On the Discontinuous Character of Annuity Curves. III. Two Forms of Viability Variation in *Drosophila melanogaster*

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Abstract—The validity of the two-component structure of annuity curves in *Drosophila melanogaster* (line Canton-S) observed in our previous studies has been confirmed. The structure is formed by a combination of a stable five-phase pattern and the variability of phase duration (the latter determines the variability of insect viability). Both the stable and the variable component were preserved over 60 generations of flies. The unconventional type of variability addressed in the present study exists along with the well-known type of variability related to the stochastic variation of the annuity curves. The variance of viability that characterizes the reaction norm remains unchanged in a series of generations as well. Thus, both types of variability can be classified as genetically determined phenomena.

Our previous studies [3, 6] revealed the presence of five distinct parts (phases) on the annuity curves of *Drosophila melanogaster* males (line Canton-S): Ph1, the initial phase of a very slow increase in mortality; Ph2, the short-term transitional phase of intense dying off in the cohorts; Ph3, the phase of a relative decrease in the rate of age-related deaths; Ph4, the period of a sharp increase in the death rate in the cohort; and Ph5, the final short period characterized by a decrease in death rate.

The independent nature of each phase was evident from a sharp change in the slope of the following section of the curve. Regression analysis showed that all these phases were linear. The discontinuous character of fruit-fly annuity curves was reproduced in five replicate experiments conducted over the past 2 years. The data collected during the study were not amenable to interpretation within the framework of the procedure developed by Gompertz [6]. The stable and reproducible character of the five-phase pattern of the annuity curves observed in the present study was indicative of rigid genetic determination of the phenomenon observed [6].

However, the possibility of changes in the phase duration turned out to be programmed in the structure observed, and this phenomenon had an effect on the viability of the individuals. Variability was observed as the replicate experiments were compared, regardless of the identical conditions of fly rearing. A heritable program that allows for individual viability variation in a certain range has been postulated to have merged at the background of the five-phase pattern subject to rigid genetic determination; however, the signals that affect the variation remain unidentified [3]. The present study reports a more detailed investigation of the dynamics of age-related mortality in *Drosophila*.

MATERIALS AND METHODS

Both the material collected during earlier studies and the experimental data obtained during the present study were analyzed. Wild-type *Drosophila* of the line Canton-S were the object of research. The flies were reared on a standard medium that contained sugar, yeast, semolina, and raisins. The flies hatched from pupae during a single day were collected and transferred to new tubes, where the male and female insects were reared separately for 2 days. These flies were allowed to lay eggs during one day, and all eggs were collected. The flies that emerged from the eggs during one day were transferred to tubes of 1 cm in diameter; the tubes contained a nutrient medium of agar—agar and sugar covered by a freshly prepared suspension of yeast in distilled water. Ten male flies were placed into each tube. The culture medium was changed once a week. Insect mortality was monitored starting from 3 days of age. The annuity curves were constructed after the death of the last individual in the cohort. The flies were reared at 25°C and 12 h of light per day. The present study reports five experiments performed over 2 years and an additional experiment performed 6 months (12 generations) after the end of the fifth experiment.
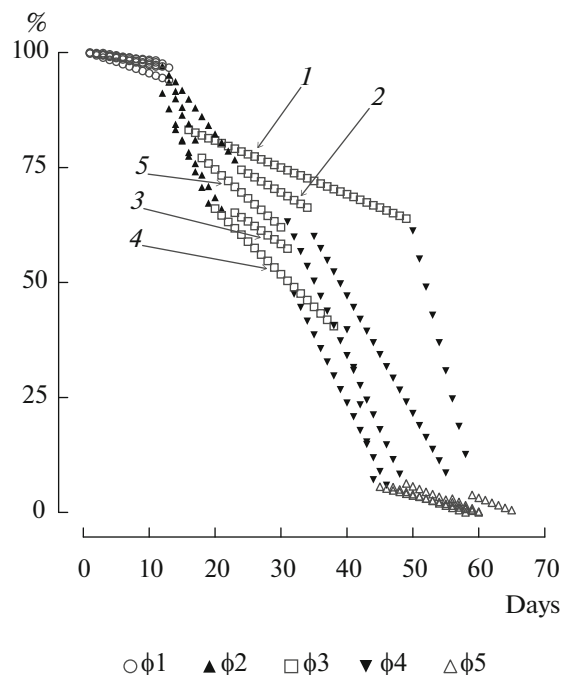
Fig. 1. Linear approximation of fruit-fly annuity curves registered in five consecutive experiments. The cohort size in the different experiments was the following: (*1*) 800, (*2*) 800, (*3*) 1200, (*4*) 800, and (*5*) 400 individuals (data from [3]). See text for the description of the phases (Ph1—Ph5) shown here and in Figs. 2 and 3.

The flies were kept in breeding stocks between the experiments. The monitoring period spanned approximately 60 generations of insects (from zygote to sexual maturity). A parallel observation approach that allows for an intuitively unambiguous assessment of actual biological variability was used to analyze the results. The approach consisted in the separation of large cohorts into relatively small ones [3]. The terms "aggregate" and "individual" were used to identify the respective cohorts and annuity curves.

RESULTS AND DISCUSSION

The "aggregate" annuity curves of *Drosophila* obtained in the five replicate experiments performed earlier (over two years) are shown in Fig. 1. The figure illustrates the two-component character of the annuity curves that was preserved over the lifespan of the cohort in all cases. The curves from each experiment apparently have a stable component (five distinct phases) and a "plastic" component (variability of phase duration). The "plastic" component determines the variation of the area under the annuity curves that is related to the variability of the dynamics of age-related mortality of the flies. Larger areas obviously correspond to higher lifespans of the flies in the population. For example, the largest area under the curve observed in experiment 1 corresponds to the highest

viability of the flies [3]. An additional experiment (experiment 6) performed in the present study confirmed the validity of the structure observed (see below).

The duration of the experiment corresponded to 60 generations of flies, this being essential for the correct understanding of the results of the study. Therefore, the curve shape observed can be regarded as a genetically determined structure. The conclusion regarding the genetic determination can be applied to the type of variability of fruit-fly lifespan described above, this being especially important in the context of the present study.

Let us assess the dependence of the parameters of the *Drosophila* annuity curves observed on the number of individuals in the cohort.

As shown in Fig. 1, both the stable component of the structure ("five-phase" character) and the "plastic" component (variability of phase duration) did not depend on sample size in the range of sample sizes used (400−1200 flies). The curve structure was conserved for all curves, regardless of the time of a specific experiment.

Further analysis of the dependence of the structure on sample size involved a methodological approach that consisted in the division of large ("aggregate") cohorts into relatively small "individual" cohorts. Experiment 1 involved the division of an "aggregate" cohort of 800 individuals into 2, 4, 8, and 16 parts to obtain "individual" cohorts of 400, 200, 100, and 50 flies, respectively (Fig. 2).

Experiment 6 involved a similar division (Fig. 3). However, the opportunities for the division of the aggregate cohort of 500 flies were limited, and, therefore, the "aggregate" annuity curve (Fig. 3a) was compared to the annuity curves of "individual" cohorts that included 250, 100, and 50 individuals (Figs. 3b−3d).

The patterns illustrated by the abovementioned figures are similar. Variability of the "individual" annuity curves is apparent in both cases, and the amplitude of the variation increases dramatically upon the decrease in the number of individuals in the cohort. The corresponding value is highest at the smallest sample size (50 flies). However, the "individual" curves apparently reproduce the stable five-phase structure characteristic of the "aggregate" curves regardless of the "individual" cohort size, even in case of a very large variation amplitude (Figs. 2, 3c, and 3d). Five distinct regions (phases) are evident in the majority of "individual" curves, as well as in the "aggregate" curve (see above). Interphase inflection points are distinct as well. Every subsequent phase on the "individual" curves (as well as in the "aggregate" curve) differs from the preceding one with regard to the slope of the annuity curve.

The "phase-duration variability" parameter was reproduced as well. The abovementioned figures show that the duration of the phases varied, both between
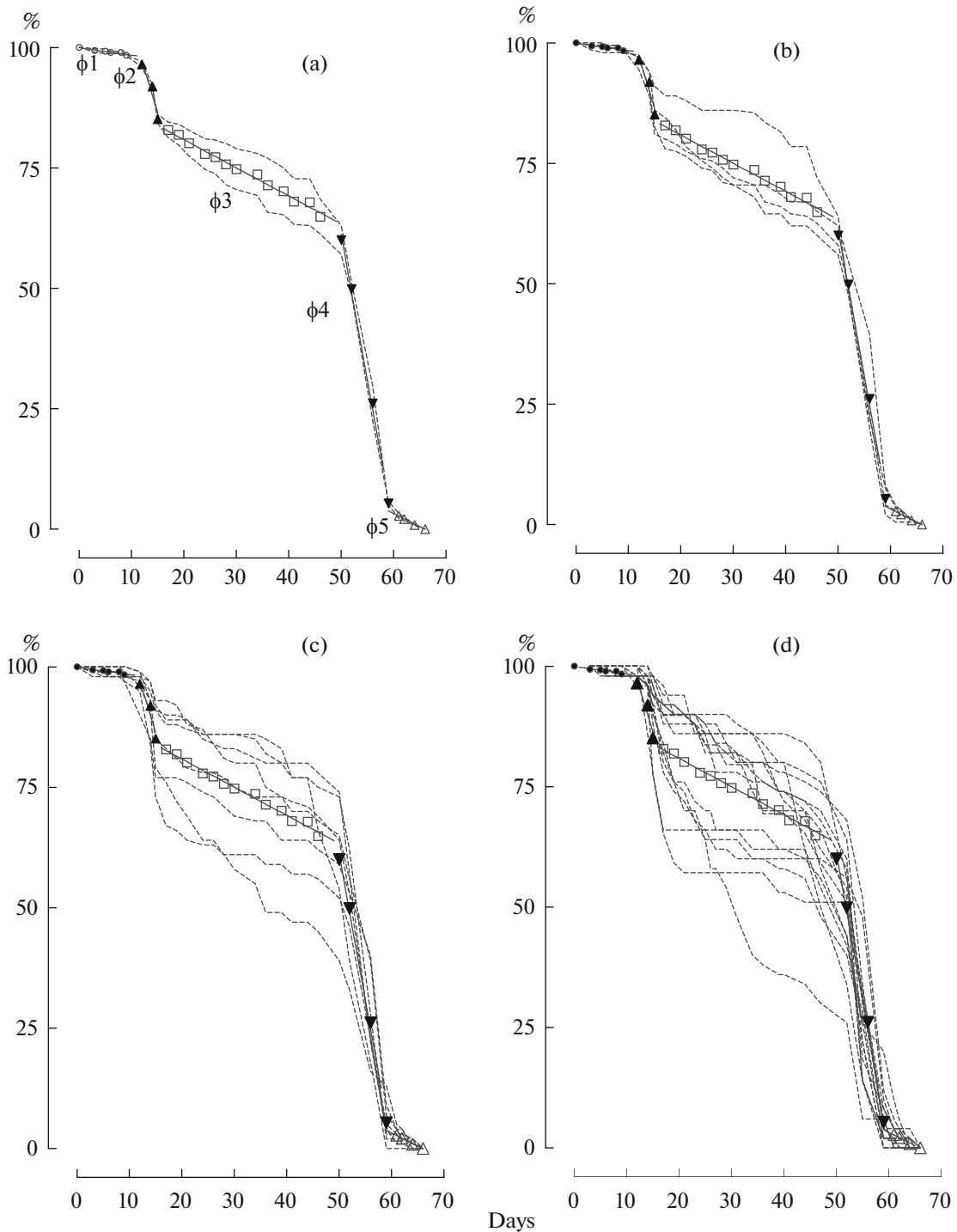
Fig. 2. Annuity curves of "individual" cohorts of (a) 400, (b) 200, (c) 100, and (d) 50 individuals. A linear approximation of the phases of the annuity curve of an "aggregated" cohort of 800 individuals is shown by the solid line.

the "aggregate" annuity curves recorded within a 2.5-year period and between the "individual" curves.

Thus, the data presented in Figs. 1−3 support the independence of the two-component structure of *Drosophila* annuity curves on cohort size in a very wide range of sample sizes (50−1200 individuals).

The independence of the structural components of the curves of cohort sizes (even in very small samples of 50 flies) and the reproduction of a characteristic structure over many generations allow for the conclusion that absolutely all individuals have an effect on both components of the structure (the stable one and the "plastic" one) at the individual level. It seems rea-
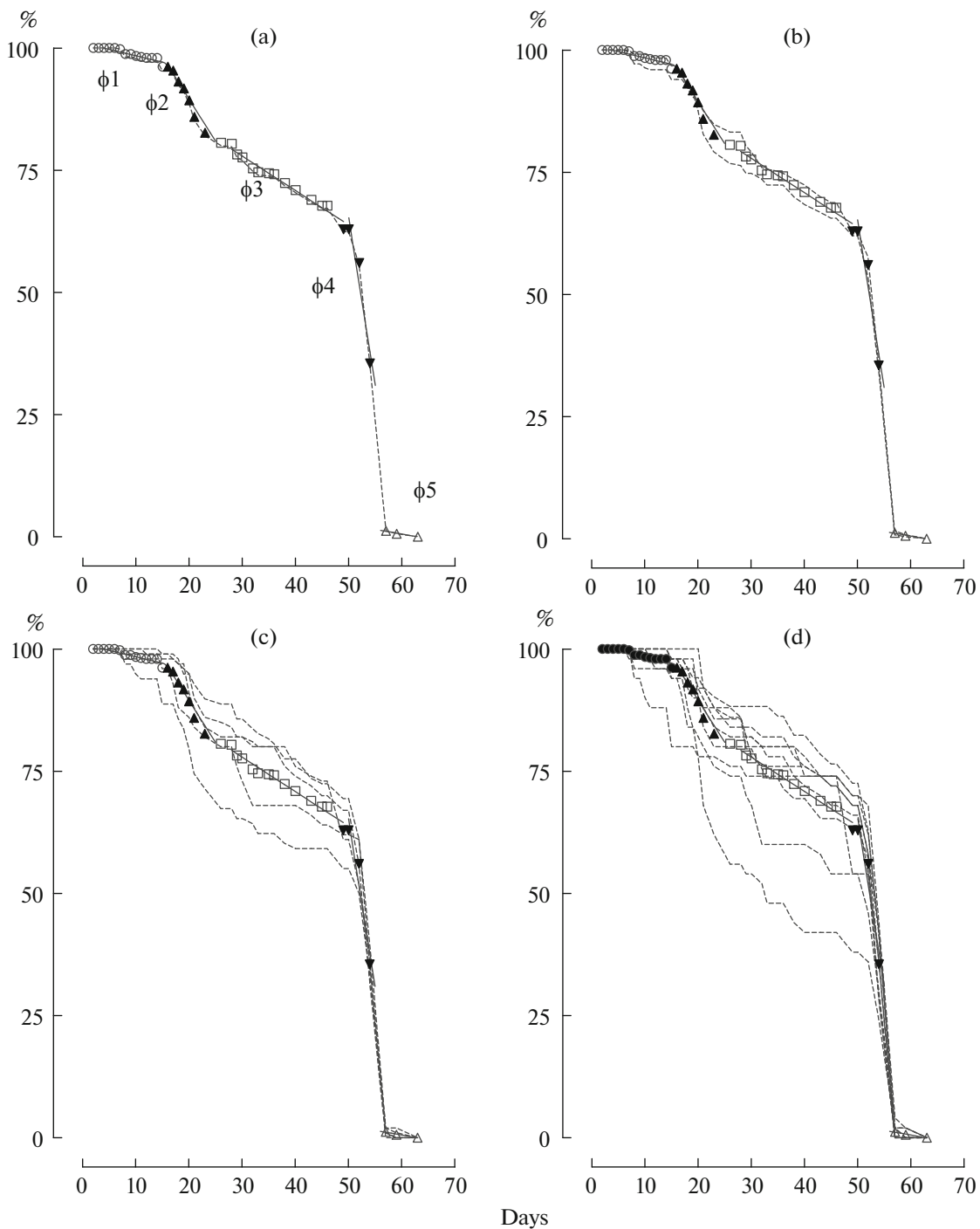
Fig. 3. Annuity curves of an "aggregated" cohort of (a) 500 and "individual" cohorts of (b) 250, (c) 100, and (d) 50 individuals. A linear approximation of the phases of the annuity curve of an "aggregated" cohort is shown by the solid line.

sonable to suppose that the population of flies is homogeneous in this regard. The genetic material of each *Drosophila* encodes a program for the formation of a two-component structure.

Let us now consider another type of variability of age-related mortality of *Drosophila*, namely, the one related to stochastic factors that should be analyzed in

order to address the regularities that underlie aging [5]. The completely trivial dependence of the degree of variability of the survival (inferred from the annuity curves of the flies) on sample size shown in Figs. 2 and 3 points at the variability of the individual viability of the flies (in contrast to the case presented above) and the heterogeneity of the fly population. It seems safe to

state that the variance observed is related to random (stochastic) selection of individuals with different lifespans in the same cohort. However, the method of monitoring a set of "individual" curves used in the present work reveals a situation that does not have a trivial explanation. For instance, Figs. 2 and 3 show that the variability of the lifespan in the "individual" cohorts is the highest for a certain fragment of the annuity curves of *Drosophila*, namely, for Ph3. The increase of the degree of variance observed upon the decrease of the sample size (Figs. 2, 3) is largely confined to this phase. This situation is clearly illustrated in Fig. 2a. The variation between the "individual" curves is apparently restricted to Ph3 in case of a sufficiently large sample. The period of aging-related dying off during Ph3 can be regarded as a "critical" period that determines the effect of the stochastic variability factor on the viability of the flies.

Further analysis of the regularities governing the variability of fruit-fly lifespan in six consecutive experiments involved the construction of graphs that illustrated the age-related dynamics of standard deviation (*SD*) assessed for the "individual" cohorts of 100 flies. All graphs were plotted in the same coordinates (Fig. 4). As is evident from the figure, the age-related dynamics of viability variation has a discontinuous character as well. The distinct fragments (phases) characterized previously are clearly visible, as well as the variability of phase duration between the experiments.

We consider the estimates of the maximum values of standard deviation shown in table to be of especial interest.

The data presented in table reveal the absence of significant changes in the maximal value of variance of the viability of the flies during 2.5 years of experiments, which corresponded to approximately 60 generations of insects.

Thus, the results of six consecutive experiments point to the validity of the two-component structure of *Drosophila* annuity curves. The structure is represented by a combination of a stable five-phase pattern of the curves with a "plastic" component that reflects the variability of individual viability of the flies in the populations. We concluded that absolutely all individuals are involved in the control of both components of the structure (the stable one and the "plastic" one) at the individual level. The fly population is homogeneous in this regard.

The estimated number of generations of the flies involved in the experiments during the entire study period was 60. Therefore, the structure observed can be regarded as a genetically determined characteristic that emerged in the course of evolutionary processes.

Given the above, we assume that a (generally) similar structure of annuity curves will be observed in other species as well. The stable component is very likely to be related to the sequential aging-related degradation of target tissues with dissimilar aging rates in
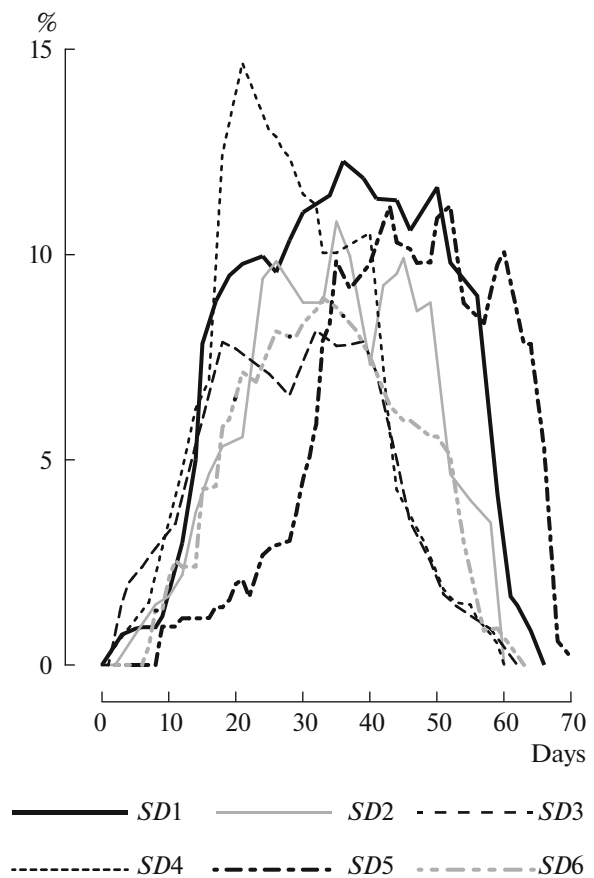

Fig. 4. Age-related dynamics of the maximal standard deviation of the viability of male fruit flies *Drosophila melanogaster* of the line Canton-S.

all organisms [1, 2, 4]. If this assumption proves true, the data on the discontinuous character of annuity curves in *Drosophila* is likely to specify the general regularities that underlie the aging of living organisms. In other words, we believe that these data will shed light on the relationship between aging-related disorders and specific periods in the lifespan of an organism, as

Point and interval estimates of the maximum values of standard deviation (*SD*) of the lifespan of *Drosophila melanogaster* individuals

Number of experiment	Number of cohorts (of 100 individuals each)	SD_{max} (%), 95% CI
1	8	$_{4.8}12.27_{14.9}$
2	6	$_{7.15}10.81_{22}$
3	12	$_{6.76}8.15_{20.8}$
4	8	$_{9.77}14.67_{29.86}$
5	8	$_{6.69}11.19_{32.11}$
6	5	$_{5.36}8.94_{25.69}$

well as between these disorders and the degradation of certain "key" systems of cells and tissues.

The study that was performed showed that special attention should be paid to the "plastic" component of the structure that reflects a special type of variability of *Drosophila* lifespan. Absolutely all individuals contribute to this type of variability. It seems reasonable to suggest that the fly population is homogeneous in this regard. The genetic material of each individual encodes the traits necessary for the formation of a two-component structure.

The material presented in our study is in agreement with reports by other researchers [8−10] that revealed considerable spontaneous variation in life expectancy between the different generations of fruit flies reared under standard conditions. The results of our studies provide certain theoretical foundations for these data, although the origin of variability of this type remains completely unknown.

Let us now consider another type of variability of *Drosophila* annuity curves determined by the variance factor. The individual viability of fruit flies varies in this case (in contrast to the case discussed above), since the populations are known to be genetically heterogeneous [7]. The original method of monitoring the set of "individual" curves allowed for the discovery of the unequal variance of the mortality at the different parts of the annuity curves.

This observation points at the distinct morphophysiological features of the "target" tissues in the different phases and opens broad perspectives for research on the targeted effects of antiaging treatments on specific "key systems."

Some of the results of these studies will be reported in our next communication.

The most important conclusion concerning the second type of variability was drawn from the comparison of the maximal values of the standard deviation for all the experiments. The stable value of this parameter was conserved over tens of generations. The range of variation was narrow, similarly to the "normal" body temperature in humans.

The situation described above allows for the assumption that this form of lifespan variability in *Drosophila* melanogaster of the line Canton-S is related to the norm of reaction of the organism [11] and is under genetic control, similarly to the form discovered previously.

It is logical to suggest that the pattern observed during the analysis of fruit-fly lifespan should be of general biological significance.

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