March 30, 1955

Dear Bruce:

I am afraid that despite having focussed all my energy on trails for the last three months (not to mention what I had spent last year) that I have not been able to come up with anything more than negative results, that is findings which do no more, to my mind, than make it still impossible to choose among any of the interpretations. There has been a by product of some technical improvements, however, which may eventually help bring a more definite solution. These would include the almost trivial expedient of concentrating the treated bacteria, which makes the rapid isolation of numerous motile initials very easy, and pour-platings of numbers of initials, or of single clones.

The principal negative finding is that, for a sample of a given collection of initials, the incidence of trails depends directly on the concentration of the motility agar. This seems to me to lead directly to the notion of an accidental determination of trails/no trails. I would agree that it is likely that a many-chained clone has a greater likelihood of producing a trail: if you look at a dispersion of ###/ Fla$^+$ bacteria in motility agar, you will see that a large proportion of bacteria are enmeshed, while others swim more freely in the appatent interstices. If an initial produces several motile offspring, there will obviously be a higher chance that one of them will be able to swim free. I have not noticed any consistent differences in motility that would help distinguish many-chained from single-chained cells, but I have not had much difficulty of the sort you mention, of immobilization on the beach. (When a cell gets stuck, I have found it easy to dislodge it by prodding the cell not with the capillary tip, but with a droplet of oil pushed in and out against the cell). I have to do more experiments on the quantitative variation, but the following table will illustrate the effect of diluting the motility agar (standard .4% agar, 8% gelatin) with nutrient broth. (The plates were incubated about 8 hours, then brought to room temperature to limit the extension of swarms). The input in each case was 0.1 ml of a suspension of 500 motile initials /### (probably closer to 550, since not every collected cell is necessarily counted in a rapid harvest) in 1 ml broth.

Medium	single cels.	clusters (2-5 or 10)	trails	swarms	total
MGA standard	53	2	2	2	59
70% + 30% broth	39	18	11	3	71
60% + 40% broth	19	11	19	3	52

In other experiments, I have had anywhere from 50 - 95% trails per total viable cells, while standard MGA has generall given from 5-15%. In the more dilute agar, the trails are not only more numerous, but individually more prolific, becoming more inflorescent than linear. I am very doubtful that one can establish a reasonable standard for a "trail-former" when the incidence is so dependent on the details of the medium. Platings of single clones have given a similar picture, with several instances of 2 - 7 well developed trails per clone (in dilute agar), which is only a corollary of the above.

Evidently, the pedigree analysis will have to be done the hard way. I am concerned that the expressivity of motility is so variable, especially whn the clones resume the lag-log phase, during which they tend to become more sluggish as they enlarge. I have in mind to look for modifications of the medium which will facilitate the diagnosis of motility. For example, in staled broth, TM2 has a very erratic behavior, with frequent stops and reversals, while in fresh medium the same cells move in a more persistent dignified manner, changing course usually only after a collision. It is not a pH effect; I don't know what it is. I only mention this to indicate what may be possible; the particular observation is not of much use, though I think a larger fraction of cells do score as motile in the staled medium.

At any rate, I must confess to some uneasiness in working under the tension of your own discomfiture at being held up. I do fully sympathize with you in wanting to get an account off our respective chests, but my own conclusion is that a definitive publication would be ill-advised right now. Rather than continue in haste to push for a definite answer, I revert to a previous proposal to write a shorter preliminary account which covers what we would agree on together. It will then be for either or both of us to stick out our singular necks. I am preparing a new draft, and will send it (and some illustrative photographs) at the earliest opportunity. (Unfortunately we ran out of gelatin about 2 weeks ago, and have not yet been able to replenish. Difco remains the only staisfactory brand; I don't know why, though for many purposes the medium without gelatin is satisfactory). I still think that the Proc Nat Acad Sci would be the best vehicle for this kind of paper; there will be no difficulty in getting it in, and since the work was firmly founded on your phenomenally productive, if brief, stay here, why all the more reason! I am afraid that the JGM reaches very few geneticists, and Genetics , J. Genetics, Heredity all have too strong an atmosphere of definitive archives. Nature and Science, on the other hand, should be for even more discursive accounts. J. Bact. would not be out of the question, but PNAS seems to me just about right. Would Proc Roy Soc be entirely out of the question, or is it difficult and time consuming to publish there? Anyhow the substance is more important than the vehicle, so now, my own notions having been better fixed, I will get to my own part.

In re terminology, I have so far had the most enthusiastic response to "chains" and "catenate" (as I hope I mentioned, these are not my own invention, but J. Crow's). Several have suggested sticking to the English chain in preference to Latin catena, whichi is all right with me, if expressions like chain (or chainly) inheritance, and single, few-, many-chain(ed) cells will go. The English adjectives in ly or ed tend to have a narrow reference than the Latin in ate, etc., but there is no real reason for this. The most sensible adjectival form is the same as the noun, chain, but there are too many pedants who object to that much flexibility.

Since I burdened you with the news of Mr. Wright in the first place, I should give you the better picture we have now. After remaining comatose for two weeks (sic), he then made a sudden and dramatic recovery. He walked out of the hospital yesterday, is improving considerably in compensating for residuals (principally left arm and hand) and ought to be back at work this summer. He may still have a difficult adjustment, depending how far his (now) monoplegia recedes. In all, he has come out so far incredibly better than we had had any reasonable basis to hope.

With Esther's best too,

Yours sincerely,

P?S. You did promise to send me a more detailed argument on the transmission of E in the pedigrees