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My dear Professor,

I must apologise for the delay in answering your letter ; I hope this has been of no inconvenience to you.

It is rather sorry to resign a post from a Department like yours; but both financial reasons and the changes which have occurred in my family in the meanwhile still keep me home. I am not in a too bad position here, but I feel sorry to have lost the permanent opportunity, which I had in Cambridge, of your advice and help. Shall I write directly to the Secretary of the General Board or to other officers of the University about my resignation ?

As to Cynthia, I should be extremely glad to have her here, as long as she would like to be; but in order to be able to do this, we may need your direct help. The present difficulty is that the Institute being in some financial troubles, fellowships for guests have been abolished, shortly after I came to Cambridge. I Hoped there might be exceptions to the rule but it does not seem likely for this year. We still accept of course guests but the difficulty is to find the money for living expenses. I am trying other ways ; probably the surest would be to obtain for her grants through one of the usual channels, bur for this she would need I imagine your direct help.

I should very much like to have your opinion on an experiment which I have made to test the problem of the origin of resistant mutants. It is described in the enclosed sheets and photograph. I shall give a paper about it at a Symposium in Rome end of this month, and at which Hinshelwood is expected to take part.

The other photograph which I am enclosing is only sent because you may like it; it seems to me a nice pictorial representation of a latin square. The experiment was a bioassay curve with six different concentrations of vitamin B 12, arranged in a latin square on a large agar plate seeded with a B12 deficient mutant of E.coli .

Re the K-12 work : the programme of the latin-square-designed crosses is being carried on as follows. A TLB_1- strain carrying several sugar deficiencies was made streptomycin resistant on one hand; on the other hand it was by successive selection of spontaneous T+, L+, B_1+ mutants made prototrophic (TLB_1+). The two

strains can be crossed together on minimal agar + streptomycin. By back mutation of the sugar fermentations (which more easily accomplished than with any other type of marker), without mutagens) I am now obtaining the necessary sixteen strains. Six strains for three complementary crosses have already been secured. The difficulty, yet unin-vestigated, is whether the socalled back mutation for sugars is a change occurring always at the same locus.

I have not yet clear enough ideas in my mind to tell you about the biochemical basis of polygene action. I have carried somewhat further the chloromycetin story and shown terramycin resistance to behave similarly to it, with the addition that there is some cross resistance to chloromycetin and terramycin. But two major problems concerning chloromycetin remain unsolved: how two independent first steps add up their resistances, and the reason for the negative interaction observed in crosses between high-resistants. As to the latter question, all I can say is that they often are very poor growers; and that on crossing them to sensitives no clear cut higher resistance is obtained. I should like to know more, if possible, about these two questions before Mac and I send a paper to Heredity about it. I am told DDT-resistant flies pose some problems not far from these.

Yours sincerely

Luig Cavalli-

ABSTRACT FROM A PAPER TO BE GIVEN AT A SYMPOSIUM ON " GROWTH AND GROWTH INHIBITION " ROME~~XT~~ END OF JUNE.

Ten 5 cc. broth cultures are inoculated with 1000 sensitive cells of E.coli #30 and incubated to saturation of growth . They are then centrifuged, the sediment is collected in 0.2 cc of saline and the whole amount of each culture is halved, each half being plated on chloromycetin-agar containing 25 micrograms/ml of the drug. The number of cells per culture was 1.8×10^{10}.

The two plates; A and B obtained from each culture are incubated and colonies counted after 24 and 48 hours. Data are given in table I, first five columns, and show an obvious correlation between numbers of resistant mutants in plates from the same culture, which can be tested by the ratio of the variance between cultures to that within cultures, giving $F = 14.6$ for 9/9 df, which is significant at the .1% level. These data are for 24 hours only; at 48 hours ~~there are~~ two plates ~~which~~ were not counted because of counting difficulties, for which a fuller explanation is given below.

When the ~~usual~~ χ^2 test ~~of~~ the technique of counting is applied to these data, a far too high value to be accounted for by chance is found ($\chi^2 = 51.44$ for 10 degrees of freedom).

The reason for this departure from an entirely correct technique is to be sought in difficulties of counting the resistant colonies, due essentially to their variability of size. On the first day the size of the resistant colonies is very small, usually below 1 mm ∅ ; many subvisible colonies can be seen at some magnification, apparently with a nearly continuous transition . On some plates, however there are clear cut categories of size of the resistant colonies arising from the same culture, suggesting the origin of these mutants in clones, by mere inspection of the plates. On the second day the originally small colonies have increased to normal size and many more have appeared. Some more appear on the 3rd day and probably more would on the fourth, were not the plates too dry. No appreciable inactivation of the drug takes place in these conditions; instead it is observed (see ~~data~~ later and Table I) that colonies developing on the second day are less resistant than those developing on the first, and therefore have probably a longer lag or a slower growth rate in the given drug concentration, in respect of the earlier resistants.

There is thus a variation of size , especially of the colonies near the visibility limit - partly shown by the accompanying photograph of a plate after 48 hours of incubation - which makes the count difficult and adds extra variance to that due solely

to the effects of chance of distribution of the mutants.

Inspite of this extra variance, the test summarised at the beginning is a valid indication of the presence of a large variability of the numbers of resistant mutants in parallel independent cultures (the "fluctuation test").

A further type of analysis is made possible by the fact that resistant colonies vary in degree of resistance. It has been shown by earlier work that such differences are inherited ; and that they are often due to different mutations. Hence, on the hypothesis of mutation and selection, there should be found a difference of the average degree of resistance of colonies from independent cultures, as they are likely to have arisen by mutations determining a different degree of resistance. In other words colonies from the same culture should resemble each other more closely than any two colonies taken at random from independent cultures.

This test was carried out by taking two colonies of those developed after 24^h and 2 of those developed after 48^h only, from each of the 20 plates ; each was isolated and tested for degree of resistance by streaking on plates with increasing amounts of chloromycetin in agar. The degree of resistance in table II, last columns is scored as follows :

Resistance score	0	1	2	3	4	5	6
Max. tolerated conc., µg/ml	25	25	32	40	50	63	79

An analysis of variance (table II) shows that the average degree of resistance varies significantly between cultures, but not between plates within cultures (the last being a test of the technique) both for 24^h, 48^h cultures and the total.

This amounts to saying that there is a positive correlation between relatives (the galtonian test of inheritance) i.e. that there must have been, according to the simplest explanation common ascendance, which is the same as the theory of mutation and selection.

It should not be forgotten that both the fluctuation test and that of the correlation between relatives, rest on the same assumption, i.e. that of the appearance of the mutants in unicolones; and are also open to the same objection, which was advanced by Hinshelwood and by Eriksen, namely that inappreciable differences in the environmental conditions of the parallel cultures with which the experiment is conducted might well lead to the observed discrepancies in number and quality of the resistants among cultures.

The fact that the quality of resistance here investigated was its degree, we can say its quantity, makes a further criterion possible, by a combination of both the fluctuation test and that of the correlation between relatives. In fact if environmental conditions favor or disfavor future resistance in independent cultures there would be reason,

on a hypothesis of physiological adaptation, for both the number of resistant individuals and their avergge resistance to be affected proportionally by those conditions. Hence a high positive correlation between the number of resistant mutants and their average degree of resistance is expected under the hypothesis of physiological adaptation, while a correlation of zero would be the consequence of mutation and selection.

Such correlation here is $r = -0.16$ for 24^h values, and approximately $r = -0.03$ for 48^h values (using logs of No.s of mutants). Although the sample is not large, the conclusion seems unescapable that everything points out to the theory of mutation and selection.

TABLE I

Numbers of resistant mutants and degree of resistance of x random samples of them from ten independent cultures of E.coli #30 plated on chloromycetin 25 ug/ml.

Culture No	Number of mutants at 24h A	B	at 48h A	B	Resistance scores of mutants Plate A 24h	48h	Plate B 24h	48h	Mean resistance 24h	48h	A	B	Tot.
1	110	155	199	250	4 ,4	4 ,4	4 ,4	4 ,4	4.0	4.0	4.0	4.0	4.0
2	33	22	159	53	3 5	3 0	4 4	2 0	4.0	1.2	2.8	2.5	2.6
3	22	34	580	356	2 4	4 4	4 2	2 0	3.0	2.5	3.5	2.0	2.7
4	14	6	58	60	5 6	4 2	4 4	4 4	4.7	3.5	4.2	4.0	4.1
5	8	6	93	97	4 4	4 4	4 4	4 4	4.0	4.0	4.0	4.0	4.0
6	19	14	148	190	4 4	2 2	2 3	3 2	3.2	2.2	3.0	2.5	2.7
7	245	150	n.c.	352	3 3	3 3	3 3	3 3	3.0	3.0	3.0	3.0	3.0
8	3	9	58	112	4 4	2 2	5 3	2 2	4.0	2.0	3.0	3.0	3.0
9	18	19	95	180	5 5	2 2	3 4	4 4	4.2	3.0	3.5	3.7	3.6
10	4	18	119	n.c.	5 4	4 3	5 5	5 3	4.7	3.7	4.0	4.5	4.2
							means:		3.9	2.9	(signif.at 1% level)		

TABLE II

Analysis of variance

Source of the variation	24h values df	Mean square	F	48h values df	Mean square	F	Total df	Meansquare	F
Between cultures	9	1.6111	3.222+	9	3.3917	5.025++	9	3.3625	2.754++
Between plates within cultures	10	0.7000	1.4000	10	1.4750	2.185	10	0.5875	<1
Within plates	20	0.5000		20	0.6750		60	1.2208	

\+ signif. at P =5% level

++ signif.at P =1% level.