April 11, 1955

Dear Bruce:

I am just in the middle of manuscripting, but hasten to answer yours of the 6th. I finally got our 35mm camera setup to work smoothly, and am enclosing a (duplicate) negative which illustrates the plating of a single clone in MGA (motility gelatin agar, standard) diluted 40% with Penassay, and incubated about 16 hours. You won't be able to count all the colonies in the trails, but the two largest had about 150, 100 respectively (sic). I think this answers what you asked for; the result is not at all unusual for these platings, but this happens to be about my best picture.

However, I do not think this is a very strong argument. It may mean that each of two subclones was plurbicatenate, but you already postulated that the sib of an E cell might give up to 10 chains, which could amply account for the trails of this magnitude. So, don't misunderstand me, I do not think to have critical evidence against two orders of chains. But I hold that it is very difficult to get decisive evidence for it. Unless you have some reliable technique for always finding the E cell in a clone, you can't be sure that there will be no more than 1. For a time you held that the platings in MGA identified the unique E cell, but this is hard to insist upon in view of the effect of diluting the agar. Even in platings in MGA, of single clones, I would find every transition between clusters and trails; an occasional clone that gave more than one trail (arbitrarily categorized) might be ascribed to accidental variations in the fluidity of the agar. So I don't think this line of evidence is crucial, and the two orders of chains remains, in my mind, one of a few plausible hypotheses.

Perhaps your pedigree experiments are more decisive, though it is hard to see how they can distinguish between a usual and invariable disparity in partition of chains among subclones without a technically unfeasible amount of work. I will send my own draft by, I hope, the end of the week for your more detailed comment.

As to trails in H_1 tranductions, I must have mentioned (or did I) at one time having seen them in ^{1} S. abony —x S. miami (in presence of anti a, 1,5). This was almost two years ago, but I have not noticed them again (and it might be tricky to, in view of the usual slight spread of the recipient) nor followed them up. It may mean that only one antigen is ever expressed at one time, even in a temporary heterogenote. You may redall that in the duplication stock (CDC 157), which is $H_1^b\ H_1^{1,2}$ (sic) only one of the H_1 loci is expressed at any moment; the same is true for various derivatives, e.g. $H_1^a\ H_1^c$, $H_1^b\ H_1^i$. The trails may be H_1^b frag,/ H_1^a cells in which the fragment is controlling the phase.

Have I sent you the squibs enclosed? Iino is putting some steam again on the phase variation story.

I'll write again in a few days when I've done with the ms.

Yours sincerely,

Joshua Lederberg