Experimental Studies on the Duration of Life. XIII. The Influence of Different Feeding during the Larval and Imaginal Stages on the Duration of Life of the Imago of Drosophila melanogaster

Author(s): W. W. Alpatov
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EXPERIMENTAL STUDIES ON THE DURATION OF LIFE

XIII. THE INFLUENCE OF DIFFERENT FEEDING DURING THE LARVAL AND IMAGINAL STAGES ON THE DURATION OF LIFE OF THE IMAGO OF *DROSOPHILA MELANOGASTER*[1]

W. W. ALPATOV[2]

I

ALTHOUGH the fruit-fly has been for almost thirty years a favorite object of experimental entomology (see Castle, Clark, Mast and Barrows, 111), the first accurate paper on duration of life of this insect was published only in 1921 (Pearl and Parker, 21), opening a new period in the history of the experimental study of duration of life in general and that of insects in particular. Sufficient time has passed since that publication to justify the making of a short review of all attempts in this field. This is done in summarized form in Table I.

It can be seen from this table how diversified are the factors which have been studied in their application to the duration of life. At the same time it is evident that all these efforts are far from being sufficient to give us a complete picture of the relationship of different factors to the duration of life. For instance, a whole group of external factors like radiant energy of different wave-length, electricity and magnetism has never (with one exception—Northrop) been studied in relation to longevity. The feeding, and particularly the influence of different foods on duration of life, has also never been tested on *Drosophila*. Other insects like bees (Phillips, 118) proved to be excellent material for studies of influence of different foods on duration of life. Another point to be emphasized is that even in the cases of thor-

[1] From the Institute for Biological Research of the Johns Hopkins University.

[2] Research fellow of the International Education Board.

TABLE I

REVIEW OF THE QUANTITATIVE DATA RELATING TO THE INFLUENCE OF DIFFERENT FACTORS UPON THE DURATION OF LIFE IN *Drosophila melanogaster*

Groups of factors	Factor	Author	Year	Influence found or not and its character
Genetic factors	Mutation	Hyde	1913	Shortens the duration of life.
	Line breed strains	Pearl and Parker	1922	Short and long lived lines.
	Mutations	Pearl, Parker and Gonzalez	1923	Shorten the duration of life.
	Mutations	Gonzalez	1923	Shorten the duration of life.
Temperature	Temperature of imaginal life	Loeb and Northrop	1917	Low temperature increases the duration of life.
	Temperature of imaginal life	Alpatov and Pearl	1929	Low temperature increases the duration of life.
	Temperature of the development	Alpatov and Pearl	1929	Low temperature increases the duration of life.
Humidity	Ventilation	Pearl and Parker	1922	Live longer in ventilated vials.
	Absorbent paper in food	Pearl and Parker	1921	No effect.
Light	.....	Northrop	1925	Above 100 meter candles the duration of life is rapidly shortened.
Clipping the wings	.....	Pearl, Miner and Parker	1927	No effect.
Narcoties	Etherization	Pearl and Parker	1922	No effect.
Food and feeding	Starvation and 5 per cent. alcohol	Sekla	1928	Alcohol prolongs the duration of life.
	Starvation with water	Lutz	1915	Shortens the life.
	Complete starvation	Pearl and Parker	1924	Shortens the life.
	Intermittent starvation	Kopeč	1928	Shortens and (?) prolongs the duration of life.
	Prolongation of larval life by underfeeding	Northrop	1917	No effect on imaginal life.
	Embryonic juice and larval pulp	Pearl and Parker	1922	No effect.
	Agar + water, salt and dextrol or "glucose-agar"	Loeb and Northrop	1917	Life longest on "glucose-agar."
Aseptic culture	Aseptic larval and pupal life	Steinfeld	1928	Aseptic conditions prolong the duration of life.
	Aseptic whole life			
Density of population	Density of population	Pearl and Parker Pearl and Parker Pearl and Parker	1922 1923 1927	There is an optimal density of population.
Duration of embryonic period (egg-larval period)	.....	Lutz	1915	Negatively related to the duration of life.

ough study of the influence of a particular factor on the duration of life the character of the functional relationship between the particular factor and the duration of life has usually remained undiscovered. Exceptions are such factors as temperature (Loeb and Northrop; Alpatov and Pearl), density of population (Pearl, Miner and Parker, 117) and light (Northrop, 115). Unfortunately, the last paper gives only a very abbreviated summary of the results, without showing the original data or even the calculated constants.

These remarks give a certain justification for showing in Fig. 1 curves based on data published by Kopeč (114).

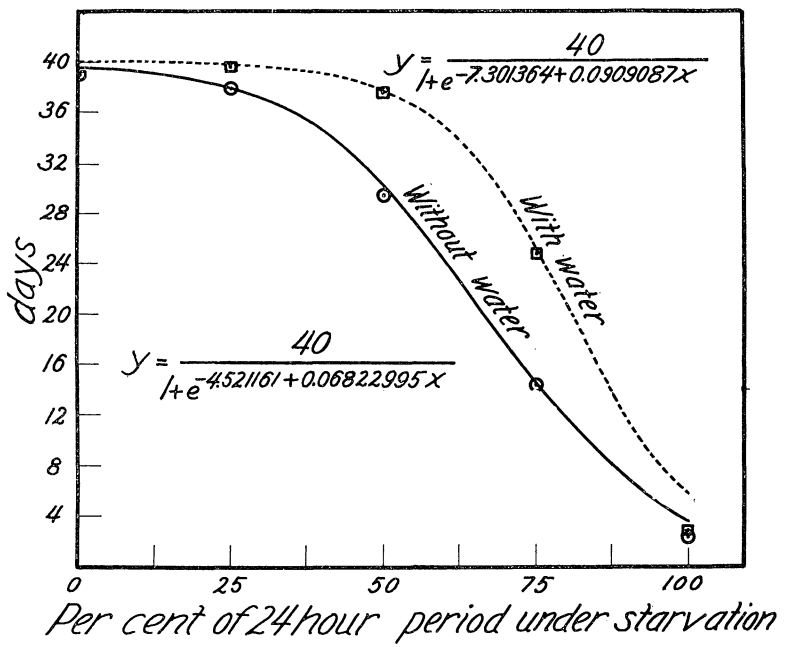

FIG. 1

The upper curve represents the relation between the duration of life at different grades of intermittent starvation with water, and the lower one represents the same but for experiments when water was not supplied. The period of transferring the flies from food in empty bottles was equal to twenty-four hours—flies being kept without food six, twelve and eighteen hours—which we

expressed as 25 per cent., 50 per cent. and 75 per cent. starvation. The curves, which fit very well, particularly the left portion of the observed points, belong to the type of logistic curves.[3] As far as the right-hand portion of the observed data is concerned (near the very high degree of starvation) there is not sufficient evidence to be sure that there is here a trend corresponding to the branch of logistic curve with a decreasing slope. But on the whole these curves differ entirely from the exponential curve which represents the relation of duration of life to temperature (Alpatov and Pearl, 110) as well as that of duration of life and density of population (Pearl, Miner and Parker, 117).

II

Our present investigation is to be considered as a preliminary study for a future detailed investigation of the influence of nutrition on the duration of life of *Drosophila.* Our material is based on three independent duration of life experiments. The first of these consisted in producing small size flies by taking the larvae from the food before the normal end of larval feeding and testing their longevity. The two others give information concerning the duration of life of flies kept on synthetic food without yeast, and the influence of changing food every day as compared with every second day.

The preparation of the material for the first experiment consisted in putting 0–4-hour-old larvae collected according to a method previously described (108) in half-pint bottles containing 100 cc synthetic medium, with yeast planted the day before. One hundred larvae of wild flies line 107 were put in each bottle. The larvae were kept at a temperature of 25° C. Thirteen bottles with 100 larvae in each bottle produced 1,033 adult flies—535 females and 498 males. That means that 79.5 per cent. of the larvae reached the adult stage, the number

[3] The method used to fit the logistic curves is described by Reed and Berkson (p. 767, 119).

of males being equal to 93.1 of the females. The control flies emerging from normally fed larvae were placed in one-ounce duration of life bottles, fifteen males and fifteen females in each, except one which had ten males and twenty females. The total number of flies for which death has been recorded may be found in Table III.

The underfed larvae were kept in the same way as the normally fed until they had fed fifty-nine hours. At that time they were taken from the food and placed in bottles with plain 2 per cent. agar-agar washed in distilled water. The mouths of the bottles were covered with 40 mm watch glasses sealed with plasteline until the moment of pupation when the watch glasses were again replaced by the usual cotton stopper. This was done to prevent the larvae from crawling out as they are apt to do before pupation. Out of 1,745 larvae put on plain agar-agar 1,243 adult flies emerged—651 females and 592 males—which shows that out of fifty-nine-hour-old larvae only 71.2 per cent. reached the final stage. In other words, there is a comparatively high mortality among the larvae unable to pupate. The males were equal in number to 90.9 per cent. of the females. The same density in duration of life bottles as in the controls was used for underfed flies, except in one bottle where thirteen males and seventeen females were kept together, and two others with thirty females each. The density in the rest of the bottles was fifteen males and fifteen females per bottle. The experience of this institute does not give any conclusive indications about the influence of celibacy on duration of life. Therefore we assume the right to include in our life table calculations the records of these sixty females kept without males. Records of the number of dead males in five bottles and of females in three bottles were larger than the original number of flies put in the bottles in the beginning of the experiments and were therefore discarded from the calculations. Food was changed every day except Sundays. We will not go into the history of the question of influence of underfeeding

of larvae upon the size of the imago, another paper (109) being partly devoted to this question.

III

Table II represents the basic biometrical constants of the measurements of the wing length and width of our underfed and normally fed flies. The measurements were made according to the scheme described before (110). Two most important facts have to be emphasized. First, that the reduction in the size of the wing is much more pronounced in females than in males. It can be judged from the values of the ratios and also from the expression of the length of wings of underfed flies in proportion to the length of wings of normally fed ones. This expression for females is equal to 83.3 and for males it is equal to 92.0. This peculiarity has its explanation in the fact that the difference in size between larvae which will produce males and females becomes more and more pronounced as the larvae approach the pupal stage. In other words, the larvae which will produce males are closer to the final larval size at a given moment of the larval life than the larvae which will become female imagoes. Therefore, younger larvae taken from the food produce males which are closer to normal males than the corresponding females to normally fed females. The second striking difference is the much larger variability of the underfed flies. This greater variation of experimental flies is in accord with a long-known fact that abnormal, unfavorable conditions increase the variation (see Pearl, 116). It is interesting to note that in case of wing width the underfed males have even broader wings than the corresponding females. The sex difference between the underfed flies is almost negligible as compared with that in normally fed flies. Tables III and IV and Figs. 2 and 3 represent the results obtained. The underfed females show the same duration of life as the control ones. The males show a longer duration, although the difference is statistically not very signifi-

TABLE II

BIOMETRIC CONSTANTS OF FLIES DEVELOPED FROM UNDERFED AND NORMALLY FED LARVAE IN MILLIMETERS

		Females from normally fed larvae	Difference and Ratio $\frac{\text{Diff.}}{\text{P.E.}}$	Females from underfed larvae	Males from normally fed larvae	Difference and Ratio $\frac{\text{Diff.}}{\text{P.E.}}$	Males from underfed larvae
Length of the wing	Mean	1.738 ± .005	.291 ± .013 R = 22.4	1.447 ± .012	1.532 ± .004	.123 ± .008 R = 15.4	1.409 ± .007
	Standard deviation	.0398	—	.1246	.0358	—	.0770
	Coefficient of variation	2.29 ± .21	—	8.61 ± .60	2.34 ± .18	—	5.46 ± .37
	N	25	—	47	40	—	50
Width of the wing	Mean	.9969 ± .0030	.1844 ± .0076 R = 24.3	.8125 ± .0070	.8962 ± .0029	.0800 ± .0052 R = 15.4	.8162 ± .0043
	Standard deviation	.0223	—	.0709	.0270	—	.0447
	Coefficient of variation	2.24 ± .21	—	8.73 ± .61	3.01 ± .23	—	5.48 ± .37
	N	25	—	47	40	—	50

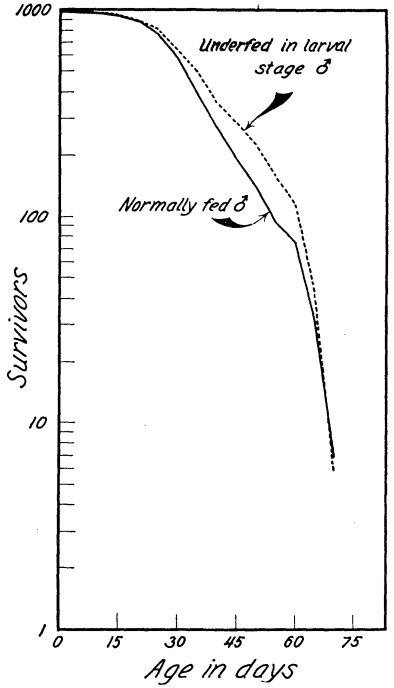

FIG. 2

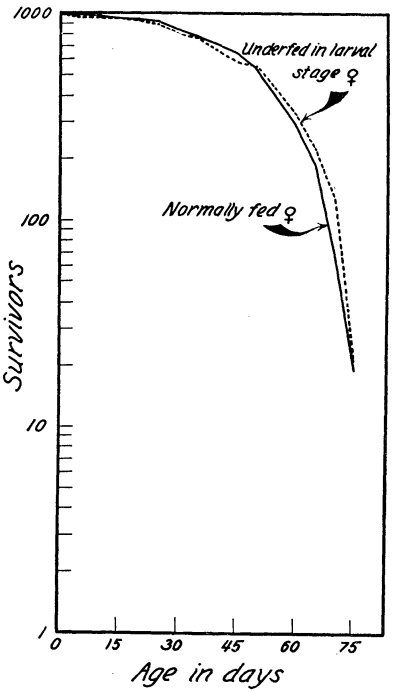

FIG. 3

cant. The conclusion which may be drawn is that in spite of the very significant reduction in size (for the female wing length 16.7 per cent., wing width 18.5; for the male wing length 8.0, wing width 8.9) the duration of life remains about the same. Comparing these results with the conclusions drawn in a paper on temperature (110) where it was shown that cold flies, characterized by larger body size, have at the same time a greater longevity than the control ones (in this case the reduction in size for four characters was for males equal to 10.1 per cent., and to 7.8 for females) it must be admitted that the size of body itself is not the fundamental factor determining the duration of life. The same somatological effect—reduction of body size—when produced by different factors (in our first case by temperature, in the present case by undernourishment) gives quite different results.

TABLE III

SURVIVORSHIP DISTRIBUTION AND BIOMETRIC CONSTANTS OF FLIES UNDERFED AND NORMALLY FED IN THE LARVAL STAGE

Days	Underfed flies		Normally fed flies	
	Male	Female	Male	Female
0– 4	1,000	1,000	1,000	1,000
5– 9	987	976	989	989
10–14	979	967	975	981
15–19	949	950	945	965
20–24	887	935	897	960
25–29	782	886	814	928
30–34	646	803	593	846
35–39	500	762	393	785
40–44	361	677	271	718
45–49	288	585	192	651
50–54	223	523	140	534
55–59	158	426	95	398
60–64	115	327	75	290
65–69	42	220	32	187
70–74	6	128	7	69
75–79	0	19	0	21
80–84	—	0	—	0
Mean	37.11 ± .52	48.42 ± .54	34.59 ± .44	49.11 ± .50
Standard deviation	15.26	18.41	13.59	16.12
Coefficient of variation	41.12 ± 1.14	38.02 ± .89	39.29 ± 1.02	32.82 ± .79
Absolute number of flies	397	532	444	474

We want to emphasize the danger of fallacious conclusions. It is not entirely improbable that a little greater duration of underfed males and an equal duration of underfed females originated as a result of a certain selective process. Only the strongest larvae succeed in the struggle for life without a sufficient supply of food. Table IV contains also some indices calculated by the well-known approximate formula recommended by Johannsen (113, p. 706). There is a slight reduction of the sex differences in duration of life among underfed flies as compared with normal ones.

TABLE IV

AVERAGES AND INDICES OF THE DURATION OF LIFE OF FLIES UNDERFED IN LARVAL STAGE AND NORMALLY FED

	Underfed in larval stage	Difference and Ratio	Normally fed in larval stage
	Average duration of life		Average duration of life
Female ...	48.42 ± .54	.69 ± .74	49.11 ± .50
		R = .93	
Male	37.11 ± .52	2.52 ± .68	34.59 ± .44
		R = 3.7	
	Indices		Indices
Male	76.65 ± 1.37	6.22 ± 1.79	70.43 ± 1.15
		R = 3.5	
Female ...	100	—	100
Male	107.30 ± 2.02	8.71 ± 2.51	100
Female ...	98.59 ± 1.49	R = 3.5	100

IV

The next experiment originated from a purely practical question. In our experiments on egg production of the fruit-fly we used the synthetic food as a substratum given to females on which to deposit eggs. To strew yeast on the surface was a great nuisance because it hampered us in counting the eggs. We finally decided to put some drops of yeast suspension on the surface of the synthetic food. But the question whether the presence of yeast influences the duration of life of adult flies yet remained unsolved. The question is, in other words, whether the carbohydrates available in synthetic food (the new synthetic medium contains 8.3 per cent. of cane sugar) are sufficient to keep up the life of an adult *Drosophila*.

We must admit that the experiment was not perfect from one point of view. The flies used came from ordinary room temperature mass culture, while undoubtedly it would be much more desirable to work with flies developed under complete absence of any micro-organisms. In the beginning of the experiment the surface of the food in bottles without yeast remained during twenty-

four hours just as shiny as at the moment of putting the flies in the bottles. Toward the middle of the experiment there could be observed in bottles a certain kind of micro-organism growth. But this slight growth was entirely different from the usual growth of yeast on the surface of the synthetic medium.

The flies used in this experiment were taken from wild line 107 on the fifth and sixth days after the beginning of the emergence in the corresponding bottles. Naturally their age at the moment of the beginning of the experiment was equal to 0–24 hours. The food in experimental and control sets of bottles was changed every day including holidays. The density was twenty-five males and twenty-five females per bottle. In the series kept without yeast we included also a bottle with thirty-two females kept without males, and a bottle with fifty females which were put without males according to the record taken at the moment of starting the experiment,

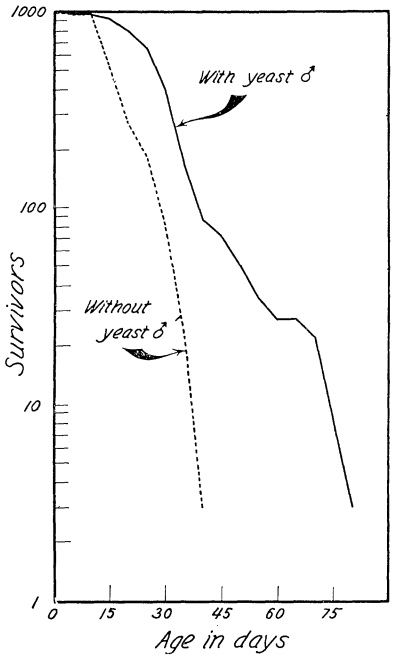

FIG. 4

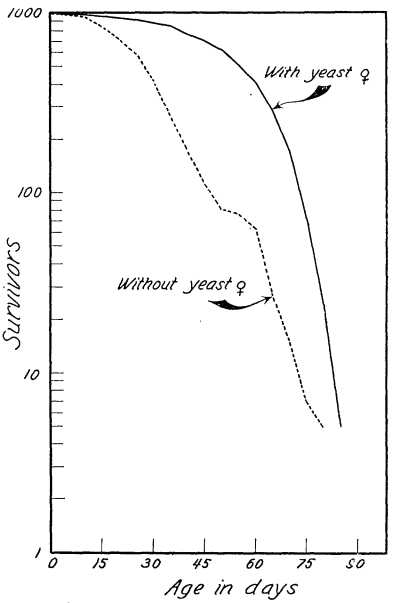

FIG. 5

but according to the subsequent observations on the process of dying out it turned out that the bottle contained also eight males. The series with yeast, besides normally populated bottles (twenty-eight males and twenty-five females), includes one which had thirty-two females kept separately, and another which had twenty females and twenty males.

The results are brought together in Tables V and VI and represented graphically on Figs. 4 and 5. It can be seen at once that the flies kept without yeast live a much

TABLE V

SURVIVORSHIP DISTRIBUTION AND BIOMETRIC CONSTANTS OF FLIES KEPT ON SYNTHETIC FOOD WITH AND WITHOUT YEAST

Days	Food without yeast		Food with yeast	
	Male	Female	Male	Female
0– 4	1,000	1,000	1,000	1,000
5– 9	983	986	978	985
10–14	969	955	973	980
15–19	523	842	924	960
20–24	276	722	808	948
25–29	186	590	655	933
30–34	82	420	400	895
35–39	23	271	171	845
40–44	3	175	88	772
45–49	0	113	73	717
50–54	—	82	51	625
55–59	—	77	35	517
60–64	—	63	27	412
65–69	—	27	27	289
70–74	—	15	22	171
75–79	—	7	8	71
80–84	—	5	3	23
85–89	—	0	0	5
90–94	—	—	—	0
Mean	17.725 ± .258	29.25 ± .48	28.715 ± .417	53.24 ± 1.80
Standard deviation	7.220	14.625	11.92	17.58
Coefficient of variation	40.73 ± 1.03	50.00 ± 1.43	41.51 ± 1.19	33.02 ± .87
Absolute number of flies	356	417	372	399

TABLE VI

AVERAGES AND SEX INDICES OF THE DURATION OF LIFE OF FLIES KEPT WITHOUT YEAST AND WITH YEAST

	Without yeast Average duration of life	Difference and ratio	With yeast Average duration of life
Females	29.25 ± 0.48	23.99 ± 1.90 R = 12.63	53.24 ± 1.80
Males	17.725 ± 0.258	10.99 ± 0.490 R = 22.43	28.715 ± 0.417
	Indices		Indices
Males	60.60 ± 1.33	6.66 ± 2.39 R = 2.79	53.94 ± 1.98
Females	100	.....	100
Males	61.73 ± 1.26	6.79 ± 2.41	100
Females	54.94 ± 2.06	R = 2.81	100

shorter time than the control ones. If we compare the sex index of duration of life (meaning the male duration of life expressed in per cent. of the female) of our second experiment with those of the first (underfed and normally fed flies) we observe that in the former the index is significantly higher than in the latter. We have no data to explain this difference, leaving it for further investigation.

Up to the present time most of the authors working on nutrition of flies have concentrated their whole attention on the requirements of larvae for different nutritive substances. Little attention has been paid to the adult form. There are meager data published by Guyénot (112), Loeb and Northrop (16), Vinokuroff (70) and Glaser (75, 76). The first of these authors was mainly interested in the influence of different kinds of food on reproduction. Loeb and Northrop could not show any difference between the duration of life of flies kept on glucose-agar with yeast and without it. Vinokuroff's data show that the average duration of life of flies kept on sugar with addition of peptone is higher than without it (22 days against 17.6). Glaser's conclusions based on a very small number of experimental animals are not very definite.

His statement is: "On a diet of sucrose and bouillon, sucrose and blood serum, glucose and bouillon, glucose and blood serum, the longevity and degree of egg deposition reach their maximum." This means that carbohydrates alone are not sufficient for adult insects which live longer on food with the addition of proteins. In our case very likely such proteins have been supplied by the growing yeast cells. We are perfectly well aware of the fact that a whole series of experiments would be needed to clear up entirely the question whether the proteins could be given in another form than living yeast cells.

V

The last experiment with different feeding arose as a side issue of an attempt to determine the influence of light and darkness, as well as of intermittent light, on the duration of life of *Drosophila*. All three groups were

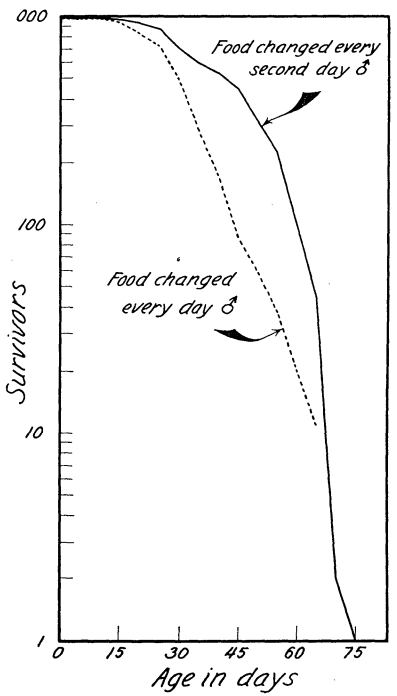

FIG. 6

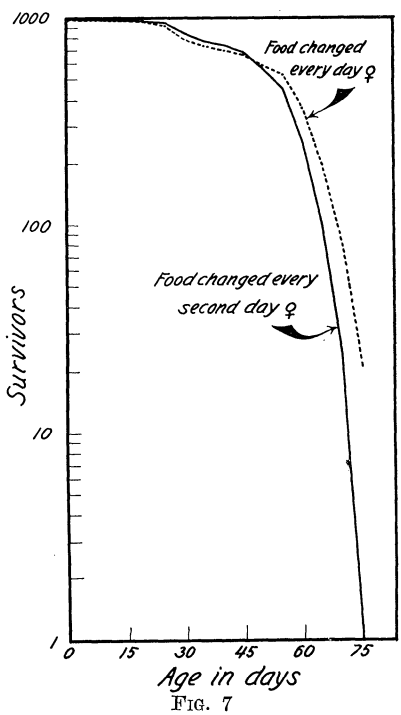

FIG. 7

kept in one incubator and in each of these groups half of the bottles were changed every day (except Sunday) and the other half three times a week. The differences in duration of life under different conditions of illumination were found to be statistically insignificant, which permitted the combining of all flies with similar conditions of food changing, and the comparison of their duration of life with each other. The density in this experiment was twenty-five males and twenty-five females per bottle. Tables VII and VIII and Figs. 6 and 7 show that there is no influence on female duration of life in experimental and control groups. On the other hand, the males in the

TABLE VII

SURVIVORSHIP DISTRIBUTION AND BIOMETRIC CONSTANTS OF FLIES KEPT ON FOOD CHANGED EVERY DAY AND EVERY SECOND DAY

Days	Food changed every day		Food changed every second day	
	Male	Female	Male	Female
0– 4	1,000	1,000	1,000	1,000
5– 9	996	997	999	998
10–14	992	992	992	993
15–19	950	981	970	984
20–24	836	967	938	978
25–29	722	936	878	961
30–34	491	802	701	848
35–39	280	750	601	791
40–44	173	715	538	756
45–49	86	677	447	686
50–54	59	598	329	571
55–59	38	538	228	468
60–64	20	378	92	251
65–69	12	200	43	101
70–74	0	84	2	24
75–79	.....	21	1	1
80–84	.....	0	0	0
Mean	30.775±0.274	50.68±0.42	41.295±0.354	49.555±0.341
Standard deviation	11.03	16.77	14.66	14.22
Coefficient of variation	35.84 ±0.71	33.09±0.64	35.50 ±0.68	28.70 ±0.52
Absolute number of flies	735	732	780	793

TABLE VIII

AVERAGES AND INDICES OF THE DURATION OF LIFE OF FLIES KEPT IN BOTTLES WITH FOOD CHANGED EVERY DAY AND EVERY SECOND DAY

	Every day change Average duration of life		Every second day change Average duration of life
Females ...	50.68 ± .42	1.125 ± 0.54 R = 2.1	49.555 ± .341
Males	30.775 ± .274	10.520 ± .448 R = 23.5	41.295 ± .354
Males	60.72 ± .74	22.6 ± 1.18 R = 19.2	83.33 ± .92
Females ...	100	.....	100
Males	74.52 ± .92	27.75 ± 1.43	100
Females ...	102.27 ± 1.10	R = 19.4	100

group where the food was changed every day show a very low longevity as compared with the controls. There is no doubt about the statistical significance of this difference in this particular experiment. It is remarkable that the duration of life sex index is extremely high for the males in the control group. Even if we compare the male mortality in the group in which the food was changed every day with the control group of our first experiment, and with flies normally fed in the larval stage, the difference remains significant, showing exceptionally low duration of life of males in case the food is changed every day.

Discussing the previous experiment we tried to point out that yeast plays an important rôle in prolongation of the life of *Drosophila melanogaster*. Very likely it is not the dry yeast cells which are eaten by the flies but fresh yeast growth which appears in abundance particularly twenty-four hours after the preparation of the food. Changing the food every day we evidently kept our experimental flies on a kind of intermittent partial starvation, depriving them of luxuriantly grown yeast colonies. This is one of the most plausible explanations of the shorter duration of males on food changed every day.

Why the female did not show the same effect can be perhaps answered by taking into account the differences in female constitution and physiology. The fat body, which represents the place of storing the nutritive substances in insect organisms, is much better developed in females than in males. Besides that, the egg-producing activity in females must react quite differently to the same external factor which first seemed to be responsible for the reduction of male life in case of every day change. A question may arise whether the effect is due to the influence of a purely mechanical shaking, which takes place much more often in the group changed every day as compared with the controls. This difference must be particularly pronounced in the beginning of the experiment. Afterwards the taking out of dead flies even without changing food requires shaking them out and back. We do not think this factor is to be taken into very serious consideration because the handling of the flies has been done by ourselves (W. W. A.) with extreme care and attention. On the other hand, against the possibility of such an explanation is the fact that the whole span of life of control flies is much longer than that of the experimental flies, showing that the process of dying was going differently all the time even when the difference in frequency of shaking the bottles belonging to the two groups disappeared almost entirely.

Summary

Summarizing, it has been shown in this paper that:

(1) The relation between the duration of life and different factors is expressed by quite different types of curves. Temperature and duration of life are connected by a simple exponential curve, while starvation (data from Kopeč) and duration of life at different grades of intermittent starvation can be represented by the upper part of a logistic curve.

(2) *Drosophila* females emerged from larvae taken from the food before the end of the normal larval feed-

ing do not differ in their duration of life from the controls. The males show even a longer (although statistically not very significant) duration of life as compared with the controls. This shows that a reduction in body size does not lead inevitably to a reduction of the duration of life, as has been the case with "room" and "cold" temperature flies which differed in size of the body and of the duration of life.

(3) Keeping flies on synthetic food with yeast and without it indicates that absence of yeast reduces greatly the duration of life of males and females. Very likely the carbohydrates available in the synthetic food are not sufficient for nutrition of adult *Drosophila,* and additional substances included in living yeast cells are required.

(4) Changing synthetic food with yeast every day and every second day indicates that the female duration of life is not affected by this procedure, while males in this experiment show a much shorter duration of life when transferred to new bottles every day. This difference may perhaps be attributed to sex differences in metabolism, or food requirements of male and female organism. Every day food changing can possibly be considered as a partial starvation, because yeast shows growth only after twenty-four hours, and changing food every day does not permit the flies to have yeast growth in such abundance as in the case when the bottles are changed every second day. It is also possible that the results for the males in this particular experiment are not typical.

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