tion after about 15 min of the first session and that the final level was reached by the second or third session.

The third column of graphs is a check on possible artifacts in the results. Under the first procedure, the pigeon moved away from the rubber strip after every peck in order to go to the feeding device. Under the second procedure, only a small fraction of the pecks were followed by a movement to the feeding device. It seemed possible that the return to the rubber strip after eating caused a certain amount of variability in the location of responding. This factor would dominate the first procedure, but would be only negligible in the second. The third column of graphs is therefore based on only those pecks, under the variable-interval schedule, that immediately followed eating. Since there were 60 reinforcements in each session, this sample included 59 pecks per session. The distributions of responding in the third column show as much stereotypy as those in the second. It is therefore evident that the increase in stereotypy produced by intermittent reinforcement is due neither to the animal's being forced to move around in the situation nor to the large difference between the numbers of responses that entered into the computation of the first and second columns of graphs.

The duration of sessions under continuous reinforcement was about 10 min; under variable-interval reinforcement it was about 3 hr. This difference in time may have been responsible for the difference in variability under the two procedures. The distributions of responding during the first 10 min of sessions under variable-interval reinforcement make this explanation implausible. The amount of variability seen here was greater than that shown in the second and third columns of Fig. 1, but was still well below the amount shown in the first column.

This experiment strongly suggests that stereotypy is enhanced by intermittency of reinforcement itself and not by some essentially trivial concomitant of the change in procedure from continuous to intermittent reinforcement. Stereotypy is to be expected when there is no benefit to the animal for moving around in the situation. The principle of reinforcement predicts that a location that is reinforced early in the animal's exposure to the procedure would gradually come to dominate the distribution of responding. Moreover, the spread of responding in extinction is empirically, although not theoretically, expected. Why responding becomes more stereotyped during intermittent reinforcement does not, however, follow easily from our present state of knowledge. The clarity of the present findings indicates that a significant but unexplored principle may be involved.

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A Bacteriophage Specific for F— Salmonella Strains

Abstract. A bacteriophage is described which grows on recipient (F—) and not on donor (Hfr, F+, or F′) salmonella. The reason for this differential is unknown, since the phage attaches to all mating types, but progeny ensue only from female bacteria. Purification of this phage has been accomplished. A procedure is described for quantitatively determining either the acquisition or loss of the F agent by bacteria.

Loeb (*1*) and Loeb and Zinder (*2*) reported on a bacteriophage, f2, which would differentiate donor (F+ or Hfr) from recipient (F—) *Escherichia coli* K-12. The phage grows only on donor bacteria. Zinder (*3*) described a staining reaction involving the interaction of *Salmonella typhimurium* strains and *Escherichia coli* strains which could be used to differentiate donor and recipient *S. typhimurium* and *E. coli.* The present report describes a bacteriophage which grow only on recipient *S. typhimurium* strains and not on donor strains. Hereafter, donor strains are referred to as males and recipients as females.

The bacteriophage SP6 was isolated from sewage some years ago by Lederberg (*4*) as one that would grow on *S. typhimurium,* and it was in the collection of salmonella phages listed by Zinder (*5*). In the course of developing some bacterial mutants that were resistant to SP6 for use as a genetic marker in studies (*3*) of recombination in *S. typhimurium* LT2, it was noted that the phage failed to grow on the male bacteria. Since LT2 is female (*3*), as are all but certain of its derivatives, it was possible to compare the growth of SP6 on a whole series of male cultures for which the immediate female ancestor was available. With ten such paired cultures, SP6 grew with equal efficiency on the females but with an efficiency of less than 10^{-6} on males. The male set included eight F+ (*6, 7*) strains, one F′ strain (*8*), and one Hfr strain (*6*). The techniques for isolating such salmonella strains were described by Zinder (*3*). It was noted that when the phage was plated at high concentration on F+ and F′ strains (but not when it was plated on Hfr strains) there was some clearing in the background of the plates but in an amount insufficient to form discrete plaques. This is because F′ and F+ strains both contain significant numbers of F— "mutants." The technique for demonstrating this will be described later.

To verify further this correlation of phage sensitivity and mating type, the inheritance of sensitivity to SP6 was studied. The criterion used to determine mating type of the progeny of a cross was based on the staining reaction. Two crosses were done with the same pairs of selective markers, except that in one the male was an F+ and in the other an Hfr which had been derived from it. In *Salmonella typhimurium* as in *Escherichia coli,* the progeny of an F+ by an F— cross are generally F+, while progeny from Hfr by F— crosses are F—. The crosses were histidine-requiring males by a leucine-requiring female. Progeny were selected on the basis of nutritional independence. The progeny were isolated, purified, and then tested for the staining reaction and phage sensitivity. There was a perfect correlation in the F status of the progeny as determined by these two criteria. Ninety-five percent of the progeny from the F+ cross were F+, and only 2 percent of the progeny from the Hfr cross had the F agent.

Another test of the correlation of phage sensitivity with mating type could be based on the fact that with *E. coli* there is efficient transfer of F by the simple growth of F+ and F— organisms in mixed culture (*6, 7*). This does not occur with Hfr cultures. With

most LT2 cultures, however, there is little if any mating in liquid culture and little if any transfer of F (*9*). However, LT2 mates and transfers F well on solid media. After mixed growth of the appropriate cultures on solid media and reisolation of the two input types on the basis of some neutral marker such as galactose fermentation, there was again a perfect correlation of the staining reaction and phage sensitivity. The F+ and the Hfr culture transferred F, as would be predicted from their mating behavior; there was a high efficiency of transfer by the F+ and a low efficiency by the Hfr.

From the foregoing it can be concluded that the bacteriophage SP6 is excluded by the presence of the F agent.

It can be shown that SP6 does not attach differentially to male and female bacteria as does f2 (*2*). When young broth-grown cultures of male and female bacteria are prepared, it is found that SP6 attaches equally well to both strains. Both strains are in fact killed, but the males do not release any progeny, nor do they lyse. The female culture lyses after a latent period of about 15 minutes, and gives a yield of some 20 infective particles per infected bacterium.

Bacteriophage SP6 has been readily purified by ammonium sulfate precipitation (2*M*) followed by alternate cycles of high- and low-speed centrifugation. There are only minor losses in viability during the course of purification. Nucleases were added during the purification; after resuspension, the final high-speed sediment was dialysed against phosphate-saline buffer at *p*H 7.2. These purified phage preparations exhibited the typical absorption spectrum of a bacteriophage solution. The ratio of the optical density at 260 mμ to 280 mμ was 1.5 with an optical density of 5.5 per 10^{12} plaque-forming units. The diphenylamine reaction for deoxypentose as calibrated with calf thymus nucleic acid gave a value of 1.6 μg of deoxyribonucleic acid per 10^{10} plaque-forming units. No significant amount of ribonucleic acid, as measured by the orcinol reaction, could be found. SP6 has an amount of nucleic acid similar to that found in the T-even coliphages (*10*).

Phage-resistant mutants can be selected from the F— strains which retain their properties as females. These fail to attach SP6 at a measurable rate. Since the resistant mutants are not killed by SP6, this resistance can be readily differentiated from the resistance exhibited by male bacteria and, as such, can be used as a genetic marker in bacterial crosses. The gene-determining phage resistance segregates quite independently of mating type.

Bacteriophage SP6 does not attach to either male or female *Escherichia coli* K-12 strains. F— *E. coli* cells which are growing in mixed culture with F+ cells would be rapidly permeated by the F agent and converted to F+. In salmonella strain LT2 it can readily be shown that there is no detectible transfer of F in liquid culture. Therefore, barring selective growth of F+ cells, any losses of F would be maintained in their true proportion in the culture. Since SP6 kills both male and female bacteria, some indirect procedure is necessary to score females in the presence of males. The procedure is as follows. Male populations are treated as desired and then plated on eosin-methylene blue medium without any sugar (*3*) at a density of 2 to 300 colonies per plate, with as many replicate plates as deemed necessary. The plates are incubated until a colony some 2 to 3 mm in diameter has formed. The colonies are then sprayed with a solution of SP6 and further incubated for 4 hours. At this time the plates are screened for mottled and unmottled colonies. The mottled ones are phage sensitive and hence F—. The use of this procedure was verified by setting up mixtures with known numbers of male and female cells. Spontaneous losses of F, which were verified by the staining reaction and by behavior in mating, have been obtained. The average recently purified F+ or F′ salmonella culture has between 1 in 1000 and 1 in 10,000 F— cells.

There are now a variety of techniques, in addition to mating behavior, by which donor and recipient bacteria in the *Salmonella* and *Escherichia coli* group can be differentiated. The one described here sharpens the analogy between the F agent and a prophage. Prophages always impose on their host bacteria immunity to superinfection by the particular phage that they represent. In addition, they may often provoke immunity to unrelated phages. Note here, in particular, the exclusion by lambda-carrying *E. coli* of the coliphage T4 (*11*). Thus "immunity" need not indicate any immediate relationship between the carried and the superinfecting element. It would therefore be premature at this time to infer any such relationship between the F agent and SP6. Further experimentation may shed some light on this point.

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Partial Metamorphosis in Anomia simplex

Abstract. Many larvae of the common bivalve, *Anomia simplex*, when grown under laboratory conditions, exhibited a partial metamorphosis. They attained a considerably larger size than that at which larvae normally set. The partial metamorphosis was also characterized by the disappearance of velum, but the retention of a functional foot. Moreover, these organisms were not able to attach to the substratum, and their shells showed a distinct demarcation line between larval and adult portions.

The plain jingle shell, *Anomia simplex*, is a common bivalve of our Atlantic coast. In Long Island Sound its period of reproduction occurs virtually simultaneously with that of the American oyster, *Crassostrea virginica*, and during a large part of the summer the larvae of both these species, which to some extent resemble each other, are found in plankton. *Anomia simplex* is one of approximately 20 species of bivalves, the larvae of which have been grown from eggs to metamorphosis at our laboratory (*1*). An interesting observation was made during our studies of *A. simplex* of what appeared to be partial metamorphosis of its larvae and is described in this report.

Metamorphosis, or setting, of *A. simplex* larvae may begin when they are only about 180 to 195 μ long. More commonly, however, this event occurs