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WITHIN- AND BETWEEN-STRAIN VARIABILITY IN LONGEVITY OF INBRED AND OUTBRED RATS UNDER THE SAME ENVIRONMENTAL CONDITIONS

ORLANDO GHIRARDI, ROBERTO COZZOLINO, DONATELLA GUARALDI and
ALESSANDRO GIULIANI

Institute for Research on Senescence, Sigma-Tau S.p.A., Via Pontina Km 30.400, 00040
Pomezia, Roma, Italy

Abstract — The analysis of 26 longevity curves of different populations of inbred (Fischer 344) and outbred (Sprague–Dawley) rats highlighted a remarkable between-populations variability in survival parameters. This variability is independent of the breeding characteristics of the strain. The two strains differed in the slope of the survival curves, with Fischer 344 rats showing a higher survival over the second year of life as well as a lower interindividual variability. A model-free approach based on principal component analysis allowed us to quantify these differences and to highlight some limitations of the classical Gompertzian approach.

INTRODUCTION

RATTUS NORVEGICUS is one of the most frequently used animal models in aging research. Variability in longevity parameters exists among different strains of laboratory rat as well as between populations of the same strain experiencing different environmental conditions (e.g., Chesky and Rockstein, 1976; Committee on Animal Models For Research on Aging, 1981). For instance, housing, climate exposure, physical activity, and dietary condition have been demonstrated to play a crucial role in affecting life expectancy, physiological deterioration, and age-related disease processes in laboratory animals (Committee on Animal Models For research on Aging, 1981; Holloszy *et al.*, 1985; Iwasaki *et al.*, 1988; Coates, 1991; Festing, 1991; Masoro, 1991; Kirkwood, 1992; Pitsikas and Algeri, 1992). This evidence implies that both genetic and nongenetic variability may result in a different pattern of biological aging among laboratory rats.

Although the genetic variability is currently monitored for both inbred rats, that is, to maintain the higher homozygosity (e.g., Festing, 1991), and outbred rats, that is, to maintain a constant level of heterozygosity (Foisil, 1989; IFFA CREDO, 1990), the phenotypic variability between rat populations belonging to the same strain has not been systematically investigated (Festing, 1993). The lack of full information on this matter is fundamentally due to the enormous cost involved in allowing animals to die naturally without being used in experiments. Consequently, longevity data reported in literature are generally referred to a relative small number of animals and never include more then one birth date-class population of the same strain kept under the same environmental conditions. A valuable compromise could be to study a colony of animals using the approach of clinical researches: individuals periodically removed from the group for experiments are considered as drop-outs in the sample group (Armitage, 1971). In this way the cost of a follow-up would be reduced, and, moreover, sampling could be enlarged. Knowledge of the between populations variability is important to compare data from different experimentations. When we perform experiments on different groups of rats having the same chronological age we presume they have (on average) the same biological status, that is, they are at the same point of their survival curve. The quantitation of between-populations variability gives us some useful information about the reliability and the confidence limits of this assumption. This kind of variability is distinct from interindividual variability, which can be assessed even in a single population of rats. The present study examines the within- and between-strain variability in longevity data among inbred and outbred rats strictly maintained under the same environmental conditions. To this end, over the years 1986–1993, a total of 26 follow-ups for longevity trends were obtained from various birth date-class populations of male Sprague–Dawley and Fischer 344 rats, as are usually used in our studies.

METHODS

The study was carried out on male rats, comprising 16 groups of Fischer 344 and 10 groups of Sprague–Dawley (Charles River, Italia), housed in a Specific Pathogen Free (SPF) area at Sigma-Tau S.p.A. laboratories. Standard monitoring of this area did not reveal any infectious event during the study period. The monitoring implies the immuno-enzymatic tests for: *Mycoplasma pulmonis*, viral hepatitis, Sendai virus, Lymphocytic choriomeningitis, Corona virus (SDA/RCV), Kilham virus, Hantaan virus, Salmonella, *Citrobacter freundi*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.

Endoparasite tests were conducted for: Flagellae, Oxyures, and Tenia. Other tests were executed for detecting cutaneous mycoses. The entire battery of tests was executed once every 2 months.

Data are referred to a total of 3802 animals, that is, 2646 Fischer rats (59–280 per group), and 1156 Sprague–Dawley rats (90–152 per group), born in the period April 1986 –January 1991. Animals were housed in transparent Makrolon cages (3 rats per cage from the age of 12 months) and received tap water and standard laboratory diet ad libitum (4RF18, Mucedola S.r.l., Italia), which contained a minimum of 16% protein, 2.5% fat, and a maximum of 7.5% fiber. The animal rooms were maintained under standard conditions of temperature (22 ± 1°C) and relative humidity (55 ± 10%), with a 12:12-h light/dark cycle (light on 07:00 a.m.), and 12–15 filtered air changes/hour.

At the onset of each data collection, the rats from each group were 12 months old when received from Charles River laboratories.

Over the study period, some animals were periodically removed from their respective groups to be used in the experiments. The animal removed represented less than 50% of the total starting number of animals, for all experimental groups.

For all groups, survival data were obtained by calculating at each age (months) the proportion of the surviving animals making, wherever necessary, the appropriate correction for drop-out individuals (see Armitage, 1971). The causes of death were not investigated in depth. Five statistical parameters for each group were evaluated:

- first quartile survival time (T_{75}), corresponding to the age at which the proportion of surviving animals per group is 75%;
- median survival time (T_{50}), that is the age at which the proportion of surviving animals for a group is 50% (in the case of a symmetrical distribution, this parameter corresponds to the life expectancy);
- third quartile survival time (T_{25}), corresponding to the age at which the proportion of surviving animals for a group is 25%;
- maximal life span (T_{max}) (the last death observed for each group);
- the interquartile range ($T_{25}-T_{75} = \Delta_{50}$), which can be considered an index of the survival curve slope.

To obtain quantitative indices summarizing the survival curves relative to each population in a model-free way, we utilized an approach based upon principal component analysis (PCA) (Jones and Rice, 1992). Each population was defined by means of 4 quartiles (namely T_{75}, T_{50}, T_{25}, and T_{max}) and of the interquartile range of the median ($\Delta 50$). This corresponds to a multivariate data matrix having $N = 26$ rows (= groups = statistical units) and $K = 5$ columns (= statistical indices = variables). The correlations among the variables make it possible to describe the data matrix by means of a number $P < K$ of components explaining most of the original variation (Lebart *et al.*, 1984; Stahle and Wold, 1988; Jones and Rice, 1992). The components are mutually independent of construction and their meaning is easily interpretable by the inspection of the correlation coefficients between the component scores and the original variables (factor loadings) (Lebart *et al.*, 1984). The inferential comparison between the component scores relative to the different strains allows the differences between the survival data to be evaluated statistically.

In addition to this model-free approach, we analyzed the differences between the survival curves of the two strains by means of the classical Gompertzian model (Finch *et al.*, 1990; Wilson, 1993). In this latter case the survival curve of each group was defined by the two parameters of the Gompertz equation (α and R_0). The Gompertz survival function was derived from the Gompertz mortality function, and can be obtained directly from survival data as reported by Wilson (1993). The Gompertz survival function is:

$$S = \exp[(R_0/\alpha)(1 - e^{\alpha t})]$$

The Marquardt–Levenberg algorithm (Marquardt, 1963) for nonlinear regression analysis, available on SigmaPlot®, was used to calculate the best fit values of R_0 and α for each group.

The inferential comparisons were performed on the values of these parameters. The two approaches were compared by correlating the component scores with the values of the Gompertz parameters.

Unpaired two-tailed Student's t-test was used as statistical inference method for all comparisons between strains. The F-test was used to compare the two strains as for the degree of within strain variability for all considered parameters. Correlations were computed using the Pearson's r coefficient.

RESULTS

Model-free analysis

Data from both strains are summarized in Table 1. Fischer 344 reached the T_{75} quartile 2.5 months later than the Sprague–Dawley and this difference was statistically significant. Instead, the T_{50}, T_{25}, and T_{max} were reached at similar ages with no statistically significant differences. As for the Δ_{50} parameter, the statistical analysis revealed significant differences between the two strains, indicating for Fischer 344 rats a sharper curve slope (see Fig. 1). This finding, together with the evidence from T_{75} parameter, indicates a higher survival rate over the second year of life for this strain. Principal Component Analysis of the five parameters describing the survival curve for each group of rats evidenced two components explaining the 90% of total variability (Table 2). Component 1 (PC1) was mainly related to the parameters T_{75}, T_{50}, T_{25}, and T_{max}: the location on the scale of age of the survival curve; component 2 (PC2) was mainly related to Δ_{50}: the curve slope. As reported in Table 1, inferential analysis of PC scores revealed that Fischer 344 and Sprague–Dawley groups differed significantly for PC2 but not for PC1.

Gompertz analysis

For each group of rats, the Gompertz parameter α and the initial mortality rate R_0 were calculated through the Gompertz survival function.

Fischer 344 and Sprague–Dawley groups differed significantly for α and R_0 parame-

TABLE 1. DESCRIPTIVE AND INFERENTIAL STATISTICS FOR LONGEVITY PARAMETERS (MONTHS), GOMPERTZ PARAMETERS, AND PRINCIPAL COMPONENTS (PC) FOR SPRAGUE–DAWLEY AND FISCHER 344 RAT GROUPS

Parameters	Sprague–Dawley (n = 10)		Fischer 344 (n = 16)		Statistical inference*
	Mean ± SD	Coef. Var.	Mean ± SD	Coef. Var.	p =
T_{75}	21.1 ± 2.4	0.113	23.6 ± 2.1	0.090	0.0083
T_{50}	25.1 ± 2.2	0.089	26.1 ± 1.9	0.074	0.2219
T_{25}	28.0 ± 1.8	0.064	28.1 ± 2.3	0.082	0.8810
Δ_{50}	6.9 ± 1.6	0.239	4.5 ± 1.1	0.250	0.0001
T_{max}†	31.7 ± 2.9	0.093	30.2 ± 3.4	0.113	0.2588
R_0	$0.55 \times 10^{-3} \pm 0.57 \times 10^{-3}$	1.029	$0.048 \times 10^{-3} \pm 0.043 \times 10^{-3}$	0.893	0.0015
α	0.248 ± 0.044	0.179	0.351 ± 0.070	0.198	0.0003
PC1 scores	−0.298 ± 0.970		0.186 ± 1.003		0.2377
PC2 scores	0.853 ± 0.775		−0.533 ± 0.720		0.0001

*Unpaired two-tailed Student's t test.
†Absolute maximum survival to 36 months was observed both for Sprague–Dawley and Fischer 344 rats.
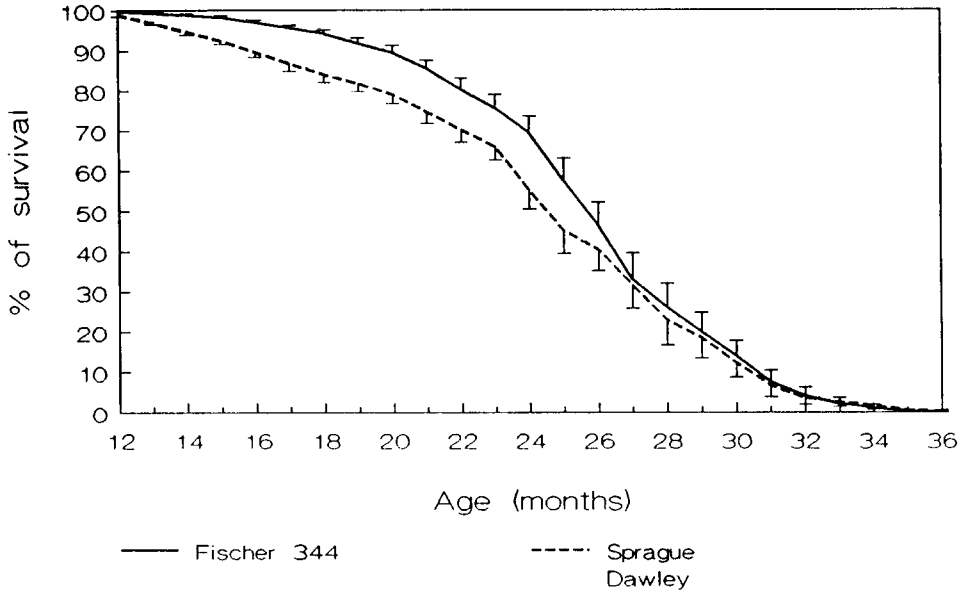
FIG. 1. Average survival curves for the two strains together with the standard errors at the individual ages.

ters (Table 1). Statistical analysis revealed a significant correlation between α values and PC2 scores ($r = -0.862$; $df = 24$; $t = -8.340$; $p < 0.0001$). This is consistent with the interpretation of both Δ_{50} and α values as the derivative of the survival curve. As for the initial mortality rate R_0, statistical analysis revealed a significant correlation between R_0 values and PC2 scores ($r = 0.709$; $df = 24$; $t = 4.924$; $p < 0.0001$), but not between R_0 values and PC1 scores ($r = -0.381$; $df = 24$; $t = -2.019$; NS). The correlation between the two parameters of the Gompertz curve is a well-known phenomenon (Riggs, 1991). However, it is important to consider that the dimensional scale of R_0 data from our sampling groups was very small and, moreover, highly variable.

Within-strain variability

The between-groups/within-strain variability of Fischer 344 and Sprague–Dawley was compared for each considered parameter (T_{75}, T_{50}, T_{25}, Δ_{50}, and T_{max}). *F*-tests did not reveal significant differences between Fischer 344 and Sprague–Dawley. The entity

TABLE 2. MATRIX OF FACTOR LOADINGS

	PC1	PC2
T_{75}	0.922	-0.369
T_{50}	0.971	-0.020
T_{25}	0.889	0.365
T_{max}	0.700	0.408
Δ_{50}	-0.265	0.946
% Explained variance	62.8	26.6

of the between-groups variability is biologically relevant and of the same size in the two strains. The between-groups variability is measured by the coefficients of variation relative to the different parameters (Table 1). The relevance of this variability is easily appreciable if we consider that T_{50} goes from 23.1 to 29.6 in the Fischer rats and from 22.7 to 28.9 in the Sprague–Dawley strain (Fig. 2). This variability has nothing to do with interindividual variability that is measured by the Δ_{50} and α values, where high values of Δ_{50} and low values of α correspond to high levels of interindividual variability. The interindividual variability is significantly lower in Fischer rats than in Sprague–Dawley. However, a striking result emerged: Fischer rats tended to modify their parameters as a function of their actual birth date. In fact, statistical analysis revealed a significant correlation between birth dates of groups and scores from PC1 ($r = -0.830$; $df = 14$; $t = -5.576$; $p < 0.0001$), but not between birth dates of groups and scores from PC2 ($r = -0.388$; $df = 14$; $t = -1.577$; NS). Consistent results were obtained with the PC1 and PC2 related parameters (T_{75}, T_{50}, T_{25}, and Δ_{50}, α), respectively.
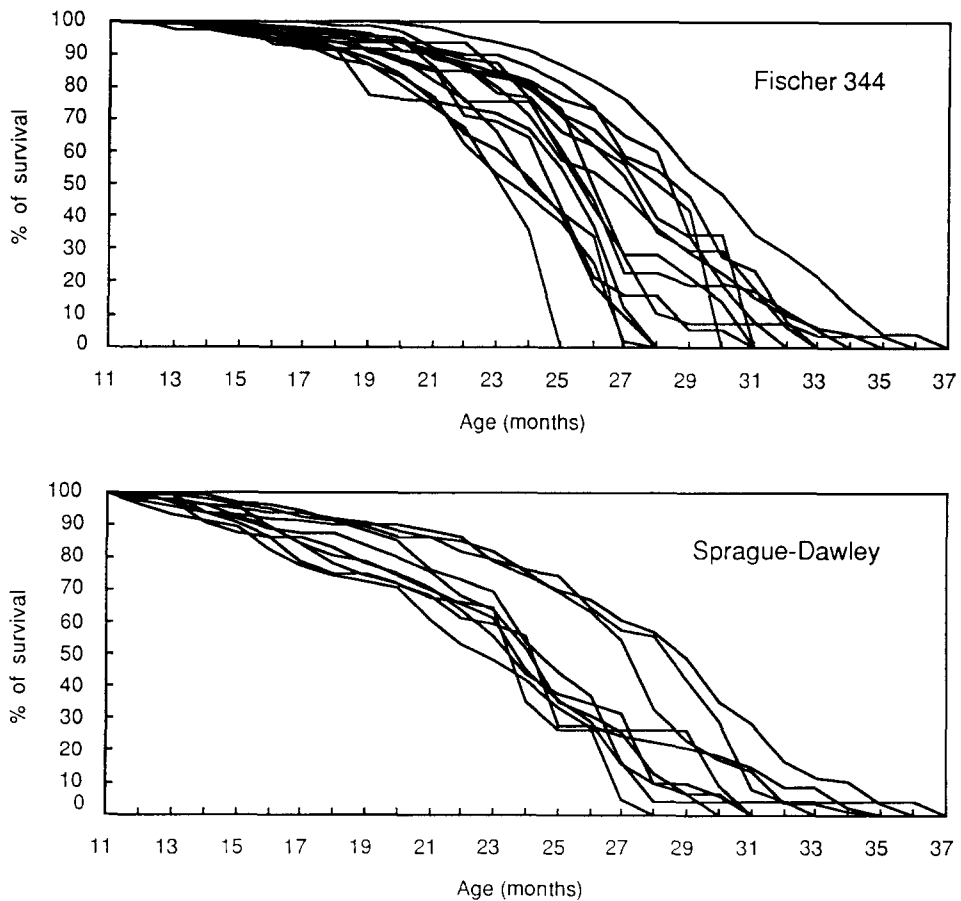

FIG. 2. Survival curves relative to the individual groups of Fischer 344 and Sprague–Dawley rats.

Regarding Sprague–Dawley rats, birth dates did not correlate significantly with PC1 scores and related parameters, nor with PC2 scores and related parameters. These results point to a general shifting over the years of the survival curves of Fischer 344 toward a decreasing life span, together with a maintenance of their slopes.

DISCUSSION

The overall results from the present study demonstrate that Fischer 344 and Sprague–Dawley rats maintained under the same environmental conditions have a similar life span. However, for Fischer 344, aging processes seem to begin later than Sprague–Dawley. The disposable soma theory predicts that life span is regulated through the efficiency of key maintenance processes (Kirkwood, 1992). The survival over the second year of life (in our case measured by T_{75}) was significantly higher for Fischer 344 than for Sprague–Dawley rats. This indicates that the efficiency of repair and homeostatic processes, as well as the good maintenance of physiological status, last longer for Fischer 344 than for Sprague–Dawley rats.

This work raises some problems of a methodological and a biological nature. From a methodological point of view it is worth noting that any nonlinear model, for example, the Gompertz equation of survival, implies a discrepancy between the model fitted to the average values (in our case the survival data averaged over all the groups) and the average of the individual Gompertz parameters (in our case the Gompertz equations relative to the individual groups). In our case the mean value of the α parameter (averaged over all the individual fittings) is 0.351 for the Fischer rats (see Table 1), while the α value deriving from the fitting of the average survival data (Fig. 1) is 0.293. The same discrepancy is observed for the other strain. The amount of this discrepancy depends on the between-groups variability: in this case, this variability is not negligible and implies substantially different estimates of the survival parameters (Fig. 2). It is important to stress that this behavior does not depend on the fitting of the Gompertz equation to real data because the same discrepancy can be observed in simulated curves too (data not shown).

The PCA approach, due to its model-free character, allows this problem to be overcome from the purely data analysis perspective. In any case the "biological" aspect of this problem persists, casting doubts on the reliability of the survival parameters estimates. The average values of the survival parameters we found in this study are in substantial agreement with the literature ones. Table 3 reports some literature data referring to animals maintained in environmental conditions comparable to ours.

When dealing with the issue of biological variability it is important to keep separate the hierarchical levels to which the observed variability pertains. There are three basic levels of variability that are in principle mutually independent: intraindividual variability, between-individuals variability, and between-populations variability. The first level deals with the temporal variability of physiological signals like EEG and EKG (Chialvo et al., 1990) relative to a single animal, the second level pertains to the differences between individuals of the same population and it is the most studied variability in biomedicine (Phelan and Austad, 1994), the last one is linked to differences between populations and it is the kind of variability mainly exploited by ecological studies.

The original contribution of this work consists, in our opinion, of the highlighting of a level of between-populations variability typical of species living in natural conditions but that is surprising in animals kept under strictly controlled conditions.

TABLE 3. REPORTED LONGEVITY DATA OF BARRIER MAINTAINED, AD LIBITUM FED, AND SOCIAL HOUSED MALE FISCHER 344 AND SPRAGUE–DAWLEY RATS

	Longevity Parameters (months)				
Strain	T_{75}	*median* (T_{50})	T_{25}	T_{max}	*Reference*
Fischer 344	23.7	26.1	28.1	30.1 oldest: 36	Sigma-Tau laboratories (Average 1986–1993)
	21.8	24.6	26.6	30.1	IFFA Credo, 1990
		29.0		35.0	Coleman *et al.*, 1977
		27.5			Sass *et al.*, 1975
		28.0			Hoffman, 1979
Sprague–Dawley	21.1	25.1	27.9	31.7 oldest: 36	Sigma-Tau laboratories (Average 1986–1993)
	22.0	26.5	30.5	36.0	IFFA Credo, 1990
		30.0			Adelmann *et al.*, 1978

The inbred or outbred character of the strain exerts its influence on the interindividual variability (Δ_{50} and α parameters) but has no visible influence on the between-populations variability.

Particularly in aging research, the choice of an inbred or outbred strain depends upon the desired level of variability between subjects. Keeping in mind that the equation inbred = low phenotypic variability can be misleading (Phelan and Austad, 1994), in any case the characteristics of biological material are assumed to be maintained strictly constant between different populations of the same strain (low between-populations variability). We purposely avoid discussions about the causal factors explaining our results: the main point of this article is not to explain why, but to measure how much different populations of the same strain can differ in their life expectancy. The populations we analyzed can be considered a typical sample of the biological material used in aging research: the animals were kept in SPF conditions by Charles River and by our Institute, they experienced the same dietary conditions throughout their life (during his life span a rat experiences at least 10 different food lots, so this source of variability cannot generate any systematic differences between populations). Other noncontrollable variations regarding holding sites, etc. during the 7 years of the overall duration of the study were randomly scattered among the populations: so it is extremely difficult (and perhaps without any real meaning) to emphasize one or more of these variations as causal factors of the observed variability. In any case, high variability is characteristic of the great majority of aging subjects and this must be kept in mind when comparing different studies.

In conclusion, in association with the standard genetic quality control currently adopted by the principal breeding laboratories (Charles River and IFFA Credo), the results from this study emphasize the necessity of keeping under control the nongenetic variability also to achieve a rigorous phenotypic quality control of the fundamental parameters linked to biological aging processes, such as longevity curves (see also Festing, 1993).

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