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NEW METHODS AND MODELS OF DEVELOPMENTAL BIOLOGY

Graduated Change of Life Expectancy in Mice in Ontogenesis

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Abstract—Life expectancy of descendants of a normal female mouse and a male with an inherited growth inhibition mutation discovered in a laboratory population was investigated. The hereditability of the characteristic allows us to consider it a result of mutation. It was shown that, in mice, the curve of dependence of life expectancy on their serial number in a row of increase in life expectancy (curve of rank distribution) has step-like shape for mutant males and females, as well as for males with normal development. The first grade of mice death on the curve of rank distribution was observed at one month after their birth and was characteristic only of males and females with a mutation during the period of maximum lag in weight as compared with their normal relatives. The surviving mutants catch up to the normally developing individuals within two months and externally become indistinguishable from them. The subsequent grades of death in mutants and normal males coincide on the time axis. The steps are absent on the rank curves of life expectancy of normally developing females. The time intervals between the steps are reproduced in parallel groups of mice and, hence, are not casual deviations from theoretical curves and are of a regular nature. The discovered phenomenon is interpreted within the scope of a hypothesis about the realization of the genetic program of ontogenesis, which provides periodic change of vitality stages with stages of sensitivity to external risk factors, which increase the probability of death, by mice. Absence of such stages in the group of normally developing females can be explained by shifts in development, which are produced by the irregular performance of reproductive functions.

Keywords: life expectancy, mice, growth inhibition, rank distribution, stages of development, death rate intensity, Gompertz–Makeham formula, hypothesis

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INTRODUCTION

Almost two centuries ago, an employee of the insurance company Gompertz (Gompertz, 1825), while studying the mortality rate statistics of the population, discovered that the death rate intensity in middle age (for modern population of industrially developed countries, from 35 to 90 years old) can be characterized by exponential dependence on age,

$$\mu(x) = dl_m(x)/[(l_0 - l_m(x))dx] = R\exp(\alpha x), \qquad (1)$$

where $\mu(x)$ is the function of the death rate intensity; x is time; $l_m(x)$ is the death rate function, i.e., the number of people that died by this age; $dl_m(x)/dx$ is the derivative, which expresses the rate of dying of the individuals of the given age; l_0 is the total number of the dead and surviving individuals; $l_0 - l_m(x)$ is the survival function, which is additional to the death rate function; R, α is the experimentally determined parameters of the Gompertz formula.

Compared with other ways of analytical description of death rate (Gavrilov and Gavrilova, 1991), the Gompertz formula became more widespread since taking its logarithm yielded a direct line, which made it possible to confine oneself to two parameters:

$$\ln\mu(x) = \alpha x + \ln R.$$

To decrease the lower limit of applicability of the Gompertz formula to 25 years old, Makeham (Makeham, 1860) introduced an additional parameter A into the formula (1):

$$dl_m(x)/[(l_0 - l_m(x))dx] = A + R\exp(\alpha x). \qquad (2)$$

To decrease the dispersal of points on the experimental curve of death rate intensity, the initial statistical data are smoothed out before differentiation by averaging out Δx with similar time intervals. For human populations, intervals of one to five years are often used. In the case of the wave-like nature of the curve, such averaging can eliminate oscillations with periods of up to 3 or 15 years, respectively.

Unlike in humans, the data on the mortality rates in sparse homogeneous populations of laboratory animals do not correspond well with the theoretical curves built using the Gompertz–Makeham model (Anisimov, 1979, 2008; Popovich, 2003). Attempts to achieve good correspondence with the theoretical curves proved unsuccessful even while using the smoothing procedure (Lamb, 1980). Despite this, the observed deviations in the experimental death rate values from the theoretical

curves are still considered random and are not of much interest to specialists.

The aim of this study is to compare the shapes of experimentally obtained and built theoretically curves, using the Gompertz–Makeham model, which characterizes the mortality rate of the mice that inherited the growth inhibition characteristic and of those that did not inherit it; to reveal the degree of regularity or randomness of the observed deviations and, possibly, to explain them using a more appropriate hypothesis.

MATERIALS AND METHODS

The study was performed on the descendants of mice obtained from cross-breeding a normal female of a heterogeneous laboratory population with an admixture of the Swiss Webster line (Andreevka Branch, Scientific Center of Biomedical Technologies, Russian Academy of Medical Sciences) and a male with a growth inhibition. The hereditability of the characteristic allows us to consider it a result of mutation. The mutant male was discovered accidentally among the offspring of a normal male from the same population, which was fed a 0.01 M solution of silver nitrate for 1.5 months, starting from the age of 28 days, and crossed with an intact female from the same line a month after the end of the procedure. The mice were kept in a vivarium on standard laboratory diet balanced in proteins, lipids, and carbohydrates, with the necessary set of vitamins and microelements. The data on the life expectancy of all 118 mice of both sexes were obtained by registering their births and natural deaths for three years and nine months at different times. The number of individuals used for building the curves of rank distribution corresponds with the number of points on the plots. The plots were built and transformed in the Origin computer program.

RESULTS AND DISCUSSION

General Characteristic of Mice with a Growth Inhibition Mutation

The difference in the weight increase kinetics in the first male with a growth inhibition and the weight of normally developed mice from the same brood is given in Fig. 1. It was discovered that the females with a growth inhibition obtained in the subsequent generations after crossing with both mutant and normal males either did not give any offspring or the young died one–two days after birth. Thus, the mutation was transferred to the subsequent generations only through crossing mutant males and normally developed females. Crossing normal males and females obtained from mutant males and normal females led to the birth of normal individuals. This indicates the nonrecessive nature of the mutation. At the first and subsequent crossings of mutant males and normal females, from 10 to 70% of individuals in the brood were mutants with a growth inhibition represented by both sexes. However, the average number of mutants in the brood did not exceed 30%, which was insufficient to consider the mutation dominant. Hence, the mutation could be of a nonchromosome nature.

When comparing the changes in the weight of mutants and normally developed individuals in the broods obtained from crossing mutant males with normal females, we found that mutants usually catch up with normal individuals in height in the course of the first two months of development. As Fig. 1 shows, maximum differences in weight were observed approximately a month after birth. In most cases, by the middle of the month, the weight of the mutants ceased to increase or even started to decrease. One can also notice that the period of growth inhibition in mutants corresponds with the period of rapid weight growth on the growth curve of normally developed mice, which falls on days 15 to 30 of development.

Another important particularity of mutant mice is the fact that many of them were incapable of overcoming the period of growth inhibition and died within 25–35 days after birth. It is known from literature that this age is characterized by maximum sensitivity to the influence of ionizing radiation in normal mice (Grosfill, 1959). Hence, the growth inhibition mutation reveals the latent stage of elevated death risk, which occurs at one month of postnatal mice development.

A comparison of the weight increase in mice and of the human weight increase curve obtained in the first half of the last century by Shmalhausen (Shmalhausen, 1984) shows that the section of rapid increase in mice (15–30 days) coincides with the section of rapid increase in weight in humans, which falls on the period of development from 12 to 19 years.

The maximum point on the first section of the curve of the death intensity logarithm in humans (Lamb, 1980), which is not described by the Gompertz–Makeham formula, corresponds with 20 years, i.e., almost coincides with the end of the period of rapid increase in the weight on the weight change curve in humans. This fact allows us to conjecture that the areas of maximum death risk at the initial life period correspond with similar stages of development in mice and in humans.

Graphic Presentation of Data on the Death Rates in Mice

The areas of elevated death rates discovered in the initial period of mutant development led to the idea of tracing the life expectancy of the individuals with growth inhibition and the normally developed ones in the course of the entire period of specific life expectancy and to reveal the degree of correspondence of theoretical curves to the experimental data. For this, it is necessary to choose a way of optimum graphic representation of theoretical and experimental data. The data could be represented as logarithms of experimental and theoretical values of death rates as accepted in demography in accordance with the Gompertz–

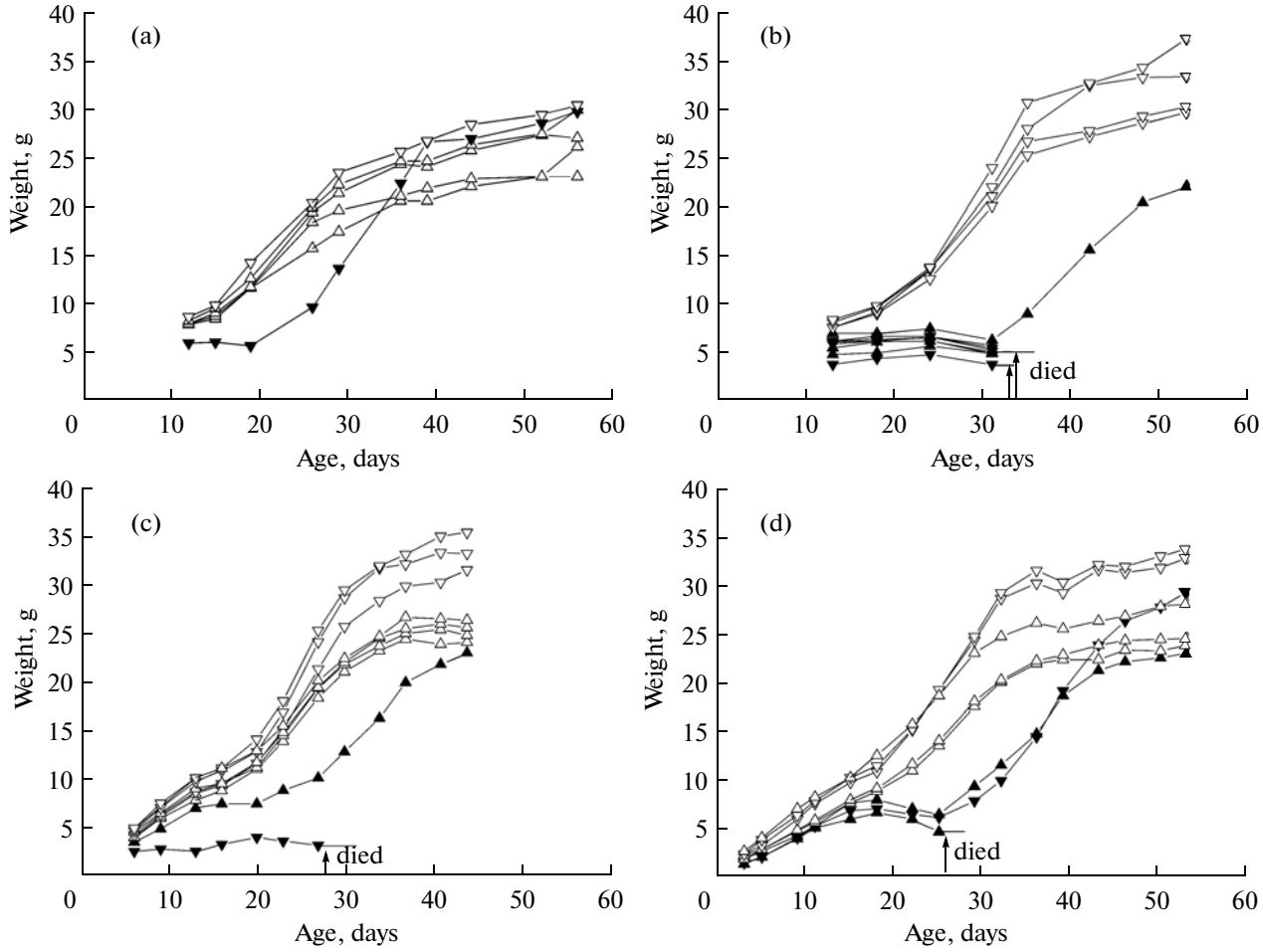

Fig. 1. Changes in the weight of mutant mice and normal mice in the beginning period of ontogenesis. Broods: (a) with the first mutant male (date of birth, July 8, 2007); (b) with one surviving mutant female (date of birth, January 4, 2008); (c) with one dead mutant male (date of birth, August 20, 2008) and (d) with one dead mutant female (date of birth, March 2, 2008). Triangles with tops pointing downward are males. Triangles with tops pointing upward are females. Dark triangles are mutant mice. Light triangles are normal mice.

Makeham formula. However, to build the experimental curves of death rates intensity, it is necessary to calculate the derivatives of the death rate curves from averaged data, which requires greater volume of initial statistical material. Since the number of the studied mice is limited in our case, it made sense to perform the comparative analysis on the curves of living or mortality, whose functions do not contain a derivative. But when plotting these curves, the number of the surviving or dead individuals is also traditionally averaged by discreet time intervals. The solution may be found in rejecting the averaging procedure. However, such a refusal to use a traditionally applied procedure requires substantiation.

The values of the independent variable used in the experiments are determined by the researcher. In natural sciences, these values are usually chosen in such a way as to be distributed evenly on the horizontal axes of the plots. In those cases when a dependent variable that is not controlled by the researcher is placed on the horizontal axis according to tradition (for example, it is the life expectancy of mice), uniform distribution is achieved by averaging the values obtained in the experiment by the identical discreet intervals of the dependent variable.

The use of averaging of data is based on the conviction that it allows the researcher to get rid of random deviations and, thus, to reveal the true nature of the experimental dependencies. However, to obtain a reliable result, it is necessary to have criteria that would allow us to distinguish regular changes from random ones. A universal criterion of the regularity of observed changes is their reproducibility in independent experiments. Neglecting this criterion turns the averaging of experimental data into just a way of simplifying information, which has loss and distortion of the information as its downside. In fact, if every point of the curve is obtained by averaging out ten experimental points, the initial information is reduced ten-fold. The average value does not represent any of the really obtained

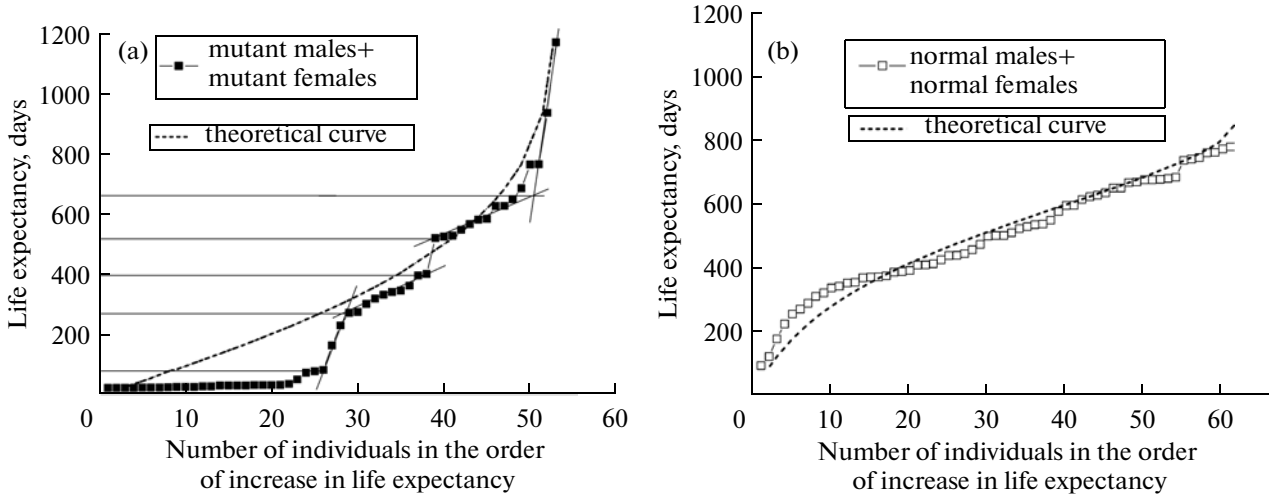

Fig. 2. Plots of rank distribution of life expectancy of (a) mutant mice and (b) normal mice. Theoretical curves are calculated at the values of the parameters of the Gompertz–Makeham formula: (a) $A = -1.3 \times 10^{-3}$; $R = 2.07 \times 10^{-3}$; $\alpha = 1.18 \times 10^{-3}$; (b) $A = 9.413 \times 10^{-5}$; $R = 2.849 \times 10^{-4}$; $\alpha = 4.68 \times 10^{-3}$.

values and, therefore, the information is distorted. For example, the five-year interval often used in demographical studies for averaging out the human death rates completely excludes finding any objective patterns within this interval.

Distortion and loss of information after averaging it out was first consciously used by astronomers (*Mathematical Encyclopedia*, 1984) to describe economically large amounts of data from observations using simple analytical functions with a limited number of parameters. Afterward, these methods seeped into other areas of science. However, at present, owing to the accessibility of computing machinery, the problem of economic recording of scientific information has lost its actuality. So, if there is no special necessity, it is expedient to avoid the averaging of initial information, which leads to its loss and distortion.

Since at small volumes of experimental data, averaging by identical discreet intervals is impossible, their uneven distribution along the horizontal axis leads to the formation of depressions, which create an impression of an inaccurately performed study. In our case, it is possible to eliminate these difficulties if we use the $x(l_m)$ function of rank distribution, which is reverse to the mortality function $l_m(x)$, for the graphical representation of the experimental data. The latter is additional to the function of survival $l_0 - l_m(x)$ usually used by demographers. In the function of rank distribution, the number of individuals placed in the order of increase in the life expectancy, serves as an independent variable, while life expectancy is the dependent variable. This provides even placement of the values of the independent variable on the horizontal axis. It should be emphasized that when passing to the reverse function, the information density of the plot is not decreased since the number of experimental points remains unchanged.

Graphically, the transition from the mortality function to the function of rank distribution is accomplished through simple change of places of the coordinate axes. The curve obtains a form that symmetrically mirrors the initial one.

The theoretical shape of the curve of the rank distribution function is obtained from the theoretical plot of the mortality function developed by integrating the Gompertz–Makeham formula (2) with the initial conditions $x = 0$ and $l_m = 0$:

$$l_m(x) = l_0\{1 - \exp[R/\alpha(1 - \exp(\alpha x)) - Ax]\}. \quad (3)$$

The parameters of the Gompertz–Makeham formula, which are part of it, were calculated by four experimental values of the survival function $l_0 - l_m(x)$ by the method described by L.A. Gavrilov and N.S. Gavrilova (Gavrilov and Gavrilova, 1991).

Patterns of Mice Death in Ontogenesis

Figure 2 gives the curves of rank distribution of the life expectancy of normal and mutant mice. As Fig. 2a shows, the two distinct stages in the intervals of life expectancy developed on the curve of rank distribution, which describes the mortality rates in the population of mutant mice, are from 80 to 270 days and from 400 to 520 days with minimum death rate (horizontal lines) and areas with elevated mortality rates, which divide these stages. At the same time, on the curve that describes the mortality rates of normal mice (Fig. 2b), analogous stages are developed quite weakly. The implication is that the life of mutant mice is divided in time into the stages of stable development, when the probability of death has minimum values,

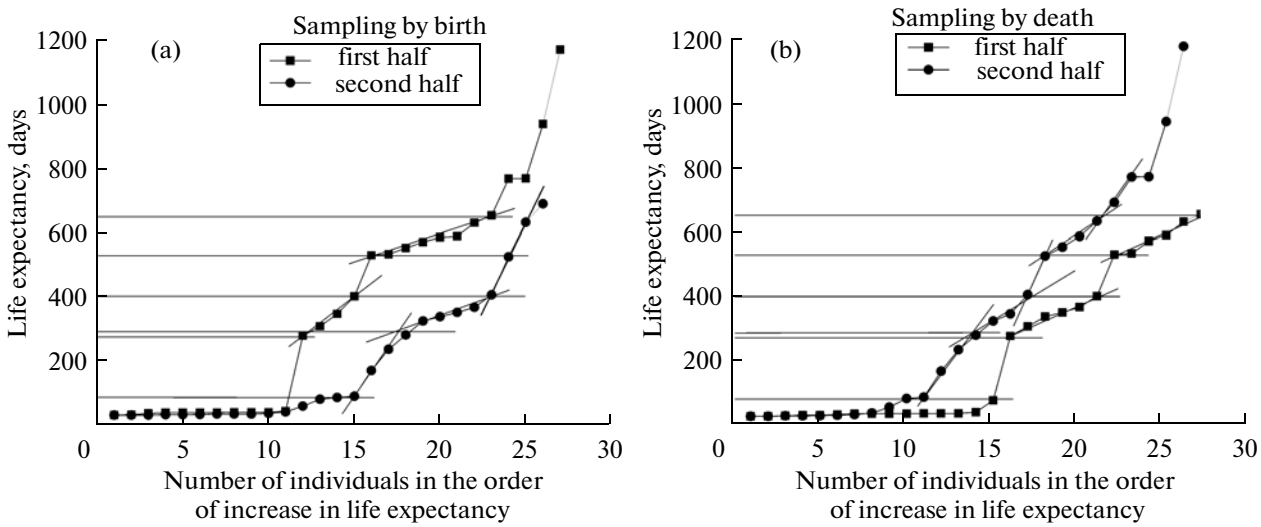

Fig. 3. Plots of rank distribution of the life expectancy of mutant mice divided into two subgroups: (a) by date of birth (27 individuals born from July 8, 2007, to June 27, 2008, and 26 individuals born from June 27, 2008, to March 17, 2011) and (b) by the date of death (27 individuals that died from February 5, 2008, to November 20, 2009, and 26 individuals that died from June 27, 2008, to April 11, 2011).

and stages of elevated risk, when the probability of death increases considerably. The maximum life expectancy for both groups of mice, which is determined by the position of points on the ends of the curves, proved to be almost identical. This means that the delay in development that the mice experienced at the beginning of life has no noticeable influence on the duration of subsequent life.

Figure 2 gives the theoretical curves of rank distribution (designated with dotted lines). A comparison of these curves with the experimental ones reveals qualitative differences in their shape. Theoretical curves have only one inflection in the middle part of the lines, while the steps of the experimental curves form several distinct inflections. This means that the theory of the Gompertz–Makeham formula, the transformation of which yielded the theoretical curves, does not correspond to the obtained experimental results.

As follows from the previous section, the criterion of reliability of the experimental data consists in their reproducibility. To prove that deviations of the experimental curve from the theoretical ones built using the parameters of the Gompertz–Makeham formula are reproducible and, therefore, the steps observed on the experimental curves are not accidental, the following study was performed. A group of mutant mice placed in the row of sequence of their births was divided into two equal subgroups. One subgroup consisted of the mice that were born earlier than the date that divided these two groups, while the other subgroup consisted of mice that were born after this date. For each group, rank distribution curves of the life expectancy were built (Fig. 3a). Similar distinction with building corresponding curves was carried out by terms of death (Fig. 3b). Since the curves for different subgroups may be interpreted as the result of two independent experiments, the closeness of the step levels on the curves obtained in these experiments indicates that the steps are of a regular nature.

To obtain additional evidence that the observed deviations are regular, another study was performed. Groups of mutant and normal individuals were divided into subgroups by gender. The corresponding curves are given in Figs. 4a and 4b. As one can see from the figure, the characteristic steps on the rank distribution curves of mutant males and females by life expectancy not only preserved their position but even obtained a more distinct outline. At the same time, the division of normal mice by gender led to the formation of distinct steps on the curves of normal males analogous to those on the curves of mutant mice. The only thing that differentiates them from the steps on the curves of mutant mice is a certain elevation. On the curve of normal females, these steps are manifested, although very weakly, but still somewhat more distinctly than on the initial curve (compare Figs. 2b and 4b). The coincidence of the curves of rank distribution in mutant males and females and the difference in normal males and females may be explained by the fact that mutant females cannot bear offspring and are similar to males in this respect. Unlike the males, normal females possess an entire set of physiological functions, which make it possible to bear and feed offspring. It is possible that some of these functions promote a shift in time of the stages of stable development and elevated death risk directly or indirectly, which is manifested in the flattening of the rank distribution curves. In addition, it was noted that, unlike in normal males, mutant males and mutant females, the larger part of normal females dies of tumors.

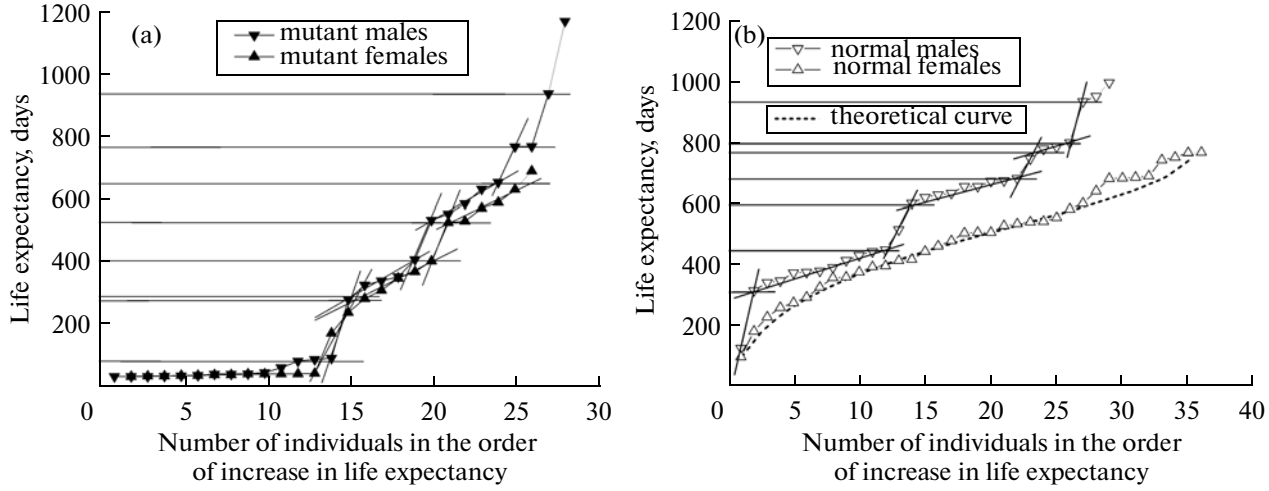

Fig. 4. Plots of rank distribution of the life expectancy of (a) mutant males and females and (b) normal males and females. Theoretical curve for the females was calculated at the values of the parameters of the Gompertz–Makeham formula: $A = 2.201 \times 10^{-4}$; $R = 2.295$; $\alpha = 6.25 \times 10^{-3}$.

The closeness of the step levels on the curves of rank distribution in mutant mice of both sexes and in normal males indicate that the steps serve as stable characteristics of mice development in ontogenesis, which is not significantly influenced by the growth delay mutation.

The reproducibility of the same steps on the curves of rank distribution in the described methods of presentation of the experimental data indicate their regular nature. This allows us to draw a conclusion that the deviations in the shape of the experimental curve from the theoretical one built using the parameters of the Gompertz–Makeham formula observed in the form of steps should not be considered as accidental errors in the determination of the initial data but instead as the inadequacy of the Gompertz–Makeham theoretical model.

The fact that the division of mice into groups with identical biological characteristics does not lead to a loss of characteristic steps on the respective curves but, on the contrary, promotes their revelation led to the thought that they must be masked on the curves composed of heterogeneous mice. The process recalls the phenomenon of interference of coherent light beams in the optics. The higher the homogeneity of sinusoidal harmonics in the beams, i.e., their coherence, the greater the interference streaks are manifested; the lesser the coherence, the lesser is their manifestation. As the loss of coherence progresses, they transform into a heterogeneously lit field. Using this analogy, groups of individuals with close step levels on the curve of rank distribution by life expectancy can be called coherent.

As noted above, the rank distribution curves of mutant males and females almost coincide (Fig. 4a), and, therefore, these groups of mice are highly coherent. At the same time, the position of inflections on the curve of rank distribution of normal males (Fig. 4b) somewhat differs from the position of identical inflections on the curves of rank distribution of mutant mice of both sexes (Fig. 2a) and, therefore, the respective groups of mice are less coherent. To demonstrate the dependence of flatness of the curves of rank distribution on the degree of coherence between the distinguished groups of mice, the curves of rank distribution for the various combinations of these groups were built.

As was expected, the steps on the curves of rank distribution built on the basis of the data on the life expectancy of the group of mice obtained through mixing less coherent groups, namely, normal males with mutant males (Fig. 5a) and normal males with mutant females (Fig. 5b), are manifested almost identically but noticeably weaker than on the curve built for the group of mice that consists of more coherent groups: mutant males and mutant females (Fig. 2a).

Due to the fact that the differences in coherency in the groups of normal males, mutant males, and mutant females remains unchanged, the degree of markedness of steps on the curve of rank distribution built for the merged groups also remains practically invariable (Fig. 5c).

On the contrary, merging these three groups (Fig. 5c) with the noncoherent group of normal females (Fig. 4c), whose rank distribution curve has quite indistinct inflections, smooths the resulting curve almost completely (Fig. 5d), making it very similar to the theoretical curve built using the parameters of the Gompertz–Makeham formula.

The analogy with the interference allows us to explain why the curves of human death intensity built by demographers on the basis of large statistical samplings is described well by the Gompertz–Makeham formula in its middle part, whereas analogous curves

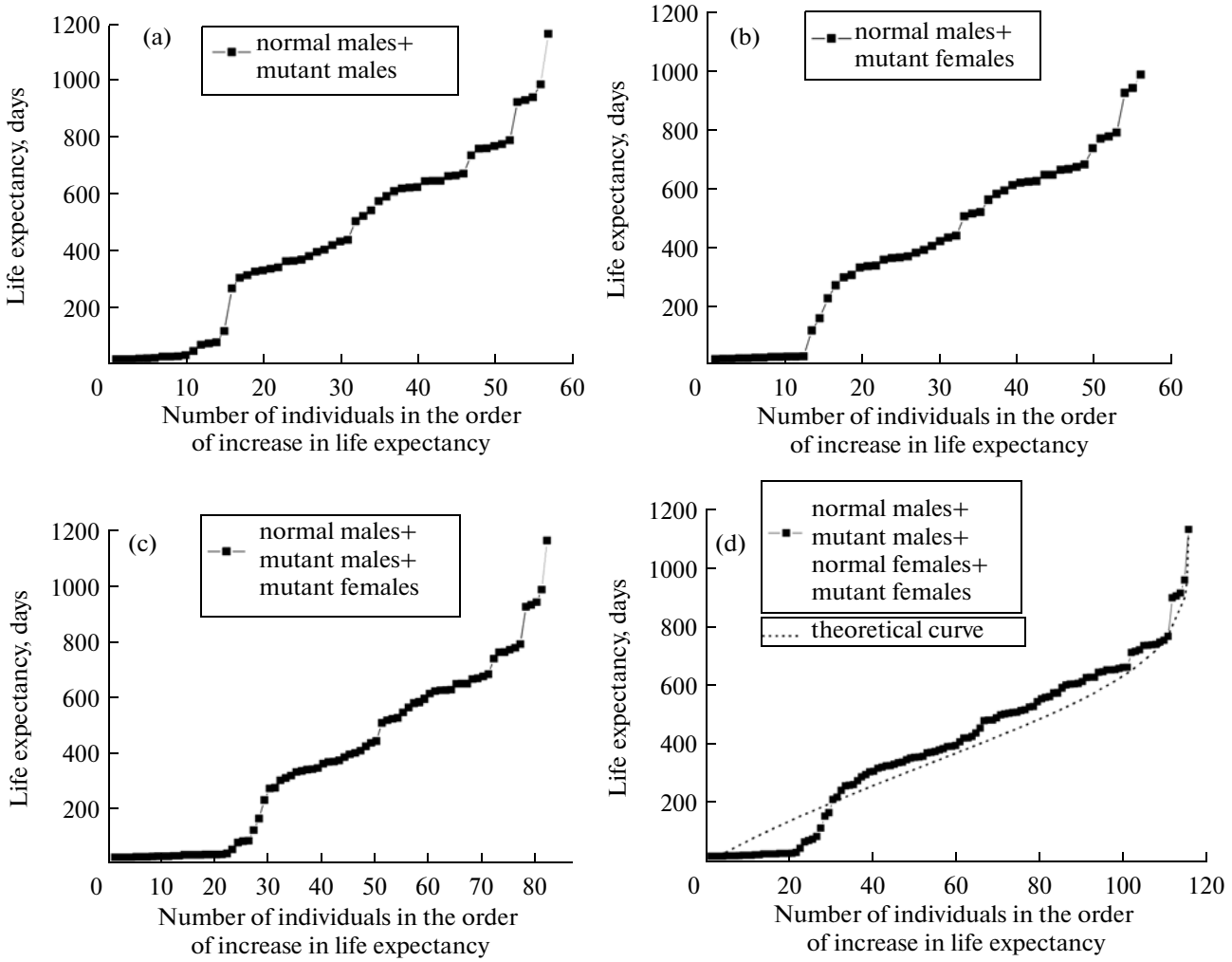

Fig. 5. Plots of rank distribution of the life expectancy of four groups of mice merged in various combinations: (a) normal males and mutant males; (b) normal males and mutant females; (c) normal males, mutant males, and mutant females; and (d) all mice. Theoretical curve for all mice was calculated at the following values of the parameters of the Gompertz–Makeham formula: $A = -9.84 \times 10^{-4}$; $R = 1.03 \times 10^{-3}$; $\alpha = 2.86 \times 10^{-3}$.

for experimental animals built using small samplings (Lamb, 1980) is described unsatisfactorily. The logarithmic form of the experimental curve of death intensity for people in the period until 30 years old has a wave-like aspect (Gavrilov and Gavrilova, 1991; Lamb, 1980). For ages older than 90 years, the curve also has inflections (Gavrilov and Gavrilova, 1991; Vaupel et al., 1998). The latter fact is little studied so it is not usually associated with the first. Another reason why demographers neglect the deviations of the statistical curve of death intensity from the theoretical curve at the beginning and end sections is because the total part of the dead (excluding the mortality rates from one year old) at these sections does not exceed 3% from the total death rates. Unlike demographers, gerontologists that study the nature of death should be interested in detailed information about the inflections on the mortality rate curves.

Joint consideration of inflections at the beginning and end parts of the curve allows us to assume that the curve is composed of periodic functions that describe mortality rates of noncoherent groups of individuals. The respective harmonics begin with one phase, which gives the total curve a wave-like aspect at its initial section. At the end of life, when their phases coincide again, the curve obtains its former wave-like form.

Individual programming of the course of physiological processes in time is characteristic of living organisms. Examples are menstruation in women, stages of transition to sexual maturity, terms of pregnancy, and start of menopause. Death may be considered the final stage of physiological development of the organism.

However, unlike the processes enumerated above, the death of individuals in a population is not a phenomenon strictly determined in time and so allows the

influence of random environmental factors such as disease agents, improper diet or unfavorable environmental conditions to be the cause of it.

On the other hand, the existing statistical data on the determinancy and narrowing of time frames of disease incidence and mortality in pairs of monozygotic twins, unlike in hetero-ovular twins, the examples of concurrence of the end of the life cycle with the ending of the fertilization processes in males or offspring-bearing in females (salmons, ephemera butterflies, annual plants, etc.), and the change of periods of stable development by periods of elevated death risk in mice described above induce us to see the cause of death in the completion of the inner program of development of the organism.

The data that testify in favor of the inner programming of life expectancy or its change under the influence of external random factors can be brought to conformity within the frame of the hypothesis that it is not death itself that is programmed but its probability determined by periodic change in the sensitivity of the organism to unfavorable environmental influences. According to this hypothesis, if one possesses information about the time when the probability of death reaches maximum values as a result of inner development, one can take measures to exclude the influence of the environment, which aggravates the danger, and, thus, to provide the transition to the next stage of stable development.

If proved accurate in relation to the human organism (namely, the presence of such stages in the human ontogenesis), this hypothesis would open the prospect of solving the problem of extending life expectancy to the specific maximum (approximately 105 years) in all individuals living now. Attempts to find proof of existence of critical stages of development based on counting the existing statistical data are already being made (Frank, 2001). In the case of success, the observed mass death of males of cardiac infarctions at the age of 60 can be interpreted from the viewpoint of this hypothesis as the result of reaching by a part of the population of the critical stage of age physiological readjustment, which, in the current socio-economic conditions, they are not able to overcome in order to pass to the new stage of stable development.

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