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The longevity of *Caenorhabditis elegans* in soil

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Relatively simple model organisms such as yeast, fruit-flies and the nematode, *Caenorhabditis elegans*, have proven to be invaluable resources in biological studies. An example is the widespread use of *C. elegans* to investigate the complex process of ageing. An important issue when interpreting results from these studies is the similarity of the observed *C. elegans* mortality pattern in the laboratory to that expected in its natural environment. We found that the longevity of *C. elegans* under more natural conditions is reduced up to 10-fold compared with standard laboratory culture conditions. Additionally, *C. elegans* mutants that live twice as long as wild-type worms in laboratory conditions typically die sooner than wild-type worms in a natural soil. These results indicate that conclusions regarding extended longevity drawn from standard laboratory assays may not extend to animals in their native environment.

Keywords: ageing; senescence; *Caenorhabditis elegans*

1. INTRODUCTION

Caenorhabditis elegans is a popular model organism for use in ageing studies, largely because of the identification of numerous long-lived mutants (Johnson *et al*. 2000). Such mutants may help to identify genes specifically involved in ageing, and an extensive research effort is underway to elucidate the mechanisms by which these mutations extend longevity (Guarente & Kenyon 2000). In these studies, *C. elegans*' longevity is typically compared between strains grown either in liquid culture or, more commonly, on agar plates. Because *C. elegans* evolved as a soil-inhabiting nematode, it is unclear whether results from longevity comparisons using laboratory culture conditions will be representative of those expected in a more natural environment. To study this issue, we compared the longevity of wild-type and a long-lived *C. elegans* mutant under both standard laboratory culture conditions and in soil and sand environments.

2. MATERIAL AND METHODS

Soil from which *C. elegans* had previously been isolated (*C. elegans* strain TR403) was obtained and assayed for its native microfauna. An initial characterization of the soil fauna revealed a variety of soil invertebrates, including numerous microbivorous, fungivorous and plant-feeding nematodes. To determine the survivorship pattern of *C. elegans* in this natural soil type, a known number of young adult *C. elegans* were added to soil samples. These samples were then assayed at regular intervals for *C. elegans* survivorship. To ensure that only individuals from the initial population of worms were recovered, we used *C. elegans* strains possessing a mutation affecting sperm motility. Such mutants grow, develop and age normally, but do not produce progeny when maintained at standard culture temperatures (Ward & Miwa 1978). Worm longevity of the strains placed in soil was compared with that of the same strains reared under typical laboratory culture conditions. To minimize the effects of microinvertebrate or other biotic predation that might affect *C. elegans* survival, we also assayed survivorship using soil which had been heat pasteurized to kill the native fauna prior to the addition of worms.

The *fer-1 wv01* strain was considered the wild-type and *daf-2 wv06* (a double mutant of *daf-2 e1368* and *fer-1 wv01*) the long-lived strain. This *daf-2* mutant is considered the most similar to wild-type in terms of growth, development and fecundity (Gems *et al*. 1998). Soil used in the study came from Madison, Wisconsin, USA. The native soil bacterial fauna was enriched with *E. coli* 2 days before adding worms. This was done to ensure that a bacterial food supply to which the worms were well adapted was present in the soil or sand environments. Heat-pasteurized soil was sealed in a container and heated to 65 °C for 24 h prior to the addition of *C. elegans*. On day 0 of the experiment, approximately 300 1-day-old adult worms were added to a 2 ml soil or sand sample contained in a 15 ml plastic centrifuge tube. A sub-population of these worms was placed on 3.5 cm Petri dishes containing nematode growth medium (NGM) agar inoculated with *E. coli* (Brenner 1974), and assayed for longevity. The *E. coli* strain NA22 was used as a supplemental food source for the worms in both the soil and sand environments. This *E. coli* strain was used for the food source in these environments because the *E. coli* strain commonly used to grow *C. elegans* in the laboratory, OP50, requires an external source of uracil to grow well. It is unlikely that the soil would have a readily available source of uracil. Longevity assays of the worms were also conducted on NGM plates spotted with OP50. This *E. coli* strain was used to allow the worms to be more easily tracked on less dense bacterial lawns, and with allow the comparison of longevity data from these experiments with previous longevity assays conducted in the laboratory on *C. elegans*. It was determined that the survivorship of *C. elegans* on agar plates was essentially identical irrespective of whether they were grown on OP50 or NA22 as a food source (see Electronic Appendix).

Groups were maintained at 23 °C and worms from six soil or sand samples were extracted at each time point using a colloidal silica method (Donkin & Dusenbery 1993). Soil extraction 3 hours after addition of the worms recovered around 80% of the initial population, of which more than 95% were alive. A worm was scored as dead when it was not moving and did not respond to mechanical stimulus.

Soil is a complex environment in which many factors aside from predation (e.g. the production of toxic compounds by soil bacteria) can influence *C. elegans*' longevity. To minimize the impact of such biological factors, we examined the longevity of *C. elegans* in autoclaved, acid-washed sand. To ensure food availability, the soil or sand samples were supplemented with *E. coli*. To minimize the biological activity of the *E. coli*, it was heat killed at 65 °C before being placed in the sand. To ensure that *E. coli* was not limiting as a food source, an amount far in excess of that which would be eaten by the several hundred worms was added to each sample. This is analogous to the conditions to which *C. elegans* are subjected during standard longevity assays in the laboratory, in which worms are on agar plates with many times their body mass of *E. coli*. We also assayed the longevity of a *C. elegans* mutant that typically lives around twice as long as the wild-type in laboratory conditions (Gems *et al*. 1998) to determine if it was also relatively long-lived in a soil environment.

3. RESULTS

The median longevity of wild-type *C. elegans* is reduced by an order of magnitude in soil compared with worms maintained on agar plates (figure 1*a*). This large reduction does not appear to be caused by interactions with other soil microfauna because the longevity of wild-type *C. elegans* in pasteurized soil is the same or less than in non-pasteurized soil

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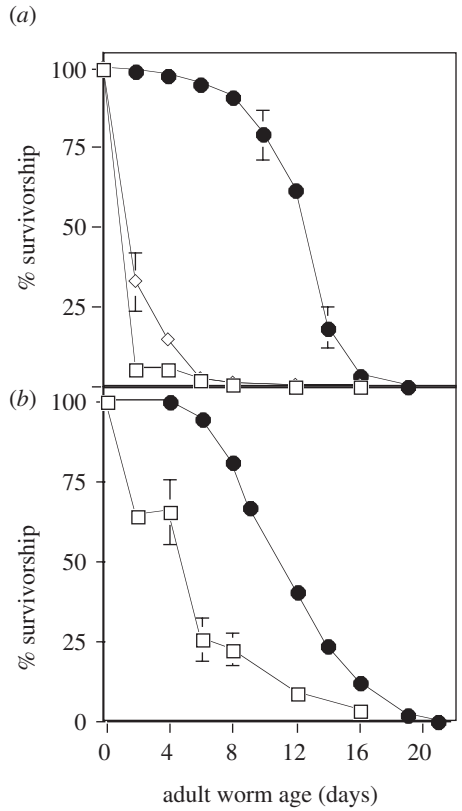

Figure 1. Longevity of wild-type *Caenorhabditis elegans* is reduced in a natural soil or sand environment compared with agar plates. (*a*) Survivorship of *C. elegans* added to a natural unheated (open diamond) or heat-pasteurized (open square) soil. The median adult survivorship in unheated soil was 1.5 days and 1.0 day for worms in heat-treated soil. The median adult survivorship of wild-type worms on agar (filled circle) was 12 days. (*b*) Survivorship of *C. elegans* in acid-washed, autoclaved sand. Median adult survivorship in sand (open square) was 4.5 days and wild-type on agar (filled circle) was 11.5 days. Median survivorship was estimated as the time at which 50% of the population remained alive. Data for the sand and soil treatments are the means and standard errors for six samples at each time point. Data of survivorship on agar are plotted for 151 and 115 worms for *a*, *b*, respectively. To determine if the sand/soil environments could support a mixed-age population, a fertile wild-type strain of *C. elegans* (N2) was added to the sand/soil environment. These worms were able to maintain reproducing populations for at least two weeks in the sand/soil environments used in the longevity assays.

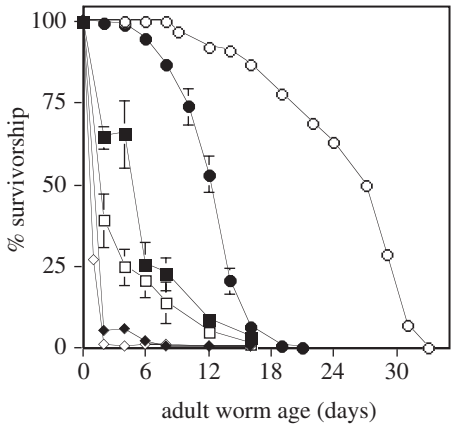

Figure 2. Longevity of a long-lived *Caenorhabditis elegans* mutant is less than wild-type in a natural soil or sand environment. The median adult survivorship of *daf-2* worms was 0.8 days in heat-treated soil (open diamond), 1.8 days in sand (open square) and 27 days on agar (open circle).The median adult survivorship of wild-type was 1.0 days in heat-treated soil (filled diamond), 4.5 days in sand (filled square) and 12 days on agar (filled-circle). The survivorship in sand of wild-type worms is significantly greater than that of the long-lived mutant for the 2 and 4 day samples (two-tailed *t*-test, $p=0.05$ and $p=0.002$, respectively, d.f.$=5$). Wild-type survivorship data on agar are combined from figure 1*a*,*b*. Survivorship data for *daf-2* agar are plotted for 104 worms.

(figure 1*a*). Worm populations in sterilized sand lived longer than worms in soil, but only about one-third as long as the comparable wild-type strain on agar (figure 1*b*). While the nominally long-lived mutant *daf-2* lived significantly longer than the wild-type when assayed on agar, *daf-2* was shorter-lived than wild-type in both the sand and soil environments (figure 2).

4. DISCUSSION

The reason for the relatively rapid mortality of *C. elegans* in a soil environment is unclear, although it appears unlikely that the worms are dying of starvation or other factors such as soil pH levels (see Electronic Appendix). Although the relatively short lifespan of *C. elegans* in soil compared with laboratory conditions may appear surprising, the shortened lifespan of worms in soil is more than sufficient for *C. elegans* to maintain viable populations. The short generation time, high rate of progeny production, and self fertilizing mode of reproduction allows even a single *C. elegans* to quickly found a large population (Wood 1988; Venette & Ferris 1998). Because *C. elegans* can produce almost all of its progeny within the first 2 days of its adult life, additional longevity adds little to its lifetime reproductive output (Byerly *et al.* 1976; Hirsh *et al.* 1976).

The relatively short longevity of *C. elegans* in soil makes it unlikely that *C. elegans* would have evolved a signalling mechanism inversely coupling longevity to its reproductive output. Although it has been proposed that such a mechanism may exist in *C. elegans* (Kenyon 2001), this hypothesis is based on longevity assays done on agar plates. Under such conditions, even wild-type *C. elegans* typically live several times longer than their reproductive period. From an evolutionary viewpoint, this extended post-reproductive longevity is puzzling. A non-reproducing worm is essentially evolutionarily irrelevant and there should be minimal selection for extended post-reproductive survivorship (Rose 1991). The high mortality rate of *C. elegans* under more natural conditions in our study suggests that few individuals would survive for long enough to benefit from any increase in late-life fecundity. Selection should favour rapidly reproducing types over survivors, as previously observed in *C. elegans*, where mutants that produce more total progeny are eventually displaced

by wild-type worms, which have a shorter generation time (Hodgkin & Barnes 1991). The apparent short longevity of *C. elegans* in nature could also help to explain why biological processes such as protein biosynthesis and turnover rates are tightly regulated during *C. elegans* development but not post-reproductively (Herndon *et al.* 2002).

Among the mechanisms postulated to be responsible for the extended longevity of long-lived *C. elegans* mutants is an increased ability to tolerate stress (Lithgow & Kirkwood 1996; Martin *et al.* 1996). Alternatively, this extended longevity has been proposed to result from a reduction in metabolic rate (Van Voorhies & Ward 1999). Factors that reduce metabolic rate also generally increase the ability of the organism to withstand stress (Sohal *et al.* 2000). Although the *daf-2* mutant studied here had a mean longevity twice that of wild-type under laboratory conditions on agar, they were shorter-lived than wild-type under more natural conditions. This indicates that any increased ability of such mutants to tolerate laboratory-induced stresses does not extend to the stresses encountered in a more natural environment. Results from similar experiments showed that under optimized laboratory conditions, populations of a long-lived *C. elegans* mutant persisted as well as wild-type worms, but were displaced by the wild-type under more stringent environmental conditions (Walker *et al.* 2000). The relatively poor survivorship of long-lived mutants under more natural conditions may also explain why such mutations are not known to occur in natural populations of *C. elegans*.

The factors responsible for *C. elegans* mortality in a natural environment appear to be different from those responsible for mortality in the laboratory. While *C. elegans* remains a valuable organism for the study of ageing, it is critical to consider its natural history when interpreting results from such studies.

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The supplementary Electronic Appendix is available at http://dx.doi.org/10.1098/rsbl.2004.0262 or via http://www.journals.royalsoc.ac.uk.