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AGE-DEPENDENT CHANGES IN MOBILITY AND SEPARATION OF THE NEMATODE *CAENORHABDITIS ELEGANS*

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INTRODUCTION

IT HAS generally been believed that a senescent state is brought about by the loss of division ability of essential cells or the disappearance of irreplaceable components.

Since aging shows considerable individual variations, it is difficult to pursue the problem in any single species. Analyses of population aging have been mainly done by measuring a decrease in population size. However, the decrease in population size involves not only senescent death but also death due to other changes, and it is not suitable for defining the aging process precisely. Therefore, the study on aging should be carried out in the populations with genetic and environmental similarity, and select several characteristics which are closely related to the senescent process.

The nematode *Caenorhabditis elegans* is easy to handle and it is possible to obtain many individuals with identical genetic background. Information on aging phenotypes of the nematode has been accumulated (Klass, 1977; Mitchell *et al.,* 1979). But in spite of their identical genetic background in synchronous cultures, individual differences in life span are conspicuous. The maximum life span of wild hermaphrodites at 20°C was double the minimum life span in *C. elegans.* Since aging reflects various biological histories following maturation, individual death has many causes. One way to identify sub-groups is to classify an aged population on the basis of other aging parameters. Previously one of the authors (R.H.) has reported behavioral changes accompanying aging of *C. elegans* (Hosono, 1978a, b). In this report, we classified the worm population on the basis of difference in movement activity and examined some aging parameters of these fractionated populations.

MATERIALS AND METHODS

Caenorhabditis elegans, var. Bristol, strain N2, was used throughout the experiments. Detailed methods for handling the nematode and its culture conditions were as described by Brenner (1974) except where otherwise noticed. The nematode was grown at 20°C in a Petri dish filled with nematode-growth (NG) agar containing a lawn of *Escherichia coli* as food. For synchronous cultures, young adults were allowed to lay eggs on fresh NG agar plates overnight. The plates were washed 7 times with M9 buffer to remove all worms; the eggs stuck to the agar. Larvae hatched for 2 h were collected by gently washing the plate with M9 buffer and centrifugation for 2 min at 2000 rev/min. The collected larvae were transferred to agar plates (3.5 cm diameter).

Individual worms were classified into 3 groups on the basis of movement activity; type I, type II, and type III (see under "Results"), and transferred to other plates using a tooth-pick.

For the examination of pumping activity of individual worms, a worm was put on the agar containing the bacterial lawn and the frequency of the pumping movement of the metacorpus was measured. To measure resistance of *C. elegans* to nile blue, the worms were incubated in either 0.1 or 1.0% dye solution ($C_{20}H_{20}ClN_3O$, Tokyo-kasei Co.) for 2 h at room temperature. After the suspension, the worms were washed with M9 buffer and viability was examined by inspection under a dissecting microscope.

RESULTS

To follow the viability of the hermaphrodite *C. elegans,* newly hatched larvae were grown on NG agar plates. A typical pattern of the survival curve is shown in Fig. 1a. The worms died between 6 and 23 days after the hatch with a mean life span of 13 days. The primary reason for this large variation among individuals is unknown. As a parameter of aging, the undulatory movement of worms on the agar was used; mobility was classified into three groups. Type I: worms progress with rhythmic sinusoidal movement. Type II: worms are able to progress, but their movement is irregular and not active. Type III: worms are unable to progress, but move their head or shrink their bodies in response to a gentle touch with a tooth-pick. Worms not responsive to the stimulus were scored as dead. The solid symbols in Fig. 1a represent the % distribution of type I, II, and III over the

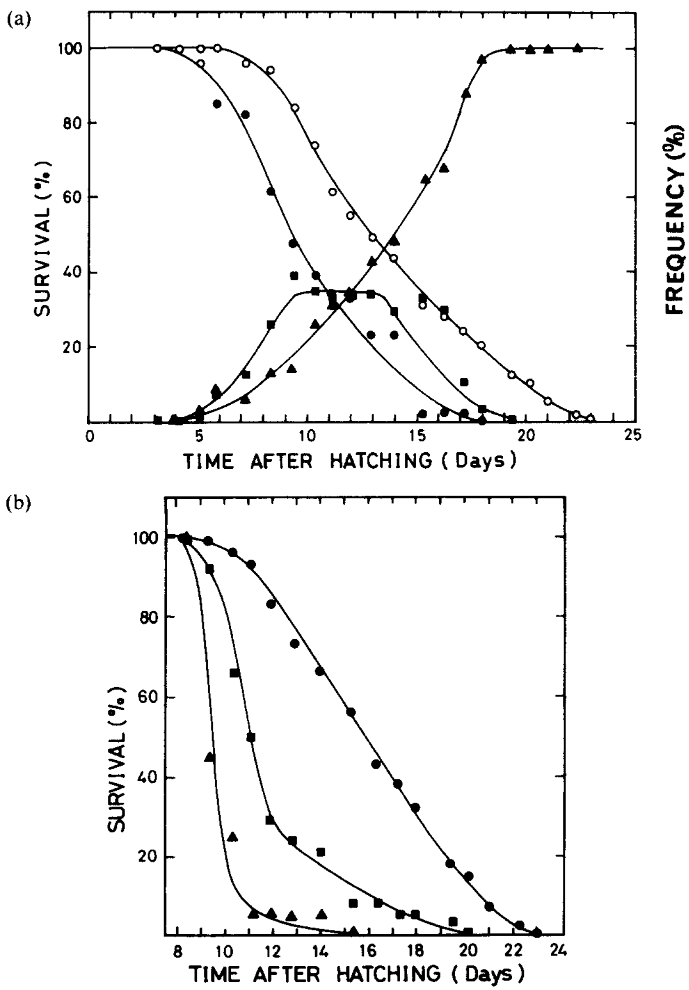

FIG 1. The survival curves of *C. elegans* at 20°C. (a) The nematodes were grown synchronously on NGM 3-cm Petri plates and transferred daily to fresh plates. Worms constituting the population were classified based on the movement activity. The open symbol (○) gives the survival of the whole population, and closed symbols give the frequency distribution of type I (●), type II (■) and type III (▲) worms over the total surviving worms. The starting number of worms tested was 200. (b) The residual survival curves of type I (●), type II (■) and type III (▲) worms from the 8-day old population. The number of type I, II and III worms were 20, 38 and 100 at the fractionation

total survival worms. It is obvious that the population of type I worms decreased almost parallel with and in advance of the decrease in the total viability, and that the senescent processes in the nematode progress in order of types I, II, and III.

In order to know the remaining life spans of each type, 8-day old worms were separated on the basis of their movement phenotype, and their viability were followed (Fig. 1b). The mean life spans of type I, II and III worms were 7–8, and 1–2 days, respectively. Thus, the mean duration of type II state was estimated to be 1–2 days.

TABLE 1. THE ACTIVITY OF THE SINUSOIDAL MOVEMENT OF AGED *C. ELEGANS*

	Frequency of sinusoidal waves (Number/30 s)	
	8-day old worms	11-day old worms
Type I	30.3 ± 6.2 (*n* = 12)	25.1 ± 8.0 (*n* = 9)
Type II	26.1 ± 4.6 (*n* = 11)	25.7 ± 6.6 (*n* = 11)
Type III	0 (*n* = 6)	0 (*n* = 12)

Worms were transferred from NG agar to M9 buffer and the frequency of the sinusoidal wave was counted under a dissecting microscope (Hosono, 1978b). *n*, number of worms assayed.

The physical movement mentioned above is closely related to the sinusoidal movement along the worm's body. In young adult nematodes (60 h in 20°C-growth), the frequency of the sinusoidal movement in liquid media was maximum (54/min); the frequency decreased with age (Hosono, 1978b). When the activity of the sinusoidal movement in three types of 8-day and 11-day old worms was compared, the frequencies of type I and II worms were approximately half of the maximum level, whereas no movement was observed in type III worms.

Properties of each movement phenotype were also compared with other criteria. *C. elegans* has a muscular pharynx consisting of two expansions, a metacorpus and a terminal bulb. After the larval stages, the worm sucks foods by rhythmic constrictions of the pharynx, called pumping movements, grinds them and sends them into the intestine. As seen in Fig. 2, the pumping frequency of pharynx increased up to 80 h after hatching

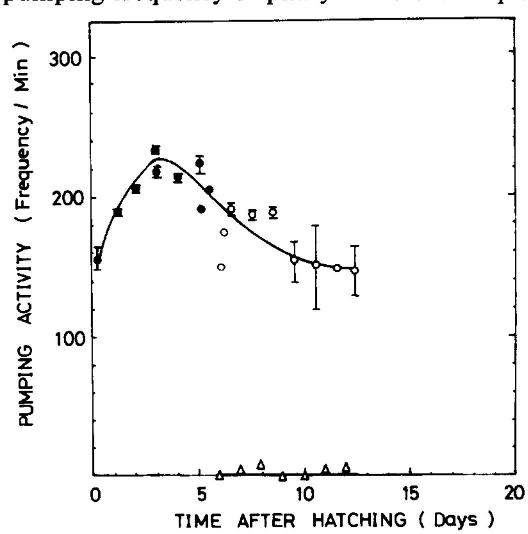

FIG. 2. Changes in pharyngeal pumping rate with age. ○; type I, △; type III worms. Differences in movement phenotype in larval and young adult age (●) were not observed. Each value represents a mean value from seven worms, and standard deviation is indicated by the vertical bar.

in parallel with growth. Although the pumping activity decreased thereafter, the frequency was maintained a level two-thirds of maximum in type I worms. Type II worms, however, lost their ability to move their pharynx.

For ecological study, several dyes have been used to distinguish dead from living nematodes (Ogiga and Estey, 1975). We found nile blue has a nematocidal effect. The effect was more severe in newly hatched larvae and aged lethargic worms than in healthy adult. The sensitivity of aged *C. elegans* to the dye is shown in Table 2. Type I worms were completely resistant when exposed to nile blue solution for 2 h. Type II was viable in 0.1% nile blue solution, but 20–30% of the type lost their viability in the 1.0% solution. All the type III worms were killed in the 1.0% nile blue solution.

TABLE 2. SENSITIVITY OF AGED *C. ELEGANS* TO NILE BLUE (NB)

		Percent survival	
		in 0.1% NB	in. 1.0% NB
8-day old	Type I	100	100
	Type II	100	70
	Type III	20	0
11-day old	Type I	100	100
	Type II	100	86
	Type III	11	0

On the criteria of sinusoidal activity and resistancy to nile blue, any distinction between type I and II was unclear but the distinction between type II and III was apparent, supporting the observation that aging progresses from type I to III in movement phenotype.

DISCUSSION

Senescence shows remarkable individual differences. One test for the study of senescent processes is to classify the population into groups with the same phenotype which age in the same way.

In the present study we have classified worms based on the difference in movement phenotype. As *C. elegans* locomotes by producing sinusoidal waves along its body, it is not difficult to discriminate worms moving abnormally. *C. elegans* becomes sluggish and decreases the frequency of sinusoidal propulsion with age, then gradually loses the ability to move rhythmically, and finally ceases to move at all. Mean life spans of type I, II and III worms were 7–8, 3–4, and 1–2 days, respectively and type I worms produced type II and type III worms with culture time, while type III worms died soon without producing type I and II worms. Therefore, these three types of worms are not the fixed subpopulations, and aging of nematodes seems to proceed in the order of type I, II and III. It is probable that many intermediate steps exist between type I and III. However, it is difficult to distinguish further differences based on the movement phenotype, unless other aging parameters are also used.

We have previously investigated developmental and aging changes of some behavioral parameters such as sinusoidal movement in liquid (Hosono, 1978b), locomotory movement on the agar plate and feeding which were measured by the incorporation of labelled *E. coli* into acid-insoluble fractions (Hosono, 1978a). These activities increased in parallel with the growth up to young adulthood corresponding to the mid-stage of egg-laying behavior, then decrease rapidly, while changes in movement became apparent at the end of the egg-laying stage. These processes of aging are probably not due to a stoppage of feeding behavior, because, as measured by pumping activity, both type I and most of the type II worms kept their ability to move their pharynx during these periods. Type III and some type II were not able to move their pharynx and these worms also showed a decline in resistance to nile blue.

The findings that parameters such as movement activity, pumping rate, frequency of sinusoidal waves and sensitivity to nile blue were correlated to the life spans of these fractionated populations suggests that these functions are good aging indices in nematodes.

Biochemical study of mass cultured nematodes does not give us precise information, since the population is comprised of worms heterogenous in terms of life span. Separation of worms into relatively age-homogenous populations enabled us to investigate the relationships between various aging indices and to characterize the aged worms with biochemical as well as morphological approaches.

SUMMARY

Easy classification and separation of aged *C. elegans* was performed on the basis of the difference in the movement activity; that is, sluggish but rhythmically locomoting worms (type I), worms that locomote but are irregular in movement (type II), and worms that are defective in locomotion but responsible for stimulus for touch (type III). The residual life span after the separation was also followed, in addition to the pumping frequency, the sinusoidal movement activity in the liquid and the resistancy to nile blue of each type. These parameters were well correlated to the length of life span of each type, and aging of the nematode seems to proceed in the order of type I, II and III.

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