

May 16, 1954

Dear Bruce:

At last a tardy and hasty reply to yours of the 20th ult., which I found on return from Oak Ridge. Your letter and the discussion there provoked a review of notes and some new experiments, and some new results with coli (see later) were particularly distracting, to delay this reply. I trust you will have returned from your vacation, (which was another reason I did not reply more urgently). I will try to go over your letter point by point or I will never get on to all of it.

20 - 22°

i): my experiments are, of course, various combinations of short and long intervals of growth and separation. Sometimes I let the initial cell go through about 15 generations before separating the descendant motiles, in others the clones were dissected early or middle or late. I had been trying to get the whole Gestalt before inquiring into details. Chambers at room temperature, occasionally refrigerated (no obvious harm or change) or incubated for an interval at 30 or 35 C. ii) Same method as for original isolation of motiles. A large empty drop is placed and fused with the large clone, and this stirred somewhat with the pipette. Motile cells almost invariably work their way into the trap drop. (My note on simple method has nothing whatever to do with pedigree experiments, but is just an expedient for getting single cell cultures for "pure line" work without much fuss). iii) My starting material consists of overnight 37° Penassay cultures mixed 1:1 with lysate or diluted lysate in same broth. My summary shows that of 11 initials yielding swarms, 4 ~~were~~ gave mixed clones. Of these 4, 2 could be told, and did, give semiclones as well as clones (but only 1 and 2 respectively). I am convinced that the paucity of mixed progeny is due simply to the pedigrees usually starting only at the 2d or 2d generation, and until this problem can be circumvented, there may not be much point in collecting more data.

p.2 I am pretty well agreed with you on interpretations, but do not put much weight now on macroscopic observations. I am convinced that chemotaxis plays an uncontrolled role in the initiation of visible trails. Some diffusion of cells in agar is quite possible, with or without phenotypic lag, but I agree there is the appearance of clustering suggesting some delay (though this is not obvious microscopically). p.3 No cases here of swarm from trail. Could yours have been spontaneous reversion? Tracy Bonehorn also suggested continuing activity of non-replicative gene, which fuses two hypotheses. Hotchkiss also raised question ~~that~~ about the motile cell of a dividing T cell remaining motile, which has been my experience. This means either a very prompt response of phenotype to genotype, or an identification or binding of the particle to the flagellar apparatus itself. p.3-4: as to this test (distribution of numbers) my data are not too well arranged for this, and I have little enough information on the most spectacular cases of multiple semiclones; these are so rare that one can imagine almost any rules for the parcelling out. Certainly it is irregular: one could never get even a 20:0 or 20:1 split at the first fission on a random distribution basis, but this might mean merely a splintering from the large bundle (on that notion). Here are some sample data on distributions (each parenthesis = 1 initial; split among subclones as indicated, i.e., number of semiclones in each): (4:8) (7:13) 30:6 (100:2) (0:0:0:0:1:1:1:2:3:4:15) (0,8,6,1) (1:(20:11 subsibs):1:1) (0:0:at least 10:100) (4:6). Other clones ~~were~~ dissected early had only a few odd semiclones. But the important clues may be in the missed first divisions, which might also account for ~~sp/wavy~~ such a disparate distribution of semiclone yields per apparent initial. I think especially important would be cases including swarm-equivalent progeny. My comment on serum inhibition was correctly quoted, but my serums may not have been clean, and this was not well controlled. Your findings noted.

. the instant I am still somewhat distracted by some writing, but also by
2 conjugations. The trick, learned from Salmonella, is to use Hfr motile X
- non-motile. Pairs, in numbers sufficient for incidence of recombination, are
not too difficult to find, and trap, by virtue of their erratic motion, They
generally look like: disjoin after 1- 2 hours, at room
temperature, with up to one intervening fission. The majority of F- exconjugants
yield recombinants + parent. Main trouble: fragility of the pairs-- many of them
suffer from handling in transfer and are likely to die if forcibly separated too
early. But at best, both exconjugants survive.

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*Yours,
Ed. James*

P.S. The Oak Ridge meetings were mildly interesting, but rather misdirected: everyone, including myself, was discussing crossing-over in some molecular terms or other, and ignoring the certainly more complex structure of the chromosomes (which undergo crossingover) altogether. I saw Jim Watson again for the first time since he graduated from Illinois-- he has changed somewhat, but gave an unexpectedly reserved (in light of rumor) sort of discussion of DNA structure. The necessary implications for replication, mutation, crossing over, etc., are almost nil, and he was, I think wise, to give a straightforward account emphasizing the probably sound at the expense of the obvious (and probably deceptive) speculation. The DNA molecule is far from the chromosome, though the phagocytists are especially tempted to extrapolate right away. Mostly, the meeting was too concrete: there is a tremendous amount of existing information on C-O and it would have been wiser to assimilate this than add a few indecisive fragments. Hotchkiss gave an account of his interesting linked transduction (Dtl - 3) which seems entirely convincing, especially after the backcross data. There was still too much emphasis on DNA molecules, rather than genetic fragments-- Lerner (Buck's lab) is a priori convinced he'll have no trouble fractionating the different DNA's, but he doesn't seem to have done it yet.

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