding by the donor strain and this was accomplished by replicating the above master plates onto a minimal medium supplemented with the growth requirements of the donor strain.

Cross-streak method. The procedure is essentially that described by Berg and Curtiss (11). Young logarithmic phase donor cells were diluted to a concentration of 2×10^2 cells/ml in L broth. One ml aliquot portions were then pipetted into 200 Wasserman Tubes and incubated at 37C for 8 hr without shaking. After 8 hr incubation the cell concentration in most tubes was approximately 1×10^8 cells/ml. Approximately 0.2 ml of a log phase broth culture of the appropriate recipient tester stock was applied in two parallel vertical lines to the surface of a properly supplemented E minimal agar plate. After the streaks had dried, loopfuls of the donor cultures were streaked

horizontally across the recipient. Using this procedure 20 cultures could be tested per plate. After 72 hr incubation at 37C the number of recombinant colonies in each streak was scored. In many instances the donors were pretreated with NTG or UV light immediately prior to being pipetted into the Wasserman tubes.

Transduction. Transduction studies were made with P35 phage. This phage was isolated from S. pullorum by zygotic induction. Each donor lysate was prepared after the phage was purified by three separate plaque isolations on the donor strain. An isolated plaque was fished into a tube of L broth containing log phase cells of the donor (1×10^6 cells/ml) and incubated overnight at 37C with aeration. The phage bacterial mixture was then centrifuged at 10,800 x g for 10 min and the supernatent stored over chloroform at 5C. Transduction was effected by mixing an overnight culture of 1×10^9 cells/ml recipient bacteria with phage at a multiplicity of infection of one. The mixture was incubated at 37C for 15 min after which 0.1 ml aliquot portions were pipetted into 3 ml of E minimal soft agar maintained at 45C and then plated on E minimal agar, supplemented where necessary. The recipient was tested for reversion, and the phage for contamination. The frequency of transduction was defined as the number of transductant colonies per absorbed or input P35 phage.

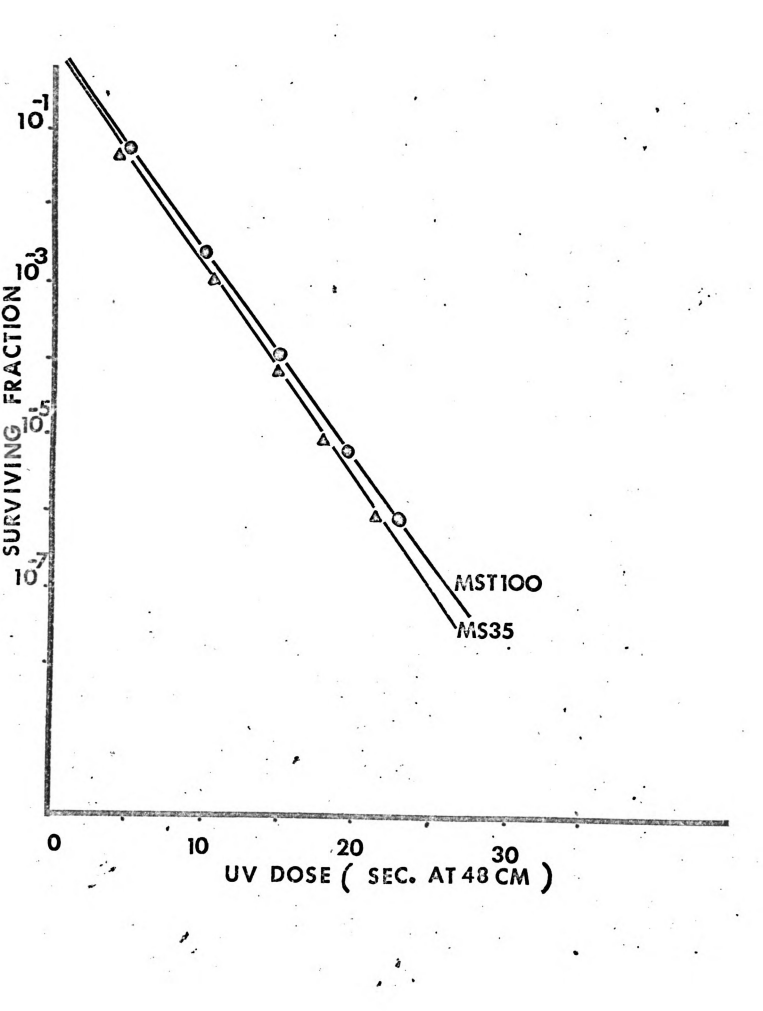
RESULTS

Part I. Feasibility of a conjugation system in Salmonella pullorum

In order for a conjugation system to be developed in a species of bacteria it is necessary that the cells possess the necessary enzymes to catalyze recombination and the ability to accept, maintain and transfer fertility factors. In addition, the discovery of a conjugation system in another genus or species is facilitated if the population is a homogenous recipient type.

Ultraviolet sensitivity. Since there exists a definite correlation between the ability of a strain to mediate genetic recombination and the ultraviolet light sensitivity of the strain, the UV sensitivity of S. pullorum MS35 was compared with that of a good recipient strain, S. typhimurium MST100. Log phase cells of each strain were resuspended in minimal A broth and irradiated with shaking at a distance of 48 cm from the lamp. From the survival curves shown in Figure 2 it appears that S. pullorum compared to S. typhimurium is not unduly sensitive to UV light. This indicates that S. pullorum possesses at least some of the enzymes required for recombination.

Figure 2. Comparative survival of S. pullorum MS35 and S. typhimurium MST100 exposed to various UV doses.


SURVIVING FRACTION
10⁻¹
10⁻³
10⁻⁵
10⁻⁷
MST100
MS35
0
10
20
30
UV DOSE (SEC. AT 48 CM)

Hybrids. A number of different S. typhimurium Hfrs were mated with S. pullorum recipients. These matings were done to determine the ability of S. pullorum recipient strains to form recombinants with S. typhimurium Hfrs and to produce recombinants that could be genetically analyzed. The data obtained from these matings are listed in Table 5. In the case of SA535 and SB172 good recombination frequencies were found with both S. typhimurium and S. pullorum recipients. The hybrids produced in these matings were stable, prototrophic and infertile. The latter conclusion was based on the insensitivity to MS2 phage and the absence of F-prime factors. The hybrids obtained from the SB172 x MS367 mating were analyzed for their inheritance of unselected donor markers and the data are presented in Table 6. There does not appear to be a high co-inheritance of any donor markers like that previously described for some recombination-less strains of E. coli (75). When the S. typhimurium Hfrs SA534, SA536 and 219 were mated with S. pullorum recipients very low recombination frequencies were obtained. A possible explanation for this might be gross gene transpositions in the lead region of the transferred DNA.

Recipient nature of S. Pullorum. To determine the recipient nature of S. pullorum approximately 100 cells of the recipient strains, E. coli, S. typhimurium and S. pullorum were spread on single L agar plates. The plates were

Table 5. Recombination frequencies between S. pullorum and S. typhimurium cultures.

Mating[a]	Marker Selected	Frequency (Recombinants per Donor Input)
SA534 x MST119	pro^+_+	7×10^{-3}
	trp^+_+	5×10^{-6}
SA534 x MS367	thr^+_+	4×10^{-7}
	pro^+	$<1 \times 10^{-8}$
SA535 x MST120	$cysA^+_+$	8×10^{-3}
SA535 x MS363	$glyA^+_+$	2×10^{-4}
SA535 x MS367	ilv^+_+	5×10^{-7}
	thr^+_+	2×10^{-7}
	pro^+_+	4×10^{-8}
	his^+	$<1 \times 10^{-8}$
SA536 x MST119	pro^+_+	6×10^{-4}
SA536 x MS367	ilv^+_+	2×10^{-8}
	thr^+_+	1×10^{-7}
	pro^+_+	2×10^{-8}
	his^+	$<1 \times 10^{-8}$
SB172 x MS367	ilv^+_+	3×10^{-2}
	thr^+_+	4×10^{-4}
	pro^+_+	3×10^{-4}
	his^+	1×10^{-7}
219 x MST120	$cysA^+_+$	1×10^{-2}
219 x MS363	$glyA^+$	2×10^{-7}
MS807 x MST119	his^+_{+b}	$<1 \times 10^{-8}$
	thr^+_+	$<1 \times 10^{-8}$
	pro^+_{+c}	$<1 \times 10^{-8}$
MS35 x MST119	thr^+	$<1 \times 10^{-8}$

[a]Procedures for these crosses are described in Materials and Methods.

[b]On each plate plaques were observed at a frequency of 4×10^{-6}/donor cell. One was selected, purified and designated P35 by W. Olsen of this laboratory.

[c]No phage located on these plates.

[d]The recombinants from each mating were found to be both infertile and MS2 insensitive.

Table 6. Inheritance of unselected markers by recombinants selected from a cross between SB172 and MS367.

Unselected Phenotype	Selected Phenotype			
	$20^{a}_{Ilv^+}$	20_{Thr^+}	20_{Pro^+}	20_{His^+}
Ilv^+	--	2/20[b]	2/20	0/20
Thr^+	0/20	--	10/20	2/20
Pro^+	0/20	8/20	--	4/20
His^+	0/20	0/20	0/20	--

[a]Number of recombinants analyzed.

[b]Number possessing unselected marker per number possessing the selected marker.

incubated 24 hr at 37C after which the developed colonies were replica plated onto plates of E minimal agar which had previously been spread with 10^9 cells of an appropriate donor strain. The replica plates were incubated at 37C for 48 hr and then observed. The results are presented in Table 7. Since 74-100% of the S. pullorum recipient cells formed recombinants with the Hfrs it may be said that S. pullorum is a uniform recipient population in its initial ability to mate with both S. typhimurium and E. coli Hfrs. Thus it is evident that the recipient ability of S. pullorum, like S. typhosa, is not confined to special cells, as has been shown with S. typhimurium (84).

Since S. pullorum is able to repair UV damage, is able to form stable and normal recombinants with S. typhimurium Hfrs, is a uniform recipient population, is transduced with P22 phage, and accepts and transfers episomes at high frequencies, it is a likely species for the establishment of a conjugation system.

Part II. Isolation and characterization of donor strains of S. pullorum

Isolation of donor strains. The male specific bacteriophage MS2 was used to detect the presence of the F factor in S. pullorum. MS2 does not form plaques on F^+ and F-prime strains of S. pullorum. However, it is absorbed at a low frequency by these strains and appears

Table 7. Recipient ability of S. pullorum for the respective recombinant genotypes.[a]

Donor	Recipient	Marker Selected	Number of Colonies Observed	Per cent of Colonies Forming Recombinants	Stability[d] of Recombinants
SB172	MS352	ilv^+	1000	100	stable
SB172	MST119	thr^+	200	100	stable
MST172[b]	MS352	ilv^+	400	<1	--
MST172	MST119	thr^+	200	<1	--
AB311	MS364	his^+	450	74	unstable
AB311	AB113	his^+	420	100	stable
MSE311[c]	MS364	his^+	200	<1	--
MSE311	AB113	his^+	200	<1	--

[a]At least 10 recombinants from each mating were tested for sensitivity to MS2 phage. None of the purified recombinants were sensitive to MS2.

[b]MST172 is an MS2 insensitive variant derived from SB172.

[c]MSE311 is an MS2 insensitive variant derived from AB311.

[d]Overnight L broth cultures of each recombinant were streaked onto L agar and incubated at 37C for 24 hr. Patches of individual colonies were made on an L agar plate, which was incubated for 24 hr. at 37C. The developed patches were then replica plated to media selective for the recombinant genotype.

to propagate despite the failure to form plaques. These results are quite similar to those described with Proteus mirabilis carrying an F-factor originating in a K-12 strain.

F-prime factors which carry Salmonella genetic material are labeled "FT" followed by a number; the "T" indicates that the particular chromosomal gene on the F factor is a Salmonella gene (95). In the case of FT80 (his^+), the histidine gene may be of an E. coli origin (personal communication with P. Hartman). From one S. abony Hfr strain (SW1444) an F-prime factor called FT59 was obtained (78). The factor carries pyrB, is readily transmissible and converts bacteria acquiring it into donors of the same type as SW1444 (O-thr-leu--pro ------purA). The FT71 factor was isolated by Sanderson (94,95) from S. typhimurium SU422 (Hfr B2). This factor carries the entire tryptophan operon and converts bacteria acquiring it into donors of the same type as Hfr B2 (O-his-str----leu----cysB). The origin and direction of chromosome mobilization of the F-prime factors used in this research are illustrated in Figure 1.

The F^+ factor was introduced into S. pullorum by mating MS35 with E. coli W6 and S. abony SH634. Log phase cultures of the donor (1 x 10^8 cells) were millipore-mated with the recipient (1 x 10^8 cells) for 30 min at 37C. The membranes were inserted into nutrient broth dispensed

in fluted test tubes and agitated with a Vortex Jr. mixer to remove the cells from the filter and to separate the mating pairs. The W6XMS35 mating mixture was plated on dry EMB-lac plates and the SH634XMS35 mating mixture plated on dry L agar plates. The plates were incubated at 37C for 24 hr. S. pullorum colonies were differentiated from E. coli W6 colonies on EMB-lac agar by their color since they do not ferment lactose. S. pullorum colonies are much smaller than SH634 colonies on L agar. S. pullorum colonies were then picked and inoculated into tubes of L broth, incubated overnight as a still culture at 37C and tested for sensitivity to MS2 phage. Tubes containing MS2 sensitive cells were then subcultured to SIM media and observed for hydrogen sulfide production and motility. These cultures were also tested for the presence of indole. The cultures appearing to be S. pullorum were then streaked on L agar and incubated 24 hr at 37C. After incubation individual clones were tested for their response to Group D antisera and their requirement for cysteine and leucine. MS800 and MS801 resulted from these efforts. In Table 8 can be seen a partial characterization of S. pullorum, S. typhimurium, S. abony and E. coli.

Isolation of S. pullorum strains carrying the F-lac$^+$ factor was carried out by mating MS35 with SB394 and AB785. In each instance log phase cultures of the donor and recipient were mated on millipore filters for

Table 8. Partial characterization of S. pullorum, S. abony, S. typhimurium and E. coli.

Strain	H_2S Production[a]	Indole Production	Motility	Response to Salmonella O antiserum Group D[b]
MS35	-	-	-	+
W6	-	+	+	-
AB785	-	+	+	-
3349	-	+	+	-
SH59	+	NU[c]	NU	-
SH634	+	NU	NU	-
SB394	+	NU	NU	-
SB890	+	NU	NU	-
SU694	+	NU	NU	-
SA534	+	NU	NU	-

[a]Procedures for the detection of H_2S production, indole production and determination of motility are described in Materials and Methods. + = positive response, - = negative response.

[b]Antisera reaction determined by slide agglutination.

[c]NU = not utilized for differentiation.

30 min and then the cells were resuspended in nutrient broth. The suspension was then plated on A minimal agar supplemented with cysteine, leucine and lactose. After incubation at 37C for 96 hr the recombinants were restreaked on the same selective medium and reincubated. The purified recombinants were then tested for H_2S production, indole production, motility, slide agglutination with group D antisera, MS2 sensitivity, F-lac$^+$ transfer and auxotrophic requirements for cysteine and leucine. The outcome of this work was MS802 and MS803.

The F-his$^+$ factor was introduced into S. pullorum by mating MS37 with 3349 , SB890. Log phase cultures of donor and recipient were mated on millipore filters for 30 min and then plated on E minimal agar supplemented with cysteine and leucine. The recombinant cells were purified and analyzed in a manner analogous to that previously described for strains MS802 and MS803. MS804 and MS805 were isolated and characterized by this manipulation.

A mating between SH59 and MS35 was employed to introduce the FT59 (pyrB$^+$) factor into S. pullorum. The mating procedure, isolation and purification procedures are essentially the same as those previously described for isolation of strain MS801. From this mating, strain MS806 was isolated.

Donor strain MS807 carrying the FT71 (*trp*$^+$) factor was isolated after a 60 min millipore mating between SU694 and MS36. MS807 was purified in a manner analogous to MS805. One hundred purified recombinants were tested for their sensitivity to MS2 phage and their ability to transfer the FT71 factor. Approximately 90% were sensitive to MS2 phage but only one of the recombinants was able to transfer the FT71 factor as indicated by the cross-streaking method.

The FT76 and FT77 factors were introduced into *S. pullorum* by mating SA523 and SA532 respectively with MS82 on millipore filters. After 30 min incubation at 37C the mating mixture was plated on E minimal agar supplemented with glycine and leucine. The resultant donor strains, MS808 and MS809, were analyzed and purified by techniques previously described.

Gene transfer. In order to determine the ability of donor strains to transfer genes to appropriate recipient strains, overnight aerated L broth cultures of the donor and recipient strains were diluted 1:20, incubated 4 hr at 37C without aeration and 1×10^9 donors were mixed with 1×10^9 recipients and mated for 3 hr on a millipore filter. The results of these matings are shown in Table 9. It is obvious that detectable chromosome mobilization occurred only with F-prime strains carrying Salmonella genes and

Table 9. Gene transfer.

Cross	Selected Recombinants	Frequency (Per Initial Donor Cell)	Cross	Selected Recombinants	Frequency (Per Initial Donor Cell)
MS800 x MS369	pro^+	$<1 \times 10^{-8}$	MS803 x MS369	pro^+	$<1 \times 10^{-8}$
	ilv^+	$<1 \times 10^{-8}$		ilv^+	$<1 \times 10^{-8}$
	thr^+	$<1 \times 10^{-8}$		thr^+	$<1 \times 10^{-8}$
	his^+	$<1 \times 10^{-8}$		his^+	$<1 \times 10^{-8}$
	gal^+	$<1 \times 10^{-8}$		gal^+	$<1 \times 10^{-8}$
MS800 x MS363	$glyA^+$	$<1 \times 10^{-8}$	MS803 x MS363	$glyA^+$	$<1 \times 10^{-8}$
MS800 x MS354	met^+	$<1 \times 10^{-8}$	MS803 x MS354	met^+	$<1 \times 10^{-8}$
MS800 x MS357	$thyA^+$	$<1 \times 10^{-8}$	MS803 x MS357	$thyA^+$	$<1 \times 10^{-8}$
MS800 x MS355	trp^+	$<1 \times 10^{-8}$	MS803 x MS355	trp^+	$<1 \times 10^{-8}$
MS801 x MS369	pro^+	$<1 \times 10^{-8}$	MS804 x MS369	his^+	1.5×10^{-1}
	ilv^+	$<1 \times 10^{-8}$		pro^+	$<1 \times 10^{-8}$
	thr^+	$<1 \times 10^{-8}$		ilv^+	$<1 \times 10^{-8}$
	his^+	$<1 \times 10^{-8}$		thr^+	$<1 \times 10^{-8}$
	gal^+	$<1 \times 10^{-8}$		gal^+	$<1 \times 10^{-8}$
MS801 x MS363	$glyA^+$	$<1 \times 10^{-8}$	MS804 x MS355	trp^+	$<1 \times 10^{-8}$
MS801 x MS354	met^+	$<1 \times 10^{-8}$	MS804 x MS363	$glyA^+$	$<1 \times 10^{-8}$
MS801 x MS357	$thyA^+$	$<1 \times 10^{-8}$	MS804 x MS354	met^+	$<1 \times 10^{-8}$
MS801 x MS355	trp^+	$<1 \times 10^{-8}$	MS804 x MS357	$thyA^+$	$<1 \times 10^{-8}$
MS802 x MS369	lac^+	2.5×10^{-1}	MS805 x MS364	his^+	1.4×10^{-1}
	pro^+	$<1 \times 10^{-8}$	MS805 x MS355	trp^+	$<1 \times 10^{-8}$
	ilv^+	$<1 \times 10^{-8}$	MS805 x MS363	$glyA^+$	1×10^{-7}
	thr^+	$<1 \times 10^{-8}$			
	his^+	$<1 \times 10^{-8}$	MS806 x MS359	thr^+	1.5×10^{-6}
	gal^+	$<1 \times 10^{-8}$	MS806 x MS358	pro^+	$<1 \times 10^{-8}$
			MS806 x MS362	gal^+	$<1 \times 10^{-8}$
MS802 x MS363	$glyA^+$	$<1 \times 10^{-8}$			
MS802 x MS354	met^+	$<1 \times 10^{-8}$	MS807 x MS364	his^+	5.8×10^{-7}
MS802 x MS357	$thyA^+$	$<1 \times 10^{-8}$			
MS802 x MS355	trp^+	$<1 \times 10^{-8}$	MS809 x MS369	ilv^+	3.2×10^{-6}
				pro^+	1.9×10^{-6}
				gal^+	2.4×10^{-7}

Procedures for these crosses are described in Materials and Methods. These data represent the results of several experiments.

at a frequency of only one recombinant per 10^6 - 10^7 donor cell input. However, the F factors themselves were transferred at frequencies from 0.14 to 0.40 per donor cell input.

UV stimulation of gene transfer. Log phase S. pullorum donor strains MS800, MS801, MS802 and MS803 were resuspended in A minimal broth to a concentration of 1 x 10^8 cells per ml and exposed to UV light at a dosage which produced 50-75% reduction in colony forming ability. These irradiated cells were resuspended in L broth, incubated 1-3 hr at 37C and then millipore-mated with MS369 and plated on media selective for Pro^+, Ilv^+, His^+, Thr^+ and Gal^+ recombinants. No recombinants were detected.

Stability of F factors in S. pullorum. One possible explanation for the low fertility of the S. pullorum donor strains might be the spontaneous curing of the F factor in these strains. To test this possibility, overnight cultures of each donor were streaked on L agar and incubated 24 hr at 37C. Individual colonies were selected and tested either for their sensitivity to MS2 phage or the ability to transfer the F-prime factor to an appropriate recipient. The results are presented in Table 10. It appears that the episomes are very stable in S. pullorum and thus spontaneous curing of the F-prime factors does not account for infertility of the donor strains.

Table 10. Stability of episomes in S. pullorum.

Strain	Episome	Number of Colonies Observed	Per Cent Sensitive to MS2 Phage	F-prime Transfer (Per Cent of Initial Donor Cells)
MS800	F^+	50	100	NT[a]
MS800[b]	F^+	160	3	NT
MS801	F^+	20	100	NT
MS802	F-lac^+	20	NT	100
MS802[c]	F-lac^+	85	NT	22
MS803	$F_{ts}lac^+$	60	NT	90
MS804	F-his^+	20	NT	100
MS805	FT80his^+	20	NT	100
MS806	FT59$pyrB^+$	20	100	NT
MS807[d]	FT71trp^+	40	NT	100
MS807	FT71trp^+	20	NT	100
MS807[e]	FT71trp^+	50	NT	36
MS808	FT77$cysE^+$	50	NT	38

[a]Not tested.

[b]Tested after 9 months storage at room temperature.

[c]Tested after 6 months storage at room temperature.

[d]Tested after 1 month storage at 5C.

[e]Tested after 6 months storage at room temperature.

Episome transfer. Another possible explanation for the low level of chromosome transfer may be that the donor strains cannot transfer DNA efficiently. The data from Table 9 demonstrates that F-prime factors are transferred at frequencies ranging from 10-25%, but the mating periods employed were 3 hr which is extremely long. The matings therefore were repeated with 5×10^7 donor cells mixed with 5×10^8 recipient cells and mated on millipore filters for 30 min at 37C. The results reported in Table 11 show that S. pullorum can transfer and receive F-prime factors at good frequencies.

Part III. Enrichment of Donor Strains for Increased Fertility

Temperature sensitive episome. One method to increase the fertility of a donor strain would be to culture them under conditions favoring integration of the fertility factor into host cell chromosome. S. pullorum strain MS803 carries the F_{ts}lac$^+$ factor. The autonomous replication of the mutated sex factor is normal at 37C but inhibited at 42C. This was demonstrated by the fact that 99.99% of the overnight aerated L broth cultures of MS803 incubated at 42C were cured of the F_{ts}lac$^+$ factor; whereas, only 10-20% of the overnight cultures grown at 37C were cured of the F_{ts}lac$^+$ factor. I made the hypothesis that MS803 should be enriched for these cells possessing the integrated sex

Table 11. Episome transfer in S. pullorum.

Cross	Episome Transferred	Frequency (Per Cent of Initial Donor)
MS802 x MS350	F-lac$^+$	35
MS803 x MS364	F-his$^+$	29
MS805 x MS364	FT80(his$^+$)	3[a]
MS807 x MS355	FT71(trp$^+$)	2[a]
MS809 x MS81	FT77(cysE$^+$ pyrE$^+$ rfa$^+$)	11[a]
MS806 x MS350	FT59(pyrB$^+$)	39[a]
AB785 x MS35	F-lac$^+$	22

[a]MS2 sensitivity used to detect presence of F-factor in recipient.

factor after several subcultures in L broth each followed by a subculture to A minimal lactose broth. Thus, 10^3-10^4 cells MS803 were inoculated into L broth and grown overnight at 42C with aeration. A 0.1 ml aliquot of the overnight culture containing 1 x 10^7 cells/ml was then inoculated into 10 ml of A minimal broth supplemented with 0.2% lactose and Casamino Acids respectively. This culture was then incubated at 42C with aeration until it reached a titer of 10^9 cells per ml at which time it was recycled through the subculture sequence. Seventy to ninety per cent of the cells plated at the end of each subculture cycle produced lactose positive colonies. The subculture procedure was repeated several times. This culture of strain MS803 enriched for fertility was then millipore-mated with MS369 and selection made for Thr^+, Pro^+, Ilv^+, Gal^+ and His^+ recombinants. In no instance were recombinants detected, but the individual MS803 colonies still remained temperature sensitive. No significant curing of F-*lac*$^+$ factors, either F-*lac*$^+$ or F_{ts}*Lac*$^+$ was obtained during the course of this research, when either Acridine Orange or Acriflavin were used to cure the cells of the F-prime factors.

Fluctuation method for the isolation of donor strains. A modified Luria-Delbrück fluctuation method (77) was employed to enrich for strains transferring chromosomal genes. In each instance an overnight L broth culture of the donor

was diluted to a concentration of 200 cells per ml and 1 ml aliquots were pipetted into 100-200 Wasserman Tubes. These tubes were incubated at 37C until there were 1 x 10^8 cells per ml. A loopful was taken from each tube and streaked across a suitable recipient on a properly supplemented E minimal agar plate. The plates were incubated 72 hr at 37C and the tubes were refrigerated at 5C for the same period. The tube showing the most recombinants was selected and 10^3-10^4cells from this tube were inoculated each into 100-200 tubes. Each tubes was in turn incubated at 37C until the average cell concentration in the tubes was approximately 1 x 10^8 cells per ml; the cells were then mated with an appropriate recipient as described previously. Upon completion of the enrichment cycles the enriched strains were millipore-mated for 3 hr with the appropriate recipient strain. An overnight culture of a non-enriched donor strain was also mated with the same recipient as a control. From the data in Table 12 it is evident that there is little if any enrichment of fertility in the donor strains. A chi square test was done on the data of the MS807 x MS354 mating described in Table 12 to determine if the results significantly differed from that expected due to randomness. A χ^2 value of 365 was obtained with the probability of this χ^2 being less than 0.005, therefore indicating non-random fluctuation. This result is not simply explained by the instability of the autonomous

Table 12. Attempt to enrich donor strains.

Donor	Recipient	Marker Selected	Number of Tubes/ Enrichment Cycle	Number of Enrichment Cyles	Total Enrichment[a]
MS800	MS364	his^+	200	2	0[b]
MS800	MS358	pro^+	200	1	0
MS800	MS359	thr^+	200	1	0
MS800[c](NTG)	MS359	thr^+	200	2	0
MS804	MS355	trp^+	100	4	0
MS804	MS363	$glyA^+$	100	2	0
MS804	MS359	thr^+	100	2	0
MS804	MS351	tyr^+	100	2	0
MS806	MS359	thr^+	100	3	1.5
MS806(NTG)	MS359	thr^+	100	2	1.1
MS807[d]	MS354	met^+	200	2	1.1
MS807(NTG)	MS354	met^+	200	2	1.2

[a]Total enrichment defined as the $\frac{\text{Recombination frequency of the enriched culture}}{\text{Recombination frequency of unriched culture}}$

[b]No recombinants were observed.

[c]Pretreatment of culture with NTG.

[d]Chi square with $P < 0.005$. ($\chi^2 = 365$)

and integrated nature of the F-prime factor but rather indicates a more complex association of the chromosome and episome.

Replica-plating. Several attempts were made to select S. pullorum Hfrs by exposure of the cells to ultraviolet light and then replica plating the developed irradiated colonies onto a lawn seeded with appropriate recipients. In no instance were either Hfrs isolated or F-prime strains with increased fertility detected.

Part IV. Mapping Studies

Prolonged matings. In order to produce a linkage map, 5×10^8 donor cells of S. pullorum and 5×10^8 recipient cells were mixed and mated on millipore filters for 3 hr at 37C. The cells were then resuspended in nutrient broth and aliquots of the appropriate dilution plated on properly supplemented minimal media. The data are presented in Table 13. With the exception of MS807, the S. pullorum donor strains transfer chromosomal genes at a very low frequency. From the gradient of transfer observed with the MS807 x MS369 mating a preliminary linkage map was constructed as follows:

gal-1	pro-1	ilv-1	thr-1	his-1
1.0	.42	.11	4.1×10^{-3}	3.1×10^{-4}

Table 13. Fertility of F-prime donors in crosses with different recipient strains of Salmonella pullorum.

Cross	Counter-Selection	Selected Recombinants	Recombination Frequency* (Per Initial Donor Cell)	Relative Frequency
MS805xMS369	cysA cysJ strA	thr^+	6×10^{-8}	--
		pro^+	$<2 \times 10^{-8}$	--
		ilv^+	$<2 \times 10^{-8}$	--
		gal^+	$<2 \times 10^{-7}$	--
MS805xMS363	cysA cysJ strA	gly^+A	1×10^{-8}	--
MS805xMS355	cysA cysJ strA	trp^+	$<2 \times 10^{-8}$	--
MS806xMS369	cysA cysJ	thr^+	1.5×10^{-6}	1
		his^+	1.8×10^{-7}	0.12
		pro^+	$<2 \times 10^{-8}$	--
		ilv^+	$<2 \times 10^{-8}$	--
		gal^+	$<2 \times 10^{-8}$	--
MS806xMS363	cysA cysJ strA	gly^+A	3×10^{-7}	--
MS807xMS369	cysA cysJ strA	gal^+	3.6×10^{-3}	1
		pro^+	1.5×10^{-3}	0.42_{-2}
		ilv^+	2.1×10^{-4}	5.8×10^{-3}
		thr^+	7.8×10^{-5}	2.2×10^{-4}
		his^+	5.8×10^{-6}	1.6×10
MS807xMS363	cysA cysJ strA	gly^+A	1.8×10^{-6}	--
MS807xMS354	cysA cysJ strA	met^+	5.5×10^{-6}	--
MS809xMS369	glyA strA	ilv^+	3.2×10^{-6}	1
		pro^+	1.9×10^{-6}	0.59
		gal^+	2.4×10^{-7}	0.08
		his^+	2.0×10^{-7}	0.06
		thr^+	1.4×10^{-7}	0.04
MS808xMS369	glyA strA	pro^+	5.2×10^{-7}	1
		ilv^+	2.8×10^{-7}	.54
		gal^+	2×10^{-8}	.04
		his^+	1.5×10^{-7}	.29
		thr^+	1.9×10^{-7}	.37

The selective media were supplemented with leucine and all the growth factors of the particular recipient strain except that one for which selection for independence was being made. The known markers carried on the F-prime factor were added as additional supplements to the media with the exception of the MS806 x MS369 mating.

*The recombination frequency is based on a mean of at least five plates. Each mating was repeated at least three times with no significant deviation. The donor and recipient cultures were also checked for reversion.

When this map is compared with the linkage map of S. typhimurium Figure 1, it is evident that the threonine locus appears to be transposed. It is also apparent that in S. pullorum MS807 the chromsome is being mobilized in an opposite direction in comparison to S. typhimurium strains possessing the same FT71 factor. SU694 was mated with MST119 and the data are presented in Table 14. These data reconfirm the fact that in S. typhimurium the direction of transfer by FT71 is 0-his--thr--pro-----cysB. The gradient of transfer for this mating and for the other matings is shown in Table 15.

Since co-inheritance of donor auxotrophic markers might alter the gradient of transfer, the following experiments were performed:

1. MS807 was mated with MS368 with sodium azide solely used for counterselection.
2. MS810 was mated with MS374 with just the absence of histidine used for counterselection. MS807 was mated with MS369 with just the absence of cysteine used for counterselection.

In all instances the gradient of transfer was found to be 0-pro-ilv-thr-his.

The FT77 and FT76 factors in S. typhimurium mobilize the chromosome in the same order, 0-ilv----thr--pro--, as Hfr KI. In the MS809 x MS369 mating the order appears to be similar except that the threonine locus seems to be transposed. This supports the gene order previously described for S. pullorum. The recombination frequencies

Table 14. Chromosome mobilization of S. typhimurium strain possessing the FT71 (trp^+) factor.

Cross	Counter Selection[a]	Selected Recombinants	Recombination Frequency (Per Initial Donor Cell)	Relative Frequency
SU694 x MST119	ilv	his^+	2.0×10^{-2}	1
		thr^+	4.0×10^{-6}	2×10^{-4}
		pro^+	1.6×10^{-5}	8×10^{-5}

[a]The selective media were supplemented with tryptophan and all the growth factors of the particular recipient strain except that one for which selection for independence was being made.

Table 15. Gradient of transfer.

1. S. pullorum cysA1 cysJ1 leu-1 trp-2/FT71(trp$^+$) x S. pullorum strA1 his-1 pro-1 thr-1 ilv-1 gal-1
(MS807 x MS369)

gal-1	pro-1	ilv-1	thr-1	his-1	met-1	glyA1
1.0	0.42	0.14	4.1×10^{-3}	3.1×10^{-4}	1.5×10^{-3}	5.0×10^{-4}
	1.0	0.14	5.2×10^{-3}	3.9×10^{-4}		
		1.0	3.7×10^{-3}	2.8×10^{-3}		
			1.0	7.4×10^{-2}	0.7	0.2

2. S. typhimurium trpA52 cysB12 pyrF146 ilv-178/FT71(trp$^+$) x S. typhimurium his-1 thr-1 pro-1
(SU695 x MST119)

his-1	thr-1	pro-1
1.0	2.0×10^{-4}	8×10^{-5}
	1.0	0.4

3. S. pullorum cysE1 glyA3 leu-1/FT77(cysE$^+$ pyrE$^+$ rfa$^+$) x S. pullorum strA1 his-1 pro-1 thr-1 ilv-1 gal-1
(MS809 x MS369)

ilv-1	pro-1	gal-1	his-1	thr-1
1.0	0.6	8×10^{-2}	6×10^{-2}	4×10^{-2}
	1.0	0.15	0.11	7.4×10^{-2}
		1.0	.83	.58
			1.0	0.70

4. S. pullorum cysE1 glyA3 leu-1/FT76(xyl$^+$ cysE$^+$ rfa$^+$) x S. pullorum strA1 his-1 pro-1 thr-1 ilv-1 gal-1
(MS808 x MS369)

pro-1	ilv-1	thr-1	his-1	gal-1
1.0	0.54	0.37	0.29	0.04
	1.0	0.68	0.54	0.07
		1.0	0.79	0.11
			1.0	0.13

Table 15 (Continued)

4.	*S. pullorum* cysA1 cysJ1 leu-1/FT59(pyrB$^+$) x *S. pullorum* strA1 his-1 pro-1 thr-1 ilv-1 gal-1	
	(MS806 x MS369)	
	(MS806 x MS363)	
	thr-1	his-1
	1.0	0.12
	glyA1	
	0.2	
5.	*S. pullorum* cysA1 cysJ1 leu-1 his-3/FT80(his$^+$) x *S. pullorum* strA1 his-1 pro-1 thr-1 ilv-1 gal-1	
	(MS805 x MS369)	
	(MS805 x MS363)	
	glyA1	
	1.0	
	thr-1	
	0.6	

obtained in this mating are only 10^{-6} to 10^{-7} for F-prime mediated chromosome transfer and still lower, 10^{-7} to 10^{-8} for the MS808 x MS369 mating.

From the MS805 x MS369 mating the only recombinants obtained were for the threonine marker. If the FT80 factor carrying the his marker does mobilize predominantly from the histidine locus this would be good evidence for the close proximity of the threonine and histidine loci. Recombinants were also formed for the glyA marker in the MS805 x MS363 mating. It is difficult to correlate these results with the previously described gene sequence because of the low recombination frequencies involved, the fact that these genes (glyA1 and thr-1) are not in the same recipient and the fact that the origin of chromosome transfer mediated by the FT80 factor is not known.

In the MS806 x MS369 mating recombinants were obtained for only the thr-1 and his-1 markers. This supports the original assumption that threonine is transposed in S. pullorum. In the MS806 x MS363 mating recombinants at a relative frequency of 0.2 were obtained for the glyA1 marker.

When single auxotrophic strains carrying pro-2, ilv-2, thr-1, or his-1 markers are mated with the donor strains of S. pullorum, the same recombination frequencies were found as with the multiple auxotroph carrying all the mutated genes.

Linkage analysis. To further study the gene order and to learn more about F-prime mediated chromosome transfer in S. pullorum, recombinants were analyzed for their linkage to unselected markers. In each instance the cells were mated for 3 hr on millipore filters prior to being plated on selective media. The recombinants were recloned on the same selective medium and then replica-plated to determine the relevant genotype of each recombinant.

The data given in Table 16 confirm the gene order previously described from the prolonged matings of MS807 x MS369. To exclude the possibility of donor auxotrophic markers reducing the linkage between loci, a terminal marker, his-4, was used solely for counter-selection. The results of this mating are given in part A of Table 16. The linkage appears to be low, especially the thr-1 to gal-1 linkage of 21%, but even this percentage of linkage agrees with the results reported in S. typhimurium Hfr x S. typhimurium F^- matings where linkage of proximal unselected genes, other than those located close to the selected locus, is 29-40%. In E. coli, however, markers nearer the origin than the selected marker are usually 50% linked to the selected marker (95). In part B of Table 16 it is shown that his-1 is essentially unlinked to the other markers, but this probably is the result of coinheritance of cysteine auxotrophy or streptomycin sensitivity from the donor.

Table 16. Occurrence of unselected donor markers in recombinants from crosses between donor strains of S. typhimurium and S. pullorum possessing FT71trp+ and multiple auxotrophic recipient strains of S. pullorum.

A. Mating: MS810 x MS374
Counterselection: Histidine auxotrophy

Unselected Phenotype	Selected Phenotype			
	182[a] Gal+	207 Pro+	198 Ilv+	146 Thr+
Gal+	--	54[b]	29	21
Pro+	25	--	34	26
Ilv+	3	8	--	33
Thr+	<1	1.4	3	--

B. Mating: MS807 x MS369
Counterselection: Cysteine auxotrophy and streptomycin sensitivity

Unselected Phenotype	Selected Phenotype	
	76 Thr+	185 His+
Gal+	19	5.0
Pro+	28	5.5
Ilv+	33	4.9
Thr+	--	10
His+	6.6	--

[a]The number of recombinants analyzed

[b]The results are given as per cent.

The data from the MS806 x MS369 mating presented in Table 17 further indicate a linkage only of thr-1 to his-1. The linkage once again appears to be very low and also is probably the result of coinheritance of cysteine auxotrophy or streptomycin sensitivity from the donor. This mating was repeated several times with the same results.

The linkage data from the MS809 x MS369 mating are presented in Table 18. A recombination frequency of 1×10^{-7} for the threonine marker prevented a more thorough examination of its linkage to other markers. It is evident that the ilv-1 and pro-1 markers are essentially unlinked. Out of 717 recombinants analyzed only eight or 1.1% had acquired the cysE auxotrophic marker of the donor and all eight were isolated with the selected marker Ilv^+. The possibility that the $cysE^-$ genotype was being masked by the coinheritance of the FT77 factor was eliminated by the fact that only two cysteine positive recombinants out of 78 or 2.6% possessed the FT77 factor; as indicated by the cross-streaking method.

The low linkage of the His^+ recombinants to the unselected markers could be the result of coinheritance of the glyA2 marker and/or streptomycin sensitivity from the donor or the possibility that there are two or more linkage groups in S. pullorum. Since only 55 His^+ recombinants were observed it is impossible to differentiate between the various possibilities.

Table 17. Occurrence of unselected donor markers in recombinants from the cross MS806 x MS369.[a]

Unselected Phenotype	Selected Phenotype	
	132^{b} Thr^{+}	52 His^{+}
Thr^{+}	--	12
His^{+}	5[c]	--
Ilv^{+}	<1	<1
Pro^{+}	<1	<1
Gal^{+}	<1	<1

[a]Cysteine auxotrophy and streptomycin sensitivity were used for counterselection.

[b]Number of recombinants analyzed.

[c]Numbers given in per cent.

Table 18. Occurrence of unselected donor markers in recombinants from the cross MS809 x MS369.[a]

Unselected Phenotype	Selected Phenotype				
	326[b] Ilv^+	240 Pro^+	83 Gal^+	55 His^+	13 Thr^+
Ilv^+	--	2.9[c]	<1	<1	0/13[d]
Pro^+	1.5	--	58	1.8	0/13
Gal^+	<1	22	--	1.8	0/13
His^+	<1	1.3	1.2	--	0/13
Thr^+	3.7	<1	<1	5	--
Cys^-	2.5	<1	<1	<1	0/13

[a]Glycine auxotrophy and streptomycin used for counterselection.

[b]The number of recombinants analyzed.

[c]Results given in per cent with the exception of threonine selection.

[d]Ratio of threonine recombinants prototrophic for the unselected marker per total number analyzed.

The data from the MS808 x MS369 mating is presented in Table 19. The low recombination frequencies resulted in a relatively small number of markers being analyzed. These data also confirm the fact that ilv-1 and pro-1 are essentially unlinked.

Kinetic studies. Kinetic studies were done to demonstrate that the results of the gradient of transfer were in fact due to increased distances between markers and the origin and not due to some artifact of F-prime directed chromosome mobilization. The entry times for chromosomal markers and the F-factors themselves were studied by interrupted mating. Chromosomal markers were analyzed from millipore filter matings and episomal markers were analyzed from centrifuge matings.

Figure 3 shows the time of entry of gal-1, pro-1, ilv-1, thr-1 and his-1 markers from MS807 x MS369 mating. The selective medium was supplemented with leucine and tryptophan. Cysteine auxotrophy and streptomycin sensitivity were used for counterselection. This mating has been repeated four times and the same time intervals, ±1 min, were obtained in each instance. The data in Figure 3 confirm that the gene sequence is gal-1, pro-1, ilv-1, thr-1, and his-1.

Figure 4 shows the time of entry of the episomal trp^+ gene in a MS807 x MS355 mating and the time of entry of the

Table 19. Occurrence of unselected donor markers in recombinants from the cross MS808 x MS369.[a]

Unselected Phenotype	Selected Phenotype				
	20[b] Ilv^+	20 Pro^+	4 Gal^+	20 His^+	20 Thr^+
Ilv^+	--	0/20[c]	0/4	2/20	0/20
Pro^+	0/20	--	3/4	2/20	0/20
Gal^+	0/20	9/20	--	2/20	0/20
His^+	0/20	1/20	1/4	--	1/20
Thr^+	0/20	0/20	0/4	1/20	--

[a]Glycine auxotrophy and streptomycin sensitivity were used for counterselection.

[b]The number of recombinants analyzed.

[c]Ratio of recombinants prototrophic for the unselected marker per total number analyzed.

Figure 3. Time of entry of various markers from MS807 x MS369 mating. MS807 was mated with MS369 on millipore filters and transfer was interrupted at various times. A 0.1 ml of the mating suspension (3×10^7 donor cells) was plated at each time interval on media selective for Gal^+, Pro^+, Ilv^+, Thr^+, and His recombinants. Each count is the mean of five plates. The selective media were supplemented with leucine and tryptophan. Cysteine auxotrophy and streptomycin sensitivity were used for counterselection.

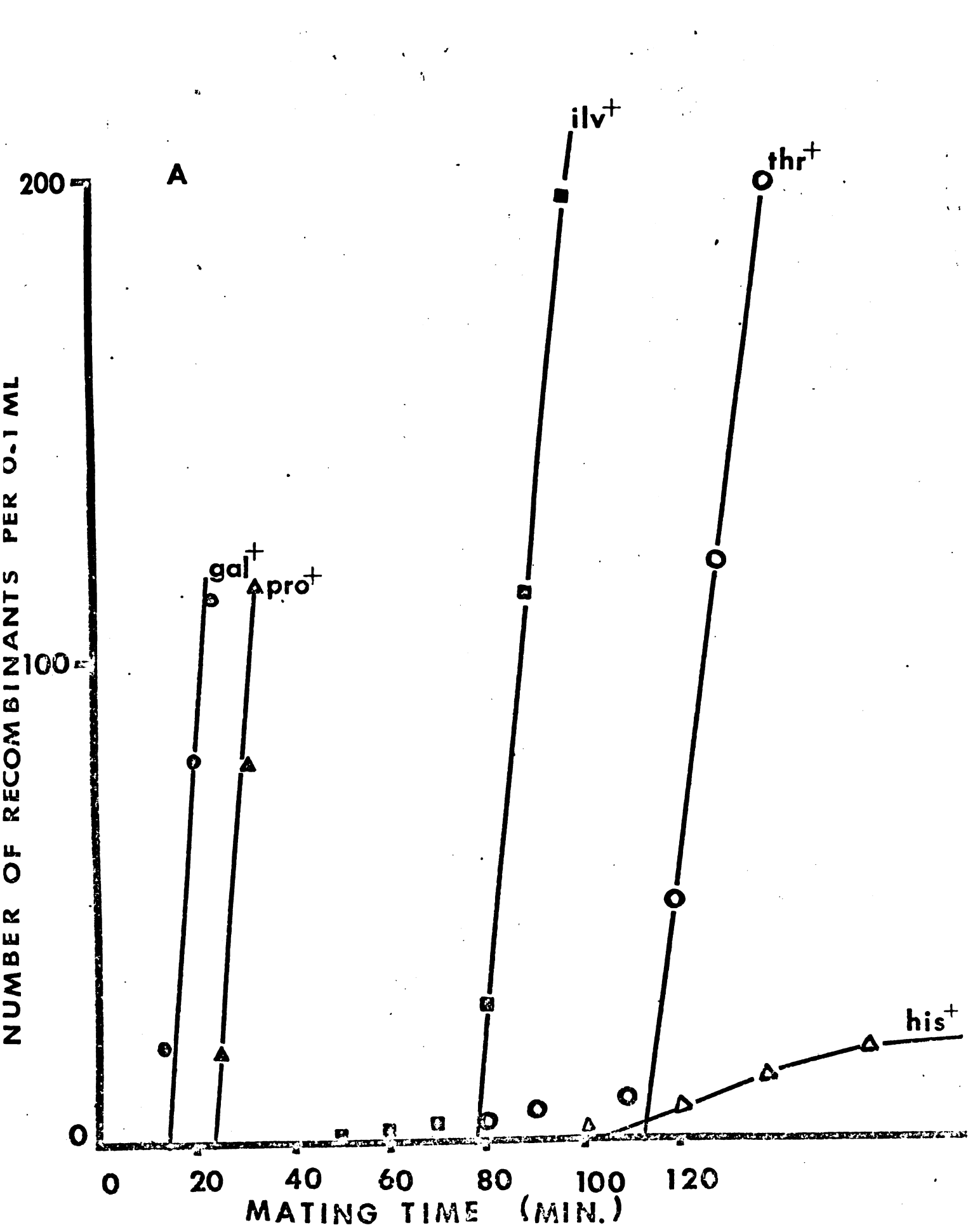
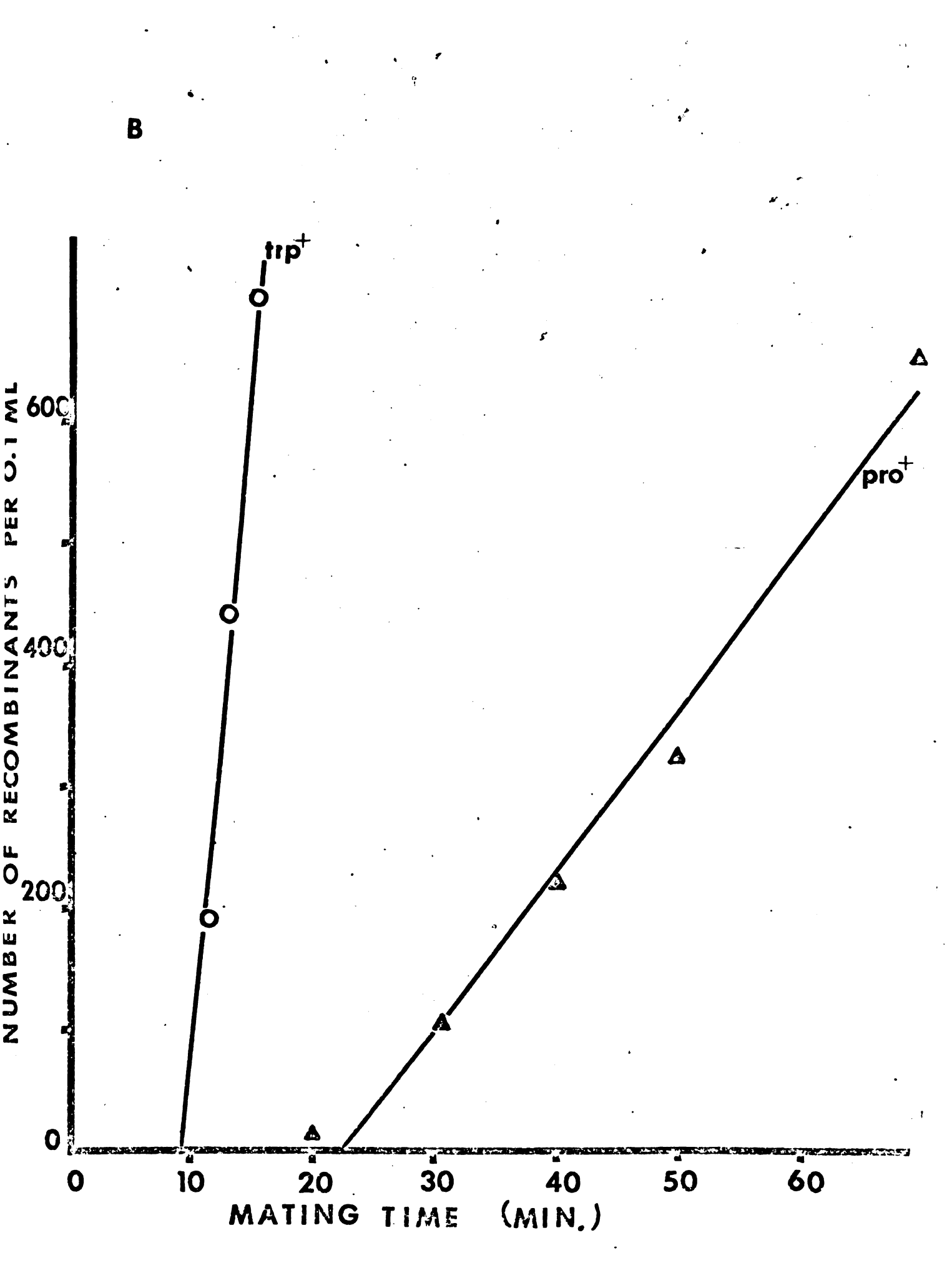

A
200
100
0
NUMBER OF RECOMBINANTS PER 0.1 ML
gal+
pro+
ilv+
thr+
his+
0
20
40
60
80
100
120
MATING TIME (MIN.)

Fig3.

Figure 4. Time of entry of the episomal *trp*$^+$ gene. MS807 was mated with MS355 and transfer was interrupted at various times. Selection was made for Trp$^+$. MS807 was also mated with MS369, transfer interrupted at various times and selection made for pro$^+$. In both instances the cells were centrifuge mated. Cysteine auxotrophy and streptomycin sensitivity were used for counterselection.


B
trp+
pro+
NUMBER OF RECOMBINANTS PER 0.1 ML
600
400
200
0
0
10
20
30
40
50
60
MATING TIME (MIN.)

chromosomal pro$^+$ gene in a MS807 x MS369 mating. Trp$^+$ was transferred at 9 min and pro$^+$ at 23 min. The transfer time of the pro$^+$ gene correlates well with the results above from a millipore mating. For S. typhimurium it has been reported that the episomal gene trp$^+$ is transferred at 7 min and the chromosomal gene his$^+$ at 15 min (94).

MS809 was mated with MS369 and the times of entry of the following markers ilv-1, pro-1, gal-1, thr-1 and his-1 determined. The times of entry are shown in Figure 5. This confirms the gene order described previously. The time interval between pro-1 and ilv-1 is only 27 min, while in the MS807 x MS369 mating (Figure 3) the time interval was 54 min. This anomaly will be taken up in the discussion. The time of entry of the episomal gene cysE$^+$ is shown to be 7 min in Figure 6.

Part V. Transduction

Transduction experiments were undertaken to determine the fine structure homologies of selected genes in S. pullorum and S. typhimurium. The genes chosen were those carried by the F-prime factors.

The phage employed in the transduction experiments was P35. This phage was isolated from the MS807 x MST119 mating previously described in Table 5. Since no phage appeared in the MS35 x MST119 mating also shown in Table 5,

Figure 5. Time of entry of various markers with a MS809 x MS369 mating. All conditions were the same as described under Figure 3 except the selective media were supplemented with cysteine and leucine. Glycine auxotrophy and streptomycin were used for counterselection.

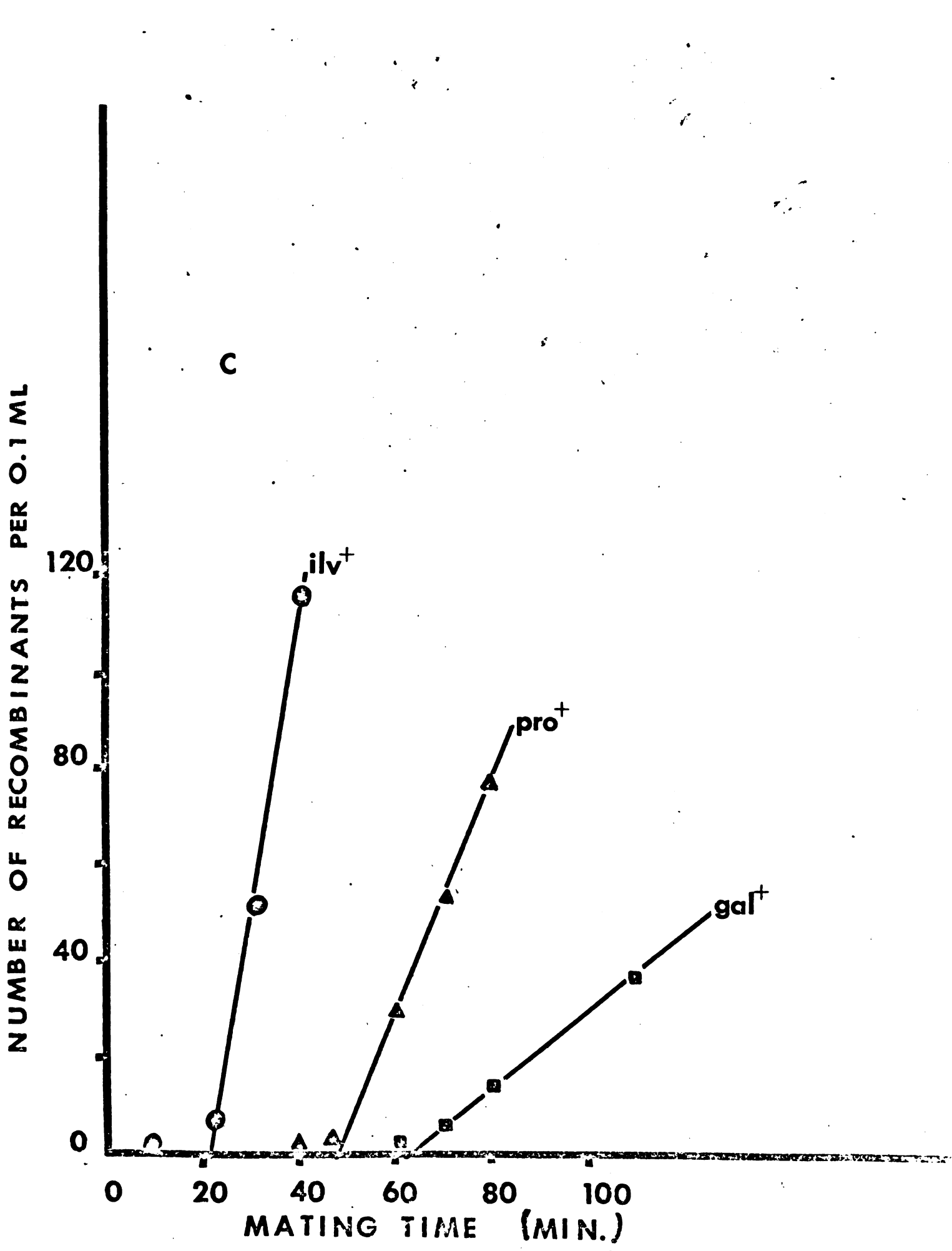

C
NUMBER OF RECOMBINANTS PER 0.1 ML
120
80
40
0
ilv+
pro+
gal+
0
20
40
60
80
100
MATING TIME (MIN.)

Figure 6. Time of entry of the episomal *cysE*$^{+}$ gene. MS809 was mated with MS90 and transfer interrupted at various times. Selection was made for cysteine prototrophy and counterselection was made with glycine auxotrophy and streptomycin sensitivity.

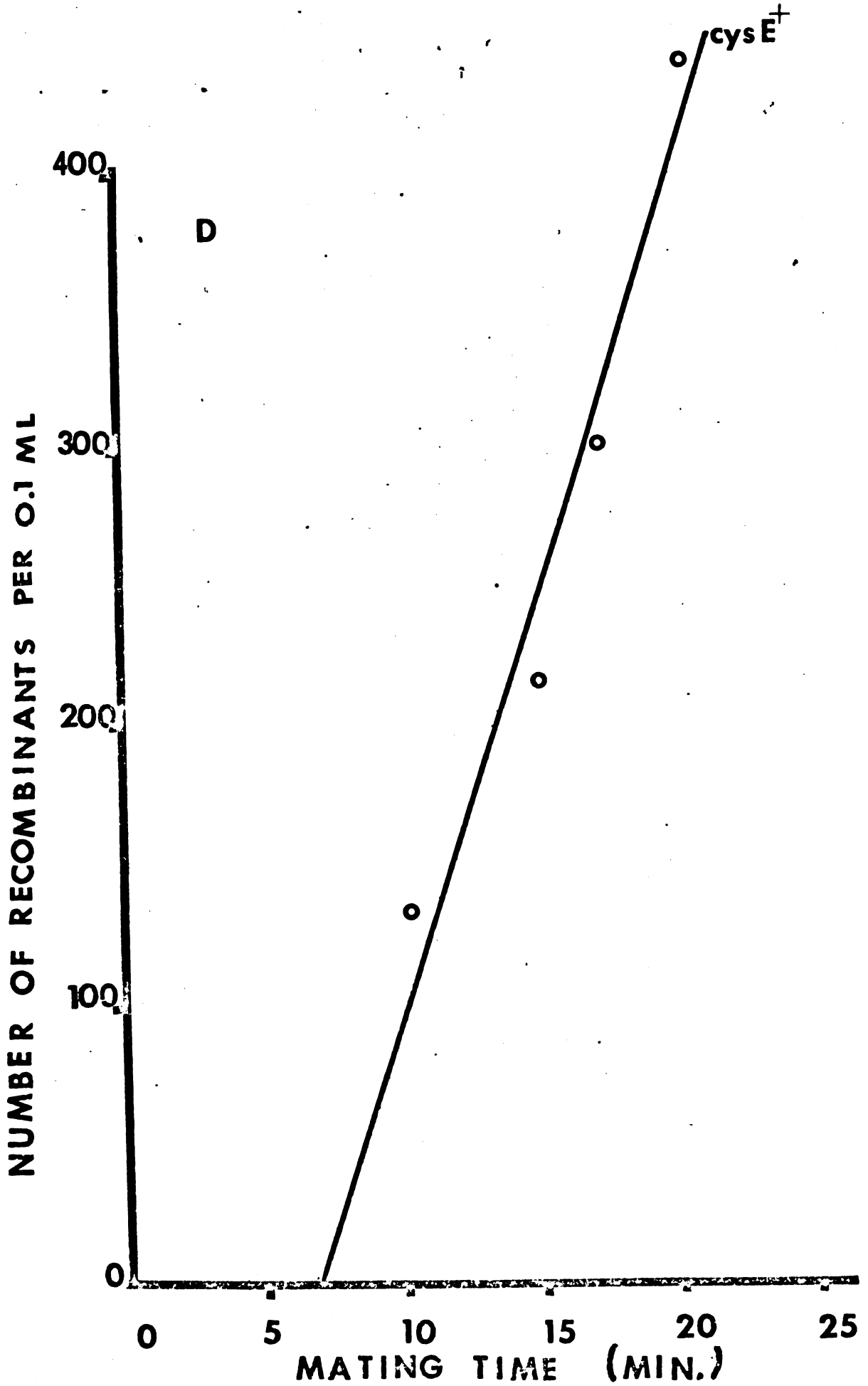

cys E+
400
D
300
200
100
0
NUMBER OF RECOMBINANTS PER 0.1 ML
0
5
10
15
20
25
MATING TIME (MIN.)

Table 20. Summary of time of entry experiments.

A.				
S. pullorum cysA1 cysJ1 leu-1 trp-2/FT71(*trp*$^+$)	MS807	Gal^+14min	Pro^+23min	Ilv^+77min Thr^+112
x *S. pullorum* strA1 his-1 pro-1 thr-1 ilv-1 gal-1	MS369			
B.				
S. pullorum cysA1 cysJ1 leu-1 trp-2/FT71(*trp*$^+$)	MS807	Trp^+9min		
x *S. pullorum* strA1 trp-1 and	MS355			
x *S. pullorum* strA1 his-1 pro-1 thr-1 ilv-1 gal-1	MS369		Pro^+23min	
C.				
S. pullorum cysE1 glyA3 leu-1/FT77(*cysE*$^+$ *pyrE*$^+$ *rfa*$^+$)	MS809	Ilv^+21min	Pro^+48min	Gal^+64min
x *S. pullorum* strA1 his-1 pro-1 thr-1 ilv-1 gal-1	MS369			
D.				
S. pullorum cysE1 glyA3 leu-1/FT77(*cysE*$^+$ *pyrE*$^+$ *rfa*$^+$)	MS809	$CysE^+$7min		
x *S. pullorum* strA2 leu-1 cysE2 ilv-3	MS90			

it has been assumed that the phage resulted from zygotic induction. Exposure of this phage, P35, to anti-P22 rabbit serum (10^{-1} dilution) for 30 min at 37C reduced the plaque forming titer of the phage lysate by approximately 99.9%. No reduction in phage titer was detected with normal rabbit serum. Thus P35 is antigenically similar to P22 phage of S. typhimurium.

For the transduction experiments 0.5 ml of an overnight aerated culture containing 1 x 10^{10} cells/ml was mixed with an equal volume of the phage lysate (1.1 x 10^{10} phage/ml). This suspension was incubated for 15 min at 37C and 0.1 ml aliquots were plated on minimal media. At least five plates were used for the plating of each phage-bacterial suspension after the proper incubation period had elapsed.

The data are presented in Table 21. It appears that the two species are homologous as judged by the recombination frequencies. The recombination frequencies using the phage grown on S. typhimurium are comparable to those obtained using the phage lysate from S. pullorum.

Tryptophan and cysteine B markers appear to be co-transducible using either S. pullorum or S. typhimurium phage lysates (Table 21, parts C and D). Sanderson found 39% linkage of cysB to trpA in S. typhimurium with P22 phage (95). No attempt was made to quantitate the relative homologies of various S. pullorum and S. typhimurium genes

Table 21. Transduction of various markers to S. pullorum.

Recipient	Marker Selected	DNase[a]	P22[b] Antiserum	Mean Number Transductants Per Plate / Absorbed Phage[c]	Transduction Frequency[d]
A. Donor lysate prepared on S. typhimurium LT2 (MST90)					
MS81	$cysE^+$	--	--	$1.0/5.5 \times 10^8$	1.8×10^{-9}
MS359	thr^+	--	--	$103/5.5 \times 10^8$	1.9×10^{-7}
MS363	$glyA^+$	--	--	$164/5.5 \times 10^8$	3.0×10^{-9}
MS357	$thyA^+$	--	--	$3.0/5.5 \times 10^8$	5.5×10^{-9}
MS354	met^+	--	--	$4.3/5.5 \times 10^8$	7.8×10^{-8}
MS353	ser^+	--	--	$10.6/5.5 \times 10^8$	1.9×10^{-7}
MS36	trp^+	--	--	$386/5.5 \times 10^8$	7.0×10^{-7}
MS355	trp^+	--	--	$82/5.5 \times 10^8$	1.5×10^{-7}
MS359	thr^+	+	--	$101/5.5 \times 10^8$	1.8×10^{-7}
MS354	met^+	+	--	$4.1/5.5 \times 10^8$	7.5×10^{-9}
MS359	thr^+	--	+	$0.2/5.5 \times 10^8$	---
MS354	met^+	--	+	$0.0/5.5 \times 10^8$	---
B. Donor lysate prepared on S. pullorum (MS359)					
MS81	$cysE^+$	--	--	$12.5/5.5 \times 10^8$	2.2×10^{-8}
MS357	$thyA^+$	--	--	$6.6/5.5 \times 10^8$	1.2×10^{-8}
MS36	trp^+	--	--	$510/5.5 \times 10^8$	9.3×10^{-7}
MS354	met^+	--	--	$61.5/5.5 \times 10^8$	1.1×10^{-7}
MS354	met^+	+	--	$57.5/5.5 \times 10^8$	1.0×10^{-7}
MS354	met^+	--	+	$0.1/5.5 \times 10^8$	---
MS359[e]	thr^-	--	--	$0.0/5.5 \times 10^8$	---

[a]The phage lysate was pretreated with 5µg/ml of deoxyribonuclease for 30 min at 37C.

[b]Phage lysate pretreated with an equal volume of anti-P22 rabbit serum (10^{-1} dilution) and incubated at 37C for 30 min. Rabbit P22-antisera was a generous gift of A. Eisenstark and normal rabbit serum a gift of V. Mallmann.

[c]The average number of colonies per plate is taken from an average of 5 plates.

[d]Transduction frequency is the calculated from c.

[e]Control for lysogenic conversion, part B.

Table 22. Cotransduction of *trp*-3 and *cysB*1.[a]

Recipient	Marker Selected	Mean Number Transductants Per Plate / Absorbed Phage	Transduction Frequency	Unselected Marker: Number of Transductants Analyzed	Unselected Marker: trp^+	Unselected Marker: $cysB^+$	Unselected Marker: Percent Co-transduction
A. Donor lysate prepared on *S. typhimurium* LT2 (MST90)							
MS103	trp^+	$63/5.5 \times 10^8$	1.1×10^{-7}	38	--	18	47
	$cysB^+$	$58/5.5 \times 10^8$	1.1×10^{-7}	24	14	--	58
	trp^+cysB^+	$34/5.5 \times 10^8$	6.2×10^{-8}	--	--	--	--
B. Donor lysate prepared on *S. pullorum* MS359							
MS103	trp^+	$134/5.5 \times 10^8$	2.4×10^{-7}	38	--	19	50
	$cysB^+$	$145/5.5 \times 10^8$	2.6×10^{-7}	29	15	--	52
	trp^+cysB^+	$82.5/5.5 \times 10^8$	1.5×10^{-7}	--	--	--	--

[a]Procedures described in Materials and Methods section.

because the extent of lysogenation of each strain is not known.

To test for stability of the transductants approximately 5 of them derived from each phage bacterial suspension were inoculated into L broth and grown overnight. The overnight cultures were then streaked onto L agar and the plates incubated 24 hr at 37C. The genotypes of the transductants were determined by replica-plating and in every instance they retained the original phenotype. Fulfillment of this criterion indicated stability which was inferred to be due to integration of the transduced gene.

The phage lysates employed were tested for sterility and the recipients tested for reversion. P22-antisera effectively reduced the recombination frequency; whereas, normal rabbit serum had no effect (data not listed). This reduction in recombination frequency correlates closely with the previously mentioned phage neutralization experiments. It should also be noted that deoxyribonuclease had no effect on the transduction frequency.

Part VI. Orientation of the *cysB trp* Region in *S. pullorum*

Sanderson (95) found that in *S. typhimurium* the gene order was *pro--gal--pyrF-cysB-trp----his*; whereas, Taylor and Trotter (109) found that in *E. coli* the order was *pro--gal--trp-cysB-pyrF---his*. This indicated an inversion

of the pyrF-cysB-trp region between the two genera. Thus a plausible explanation for the opposite direction of transfer of chromosomal genes encountered with S. pullorum strains carrying the FT71 (trp$^+$) factor in comparison to S. typhimurium strains carrying the same F-prime factor would be an orientation of the trp-cysB-pyrF region in S. pullorum similar to that found in E. coli.

To determine the orientation of the cysB trp region in S. pullorum with respect to pro--gal---his region several matings were done and these results are seen in Table 22. It is evident that MS807 (FT71 (trp$^+$)) does not transfer the cysB$^+$ gene at a detectable level to MS105. This is a good indication that the cysB trp region is inverted in S. pullorum in comparison to S. typhimurium. The other donors were apparently not capable of transferring cysB$^+$ or trp$^+$ genes into MS105 and thus linkage studies could not be done.

Part VII. Criteria of Conjugation in S. pullorum

Since 72-96 hr incubation periods were required for appearance of recombinants and there was a high concentration of cells on each plate, several precautions were undertaken to eliminate the possibility of syntropism:

1. Either streptomycin or sodium azide in addition to a distal auxotrophic marker was used for counterselection in many instances.

Table 23. Orientation of the *cysB trp* region.[a]

Mating	Counterselection Markers	Selected Markers	Recombination Frequency[b]
MS807 x MS105	cysA cysJ	$cysB^+$	$<2 \times 10^{-8}$
	leu strA	gal^+	4×10^{-3}
MS809 x MS105	glyA strA	gal^+	2×10^{-7}
		$cysB^+$	$<2 \times 10^{-8}$
		trp^+	$<2 \times 10^{-8}$
		his^+	$<2 \times 10^{-8}$
MS806 x MS105	cysA cysJ	$cysB^+$	$<2 \times 10^{-8}$

[a]The selective media were supplemented with all the growth factors of the particular recipient strain except that for which selection for independence was being made.

[b]The recombination frequency is based on the mean number of recombinants per five plates divided by the donor input.

2. In all matings a female cell with the same genotype as the donor was millipore-mated with each recipient. In no instance did the number of colonies appearing exceed the normal reversion rate of the recipient.

3. Cross-streaking did not show the heavy background growth indicative of syntrophism.

4. A large number of recombinants inherited unselected donor markers.

5. In all instances the purified recombinants were replica-plated on a medium selective for the growth of the donor genotypes.

6. A millipore membrane filter (HA 0.45μ) was interposed between donor and recipient bacteria on selective media. In the area where the donor and recipient were separated by the membrane filter no recombinants were observed.

An exhaustive analysis (prototrophy, stability, infertility and MS2 insensitivity) of recombinants arising from each mating was made. The data are presented in Table 24.

More than 1800 recombinants were selected from the previously described S. pullorum matings and in each instance they were found to be prototrophic. Because the cells tested from the colonies arising on a minimal medium are prototrophic, it is concluded that genetic exchange occurred and not some artifact created by the presence of nutrient broth in the medium and/or syntrophism.

Stability of a recombinant marker would indicate that the donor gene is physically integrated into the chromosome of the recipient. To test the stability of

Table 24. Recombinant analysis.

Cross	Recombinant Selected	Prototrophy: Number Tested	Prototrophy: Per Cent Prototrophic	Stability: Number Tested	Stability: Per Cent Stable
A. Prototrophy and Stability					
MS806 x MS367	thr^+_+	132	100	15	100
	his^+_+	27	100	18	100
MS807 x MS369	thr^+_+	146	100	31	100
	his^+_+	185	100	26	100
	pro^+_+	207	100	28	100
	ilv^+_+	198	100	32	100
	gal^+_+	182	100	15	100
MS809 x MS369	thr^+_+	13	100	10	100
	his^+_+	55	100	10	100
	pro^+_+	240	100	10	100
	ilv^+_+	326	100	10	100
	gal^+_+	83	100	10	100

Cross	Recombinant Selected	MS2 Sensitivity[a]
B. Fertility		
MS806 x MS367	thr^+_+	4/12
	his^+_+	5/11
MS807 x MS369	thr^+_+	0/10
	his^+_+	0/10
	pro^+_+	0/10
	ilv^+_+	0/10
	gal^+_+	0/10
MS804 x MS369	thr^+_+	0/10
	his^+_+	0/10
	pro^+_+	0/10
	ilv^+_+	0/10
	gal^+_+	0/10

The techniques have been previously described in Methods and Materials.

[a]Number of recombinants sensitive to MS2 phage per number tested.

the recombinants, approximately 215 purified recombinants were grown overnight in L broth and then streaked on L agar and incubated 24 hr at 37C. Five colonies were selected from each of the 215 recombinants originally isolated and streaked onto properly supplemented minimal media. In no instance was the original recombinant genotype lost. Thus, it is concluded that the recombinants are stable.

Infertility and MS2 insensitivity of the recombinants shows that the transferred gene is located on the chromosome and not on an F-prime factor. Thirty-nine per cent of the recombinants derived from the MS806 x MS367 mating were sensitive to MS2 phage, but none had the ability to transfer either threonine or histidine to a suitable recipient. In the case of the MS809 x MS369 mating, 3% of the recombinants carried the FT77 factor but none of the 50 analyzed were able to transfer the recombinant gene to a suitable recipient. In the MS807 x MS369 mating only one out of the 50 recombinants analyzed was sensitive to MS2 and none had the ability to transfer the recombinant gene to a suitable recipient as determined by cross-streaking. Since the cells were mated for 3 hr at a ratio of 1:1 and at high cell concentrations it is reasonable to expect as in the case of K-12 crosses involving F^+ and F-prime males, that the sex factor would be transferred independently of chromosomal genes to F^- cells during mating.

Thus is may be stated that the recombinants derived from the S. pullorum F-prime x S. pullorum F^- matings are prototrophic, stable, infertile and do not possess a high coinheritance of the donor markers.

DISCUSSION

Part I. Linkage Map of S. pullorum

The donor strain MS807 of S. pullorum which possesses the FT71 (trp$^+$) factor gave the greatest amount of chromosomal gene transfer. When this strain was mated with MS369 the following gradient was obtained:

gal-1>pro-1>ilv-1>thr-1>his-1 (Table 15).

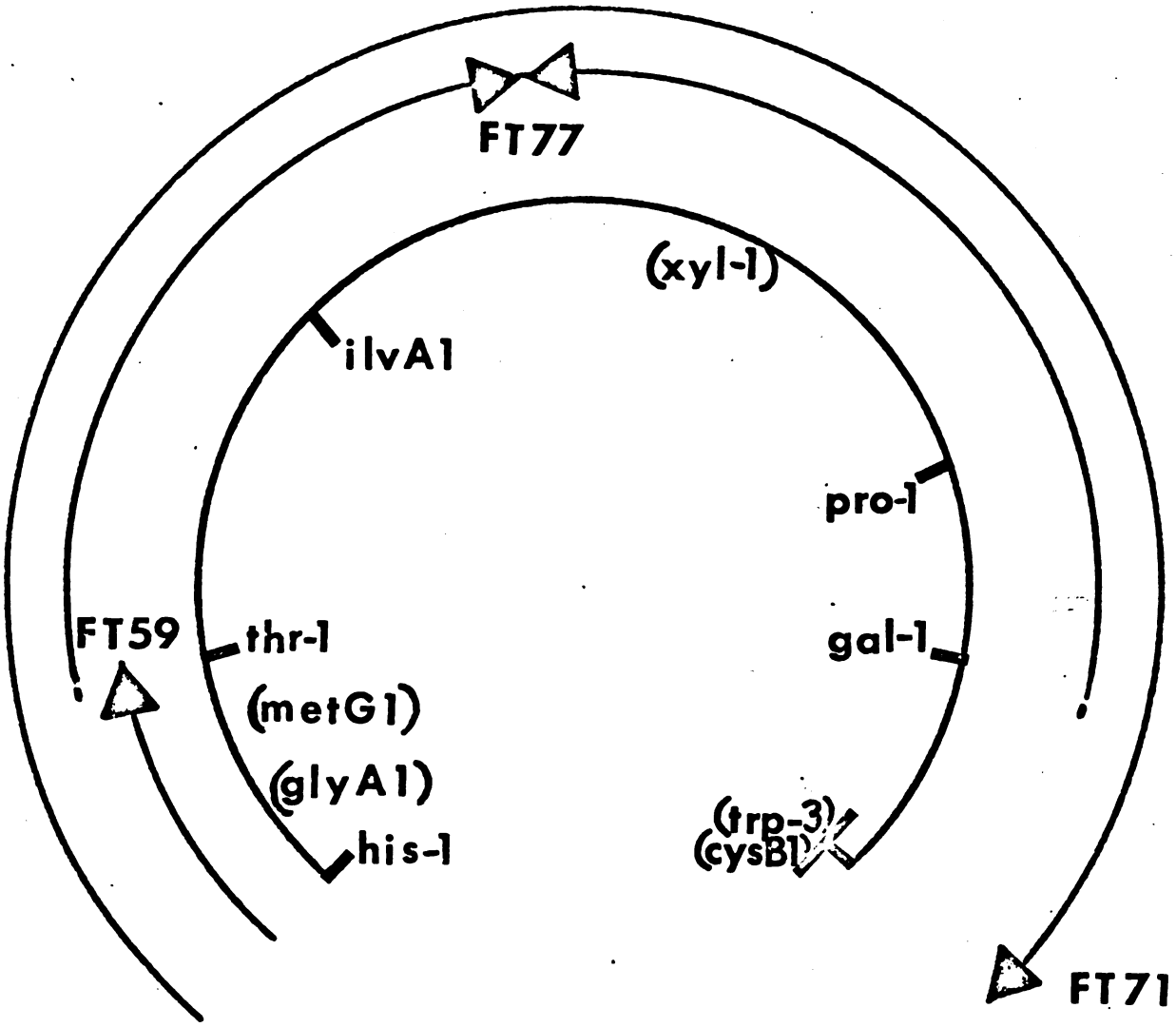
Interrupted matings of MS807XMS369 demonstrated (Figure 3) that the relative recombination frequencies of the various markers were proportional to their time of entry. The linkage studies from the MS810XMS374 mating (Table 16) also confirmed the above gene sequence. From the gradient of transfer, interrupted matings and linkage studies it is readily apparent that MS807 behaves as a stable, homogeneous population in transferring a given set of markers at high frequency and in a particular sequence.

This gene sequence was further substantiated by the gradient of transfer and linkage data obtained from the MS809 X MS369 and MS806 X MS369 matings. The gene sequence as determined by kinetic analysis, linkage, and gradient of transfer was essentially the same irrespective of the donor strain used; thus, a general linkage map in S. pullorum is

unequivocal. The linkage map of S. pullorum is shown in Figure 7.

The evidence for the location and orientation of the cysB1 trp-3 region in S. pullorum is by no means unequivocal. The location of this region is based on the assumption that the chromosome transfer mediated by the FT71 (trp^+) factor originates at this region and on the fact that no $cysB1^+$ or $trp\text{-}3^+$ recombinants were obtained from the MS806 x MS105 and MS809 x MS105 matings (Table 22). The orientation of the cysB1 trp-3 region is based on the fact that S. pullorum strains possessing the FT71 (trp^+) factor transfer the chromosome in an opposite direction to that of S. typhimurium strains possessing the same F-prime factor. This inversion is supported by the finding that no $cysB1^+$ recombinants were obtained from the MS807 x MS105 mating (Table 22). The critical test to determine both location and orientation of the cysB1 trp-3 region would be to study the linkage of the cysB1 and trp-3 markers to his-3. This has been attempted (Table 22) but no $cysB^+$ or trp^+ recombinants were obtained. The FT21 factor carries no known chromosomal loci, but mobilizes the chromosome with trp near the proximal end in S. typhimurium (116,96). It is possible that S. pullorum strains possessing this F factor might transfer the entire trp-cysB------his region and allow linkage studies of those three markers. The FT21 factor has not been introduced into S. pullorum.

Figure 7. Linkage Map of S. pullorum. The F-prime factors and their direction of chromosome mobilization are indicated in the expanded portion. The extent of chromosome mobilization is indicated by the length of the solid line. No linkage analysis was done with the gene markers in parentheses.



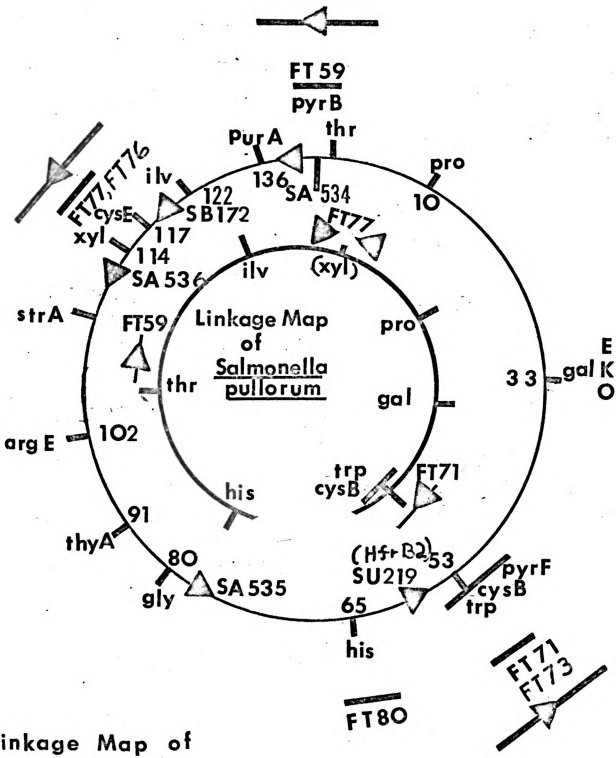
Linkage Map
of Salmonella pullorum

Part II. Comparison of *S. pullorum* to *S. typhimurium* Linkage Maps

When the linkage map of *S. pullorum* is compared with that of *S. typhimurium* (Figure 8) it is readily seen that the *cysB trp* region appears to be inversed between the two species and that the threonine locus is transposed. The transposed region probably includes the *pyrB* region since MS806 which possesses the FT59 (*pyrB*$^+$) factor seems to mobilize the chromosome in the transposed region. Inversion of the *pyrB thr* region when compared to *S. typhimurium* or *E. coli* is indicated also as MS806 x MS369 mating produces Thr$^+$ and His$^+$ recombinants but no Ilv$^+$, Pro$^+$ or Gal$^+$ recombinants.

Since MS809 which possesses the FT77 (*cysE*$^+$ *pyrE*$^+$ *rfa*$^+$) factor mobilizes the chromosome from a region located between *ilv*-1 and *pro*-1 there may be either an extension of the previously mentioned transposed inverted area to include *ilv cysE* or another transposed area; the *cysE ilv* region. The xylose marker is carried by the FT76 factor and is positioned in close proximity to the *cysE* locus; thus it is possible that this marker could also be transposed. When MS807 was mated with MS370 and the location of the *xyl*-1 marker determined by gradient of transfer and interrupted matings, it was found that the *xyl*-1 marker was located between *ilv*-1 and *pro*-1. This location of *xyl*-1 may be due either to another transposition or to an inversion associated with

Figure 8. The linkage Map of S. pullorum and S. typhimurium. The Linkage Map of the S. typhimurium is the outer circle and the Linkage Map of S. pullorum is the inner circle.



Linkage Map of

Salmonella typhimurium

the xyl-cysE-ilv, pyrB, thr segment. No linkage studies of xyl-1 to other markers were done because of the slow growth rates of S. pullorum on A minimal agar with xylose as the only energy source.

Further evidence in support of the previously mentioned gene transpositions and/or inversion was indicated from the SA534XMS367 and SA536XMS367 matings (Table 5) from which extremely low recombination frequencies for early markers were obtained. If the lead region of the donor DNA (S. typhimurium Hfr) had little homology with the recipient DNA (S. pullorum) due to a gene transposition or inversion in the recipient, then the recipient is less likely to pull the chromosome. Likewise, recombination of the hybrid merozygote may be impaired by transposition and unquestionably it will be impaired by an inversion.

When the gross arrangement of the genes on the S. pullorum and S. typhimurium are compared there seems to be a gene inversion and/or a gene transposition. However, there appears to be a high level of genetic fine structure homology between the two species as crosses between them mediated by transduction (Table 21) yield recombination frequencies which are analogous to intraspecies crosses. Also recombinants formed from the crosses are exceedingly stable. Homology was also demonstrated when SB172 was millipore-mated with MS367 for 60 min at a donor recipient ratio of 1:20 yielding a recombination frequency

for Ilv$^+$ which was very high (0.7 per donor cell) and the prototrophic recombinants were exceedingly stable. A similar result was obtained in the SA535 x MS363 mating where Gly$^+$ recombinants were selected and also found to be very stable (Table 5).

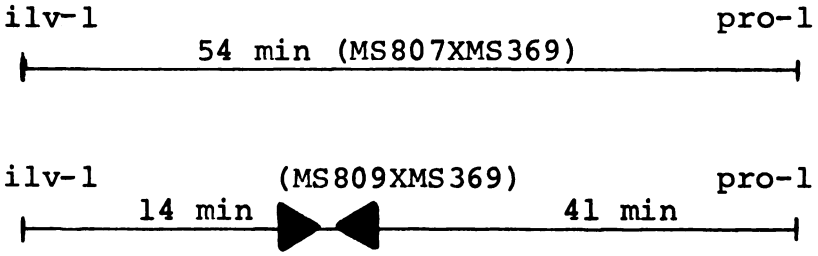
When S. pullorum (MS364) was mated with E. coli AB311 the recombination frequency for an early marker his-1 was very low (10^{-6}) and the His$^+$ recombinants were very unstable (Table 7). Similar results were obtained when E. coli AB257 was mated with S. pullorum MS369 and selection was made for Pro$^+$, Thr$^+$, and Lac$^+$ recombinants (data not presented). These results may be due to homology differences.

When the chromosome of S. typhimurium is compared with E. coli only one case of a chromosomal rearrangement has been reported (95), the inversion of the pyrF cysB trp region, but there are still numerous cases of uncertainty about gene location in both organisms. The locus of the operator in relation to the sequence of structural genes of the trp operon seems to be the same in E. coli and S. typhimurium (95).

The heterogeneity of gene sequence between S. pullorum and S. typhimurium may be the result of a diverse evolution of the two species. S. pullorum is commonly isolated from chickens (body temperature 41-43C) and S. typhimurium from mice (body temperature 36-38C).

Part III. Chromosome Mobilization Directed by the FT77 (*cysE*$^+$*pyrE*$^+$*rfa*$^+$) Factor

The FT77 factor appears to mobilize the chromosome in two directions. The basis for this conclusion follows. First, the time interval between *ilv*-1 and *pro*-1 is 27 and 54 min with the MS809XMS369 and MS807XMS369 matings respectively. If the time of entry of the episomal *cysE*$^+$ gene (Figure 6) is used as an approximation of both the "lead" and "dead" time then the approximate location of the initiation sites for chromosome mobilization mediated by the FT77 factor can be determined. With the above information the following model can be devised:



Second, the *ilv*-1:*pro*-1 ratio of the gradient of transfer with MS809XMS369 and MS807XMS369 is 0.6 (*pro*/*ilv*) and 0.14 (*ilv*/*pro*) respectively. Third, linkage of *ilv*-1 to *pro*-1 is not significant in recombinants from the MS809XMS369 mating but is significant in recombinants from the MS807XMS369 matings. Fourth, linkage of Ilv$^+$ recombinants to the *thr*-1 marker from the MS809XMS369 and MS810XMS369 matings are 3.7% and 3.0% respectively. These observations indicate counterclockwise transfer of the

chromosome by the FT77 factor. Finally, when the Pro^+ recombinants obtained from the MS809XMS369 mating are analyzed for linkage to the unselected gal-1 marker it is found that 22% of the Pro^+ recombinants are Gal^+ (Table 18). This corresponds very nicely to the linkage of the Gal^+ recombinants to the unselected pro-1 marker (MS810XMS374) which was found to be 25% (Table 16). These observations indicate a clockwise transfer of the chromosome by the FT77 factor.

Out of 717 recombinants analyzed from the MS809X MS369 mating only 8 (1.1%) had acquired the cysE auxotrophic marker of the donor and all 8 were isolated in the initial selection for Ilv^+ recombinants. This linkage is probably the result of low donor crossover between the FT77 factor and the cysE1 marker on the chromosome.

This multidirectional transfer is not a unique situation, as Clark (21) has isolated an Hfr strain that had two stably attached sex factors at different sites on the chromosome. The Hfr was able to transfer its chromosome by one or the other sex factor but not both.

Part IV. Chromosome Mobilization in S. pullorum

Integration of a sex factor is usually studied by observing its ability to mobilize the bacterial chromosome to another cell during conjugation (98). Studies concerning the conditions or mechanisms of F^+ or F prime

integration into the chromosome have yielded few clear cut results.

Chromosome transfer mediated by cells possessing the F^+ factor in E. coli, S. abony or S. typhimurium (98) is generally about 10^{-5} recombinants per donor cell. Similar matings in S. pullorum yielded no recombinants. Even pretreatment of the S. pullorum F^+ strains with ultraviolet light did not produce detectable levels of chromosome transfer. Only donor strains of S. pullorum possessing F-prime factors carrying Salmonella genes were able transfer chromosomal genes at detectable levels.

The inability of S. pullorum strains possessing the F^+ or F-prime factors (containing E. coli genes) to transfer a detectable level of chromosomal genes to S. pullorum F^- strains is difficult to rationalize for the following reasons. S. pullorum can repair UV damage and forms stable normal recombinants with S. typhimurium Hfrs at very high frequencies. One possible explanation would be that the F^+ factor and the F-prime factors carrying E. coli genes are preferentially modified such that they cannot physically integrate into the chromosome of S. pullorum. Modification of the F-lac$^+$ factor by S. pullorum has been demonstrated in this laboratory (43). It is not difficult to envision preferential modification of an F-prime factor possessing

E. coli genes and not one carrying Salmonella genes because S. pullorum appears to possess a high degree of fine structure homology to S. typhimurium and very little to E. coli.

Perhaps certain regions of S. typhimurium and S. pullorum are less homologous than others and consequently modification of S. typhimurium DNA also otcurs when it is introduced into S. pullorum. An example of this phenomenon may be seen in the comparative frequency of transduction of the cysE and trp markers. Transduction of the cysE marker between S. pullorum is 12-fold higher than between S. pullorum and S. typhimurium; whereas, a similar observation with the trp marker yields equivalent transductants. This apparent difference in homology of the cysE and trp regions of S. typhimurium and S. pullorum may be related to the difference in chromosome mobilization capacity of MS809 and MS807.

If it is assumed that the FT59 factor and the FT77 factor are modified in such a manner that they cannot integrate into the chromosome of S. pullorum, then the chromosome transfer mediated by these factors in MS806 and MS809 respectively becomes very difficult to envision. The synapsis model may be invoked as a possible explanation for the chromosome transfer directed by these factors. This model assumes that the F-prime factor attaches laterally to a site on the bacterial chromosome. The degree of association of the episome and chromosome is directly related to the relative homology

existing between the episome and chromosome. At the time of mating a specific endonuclease nicks the F-prime factor at a specific site thus initiating transfer. If the episome and chromosome are tightly synapsed then non-random breakage of the chromosome by the endonuclease would lead to regional unwinding with the creation of single stranded regions. If single stranded DNA is transferred in conjugation (24), then annealing of the DNA strand of the episome with a single strand of chromosomal DNA would lead to transfer of chromosomal DNA. This model seems to account for the inability of F^+ and F-prime factors carrying E. coli genes to transfer chromosomal genes to S. pullorum and for the low levels of chromosomal transfer mediated by FT59 and FT77. By itself this model is inadequate to account for the two-directional transfer. The two directional transfer by the FT77 factor could be explained by the above plus the fact that transfer in the opposite direction could be the result of a theoretical "transfer replicase" switching strands from the episomal DNA to the chromosomal DNA while maintaining the same 5' 3' direction.

This two directional transfer by the FT77 factor could also be the result of an inversion of the cysE, pyrE or rfa genes in S. pullorum. A crossover between a non-inverted segment on the chromosome and the homologous segment on the FT77 factor gives mobilization in one direction, and that crossover between an inverted segment on the chromosome

and the noninverted homologous segment on the FT77 factor gives mobilization in the other direction. This phenomenon has been observed with an F-*lac*$^+$ factor carrying an inverted segment (11).

According to Scaife and Gross (99) the frequency of chromosome transfer is determined by the frequency of donor crossover between the F-prime factor and the chromosome. In *S. typhimurium* the FT77 factor mobilizes the chromosome more frequently than it is transferred intact (97), indicating a high frequency of donor crossover between the F-prime factor and the chromosome. However, in *S. pullorum* the FT77 factor transfers chromosomal genes at frequencies of 10^{-6} to 10^{-7} per donor cell while the intact episome is transferred at a frequency of 0.11 per donor cell.

The insertion model is another one used to explain chromosome mobilization (19). This model is currently in vogue due to its high predictive value and the overwhelming data supporting it. Even though highly substantiated, the insertion model does not account for the residual undirected chromosome transfer by an F-*lac*$^+$/rec$^-$ cell (23), Hfr selection from an F$^+$/ rec$^-$ cell (98) and the data of Curtiss (28) showing that gene transfer by a population of F$^+$ cells is markedly higher than the concentration of stable Hfrs in a donor population.

It could also be argued that the lack of homology between the F factor and the chromosome is the cause of the

low to nondetectable levels of chromosomal transfer in S. pullorum. It has been demonstrated that the F^+ factor, even though many integration sites are known, shows a distinct preference for certain locations on the bacterial chromosome. Many investigators equate this preference with the requirement for gross homology between the episome and chromosome subsequent to integration. It must be remembered that it is not known how long the F^+ factor has been in E. coli and thus the designation of a fertility factor being F^+ or F-prime is purely academic. That is to say that the preference of the F^+ factor is possibly the result of unknown chromosomal genes on the F^+ factor and thus the requirement of homology for the integration of the episome into the chromosome is probably not stringent.

SUMMARY

The purpose of this investigation was to study chromosome mobilization in a slow growing species of Salmonella, Salmonella pullorum. Only donor strains of S. pullorum possessing F-prime factors carrying genes from either Salmonella typhimurium or Salmonella abony were able to transfer a detectable level of chromosome material. The recombinants were prototrophic, F^-, stable and did not possess a high coinheritance of donor characteristics. All matings were conducted on millipore-membranes and streptomycin was used for counterselection. The following observations were determined by gradient of transfer, interrupted matings and unselected marker analysis. The threonine locus appears to be located in close proximity to glycine A and histidine. S. pullorum F-trp strains transfer chromosomal genes in a direction opposite to that of a S. typhimurium F-trp strain. This is possibly due to an inversion of the pyrF-cysB-trp region. S. pullorum F-cysE appears either to transfer the chromosome in more than one direction or to mobilize it at more than one site as indicated by the near absence of linkage of isoleucine to either proline or galactose genes.

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