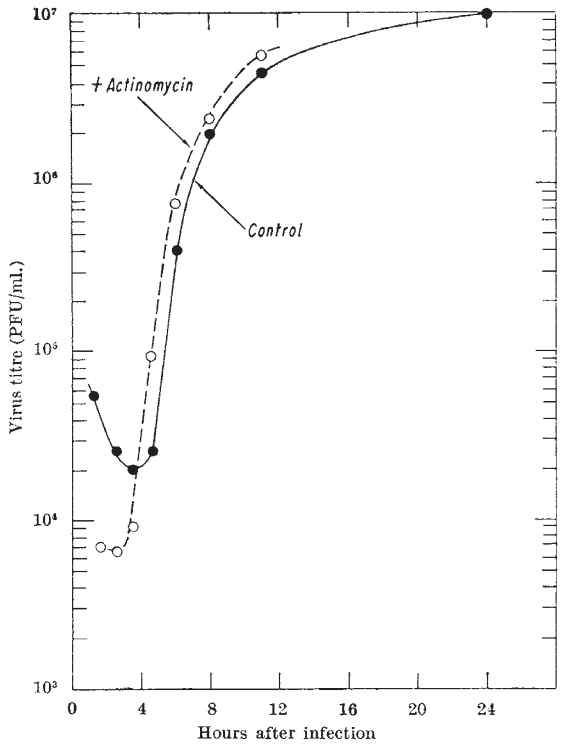

Fig. 3. Inhibition of infectious virus formation by actinomycin. A series of replicate cultures of infected cells was established at a cell concentration of 2×10^5/ml. One culture was sampled at increasing intervals after infection to obtain the infectious virus growth curve which is designated 'control'. At the indicated time after infection, one culture of the series received 0·5γ actinomycin/ml. At 24 h after infection, all inhibited cultures were assayed for infectious virus. The curve labelled 'actinomycin' represents the 24-h virus yields obtained when actinomycin was added at the times indicated by the position of the points on the curve

uridine into acid-insoluble material (Table 3)[7]. The observed reduction in total RNA synthesis may reflect a decrease in cell RNA formation together with an increase in the synthesis of virus-related RNA. Both the base composition and site of synthesis of the RNA made by vaccinia-infected cells are of interest in view of the cytoplasmic formation of both viral DNA and proteins[8]. A decrease in cell RNA synthesis would not by itself explain the cessation of cell protein synthesis in vaccinia-infected

Table 3. Reduction in RNA Synthesis after Vaccinia Infection

Hours after infection	Total CPM incorporated	% of maximum
Uninfected	2,310	100
2	2,110	91
4	1,400	61
7	730	32

At the indicated times after infection, one of a series of replicate suspension cultures each containing 100 ml. of cells at a concentration of 2×10^5/ml. received 2×10^{-5} M uridine-^{14}C (specific activity = 0·56 μc./μM). After 30 min the culture was chilled and the amount of uridine-^{14}C incorporated into RNA was determined[10].

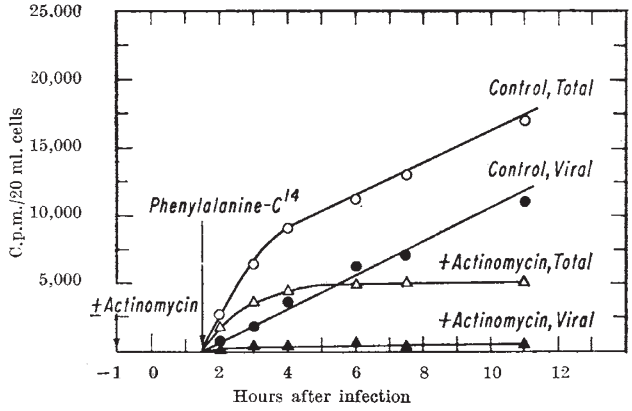

Fig. 4. Inhibition of cell protein synthesis following virus infection. One of two suspension cultures containing 2×10^5 cells/ml. was treated with 2γ/ml. actinomycin for 1 h at 37°. Each was then concentrated ten-fold, infected with an input multiplicity of 10–20 PFU/cell, washed twice in phenylalanine-deficient Eagle's medium[11] containing 20 per cent dialysed human serum, and resuspended to 2×10^5 cells/ml. in deficient medium plus 5 per cent dialysed horse serum. Actinomycin was present throughout. At 1½ h after infection, 0·01 mM phenylalanine-^{14}C (specific activity = 2 μc./μM) was added and the incubation continued at 37° C. Samples were removed at time-intervals and centrifuged. Total radioactivity incorporated into protein was determined following cold 5 per cent trichloroacetic acid extraction and solubilization in 1 N potassium hydroxide of an aliquot of each sample. Viral protein was measured by immunological precipitation of a second aliquot

cells, since protein synthesis in uninfected cells continues for 12 h or longer when RNA formation has been 98 per cent inhibited by actinomycin (Fig. 1). The finding that synthesis of cell protein decreases even when viral products are not formed in infected cells suggests that the infecting virus inhibits the ability of the RNA synthesized prior to infection to support cell protein formation. In the presence of the antibiotic only the inhibition of cell protein synthesis may occur. In its absence, the synthesis of virus-related RNA could proceed and might then direct the synthesis of viral proteins.

The foregoing observations suggest that a vaccinia virus DNA-directed RNA is synthesized in infected HeLa cells and that its synthesis throughout infection is necessary for continued viral protein and infectious virus production. Information now being sought concerning the cellular site of synthesis and chemical nature of this RNA may provide a better understanding of the processes by which virus re-directs the metabolism of cells.

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A POSSIBLE CASE OF GENETIC ASSIMILATION OF BEHAVIOUR

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CLUTTERBUCK and Beardmore[1] recently reported that if *Drosophila melanogaster* is reared on a medium adulterated with a substance to which they are aversive, such as peppermint oil, then the behaviour of the flies may alter so that they become less aversive to the adulterant. These authors found that flies reared on peppermint-flavoured medium for six generations became less averse to that substance, both measured by the relative numbers of eggs laid on it compared with the number laid on a normal medium, and also in terms of the number of flies

found on peppermint food rather than on normal food in a choice situation.

The method of testing which they used is not sensitive, owing to the great discrepancy in the number of eggs laid by different pairs of flies. We have found that in single pair matings the number of eggs laid may vary from zero to more than 100 in flies randomly picked from the same population. Hence their egg-laying results could be due to abnormal fertility in one or two flies, which could more than counterbalance a low egg-laying score on normal food by the majority. Further, their food choice test was performed over periods of 1 h and 24 h, and it is likely that with the volatile oils which they used for adulterants there would have been a considerable spread of the adulterant throughout the apparatus in that time. Their results are, however, suggestive, and we have repeated their experiment with a different design.

A stock of wild-type *Drosophila melanogaster* (Pacific) strain was kindly provided by the Department of Zoology, University of Edinburgh. These were divided into the groups shown in Table 1.

Table 1

AN	Flies averse to peppermint reared on normal food
NAN	Flies non-averse to peppermint reared on normal food
UN	Unselected flies reared on normal food
AP	Flies averse to peppermint reared on peppermint food
NAP	Flies non-averse to peppermint reared on peppermint food
UP	Unselected flies reared on peppermint food

From this Table is will be seen that there was artificial selection for aversion or non-aversion to the adulterant, and natural selection by the presence or absence of adulterant in the food. Artificial selection was carried out in the apparatus shown in Fig. 1. Flies were placed in the chamber *P* and the door *D* opened. After 2 min, *D* was closed. If aversive flies were being selected those which remained in *P* were removed, the remainder replaced in *P*, and the process repeated. This was done 5 times. The remaining flies were used as the 'aversive' (*A*) stock. If non-aversive flies were required, the flies remaining in *P* were retained each time. Each artificially selected line was therefore composed of those members of the population which remained after a 5-fold cumulative screening for the character in question.

Of such a group, mating was between 10 males and 10 females, all virgin, and approximately 4 days old.

Testing of the characteristics of the offspring was carried out in the same apparatus. 50 males and 50 females, in groups of 10 and approximately 2 days old were placed in *P*, and the number remaining in *P* after 2 min was counted. The summed scores are represented as percentage of the population showing aversion (percentage of flies which had passed beyond *D* in 2 min).

The results of artificial selection alone, natural selection alone, and the two in combination are shown in Figs. 2 and 3.

AN tends to show an overall rise over 14 generations. *NAN* shows a slight continuous upward drift. *UN* retains its original level, representing the relatively stable characteristics of the wild-type population. None of these results is particularly striking. The effects of artificial selection alone do not appear violent.

The behaviour of the graph *UP* does not seem to suggest that natural selection alone has much effect:

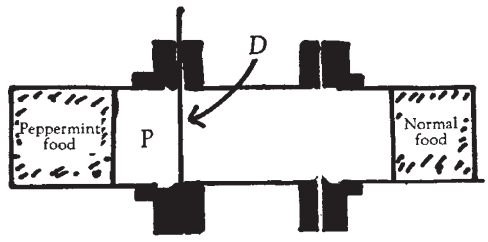

Fig. 1. Longitudinal section through testing and screening apparatus

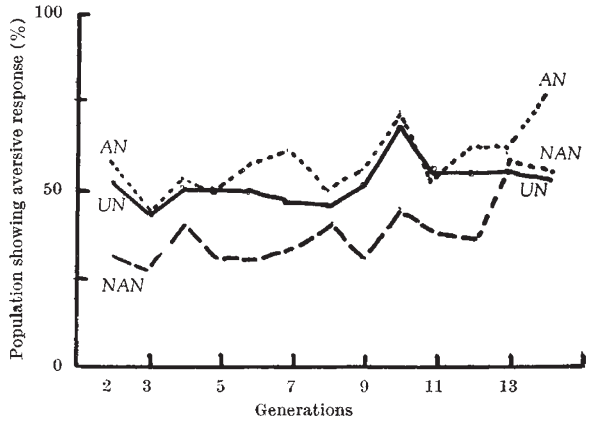

Fig. 2. Percentage of the population showing aversion at each generation. Flies reared on normal food

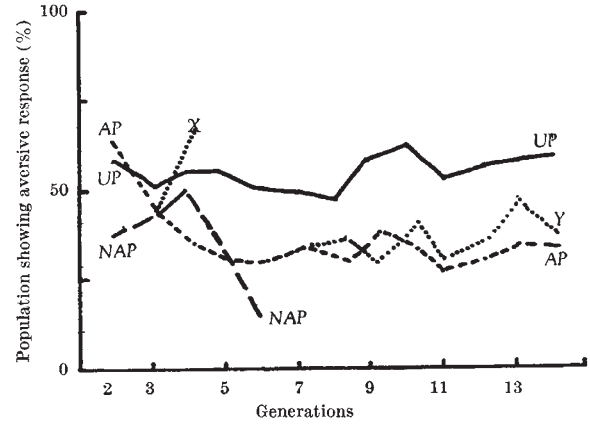

Fig. 3. Percentage of the population showing aversion at each generation. Flies reared on peppermint food

this line remains relatively stable over 14 generations at a level slightly below *UN*.

For the two lines which were subjected both to artificial and natural selection, however, the results are striking. *AP* and *NAP* both become non-aversive, though selected artificially in opposite senses. This result is not surprising for *NAP*. The data are only plotted up to F_6 since beyond this point the line had to be released from artificial selection owing to the very small numbers surviving. It appears to be extremely unfit, and afterwards had to be frequently released from artificial selection to maintain it. Beyond F_6 therefore the data for *NAP* are not reliable.

The most interesting line is *AP*. This line was selected for aversion in each generation, but becomes non-aversive when reared for several generations (approximately the same number over which Clutterbuck and Beardmore observed the effect) on peppermint. Neither artificial selection alone nor natural selection alone produced this effect (*NAN* and *UP*).

A point should be made about the method of testing. The method used means that the aversive response is partly confounded by the activity level of the flies. More active flies are more likely (other things being equal) to go beyond *D* in 2 min than less active flies. But since testing procedure was identical for all the lines the result is not artefactual and due simply to differences in activity-level in the original population. If the effect is due to activity rather than aversion, then we have altered activity by the experimental conditions. But one or other or both has been altered, which is what we are interested in.

A possible explanation might proceed along the following lines. Two processes seem to be occurring at once. If we release line *AP* from the adulterated medium at F_3 (shown by the dotted line in Fig. 3), it returns to its original

level of aversion (point X in Fig. 3). If, however, we wait until F_7 to release them, the progeny do not return to their original level (dotted line ending in point Y in Fig. 3). Moreover, the $AP(Y)$ line does not return to its original level even after a further 8 generations. The aversive character of the flies seems to have been reduced more or less permanently.

We suggest that the short-term effect should be called habituation, and that it is probably a within-one-generation effect. It seems not unlikely that flies which have spent their whole life from egg to adult in a peppermint-scented medium should habituate to it so that when tested as adults they would not be so aversive to it as wild-type flies tested when adults. On the other hand, the relatively stable, long-term effect bears a similarity, we would tentatively suggest, to genetic assimilation of a character which does not have a threshold, as was discussed for structural characters by Waddington[2]. If so, we may proceed with the explanation as follows.

Consider Fig. 4. A 5-fold screening is a rather intensive method of artificial selection, which may be expected to select only the extreme tails of the distribution marked A and NA respectively. In the total population there is a great deal of genetic variability, but comparatively little in the extremes of the distribution. Let us assume that the effect of the adulterated environment is to produce 'canalization' as described by Waddington. Then for the total population, the effect of canalization may well be small in each generation compared with the spread of genetic variability, so that the range of the latter offsets the effect of the former in such a degree that the overall characteristics of the population do not change noticeably. When we select for non-aversion and breed on peppermint (NAP) the two effects reinforce each other and the fall in aversion is precipitous and carries the population into a region where it is unfit; and in the case where we select for aversion but breed on peppermint, the following might occur. The variability is so reduced by the severe artificial selection that the effect of canalization is large with respect to it. Hence, although the artificial selection is in the opposite sense, the shift produced by canalization is not offset by the inherent variability, and the characteristics of the population slowly shift. What variability there is, however, tends to return the population toward the normal population, and so the line does not enter the unfit region which NAP enters, and instead, becomes stabilized at a comparatively low level of aversion.

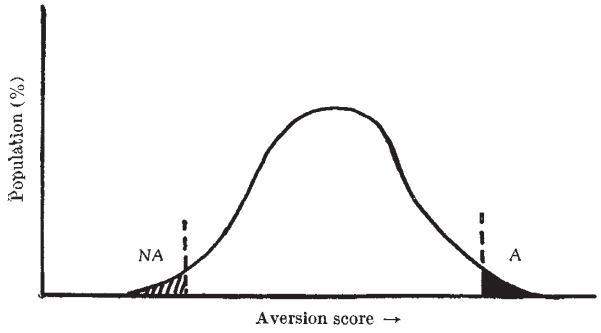

Fig. 4. Regions of population from which breeding is occurring on the basis of 5-fold screening selection

Such an explanation is amenable to experimental verification. It should follow that the rate of change of the characteristics of the population should be a function of the amount of variability in the breeding population. Thus by breeding from more or fewer pairs of flies a series of differently sloping curves should be obtained, all tending, but at a different rate, towards the non-aversive end of the distribution. We have begun experiments on this hypothesis, using an improved form of testing procedure.

We thank the Medical Research Council for financial assistance which made the work possible through the tenure of a research assistantship by one of us (K. C.) at the University of Sheffield.

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CARBON DIOXIDE: A FACTOR INFLUENCING CELL DIVISION

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A HIGH concentration of carbon dioxide promotes mesocotyl growth in etiolated oat seedlings and reduces that of the coleoptile and leaves[1–4]. To explain these growth variations it has been supposed that carbon dioxide promotes cell division in the mesocotyl rather than the content of growth hormone[5]. This speculative hypothesis has now been tested by conducting a 2 × 2 factorially arranged experiment in which 5 per cent carbon dioxide in the air, for 3 days from the time of planting the seed, was combined with exposure of the seedlings to dim red light (1 ft.-candle) for 5 min on the third day, after treatment with carbon dioxide had been terminated. From the second to the seventh day of growth, samples of seedlings were measured and a plant having lengths nearest to the mean values obtained was selected from each sample, embedded in the usual way (either whole or after cutting into a series of 10-mm segments) and sectioned. Cell sizes and numbers were recorded for each mm of the entire length of both mesocotyls and coleoptiles by counting 5 rows of cortical cells and then calculating the mean cell length from the mean number of cells/mm. Fig. 1, which is self-explanatory, shows a typical set of data for a 10-mm segment of mesocotyl.

In Figs. 2*A* and *B* appear respectively the mean lengths of the mesocotyls plotted against time, and the mean

Carbon dioxide: fourth day

Mesocotyl segment	Columns A	B	C	D	E	Mean No.	Mean length
1st mm	23·0	23·0	22·0	22·0	25·5	23·50	0·043
2nd ,,	13·5	15·5	13·0	16·5	13·0	14·30	0·070
3rd ,,	10·0	5·7	8·3	8·5	7·2	7·94	0·126
4th ,,	7·5	6·0	6·3	6·5	6·2	6·50	0·154
5th ,,	4·5	4·3	4·7	4·0	5·0	4·50	0·222
6th ,,	3·0	3·5	5·0	4·0	5·0	4·10	0·244
7th ,,	3·0	2·5	3·3	4·0	3·7	3·30	0·303
8th ,,	4·0	3·0	2·7	3·0	3·7	3·28	0·305
9th ,,	3·7	3·0	2·7	2·5	2·9	2·96	0·338
10th ,,	3·5	3·3	2·5	3·0	2·5	2·96	0·338

Fig. 1. Technique used for cell counts and for determining the distribution of cell size with increase in distance from the apex of the segment