DURATION OF LIFE OF AN ASEPTIC DROSOPHILA CULTURE INBRED IN THE DARK FOR 230 GENERATIONS.

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The idea has frequently been expressed that the resistance of organisms to disease has been developed by means of natural selection, and hence that prolonged lack of exposure to infection might lead to a race lacking in resistance. Prolonged inbreeding and the absence of light have also been supposed to affect the activities of organisms. The cultures of Drosophila freed from microorganisms by Loeb and the writer in 1916 have been continued since under aseptic conditions and have been kept in the dark. (The cultures have been exposed to diffuse daylight at intervals when being transferred to a new flask. In view of the marked effect of even short exposures to lower temperatures on the upper temperature limit of the organism, it is possible that even this short exposure to light may be of importance.) It seemed of interest, therefore, to compare the fertility, rate of growth, and duration of life of these cultures with that of a "normal" culture when exposed to the rigors and uncertainties of a non-aseptic environment. The experiment was made under favorable conditions and also under conditions in which the duration of life was greatly shortened owing to the activities of the bacterial flora, although it is not possible to say that the insects were killed directly by the microorganisms. In neither case, however, was there any significant difference between the aseptic and the control cultures.

1 It is naturally impossible to prove that the culture is free from all microorganisms. All that can be said is that it has not been possible to obtain a culture of microorganisms from the insects when placed in a variety of media under both aerobic and anaerobic conditions.


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EXPERIMENTAL.

The "aseptic" culture was the 230th generation of the insects freed from microorganisms by Loeb and the writer in 1916. They had been grown since on sterilized yeast as already described. The normal culture was a strain, obtained from Dr. R. W. Glaser, which had been grown for some time on fermenting banana. They had been grown in this laboratory for 10 generations on yeast.

Yeast Medium.—280 gm. of bakers' yeast stirred up with 500 cc. of water and 15 cc. of glacial acetic acid. 25 cc. of this suspension placed in a 500 cc. flask and sufficient cotton added to absorb the excess of water. This has been found to be a very good culture medium.

TABLE I.

Comparison of Fertility, Larval Period, and Duration of Life of "Normal" Drosophila Cultures and of a 230th Generation of an "Aseptic" Culture.

<table>
<thead>
<tr>
<th>Culture medium.</th>
<th>Drosophila culture.</th>
<th>No. of pupae per Q.</th>
<th>Days as larva.</th>
<th>Days as pupae and imagos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile yeast</td>
<td>230th generation, aseptic</td>
<td>6.0 ± 0.5</td>
<td>5.0 ± 0.1</td>
<td>27 ± 0.1</td>
</tr>
<tr>
<td>cotton.........</td>
<td>21st generation, aseptic</td>
<td>6.2 ± 0.1</td>
<td>33 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Yeast cotton...</td>
<td>&quot;Normal.&quot;.........</td>
<td>1.9 ± 0.2</td>
<td>4.6 ± 0.1</td>
<td>23 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>&quot;Aseptic.&quot;.........</td>
<td>2.8 ± 0.2</td>
<td>4.9 ± 0.1</td>
<td>19 ± 0.3</td>
</tr>
<tr>
<td>Banana.........</td>
<td>&quot;Normal.&quot;.........</td>
<td>0.35 ± 0.01</td>
<td>5.1 ± 0.04</td>
<td>7.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>&quot;Aseptic.&quot;.........</td>
<td>2.70 ± 0.2</td>
<td>5.0 ± 0.04</td>
<td>6.2 ± 0.05</td>
</tr>
</tbody>
</table>

Sterile Yeast Medium.—Same as the preceding except that the flasks were sterilized for 1 hour at 15 lb., after being plugged with cotton.

Banana Medium.—Ripe bananas were ground in a mortar and about 25 gm. of the pulp placed in each flask. It has been repeatedly observed that banana, unless inoculated with yeast, is a very poor culture medium for these insects due apparently to the growth of bacteria. In this experiment an unpleasant odor developed in a day or so and the flies died rapidly.

Mass cultures of the two strains were made on yeast medium. The succeeding generation of flies was transferred when 1 day old to a series of 10 flasks containing the proper culture medium and placed in a constant temperature room at 25°C. for 24 hours. Each flask contained 30 to 50 flies. The parent flies were then removed and the number of males and females counted. In all cases the numbers of male and female were about equal. The number of pupae formed were then counted at 24 hour intervals. The adult flies were transferred every 4 or 5 days to fresh flasks and the number of dead determined at daily intervals.
About 50 larvae or imagos were in each of the 10 flasks. The average time for each individual culture was determined and these averages were then treated as individual observations. The figures given are therefore the means of these averages. This procedure, although perhaps difficult of justification, has been adopted rather than the more usual one of considering each individual insect, since the latter method gives probable errors so small that no two series of experiments ever agree within their probable errors. When the present method is used, it is possible to repeat the experiment within the error. The explanation of this is, presumably, that there are small constant differences in the culture flasks as well as in the organisms and the latter method takes these differences into consideration as well as the individual variation in the insects.

The results of the experiments are given in Table I. It is evident that there is no marked difference between the two cultures except in the number of pupae formed per female on the banana medium. In that case the aseptic flies produced many more pupae than the controls. This may be due either to a smaller number of eggs laid or to a greater mortality of the larvae.

SUMMARY.

The number of pupae formed per female, the duration of the larval period, and the duration of the pupal-imago period of a normal Drosophila culture and of the 230th generation of an aseptic culture which had been kept in the dark have been determined. The larval period and the pupal-imago period were found to be nearly the same for both cultures under both favorable and unfavorable conditions. There is no evidence, therefore, to show that inbreeding, absence of light, or growth in the absence of bacteria for 230 generations has had any effect either on the duration of life or on the ability of the organism to resist unfavorable bacteria.