DEVELOPMENTAL BIOLOGY 51, 23-33 (1976)

The Life Cycle of the Nematode *Caenorhabditis elegans*

I. Wild-Type Growth and Reproduction

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Accepted January 29, 1976

The growth and reproduction of the small nematode *Caenorhabditis elegans* has been studied using an electronic nematode counter recently developed in our laboratory. At 20°C, the usual growth temperature, size increases in a smooth sigmoidal manner with time, linear growth being most rapid around the time of the fourth molt and nearly ceasing by the end of the period of egg-laying. Growth of populations is highly synchronous; the small residual size heterogeneity is maximal at about the time of maximal growth. The four molts do not involve major interruption of growth, but they do entail slight shape changes (elongating upon escape from the old cuticle). Egg-laying begins shortly after the fourth molt, the rate rising rapidly at first, then more gradually to a peak followed by a relatively rapid fall. Comparable measurements at 16 and 25°C establish that these are acceptable limit temperatures for work with temperature-sensitive mutants, although egg yield is somewhat reduced and the kinetics of egg-laying are altered at 25°C. Developmental chronologies for all three temperatures are presented.

INTRODUCTION

The small free-living soil nematode *Caenorhabditis elegans* is especially suitable for genetic studies of development and behavior, due to its ease of culture, short life cycle, and normally hermaphroditic mode of reproduction. Brenner and others have isolated a large number of mutant strains of *C. elegans* which exhibit a wide range of morphological and behavioral modifications from the wild-type animal (Brenner, 1974; Ward, 1973; Epstein *et al.*, 1974; Dusenbery *et al.*, 1975; Hedgecock and Russell, 1975). In general these mutations also affect the growth and reproduction of the animal, and in studying the mutants it is often important to know with accuracy their growth and reproductive changes (for example, so that mutant and wild-type can be compared at the same developmental stage or so that both mutant and wild-type can be given adequate time to grow and be properly scored in the progeny of a genetic cross). In addition, accurate characterization of a mutant's growth and reproduction can put important constraints on the interpretation of its defects, as in the case of an apparently specific behavioral mutant that turns out to have reproductive defects as well.

To facilitate studies of growth and reproduction, we have developed a machine which can quickly count and measure the size of large numbers of *Caenorhabditis elegans* (Byerly *et al.*, 1975). This paper reports the results of studies conducted with this machine on the wild-type *C. elegans*. The accompanying paper shows how these studies can be enhanced by the use of computer analysis and how they can be applied to the study of mutant strains of *C. elegans*.

METHODS

Culture of nematodes. We used the N2 strain of *Caenorhabditis elegans*, originally obtained from Dr. Sydney Brenner. All nematodes were grown in petri dishes filled with an agar medium on which a lawn of *Escherichia coli* bacteria is grown (see Brenner, 1974, from which the methods are derived). The nematodes were gen-

erally transferred from one plate (petri dish) to another in the same standard solution (0.35% Na_2HPO_4, 0.15% KH_2PO_4 and 0.2% NaCl) used by the nematode counter. In studies of egg laying rates, the egg-laying animals were transferred instead on the tip of a bent hypodermic needle (transfer time, 5 sec) to avoid the disturbance of being suspended in solution for several minutes. Synchronous populations of nematodes were normally started by transferring adult animals to a plate, allowing them to lay eggs for 4 hr (2 hr for the hatching study), and then removing them by a gentle wash which leaves the eggs behind, stuck to the agar. These eggs were then allowed to hatch and the resulting animals allowed to grow for the desired length of time. In the fourth molt study, which required optimal synchronization, populations were obtained by washing off the newly hatched worms from a plate of hatching eggs that had been washed clean of animals 1 hr earlier. Populations which were to grow for several days before measuring were transferred to new plates as necessary to prevent exhaustion of the bacterial food supply. All ages are measured from the time at which the originating eggs are laid.

Sample processing. Nematodes to be measured were eluted from the agar plate in 10 ml of the standard solution and then washed twice by centrifugation before being run through the counter. To avoid any possible osmotic effects which the standard solution might have on the size of the animal, the preparation procedure was standardized so that all samples were run at a fixed time (~5 min) after being washed off the plate; adult nematodes repeatedly measured over a half-hour period showed no significant change in size. In studying growth, animals once run through the counter were not reused although they appeared healthy and unaffected by the measurement. In all counts, the final nematode suspension was adjusted so as to contain no more than 1500 large animals or 3000 small animals per milliliter; this reduces coincidences to less than 1% of count rate. In the standard elution method, eggs stick lightly to the plate and are not eluted; therefore, when eggs were to be measured (Fig. 1), the plate was scraped with a glass rod or pipet tip to dislodge them before elution.

Optically measured length. It is very difficult to measure the length of live nematodes optically due to their movement. For careful measurements the animals must be either photographed or immobilized in some way and measured directly. All length measurements given in this paper were taken after the nematodes had been killed and fixed by incubating at 60°C for 20 min. This treatment results in a straight, easily measured carcass; however, it can affect length, e.g., gravid adults lose eggs and sometimes a whole gonad after heat-killing. Preliminary tests indicated that the lengths of nematodes that had been heat-killed were quite close to the lengths of animals of the same sample that had been anesthetized with 1% 1-phenoxy-2-propanol. Also, heat-killed lengths were found to be proportional to the lengths measured from photographs of live nematodes throughout the first 3 days of development. The long tail of the nematode was included in the length measurements.

Pumping measurements. The percentage of animals which exhibited a pumping motion of the pharynx at any particular time was determined by examining 50 animals under the dissecting microscope. Each animal was observed until it pumped or until 5 sec has passed; animals which did not pump within 5 sec were considered not to be pumping.

RESULTS

Hatching

For hatching studies, synchronous ~20°C populations of eggs laid by adult nematodes over 2-hr periods were measured in the nematode counter (Byerly *et*

al., 1975) at various times after they were laid. Up until the time of hatching the eggs give an unchanging, narrow distribution of nematode counter signals (NCS) with a mean of 3.25 channels as shown in Fig. 1. Newly hatched nematodes, however, give somewhat smaller, more widely dispersed signals, with a mean of 2.90 channels (see Fig. 1). The difference allows hatching to be quantitatively monitored with the counter. In the "9-hr" population of Fig. 1 (from eggs laid 8–10 hr before), almost no young animals are present; subsequent populations contain increasing numbers of hatched animals until, in the "12-hr" population (from eggs laid 11–13 hr before) almost all the eggs have hatched to produce young animals. Therefore, at 20°C most of the eggs must hatch between 10 and 11 hr after being laid.

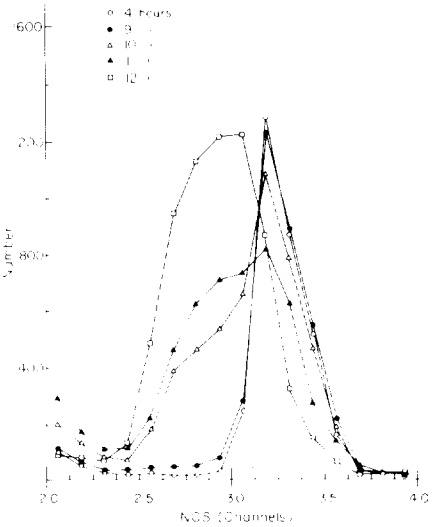

FIG. 1. Hatching of egg populations at 20 ± 0.5°C. Five samples of eggs (laid over 2-hr periods) were measured in the counter at times ranging from 4 to 12 hr after they were laid. Times are measured from the middle of each period. Mean NCS for eggs is 3.25 channels; the mean for newly hatched animals is 2.90 channels.

Growth

For growth studies, similar synchronous ~20°C populations were started from eggs laid over 4-hr periods, and the resulting nematodes were measured at various times after hatching. The size (NCS) distributions for eight typical synchronous populations, measured at various times throughout the life cycle, are shown in Fig. 2. In each case, the size distribution is reasonably narrow, indicating synchronous growth with no evidence of "laggards." Since synchronous populations almost always give roughly bell-shaped distributions like those shown, each has been characterized for comparative purposes by calculating its mean and spread (standard deviation).

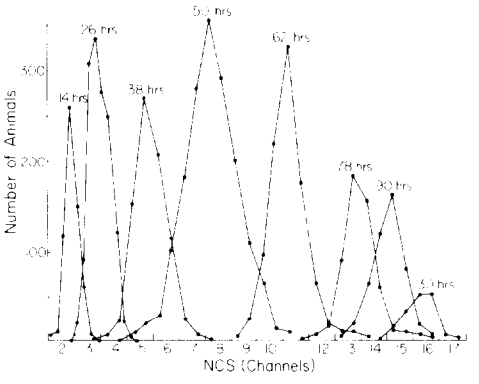

FIG. 2. Distributions of NCS for eight synchronous 20°C nematode populations measured at times ranging from 14 to 130 hr after egg-laying. The first three distributions actually involve numbers twice as large as indicated; the general trend toward smaller sample size as nematodes get larger is necessitated by the increasing difficulty of keeping nematodes well fed as their mass increases nearly 1000-fold. For comparison with Fig. 1, the channel labeled 2 includes NCS signals from 2.0 to 3.0, channel 3 those from 3.0 to 4.0, and so forth.

The increase of mean size with age is shown in Fig. 3A, together with previously obtained information (adjusted for a small difference in growth rate) on the timing of *C. elegans*' four cuticular molts (Cassada and Russell, 1975) and additional information on egg-laying, to be described in more detail below. The animals grow fairly slowly before the first molt; then growth accelerates, becoming maximal at about the time of the fourth molt or shortly thereafter; after egg-laying begins (at about 65 hr) growth begins to slow, dropping to a very low rate by the end of the

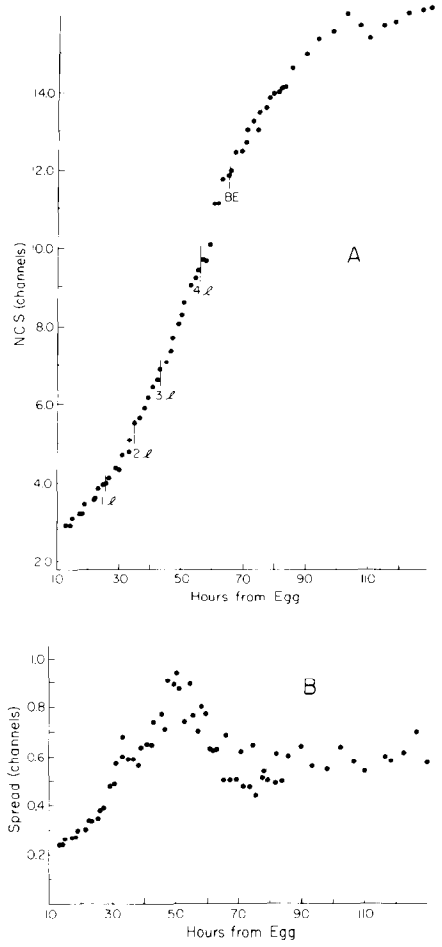

FIG. 3. (A) Growth at 19.5 ± 0.5°C as measured by the nematode counter. Each point indicates the mean size of one synchronous population, where age is calculated from the middle of the time the eggs were laid. Large enough populations have been measured each time so that statistical errors are always smaller than the expected systematic errors of 1%. The vertical lines labeled 1–4*l* indicate the times of maximum lethargus (minimal pumping) associated with the four molts. BE marks the beginning of egg-laying. (B) Population spread (standard deviation of the NCS distribution) as a function of age at 19.5 ± 0.5°C.

egg-laying period (at about 120–130 hr).

The dependence of population spread on age is shown in Fig. 3B. As might be expected, the spread is greatest at about the time when growth rate is maximal, i.e., at about 50 hr of age. The spread of the populations is not reduced by decreasing the period over which the initial eggs were laid; populations started from eggs laid over a 2-hr period have essentially the same spreads as do the populations started from 4-hr egg-laying periods (Fig. 3B).

Molting

From the relatively smooth shape of the growth curve in Fig. 3A, it appears that the four cuticular molts of *C. elegans* do not represent major growth discontinuities. However, some shape changes do appear to accompany molting, as can be seen by comparing NCS size measurements with optical measurements of length. In general, the two size measurements should be linearly related, since the nematode counter signal, originally based on nematode volume, is deliberately processed in an attempt to make the final signal proportional to length (Byerly *et al.*, 1975). That the relationship is indeed very nearly linear is shown in Fig. 4. In Fig. 4A is a growth curve analogous to that of Fig. 3A, except that length, rather than NCS, is used as the index of size; although length measurements are more laborious and have larger errors, the two growth curves correspond quite closely. In Fig. 4B, NCS and length are plotted against one another to show the nearly linear relationship directly. The deviations from linearity in Fig. 4B, although small, are significant and therefore indicate real shape changes during growth. These changes appear to be correlated in time with the four molts, indicated by arrows in the figure. To investigate the apparent correlation more closely, especially well-synchronized populations were prepared (as described in Methods) and followed through the fourth molt; Fig. 5A shows how NCS and length varied as these animals passed through the lethargus period (indicated by reduced pumping) and then shed their old cuticles. The nematode counter signal, derived primarily from the animal's volume, leveled off quite noticeably during the molt (probably due to reduced feeding) and then re-

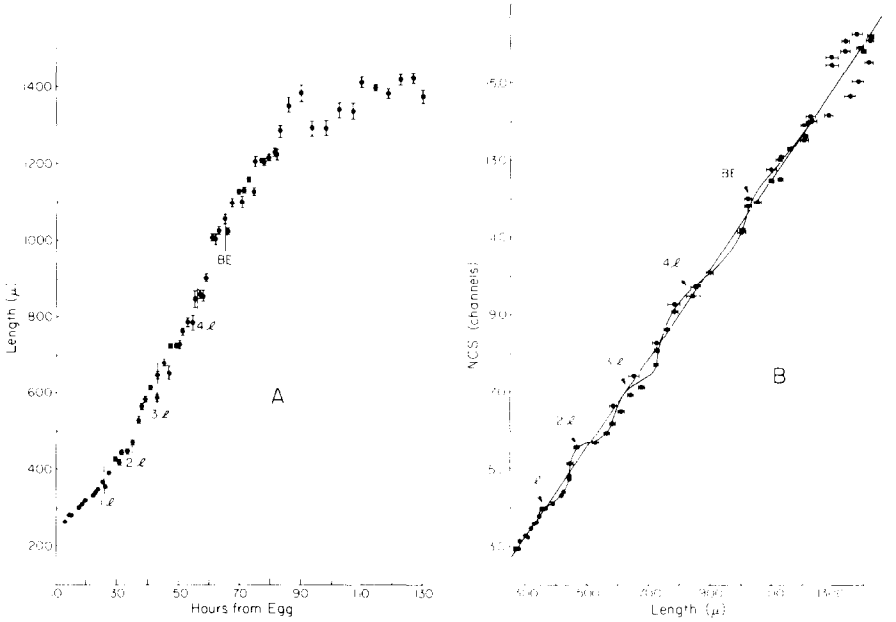

FIG. 4. (A) Growth at 19.5 ± 0.5°C as measured by length. Each point is the mean of the lengths of 16 nematodes. The indicated errors are statistical errors; 1-4*l* and BE have the same meaning as in Fig. 3A. (B) Relation between NCS and optically measured length of nematodes. The straight line drawn through the data demonstrates the basically linear relation between NCS and length. The curvy line shows that the deviations from linearity correlate with the molting events indicated by 1-4*l*. BE marks the beginning of egg-laying.

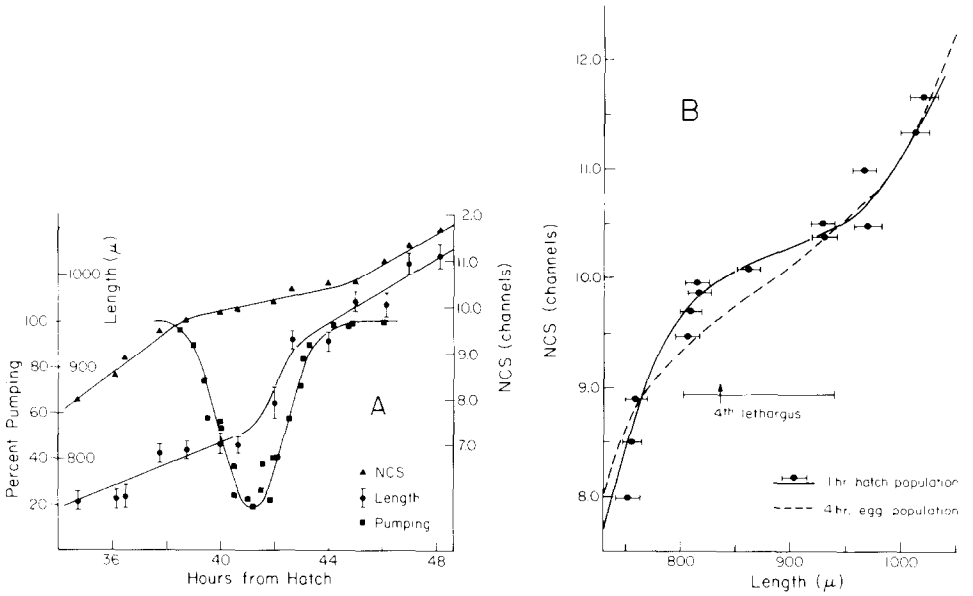

FIG. 5. (A) Pumping, NCS, and length as functions of age through the fourth molt. Populations synchronized by taking animals that hatch in a 1-hr period were used for this study. Ages are measured from the middle of the hatch period. These populations were maintained at 20.0 ± 0.3°C. (B) Relation of NCS to length at fourth molt. Data is the same as that in Fig. 5A. The solid line is drawn by eye through the data of this experiment, which comes from populations which hatched over a 1-hr period. The dashed line is the corresponding part of the curvy line in Fig. 4B, which came from populations started from eggs laid over 4-hr periods. The arrow marks the length at which the pumping was minimal, and the interval about the arrow indicates the range of lengths measured during the fourth lethargus.

sumed its former rate of rise. The length, however, continued to increase during the molt, showing a rather sharp jump when the animals resumed pumping at the end of lethargus (just when cuticular shedding has been observed in other experiments). As a consequence, the relationship between NCS and length changed quite significantly during the molt, as shown in Fig. 5B. The simplest interpretation appears to be that the animals elongate during the molt, and that a major part of the elongation occurs as they escape the confines of the old cuticle. To determine just how much linear expansion a cuticle might be capable of, we calculated for each juvenile stage and for the adult, the minimum (beginning) size, the maximum (final) size, and their ratio, as shown in Table 1. Although there is certainly some variation in the ratios, the four molts do appear to divide the *C. elegans* life span into periods with roughly equal ratios of linear growth. Whether this reflects some inherent limitation to the linear expansion of cuticles remains to be seen.

TABLE 1

SIZE INCREASES OF VARIOUS DEVELOPMENTAL STAGES[a]

Developmental stage	Minimal size (NCS)	Maximal size (NCS)	Ratio: maximal to minimal
First juvenile	2.9	4.1	1.41
Second juvenile	4.1	5.6	1.37
Third juvenile	5.6	7.0	1.25
Fourth juvenile	7.0	9.6	1.37
Adult	9.6	16.0	1.67

[a] The sizes given are averaged for growth at 16, 20, and 25°C.

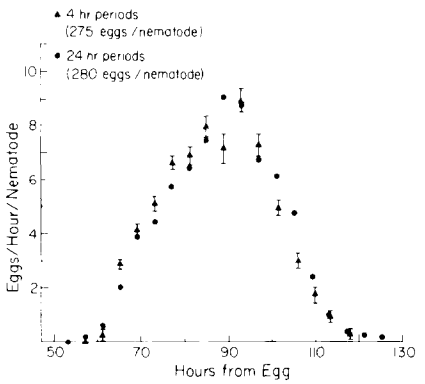

FIG. 6. Rate of egg-laying as a function of age. Each point indicates the average egg-laying rate for a 4-hr period centered about the time of the point. The triangles are derived from experiments where the layers were transferred to new plates every 4 hr. The circles are from experiments where the layers were transferred to new plates every 24 hr, and a computer fit was used to infer the average egg-laying over each of the six 4-hr periods. Both experiments were done at 19.8 ± 0.3°C, but at different times. The error bars on the 4-hr period data are calculated from the variation between the two copies done of this experiment.

Egg-Laying

C. elegans begins laying eggs a number of hours after the fourth molt. To determine how the egg-laying rate varies with the age of the adult, we transferred 10 synchronous animals from plate to plate every 4 hr at 20°C. The young animals that hatched on each plate were counted a day or two later on the nematode counter to determine the number of fertile eggs that had been laid in that 4-hr period. The triangles in Fig. 6 show the egg-laying rates determined by this method. Egg-laying begins shortly after 60 hr, climbs quickly to a rate of four eggs per hr and then rises more slowly until it reaches a maximum of about nine eggs per hr at 92 hr; it then drops sharply, and effectively ends at 120 hr. On the average, each nematode lays a total of 275 eggs.

An easier method of measuring egg-laying rates should be possible, according to the following considerations. If eggs are laid over an extended period (e.g., 24 hr) and then the resulting animals are allowed to grow for a known time, the NCS distribution of the resulting population should reflect not only how many eggs are laid during the entire period, but also how the egg-laying rate varied over the period. In brief, the eggs laid at earlier times are represented by the larger animals, while the smaller animals represent the eggs laid toward the end of the period.

The observed NCS distribution should therefore be convertible into an egg-laying rate curve for the original laying period, provided that one takes into account the known growth rate and population spread characteristics of young animals, as previously measured. In practice we have found this to be most effectively accomplished by the following computerized matching procedure. Given (a) the NCS distribution of nematodes hatching from eggs laid over a 4-hr period, and (b) how the spread (standard deviation) and mean of such a distribution change with time, a computer can quickly generate the summed NCS distribution expected for animals hatching from eggs laid over a number of successive 4-hr periods. By varying the rates of egg-laying for each period independently, different summed distributions can be generated and compared with the observed distribution, to obtain the best possible fit. The rates which generate the best-fit distribution should then correspond to the 4-hr rates which would be measured directly.

To test the validity of this approach, we allowed adults of various ages to lay eggs over 24-hr intervals, and then used the computerized matching procedure to convert the resulting NCS distributions into egg-laying rates for the six 4-hr periods within each interval. The filled circles of Fig. 6 show the egg-laying rates determined in this way; they agree quite well with the rates determined by the more directly determined method. In view of this agreement, we have used the second method in most subsequent studies of egg-laying.

The Effects of Temperature

Considerable interest exists in using temperature-sensitive mutants of *C. elegans* to probe behavior and development. Accordingly it becomes important to know how the life cycle of normal *C. elegans* varies with temperature. Practical experience in our laboratory has led to the choice of 16 and 25°C as the convenient lower and upper limits, respectively, for work with *C. elegans*. Experiments similar to most of those described above have also been performed at 16 and 25°C to determine how well the growth parameters measured at the usual 20°C growth temperature can serve to describe growth at these temperature extremes. The results are summarized in Table 2 and described in more detail below.

Growth curves at ~16 and ~25°C are presented in Fig. 7, along with the standard ~20°C growth curve for comparison. The general shapes appear similar in all three cases, with the exception that the 16 and 25°C animals apparently do not get quite as large as the 20°C ones. To test the similarity more rigorously, the 20°C growth curve was fitted to the 16 and 25°C data, using a computer fitting method which preserved the shape of the curve but allowed scale changes of time and size. The original 20°C growth curve is drawn through the ~20°C data points of Fig. 7, and 16 and 25°C fitted versions of it are drawn through the respective data points. The fits appear quite good, suggesting that general growth characteristics are quite similar over the entire temperature range. From the fitted scale changes, indicated next to the curves, growth is 53% more rapid at 25.0°C and 21% slower at 16.1 than at 19.5°C; these values fit well an exponential dependence on temperature with a Q_{10} of 2.1.

Molting times and sizes were also measured at 16 and 25°C, and are indicated in Fig. 7. Although the 25°C animals may molt at very slightly larger sizes, the overall pattern is clear; molting occurs at very nearly the same sizes at all temperatures. (The possible occurrence of molting-associated shape changes at 16 and 25°C has not been investigated.)

In contrast to overall growth and molting, egg-laying shows significant differences as a function of temperature. Egg-laying curves for 16 and 25°C are presented in Fig. 8, together with the ~20°C curve

TABLE 2

DEVELOPMENT AT DIFFERENT TEMPERATURES[a]

	"16°C" (16.0 ± 0.3°C)	"20°C" (19.5 ± 0.5°C)	"25°C" (25.0 ± 0.2°C)
Egg laid	0 hr	0 hr	0 hr
Egg hatches	16–18 hr	10–12 hr	8–9 hr
First-molt lethargus	36.5 hr	26 hr	18.0 hr
Second-molt lethargus	48 hr	34.5 hr	25.5 hr
Third-molt lethargus	60.0 hr	43.5 hr	31 hr
Fourth-molt lethargus	75 hr	56 hr	39 hr
Egg-laying begins	~90 hr	~65 hr*	~47 hr
Egg-laying maximal	~140 hr	~96 hr*	~62 hr
Egg-laying ends	~180 hr	~128 hr*	~88 hr
DFR for growth	0.79	1.00	1.53
Length at first molt	360 μm	370 μm	380 μm
Length at second molt	490 μm	480 μm	510 μm
Length at third molt	650 μm	640 μm	620 μm
Length at fourth molt	900 μm	850 μm	940 μm
Length at egg-laying onset	1150 μm	1060 μm	1110 μm
SIF	0.94	1.00	0.96
Maximal egg-laying rate	5.4/hr	9.1/hr	8.1/hr
Total eggs laid	275	280	170
DRF for egg-laying	0.66	1.00	1.33

[a] For ease of reference the temperatures are called 16, 20, and 25°C; the measured means and ranges are given in parentheses below. Unintentional slight variations in temperature sometimes occur and can influence the results; for example, the mean temperature during the measurement of "20°C" growth was 19.5°C, whereas that during the measurement of "20°C" egg-laying was 19.8°C. The size factor SIF is the size scale change applied to the 20°C growth curve to achieve the best fit to the growth curve at the indicated temperature; the developmental rate factors "DRF for growth" and "DRF for egg-laying" are the time scale changes applied to the respective curves to achieve best fits. Both types of factors bear a direct relationship to the analogous factors described in the accompanying paper (Byerly *et al.*, 1976). The data values marked by an asterisk (*) were collected from cultures grown at 19.8 ± 0.3°C and adjusted slightly (by reference to known events in the life cycle) to provide 19.5°C values.

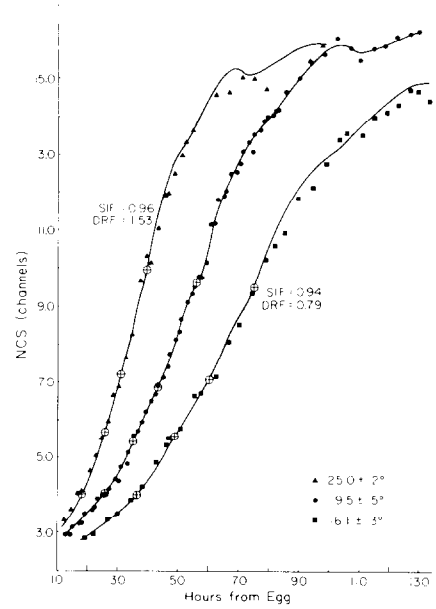

FIG. 7. Growth at 16, 20, and 25°C as measured by the nematode counter. Conditions as in Fig. 3A. The curves drawn through the 16 and 25°C data are the best fits derived from the 20°C curve by a computer fitting procedure which preserved the shape of the curve but allowed scale changes of size and developmental rate. The SIF and DRF values beside each curve indicate the scale changes which gave these best fits. ⊕ Indicates a molting period.

for comparison. While the overall shapes are somewhat similar, there appear to be discrepancies as well. These discrepancies become quite apparent when attempts are made to fit the 16 and 25°C data, using the 20°C curve as a model. The best computer-generated fits are drawn through the respective data points; for 16°C the fit is reasonable, but for 25°C the fit is clearly unsatisfactory.

Moreover, the total egg yield is markedly reduced at 25°C (see Table 2), and the overall kinetics of egg-laying, unlike those of growth, do not show an exponential dependence on temperature. In short, egg-laying is more sensitive to high temperatures than is general growth.

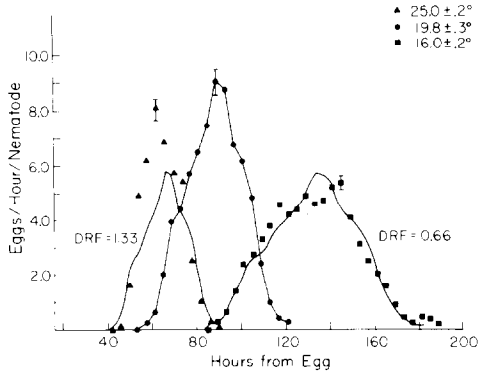

FIG. 8. Egg-laying at 16, 20, and 25°C. Parental animals were transferred every 24 hr, as in the second method of Fig. 6. The curves drawn through the 16 and 25°C data are the best fits derived from the 20°C curve by a computer fitting procedure like that of Fig. 7. The DRF values have the same meaning as in Fig. 7. The error bars drawn on the highest point of each curve show the 5% error that is estimated from the uncertainty of the fitting procedure and variation in the data. The error in low egg-laying rates is probably about 0.2 eggs per hr per nematode.

Population spread characteristics also show a temperature dependence which may be related to that of egg laying. Figure 9 shows the spreads of 16, 20, and 25°C populations, plotted as a function of size to facilitate comparison at similar developmental stages. The 16 and 20°C results are virtually identical; in both cases the spreads are maximal at or near the time of maximum growth, and drop thereafter as the animals enter the egg-laying period. At 25°C, not only do the spreads rise to a greater maximum, but they remain high throughout the subsequent egg-laying period.

DISCUSSION

The features of the *C. elegans* life cycle described above conform to the general nematode pattern (Bird, 1971, p. 80) in the sense that the growth curve is sigmoidal and relatively uninterrupted by the four cuticular molts. To our knowledge no previous growth or reproductive data have been published for *C. elegans*, but a growth curve (without data points) has been published for the closely related species *Caenorhabditis briggsae*, growing at 20°C in a rich but axenic liquid culture medium (Jantunen, 1964). The shape of the 20°C *C. elegans* growth curve corresponds very closely to that reported for *C. briggsae*, and the sizes of *C. elegans* at its molts are only slightly larger than those of *C. briggsae* (see Table 3). The most notable differences between the two curves are an approximately 15% faster growth rate

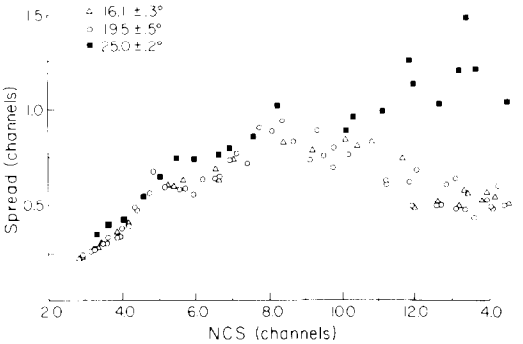

FIG. 9. Population spread as a function of size at 16, 20, and 25°C. For comparative purposes the spread is plotted against size, rather than against age, as in Fig. 3B.

TABLE 3

COMPARATIVE GROWTH OF *C. elegans* AND *C. briggsae*[a]

	C. elegans	*C. briggsae*
First-molt lethargus	15.0 hr	9.5 hr
Second-molt lethargus	23.5 hr	26 hr
Third-molt lethargus	32.5 hr	40 hr
Fourth-molt lethargus	45.0 hr	50 hr
Egg-laying begins	~54.0 hr	65 hr
Length at first molt	370 μm	315 μm
Length at second molt	480 μm	445 μm
Length at third molt	640 μm	605 μm
Length at fourth molt	850 μm	745 μm
Length at egg-laying onset	1060 μm	1050 μm

[a] Data for *C. elegans* are from this paper; data for *C. briggsae* are from the curve of Jantunen (1964). Times are to the nearest 0.5 hr, measured from hatching; lengths are to the nearest 5 μm. Both measurements are at or near 20°C, but the *C. briggsae* growth is in axenic liquid medium, while the *C. elegans* growth is on agar plates with bacteria present.

for *C. elegans*, and a difference in the lag between the fourth molt and the onset of egg laying (9 hr for *C. elegans*, 15 hr for *C. briggsae*); these differences may stem from the different growth conditions used.

The elongation observed at the molt in *C. elegans*, although not previously reported, agrees well with structural studies of molting in other species (see Bird, 1971, pp. 84–86). In these, the new cuticle laid down under the old is longitudinally buckled, so that an abrupt longitudinal extension after release from the old cuticle seems very likely. A possible consequence of the buckling is that once the new cuticle is completed, a simple increase in internal pressure might exert selectively longitudinal forces that could aid in separating the anterior cap of the old cuticle from its bulk portion.

From the longitudinal spacing of structural elements observed in new and old cuticles of other species (Bird, 1971, p. 87), it appears that these cuticles might be capable of longitudinal extension, up to a point, without the insertion of new elements. In fact, the relative length increases inferred from these observations agree fairly well with the relative length increases observed in *C. elegans* (Table 1), suggesting that this might be the mechanism of longitudinal cuticular growth in *C. elegans*. However, it should also be pointed out that other modes of nematode cuticle growth must surely occur, since some large animal parasites undergo very much greater relative increases within the adult cuticle (Watson, 1965).

The studies of *C. elegans* growth at 16 and 25°C establish that these are acceptable temperature extremes for the study of temperature-sensitive mutants, although the reduced egg yield, altered egg-laying curve, and greater population spread at 25°C make it clear that this temperature should not be exceeded. The temperature studies also point out the differing temperature dependencies of growth and egg-laying and thereby emphasize the point that these two phases of the life cycle are limited by different constraints. The transition between these phases is of some interest; from the growth curve it is clear that growth slows down shortly after the onset of egg-laying, as would be expected if the animal had relatively constant biosynthetic capacities which are being diverted from growth into egg-laying. The accuracy of this description is attested to by the calculations presented in Fig. 10; these make it clear that the fractional increase in mass generated by an animal (the combined increase in its own mass plus the mass of its eggs) remains relatively constant at 7.1–9.7% per hr throughout the period when the animal converts from pure growth to almost pure egg-laying. The diversion of biosynthetic capacity from growth to egg-laying is not particularly abrupt (except in the sense that the egg-laying rate shows an initial rapid rise) nor is it absolute, since even at the height of egg-laying more than 10% of the observed mass increase is due to growth. However, it does appear irreversible, since growth does not resume at a high rate at the end of egg-laying.

The transition which occurs at the end of

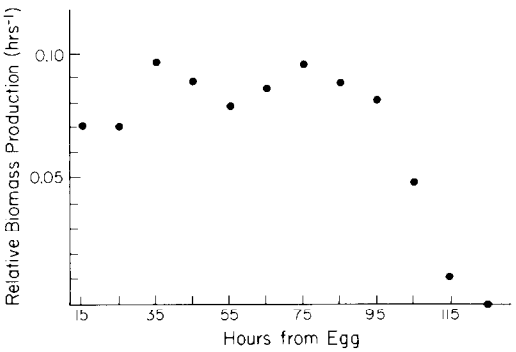

FIG. 10. Relative rate of biomass production as a function of age for nematodes at 20°C. Biomass production includes eggs laid and body growth. For this calculation the mass of an egg is estimated to be 0.035 μg and the animal's mass is assumed proportional to the cube of its length with a 1400-μm nematode having a mass of 4.3 μg.

egg-laying appears somewhat more abrupt, whether examined as in Fig. 10 or more directly by egg-laying rate as in Fig. 8. The most likely explanation for this abruptness is the exhaustion of sperm, and indeed it is possible to stimulate "exhausted" hermaphrodites to produce more progeny by mating them with males, who provide additional sperm (C. D. Johnson, unpublished results). Nonetheless, in the normal case the end of egg-laying marks the onset of apparent degenerative changes (darkening, loss of internal resolution, loss of cuticular resiliency) and with respect to the onset of this "aging" it would be of interest to know whether metabolic rate drops at this time and whether the changes can be delayed if reproduction is prolonged by mating.

We thank Carol Vermaes and Margot Szalay for providing materials, and Stewart Scherer for help in the computer fittings. This work was supported by NIH postdoctoral fellowships to L.B. (1 F02 GM55347) and R.C.C. (1 F02 NS51610), and by grants from the Sloan Foundation and the USPHS (NS09654) to R.L.R.

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