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On the Discontinuous Character of Annuity Curves. Communication 2. Analysis of the Variability of Annuity Curve Shape in *Drosophila melanogaster* of the *Canton-S* Line

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Abstract—The five-phase structure of annuity curves for fruit flies of the Canton-S line detected earlier has been observed at different cohort sizes, which is indicative of the stability of the feature in question. However, variability in the duration of each of the five phases and a related variability in the cohort lifespan was observed in five experimental replicates. The five-phase structure is assumed to be under rigid genetic control, whereas the certain variability in the phase duration (which determines lifespan variability) implemented in the program of fruit fly development is presumably affected by weak uncontrollable signals.

Keywords: Drosophila, annuity curves, discontinuity, lifespan variability
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INTRODUCTION

Our previous study, which involved repeated large-scale observations of fruit fly imagoes of the line Canton-S, revealed a clearly discontinuous structure of the annuity curves. The structure was stable throughout the lifespan of the cohorts. Five discrete parts (phases) separated by sharp inflections of the curves have been identified. The phases observed were the following:

ph1—the starting phase of a very slow increase in mortality;

ph2—a short transition phase characterized by a high cohort death rate;

ph3—a phase of a relative decrease in the intensity of age-related demise;

ph4—a period of a dramatic increase of the cohort death rate;

ph5—a short final period characterized by a decrease in mortality.

The independent character of each phase was evident from the abrupt, step-like change of the slope of the next part of the curve. Regression analysis revealed the linear character of all phases observed.

The five-phase structure of annuity curves could not be revealed by analysis of a Gompertz graph constructed in semilogarithmic coordinates. The Gompertz approach proved inadequate for an analysis of the discontinuous dynamics of age-related mortality, whereas the use of annuity curves appeared justified.

The experimental data, which demonstrated good reproducibility of the five-phase structure of annuity curves in repeated experiments, indicated the high probability of this phenomenon. The five-phase character of the increase in the aging-related mortality of fruit flies was assumed to be under rigid genetic control. A difference between the biological mechanisms that underlie the death of fruit flies during the phases (age periods) observed was addressed. The present study was focused on further analysis of this phenomenon.

MATERIALS AND METHODS

Wild-type fruit flies (the Canton-S line) were used as the objects of the study. The flies were reared on standard medium containing sugar, yeast, manna groats, and raisins. The flies that hatched within one day were collected and transferred to tubes for separate rearing of males and females for two days. Clutches of eggs from these parents were collected daily. The flies that hatched in one day were placed into tubes (with a diameter of 1 cm) containing sugar-syrup agar covered by a freshly prepared suspension of yeast in distilled water. Ten males were placed into each tube. The nutrient medium was replaced once a week. The mortality of fruit flies was assessed three times a week, starting from the age of 1 day. The annuity curves were constructed after the death of the last individual in a cohort. The flies were reared at 25°C, and the duration of the light period was 12 h. Five experiments following the procedure described above were performed over two years, with the first experiment separated from the others by the longest interval (approximately a year).

Table 1. Results of analysis of variance for a linear approximation of the five-phase structure of the annuity curve

Experiment, number of individuals	Parameter	Parts of the annuity curve (phases)				
		1	2	3	4	5
First, $n = 800$	R^2	0.91	0.91	0.99	0.99	0.99
	p	1.00	1.00	0.79	0.67	0.67
Second, $n = 800$	R^2	0.81	0.98	0.97	0.97	0.96
	p	0.90	1.00	1.00	0.71	1.00
Third, $n = 1200$	R^2	0.96	0.86	0.95	0.94	0.92
	p	0.20	0.67	1.00	0.20	0.90
Fourth, $n = 800$	R^2	0.92	0.98	0.98	0.98	0.97
	p	0.50	1.00	0.50	1.00	0.19
Fifth, $n = 400$	R^2	0.92	0.97	0.97	0.98	0.97
	p	0.50	1.00	0.50	1.00	0.22

Note. R^2—determination coefficient for the linear model; p—results of the *Run test*.

The parallel observation approach developed in [1] was used to analyze the previously discovered five-phase structure of annuity curves, since this approach allows for visual assessment of actual biological variability. The approach used in the present work consisted in the separation of large cohorts into smaller cohorts. The cohorts herein termed "combined" consisted of 400, 800, or 1200 individuals (in 40, 80, and 120 tubes, respectively). The cohorts termed "individual" consisted of 100 individuals (in ten tubes) and were used along with the "combined" cohorts.

The results were subjected to regression analysis. Stepwise removal of individual time points from the right side of the survival curve was performed to identify the period for which the model under investigation was adequate. The removal was repeated until the deviation from the model became insignificant ($p > 0.01$). *Run test* was used to assess the adequacy of the models. The *AIC* information criterion [5] was used to choose between two alternative models.

The experimental material presented in the previous communication, complemented by the results of an additional experiment, was used for the analysis.

RESULTS AND DISCUSSION

Let us first consider Table 1, which presents the results of regression analysis of a linear approximation of five discrete parts (phases) of the combined annuity curve of fruit flies (Canton-S line) for the entire set of experiments, including experiment no. 5 (which was not analyzed during the previous study).

The table demonstrates the satisfactory character of the linear approximation of all of the phases identified in all the performed experiments, since the determination coefficient of the linear model is not less than 90% in 23 of 25 cases. The *Run test* results allowed for the same conclusion, since the p values exceeded 0.2 in 95% of cases. The considered materials provide convincing proof of the five-phase character of the increased age-related mortality in fruit flies.

Let us assess the stability of the data by means of following the dynamics of extinction in "individual" cohorts composed of a relatively small number of individuals (100).

Let us consider the results of the first experiment (Fig. 1). Analysis of the dynamics of extinction of "combined" and "individual" cohorts revealed considerable variability of the "individual" curves. For instance, the mortality in the individual cohorts ranged from 14 to 42% by day 30, whereas the respective value for the "combined" cohort was 25%. It is noteworthy that the shape of the "individual" curves was virtually similar to that of the "combined" annuity curve. Five distinct parts (phases), namely, the start phase (ph1), the transition phase (ph2), the phase of a relative decrease in mortality (ph3), the phase of a sharp increase in the cohort death rate (ph4), and a final decrease in mortality (ph5), were apparent on the majority of the "individual" curves. Sharp interphase bends were apparent as well. Each subsequent phase was dramatically different from the preceding one with regard to the slope of the annuity curve, as in the case of the combined curve.

As is evident from Fig. 2, four experiments performed one year after the first experiment yielded similar results.

It is noteworthy that the variation of "individual" curves in all of the performed experiments could not be regarded as simple statistical deviation from the mean value, since the degree of variation was different for the different phases of each experiment. The variation increased slowly during ph1, attained rather high values during ph2, reached a maximum during ph3, and decreased dramatically during ph4 and ph5.

We believe that such structure of the curves is evidence of the existence of different mechanisms that underlie the aging-related death of fruit flies during the different stages of the insects' lives.

Thus, parallel observations conducted with a set of "individual" curves revealed the stability of the five-phase structure of the annuity curves and provided novel proof of the difference between the mechanisms underlying the death of fruit flies at different stages of aging.

Let us now address the issue of variability of the annuity curves. Signs of variability were apparent on all five linear fragments (phases) of the "combined" curves obtained in all experiments (Figs. 1 and 2). However, the variability detected did not affect the five-phase character of the curves. Variability was observed in every repeated experiment performed under identical cultivation conditions. The "individual" curves apparently reproduce these variability features. The decrease of cohort size did not have an effect on the above named process.

The nature of the phase components that determine the variability must be elucidated. Let us consider Table 2, which presents the parameters of phase duration and slope for all five linear phases.

A fundamental difference in the age-related dynamics of the values of phase duration and slope is apparent. For instance, the slope values alternated in a regular manner upon the transitions between the phases in every experiment (hence the five-phase structure), whereas no apparent regularities that governed the duration of the phases were observed. The changes in this parameter appeared unpredictable in every experiment. The change in phase duration did not have any effect on the general five-phase structure of the curves, in contrast to the change of the slope. One may assume that the variability of the five-phase annuity curves of fruit flies is due to changes in the durations of the individual phases that constitute the curves.

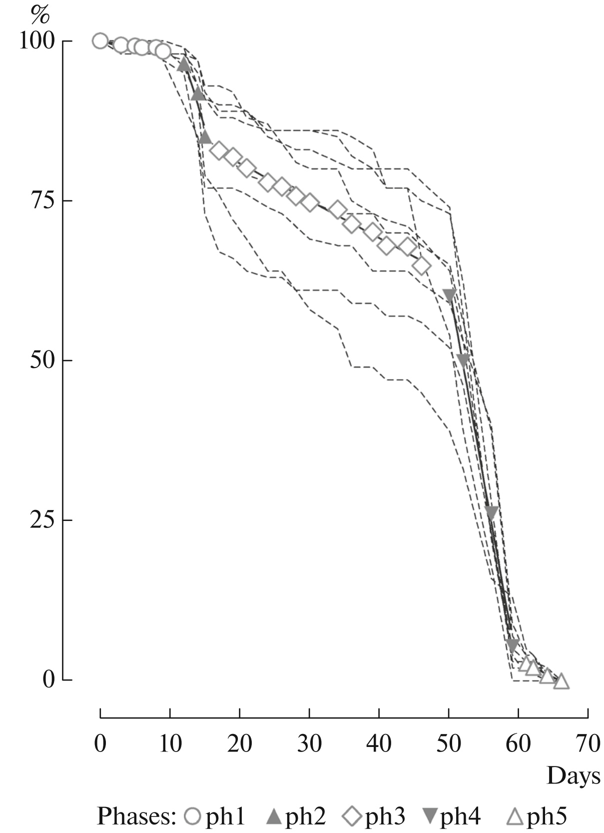

Fig. 1. Annuity curves of individual cohorts (dashed lines) and the combined cohort (linear approximation, symbols) in the experiment no. 1.

It appears necessary to address the biological significance of this parameter. The "phase duration" parameter should have a decisive influence on the lifespan of

Table 2. Slope and duration of annuity curve phases in five experiments

Experiment, number of individuals	Parameter	Parts of the annuity curve (phases)				
		1	2	3	4	5
First, $n = 800$	Slope, 95% CI	${}_{0.08}0.15_{0.22}$	${}_{-10.37}3.58_{17.53}$	${}_{0.54}0.58_{0.62}$	${}_{5.06}6.07_{7.07}$	${}_{-41}0.56_{0.70}$
	Duration, days	9	3	30	9	5
Second, $n = 800$	Slope, 95% CI	${}_{0.09}0.29_{0.48}$	${}_{1.13}1.89_{2.65}$	${}_{0.34}0.82_{1.29}$	${}_{2.09}2.57_{3.04}$	${}_{0.27}0.70_{1.12}$
	Duration, days	12	7	9	14	8
Third, $n = 1200$	Slope, 95% CI	${}_{0.37}0.49_{0.61}$	${}_{0.56}2.47_{5.05}$	${}_{1.92}0.97_{3.87}$	${}_{2.07}2.97_{3.87}$	${}_{0.24}0.50_{0.76}$
	Duration, days	11	6	5	14	9
Fourth, $n = 800$	Slope, 95% CI	${}_{0.15}0.31_{0.47}$	${}_{1.55}3.42_{5.29}$	${}_{1.19}1.42_{1.64}$	${}_{5.49}8.13_{21.76}$	${}_{0.32}0.37_{0.42}$
	Duration, days	9	10	11	5	112
Fifth, $n = 400$	Slope, 95% CI	${}_{0.16}0.25_{0.34}$	${}_{0.99}3.53_{6.08}$	${}_{1.14}1.26_{1.38}$	${}_{2.84}3.23_{3.62}$	${}_{0.44}0.58_{0.72}$
	Duration, days	11	4	12	11	15

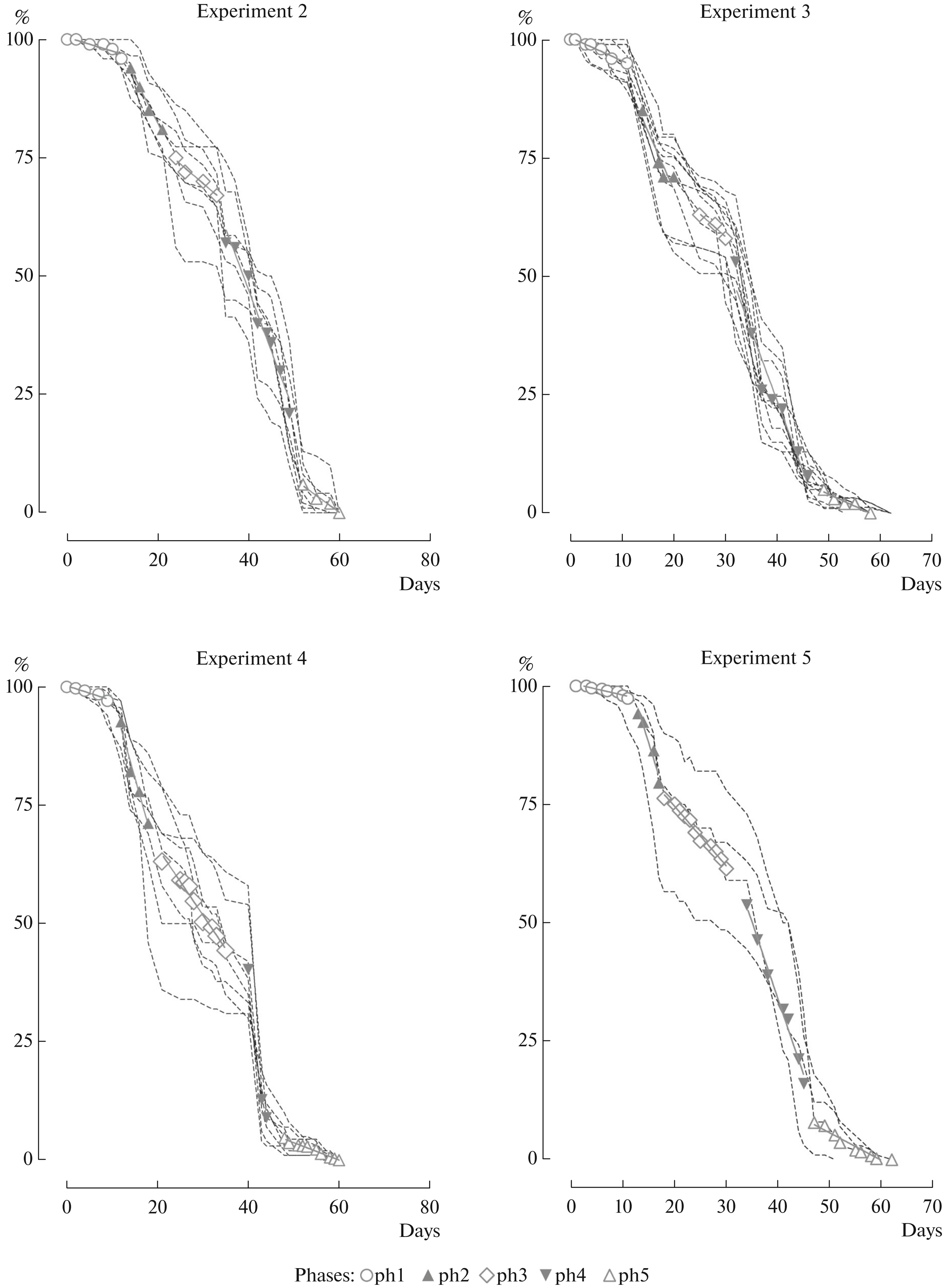

Fig. 2. Annuity curves of individual cohorts (dashed lines) and the combined cohort (linear approximation, symbols) cohort in experiments 2–5.

a biological object in the case of annuity curves of a multiphase structure. Let us consider Fig. 3, which combines the results of linear approximations of the "combined" curves from experiments 1–5.

The following reasoning will be based on the following logical postulate: the lifespan of the cohorts (and the flies) in every experiment is directly correlated to the area under the annuity curve. Extension of the phases characterized by sharp slopes results in a decrease of the area in question, whereas extension of the phases characterized by smooth slopes should result in an increase of the area. Therefore, the duration of the "abrupt" phases (ph2 and ph4) is negatively correlated with the area under the curve in each experiment, whereas that of the "smooth" phases (ph1, ph3, and ph5) is positively correlated to the area. These effects are especially pronounced in case of the most "variable" phases ph2 and ph3 (Fig. 3). The extension of ph2 upon the transition between experiments apparently results in a "dive" of the annuity curve, whereas the expansion of ph3 enables a correction of this change. As evident from Fig. 3, extension of this phase would cause an increase of the area under the annuity curve in any of the experiments.

As is shown in Table 3, the area under the annuity curve is largest for the curve with the shortest phase 2 and the longest phase 3. Thus, the variability of phase duration can have a significant effect on the lifespan of fruit flies of the Canton-S line.

The question of the lifespan of biological objects raised in the present work is of principal importance and requires further investigation. Notably, conventional approaches to the assessment of lifespan, such as median, slope of the survival curve, expected maximal lifespan, and the probability of death at a certain age [2–4], were deliberately left out upon the analysis of the effect of the specific phases on fruit fly lifespan. These approaches appear inadequate for the analysis of the variable multiphase annuity curves. We believe that the possibility of the existence of such a structure must be taken into account as the aging of fruit flies is investigated.

The analysis of the previously discovered five-phase structure of annuity curves for Canton-S fruit flies has been continued and significantly expanded in the present work. Five experiments performed over two years provided additional proof for the high stability of the five-phase structure and novel data that support the assumption of different mechanisms of fruit fly death during different phases. However, the discovered structure was characterized by a certain degree of variability manifested as selective changes in the differences of individual phases that did not affect the general five-phase structure. The multiphase character of annuity curves of fruit flies indicates the decisive importance of the "phase duration" parameter for the lifespan of a biological object. This opens up a completely novel approach to the extension of the lifespan of fruit flies through the search for means to increase the duration of the relevant phases.

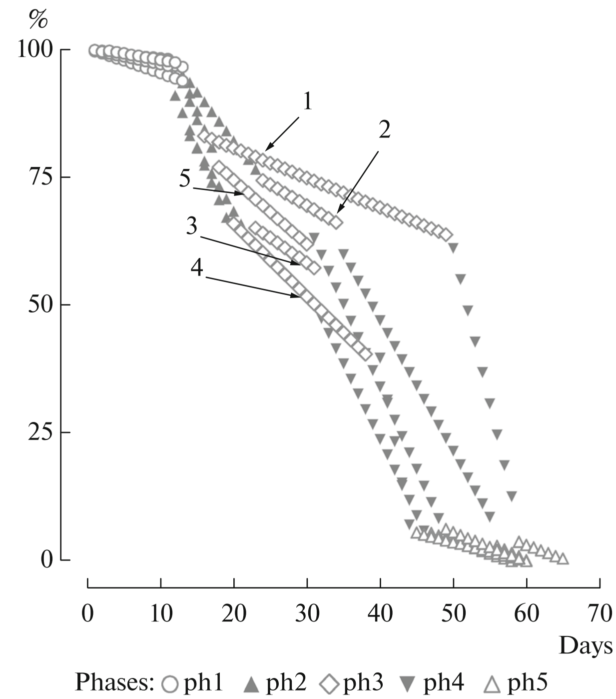

Fig. 3. Results of linear approximation of the "combined" curves from the experiments 1–5.

The results of the present study reveal the comprehensive and complex character of the system that defines the sequence of manifestation of the signs of aging in fruit flies. The discrete parts of the curve (phases) that reflect the aging-related changes in those mechanisms that underlie fly death form the constant part of this system, whereas the durations of the phases that determine the lifespan of the insects form the variable part. This system was reproduced in a stable manner as the experiments were repeated. It did not depend on the size of the cohorts used, which is indicative of the involvement of all individuals in the studied processes.

Table 3. Phase duration and area under the curve

Experiment number	ph1	ph2	ph3	ph4	ph5	Area, a.u.
First	9	3	30	9	5	3637
Second	12	7	9	14	8	3049
Third	11	6	5	14	9	2224
Fourth	9	10	11	5	12	2255
Fifth	11	4	12	11	15	2255

A hypothesis of a rigorous genetic determination of the stable part of the five-phase structure of the annuity curves of fruit flies was put forward in our previous work. The approach to the assessment of the variable features of curves included in this structure merits thorough analysis. It is necessary to determine whether these elements have a deterministic character and to identify the cause for the emergence of variability in experiments performed under standard conditions (if the elements are supposed to be determined). The variability of the lifespan of fruit flies included in the general genetic program of the increase in age-related mortality appears to be induced by some weak uncontrollable signals. The ontogenesis stages at which these signals take effect have not yet been identified.

The experimental data of the present study were obtained with a single line of wild-type *Drosophila melanogaster* (Canton-S). However, one can hardly expect that the regularities detected were conserved only in these flies in the course of evolution. The discovery of similar regularities in other species and lines of fruit flies, as well as the existence of individual structural elements of annuity curves described above in other species, appears more plausible.

We believe that the expansion of this area of research in the evolutionary aspect has great potential.

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