ISSN 0006-2979, Biochemistry (Moscow), 2017, Vol. 82, No. 7, pp. 834-843. © Pleiades Publishing, Ltd., 2017.
Original Russian Text © A. G. Malygin, 2017, published in Biokhimiya, 2017, Vol. 82, No. 7, pp. 1085-1096.

New Data on Programmed Risks of Death in Normal Mice and Mutants with Growth Delay

A. G. Malygin

Bach Institute of Biochemistry, Research Center of Biotechnology, Russian Academy of Sciences, 119071 Moscow, Russia; E-mail: agmalygin@mail.ru

Received August 30, 2016
Revision received March 23, 2017

Abstract—Study of the lifespans of normal (non mutant) mice and growth delay mutants has shown that mortality rates for both kinds of animals exhibit reproducible fluctuations. In the case of the mutant mice, the positions of peaks on the differential mortality curves (mortality rate plotted against lifespan) coincided in different-sex groups of animals and in same-sex subgroups of animals. Differential mortality curves of the mutant mice also had a peak at 1 month of age that was absent from the differential mortality curves of the normal mice. In the case of normal animals, positions of most peaks were the same in the studied independent subgroups of males, and to a lesser extent – independent subgroups of females, which might be explained by a shift in mortality peak positions due to the reproductive activity of females. Similar positions of mortality rate peaks in the differential mortality curves for animals from independent groups and subgroups indicate the existence of increased risks of death at specific ages. The observed pattern could be due to the programming in the genome of both the periods of increased risk of death and the intermitting intervals of stable development.

DOI: 10.1134/S0006297917070094

Keywords: lifespan, mice, growth delay mutation, differential mortality curves, mortality peaks, Gompertz model

The mortality patterns of normal mice and mutants with growth delay were investigated in our earlier studies [1-4]. The dependence of the number of mutant mice that died at a certain age on lifespan (mortality curve) exhibited a step at days 25-35. Similar but less pronounced steps were also observed at later ages in the mortality curves of both normal and mutant animals. This contradicts the commonly accepted gerontology concept that mortality curves for humans and animals have smooth shape and could be described by the Gompertz function [5]. It was demonstrated recently by other authors that survivorship curves of *Drosophila* flies also deviate from the Gompertz model [6]. Our previous studies were performed using a limited number of animals (53 mutant and 65 normal mice of both sexes), which was insufficient for statistical analysis of the results [1]. In this work, the studied a cohort of experimental animals was seven times larger, which made it possible to differentiate the mortality curves to plot the dependence of the mortality rate on lifespan. As a result of this procedure, previously poorly distinguishable steps of increased mortality were transformed into clearly pronounced peaks at the ages corresponding to the highest mortality rates, i.e. to the periods of the highest risk of death. The reproducibility of peaks in the differential mortality curves for independent groups of experimental animals was studied to prove the existence of specific "mortality patterns" in ontogenesis.

The aim of this study was to use a large cohort of experimental animals to corroborate our conclusion on the genetic programming of mortality fluctuations in ontogenesis that was observed earlier using a limited number of mice.

MATERIALS AND METHODS

White mice used in this study were progeny of a normal female from a heterogeneous laboratory population crossbred with the Swiss Webster line from the Andreevka Animal Facility (Research Center for Biomedical Technologies, Russian Academy of Medical Sciences) and a mutant male with a growth delay that was accidentally discovered in the offspring of a normal female and a male that had been given 10 mM $AgNO_3$ solution for 1.5 months. The mice were kept at the Animal Facility of the Bach Institute of Biochemistry on a standard chow (PK-120, GOST 51849-2001) at 18-22°C and 50-65% humidity. Both normal and mutant mice of both sexes were found in the offspring of the subsequent crossbreedings. The mutants remained significantly smaller than the nor-

mal mice during the first two months of development: the weight of mutants at days 25-35 was less than two-thirds of that of their normal littermates. About one third of the mutants died within this time interval; the weight of animals that survived reached the weight of their normal littermates during the following development. Despite no visible differences with the normal females, mutant females were either infertile or gave birth to pups that died during postnatal day 1. Due to this, new mouse generations were obtained by crossbreeding mutant males with normal females (usually littermates).

The number of mice that died by a certain age was plotted against mouse lifespan (in days). When the lifespans of several mice were the same, the distance between the corresponding values on the x-axis equaled zero, i.e. the derivative value at these points equaled infinity. To avoid this situation, the mortality curves were smoothed before differentiation by calculating the rolling average for the maximal number of the adjacent points with the same lifespan value (in our study, the number of such points did not exceed three). The rolling average was calculated by adding the lifespan values for the three adjacent points and then dividing the obtained sum by three. This decreased the total number of points on the smoothed curve by two compared to the original number of points. Averaging using more points than necessary leads to the excessive loss and distortion of experimental data; hence, in our calculations we used the minimally possible number of points. Numerical differentiation of the smoothed mortality curve was performed using the derivative calculation module of the Origin computer program. The obtained differential mortality curves (see figures) described the mortality rate fluctuations in mouse ontogenesis.

A proof for the reliability of experimental data is their reproducibility in independent experiments. To obtain independent sets of data, all the data on the mouse lifespans collected within eight years of observations were separated into four groups (mutant males, mutant females, normal males, and normal females); each group was then divided into two subgroups by two diverse different methods.

In the first case, mice in the groups were ranked according to their half-life data (middle date between mouse birth and death data) and then divided into two equal subgroups. The use of a half-life data avoided enrichment of the first subgroup and depletion of the second subgroup with data on the lifespan of long-living mice, when ranking the mice according to the birth date, and vice versa, depletion of the first subgroup and enrichment of the second subgroup with data on the lifespan of long-living mice when ranking the mice according to the date of death.

In the second case, the subgroups were formed from odd and even members of the ranked cohort, respectively.

Since subgroups obtained by one of the methods did not overlap, they could be considered independent. On the contrary, half of the mice in the subgroups obtained by the diverse methods were the same; therefore, these subgroups were not independent. The data in the subgroups obtained by the first method could be considered results of independent experiments performed at unconventional times, while data from the subgroups obtained by the second method could be considered results of independent parallel experiments performed at the same time.

An apex of an actual peak might be located at any place between two nearest experimental points to the right and left, respectively, of its visible position on the curve. For this reason, the admissible offset error could be calculated as an average between adjacent points on the curve fragments corresponding to the peaks. In our case, the admissible offset error was calculated as a sum of six intervals between adjacent points on the lifespan axis (three to the left and three to the right of the peak) divided by six. Peaks from different curves were considered the same if the distance between their apices on the lifespan axis did not exceed the sum of all errors characteristic of each of the peaks.

Peaks on the differential mortality curves were numbered. Peaks that satisfied the admissible offset error criterion were numbered similarly. Peaks in the differential mortality curves of normal and mutant mice were designated using different numerical systems due to their significant distinction. Additionally, groups of coinciding peaks in different graphs were marked with vertical lines of the same type (solid, short-dashed, long-dashed, and dotted). Peaks that were present in only one of the four graphs were not marked.

Peak numbers, their positions on the lifespan axis (days), and admissible offset errors are shown in the tables. Peaks considered the same are positioned in the tables on the same lines and under the same numbers.

RESULTS AND DISCUSSION

Dependence of mortality rate on lifespan in mutant mice. Differential mortality curves for the mutant male and female mice are shown in Figs. 1a and 1b, respectively. These curves reflect changes in the mouse mortality rate during ontogenesis.

In Fig. 1 and below, peaks corresponding to the period of increased mortality in mutant mice at days 30-35 are close to the left axis and, being incomparably higher than all other peaks, are truncated at the upper edges of the graphs. The reproducibility of this first stage of increased mortality in mutant males and females has been convincingly demonstrated earlier [1-4]. For this reason, here we omitted detailed description of the corresponding peaks in the differential mortality curves.

Differential mortality curves for the mutant males (142 animals, 13 peaks) and females (147 animals, 15 peaks) were plotted based on the data collected during 8

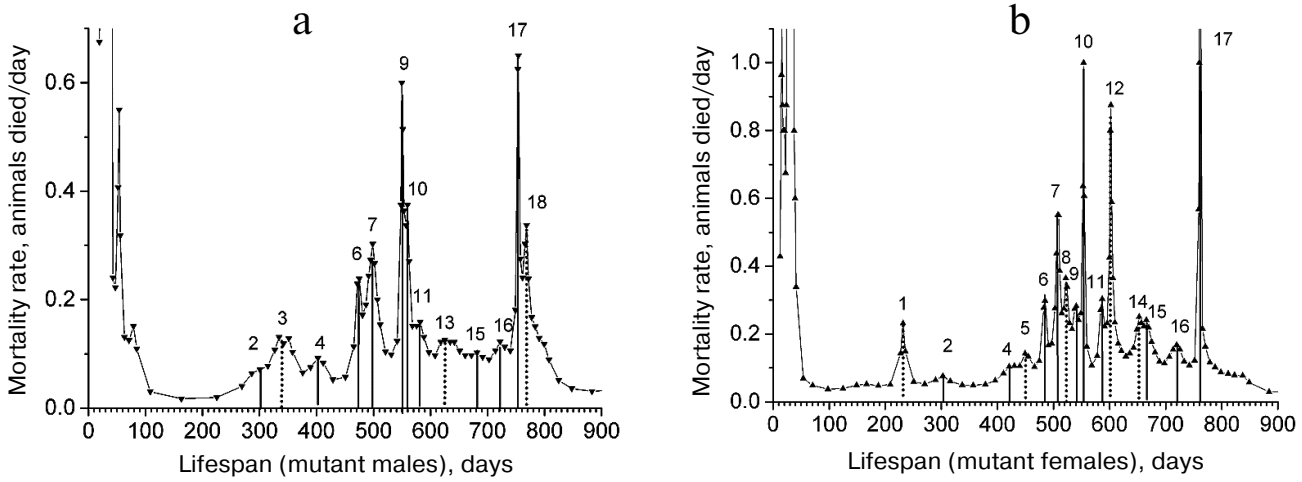

Fig. 1. Differential mortality curves for mutant male (a) and female (b) mice. Peaks with the same positions in graphs (a) and (b) are shown with solid lines and designated with the same numbers; peaks that differ in graphs (a) and (b) are shown with dotted lines and designated with the other numbers.

years of observations and are presented in Figs. 1a and 1b, respectively. Figure 1 and Table 1 show that 10 peaks (Nos. 2, 4, 6, 7, 9, 10, 11, 15, 16, and 17) have similar positions on both graphs. The percentage of these peaks of the total number of peaks is 77% in Fig. 1a and 67% in Fig. 1b. The presence of such high number of coinciding peaks not only corroborates the existence of critical developmental periods at middle and late ages, but also indicates that peak position on the lifespan axis is virtually independent of the mutant animals' sex. The exceptions were three peaks (dotted lines) for the mutant males and five peaks for the mutant females. Note a large peak (No. 12) that was typical for females only.

Table 1. Peak positions (days) in differential mortality curves for mutant male (a) and female (b) mice in Fig. 1

Peak No.	All mutant males (a)	All mutant females (b)
1	–	232 ± 14
2	301 ± 18*	303 ± 18
3	342 ± 10	–
4	402 ± 15	421 ± 12
5	–	449 ± 9
6	475 ± 7	485 ± 6
7	499 ± 4	507 ± 3
8	–	522 ± 4
9	550 ± 4	541 ± 4
10	560 ± 4	554 ± 4
11	582 ± 8	587 ± 5
12	–	602 ± 2
13	625 ± 9	–
14	–	653 ± 5
15	681 ± 10	666 ± 5
16	722 ± 10	720 ± 7
17	754 ± 4	761 ± 3
18	768 ± 4	–

Note: In this and the other tables, hyphen designates the absence of pair peaks in the graphs; asterisk marks "shoulder" peaks that could be considered individual peaks; data are presented as value ± admissible error (days) calculated as described in "Materials and Methods".

To confirm the observed patterns of peak distribution in the curves, male and female mutant mice were divided into subgroups by the two methods described in the "Materials and Methods" section.

Differential mortality curves for each of the subgroups of mutant males are shown in Fig. 2.

The data for the mutant males obtained during the first 4 years of observation (71 animals, 4 peaks) and the following four years of observation (71 animals, 8 peaks) are shown in Figs. 2a and 2b, respectively. The plotted

Table 2. Peak positions (days) in differential mortality curves in subgroups of mutant male mice (a, b, c, d) in Fig. 2

Peak No.	All mutant males			
	1st half (a)	2nd half (b)	odd animals (c)	even animals (d)
2	327 ± 37	334 ± 33*	321 ± 35	–
3	–	376 ± 23	–	353 ± 25
6	–	479 ± 14	–	481 ± 11
7	487 ± 16	506 ± 10	489 ± 20	495 ± 6
10	560 ± 4	–	557 ± 5	555 ± 19*
13	–	619 ± 15	618 ± 11	–
15	–	681 ± 13	–	–
17	751 ± 33	754 ± 5	756 ± 15	762 ± 10
18	–	777 ± 8	–	–

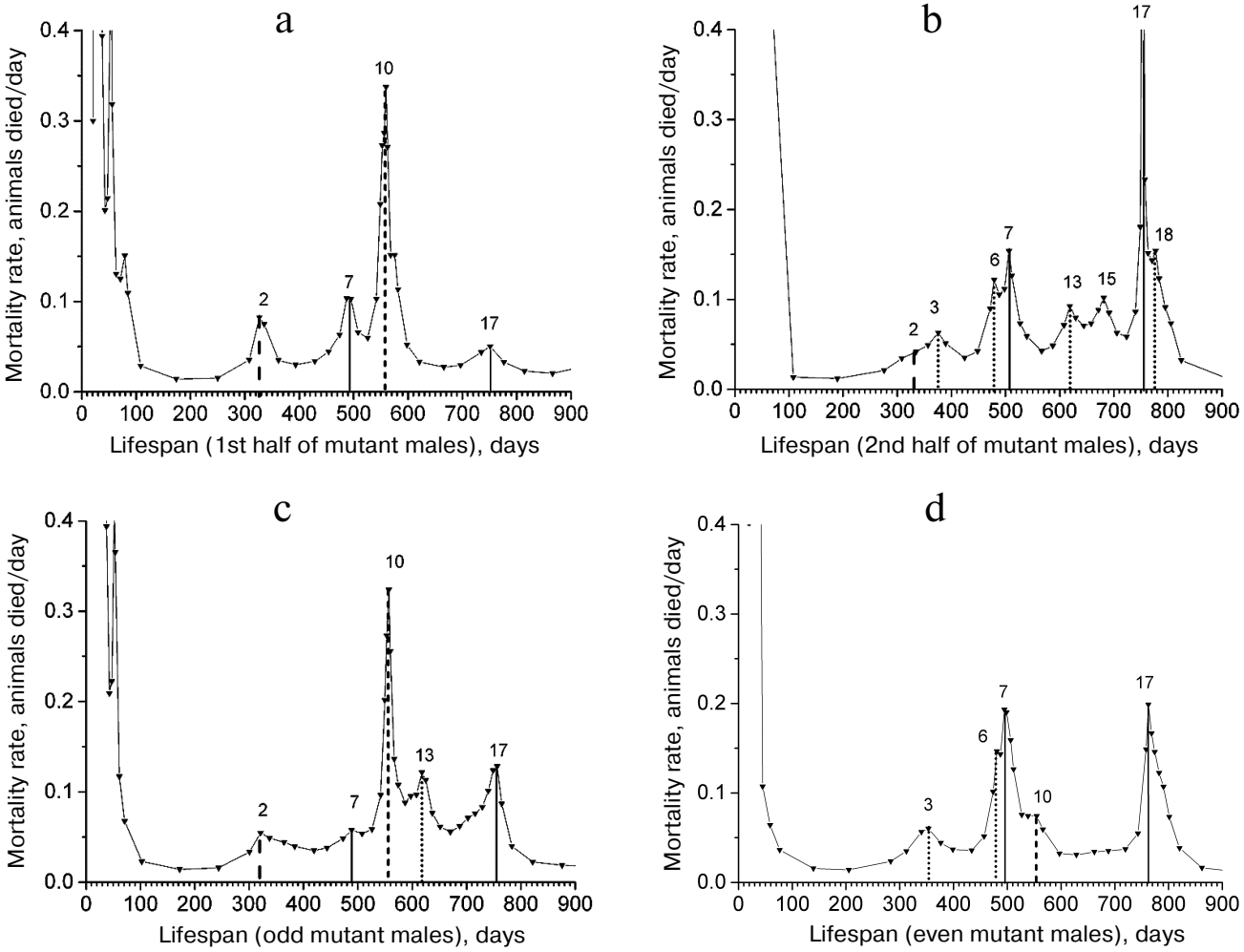

Fig. 2. Differential mortality curves for subgroups of mutant male mice: a) the first half of the analyzed mice ranked according to their half-life data; b) second half of the analyzed mice ranked according to their half-life data; c) odd animals; d) even animals. Peaks with the same positions on the lifespan axis are designated with the same numbers. Peaks observed in each of the four graphs are shown with vertical solid lines; peaks observed in both (a) and (b) graphs but absent from either (c) or (d) graphs or from both (c) and (d) graphs are shown with long-dashed lines; peaks observed in (c) and (d) graphs but absent from either (a) or (b) graphs or from both (a) and (b) graphs are shown with short-dashed lines; peaks observed in either (a) or (b) graphs and present in either (c) or (d) graphs are shown with dotted lines.

curves could be considered results of two independent experiments performed at different times.

Figure 2 (a and b) and Table 2 (columns (a) and (b)) show that three peaks (Nos. 2, 7, and 17) have similar positions in both graphs. The percentage of these peaks of the total number of peaks is 75% in Fig. 2a and 37% in Fig. 2b. The observed decrease in the number of coinciding peaks in relation to the total number of peaks during the second four years of observation could be due to the increase in the number of critical states in ontogenesis of mutant males resulting from the acquisition of additional mutations in the subsequent generations.

Differential mortality curves for the subgroups of mutant males composed of odd (71 animals, 5 peaks) and even (71 animals, 5 peaks) animals are shown in Figs. 2c and 2d, respectively. Such assignment of mice to subgroups imitates random distribution of analyzed animals; therefore, the curves could be considered results of two parallel experiments performed at the same time.

Figure 2 (c and d) and Table 2 (columns (c) and (d)) show that three peaks (Nos. 7, 10, and 17) had the same positions in both graphs. The percentage of these peaks of the total number of peaks in both Figs. 2c and 2d is 60%, i.e. the ratio of the coinciding peaks to the total number of peaks in each subgroup tends to a constant, as it should be in randomly selected samples.

The data on the lifespans of mutant females were distributed into subgroups by the same methods used for the male mice. The differential mortality curves are shown in Fig. 3.

Differential mortality curves for the subgroups of mutant females based on the data obtained during the first four years of observation (74 animals, 7 peaks) and the following four years of observation (73 animals, 7 peaks) are shown in Figs. 3a and 3b, respectively. As in the

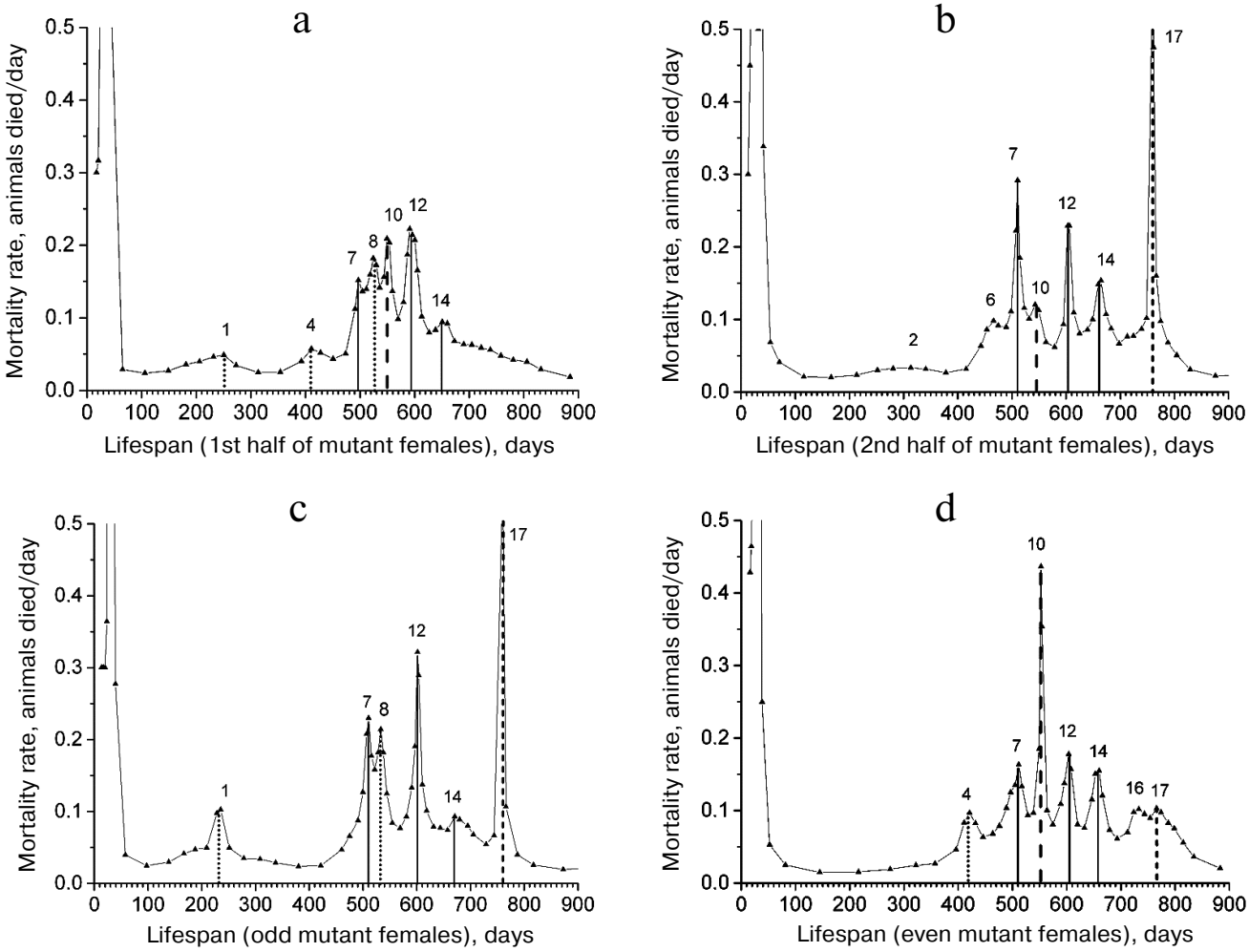

Fig. 3. Differential mortality curves for subgroups of mutant female mice: a) first half of analyzed mice ranked according to their half-life data; b) second half of analyzed mice ranked according to their half-life data; c) odd animals; d) even animals. For designations, see Fig. 2.

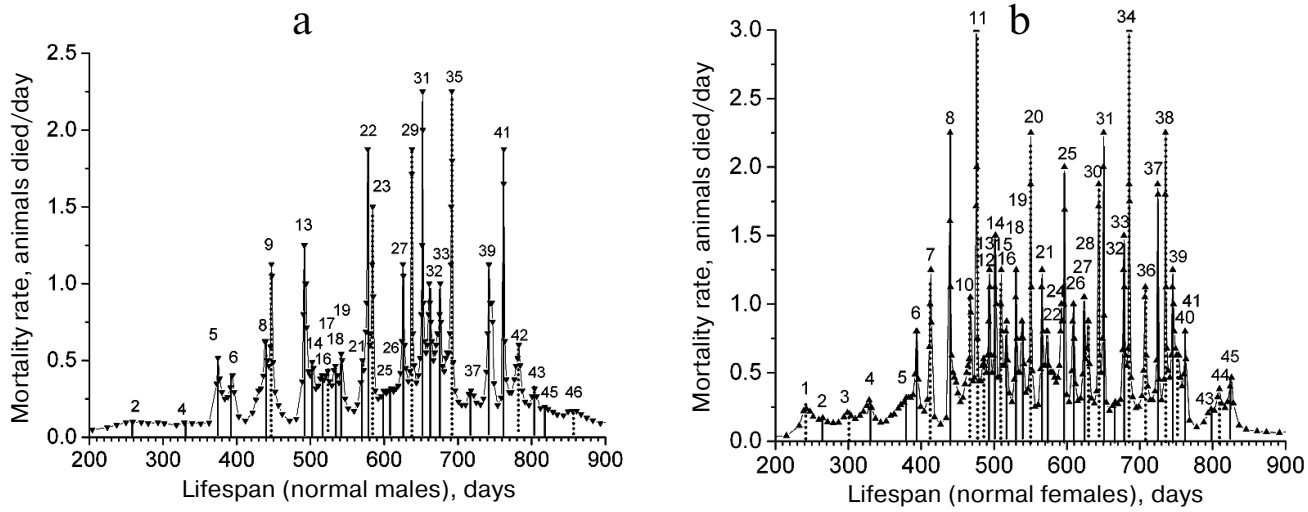

Fig. 4. Differential mortality curves for normal (a) male and (b) female mice. Peaks with the same positions in graphs (a) and (b) are shown with solid lines and designated with the same numbers; peaks that differ in graphs (a) and (b) are shown with dotted lines and designated with the other numbers.

case of mutant males, the data for the two subgroups of mutant females could be considered results of two independent experiments performed at different times.

Figure 3 (a and b) and Table 3 (columns (a) and (b)) show that four peaks (Nos. 7, 10, 12, and 14) have the same positions in both graphs. The percentage of these peaks of the total number of peaks in both Figs. 3a and 3b is 57%. The peak corresponding to the highest mortality rate (No. 16) appears only in Fig. 3b, i.e. four years after the beginning of the experiment. Note that in the case of mutant males, the similar peak was also more pronounced in graph (b) (Fig. 2b). This peak might be a result of mutation that spread among both males and females and caused a tendency for the increase in the number of critical states in ontogenesis of mice of both sexes in the following generations.

Differential mortality curves for the subgroups of mutant females composed of the odd (74 animals, 6 peaks) and even (73 animals, 7 peaks) animals are shown in Figs. 3c and 3d, respectively. As in the case of male mutants, such assignment of female mice to independent subgroups imitates random distribution of analyzed animals; therefore, the curves could be considered results of two parallel experiments performed at the same time. Figure 3 (c and d) and Table 3 (columns (c) and (d)) show that four peaks (Nos. 7, 12, 14, and 17) have similar positions in both graphs. The percentage of these peaks of the total number of peaks is 67% in Fig. 3c and 57% in Fig. 3d, i.e. random sampling allows value scattering in statistical analysis. The fact that peaks 12 and 14 are present in all differential mortality curves for the female mice and are absent in the corresponding differential mortality curves for the male mice allows considering them as characteristic properties of female ontogenesis.

Table 3. Peak positions (days) in differential mortality curves in subgroups of mutant female mice (a, b, c, d) in Fig. 3

Peak No.	All mutant females			
	1st half (a)	2nd half (b)	odd animals (c)	even animals (d)
1	250 ± 9	–	235 ± 20	–
2	–	313 ± 34	–	–
4	411 ± 27	–	–	421 ± 18
6	–	466 ± 14	–	–
7	497 ± 11	510 ± 7	510 ± 6	512 ± 8
8	524 ± 7	–	533 ± 6	–
10	549 ± 7	543 ± 11	–	553 ± 8
12	591 ± 6	603 ± 10	601 ± 6	604 ± 10
14	651 ± 13	664 ± 10	671 ± 12	660 ± 10
16	–	–	–	733 ± 12
17	–	760 ± 8	750 ± 26	760 ± 12

Dependence of mortality rate on lifespan in normal mice. After we identified the peaks of increased mortality rate in differential mortality curves of mutant mice and confirmed the reproducibility of most of these peaks in independent groups of animals, the next stage was searching for similar phenomenon in normal mice.

Differential mortality curves for normal males (209 animals, 29 peaks) and normal females (249 animals, 39 peaks) plotted using the data collected for eight years of observations are shown in Figs. 4a and 4b, respectively.

Figure 4 (a and b) and Table 4 (columns (a) and (b)) show that 23 peaks (Nos. 2, 4, 5, 6, 8, 13, 14, 16, 18, 19, 21, 22, 25, 26, 27, 31, 32, 33, 37, 39, 41, 43, and 45) have similar positions in both graphs. The percentage of these

Table 4. Peak positions (days) in differential mortality curves for normal male (a) and female (b) mice in Fig. 4

Peak No.	All normal males (a)	All normal females (b)	Peak No.	All normal males (a)	All normal females (b)	Peak No.	All normal males (a)	All normal females (b)
1	–	242 ± 7	16	516 ± 3	517 ± 2	31	652 ± 1	650 ± 2
2	259 ± 11	265 ± 7	17	523 ± 3	–	32	661 ± 1	666 ± 4
3	–	299 ± 6	18	534 ± 3	530 ± 2	33	676 ± 2	678 ± 1
4	329 ± 12	329 ± 5	19	542 ± 3	539 ± 2	34	–	686 ± 1
5	374 ± 5	379 ± 3*	20	–	551 ± 2	35	692 ± 1	–
6	394 ± 5	393 ± 2	21	570 ± 3	566 ± 2	36	–	707 ± 2
7	–	413 ± 3	22	578 ± 1	579 ± 2	37	718 ± 4	725 ± 2
8	439 ± 2	440 ± 3	23	584 ± 2	–	38	–	735 ± 1
9	447 ± 2	–	24	–	592 ± 1	39	742 ± 1	745 ± 1
10	–	468 ± 2	25	599 ± 4	597 ± 2	40	–	754 ± 2*
11	–	476 ± 1	26	609 ± 3	609 ± 2	41	762 ± 3	762 ± 4
12	–	486 ± 2	27	625 ± 2	624 ± 2	42	783 ± 2	–
13	492 ± 1	494 ± 1	28	–	630 ± 2	43	804 ± 4	798 ± 5
14	502 ± 3	502 ± 1	29	638 ± 2	–	44	–	809 ± 4
15	–	510 ± 2	30	–	644 ± 2	45	818 ± 5	826 ± 5

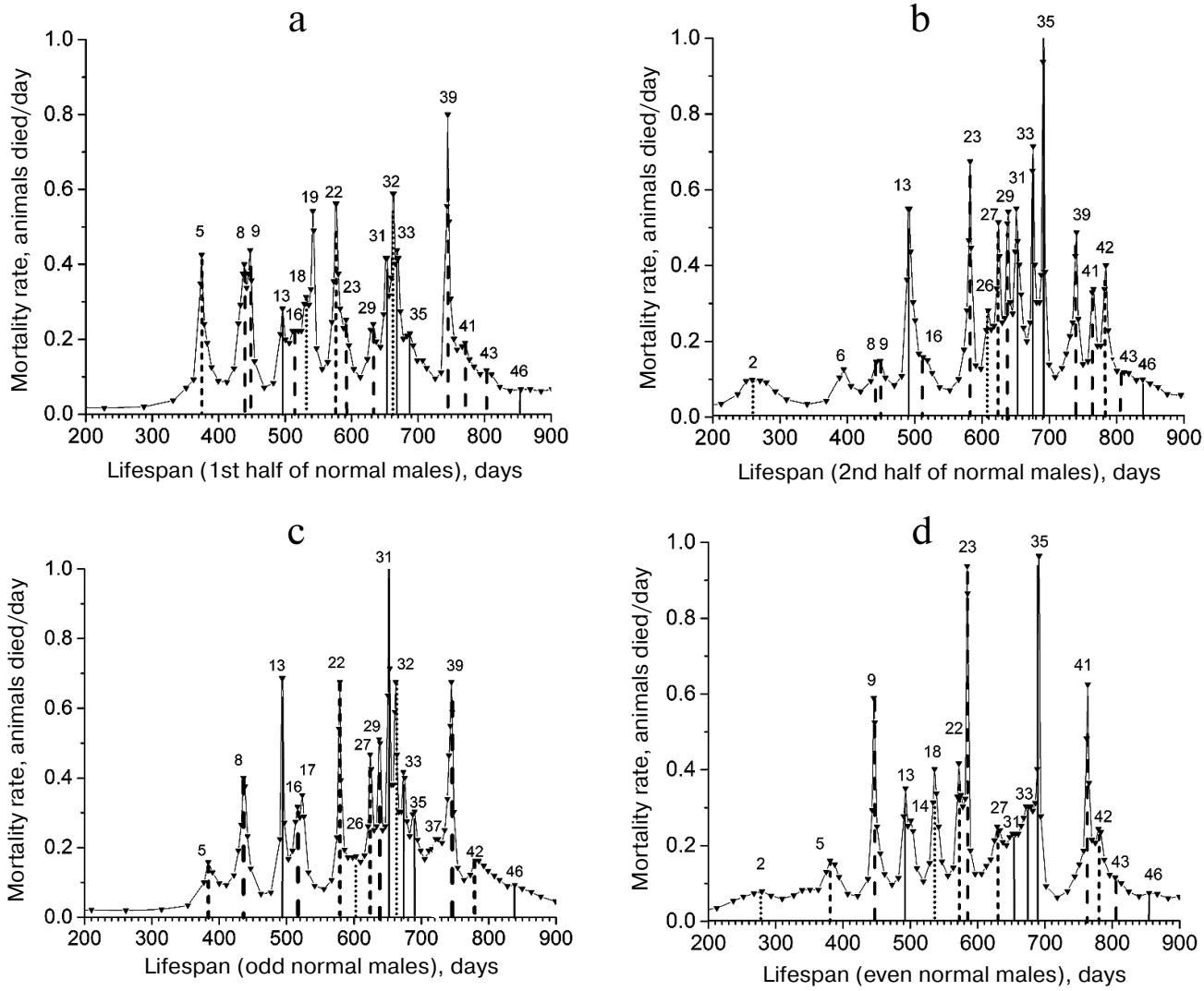

Fig. 5. Differential mortality curves in subgroups of normal male mice: a) first half of analyzed mice ranked according to their half-life data; b) second half of analyzed mice ranked according to their half-life data; c) odd animals; d) even animals. Peaks with the same positions on the lifespan axis are designated with the same numbers (note that numeration here is different from the numeration of peaks in the curves for mutant mice). Peaks observed in each of the four graphs are shown with vertical solid lines; peaks observed in both (a) and (b) graphs but absent from either (c) or (d) graphs or from both (c) and (d) graphs are shown with long-dashed lines; peaks observed in (c) and (d) graphs but absent from either (a) or (b) graphs or from both (a) and (b) graphs are shown with short-dashed lines; peaks observed in either (a) or (b) graphs and present in either (c) or (d) graphs are shown with dotted lines.

peaks of the total number of peaks is 79% in Fig. 4a and 59% in Fig. 4b. The coincidence of 60 to 80% of peaks confirms the existence of critical periods that are genetically programmed in ontogenesis in both normal males and female sand, as in the case of mutants, demonstrates that peak positions on the lifespan axis are virtually independent of the animals' sex. Only 6 peaks in Fig. 4a and 16 peaks in Fig. 4b were unique for normal males and females, respectively.

As can be seen from the figures, differential mortality curves of normal mice contain more peaks of accelerated mortality than those for the mutants. Unlike the mutants, for which the numbers of peaks in the differential curves were approximately the same for both sexes, in normal mice the number of peaks for the females considerably exceeded the number of peaks for the males. It is possible that such difference could be explained by different sizes of analyzed samples. To prevent this discrepancy and to obtain additional data that would allow proving the existence of death risk programming in ontogenesis in both normal and mutant mice, peak distribution in the differential mortality curves was studied in four subgroups of normal male mice and analogous four subgroups of normal female mice that contained equal numbers of animals. The differential mortality curves are shown in Figs. 5 and 6.

Differential mortality curves for subgroups of normal males based on the data obtained during the first four years of observation (105 animals, 18 peaks) and the following four years of observation (104 animals, 18 peaks) are shown in Figs. 5a and 5b, respectively. The curves could be considered results of two independent experiments performed at different times.

Figure 5 (a and b) and Table 5 (columns (a) and (b)) show that 13 peaks (Nos. 8, 9, 13, 16, 23, 29, 31, 33, 35, 39, 41, 43, and 46) have similar positions on both graphs. The percentage of these peaks of the total number of peaks in both Figs. 5a and 5b is 72%.

Differential mortality curves for the subgroups of normal males composed of odd (105 animals, 17 peaks) and even (104 animals, 16 peaks) animals are shown in Figs. 5c and 5d, respectively. Such assignment of mice into independent subgroups imitates random distribution; therefore, the curves could be considered results of two parallel experiments performed at the same time. Figure 5 (c and d) and Table 5 (columns (c) and (d)) show that nine peaks (Nos. 5, 13, 22, 27, 31, 33, 35, 42, and 46) have similar positions on both graphs. The percentage of these peaks of the total number of peaks is 53% in Fig. 5c and 56% in Fig. 5d.

Differential mortality curves for subgroups of normal females based on the data obtained during the first four years of observation (125 animals, 23 peaks) and the following four years of observation (124 animals, 21 peaks) are shown in Figs. 6a and 6b, respectively. As in the case of normal males, the curves could be considered results of two independent experiments performed at different times.

Figure 6 (a and b) and Table 6 (columns (a) and (b)) show that 13 peaks (Nos. 1, 6, 7, 8, 11, 16, 20, 21, 26, 28, 31, 39, and 44) have similar positions on both graphs. The percentage of these peaks of the total number of peaks is 56% in Fig. 6a and 62% in Fig. 6b.

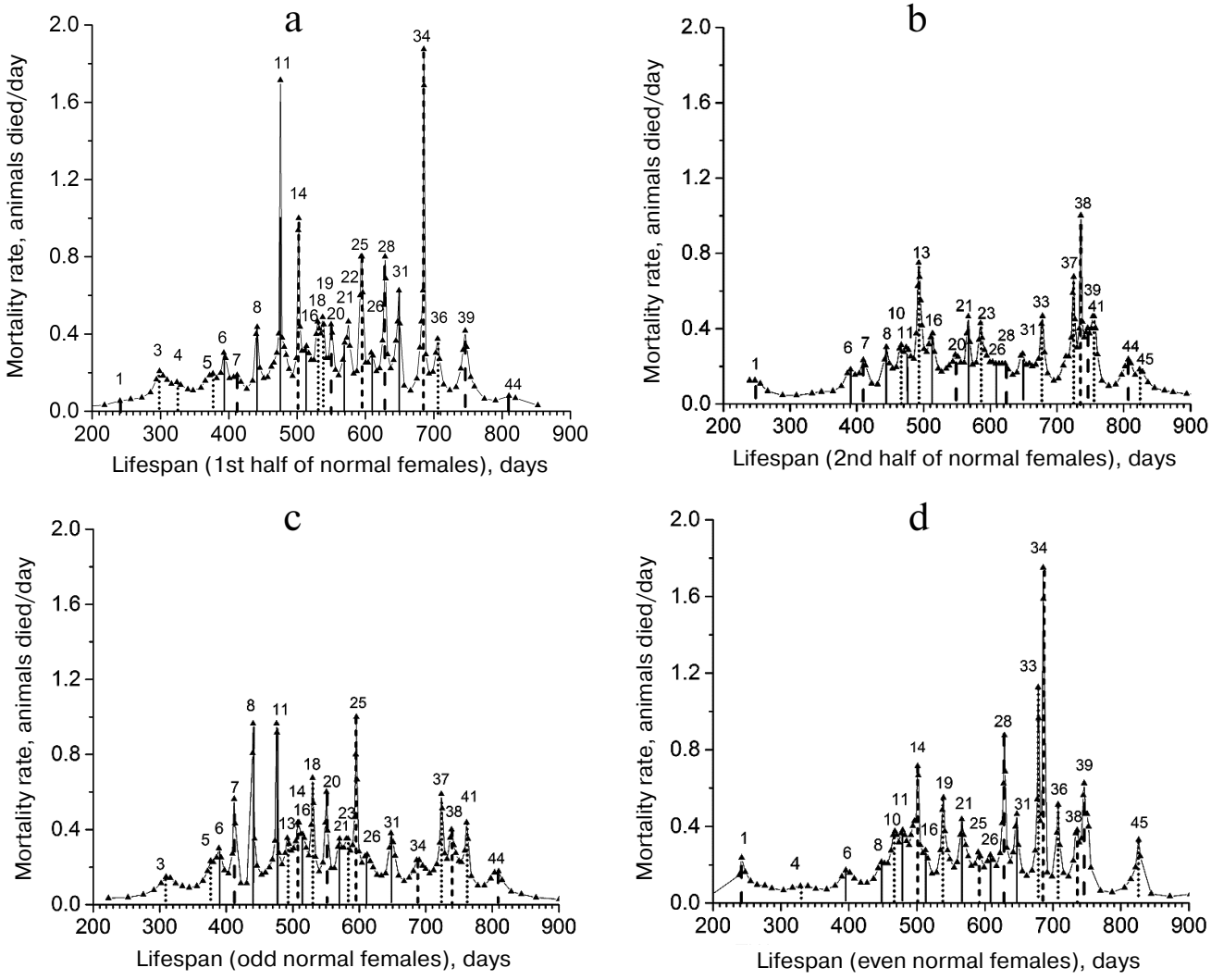

Fig. 6. Differential mortality curves in subgroups of normal female mice: a) first half of analyzed mice ranked according to their half-life data; b) second half of analyzed mice ranked according to their half-life data; c) odd animals; d) even animals. For designations, see Fig. 5.

Differential mortality curves for the subgroups of normal females composed of odd (125 animals, 21 peaks) and even (124 animals, 20 peaks) animals are shown in Figs. 6c and 6d, respectively. Such assignment of mice into independent subgroups imitates random distribution; therefore, as in the case of normal males, the curves could be considered results of two parallel experiments performed at the same time. Figure 6 (c and d) and Table 6 (columns (c) and (d)) show that 11 peaks (Nos. 6, 8, 11, 14, 16, 21, 25, 26, 31, 34, and 38) have similar positions on both graphs. The percentage of these peaks of the total number of peaks is 52% in Fig. 6c and 55% in Fig. 6d.

It is possible that lower percentage of coinciding peaks in the differential mortality curves in the independent subgroups of female mice compared to the corresponding subgroups of male mice is related to the physiological rearrangement of the female organism during pregnancy.

Table 5. Peak positions (days) in differential mortality curves in subgroups (a, b, c, d) of normal male mice in Fig. 5

Peak No.	Normal males			
	1st half (a)	2nd half (b)	odd animals (c)	even animals (d)
2	–	259 ± 13	–	280 ± 15
5	374 ± 6	–	384 ± 16	381 ± 9
6	–	395 ± 18	–	–
8	439 ± 3	443 ± 10*	436 ± 4	–
9	447 ± 5	449 ± 10	–	446 ± 6
13	496 ± 7	492 ± 3	493 ± 7	493 ± 5
14	–	–	–	501 ± 5
16	515 ± 5*	513 ± 7*	516 ± 4	–
17	–	–	523 ± 5	–
18	532 ± 4	–	–	536 ± 5
19	541 ± 4	–	–	–
22	577 ± 3	–	579 ± 4	572 ± 4
23	592 ± 6	582 ± 4	–	584 ± 4
26	–	609 ± 5	603 ± 6	–
27	–	624 ± 3	624 ± 4	629 ± 5
29	633 ± 6	639 ± 3	637 ± 3	–
31	653 ± 3	651 ± 3	652 ± 2	656 ± 4
32	662 ± 2	–	662 ± 2	–
33	668 ± 3	676 ± 3	673 ± 3	675 ± 4
35	688 ± 6	691 ± 2	690 ± 4	691 ± 3
37	–	–	725 ± 5	–
39	745 ± 5	740 ± 4	745 ± 3	–
41	771 ± 7	766 ± 5	–	763 ± 4
42	–	784 ± 5	785 ± 8	779 ± 5
43	803 ± 10	805 ± 8*	–	804 ± 9*
46	855 ± 15	839 ± 11	839 ± 12	855 ± 14

Table 6. Peak positions (days) in differential mortality curves in subgroups (a, b, c, d) of normal female mice in Fig. 6

Peak No.	Normal females			
	1st half (a)	2nd half (b)	odd animals (c)	even animals (d)
1	241 ± 21	247 ± 16	–	242 ± 15
3	299 ± 7	–	310 ± 10	–
4	324 ± 7	–	–	329 ± 13
5	377 ± 6	–	377 ± 5	–
6	393 ± 5	391 ± 8	389 ± 4	395 ± 8
7	412 ± 7	410 ± 7	412 ± 6	–
8	442 ± 5	444 ± 6	440 ± 5	448 ± 6
10	–	467 ± 4	–	468 ± 3
11	476 ± 2	474 ± 4	476 ± 3	479 ± 3
13	–	493 ± 2	492 ± 4	–
14	502 ± 3	–	507 ± 3	501 ± 2
16	514 ± 3	514 ± 4	515 ± 3*	513 ± 4
18	530 ± 3	–	530 ± 3	–
19	537 ± 3	–	–	539 ± 3
20	550 ± 4	548 ± 4	551 ± 4	–
21	568 ± 4*	567 ± 4	570 ± 4	566 ± 3
22	575 ± 3	–	–	–
23	–	585 ± 3	583 ± 3	–
25	593 ± 3	–	596 ± 2	590 ± 5
26	608 ± 4	613 ± 5*	612 ± 4	608 ± 5
28	629 ± 3	622 ± 5*	–	629 ± 2
31	649 ± 4	649 ± 5	648 ± 4	647 ± 4
33	–	678 ± 4	–	678 ± 2
34	685 ± 2	–	686 ± 5	686 ± 3
36	706 ± 5	713 ± 5*	–	708 ± 5
37	–	725 ± 3	723 ± 3	–
38	–	735 ± 2	739 ± 4	736 ± 4
39	746 ± 4	746 ± 3	–	746 ± 2
41	–	756 ± 3	761 ± 5	–
44	810 ± 16	–	809 ± 9	–
45	–	824 ± 6	–	826 ± 11

Conclusion

The presence of the same peaks in the differential mortality curves plotted for independent groups and subgroups suggests genetic programming of periods of increased death risk in mouse ontogenesis. As seen from the graphs, mortality rates at the apices of the peaks might exceed those during the periods of stable development (at the bases of the peaks) more than 10-fold. On the other hand, peaks with similar positions could considerably differ in size, which might be due to the effect of external factors on the programmed death risks and not related to the intrinsic program of organism development. The presence of a peak in a differential mortality curve and its

absence in the paired independent curve indicates that favorable external factors might decrease the probability of death during the critical period to its minimal value otherwise typical for the period of stable development.

The similarity between mouse and human genomes suggests that the observed reproducibility in the peak positions in the differential mortality curves in mice might also be found in humans, e.g. if we analyze data on the mortality of primates in homogenous populations in aperies [7]. However, genetic heterogeneity of human populations will inevitably result in smoothed mortality curves that will fit the Gompertz model [5] and considerably hinder identification of discontinuities in the curves.

It is commonly accepted that similarity between monozygotic twins is determined by the identity of their genomes. Therefore, the well-known fact that the difference in the lifespans of monozygotic twins is considerably less than that in dizygotic twins could be explained by the genomic programming of lifespan [8]. On the other hand, monozygotic twins rarely die at the same moment. The absence of rigid lifespan control by the genome in twins, as well as in mice, might be interpreted as programming of increased/decreased death risks, but not the lifespan per se.

Up to 60% of people die suddenly [9]. The most common cause of sudden death is heart failure because of myocardium infarction. Individuals that survive myocardial infarct often die within a short period of time due to repeated myocardium infarction, although some of them live for decades. One possible explanation is that the former are still in the period of critical development of heart pathology, while the latter have overcome it and entered a new period of stable development. A suitable analogy is the death of salmonids after spawning. It is known that this death results from genetically programmed accelerated aging. In some cases, the process is reversible [10], i.e. salmonids can transit from the critical state to a stable one.

If we accept that an accidental event is an unrecognized regularity, then the theory of aging as accumulation of random errors justifies the refusal of the search for the regularities in aging. The latter proves the case of those researchers who insist on the existence of lifespan genetic programming [11] and leaves to their opponents the possibility only to speculate on the absence of adequate knowledge of the programming mechanisms. On the other hand, the variability of peak sizes in the differential mortality curves explains the facts that confirm the theory that aging and death are determined by errors accumulated as a result of interactions of the organism with the environment [12].The acceptance of the theory of genetic programming of death risks in ontogenesis and the ability of environmental factors to affect these risks will allow drawing a compromise between the two opposing concepts. The appearing randomness of the action of external factors on the lifespan should be explored for hidden regularities.

Acknowledgments

The author thanks L. A. Denisova, Director of the Animal Facility, Institute of Developmental Biology, Russian Academy of Sciences, for her help with the experiments.

This work was supported by the Russian Academy of Sciences Presidium Program for the Fundamental Research "Biosphere origin and evolution of geobiological systems" (program 28, 2014; subprogram I).

REFERENCES

1. Malygin, A. G. (2012) Variations in the lifespan of mice during the periods of growth and aging, *Dokl. MOIP Sekt. Gerontol.*, **50**, 56-65.
2. Malygin, A. G. (2012) Oscillation in the survivorship of mice during growth and aging, in *Problems of Biosphere Origin and Evolution* [in Russian], Pt. 2 (Galimov, E. M., ed.), KRASAND, Moscow, pp. 635-639.
3. Malygin, A. G. (2013) Graduated change of life expectancy in mice ontogenesis, *Russ. J. Dev. Biol.*, **44**, 48-55.
4. Malygin, A. G. (2013) Age fluctuations in mortality of mice with mutation causing growth retardation, *Biochemistry (Moscow)*, **78**, 1033-1042.
5. Gompertz, B. (1825) On the nature of the function expressive of the law of human mortality and on a new mode of determining the value of life contingencies, *Phil. Trans. R. Soc.*, **115**, 513-583.
6. Mylnikov, S. V., Oparina, T. I., and Bychkovskaya, I. B. (2015) On the discontinuous character of annuity curves. Communication 1. Deviations from the Gompertz law in *Drosophila melanogaster* Canton-S line, *Usp. Gerontol.*, **28**, 624-628.
7. Malygin, A. G. (2016) Reproducible peaks in differential mortality curves of mice as a reason for searching for analogous regularities in primates, in *Fundamental and Applied Aspects of Medical Primatology: III Int. Conf.*, Sochi-Adler, August 8-10, 2016, Vol. 1, pp. 75-87.
8. Lamb, M. J. (1977) *Biology of Aging*, Blackie, Glasgow.
9. Vikhert, A. M., and Laune, B. (eds.) (1982) *Materials of the 2nd Soviet-American Symp. Sudden Death*, December 6-8, 1979, Indianapolis; Meditsina, Moscow.
10. Ziuganov, V. V. (2005) A paradox of a parasite prolonging the life of its host. Pearl mussel can cancel the accelerated aging program in salmon, *Izv. Akad. Nauk. Ser. Biol.*, **4**, 435-441.
11. Skulachev, V. P., and Longo, V. D. (2005) Aging as a mitochondria-mediated atavistic program. Can aging be switched off? *Ann. N. Y. Acad. Sci.*, **1054**, 145-164.
12. Khalyavkin, A. V. (1998) Organism–environment interaction and causes for aging, *Usp. Gerontol.*, **2**, 43-48.