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LONGEVITY IN *DROSOPHILA MELANOGASTER* AND ITS EBONY MUTANT IN THE ABSENCE OF FOOD

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INTRODUCTION

THE effect of the absence of food on longevity has received much attention in recent years. Rau (1910) made observations concerning the duration of life in the saturniid moth, *Samia cecropia,* an insect that does not take food during its imaginal stage. Working with small samples, he reported that the mean length of life of the male was approximately 11 days and that of the female approximately 8 days. Rau and Rau (1912, 1914) reported that the mean length of life in various genera of the saturniid moth ranged from 6.53 days to 15.74 days. They found that the males of *Samia cecropia* (obtained from New York) lived longer than the females, that the males and females of *Tropaea luna* and *Samia cecropia* (obtained from St. Louis) showed no significant differences in mean duration of life, while the females of *Callosamia promethea* and *Telea polyphemus* lived longer than the males. Baumberger (1914) reported studies on longevity in insects without food. Many genera and species were used in this work. The imagoes were caught in a net and for this reason the true dates of hatching and consequently the true ages of the organisms at death were not known. Baumberger, however, concluded from his data that duration of life varies inversely with temperature and is not correlated with systematic groups. Kopec (1924) worked with *Lymantria dispar,* an insect which does not take food during the imaginal stage. He reported that intermittent starvation of the larvae produced a prolongation of the larval period but did not affect the mean duration of life of the adult. Pearl and Parker (1924) reported exact quantitative studies on the duration of life under conditions of complete starvation of two varieties of *Drosophila melanogaster*. They found that under starvation the mean duration of life was almost the same in the vestigial as in the wild-type fly, although in a previous paper (Pearl and Parker, 1921) it was found that under conditions of full feeding the wild-type fly lived approximately three times as long as the vestigial. It was found that density of population had little effect on the length of life of the combined sexes under conditions of starvation. This finding is also in contrast to the results obtained when the flies were fed (Pearl and Parker, 1922). At all densities tested the females had a greater mean duration of life than the males. Lilliland (1938), working with *Drosophila pseudoobscura,* varied temperature, humidity and density of population. It was reported that without food the mean duration of life was greater at lower temperatures, lower densities and greater humidity. It was also reported that Race A lived longer than Race B. The differences were more pronounced at higher humidities.

Lutz (1915) used *Drosophila ampelophila* (*melanogaster*) in experiments dealing with duration of life. He allowed the flies to gain access to water but not to food. The results obtained when compared with more recent work (Pearl and Parker, 1924) showed that the addition of water increased the mean length of life. Loeb and Northrop (1916) performed the same experiment and reported that the mean duration of life varied inversely as the temperature between 9° C. and 34° C.

The reader is referred to Pearl (1928), Alpatov (1930), Pearl and Miner (1935) and Cowdry (1939) for more extensive bibliographies dealing with duration of life.

In the following investigation the duration of life of the wild-type fly of *Drosophila melanogaster* and its ebony mutant in the absence of food and water was studied. The wild-type fly was used as a control. The ebony mutant was used because, as was pointed out to the author by Dr. E. S. McDonough, its cultures thrived hardily under regular laboratory conditions.

The writer wishes to express his sincere appreciation to

Dr. E. S. McDonough, of the Department of Biology of Marquette University, for his constant help and encouragement throughout the course of this investigation. He wishes also to thank Dr. Raymond Pearl, of the Johns Hopkins University, for his helpful criticisms.

Materials and Methods

Experiments dealing with duration of life require that environmental factors be constant. A modified two-shelf incubator much like that of Bridges (1932) was constructed to maintain constant temperature. The thermoregulator and relay assembly was constructed according to Greiff (1939). The incubator during the course of the investigation ran constantly for five months with a temperature fluctuation of ± 0.05° C.

Pearl and Parker (1924) have pointed out the importance of controlling humidity so that there is no water present and at the same time no active desiccation of the flies. Accurate humidity control was accomplished by employing a saturated salt solution, as described by Obermiller (1924). Ammonium chloride (C.P.) was used. This solution has been reported to maintain a humidity of 79.3 per cent. at 25° C. (International Critical Tables, 1926). The solution was poured into a container and a mark made on the side of the container indicating the level of the solution. Care was taken to add only enough water to bring the solution level up to this mark. Crystals of ammonium chloride were added in excess to provide a margin of safety.

The stock cultures from which the flies used in this investigation were obtained were brought to Marquette University from Michigan State College in 1929. The flies were inbred for this investigation and the F_4 generation used. They were grown on a banana-agar medium and kept in the incubator at all times except as noted below.

The pupae comprising the F_4 generation were removed from the culture bottles by means of a flamed nichrome wire. The pupae were washed in 70 per cent. alcohol and then put into individual test-tubes which had been previously plugged with cotton and sterilized. Each tube contained a strip of slightly moistened paper toweling upon which the pupa was placed. The tubes were placed in numbered racks and examined every 12 hours.

The technique used in handling the flies while they were in the incubator was that developed by Powsner (1935). Only four racks, each containing 30 test-tubes, were removed from the incubator at one time. It was found that no individual rack was out of the incubator more than 15 minutes in 24 hours.

Data

Tables I and II give the survivorship distributions of

TABLE I

Survivorship Distributions of Drosophila in the Complete Absence of Food Based on 1,000 Flies. Sexes Combined

Age (in hours)	Wild type	Ebony
6	1,000	1,000
12	998	1,000
24	997	998
36	956	997
48	562	932
60	121	899
72	14	319
84	4	78
96	0	13
108		2
120		1
132		0
Absolute no. of flies	766	784

TABLE II

Survivorship Distributions of Drosophila in the Complete Absence of Food Based on 1,000 Flies. Sexes Separate

Age (in hours)	Wild type		Ebony	
	Male	Female	Male	Female
6	1,000	1,000	1,000	1,000
12	997	1,000	1,000	1,000
24	997	998	980	1,000
36	973	940	980	997
48	646	487	930	936
60	151	98	723	702
72	16	17	313	326
84	0	7	77	79
96		0	14	11
108			2	3
120			0	3
132				0
Absolute no. of flies	370	396	441	343

the wild-type fly and its ebony mutant. The tables were calculated on the basis of 1,000 flies and were corrected to the nearest whole number. The survivorship lines are compared graphically in Figs. 1 to 3. Figs. 4 and 5 show

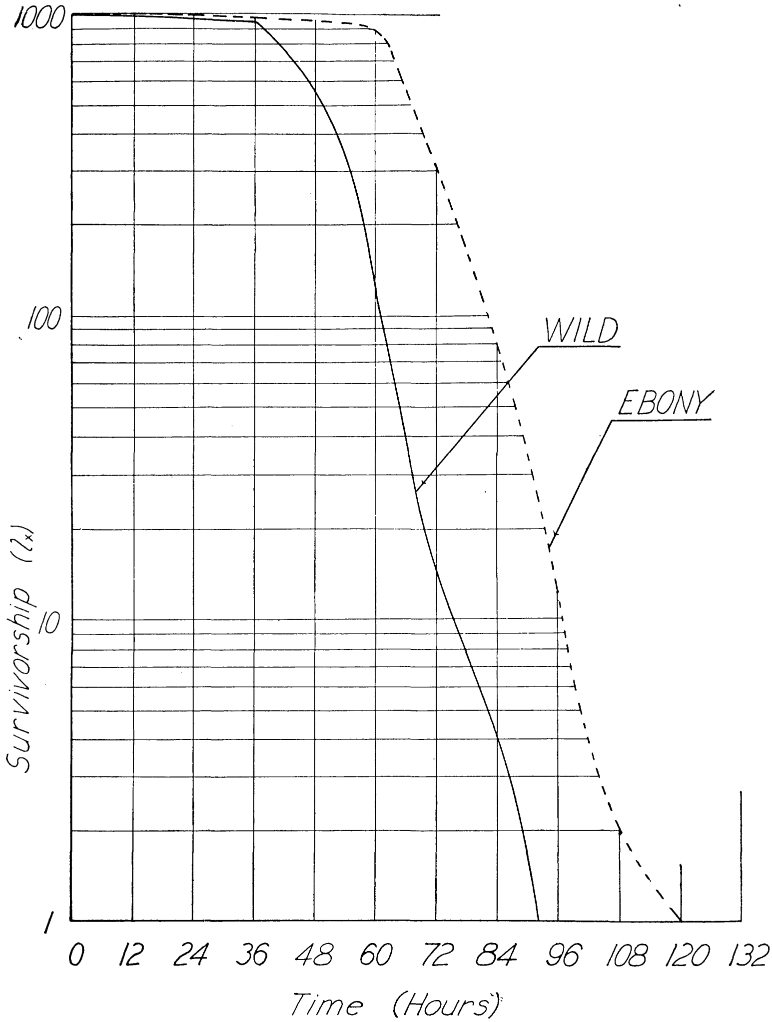

FIG. 1. A Comparison of the Wild Type and Ebony Mutant 1_x Lines of *Drosophila*.

the death curves (d_x) and the survivorship curves (l_x) placed on a relative time base for the purpose of comparison (Pearl, 1927).

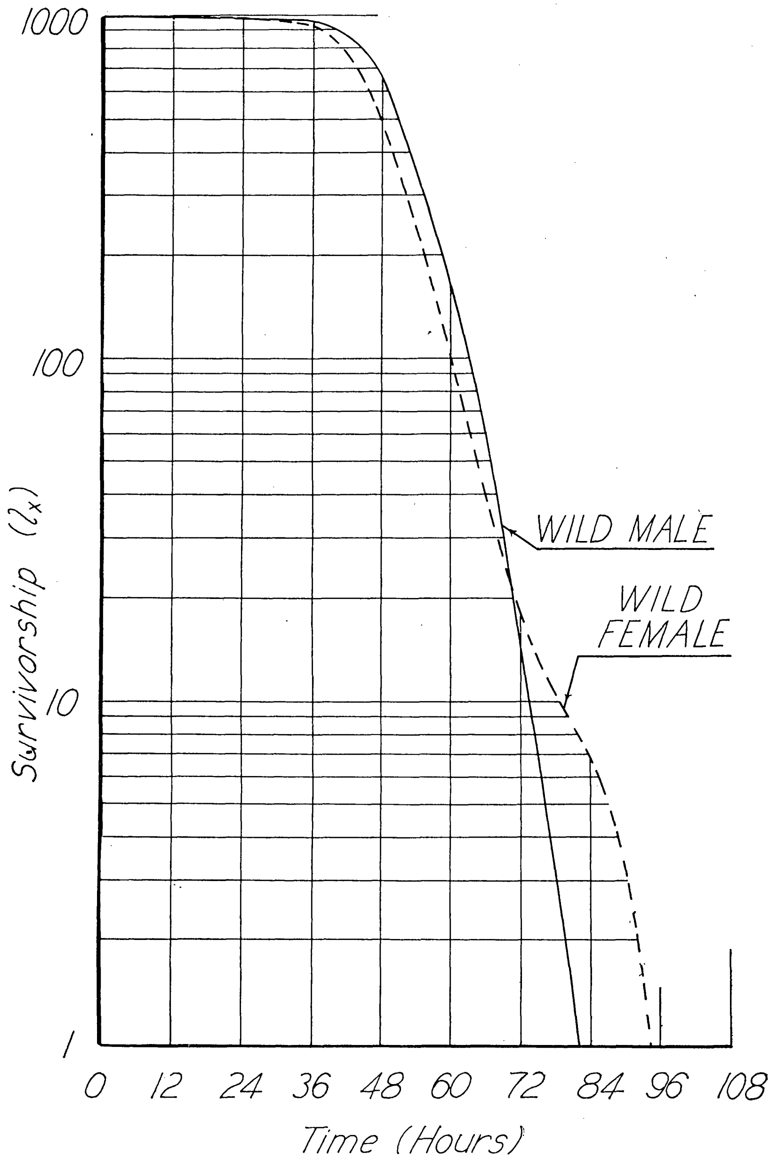

Fig. 2. A Comparison of the Male and Female 1_x Lines of the Wild-type *Drosophila.*

The chief biometric constants for this investigation are given in Table III.

The mean duration of life of the ebony fly, sexes com-

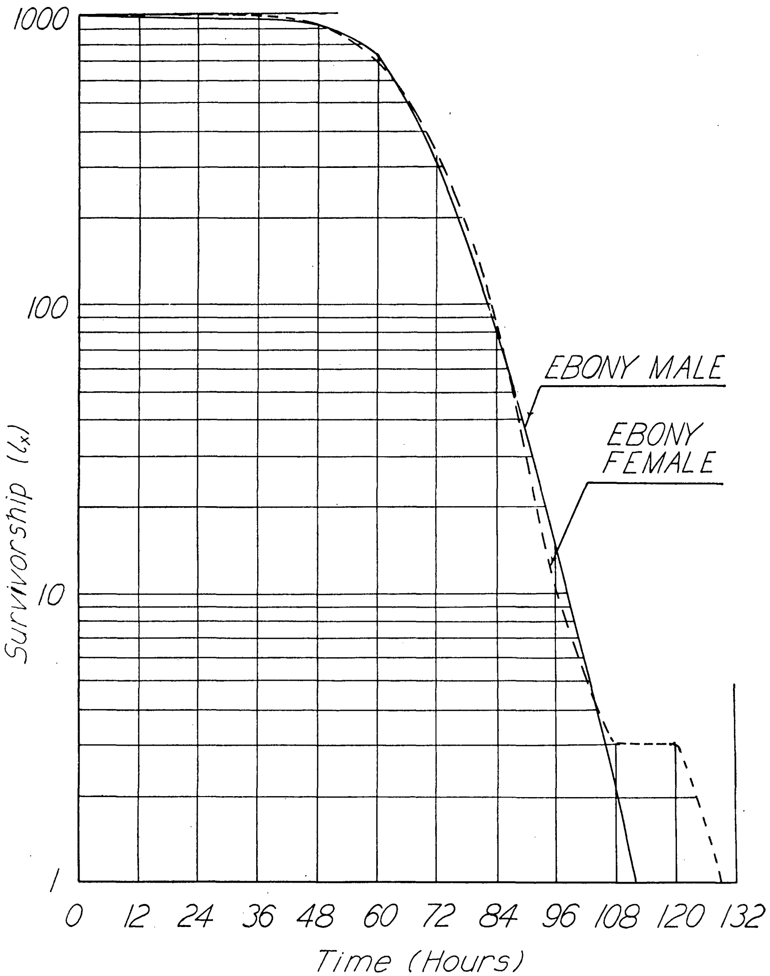

FIG. 3. A Comparison of the Male and Female 1_x Lines of the Ebony Mutant of *Drosophila.*

TABLE III

	Mean duration of life (hours)	Standard deviation (hours)	Standard deviation of mean (hours)
Wild-type fly (sexes combined) .	49.89	9.77	.353
Ebony fly (sexes combined) . . .	66.76	11.77	.420
Wild-type male	51.51	9.46	.492
Wild-type female	48.91	10.08	.507
Ebony male	66.80	12.82	.609
Ebony female	66.89	12.99	.702

bined, was found to exceed that of the wild-type fly, sexes combined, by 16.77 hours. The standard error of the

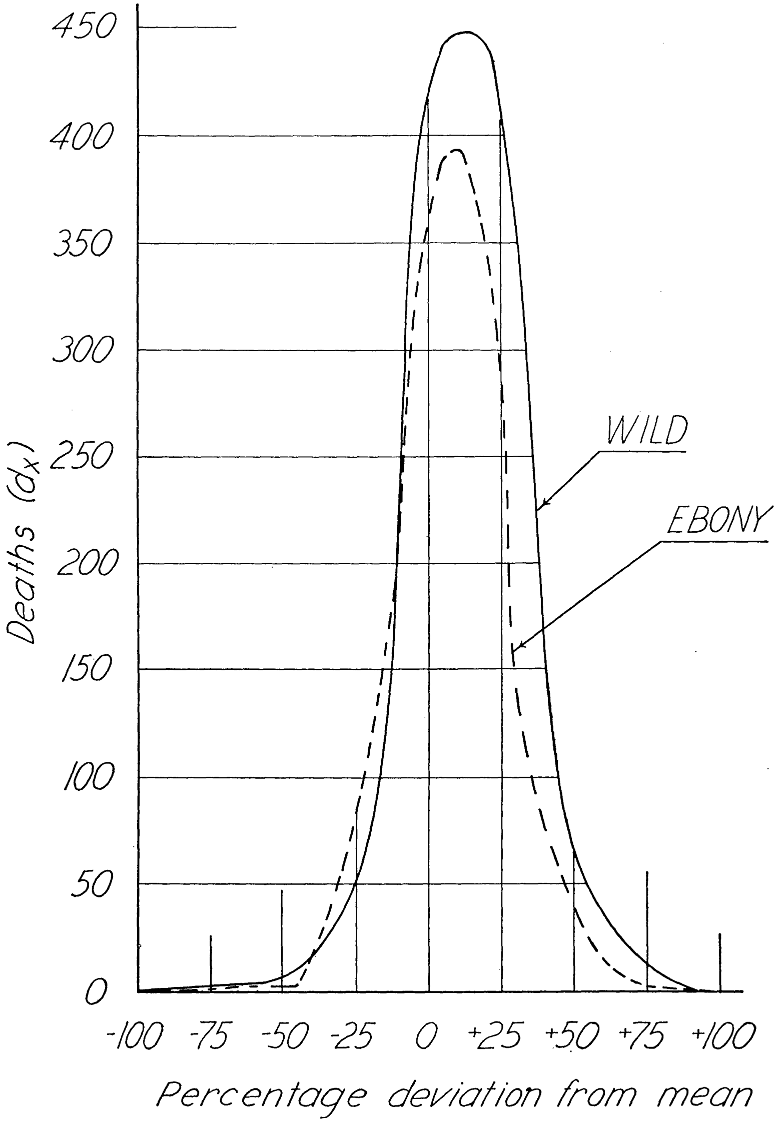

FIG. 4. Death Curves for Wild Type, Sexes Combined, and Ebony Mutant, Sexes Combined, on a Relative Time Base.

For each form represented the mean duration of life is taken as 100 per cent. on the abscissal scale, and all other ages (time duration) are expressed as percentage deviations (plus or minus) from this mean.

difference being .55 the difference was significant. In the foregoing and the following determinations of significance

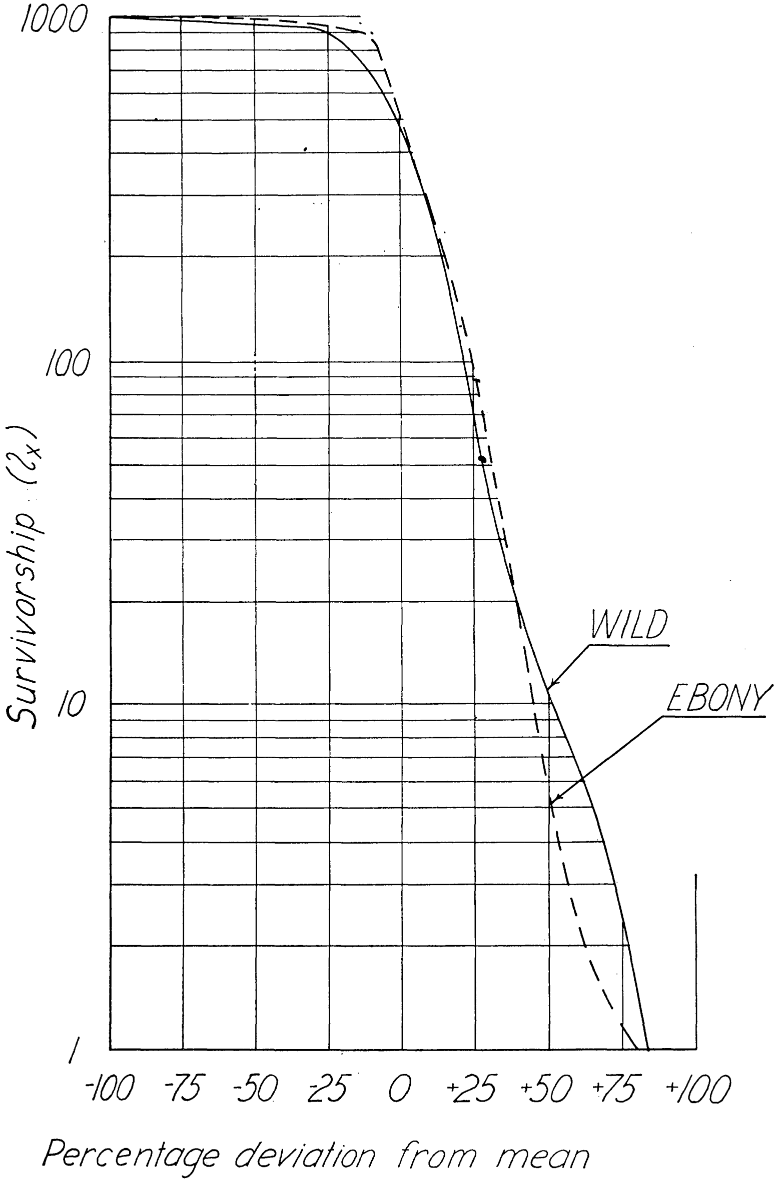

FIG. 5. Survivorship Curves for Wild Type, Sexes Combined, and Ebony Mutant, Sexes Combined, on a Relative Time Base.

For each form represented the mean duration of life is taken as 100 per cent. on the abscissal scale, and all other ages (time duration) are expressed as percentage deviations (plus or minus) from this mean.

or non-significance "Student's" t-test for unique examples was used.

The mean duration of life of the ebony female was found to exceed that of the ebony male by .09 hours. The standard error of the difference being .92 hours, the difference was not significant.

The mean duration of life of the wild-type male was found to exceed that of the wild-type female by 2.60 hours. The standard error of the difference being .707 hours, the difference was significant.

The mean duration of life of the ebony male was found to exceed that of the wild-type male by 15.29 hours. The standard error of the difference being .805 hours, the difference was significant.

The mean duration of life of the ebony female was found to exceed that of the wild-type female by 17.98 hours. The standard error of the difference being .85 hours, the difference was significant.

DISCUSSION

In this investigation the mean length of life of the ebony mutant fly under starvation was found to be significantly greater than that of the wild-type fly under starvation. The mutant ebony gene apparently affects not only the coloration of the fly but also the summation of the physiological processes so that the mean length of life increases under the conditions of the investigation. Jennings (1939), in speaking of the work on longevity performed by Gonzalez (1923), says:

> As is well known, every gene (with seemingly few exceptions) plays an essential rôle in the life and development of every cell of the body. It is not surprising therefore that changing a single gene may so alter the cellular processes as to change the length of life.

Two explanations for the difference in mean length of life under starvation are possible. Using the duration of life of the imago as an index of its "rate of living" (Pearl, 1928) we may say that the "rate of living" was slower in the ebony mutant than in the wild-type fly under conditions of starvation. It is also possible that the ebony mutant imago possesses a greater "capital" than the wild-type fly and maintains its advantage throughout life (Ashby, 1930, 1932).

Flies grown under laboratory conditions, where often there are in excess of 200 flies to a bottle, may be considered as under a condition of partial starvation. It may be reasonably stated that those flies which lived longer under a condition of complete starvation would retain this advantage under a condition of partial starvation. As was pointed out in the beginning of this report, the ebony mutant fly was observed to do better under laboratory conditions than the wild-type fly.

Pearl and Parker (1924) in reporting their work on the wild-type fly and its vestigial mutant under starvation used three different densities of population, these being fly densities of 5, 50 and 100 per container. They reported that the mean duration of life of the vestigial mutant under these conditions was much the same as the mean duration of life of the wild-type fly. Pearl and Parker, however, allowed males and females to live together for a short time. Krumbiegel (1929) reported that cohabitation of males and females shortened the mean duration of life in both sexes of Drosophila. The mean duration of life of the vestigial and wild-type fly under a density of one might give dissimilar life curves. On the other hand, it is not improbable that the effects of living together vary in different varieties of flies.

The survivorship curves for the ebony mutant fly and the wild-type fly, when placed on a relative time base, were found to be almost identical in shape (*cf.* Fig. 5), although their time placement when computed with regard to actual time is different. Pearl and Parker (1924) found that the form of the life curve under starvation was the same for the fed wild-type and starved wild-type and starved vestigial males. The life curve for the fed vestigial males, however, approached a straight diagonal on an arithlog grid.

The death curves for the ebony mutant and the wild-type fly were skewed to the left (*cf.* Fig. 4). The two limbs of the curves were very close together and showed a small degree of variation. This gave rather sharply peaked, unimodal frequency curves. Pearl and Miner (1935) state that this type of curve can not be caused by accident. It has been suggested by them that one of the causes for this type of curve is a lethal agent of environmental origin. This lethal agent must be so powerful that it transcends individual variations attempting to nullify its power. Starvation in the present investigation seems to take the place of an externally administered poison. As soon as the endogenous source of energy is lessened to an appreciable extent the toxic substances of the organism accumulate and, after the threshold of toleration is passed, death results. Schlutz, Hastings and Morse (1933) working with mammals reported that inanition may cause the physiological machine to be less efficient for delivering oxygen and removing metabolic products from the tissue.

A study of the survivorship curves of the ebony mutant and the wild-type fly (*cf.* Figs. 2 and 3) brings out a very interesting fact. The last surviving individuals were female flies. Numerous experiments on both man and rats (Bodansky, 1934) have shown that basal metabolism is lowered by starvation. Orr (1937) reported that starvation reduces oxygen consumption in both sexes of Drosophila. An explanation of the above observation may be that the basal metabolism of the female decreases more rapidly than that of the male. Thus the "rate of living" of the female would become progressively lessened and as a consequence the length of life increased.

Many workers in the field of longevity have reported that the female is longer lived than the male. Thus Pearl and Parker (1924) state:

> The normal relation between the sexes in respect of mean duration of life (females longer-lived than males) observed under full feeding, is preserved under conditions of complete starvation.

It will be recalled that Pearl and Parker worked with flies at densities of 5, 50 and 100 per container. In the author's investigation, in which the density was 1 per container, it was found that the wild-type male lived significantly longer than the wild-type female. In the work of Pearl and Parker, although the difference in the mean duration of life between male and female favored the female, this difference became progressively less as the density of population decreased. Plotting the differences of the means against the log of the density results in an approximately straight line. This line shows that there is a crossing over in the regions of low population densities and the mean duration of life of the male becomes greater than that of the female. Further experiments are being planned to check the foregoing observations.

Summary

This paper presented the results of the determination of duration of life in 1,550 adult individuals of *Drosophila melanogaster* and its ebony mutant, under a population density of one and conditions of starvation without water. The results obtained were:

(1) The mean duration of life of the ebony mutant was found to exceed in a statistically significant manner the mean length of life of the wild-type fly.

(2) The wild-type male was found, on the average, to live longer than the wild-type female. The difference was shown to be statistically significant.

(3) The mean duration of life of the ebony female was found to be greater than that of the ebony male. The difference was shown to be statistically non-significant.

(4) The last surviving individuals in both the ebony and wild-type populations were females.

(5) The life curves of the ebony and wild-type flies were found to have the same shape when placed on a relative time base.

LITERATURE CITED

Alpatov, W. W.
1930. Am. Nat., 64: 37–55.

Ashby, Eric
1930. *Annals of Botany,* 44: 457–467.
1932. *Annals of Botany,* 46: 1007–1032.

Baumberger, J. P.
1914. *Ann. Ent. Soc. Amer.*, 7: 323–353.
Bodansky, Meyer
1934. "Introduction to Physiological Chemistry." New York: John Wiley and Sons, Inc. Pp. xi + 662.
Bridges, C. B.
1932. AM. NAT., 66: 250–273.
Cowdry, E. V. (ed.)
1939. "Problems of Aging." Baltimore: Williams and Wilkins Company.
Gonzalez, B. M.
1923. AM. NAT., 57: 289–326.
Greiff, Donald
1939. *Science*, 89: 468.
Jennings, H. S.
1939. "Senescence and Death in Protozoa and Invertebrates," "Problems in Aging." Ed. by E. V. Cowdry.
Kopec, S.
1924. *Biol. Bull.*, 46: 1–21.
Krumbiegel, Ingo
1929. *Zool. Jahrb. Abt. Anat. u. Ontog. Tiere*, 51: 111–162.
Lilleland, Ole
1938. *Biol. Bull.*, 74: 314–318.
Loeb, J. and J. H. Northrop
1916. *Proc. Nat. Acad. Sci.*, 2: 456–457.
Lutz, F. E.
1915. *Bull. Amer. Mus. Nat. Hist.*, 34: 605–624.
Obermiller, J.
1924. *Zeitschr. f. Physik. Chem.*, 109: 145.
Orr, P. R.
1937. *Physiol. Zool.*, 10: 235–243.
Pearl, Raymond
1927. *Science*, 65: 237–241.
1928. "The Rate of Living." Pp. vii + 185. New York: Alfred Knopf.
Pearl, R. and J. R. Miner
1935. *Quart. Rev. Biol.*, 10: 60–79.
Pearl, R. and S. L. Parker
1921. AM. NAT., 55: 481–509.
1922. AM. NAT., 56: 312–322.
1929. AM. NAT., 63: 193–218.
Powsner, L.
1935. *Physiol. Zool.*, 8: 474–520.
Rau, P.
1910. *Trans. Acad. Sci. St. Louis*, 19: 21–48.
Rau, P. and N. Rau
1912. *Jour. Exper. Zool.*, 12: 179–204.
1914. *Trans. Acad. Sci. St. Louis*, 23: 1–78.
Schlutz, F. W., A. B. Hastings and M. Morse
1933. *Amer. Jour. Physiol.*, 104: 669–675.