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

when the individuals are approximately 200 μ long, although the largest swimming larvae found in our cultures measured 215 by 210 μ.

Normally, larvae of *A. simplex* approaching metamorphosis drop to the bottom and begin crawling by means of a strong foot, which is developed during the late swimming stages. Upon finding a clear hard area, such as an oyster shell or rock, the metamorphosing organisms attach to it by means of a special byssus that later becomes calcified. From then on a young *Anomia* is unable to move, and it soon completes the process of metamorphosis which, in part, consists of a resorption and disappearance of the foot and velum. Meanwhile, well-developed gills become visible through the semitransparent shell.

In our cultures, however, we observed hundreds of individuals, which were considerably longer than 215 μ, but which were, nevertheless, still crawling on the bottom by means of a ciliated foot. The largest individual, which possessed such a well-developed foot but no velum, measured 577 by 514 μ. All these animals had certain features in common, namely that, although the gills were already well formed and the velum was entirely absent, the powerful functional foot was still present and the animals remained unattached. The last two conditions clearly indicated that the young mollusks had not yet entirely metamorphosed.

Closer examination of these individuals showed that they all had another peculiarity in common—the presence of a definite narrow band in their shells which, apparently, indicated the edge of the prodissoconch, or larval shell, and the beginning of the dissoconch or post-larval shell (Fig. 1). The dividing band was probably formed during an important event in the life of the young mollusks, possibly at the time the velum was resorbed or when some other equally important anatomical or physiological change occurred. Measurement of the inner, prodissoconch, shells surrounded by this band usually gave a length of approximately 200 μ, the size at which setting or metamorphosis of the majority of normal larvae occurs. This older portion of the shell, surrounded by the band, was usually somewhat darker than the outer, more recently formed portion.

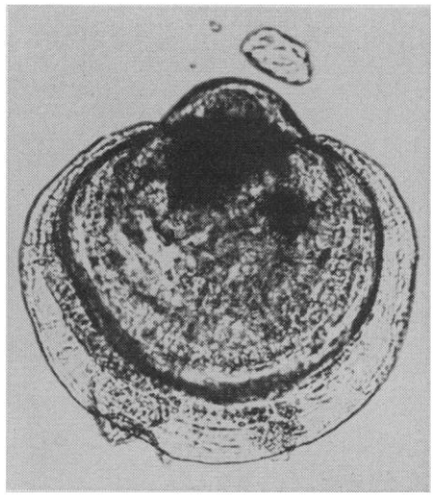

Fig. 1. Photomicrograph of young *Anomia simplex* during the stage of delayed metamorphosis. A definite line dividing prodissoconch and dissoconch portions of the shell and part of the protruding, still functional foot are visible. The size of the organism is about 255 μ.

Fortunately, we also observed several recently attached individuals which, before setting, had passed through the stage of partial and delayed metamorphosis but which, nevertheless, finally succeeded in attaching to the substratum. In all cases a formerly large foot was in the latest stages of resorption, thus indicating that these young mollusks were completing an abnormally prolonged metamorphosis.

Several reasons may be advanced to explain why some individuals could not normally metamorphose at the proper time. One possibility is that either the absence or presence of some substance in the water, in which the larvae were grown, interfered with the normal progress of metamorphosis. The second, and perhaps more probable explanation, is that the normal setting of larvae was delayed because they could not find the proper substratum for attachment. The importance of such environmental factors, often affecting settling and metamorphosis of many invertebrates in aquatic communities, was well reviewed by Wilson (*2*).

In some of Wilson's early experiments it was clear that the condition which caused the delay in metamorphosis of larvae was the lack of sand on the bottom. Wilson suggests that phenomena of this kind may be general, and the choice of the substratum on which the metamorphosing organisms wish to settle depends as much on its microbiological characteristics as upon its chemical and physical characters. Since, in our experiments, all the vessels in which the cultures of *A. simplex* were grown were of glass, perhaps the smoothness of the material and its composition did not offer the necessary chemical or physical stimulus for normal metamorphosis. However, although the explanations are plausible, they do not appear to be well based because they fail to explain why only certain individuals in the cultures behaved in an abnormal way, while others, usually the majority, metamorphosed normally at the expected size. Nevertheless, our observations of partial metamorphosis of *A. simplex* suggest many interesting experiments on this and related species that may help us to understand and explain certain aspects of the importance of various chemical and physical factors in the environment, especially of the substratum, on metamorphosis of lamellibranch larvae.

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8 March 1961