PATTERNS OF SEXUAL RECOMBINATION IN ENTERIC BACTERIA[1]

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STRAIN K-12 of *Escherichia coli* has played a preeminent role in the study of bacterial sexuality. New knowledge of the mechanism of sexual differentiation and the development of more sensitive techniques and test strains have subsequently brought many more bacteria within the orbit of this breeding system. The immunogenetics of Salmonella poses many interesting problems (LEDERBERG and IINO 1956; IINO 1958, 1961a,b; LEDERBERG 1961) that could be only partly analyzed by methods of phage-mediated transduction. This paper presents a survey of crossing behavior in Salmonella and some other enteric bacteria which was conducted as a basis for the further study of flagellar phase variation in Salmonella.

Sexual recombination in *E. coli* is dependent on a fertility factor F which confers the property of maleness on cells carrying an F particle either in the cytoplasm or fixed to the chromosome (LEDERBERG, CAVALLI and LEDERBERG 1952; JACOB and WOLLMAN 1961). The impact of F is expressed in at least two ways: the modification of the cell surface allowing for the conjugation reaction of an F^+ with F^- acceptor cells, and the impulse to the chromosome to migrate from the male partner via the conjugal bridge to synapse and crossover with the corresponding chromosome of the female partner. Even in the F^+ cell where the F particle is characteristically extrachromosomal, it probably forms at least a temporary association with the chromosome in those cells actually involved in conjugation. In general, the point on the chromosome at which the F particle is located tends to be the last segment to be transferred during an orderly progressive process of conjugal exchange, perhaps on account of a fixation of the F factor that binds the chromosome to a position on the cell surface whose modification is involved in the formation of the conjugal bridge.

The F particle sometimes acquires a translocated fragment of chromosome, a few recognizable markers now sharing the contagious transmission of the F element (JACOB and ADELBERG 1959; HIROTA 1959). These compound F elements, designated F′ (F prime) have the advantage that their transmission can be more readily followed through the diagnosis of the translocated markers. They

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also confer a very efficient transfer of the translocated markers. Following the preliminary reports of fertility of *E. coli* with Salmonella (BARON, SPILMAN and CAREY 1959; BARON, CAREY and SPILMAN 1959a,b; MIYAKE and DEMEREC 1959; ZINDER 1960a,b) we thought to explore the range of sexual competence in Salmonella by following the transmission of an F′ that efficiently transfers the *Lac* markers.

MATERIALS AND METHODS

The cultures used in this investigation are listed in Table 1.

TABLE 1

Strains used

Our strain number	Description*	References
Escherichia coli, K-12 derivatives		
W 6	$F^+Lac^+M^-$	LEDERBERG, *et al.* (1952)
W 1895	$Hfr_1Lac^+M^-$	CAVALLI, LEDERBERG and LEDERBERG (1953)
W 3287†	$F_{13}{}^+Lac^+M^-S^r$	
W 3637	$Lac^+M^-S^r$	ØRSKOV, *et al.* (1961)
W 3747	$F_{13}{}^+Lac^+M^-$	HIROTA (1959)
W 3876	$♀_3Lac^-S^r$	RICHTER (1961)
W 4145	$Lac^-{}_{85}$	COOK and LEDERBERG (1962)
W 4678	$Lac^-{}_{85}P^-S^r$	COOK and LEDERBERG (1962)
W 4680	$Lac^-{}_{39}S^r$	COOK and LEDERBERG (1962)
Salmonella		
TM 2	*typhimurium* (Lilleengen No. 85) its derivatives: S^r(SW 1342), $F_{13}{}^+$(SW 1346)	STOCKER, ZINDER and LEDERBERG (1953)
SW 685	*paratyphi B* (derivative of SW 543 of STOCKER *et al.*, originally Kauffmann No. 223) its derivatives: S^r(SW 1390), $F_{13}{}^+$(SW 1343)	STOCKER, *et al.* (1953)
SW 753	*bovis-morbificans* 3640	EDWARDS and BRUNER (1942)
SW 764	*enteritidis* 1891	EDWARDS and BRUNER (1942)
SW 776	*london* 1446	EDWARDS and BRUNER (1942)
SW 777	*give* 316	EDWARDS and BRUNER (1942)
SW 779	*muenster* 4546	EDWARDS and BRUNER (1942)
SW 787	*senftenberg* 3007	EDWARDS and BRUNER (1942)
SW 790	*aberdeen*	EDWARDS and BRUNER (1942)
SW 791	*poona*	EDWARDS and BRUNER (1942)
SW 795	*hvittingfoss*	EDWARDS and BRUNER (1942)
SW 803	*abony* 74 its derivatives: S^r(SW 1353), F^+(SW 1351, 1364, 1463), $F_{13}{}^+$(SW 1485), $F_{13\ stable}{}^+$(SW 1365, 1486), Hfr(SW 1462), M^-S^r(SW 1361), P^-S^r(SW 1355), Gal^-H^-i:1,2 S^r(SW 1464), $Mal^-Ara^-S^r$ (SW 1417), etc.	EDWARDS and BRUNER (1942)

TABLE 1—Continued

SW 1214	*typhimurium* TM-9S^r-2 its derivatives: T^-Tyr^-(SW 1259), $F_{13}{}^+T^-Tyr^-$(SW 1372)	BARON, *et al.* (1959b)
SW 1338	*adelaide*	NOSSAL and LEDERBERG (1958)
SW 1394	*java* No. 5, obtained from F. ØRSKOV as fertile with Hfr *coli*	ØRSKOV, *et al.* (1961)
SW 1395	*miami* No. 187 obtained from F. ØRSKOV as fertile with Hfr *coli*	ØRSKOV, *et al.* (1961)
Shigella		
W 1779	*sonnei* S3 (P9) is Niacin$^-$ its derivatives S^r(W 4973), $F_{13}{}^+$	FRÉDÉRICQ (1948)
H 1	*flexneri* its derivatives: S^r, $F_{13}{}^+$	
Klebsiella		
K 1		
Serratia marcescens		
SM 6	its derivative: $F_{13}{}^+$, very unstable	FALKOW, *et al.* (1961)
SM 6-S^r-11	S^r	FALKOW, *et al.* (1961)
W 2745	fecal isolate, also listed as CDC 184/55	WAISMAN and STONE (1958)

* All stocks prototroph, streptomycin sensitive, *Lac*$^-$, and without a demonstrable F factor if not otherwise indicated. Abbreviations: S^r=resistant to streptomycin 200μg/ml *Lac*=lactose, *Gal*=galactose, *Ara*=arabinose, *Mal*=maltose, $^+$=fermenting, $^-$=nonfermenting. Growth factor requirements: M^-=methionine, P^-=proline, H^-=histidine, T^-=threonine, Tyr^-=tyrosine requiring. Mating types: F^-, F^+, Hfr, $F_{13}{}^+$, $F_{13\text{stable}}{}^+$ are described in the text. The references should be consulted for the presence in some strains of additional markers immaterial to the present work.

† Test strain used by ØRSKOV, *et al.* (1961) W 3287 and 3747 both acquired their F_{13} from a strain W 3213 isolated in 1955 by LEDERBERG (unpublished) as an unstable, hyperfertile male derivative of W 6.

Cultural procedures are detailed elsewhere (LEDERBERG 1950). "EM" agar (EMS agar without succinate) was frequently used as a combined selective and indicator medium. It is a synthetic medium with a given sugar as sole carbon source, and also contains eosin and methylene blue to delineate prototrophic sugar-positive colonies. Recombinants were selected on minimal agar plates at the intersection of drops or streaks of the parent cultures, or from cell suspensions mixed in broth for 30 minutes at a density of about 5×10^8 male and 2×10^7 female cells per ml. The plates were scored after two days incubation at 37°C.

To test for F′ infection, spot tests equivalent to crossing tests were made on lactose selective media. For greater encouragement of F′ transfer, mixed cultures were incubated in broth either for 30 minutes or overnight, centrifuged and spread on EM *Lac* plates. In the latter case, 10^8 cells of the minority parent were plated. Since in this experiment, the F′ can migrate not only from the donor to recipient, but also from one infected recipient to others, it is not possible to give precise frequencies of infection and the results are expressed as plus or minus.

Several mutually confirmatory tests were routinely conducted for the successful transfer of F to a new Salmonella culture (A) by crossing with known female indicator strains, (B) by infective transfer to known female strains, (C) by transfer to a special indicator strain, $♀_3$ (RICHTER 1961) which is especially advantageous as the acquisition of F results in an unusually fertile $♂_3$ that can be

efficiently detected *in situ* (SNEATH and LEDERBERG 1961), (D) by a staining reaction on EMB agar plates (compare ZINDER 1960b): F^+ strains of many Salmonella types can be distinguished on EMB agar without fermentable sugar giving purplish as compared to white or bluish colonies of F^-. The color difference was best seen after 18 hours at 37° followed by 24–48 hours at room temperature, the plates being observed by oblique lighting.

For the disinfection of F by acridine orange (HIROTA 1960) these conditions were used: overnight incubation in acridine orange-nutrient broth, pH 7.6 starting from a small inoculum of 100–10,000 cells/ml.

For f-agglutination test, the Hfr and F^- sera described by ØRSKOV and ØRSKOV 1960, and kindly furnished by them were used according to their instructions in both slide and tube agglutination tests.

EXPERIMENTS AND CONCLUSIONS

Many previous attempts to demonstrate sexuality in Salmonellas were unsuccessful (ZINDER and LEDERBERG 1952). Later successes depended on a fortunate choice of Salmonella strain as the initial female parent and on the use of appropriate highly fertile male strains of *E. coli* in conjunction with suitable diagnostic markers. We are particularly indebted to DR. L. S. BARON and DR. N. ZINDER for early information on their findings. Further matings in Salmonella depend on the successful transmission of the F particle, whose provenience was usually strain K-12 of *E. coli*, to competent Salmonellas which would then act as males.

The first report of *E. coli* × Salmonella, (BARON *et al.* 1959a) involved the unique strain of *Salmonella typhimurium*, TM 9-S^r-2 which was highly fertile with *E. coli* W1895, an Hfr_1 male. However, the progeny of this cross were generally interfertile with many other Salmonellas. The immunogenetic factors in which we are especially interested, H_1 and H_2, had not been definitively mapped, nor could we succeed in demonstrating the segregation of H_1 or H_2 in these crosses which did involve a substantial segment including the *Lac* marker. This provocative cross was therefore futile for these purposes and further work was focused on achieving (a) general fertility of Salmonella × Salmonella matings and (b) segregation of a wide range of markers, especially H_1 and H_2. To establish appropriate strains it appeared necessary first to introduce a typical infectious F particle. In due course it was found possible to do this with a number of Salmonellas.

However, the initial survey stressed the behavior of the technically favorable F_{13} particle which is readily recognized by the associated transmission of the lactose positive phenotype. This character is especially apt for work with Salmonella as most naturally occurring serotypes of Salmonella are inherently lactose negative. They therefore require a minimum of prior laboratory manipulation to make them ready for experimental tests. The experimental regime was to cultivate an auxotrophic F_{13}-*Lac*$^+$ donor strain with a prototrophic *Lac*$^-$ acceptor strain and then selectively search for prototrophic *Lac*$^+$ progeny by plating on EM lactose agar.

In K-12, F_{13} infection leads to the establishment of moderately stable heterogenotes, i.e., partially diploid cells carrying the F_{13} with its attached segment in addition to the original haploid chromosome. The heterogenotic state is revealed by subsequent segregation of new phenotypes: *Lac*$^-$ F$^-$ (F′ lost); *Lac*$^-$ F$^+$ (F′ particle broken with disappearance of *Lac*$^+$ but retention of F$^+$); *Lac*$^+$ stable (by integration of *Lac*$^+$ into the chromosome), in addition to the parental *Lac*$^+$ F′ type.

All *Lac*$^+$ progeny from F_{13}·*Lac*$^+$-infected Salmonellas, and more than 1,000 have been purified and examined on EMB lactose agar, have been heterogenotic in respect to *Lac*. Furthermore, they have been much less stable than corresponding K-12 F_{13}·*Lac*$^+$, the degree of stability varying with different recipient species. The segregants have all been *Lac*$^-$, either F$^-$ or F$^+$; in no case have stable *Lac*$^+$ been observed which would correspond to the integration of the *Lac*$^+$ fragments in the chromosome. In Table 2 are given the proportions of *Lac*$^-$ segregants when F_{13}-infected clones are transferred in broth. In K-12, an occasional clone is

TABLE 2

Segregation of Lac$^-$ *from F′ ·* Lac$^+$*-infected clones*

Strain	Time of growth in broth	Infected with F_{13}: Relatively stable clones, No. of clones	Infected with F_{13}: Relatively stable clones, Percent *Lac*$^-$	Infected with F_{13}: Unstable clones, No. of clones	Infected with F_{13}: Unstable clones, Percent *Lac*$^-$	Infected with $F_{13\ stable}$, No. of clones	Infected with $F_{13\ stable}$, Percent *Lac*$^-$
Salmonella abony SW 803	1 day	1	0.5	11	4–50	12	<1
	5 days		1		>99		<1
S. typhimurium TM 2	1 day	0	.	6	1–8	6	<1
	5 days		.		98,99,>99		<1
S. java SW 1394	1 day	3	1	3	3–30	6	<1
	5 days		1–10		99		<1
S. miami SW 1395	1 day	4	<1	2	<1	6	<1
	5 days		<1		6,20		<1
E. coli K-12 W 4678	1 day	5	<1	1	6	6	<1
	5 days		<1		>99		<1

Purified F′ containing clones were grown in broth with daily transfers to fresh medium, and periodically plated on lactose-indicator media for counting the proportion of *Lac*$^-$ to total colonies. One day's growth corresponds to 20 generations.

observed from which F_{13} has disappeared, while most continue to segregate *Lac*$^-$ at a frequency of less than one percent, suggesting a stable equilibrium between the F′ particle and its host cell. In most Salmonellas on the other hand, F_{13} gradually disappears. Because it would have had ample opportunity of infecting new cells and spreading through the culture this would suggest that cells that have lost F_{13} remain immune to it, or that F_{13} multiplies more slowly than the host and is gradually diluted out. The first possibility is contraindicated since isolated *Lac*$^-$ segregants from such cultures are readily reinfectible with F_{13}.

Some clones of Salmonellas, as shown in Table 2, show more stable associations of F_{13}. In fact, *Salmonella miami*, which is also rather easily infected with F from

K-12, gives a majority of stable clones. One such was also picked in *S. abony* after 13 *Lac*$^+$ reisolations and subjected to further study. In this case the F_{13} seems to have been modified permanently: infecting almost any strain it would give a heterogenote in stable equilibrium continuously segregating *Lac*$^-$ at a low rate of about 0.5 percent. This F_{13} mutant was called $F_{13\ stable}$ and for infectibility study was transferred into suitable Salmonella and *E. coli* stocks.

F-infectibility of various enteric bacteria: A number of Salmonella, Shigella, Serratia and Klebsiella strains were tested with F_{13} and $F_{13\ stable}$ from K-12 and from *Salmonella abony*. As usual *Lac*$^+$ transfer was used as an indication of the

TABLE 3

F' · Lac$^+$ *infectibility of various enteric bacteria*

Recipient *Lac*$^-$		Donors *Lac*$^+$ *M*$^-$			
		1	2	3	4
		K-12 F_{13}	K-12 $F_{13\ stable}$	*S. abony* F_{13}	*S. abony* $F_{13\ stable}$
E. coli K-12	W 4145	10^{-2}	10^{-1}	10^{-2}	10^{-1}
Salmonella					
Group B					
abony	SW 803	10^{-6}	10^{-5}	10^{-3}	10^{-2}
typhimurium TM 2		10^{-7}	10^{-6}	10^{-4}	10^{-3}
TM 9-S^r-2	SW 1214	10^{-3}	10^{-2}	10^{-4}	10^{-2}
paratyphi B	SW 685	+	. .	10^{-6}	$>10^{-5}$
java	SW 1394	10^{-7}	. .	. .	10^{-5}
Group C					
bovis-morbificans	SW 753	+	. .	. .	10^{-5}
Group D					
enteritidis	SW 764	—	+	10^{-7}	10^{-6}
miami	SW 1395	10^{-5}	.	. .	10^{-2}
Group E					
london	SW 776	10^{-8}	. .	. .	$>10^{-5}$
give	SW 777	—	+	10^{-6}	10^{-4}
muenster	SW 779	10^{-8}	10^{-6}	10^{-4}	10^{-3}
senftenberg	SW 787	10^{-7}	10^{-6}	. .	10^{-2}
Groups F,G,I,etc.					
aberdeen	SW 790	10^{-7}	. .	. .	$>10^{-5}$
poona	SW 791	—	+	10^{-7}	10^{-6}
hvittingfoss	SW 795	10^{-7}	. .	. .	$>10^{-5}$
adelaide	SW 1338	—	+	10^{-7}	10^{-7}
Shigella					
flexneri	H 1	10^{-3}	10^{-2}	10^{-3}	10^{-2}
sonnei	SW 1779	. .	. .	10^{-3}	10^{-1}
Klebsiella	K 1	+	. .	. .	+
Serratia					
marcescens	SM 6	+	+	+	+
	SM 6-S^r-11	10^{-7}	10^{-7}	10^{-7}	10^{-7}
	SW 2745	10^{-6}	10^{-6}	. .	10^{-6}

Donor (100 parts):recipient (1 part) mixtures were plated after 30 min contact in broth on minimal lactose agar. Number of infected cells (=colonies growing) is expressed as fraction of recipient cells plated. If this test was negative, a prolonged time of incubation was used, and approximately 10^8 recipient cells were plated; the results in this case are given as + or —.

F′ infection. The results are shown in Table 3. In column 1 are given the frequencies of infection with K-12 F_{13}^+ as donor. Large differences in the infectibility of the various species are evident, ranging from 10^{-2} to less then 10^{-8} under the experimental conditions. Eighteen of 23 strains tested could, however, be infected.

With $F_{13\ stable}$ (column 2) frequencies are augmented 10 to 100-fold except for the Serratia species. Thus $F_{13\ stable}$ originally adapted to *S. abony* has an advantage in other Salmonellas and also in Shigella and *E. coli* K-12. The same advantage is seen as the difference between columns 3 and 4 where F′-infected *S. abony* was the donor.

E. coli K-12 can be compared directly with *S. abony* as an F donor: columns 1 and 3 *versus* 2 and 4 of Table 3. When *S. abony* is the donor, every one of the 23 strains tested can be infected with F_{13}. K-12 is equally well infected from either donor, but in all Salmonella × Salmonella combinations there is a difference of 10^2–10^4 in favor of the Salmonella donor. This may be attributed to a specific surface compatibility of Salmonellas in conjugation with other Salmonella.

A comparison of reciprocal crosses suggests a complex pattern of breeding compatibility: Therefore, the reciprocal infections were expanded to include additional donors (Table 4). The first two columns come directly from Table 3, the third column refers to *S. typhimurium* TM2 as the donor. This strain, which is widely used in transduction studies in combination with phage P22, is a very poor donor of F′ either in homologous or heterologous combinations. However, F_{13} is quite stable in TM2 in these conditions and one can only guess that the effectiveness of the F particle in altering the surface for male conjugal function varies from one background genotype to another. In the fourth column is represented *S. typhimurium* TM9-*S*^{r}*-2*, the recipient strain of BARON *et al.* 1959b, which is a good donor both to F^- forms of the same strain and to K-12. The same pattern is shown by *S. paratyphi B*. The Shigella strains were very effectively infected from F′ K-12 and Salmonella, as well as in the homologous combination (frequencies 10^{-3}–10^{-4}), while Serratia is very poorly infected in all combinations.

TABLE 4

F_{13} infection in homologous and heterologous combinations

	Donors: F_{13}-infected clones of							
	E. coli	Salmonella				Shigella		Serratia
Recipients	K-12	*abony*	TM 2	TM 9-*S*^{r}*-2*	*para-typhi B*	*flexneri*	*sonnei*	*marcescens*
E. coli K-12	10^{-2}	10^{-2}	10^{-6}	10^{-2}	10^{-4}	$-(<10^{-7})$	..	10^{-6}
Salmonella abony	10^{-6}	10^{-3}	10^{-6}	10^{-7}	10^{-6}	10^{-7}	10^{-4}	10^{-6}
TM 2	10^{-7}	10^{-4}	10^{-6}	10^{-7}	10^{-7}	..	..	..
TM 9-*S*^{r}*-2*	10^{-3}	10^{-4}	10^{-6}	10^{-3}	10^{-7}	..	..	..
paratyphi B	$+(<10^{-8})$	10^{-6}	10^{-6}	..	10^{-4}	..	..	..
Shigella flexneri	10^{-3}	10^{-3}	..	..	..	10^{-4}	10^{-3}	..
sonnei	..	10^{-3}	..	..	..	10^{-4}	10^{-3}	..
Serratia marcescens	10^{-7}	10^{-7}	..	..	..	..	..	$-(<10^{-6})$

Infection done by growing together (30 min) 100 parts of donor and one part of recipient, plated on minimal-lactose-streptomycin medium. Number of growing colonies expressed as fraction of recipient cells. Homologous combinations are in squares along a diagonal.

There thus seem to be three relevant genotypic statements for a given mating test: (1) female strain, (2) the strain which has acquired an F particle to become male, and (3) the quality (origin and history) of this F particle. To recapitulate, K-12 seems to be a universally good recipient for F infection; one is bound to recall that the F particle used in all these experiments originates in this strain. No universally competent donors have been found. Most strains are effective donors in a homologous combination and to strain K-12. Their ability to accept F from K-12 or from other species varies subject to alteration by complementary mutants like the aforementioned TM9-*S*^{r}-*2*. These can be selected for by crossing *E. coli* with the species in question. BARON has described these variants which are more fertile than the original population as "F$^-$ mutants" from an F^0 status (BARON *et al.* 1959b). However, this designation applies peculiarly to the reaction with K-12 as the source of F.

While more compatible mutants must be assumed to occur, they are not the principal factor in the frequency of F transfer. Disinfected *Lac*$^-$ segregants obtained from a number of F_{13}·*Lac*$^+$ heterogenotes of *E. coli* × Salmonella exhibit the same fertility as the original Salmonella strain. This was also true of *Lac*$^-$ segregants from *S. abony Lac*$^+$ derived from a cross with *E. coli* Hfr W1895 (Table 5).

Infection with wild-type F: There is no prior basis to expect different compatibilities of F from F_{13}, but more effective methods are needed to detect the F-infected cells. The presence of standard F need not always be manifested by observable fertility of the F-carrying strain in a new species, and, as in Shigella (LURIA and BURROUS 1957) may have to be demonstrated by transfer back to

TABLE 5

Fertility of Salmonella clones after a previous mating with E. coli *K-12*

Recipients	Frequency of *Lac*$^+$ progeny from crosses with donors: K-12 Hfr W 1895	K-12 $F_{13}$$^+$ W 3747	*S. abony* $F_{13\ stable}$$^+$ SW 1365
S. abony			
clone 1	. . .	10^{-6}	10^{-2}
clone 2	. . . .	2×10^{-6}	3×10^{-2}
Lac$^-$ segregant from			
clone 1 $F_{13}$$^{+*}$	. . . .	5×10^{-7}	3×10^{-2}
clone 2 $F_{13}$$^{+*}$	. . . .	10^{-6}	10^{-2}
clone 1 *Lac*$^+$†	. . . .	2×10^{-6}	10^{-2}
S. typhimurium TM–9–*S*^{r}–*2*			
clone 1	10^{-4}	2×10^{-3}	2×10^{-2}
clone 2	5×10^{-5}	4×10^{-3}	10^{-2}
Lac$^-$ segregant from			
clone 1 $F_{13}$$^{+*}$	2×10^{-4}	3×10^{-3}	3×10^{-2}
clone 2 $F_{13}$$^{+*}$	2×10^{-5}	3×10^{-3}	10^{-2}

* From a cross with W 3747.
† From a cross with W 1895.
Lac$^+$ progeny was selected on minimal lactose streptomycin plates after 30 min incubation of mating mixtures with a 100-fold donor excess in broth. Results are expressed as fraction of *Lac*$^+$ of recipient cells plated. As recipients we used two single colony isolates of *S. abony* and *S. typhimurium* each, and a *Lac*$^-$ segregant from *Lac*$^+$ derivatives of these obtained after crossing with either F_{13} or Hfr K-12.

E. coli. Unlike the F-associated characters which mark the F′ particles, standard F confers no advantage we might readily use to select a small number of infected cells. However, we have to rely on the contagiousness of F to gradually enrich for F^+ cells in mixed populations even though its rate of spread might be eventually limited (Table 2).

For the detection of F in new strains back transfer to *E. coli* was customarily used as an ultimate criterion and was greatly facilitated by the use of the $♀_3$ detector (RICHTER 1961). The color differential on EMB agar, which was more reliable in *S. abony* than in *S. typhimurium*, also was particularly helpful in detecting the segregation of F^- in F^+ clones. F^- could always be found to the extent of at least one percent in F-infected *S. abony*. The color test corresponded very well with other tests for F as repeatedly confirmed (Table 6). However, it can be easily confused with other sources of color variation (e.g. S→R (smooth to rough) shows a color difference in this system) and it cannot be relied upon for the diagnosis of F independently of other evidence.

Experiments on the transfer of F are summarized in Table 7. F is quickly transmitted from *S. abony* to the homologous recipient as well as to *S. muenster*

TABLE 6

Correlation of different tests for the presence of F

Stock		No. of colonies	Infective transfer to $♀_3$	Agglutination in Hfr serum 1:80	Agglutination in F^- serum 1:10	Staining on EMB		
			++	++	+	+	?	—
S. abony F^-		95	0	0	0	0	9	86
F^+		36	36	36	0	31	5	0
F_{13}		5	5	5	0	5	0	0
Hfr		1	1	1	0	1	0	0
S. typhimurium TM-9-*S*^{r}-*2*	F^-	7	0	0	0	0	7	0
	F^+	8	8	0	0	0	8	0

Single colonies from EMB plates, where the staining reaction (purple +, white —) was scored, were picked up in broth and grown overnight. Drops of these were tested by infective transfer to $♀_3$, and in tube agglutination tests. For details see Materials and Methods.

TABLE 7

Infection of wild-type F into various species

Time of mixed culture (number of transfers, each ≅ 9 generations)		Donor K-12 F^+ W 6				Donor *S. abony* F^+ SW 1364			
		1	2	3	7	1	2	3	7
Recipients									
E. coli	W 4145	++	++	++	++	++	++	++	++
S. abony	SW 803	—	+	++	++	++	++	++	++
S. typhimurium	TM 2	—	—	—	—	—	+*	+*	+*
S. muenster	SW 779	—	—	—	—	++	++	++	++
Serratia marcescens	SM 6-*S*^{r}-*11*	—	—	—	—	—	—	—	—

* Weak reaction.

Donor (in 100-fold excess) and recipient grown together in broth with serial transfers of 0.1 ml to 50 ml of fresh broth. F character of recipient cells reisolated at various times is tested by infective transfer to $♀_3$ (see Materials and Methods), recorded as ++ if majority of cells are F^+, as +, if less than ten percent are F^+, and —, if none of 500 cells tested are male.

and *E. coli* but only slowly to *S. typhimurium* TM2. However, the assay of TM2 F^+ might be hindered as already noted with F_{13}. *S. abony* is also infected from *E. coli* K-12 although more slowly than from the homologous donor. We did not demonstrate infection of *Serratia marcescens*. All in all these results agree with the data from F_{13} infection studies taking into account that wild-type F is less readily detected than F_{13}.

Properties of F-infected strains: Most of these studies have been carried out in *S. abony*; the male and female cultures have behaved rather as in *E. coli* K-12. The F^+ strain is infective at high efficiency to the homologous strain, less to others (Tables 4 and 7). F^+ can be disinfected with acridine orange but higher concentrations are required than with K-12 and full disinfection of the culture is not achieved (Table 8). In *E. coli*, female cells have been observed to move less rapidly than males (SKAAR, RICHTER and LEDERBERG 1957). Although the difference in motility is not impressive, it permits the practical selection of F^- by passing the stock through one or several motility agar columns of 5 cm. In *S. abony* the inhibition of motility by F^+ is much more marked, in fact, male cultures are usually very poorly agglutinated by antiflagellar antiserum (which does not bode well for the use of F-mediated crossing for studies of the immunogenetics of the H antigen). The agglutination can be restored by selection through 5 cm of motility agar, but the F character is usually lost at the same time.

ØRSKOV and ØRSKOV (1960) have demonstrated a new antigen on the surface of male *E. coli* by means of specific agglutinating antisera (F^+ or Hfr sera). This Hfr serum also agglutinates *S. abony* males to a titer of less than 1–100 (36 F^+, five F_{13} and one Hfr were tested). The reaction of these with F^- serum as well as the reaction of eight female strains with both these sera were negative in a serum dilution of 1–10 (Table 7).

The f^+ antigen could not be detected in F^+ *S. typhimurium* TM9-S^r-*2*.

Colonies of male strains also tend to be rougher than female ones. This has been described by MACCACARO (1955) for K-12 and has been our experience in *S. abony* as well. The effect might be partly explained by the selection of rougher

TABLE 8

Effect of acridine orange on F-infected coli *and Salmonella cultures*

Experiment	Stock	Fraction of F^+ cells after overnight growth with the concentrations of acridine indicated: 0	20 μg/ml	40μg/ml	80 μg/ml	160 μg/ml
I	*E. coli* F^+	140/140	20/456	9/306	2/201	growth inhibition
II	*E. coli* F^+	97/97	44/71			
I	*S. abony* F^+	158/158		80/89	29/81	
II	*S. abony* F^+	199/201	161/162	258/260		53/133
I	*E. coli* $F_{13}{}^+$	98/98	24/36	3/60		
II	*S. abony* $F_{13\ stable}{}^+$	782/795	165/169	88/106	60/161	37/116

All the cultures were recently infected with the F agent in question. They were grown from a small inoculum of approximately 10^4 cells overnight in 1 ml of nutrient broth pH 7.6 with varying concentrations of acridine orange, streaked out on EMSLac plates and replica plated on F indicator $♀_3$ *Lac*-S^r + F-*M*-*Lac*+S^r spread on EMLacSm; (for details see Materials and Methods). Number of colonies giving a + reaction on these plates is given as fraction of total number of colonies tested. Blank entries signify not done.

cells, which probably are more effective recipients (ØRSKOV and ØRSKOV 1961) but was also observed as an immediate consequence of F transfer.

F and F_{13} both make *S. abony* able to donate chromosomal markers to acceptor cells. The recombinants appear at a low frequency, about 5×10^{-7}, observed as 10–20 recombinants in a simple spot test (Table 9). Recombinants have thus been obtained for various auxotrophic markers, sugar fermentation markers and H antigen markers (linked to the histidine or methionine markers). Thus larger segments of the chromosome can be transferred than in phage-mediated transduction. Hfr variants showing a more stable attachment of the F particle to the *S. abony* chromosome can also be obtained. A more comprehensive account of such strains and their application to immunogenetic studies will be forthcoming.

DISCUSSION

With the use of a wide range of fertile males and more effective methods of detection, the scope of conjugal interaction among enteric bacteria has continuously increased. In the present report it has been possible to establish interspecies hybrids by means of a mutant fertility factor F′ in every one of 23 strains of Salmonella, Shigella, Serratia and Klebsiella tested. This experiment has been paralleled or anticipated by several other authors and will doubtless continue to constitute the basis of exciting work on the molecular basis of evolutionary differentiation. An outstanding example of the materialization of such a hope is the study of the DNA of intergeneric hybrids of *S. typhi* × *Serratia marcescens* (MARMUR *et al.* 1961; FALKOW *et al.* 1961). In these hybrids, the DNA pycnogram shows a new band whose density suggests that it can be attributed to the F fragment ultimately derived from *E. coli*, whose DNA has a different base composition and characteristic density than that of *S. marcescens*. In this particular hybrid combination the exogenotic material associated with the F particle appears not to have successfully integrated with the acceptor chromosome. A failure of integration is also indicated in ZINDER's (1960a) studies on phage-mediated transduction from *E. coli* × Salmonella hybrids, the genes of *E. coli* origin being poorly transduced to Salmonella by phage grown on the hybrid. It is often per-

TABLE 9

Fertility of Salmonella abony *F+*

Recipient strain	Marker scored	Medium	Number of colonies growing in drops with F⁻S^{s}SW 803 (=control)	F⁺S^{s}SW 1351	F⁺S^{s}SW 1463
SW 1361	*M*	DOSm	0	10	15
1355	*P*	DOSm	4	15	25
1464	*H*	DOSm	5	20	20
1464	*Gal*	EMGalSm + Histidine	0	10	8
1417	*Mal*	EMMalSm	0	15	15
	Ara	EMAraSm	0	12	15

All crosses were done by dropping approximately 3×10^7 of both recipient and donor bacteria from an overnight broth culture onto the selective plates. Number of colonies growing within each drop after 48 hours' incubation is given in the table, as mean values of 3–100 experiments.

plexing to determine whether, from a genetical standpoint, a stable association with integration of the genetic material into the chromosome has taken place or whether the cell remains heterogenotic. A good indication of integration would be the transfer of genes of Salmonella origin with equal efficiency as those of *E. coli* origin.

ØRSKOV, ØRSKOV and KAUFMANN (1961), concur in reporting the fertility of a wide range of Salmonella serotypes with a culture designated as W3287, K-12, Hfr. This strain is closely related to strain W3747 used in the present investigation and like it, carries the F_{13}-*Lac*$^+$ fragment (Table 1).

SUMMARY

Twenty-three strains of Salmonella, Shigella, Serratia and Klebsiella have been tested for infectibility by the sex-fertility factor, F, from *Escherichia coli* K-12. Large differences were observed in the ability of the various strains to be infected with F, due partly to differences in their ability to support the growth of F, partly and perhaps mainly to differences in their mating ability. Apart from the requirement for F-determined maleness of one partner specific compatibilities were observed in several cases, homologous strains showing the highest degree of F transfer and fertility. In addition, the F factors varied in their capacity to infect Salmonella strains and all 23 strains could be infected with a mutant F factor designated $F_{13\ stable}$.

The F factor introduced from *E. coli* confers on the infected cells very much the same properties of sexual compatibility as it does in *E. coli* K-12. In this way it is possible to obtain a complete sexual recombination system in *Salmonella abony* and other serotypes.

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