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Comparative demography of isogenic populations of *Caenorhabditis elegans*

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Abstract

Demographic characteristics of the bacterial-feeding nematode *Caenorhabditis elegans* were determined in two long-lived mutant strains, TJ1052 (*age*-1), CB4876 (*clk*-1), and a wild-type strain, N2. Within each strain, there was little correlation between longevity and reproduction for individuals that lived longer than 10 days. Long-lived mutant strains produced fewer eggs than the wild type. Mean total life spans were 13.2 days for the wild type, 21.9 days for *age*-1, and 15.8 days for *clk*-1; maximum life spans were 24 days for the wild type, 47 days for *age*-1, and 32 days for *clk*-1. Differences in total life span resulted primarily from longer post-reproductive survival. The mean post-reproductive life spans were longer than the wild type by 183% in *age*-1 and 60% in *clk*-1. We conclude that (i) post-reproductive survival is not correlated with egg production within isogenic populations of *C. elegans*, and (ii) the relationship between reproduction and longevity differs among isogenic populations with specific longevity genes.

Keywords: Bacterial-feeding nematode; *Caenorhabditis elegans*; Demography; Life span; Longevity genes; Reproduction

1. Introduction

The bacterial-feeding nematode *Caenorhabditis elegans* is an important model in biogerontology with many longevity mutants having been reported and described (Kenyon, 1997; Murakami and Johnson, 1998; Wood, 1998; Vanfleteren and Braeckman, 1999). Strains that carry longevity mutations in genes that regulate two different pathways have been well characterized (Dorman et al., 1995; Johnson, 1990; Kenyon et al., 1993; Lakowski and Hekimi, 1996; Wong et al., 1995). In the *age*-1 mutant, an insulin-like

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signaling pathway affects both longevity and reproduction (Tissenbaum and Ruvkun, 1997). In *clk*-1 mutants, a single gene appears to extend lifespan by altering both developmental and behavioral timing. Individuals exhibit slower development, slower movement, and depressed rates of pharyngeal pumping and digestive function (Ewbank et al., 1997; Lakowski and Hekimi, 1996; Van Voorhies and Ward, 1999).

Detailed comparative demographic analyses are lacking for strains with extended life spans although there are data on survival and brood size for individual strains (Dorman et al., 1995; Friedman and Johnson, 1988; Larsen et al., 1995; Wong et al., 1995). In this paper, we report the survival, egg production, and life table parameters of three *C. elegans* genotypes, the wild type and two mutant strains in which lifespan is extended through changes in different pathways. Our objectives were to compare the following characteristics of *age*-1, *clk*-1 and wild-type strains: (1) the relationship between reproduction and longevity for hermaphrodites within isogenic populations; (2) lifetime egg production; and (3) post-reproductive life span of hermaphrodites.

2. Methods

2.1. Experimental cohorts

Three *C. elegans* strains and *Escherichia coli* strain OP50 were obtained from genetic stocks maintained at the *Caenorhabditis* Genetics Center (University of Minnesota, St. Paul). The strains were: (1) Bristol N2 of the wild type; (2) TJ1052 of genotype *age*-1 (*hx*546) II; and (3) CB4876 of genotype *clk*-1 (*e*2519) III. *C. elegans* was grown on a hardened nematode growth medium (NGM) agar surface with a limited supply of *E. coli* as food (Brenner, 1974). NGM (9 ml) was pipetted into each petri dish (Fisher, 60 × 15 mm), seeded with *E. coli* and stored overnight at room temperature before nematodes were added. A single newly hatched juvenile was placed in each petri dish. Adults were transferred each day during the reproductive period to new dishes of NGM seeded with *E. coli.* Platinum wire, trimmed into a microspatula and flame-sterilized between transfers, was used to move the adults. The petri dishes were incubated in the dark at 20°C. During the course of the experiment, about 2% of the worms were either accidentally killed or died due to internal egg hatching. They were excluded from subsequent data analyses and the final cohort size was 48 individuals of each genotype.

2.2. Data collection and analysis

Nematode survival was monitored daily. Daily nematode fecundity was determined by counting the number of vermiform nematodes 24 h after transfer of cohort adults. Cessation of egg production signaled the beginning of the post-reproductive period. Immobile nematodes were considered dead if they did not respond when the medium was disturbed and then did not respond when touched. Daily egg production of individuals, and cohort survival, were plotted as color-coded event-history graphs (Carey et al., 1998a). Demographic analyses, including computation of life schedule, life expectancy, life endurance, life span, gross and net reproductive rate, expectation of reproduction, age-specific reproduction, followed Carey (1993, 1995), Carey et al. (1998b) and Ferris et al. (1996).

3. Results

3.1. Event-history graphs

Age at first egg production was consistent among and within *C. elegans* strains (Fig. 1) with the "reproductive window" spanning days 3–10. Patterns of egg production were similar both within and among *C. elegans* strains with highest production periods generally occurring during the first three days of egg production. There were more instances of high-production of eggs among individuals of the wild-type strain than among individuals of the long-lived mutants.

Survival rates of the three isogenic populations were similar during the first seven days. Strain survival rates diverged after cessation of egg production. We measured "worm-days" as areas under the population survival curve. The proportion of total worm-days for which there was no egg production was 71% for *age*-1, 64% for *clk*-1, and 52% for the wild type (Fig. 1). The *age*-1 mutant exhibited the longest post-reproductive life span and the wild type exhibited the shortest. The differences in post-reproductive survival accounted for the differences in total life span among strains.

3.2. Characterization of adult life-span extension

Mean total life span was 21.9 days for *age*-1, 15.8 days for *clk*-1, and 13.2 days for wild-type nematodes (Table 1). Mean post-reproductive life span was greater than that of the wild type by 183% in *age*-1 and 60% in *clk*-1. Post-embryonic survival of longest-lived individuals was 47 days in *age*-1, 32 days in *clk*-1, and 24 days in the wild type (Table 1, Fig. 1). There was little difference in the age of first or last reproduction among *C. elegans* strains. Expectation of life (e_x) at the beginning of egg production was greater than that of the wild type by 8.7 days in *age*-1 and 2.6 days in *clk*-1. The shortest life expectancy at the beginning of both reproductive and post-reproductive periods occurred in the wild type; the longest in *age*-1. The longevity of 50% of individuals in a cohort ($l_x = 0.5$) was 18 days for *age*-1, 15 days for *clk*-1 and 12 days for the wild type (Table 1). The longevity of 10% of individuals ($l_x = 0.1$) was 40 days for *age*-1, 24 days for *clk*-1 and 19 days for the wild type.

3.3. Comparison of longevity and reproduction among strains

Average number of eggs produced per adult per day, and both gross and net reproductive rates of wild-type *C. elegans*, were higher than in the two mutant strains (Table 2). Number of progeny of all strains peaked on day 4, although *age*-1 produced a greater proportion of its total progeny on day 3 and late in the reproductive period than the other strains (Fig. 2). After day 3, expectation of reproduction in *age*-1 was lower than in the wild type (Table 2). In *age*-1, a large percentage of nematodes produced a small brood ($\leqslant$200 eggs), compared to other genotypes, and fewer nematodes a large brood ($\geqslant$201 eggs). Net reproductive rate was lower than that of the wild type by 47% in *age*-1 and 35% in *clk*-1 (Table 2).

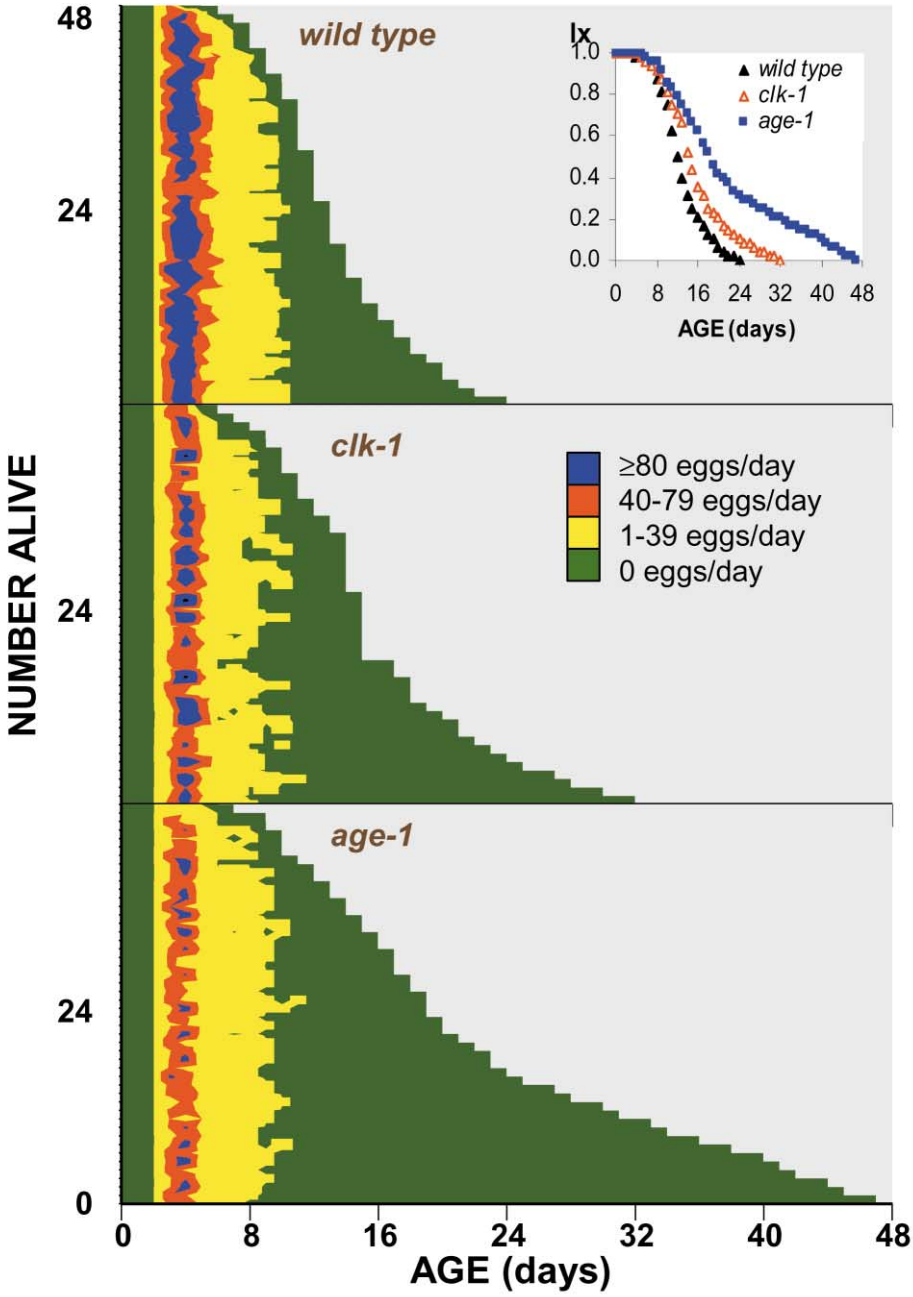
wild type
clk-1
age-1
lx
1.0
0.8
0.6
0.4
0.2
0.0
0 8 16 24 32 40 48
AGE (days)
≥80 eggs/day
40-79 eggs/day
1-39 eggs/day
0 eggs/day
NUMBER ALIVE
48
24
24
24
0
0 8 16 24 32 40 48
AGE (days)

Table 1
Survival and life span (days) of wild-type *C. elegans* and its mutants, *age*-1 and *clk*-1

	Genotypes		
Life history trait	Wild type	*clk*-1	*age*-1
Schedule			
First reproduction	3	3	3
Last reproduction	8.4 ± 0.2[a]	8.0 ± 0.3	8.3 ± 0.2
Oldest worm	24	32	47
Expectation of life at age x (e_x)			
e_0	12.7	15.3	21.4
e_3	9.6	12.3	18.4
e_{10}	4.3	7.1	13.6
Life endurance			
$l_x = 0.9$	7.5	8.2	9.2
$l_x = 0.5$	12.0	14.5	18.3
$l_x = 0.1$	19.0	24.0	40.0
Life span			
Reproductive	6.4 ± 0.2	6.0 ± 0.2	6.3 ± 0.2
Post-reproductive	4.8 ± 0.6	7.8 ± 0.8	13.6 ± 1.5
Total[b]	13.2 ± 0.6	15.8 ± 0.9	21.9 ± 1.6

[a] Mean ± standard error ($n = 48$).
[b] Total life span is the sum of the developmental, reproductive, and post-reproductive life periods.

3.4. Relationship of longevity and reproduction within each strain

Lifetime egg production decreased with longer lifespan of strains (Fig. 3). Since the asymptote was reached around day 10 for all three genotypes, rates of egg production during the reproductive period were lower in the long-lived mutants. However, reproduction was positively correlated with total life span only in individuals that did not survive beyond the reproductive period. There was little correlation between lifetime egg production and total life span for individuals that survived into the post-reproductive period, that is individuals that survived beyond 10 days.

4. Discussion

In a previous experiment investigating the same isogenic populations of *C. elegans*, but in smaller feeding arenas (24-well Costar culture plates) and at a slightly higher temperature (22°C), we found: (1) mean total life span was 12.8 days for the wild type, 20.2 days

Fig. 1. Event-history diagrams for longevity and reproduction in three *Caenorhabditis elegans* genotypes, *age*-1, *clk*-1, and wild type. Each cohort consisted of 48 nematodes. *X*-axis represents nematode life course. *Y*-axis represents a cohort survival schedule created by rank-ordering individual life course from shortest to longest. Color-coded segments depict the reproduction at each age interval. Inset: Survivorship curves for cohorts of 48 individuals of *age*-1, *clk*-1, and wild-type *C. elegans.*

Table 2
Reproduction of the wild-type and two long-lived genotypes of *C. elegans*

Reproduction parameter	Genotypes		
	Wild type	*clk*-1	*age*-1
Average eggs/day (R)	37	21	19
Gross reproductive rate (GRR)	278	177	146
Net reproductive rate (R_0)	271 ± 7.3[a]	175 ± 7.2	145 ± 4.4
Expectation of reproduction (R_x) — % of zero-day			
≥ Day 4 (R_4)	76	80	69
≥ Day 6 (R_6)	17	11	12
≥ Day 8 (R_8)	4	2	2
Lifetime egg production — % of cohort			
≤ 100	2	4	8
101–200	4	67	88
≥ 201	94	29	4

[a] Mean egg production/adult ± standard error.

for *age*-1, and 13.7 days for *clk*-1; (2) mean post-reproductive life spans were longer than that of the wild type by 142% in *age*-1 and by 24% in *clk*-1; and (3) net reproductive rate was lower than that of the wild type by 21.8% in *age*-1 and 13.6% in *clk*-1 (unpublished results). Those data generally support our findings in this study which was conducted in petri dishes at 20°C. Fecundity rates of all three genotypes were lower in the 24-well plates than in petri dishes, suggesting that, as observed by other researchers (Klass, 1977; Johnson, 1983), the abundance of bacteria as a food resource in monoxenic cultures affects nematode brood size.

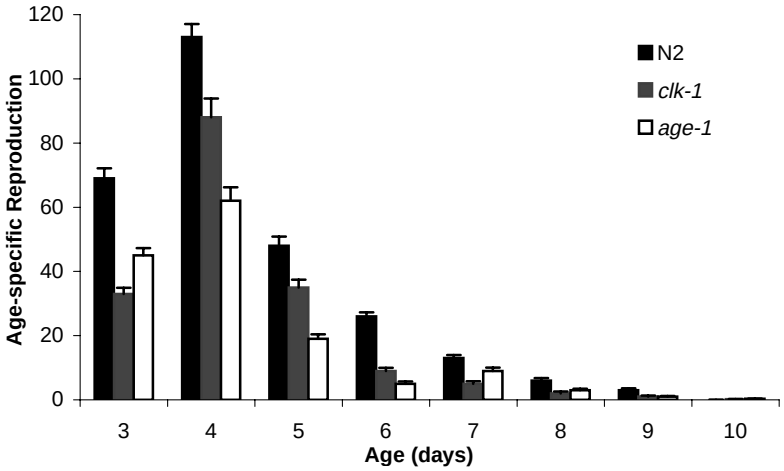

Fig. 2. Age-specific schedule of reproduction for wild-type *Caenorhabditis elegans* and its mutants *age*-1 and *clk*-1. Bars indicate standard errors.

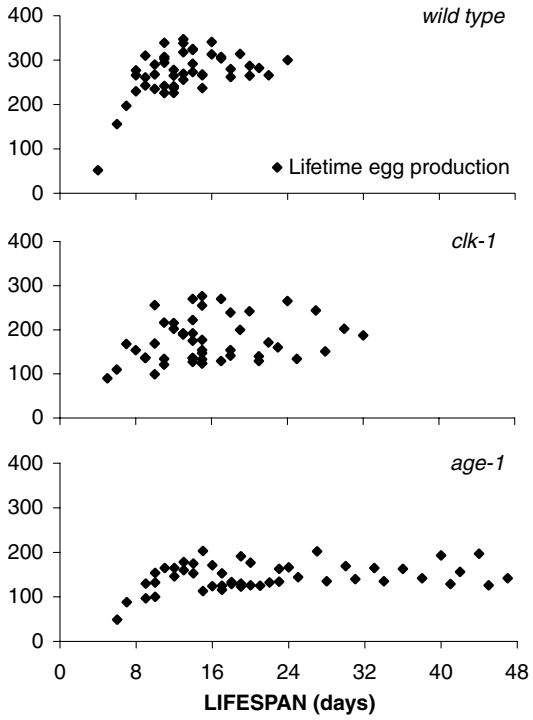

Fig. 3. Lifetime reproduction versus life span in 48-nematode cohorts of *Caenorhabditis elegans* genotypes *age*-1, *clk*-1, and wild type.

Results from both well and petri dish experiments agree with studies by Klass (1977) who observed no significant correlation between life span and number of eggs produced by individual wild-type worms. One possibility is that energy use is more efficient in reproduction than in somatic growth (Calow, 1983a). Also there may be an increase in the assimilation:respiration ratio during the reproductive period, resulting in resource intake in excess of somatic requirements, rather than a trade off between gamete production and somatic maintenance (Calow, 1983b; Wharton, 1986). Nevertheless, there is compelling recent evidence for regulation of longevity by signals from the reproductive system. Through laser ablation experiments, Hsin and Kenyon (1999) suggest that lifespan of *daf*-2 mutants is regulated by the integration of signals from the germinal and somatic regions of the gonad that affect the *daf*-2/*daf*-16 gene pathways.

The survival function of wild-type *C. elegans* hermaphrodites (Bristol N2 strain) is a typical sigmoid curve (Fig. 1) (Gems and Riddle, 2000; Tissenbaum and Ruvkun, 1997). Both longevity genes in this study have alleles of different strength and characteristics, and lifespan is extended by different mechanisms (Johnson, 1990; Kenyon, 1997; Vanfleteren and Braeckman, 1999; Wong et al., 1995). The observation that the post-reproductive periods of the two *C. elegans* mutants are longer than that of the wild type is consistent with studies of other *age*-1 strains (Friedman and Johnson, 1988).

Self-fertilized wild-type *C. elegans* produce about 280 eggs (Hirsh et al., 1976), with populations averaging as few as 104 and as many as 342 eggs at 20°C (Friedman and Johnson, 1988). The brood size for the laboratory wild-type strain N2 and 15 natural isolates ranged from 250 to 350 progeny per adult (Hodgkin and Barnes, 1991). We observed considerable heterogeneity of reproduction and survival within isogenic strains. Lifetime egg production for individuals surviving to any age within a cohort, including those that died during the reproductive period, was variable, ranging from 0 to 347. We found a positive relationship between total egg production and longevity for individuals that did not survive until the end of the reproductive period. That is, the longer the nematode survives during the reproductive period, the greater the number of eggs produced. However, there was no correlation between egg production and life span for *C. elegans* after reproduction was completed.

Different strains of the longevity mutants, TJ401 (*age*-1) and MQ130 (*clk*-1), also produced fewer eggs than the wild type (Wong et al., 1995; Friedman and Johnson, 1988). The *age*-1 and *daf*-2 genes function in a common pathway to regulate longevity of *C. elegans* (Dorman et al., 1995), and Larsen et al. (1995) observed that brood size was significantly smaller in CB1370 (*daf*-2) than in the wild type. The trade off between individual longevity and fitness is apparent. We hypothesize that a mixed population of the three genotypes would become dominated by the wild-type strain due to its more rapid reproductive rate. Of particular interest would be the cost to the population growth rate of competition for resources between reproductive and post-reproductive individuals.

The phenotypic expression of egg production or longevity in the different strains may also be environmentally mediated (Byerly et al., 1976; Johnson, 1983; Kenyon, 1997; Murakami and Johnson, 1996; Shook and Johnson, 1999). Metabolic rates of these poikilothermic organisms are temperature-dependent (Ferris et al., 1995; Venette and Ferris, 1997). Conceivably, the longevity strains may differ from the wild type in temperature minima and optima for reproduction. In that case, physiological time would progress at different rates in relation to chronological time for the mutants and an argument could be made for comparing reproduction to longevity relationships while maintaining each strain at its optimal temperature. Additionally, because the total number of sperm produced limits maximum hermaphroditic egg production of each strain (Ward and Carrel, 1979), experiments with males present might have different results.

Understanding of the mechanisms by which life is extended in these strains is gradually emerging. Respiration capacity of mitochondria is suppressed by *clk*-1, while overexpression of the gene increases respiration, prevents physiological slowing and shortens lifespan (Felkai et al., 1999). In other studies, metabolic rates of mutant strains were lower than those of the wild type under the same environmental conditions. Consequently, Van Voorhies and Ward (1999) suggested that longevity in some mutants of *C. elegans* may be a consequence of reduced metabolic rates rather than lifespan-extending changes in genetic pathways with physiological processes occurring at normal rates. Although respiration rates were slightly depressed in *clk*-1, metabolic potentials, measured as ATP levels, were initially similar in *clk*-1 and wild type nematodes but did not decline at the same rate with age. It is suggested that the slight reduction in metabolic rates may reduce oxidative damage and so contribute to a slower rate of aging (Braeckman et al., 1999; Vanfleteren and Braeckman, 1999; Vanfleteren and De Vreese, 1995).

In summary, event-history diagrams depict survival and reproduction of three *C. elegans* genotypes, *age*-1, *clk*-1, and the wild type. In comparative biodemographic analyses, the relationship between reproduction and longevity differed among isogenic populations with specific longevity genes. Longevity mutants produced fewer eggs and spent a smaller proportion of their life span in the reproductive phase, and a greater proportion in the post-reproductive phase, than individuals of the wild-type strain.

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