March 28, 1954.

Dear Bruce:

Thanks ever so much for writing so detailed an account as your letter of the 16th. You may have found my last letter (March 1) rather puzzling, with its account of some of the micromanipulations here, if you did not keep in mind that the last I had heard from you on these was several months ago, and at that time all you had to say was that there was an apparently irregular pattern of replication. Reading your most recent letter, I am in fact astonished (and delighted) at how closely we had converged, on such technical features as the trapping-drop for isolation, all the way through the experimental details, and finally including the "polyteny" interpretation! The latter is especially gratifying, as I thought this at first a rather wild idea, but if we have both fallen into it, essentially independently, there may be something in it.

As I see it, irregular replication can of course account for the results in an ad hoc way, but does not seem to suggest any further directions, and if for this reason only ought to be adopted as a last resort, I can see no occasion for phenotypic lag in the usual sense, if only that observations on most semiclonal lines show an immediate and precise delineation of motile from non-motile daughter, and because the apparent carryover is seen only in early, never (?) late generations. If the multiple semiclones result from first division, i.e., partition rather than replication, without multiplication, we can then enquire: what is being partitioned? Ex hypothesi, the particles do not reproduce in the transformed cells. Were they all genes in the donor cell, the reproductive capacity having been lost as an accident of transduction (abortive transduction hypothesis) or were they non-reproducible particles in the donor cell itself, i.e., the hypothetical immediate products of gene action. The latter notion might be more easily reconciled with pedigrees like le-99a etc. I.E., I would expect the polytenic status to be more or less uniform from one donor to another, and not to be profoundly disturbed by transduction, and not to reach immense values, while the extent of accumulation of particulate, non-reproductive gene products might be expected to be less uniform.

Like yourself, I have had a few rather disturbing pedigrees showing either very many semi-clonal cells (~ 100), or division occurring quite late in a given pedigree (my latest is not earlier than the 14th, while yours must have been in the neighborhood of 20 to 25); on the other hand, I have carried one semi-clone to the 59th generation, in a pedigree where no further division was seen after the 10-14th generation, so there is certainly a clear distinction between the early and late behavior of the "particles", so clearcut as to seem somehow contradictory to the idea of irregular replication. I admit both processes may operate, but do not like to multiply hypotheses, and if we admit this it can shoulder the whole burden itself. But like yourself, I have found most pedigrees to show quite limited "replication", generally completed well before the 10th generation, and often much earlier. So there is actually very little disagreement between us on experimental findings; I had no idea you had gotten so far, and it is quite startling to see the concordance,

The only point where I do depart from your account is the segregation of non-motiles from swarm-equivalents. I do not have very many of these, as they are quite infrequent in my material (and none yet have turned out to be the complementary cross-overs I was initially searching for), but at least half the "swarms" have been associated with non-motiles. I think the difference might be due in part to my use of lag phase cells, from which motiles appear in about 2 hours at room temperature, i.e., at about the first fission. My material is now entirely SW-543 --x SW-666, and I have had very little trouble with lysis, etc. The lower temperature may have something to do with it. SW-543 x-- SW-666, on the other hand has been very discouraging: low yields of motile individuals, and low viability of them when found. The other thing is the single instance

not yet repeated, of a motile clone and numerous semiclones from one individual. If this should ever be found from a late generation, we might have to accept the irr, repl. hypothesis after all, and would certainly have to reject "gene products".

In re mapping, I understand your argument now. I don't know what to think of the first postulate, that an overlapping entire segment is always transduced in the first instance: these experiments may provide the evidence for it. Larry and Esther have been setting up various trials on this point with the K-12 transduction, but no decisive results. At least a fair fraction of the lambda particles would, however, have to carry at least two loci, judging from transductions to double Gal- mutants, but whether this is true of most or all is not yet settled. Mapping is, if anything rather more difficult here (on the transductions, not the recombination analysis) owing to the interim "heterozygous" condition.

In your analysis, you are I take it ignoring double crossovers completely. I wonder if they would not complicate the picture, especially for the qualitative approach. I think it still <u>absolutely essential</u> to make quantitative measurements on the incidence of single and doubles, although one cannot, of course, directly compare different systems (Cf., e.g., SW-666 x-- TM2, and SW-666 x-- SW-623). The comparisons of Fla_x^- --x... with Fla_x^+ --x... seem to me by far the most reliable evidence, if done quantitatively. Without counts, once cannot assess the statistical significance of the assertion that 543 --x 28 gives no singles, as compared with $543Fla^+$ --x 28. But a story like this often hangs on its whole consistency as much as on the rigor of its details.

As to publication, you can of course quote anything you like that may seem useful. But I can hardly join in the authorship of your projected paper on the mapping, as I have done nothing <u>constructive</u> on it that is not already in print. But may I make the counterproposal of such an arrangement for an ultimate definitive account of the cell pedigrees? I am sure both of us will want to talk about it to sharpen our ideas; I hope before we go too far out on the limb that one or both of us can think of some more decisive experiments to choose among the hypothetical explamations. If nothing else, it seems to me that the concordance of results on several systems has been indispensable.