January 26, 1955

Dear Bruce:

Hallo! I hope you are going to be patient with me about this manuscript. It is no exaggeration that I have dropped everything else abruptly in order to concentrate on it, and try to resolve my ambiguous thoughts about the problem.

Let me say to start that my own experiments, with —x SW666 almost exclusively, papallel your results quite closely. I have not been so fortunate as you, however, in being able to trace the descendancy of "E" cells over many generations, as you have. This must be due to the rather lower incidence of "E" cells in this system, and to what seems a rather lower number of motile progeny, on the average. What data I do have (I've already sent you some) do indeed support the unequal distribution of such progeny among sibs, but some of the numbers are on the shaky side of 10. [At least for now, do let me speak of a polycatenate = E, oligocatenate for the cells or clones with fewer motiles and (uni)-catenate for the strict "unilinear" case]. Most of my earlier experiments were devoted to studying the life expectancy of single chains, for example one was followed to the 59th (sic) generation after its initiation, which was certainly <u>not less</u> than 45 generations after its separation as a single chain. That is, I was primarily concerned in getting as rigorous proof as possible of the particulate and non-reproductive character of the "<u>m</u>otility-<u>c</u>onferring-<u>p</u>article"; this may be taken as amply settled on both sides of the Atlantic. In my experience, however, I have almost never seen further increase in the number of chains after about the 13-14th generation, which is simply to say I had never caught the polycatenate cell among the numerous chains already produced.

There are just two questions I am not altogether happy about: is there actually an increase in the numbers of the mcp's, in accord with your hierarchy of primary and secondary chains, or is it still possible that all the mcp's are already formed in a polycatenate cell, and are then distributed albeit non-randomly at successive divisions; and, how certain is the correspondence between polycatenate and trail-forming cells? As to the first, I am not greatly bothered by the disproportions; owing to the small numbers involved there is no good evidence that the apportionment from <u>oligoc</u>atenates is random either (how are your data on this?; my own offer examples like 6:1:0:0 ; 7:1 ; 7:1:1:0 ; 7:4:0:0, which should be none too frequent on a random basis.) And, indeed, I am rather more sympathetic than you to Bisset's notion; some time ago I had done some experiments on TZ-labelled cells which suggested that they regularly grew/from the pole opposite the TZ granule, which accounts for (and divided) the subpolar position being maintained. In any event, it is too uncertain that the mcp's are instantly flagella for our observations to be decisive. Concerning the trails, I would not yet reject the role of accidental factors, as you state it at the bottom of 4g - 4h, and I think it will be necessary to get more direct proof that unicatenates are not, and polycatenates are, able to form trails. In your experiment, is the Poisson distribution applicable? That is, was the number of motile cells per drop uniform or normally distributed, etc. What was the number? I have been transferring drops with just one cell each and have about a 50-60% successful recovery, but as told you before, virtually no trails.

(Index Nos: for
conv. reference)

Bruce, I would like you to bear with me on two possible courses, which I should like to try simultaneously. One is a terser account, to be considered
9) preliminary, and designed say for the Proc Nat Acad Sci US,(where it can be published within 10 - 15 weeks) where we can summarize our accordant data, and emphasize especially the unicatenate end of the story (which is the most interesting to my mind, perhaps because least speculative). You can then follow this up at your own convenience, and feel no hindrance from my part. Alternatively,
10) I will try to continue some more experiments, including a look at your own —x SW541 material, for example, to try and convince myself more definitely of your choice among the alternative interpretations, one way or the other. I have in mind particularly to look more closely at trails and the chemotactic and physical factors that might be involved. Since an accidental experience
11) with phenol some time ago, as I may have written, I have no doubt of the importance of tactic behavior, be it "apobatic" or "strophic".

One other question: one way of correlating trails, swarms, and x-catenates would be by considering the frequency of swarms as a common denominator. I think
12) you will agree that every cell that engenders a motile clone should be detected as a swarm. Have you detailed data on the ratio of trails: swarms on one hand, and poly-: oligo-catenate: motile clones on the other? I have to collect my own scattered pedigrees on this point, but offhand I would judge that they <u>do</u> agree
13) with your notion. I think I should like to take another look at —X SW967, which forms such beautiful trails.

Query? Have you any more recent quantitative data on incidence of swarms and trails per phage? In your "report" you estimated, for TM2 —x SW541 an efficiency of better than 10^{-4} ! I don't recall any direct comparison; this must have been what impelled me to make SW-665 (= SW541 Xyl$^-$), transduction to which proved to be only the usual, about 10^{-6}, for Xyl$^+$

Do you account directly for the small incidence of polycatenates among the initial isolations? Do you think the others are all oligocatenate <u>sibs</u>?

Just to be the devil's advocate, let me propose an alternative version(s).

1) in re trails, that owing to the lack of a chemotactic impulse, most motile cells are content to swim and grow on the surface. Very few start a trail, and those that do only after all of the motile cells are unicatenate. Ans: if trail-formers are not distinctive polycatenate cells, it should be possible to modify the incidence of trails either by manipulating the medium, or with chemotactic pressure. Will do.

2) in re polycatenates:"let us not multiply particles without necessity". We can readily presume that, in transduction, some fragments implant; others do not and are lost. Meanwhile, even in the latter (which might be expected to be most frequent) the gene has left its product. The product is not particulate (necessarily) but is sooner or later assimilated into particles (flagella). The product is, however, not soluble and is disproportionately distributed. [Even simpler would be the accumulation of flagella themselves, but it may be awkward to think of accumulating as many as 100+ flagella. What do you think? What is the maximum number of chains you have observed?]. If you like, the intermediate product (essentially equivalent to your "E particle") might be an enzyme which could function only when present above a certain threshold amount.

1) and 2) are not directly dependent on one another. If I may state a general outlook on the problem, it would be possible to postulate any number of elements in the path from gene to flagella or motility; the genetic literature is

full of hypothetical hierarchies, and you can take your choice whether the intermediate elements do or do not themselves replicate (cf. Sewall Wrights reviews, Amer. Natural., 79:289; Ann. Rev. Physiol. 7:75; Physiol. Rev. 21:487, and Spiegelman's fantasies in CSH 1946 at p.271). The present case is unusually simple in some respects, but we have an unknown numbers of parameters in the way that the transduced fragment might function. I want to distinguish, if we can, between what is reasonable, and what is reasonably certain.

If I can find the time, on top of the two "courses" supra, I will try to set up another tape by way of verbal commentary on the ms. Perhaps one reason for hesitancy in using the tape is that I did not know whether you could conveniently listen in private, as some comments are likely to be designed as more intimate conversation.

Yours,

Joshua Lederberg