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TWO PHASES OF AGEING IN *DROSOPHILA SUBOBSCURA*

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INTRODUCTION

Drosophila, like other poikilotherms, lives for a shorter time at high temperatures and for a longer time at low temperatures. This has commonly been explained by assuming (e.g. Pearl, 1928) that the rates of ageing processes, like those of chemical reactions, are temperature-dependent. Earlier experiments on *D. subobscura* (Maynard Smith, 1958) cast doubts on this interpretation. It was found that if young adult flies were kept at 30° C. for periods equal to about half their expectation of life at that temperature, and were then transferred to 20° C., they died at the same chronological age as did flies kept continuously at 20° C.

These results were incompatible with the assumption that differences in longevity at different temperatures are due to the effects of temperature on the rate of a single ageing process. It was therefore suggested that the causes of ageing at the two temperatures are different. But the results can be explained in another way, as follows. It is supposed that the rate of ageing is, at least approximately, independent of the temperature. Ageing leads to a continuous decline in the ability of individuals to withstand the various factors, internal or external, which may cause death; it is convenient to refer to this as a decline in 'vitality'. The decline continues until the vitality falls below some threshold level appropriate to the temperature at which the animal is living. Once this threshold is passed, the individual starts to die. The level of vitality necessary for survival is higher at higher temperatures, so that differences in longevity at different temperatures are due, not to differences in the rate of ageing, but to differences in the level of vitality necessary for survival at different temperatures.

This hypothesis would explain the earlier results, because individuals transferred from a high to a low temperature before their vitality had fallen to the threshold level appropriate to the higher temperature would be physiologically the same age as flies of the same chronological age kept continuously at the lower temperature. But the hypothesis also predicts that flies which are kept at a low temperature for periods equal to or greater than their expectation of life at some higher temperature, and which are then transferred to the high temperature, should start to die immediately. More generally, for every day an individual is kept at a low temperature, its expectation of life at a high temperature should be reduced by one day.

In this paper experiments are described which confirm this prediction over a wide range of temperatures, although at very low temperatures it appears that the rate of ageing is itself reduced.

METHODS

The flies used were F_1 hybrids between the **B** and **K** inbred lines of *D. subobscura.* They were raised at 20° C. in half-pint milk bottles on a food medium of maize meal, agar and molasses, with 0·5 % dead yeast and two drops of live yeast suspension added. The adults were always kept for 4 days at 20° C. before being transferred to other temperatures. Adults were removed from the culture bottles on the day of emergence and kept subsequently in pairs in 3 by 1 in. diameter vials containing a similar food medium. They were transferred to fresh food vials at regular intervals of 4 days at 20° C. or less, of 2 days at 26° C. and of 1 day at temperatures higher than 26° C.

RESULTS

Table 1 gives the mean longevities of flies kept continuously at various temperatures. Fig. 1 gives the results of four experiments in which flies were kept initially at a low temperature, and then transferred to a higher temperature until they died. The details are given in Table 2. In no case was there any appreciable mortality at the low temperature before transfer to the higher temperature.

Table 1. *Mean survival times in days at various temperatures*

Temperature (° C.)	Males	Females virgin	Females mated
3	224	—	—
15	146	> 100*	—
20	83·4	—	58·8
26	35·4	—	—
28·3	—	35·7	—
30	6·4	—	14·0

* A group of twenty virgin females was kept with no deaths for 100 days at 15° C.

Table 2

Exp.	No. of flies given each treatment	Temperature (° C.) Before transfer	Temperature (° C.) After transfer	Age in days at transfer
1	{10 ♂♂ / 10 mated ♀♀}	20	30	4–48
2	10 ♂♂	20	26	4–48
3	16 virgin ♀♀	15	28·3	4–40
4	16 virgin ♀♀	3	28·3	4–40

If the rate of ageing is in fact independent of the temperature, the curves in Fig. 1 should follow the broken lines, which have been drawn with a slope of $-1{\cdot}0$. The first three experiments agree closely with this prediction during the early part of the life span. Thus in Expt. 2, males transferred to 26° C. when aged 4 days died at a chronological age of 39·4 days, after 35·4 days at 26° C., whereas males transferred from 20 to 26° C., when aged 24 days, died at approximately the same chronological age of 40·2 days, after only 16·2 days at the higher temperature; the longevity of males kept continuously at 20° C. was over 80 days.

But after this initial period, the survival times at high temperatures declined slowly with increasing chronological age at transfer. The explanation for this is probably as follows. Flies whose vitality has fallen below the threshold necessary for survival at a high temperature start to die if transferred to that temperature. But the process of dying may take an appreciable time to reach completion; in fact, it appears to take about 16 days in males kept at 26° C. and about 3 days in males kept at 30° C. Thus the rate of this dying process is highly dependent on temperature.

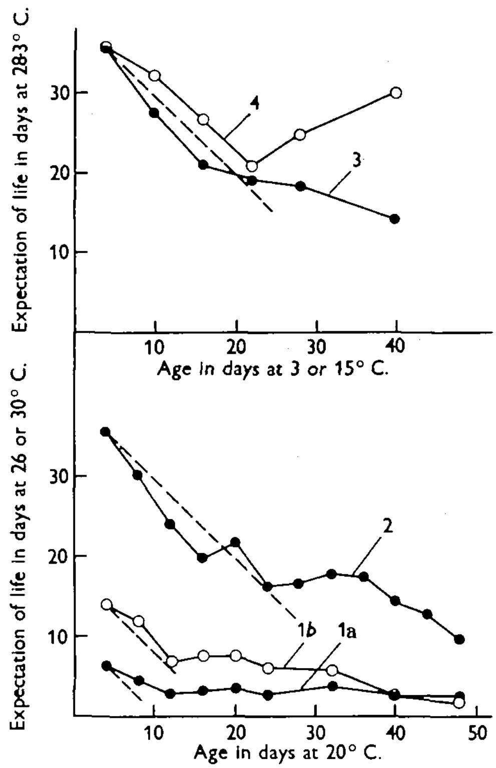

Fig. 1. Mean survival times in days at a high temperature of flies kept previously for varying periods at a lower temperature. 1 *a*, 1 *b*, males and females respectively, transferred from 20 to 30° C.; 2, males transferred from 20 to 26° C.; 3, females transferred from 15 to 28·3° C.; 4, females transferred from 3 to 28·3° C. The broken lines have a slope of −1·0, predicted if the rate of ageing is independent of the temperature.

Expt. 4, in which flies were kept at 3° C. before transfer to 28·3° C., gave an unexpected result; the survival times first decreased and then increased again with increasing chronological age. But it was suspected that this might be a spurious result due to an incorrectly low value of the survival time of the group of females transferred at an age of 20 days. It was therefore decided to repeat this experiment, and at the same time to correct a weakness of Expts. 1 to 3. In these experiments there was no replication of groups given a similar treatment. Consequently there was no method of deciding whether slight changes of temperature or differences between successive batches of food had influenced the results.

In Expt. 5, results of which are given in Table 3, five batches of virgin females were raised in different sets of culture bottles at different times, over a period of about

Table 3. *Expectation of life in days at 28·8° C. of virgin females kept for varying periods at 3 and 15° C.*

Batch	Chronological age in days at 3° C.				Chronological age in days at 15° C.			
	4	20	40	60	4	20	40	60
a	27·86	—	—	—	27·86	—	—	—
b	—	—	24·85	21·0	—	—	8·20	5·95
c	27·60	22·55	21·65	20·34	27·60	18·35	8·60	8·30
d	28·50	19·75	—	—	28·50	15·56	—	—
e	24·45	18·40	—	—	24·45	9·70	—	—
Mean	27·10	20·23	23·25	20·67	27·10	14·54	8·40	7·12

60 days. Groups of females were transferred to 28·8° C. at chronological ages of 4, 20, 40 and 60 days, having been kept from the age of 4 days either at 15 or at 3° C. Each experimental treatment was replicated at least once, using females from different batches.

Considering first females kept at 3° C., the initial decline in survival time followed by an increase was not repeated, and it is concluded that this earlier result was spurious. The survival times of females kept at 3° C. for from 20 to 60 days were only slightly less than those of control females transferred to 28·8° C. 4 days after emergence. It is concluded that at 3° C. the rate of the ageing process is appreciably slower than it is at higher temperatures.

The results for females kept at 15° C. agree reasonably well with Expts. 1 to 3, although unfortunately there was a rather poor agreement between replicates for females transferred from 15° C. when aged 20 days. From Table 3, the best estimate that can be made of the ratio of the rate of ageing at 28·8° C. to that at 15° C. is $16 \div (27{\cdot}1 - 14{\cdot}54) = 1{\cdot}27$, with a standard error of 0·31. This should be compared to the ratio of over 4:1 for the longevities at the two temperatures. Expts. 1 to 3 suggest a ratio closer to unity, but no estimate can be made of the standard error.

The results of all these experiments are consistent with the hypothesis that the rate of ageing is independent of the temperature from 15 to 30° C. More accurate measurements might show a slightly higher rate at higher temperatures, but the differences in rate are certainly far too small to account for the differences in longevity of flies kept continuously at different temperatures.

REVERSIBILITY OF THE 'DYING' PROCESS

It has been suggested that the lifespan of an individual can be divided into two phases, an irreversible process of ageing whose rate is approximately independent of the temperature, followed by a process of dying in individuals whose vitality has fallen below the threshold appropriate to the temperature at which they are living. The rate of the dying process appears to be highly dependent on temperature. The question arises, are the changes which occur during the dying process at a high temperature reversed if the individual is transferred back to a low temperature?

Four-day-old males survived at 30° C. for a mean of 6·4 days, 8-day-old males for 4·9 days, and males aged from 12 to 64 days for approximately 3 days. This suggests that freshly emerged males are only just capable of maintaining a steady state at 30° C., and that after a few days their vitality has fallen below the appropriate threshold. The 3-day survival time of older males then represents the duration of the dying phase at 30° C.

Two groups of ten males each were kept for 20 days at 20° C. and then transferred to 30° C. The first group was kept at 30° C. until death, which occurred on the third or fourth day after transfer. The second group was kept alternately for one day at 30° C. and for 3 days at 20° C. They died after total periods at 30° C. of from 9 to 17 days, at chronological ages varying from 53 to 85 days.

This result suggests that the changes which occur during the dying phase and which are ultimately responsible for death can be reversed in individuals transferred to lower temperatures.

CONCLUSIONS

The experiments described above measure the rate at which the expectation of life at a high temperature declines in flies living at a lower temperature. It has been found that over the range 15–30° C., for every day that an individual spends at a low temperature, its expectation of life at a higher temperature is reduced by approximately 1 day. This confirms the hypothesis that the rate of ageing is approximately independent of the temperature over this range, an idea which was first put forward to explain the fact that the exposure of young flies for an appreciable time to a high temperature does not alter their expectation of life at a lower temperature.

A number of rhythmical processes, in *Drosophila* and in other poikilotherms, are known to have periods which are approximately constant over a similar range of temperatures. For example, Pittendrigh (1954) has shown in *D. pseudoobscura* that there is a rhythm of emergence of adults from the pupa, which has a period of approximately 24 hr. over the range 16–26° C., in the absence of any rhythmical environmental stimulus. Unfortunately, there is no reason to suppose that the process of ageing has anything in common with such rhythmical processes, other than its temperature independence.

It is concluded that the life span of a fly can be divided into two phases, referred to as 'ageing' and 'dying'. These may correspond to the processes of 'induction' and 'development' suggested by Neary (1960) from a study of mice. The rate of the ageing process is approximately independent of temperature from 15 to 30° C., but is considerably slower at 3° C. The dying process is initiated when ageing has caused the vitality of the individual to fall below the threshold level necessary if a steady state is to be maintained. Both the level of this threshold and the rate of the dying process are highly dependent on temperature. The damage which occurs during the dying process at a high temperature can be repaired in flies transferred to a lower temperature.

SUMMARY

1. Male and female *D. subobscura* were kept for varying periods at low temperatures (3–20° C.) and then transferred to a higher temperature (26–30° C.) and kept there until they died.

2. It was found that during the early part of the life span, over the range 15–30° C., every day spent at a low temperature reduced the expectation of life at a higher

temperature by approximately 1 day. Later, when the expectation of life at the higher temperature had fallen to about half its initial value, little further change in this expectation occurred with increasing age at a lower temperature.

3. It is concluded that the life span can be divided into two phases, (i) an irreversible 'ageing' process whose rate is approximately independent of temperature from 15 to 30° C., but which is slower at 3° C., and (ii) a 'dying' process which is initiated when ageing has proceeded to a stage at which the individual is no longer capable of maintaining a steady state at the temperature at which it is living, although the same individual would be capable of maintaining a steady state at some lower temperature.

4. The rate of the dying process is highly dependent on temperature, and it can be reversed in flies transferred to lower temperatures.

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