THE EFFECTS OF TEMPERATURE AND OF EGG-LAYING ON THE LONGEVITY OF *DROSOPHILA SUBOBSCURA*

BY J. MAYNARD SMITH
Department of Zoology, University College London

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1. INTRODUCTION

If an outbred population of adult *Drosophila* are kept from the time of emergence in a uniform and favourable environment there is a fairly protracted initial period during which very few individuals die, followed by a period during which the force of mortality rises rapidly until all individuals are dead. Similar life tables can be obtained for most animal species, provided that the environment is favourable and the population is neither genetically very diverse nor excessively inbred.

Such results show that progressive changes take place in individuals, starting at the time of emergence, and that these changes ultimately result in death or render individuals increasingly susceptible to various extrinsic causes of death.

As would be expected, in poikilotherms such changes proceed more rapidly at higher temperatures, as is shown by the decrease in the expectation of life with increasing temperature. It was the purpose of the present investigation to discover how far the processes responsible for death in *D. subobscura* are the same at different temperatures, differing only in the rate at which they proceed, and how far different processes are concerned at different temperatures. The results obtained strongly suggest that different processes are responsible for ageing at different temperatures; they also indicate a connexion between the rate of egg-laying and the rate of ageing, and this possibility has been confirmed by a study of ageing in virgin females and in females lacking ovaries.

2. METHODS

The flies used in these experiments (except for those described in §7) were first generation hybrids between the **K** and **NFS** inbred lines of *D. subobscura* (Maynard Smith, Clarke & Hollingsworth, 1955).

All flies were raised in half-pint milk bottles on a food medium of maize meal, agar and molasses, with two drops of living yeast suspension added, and at a temperature of 20° C. unless otherwise stated. On the day of emergence adults were removed from the culture bottles and kept subsequently in mated pairs in 3 in. by 1 in. diameter vials containing a similar food medium. They were transferred without etherization to fresh food vials at regular intervals, of 4 days if kept at 20° C., of 2 days at 25° C., of 1 day at 30·5° C. and of 12 hr. if kept at 33° C. Deaths were recorded only at the time of transfer (except at 33° C., when they were recorded at 3-hourly intervals) and for purposes of calculation were assumed to have taken place mid-way between successive transfers.

3. SURVIVAL TIMES AT DIFFERENT TEMPERATURES

The mean ages at death (measured from adult emergence) of adults kept continuously at 20°, 25°, 30·5° and 33° C. are given in Table 1.

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

Temperature (° C.)	Males		Females	
	No. of flies	Survival time in days	No. of flies	Survival time in days
20	50	67·4 ±2·46	50	55·9 ±2·58
25 raised at 15° C.	25	29·5 ±1·07 } 27·0	25	40·5 ±1·68 } 35·5
25 raised at 25° C.	25	24·6 ±1·10 }	25	30·6 ±1·65 }
30·5	50	7·58±0·28	50	17·6 ±0·65
33	10	0·79±0·08	10	0·82±0·05

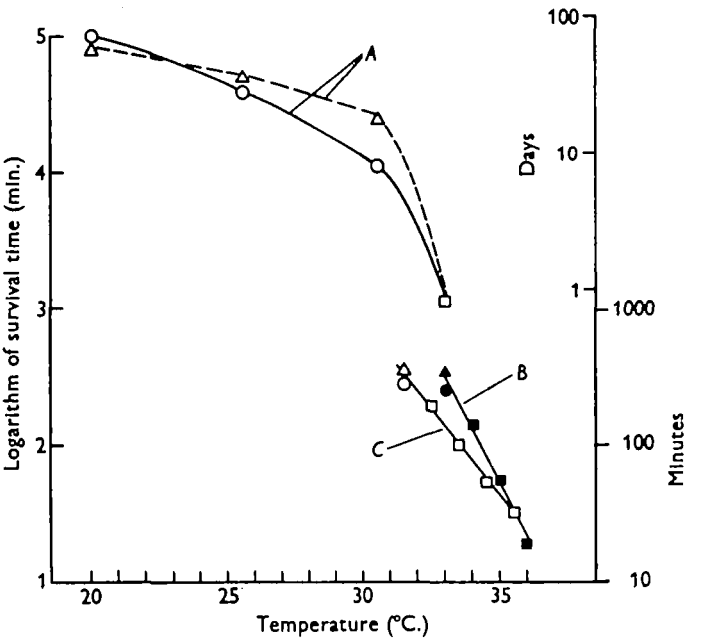

Fig. 1. Survival time of flies at different temperatures. *A*, In food vials; *B*, in saturated air; *C*, in dry air; △, ▲, females; ○, ●, males; □, ■, sexes combined.

In Fig. 1 these values are plotted on a logarithmic scale, together with the results of earlier experiments (Maynard Smith, 1957) on F_1 hybrids between the **B** and **K** inbred lines exposed to high temperatures without food or water in dry and in saturated air. It is known (Maynard Smith, 1956*b*) that at these high temperatures the survival times of **B/K** and of **K/NFS** hybrids do not differ significantly.

There is a rather sudden change in the slope of the curve in Fig. 1 at about 31° C. This suggested that the causes of death at high temperatures might be different

from those acting below 31° C. This is confirmed by two other differences between the factors influencing survival time at high and at low temperatures.

First, flies raised during pre-adult life at 25° C. survive at 33·5° C. in dry air for about twice as long as do flies raised at 15° C. There is a similar, although smaller, difference between the survival times of the two groups at 34·3° C. in saturated air. But precisely the opposite is true of survival at 25° C. As shown in Table 1, flies raised at 15° C. live for longer as adults than do flies raised at 25° C. Hence the process of acclimatization in larvae raised at 25° C. enables them to live for longer at temperatures above 33° C., but not at 25° C.

Table 2. *Mean total survival times in hours at* 33° C. *in food vials*

	Males	Females
Kept continuously at 33° C.	18·85	19·55
Kept alternately for 8 hr. at 33° C. and for 16 hr. at 20° C.	28·45	26·05

A second and more important difference concerns the reversibility of changes occurring at temperatures above and below 31° C. It was found (Maynard Smith, 1957) that if flies are exposed to a high temperature (33·5° C. in dry air or 34·3° C. in saturated air) for 50 min. (i.e. for about half their expectation of life at that temperature) and are then kept for 3 hr. at 20° C. in a food vial, their survival times when they are again exposed to a high temperature are as great as those of flies not previously exposed. (In fact, they live for longer in their second exposure, but this only strengthens the argument.) It follows that whatever damage was done during the first exposure can effectively be repaired during the intervening period at 20° C., and in this sense the changes which occur at high temperatures are reversible. The same is in part true of the changes which kill individuals kept in food vials at 33° C., as is shown in Table 2. Although the changes which occur in 8 hr. in a food vial at 33° C. are not completely reversed, some recovery does take place during the intervening periods of 16 hr. at 20° C. It will now be shown that this is not true of the changes which occur at 30·5° C.

4. IRREVERSIBILITY OF CHANGES OCCURRING AT 30·5° C.

If the changes which occur at 30·5° C. and which ultimately are responsible for death at that temperature were in part reversible, or if the damage caused could be repaired, then it would be expected that the total survival time at 30·5° C. would be greater for flies exposed intermittently, with intervening periods at 20° C., than for flies exposed continuously. Table 3 shows that there is little sign of such reversibility.

In the case of males there is evidence for a small degree of recovery, since the first 8-day interruption at 20° C. did slightly increase the further expectation of life at 30·5° C., although the second 8-day interruption did not. In the case of females flies exposed intermittently had total survival times at 30·5° C. which were if anything slightly shorter than those of flies exposed continuously.

It is concluded that, at least in the case of females, the changes responsible for death at 30·5° C. cannot to any appreciable extent be reversed or repaired at 20° C.

Table 3. *Expectation of life at* 30·5° C.

	No. of flies	Further expectation of life at 30·5° C. days
Females		
(1) Exposed continuously to 30·5° C.	50	17·60±0·65
(2) After 5 days at 30·5° C.		
(*a*) Exposed continuously	50	12·60±0·65
(*b*) 8-day interruption at 20° C. after 5 days at 30·5° C.	25	11·02±0·28
(3) After 13 days at 30·5° C.		
(*a*) Exposed continuously	44	5·82±0·49
(*b*) 8-day interruption at 20° C. after 5 days at 30·5° C.	25	3·02±0·28
(*c*) two 8-day interruptions at 20° C. after 5 and 13 days at 30·5° C.	22	5·23±0·32
Males		
(1) Exposed continuously to 30·5° C.	50	7·58±0·28
(2) After 5 days at 30·5° C.		
(*a*) Exposed continuously	49	2·64±0·27
(*b*) 8-day interruption at 20° C. after 5 days at 30·5° C.	44	5·23±0·38
(3) After 8 days at 30·5° C.		
(*a*) Exposed continuously	21	1·40±0·35
(*b*) 8-day interruption at 20° C. after 5 days at 30·5° C.	36	2·97±0·36
(*c*) Two 8-day interruptions at 20° C. after 4 and 8 days at 30·5° C.	25	2·42±0·23

5. THE EFFECTS ON THE EXPECTATION OF LIFE AT 20 °C. OF A PREVIOUS EXPOSURE OF ADULTS TO 30·5 °C.

Since the changes responsible for death at 30·5° C. are not reversed at 20° C., it seemed possible that the same processes might ultimately be responsible for death at the two temperatures. If the ageing processes were in fact identical a simple relationship would hold for total life span, whereby a period of n days at 30·5° C. would reduce the expectation of life at 20° C. by $n \times 55{\cdot}9/17{\cdot}6$ days for females, and by $n \times 67{\cdot}4/7{\cdot}58$ days for males. Although slight differences between the ageing processes at the two temperatures might upset this simple relationship, it would still be expected that exposure to 30·5° C. would reduce the expectation of life at 20° C.

Experiments in which flies were kept for varying periods at the two temperatures do not confirm this simple hypothesis. Fig. 2 and Table 4 show the results of exposing young adult females to 30·5° C. for 5, 8 and 12 days (i.e. for 28, 45 and 68 % of their expectation of life at that temperature) and then keeping them at 20° C. until they died. So far from reducing their expectation of life this exposure in fact increased it, by as much as 50 % in the case of females exposed for 8 days. These results are fully confirmed by a similar experiment to be described in §7.

A similar experiment on males (Table 5 and Fig. 3) gave slightly different results. A group of males were exposed to 30·5° C. for 5 days, i.e. for 66% of their expectation of life at that temperature. Four males died almost immediately after their

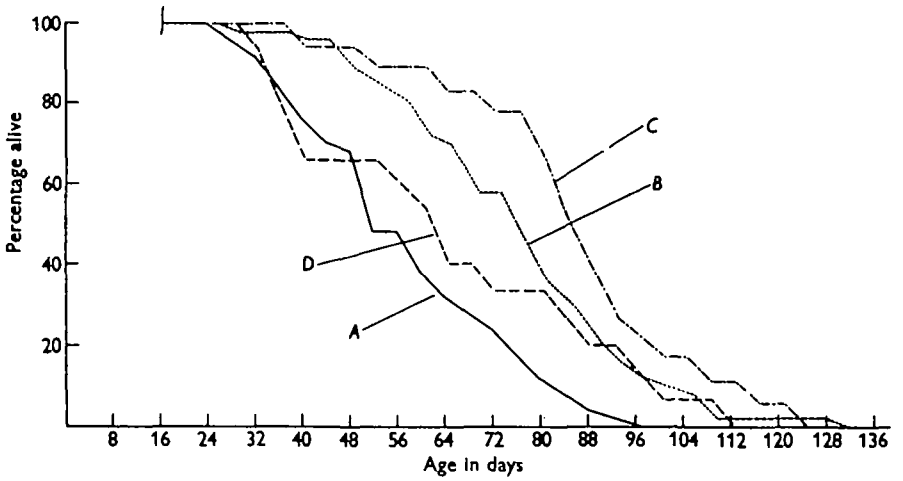

Fig. 2. Survival time at 20° C. of females previously exposed to 30·5° C. *A*, Unexposed; *B*, exposed for 5 days; *C*, exposed for 8 days; *D*, exposed for 12 days.

Table 4. *Expectation of life in days of females kept at* 20° C.

	No. of flies	Further expectation of life in days at age 17 days
Kept continuously at 20° C.	50	38·9±2·6
Exposed to 30·5° C. for 5 days (6th to 10th day after emergence)	47	57·2±3·0
Exposed to 30·5° C. for 8 days (6th to 13th day after emergence)	18	67·8±4·9
Exposed to 30·5° C. for 12 days (6th to 17th day after emergence)	15	50·0±6·6

Table 5. *Expectation of life in days of males kept at* 20° C.

	No. of flies	Further expectation of life in days at age 10 days	No. of flies	Further expectation of life in days at age 14 days
Kept continuously at 20° C.	50	57·4±2·5	50	53·4±2·5
Exposed to 30·5° C. for 5 days (6th to 10th day after emergence)	47	51·5±3·6	43	53·4±2·8

return to 20° C. If these are ignored, as in the last column of Table 5, the expectation of life of the remainder at 20° C. did not differ from that of the unexposed controls. Thus the simple additive assumption outlined above is again contradicted by the results, but in this case there is no prolongation of life due to exposure; these findings are again confirmed by an experiment to be described in §7.

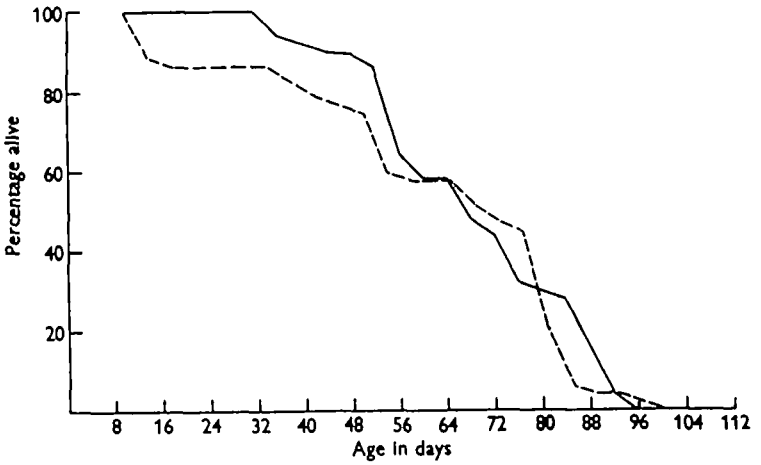

Fig. 3. Survival time of males at 20° C. Full line, unexposed; broken line, exposed for 5 days to 30·5° C.

6. OTHER EFFECTS OF EXPOSURE TO 30·5° C.

(*a*) *Male mating behaviour and fertility*

Twenty-five males were kept for 5 days at 30·5° C. and paired with virgin **B/K** females immediately after being returned to 20° C. Nineteen of these pairs were seen to mate normally within 1 hr. Eggs were collected from the nineteen mated females, with the results shown in Table 6. Exposure to 30·5° C. did not seriously impair the mating ability of these males, nor, with the exception of one mating, did it greatly reduce their fertility.

Table 6. *Fertility of flies after exposure to* 30·5° C. *for* 5 *days*

	No. of females	No. of eggs		
		Laid	Hatched	Unhatched
Females exposed and then mated	9	93	85	8
Females mated and then exposed	20	216	191	25
Females mated to exposed males	18	187	182	5
	1	16	0	16

(*b*) *Female mating behaviour and fertility*

Twenty virgin females were exposed for 5 days to 30·5° C., and paired with **B/K** males immediately after their return to 20° C. In no case did mating take place within 1 hr., and the females were seen to extrude their ovipositors towards approaching males, a signal normally characteristic of inseminated females (Maynard Smith, 1956*a*). These twenty pairs were kept in the dark (in which mating does not take place) and tested in the light for 1 hr. daily for the next 12 days. Seven pairs mated, apparently normally, during the next 4 days and two more mated on the

tenth day. The remaining eleven females did not mate and continued to extrude their ovipositors. These eleven females were dissected; as expected, they were found not to contain sperm. The state of development of their ovaries was variable but the ovaries were always smaller than those of unexposed females. This means that there was a regression of the ovaries during exposure to 30·5° C., since the females had been kept for 5 days at 20° C. before exposure and at that age their ovaries would have been fully developed.

This experiment was repeated with 'ovariless' females from the *grandchildless* stock (Spurway, 1948). It was found that 'ovariless' females do not mate for 2 days after emergence, mate readily 4 days after emergence, extrude their ovipositors at an approaching male once they have been inseminated, and that after 5 days at 30·5° C., even if virgin, they behave like fertilized females, extruding the ovipositor and refusing to mate. It seems therefore that exposure of normal females to 30·5° C. causes a partial regression of the ovary and produces a long-term change in behaviour, similar to that caused by the stimulus of insemination, but that the change of behaviour is not caused by changes in the ovary since an exactly similar change in behaviour occurs in 'ovariless' females.

Eggs were collected from the nine exposed virgin females which did mate, and also from twenty females which mated before being exposed for 5 days to 30·5° C. The results are shown in Table 6. A high proportion of the eggs laid by exposed females hatch and the second experiment shows that sperm stored in a female's receptacle is not seriously damaged by exposure to 30·5° C.

(*c*) *Female productivity*

Since it is known that about 90% of the eggs laid by exposed females hatch, a rough estimate of the rate of egg-laying can be obtained by keeping such females in food vials for a day and counting the number of adult offspring which emerge. The 'mean productivity' (i.e. the number of adult offspring which emerged from a day's lay of eggs, divided by the number of surviving females) was measured for a group of twenty-two females, mated to **K/NFS** males and then exposed to 30·5° C. for 5 days; measurements were made at intervals until the females were 63 days old. In Fig. 4, the productivity of these twenty-two females is compared with that of eleven unexposed females measured in a similar manner.

No offspring were obtained from the exposed females for the first few days after their return to 20° C., and tests on other females showed that no eggs are laid during this period. But from the 12th to the 30th day after their return to 20° C. all females were producing at least a few offspring. However, the productivity of the exposed females was always lower than that of the controls, and this almost certainly reflects a lower rate of egg-laying.

7. THE RATE OF EGG-LAYING AND THE RATE OF AGEING

Since the expectation of life of females, but not of males, was increased by exposure to a high temperature, and since that exposure also caused a partial regression of the ovaries and a permanent reduction in the rate of egg-laying, it seemed possible

that the latter changes were the direct cause of the former. That is, unexposed females may die sooner because they lay eggs more rapidly.

Therefore two other ways of reducing the rate of egg-laying by females were investigated. The first is to keep the females unmated; virgin females do lay eggs, but at a greatly reduced rate (Maynard Smith, 1956*a*). The second is to use females without ovaries. Females homozygous for the mutant *grandchildless* lay eggs which develop into adults without gonads. The testes in this species have an orange sheath, and can be seen through the body wall. Thus a female can be recognized as *gs*/*gs* because her sons have no testes; it can then be assumed that her daughters have no ovaries, without dissecting the daughters. The stock which segregates for *gs* is necessarily highly inbred. Therefore virgin females from this stock were crossed to **K** males, and outbred 'ovariless' females obtained from the F_1. As normal controls, offspring from similar crosses in which the female parent proved not to be *gs*/*gs* were used.

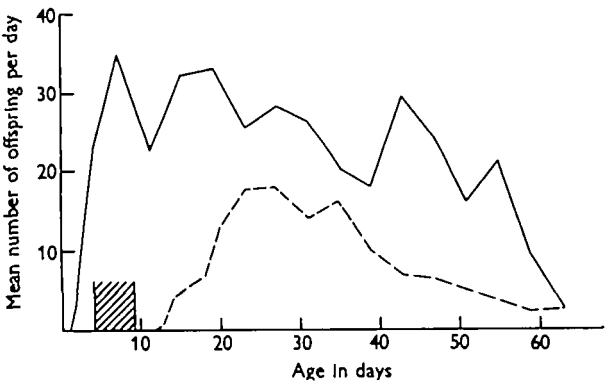

Fig. 4. Full line, mean productivity of eleven females kept continuously at 20° C.; broken line, mean productivity of 22 females exposed for 5 days to 30·5° C.

Life tables were obtained for the following groups of flies: (i) normal mated females; (ii) normal virgin females; (iii) 'ovariless' females; (iv) normal mated females exposed to 31° C. for 5 and for 6 days; (v) 'ovariless' females exposed to 31° C. for 3 days; (vi) normal mated males; and (vii) normal mated males exposed to 31° C. for 3 and for 4 days.

Two virgin females were kept in each vial; all other vials contained a male and a female.

The fact that flies were exposed to 31° C., and not to 30·5° C. as before, was due to an error; the periods of exposure were made correspondingly shorter. 'Ovariless' females were exposed for a shorter time than normal females because preliminary experiments suggested that their expectation of life at high temperatures was lower.

The results are given in Fig. 5 and Table 7. As before, a few males died immediately after their return to 20° C., but if these are ignored exposure for 3 days to 31° C. did not alter the expectation of life at 20° C. although exposure for 4 days did slightly reduce it.

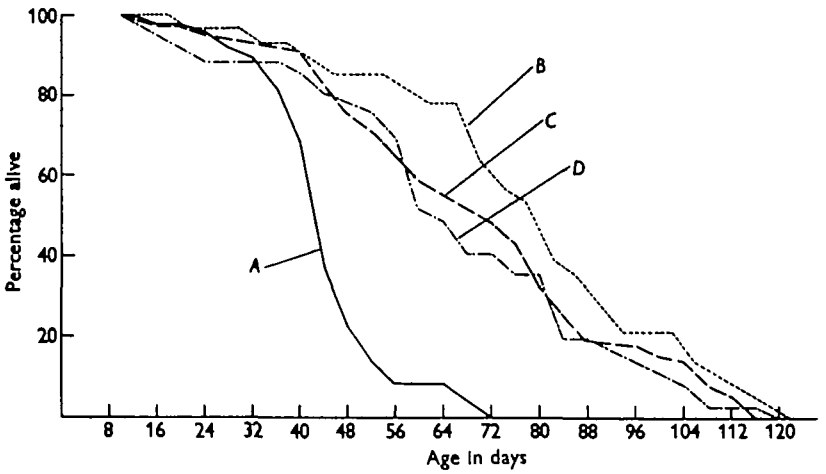

Fig. 5. Survival time of females at 20° C. *A*, Mated females; *B*, 'ovariless' females; *C*, virgin females; *D*, mated females exposed for 5 or 6 days to 31° C.

Table 7. *Expectation of life in days of flies kept at* 20° C.

	No. of flies	Further expectation of life in days at age 10 days	No. of flies	Further expectation of life in days at age 20 days
Males				
Kept continuously at 20° C.	79	41·1 ± 1·7	76	32·6 ± 1·6
Exposed to 31° C. for 3 days	39	36·3 ± 3·5	31	34·8 ± 1·8
Exposed to 31° C. for 4 days	26	23·8 ± 3·6	17	24·4 ± 3·2
Mated females				
Kept continuously at 20° C.	48	33·1 ± 1·6		
Exposed to 31° C. for 5 days	23	61·2 ± 5·7		
Exposed to 31° C. for 6 days	14	45·2 ± 5·7		
Virgin females				
Kept continuously at 20° C.	89	58·7 ± 2·7		
'Ovariless' females				
Kept continuously at 20° C.	28	67·6 ± 4·7		
Exposed to 31° C. for 3 days	22	64·2 ± 5·4		

The expectation of life of normal females was again significantly increased by exposure to 31° C. Both virgin and 'ovariless' females lived for longer than the controls and closely resembled females exposed to a high temperature. Finally, the expectation of life of 'ovariless' females was not increased by exposure to 31° C. In this respect 'ovariless' females resemble males and not normal females.

It is therefore reasonably certain that the hypothesis outlined above is correct; the expectation of life of females can be increased by reducing the rate of egg-laying, either by keeping them unmated, or by using 'ovariless' females, or by exposing normal females to a high temperature for a period sufficient to cause a partial regression of the ovaries. The failure to increase the life span of 'ovariless' females by exposure to a high temperature seems decisive in showing that the prolongation of the life of normal females by such exposure is the direct result of the effect of high temperature on their ovaries, and so on the rate of egg-laying.

8. DISCUSSION

There is other evidence that in insects the longevity of females is reduced by laying eggs. Bilewicz (1953) found that in *D. melanogaster* virgin females lived for approximately twice as long as mated females but laid about the same total number of eggs in a lifetime; unmated males also lived longer than mated ones but the difference was very small. Similarly, Griffiths & Tauber (1942) found that virgin females of *Periplaneta americana* laid eggs less rapidly and lived for about 60% longer than did mated females. Rockstein (1957) found that the longevity of female houseflies, but not of males, was increased by adding powdered milk to a diet of sugar and water. Since the females on sugar and water only laid at least some eggs, this last observation suggests that in an egg-laying female various substances may be utilized more rapidly than they can be assimilated or synthesized and that this may accelerate ageing.

The other main point of interest concerns the causes of ageing at different temperatures. It seems reasonable to regard the changes which ultimately kill individuals kept at 30·5° C. as processes of ageing, since they are not reversible at 20° C. and since they take an appreciable time to reach completion (17·6 days for females as compared to 55·9 days at 20° C.). Yet these changes are not the same as those responsible for ageing at 20° C., since if they were the same, exposure to 30·5° C. would reduce the further expectation of life at 20° C. This conclusion holds both for males and for females. It follows that different processes are responsible for ageing and ultimately for death at the two temperatures.

9. SUMMARY

1. The adult life span of *Drosophila subobscura* has been measured at temperatures varying from 20° to 33° C. A sharp increase in the slope of the curve of log survival time against temperature occurs at temperatures above 31° C.

2. Changes which occur in individuals at 33° C. or above are reversible, at least in part, at 20° C.; but changes occurring at 30·5° C. are irreversible in the sense that the total survival time at 30·5° C. is not increased by intervening periods at 20° C.

3. Exposure of young adult flies to 30·5° C. for a period of about half their expectation of life at that temperature significantly increases the further expectation of life of females at 20° C. but does not alter the expectation of life of males. Such exposure causes a partial regression of the ovaries of females, a permanent change in their behaviour and a reduction in their rate of egg-laying; exposure does not alter the behaviour or seriously reduce the fertility of males.

4. 'Ovariless' females and virgin females live for significantly longer than do normal mated females. The expectation of life of 'ovariless' females at 20° C. is not altered by exposure to 30·5° C. It is concluded that egg-laying accelerates the ageing of females at 20° C., and that the prolongation of life of females exposed to 30·5° C. is due to the reduction in the rate at which such females subsequently lay eggs.

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REFERENCES

BILEWICZ, S. (1953). Experiments on the effects of reproductive functions on the length of life of *Drosophila melanogaster*. *Folio Biologica*, **1**, 177–94.

GRIFFITHS, J. T. & TAUBER, O. E. (1942). Fecundity, longevity, and parthenogenesis of the American Roach, *Periplaneta americana* L. *Physiol. Zoöl.* **15**, 196–209.

MAYNARD SMITH, J., CLARKE, J. M. & HOLLINGSWORTH, M. J. (1955). The expression of hybrid vigour in *Drosophila subobscura*. *Proc. Roy. Soc.* B, **144**, 159–71.

MAYNARD SMITH, J. (1956*a*). Fertility, mating behaviour and sexual selection in *Drosophila subobscura*. *J. Genet.* **54**, 261–79.

MAYNARD SMITH, J. (1956*b*). Acclimatization to high temperatures in inbred and outbred *Drosophila subobscura*. *J. Genet.* **54**, 497–505.

MAYNARD SMITH, J. (1957). Temperature tolerance and acclimatization in *Drosophila subobscura*. *J. Exp. Biol.* **34**, 85–96.

ROCKSTEIN, M. (1957). Longevity of male and female houseflies. *J. Geront.* **12**, 253–6.

SPURWAY, H. (1948). The genetics and cytology of *Drosophila subobscura* IV. An extreme example of delay in gene action causing sterility. *J. Genet.* **49**, 126–40.