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Facultative Vivipary is a Life-History Trait in *Caenorhabditis elegans*

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Abstract: Organisms partition their resources among growth, maintenance, and reproduction and, when resources become limiting, the allocation to one process necessitates reduced allocation to others. When starved, *Caenorhabditis elegans* adults retain progeny internally which then consume the parent body contents, and some of those larvae use the resources to reach the resistant, long-lived dauer stage. If starved under similarly extreme conditions, larvae from eggs laid outside of the body are unable to develop into dauers. We interpret this switch from ovipary, or laying eggs, to bearing live young as facultative vivipary. This switch is induced by starvation of late fourth-stage larvae, young adults, or gravid adults. In *C. elegans*, vivipary is the altruistic allocation of all available parental energy and nutrients to progeny, with the associated costs to adult hermaphrodites of truncated life span and fecundity. As a life-history trait, facultative vivipary is a survival-enhancing response to stress that may provide insights into the evolution of reproduction and longevity.

Key words: Bagging, *Caenorhabditis elegans*, life history, longevity, reproduction, survival strategy, vivipary.

The bacterivorous nematode *Caenorhabditis elegans* is a model organism for developmental genetics (Brenner, 1974; Hodgkin et al., 1998; Hope, 1999; Wood, 1988). Its life cycle includes a survival and dispersal stage, the well-documented resistant, long-lived dauer larva (Klass and Hirsh, 1976; Riddle and Albert, 1997). Cues that trigger dauer formation include the ratio of food signal to nematode pheromone, specifically a high ratio of nematode pheromone to food signal in the environment (Golden and Riddle, 1982). The dauer stage facilitates dispersal (Riddle et al., 1997) but is not inducible under complete starvation (Cassada and Russell, 1975), when such a response would seem highly appropriate.

Caenorhabditis elegans is commonly regarded as a cosmopolitan, soil-dwelling, bacterial-feeding nematode, although its life history in nature is little explored (Delattre and Felix, 2001; Hodgkin, 2001). Theories of life-history evolution consider that organisms partition limited resources to growth, reproduction, and maintenance and, when resources are limited, an increased allocation to one process necessitates reduced allocation to the others (Partridge, 1989; Reznick, 1992). An example of a life-history trait that has received relatively little attention in *C. elegans* is egg retention with internal hatch, called bagging or *endotokia matricida* (Johnson, 1984; Mitchell et al., 1979; Samoiloff, 1980). We have proposed and tested the hypothesis that internal hatching of larva presents a possible fitness advantage to hermaphrodites by supporting dauer development under extreme nutrient limitation (Chen and Caswell-Chen, 2003).

Here we report the results of our research designed to assess bagging as a plastic life-history trait and part of *C. elegans* life cycle. We tested the following specific hypotheses: (i) bagging happens in a range of stressful environments; (ii) bagging occurs in many wild-type *C. elegans* and is not peculiar to the standard laboratory wild-type N2 strain; (iii) bagging occurs in longevity mutants; (iv) bagging is inducible; (v) bagging results in healthy progeny; (vi) larvae from bagging are able to enter the dauer stage under the same conditions as larvae from laid eggs; and (vii) if larvae consume the parent body contents, they are able to enter the dauer stage under complete starvation, conditions under which larvae from laid eggs can not become dauers.

Materials and Methods

General: Cultures of *C. elegans* used in this research were obtained from the *Caenorhabditis* Genetics Center (CGC), University of Minnesota, St Paul. NGM agar (Brenner, 1974) was seeded with *Escherichia coli* strain OP50. Except where stated otherwise, the N2 strain (ancestral) was used. Artificial tap water (ATW) (Greenaway, 1970) was made by adding 0.021 g NaCl and 0.003 g KCl to 1,000 ml purified water (Milli-Q water system, Bedford, MA), autoclaving 20 minutes at 121 °C, allowing the solution to cool, adding 0.1 g $CaCO_3$ and 0.034 g $MgCO_3$ (dissolved in 2.0 and 0.81 ml 1 M HCl, respectively), and 0.1 ml 1 M NaOH.

Bagging under stress: Starvation stress was assessed in aqueous, agar, and soil environments. Water was Milli-Q (16 megaohm) water (Millipore, Bedford, MA), deionized water, city tap water, laboratory tap water, 0.22 µm-filtered laboratory tap water (Millipore, Bedford, MA), and ATW. M9 buffer was standard (Brenner, 1974). Two hundred eggs were placed on NGM with OP50 at 20 °C. The cohort became adults in 3 days, and 1 day later the culture was exhausted and bagging adults were observed. One ml of ATW was added to the exhausted culture and swirled, collected, centrifuged at 10,000 g for 10 minutes, and the resulting supernatant was stored at 4 °C and centrifuged before use. One ml of ATW was added to a 3-day growth of OP50 on NGM at 25 °C, swirled for 1 minute, collected, and filtered by a 0.2-µm Nalgene Filter to obtain a bacterial filtrate.

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Occurrence of bagging in wild-type worms: The occurrence of bagging was assessed in *C. elegans* strains CB4855 (Tc1 pattern VI), PB303, RC301 (Tc1 pattern HCF), and TR389 (Tc1 pattern I); and also in *C. briggsae* strain AF16. Cohorts or individuals of energetic gravid adults were obtained as follows: 0.1 ml of *C. elegans* cryopreserved at –80 °C was pipeted onto NGM culture streaked with *E. coli* OP50 in 60 × 15-mm petri dishes. Dishes were incubated in the dark at 20 °C for 3 days, and freshly laid eggs were transferred onto fresh NGM with OP50. In 3 days at 20 °C, the eggs developed into adults that were used in the experiments. One or five of the energetic gravid adults were placed in 10 ml liquid in a 60-mm tissue culture dish with 2-mm grids (Corning Inc., Corning, NY). After 2 to 3 days of incubation in the dark at 20 °C bagging worms were counted (n = 20, two trials).

Response of daf and clk mutants to starvation: The strains assessed included dauer defective strains CB1376, *daf-3* (*e1376*) X; CB1386, *daf-5* (*e1386*) II; CB1377, *daf-6* (*e1377*) X; CB1387, *daf-10* (e1387) IV; DR1407, *daf-12* (*m583*) X; CF1038, *daf-16* (*mu86*) I; and DR476, *daf-22* (*m130*) II; dauer constitutive strain TJ1052, *age-1* (*hx546*) II; and *clk* strain MQ130, *clk-1* (*qm30*) III. The protocol used to assess the frequency of bagging was as described.

Inducibility of bagging: The dauer stage is inducible, and the following experiments were conducted to determine whether a parallel pattern occurs with respect to bagging. Experiments were initiated by monitoring the development of post-dauer *C. elegans,* including fourth-stage larvae, young adults, and mature adults. Individual dauer larvae were placed onto NGM with OP50, and worm length was recorded every 2 hours. Worms at a post-dauer interval of 2 hours were individually placed into wells containing 200 µl ATW, and the subsequent occurrence of bagging was monitored. Nematodes were observed using a Nikon Diaphot inverted microscope (Nikon Inc., Garden City, NY), and nematode stages were identified based on morphological characteristics and body length.

Health of progeny from bagging: The life span and fertility of progeny arising from eggs laid externally were compared with that from progeny arising from bagging. The ovipary-cohort consisted of eggs laid by energetic adults on NGM with OP50, and the bagging cohort consisted of larvae that emerged from adults after bagging. The eggs and larvae were individually transferred onto NGM with OP50 and incubated in the dark at 25 °C. Individual first-stage larvae (L1) were placed onto NGM with OP50 and transferred to fresh NGM daily until egg production ceased, and worms were monitored until they died.

Dauer formation under starvation: To further test the hypothesis that larvae from bagging become dauers by using the parent body contents as the nutrition necessary to reach the dauer stage, adults containing larvae were placed into ATW augmented with aqueous rinsate from an exhausted NGM-OP50 *C. elegans* culture (containing putative low food and high dauer pheromone signals to stimulate dauer formation) and a suspension of OP50 bacterial cells (ca. 8×10^7 cells) in Costar 48-well plates (Corning, Inc, Corning, NY). Such conditions result in freshly laid eggs yielding dauer larvae (Cassada and Russell, 1975). A supernatant was obtained by adding 1.0 ml of ATW to an exhausted NGM culture with bagging adults, agitated for 1 minute, centrifuged at 10,000 g for 10 minutes, stored at 4 °C, and centrifuged before use. Dauer larvae were identified and counted based on morphology and depicted as the percentage of total larvae. On day 12, dauer stages were confirmed by the resistance of larvae to 30-minute exposure to 1% sodium dodecyl sulfate (SDS).

Use of parent body contents for dauer formation: This was assessed by experiments to determine whether larvae from bagging reached the dauer stage without any nutrition beyond that provided by the parental body. ReNu (Bausch & Lomb, New York, NY) contact lens cleaner was used to surface-sterilize worms in experiments on eliminating food sources. It was anticipated that such surface sterilization would not be particularly harsh for *C. elegans* (Burger et al., 1994). Longer exposure (15 to 20 hours) to surface sterilization resulted in some larvae remaining in the parent body as long as 2 days. In this study, a series of experiments was completed under different conditions to expand on our other research (Chen and Caswell-Chen, 2003).

Energetic gravid adults from NGM with OP50 were surface-sterilized by placing them in 20 ml of ReNu contact lens cleaning solution in a sterile 60 × 15-mm petri dish with 2-mm grids for 15, 17, 18, 19, or 20 hours at 20 °C or 25 °C. Adults laid eggs in the dish during the time in ReNu before being individually transferred into 50 µl ATW in a 96-well Falcon tissue culture dish (Becton, Dickinson and Co., Franklin Lakes, NJ). Each plate was sealed with Parafilm M (Pechiney Plastic Packaging, Chicago, IL), stored in a box with cover, and incubated in the dark at 20 °C or 25 °C. The number of larvae and dauer larvae were counted daily, and every 2 days the water from three wells was plated and bacterial CFUs counted. The experiment was repeated.

Results

Bagging under stress: The frequency of bagging in wild type worms was 90% or greater under starvation in six different sources of water, in M9 buffer, on NGM agar, in aqueous rinsate from exhausted NGM cultures, in bacterial filtrate from a 3-day NGM culture of OP50, in ReNu, and also in soil (Table 1). The larvae retained in the parent body consumed the internal body contents, leaving only the cuticle, until the progeny eventually exited the parent body through the vulva (Fig. 1). Bagging was also observed in N2 strain worms exposed to

TABLE 1. Frequency of bagging in the standard wild-type strain N2 of *Caenorhabditis elegans* when starved in aqueous environments.

Stressful environments	Bagging (%)
MQ purified water	100
Deionized water	98
Normal tap water	100
Industrial tap water	100
Filtered tap water	100
M9 buffer	100
Supernatant	100
Bacterial filtrate	100
ReNu & ATW	96

Frequency of bagging was assessed after 2 to 3 days under stress ($n = 20$, two trials). Adults were in ReNu solution for 18 hours and then transferred into ATW ($n = 92$, two trials).

antimicrobial agents, high salt concentrations, and antagonistic bacteria (*data not shown*).

Occurrence of bagging in wild-type worms: To assess whether the phenomenon was specific to laboratory standard strain N2, four other wild-type strains of *C. elegans* were subjected to starvation. The frequency of bagging was high in all cases (Table 2). The related nematode *C. briggsae* also revealed a relatively high frequency of bagging in ATW (Table 2).

Response of daf and clk mutants to starvation: Starvation of the dauer defective strains *daf*-3, *daf*-5, *daf*-6, *daf*-10, *daf*-12, *daf*-16, *daf*-22, and the dauer constitutive strain *age*-1 resulted in bagging the same as in the wild type

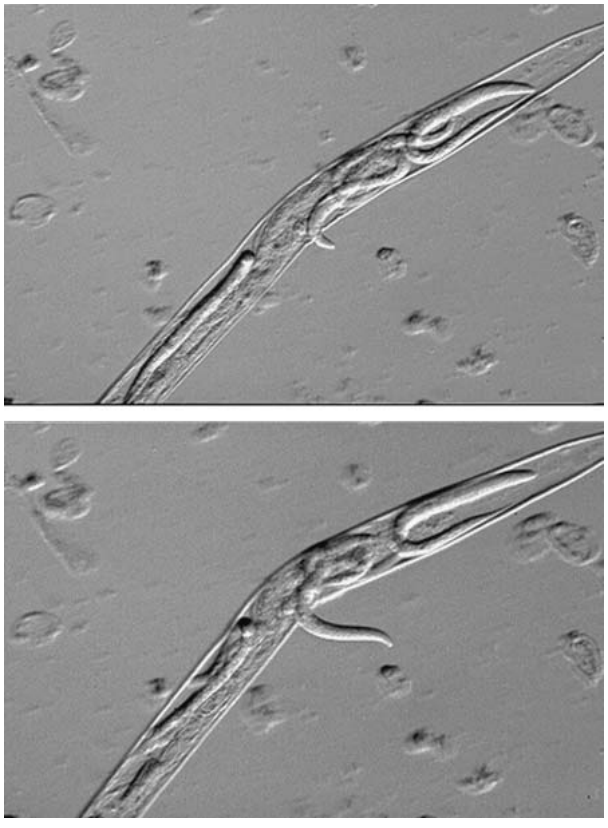

FIG. 1. Photomicrograph of active *Caenorhabditis elegans* larvae emerging from parent body.

TABLE 2. Occurrence of bagging in wild-type *Caenorhabditis elegans* and *C. briggsae* as a response to starvation.

Strain	Geographic origin	Artificial tap water (ATW)	Other water[a]
CB4855	California	100% ($n = 50$; 5 trials)	97% ± 4.5
N2	England	100% ($n = 100$; 10 trials)	99% ± 2.2
PB303	Ohio	100% ($n = 50$; 5 trials)	99% ± 2.2
RC301	Germany	100% ($n = 50$; 5 trials)	98% ± 2.7
TR389	Wisconsin	100% ($n = 50$; 5 trials)	96% ± 5.5
AF16[b]	India	71% ± 1.9 ($n = 21$; 2 trials)	—

[a] Purified water, deionized water, city tap water, laboratory tap water, and filtered laboratory tap water. The data are mean percent ± standard deviation.
[b] *C. briggsae*

(*data not shown*). The *clk-1* strain MQ130, however, showed a delay in the occurrence of adult death due to bagging and also an extension of adult life when starved (Fig. 2).

Inducibility of bagging: To establish the nematode stages that might respond to induction, late stage-four larvae, young adults lacking eggs, and gravid adults were transferred to water and observed. In each case bagging occurred (Fig. 3).

Health of progeny from bagging: To evaluate whether progeny produced by bagging were less viable than progeny from eggs laid outside of the body, the survival and lifetime egg production were compared in nematodes from bagging and from laid eggs (Fig. 4). Individual L1 were placed onto NGM with OP50 and transferred to fresh NGM daily until egg production ceased. The nematodes were examined daily until they died. The progeny had similar life spans and fertility (those from bagging lived 11.2 ± 1.0 days with 197 ± 17 eggs/adult at 25 °C, compared to 9.7 ± 0.8 days and 206 ± 9 eggs/adults (mean ± SE) for those from laid eggs).

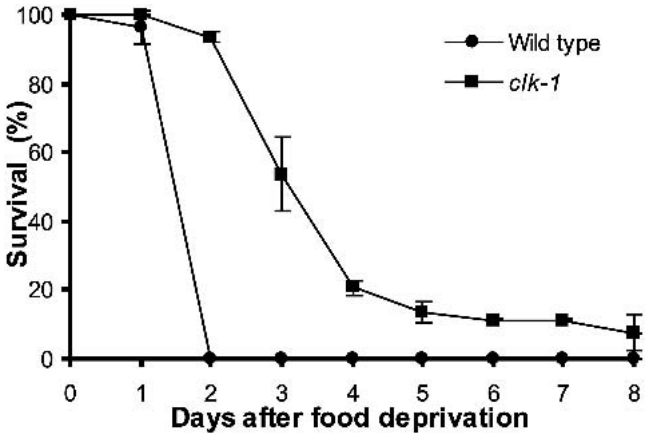

FIG. 2. Bagging in *Caenorhabditis elegans* strain *clk-1* when starved. One or two gravid adults were transferred into a well in 48-well plates kept at 25 °C, wells containing 0.5 ml M9 buffer. Experiments were repeated ($n = 26$ for strain MQ130, square; $n = 14$ for wild type strain N2 , circle) (error bars are standard deviations). Bagging caused 99% of deaths among wild-type adults. Among *clk-1* adults between day 2 and 7, bagging caused about 72% of deaths, and further bagging was not observed after 7 days. One worm lived to 20 days. Similar results occurred with adults in ATW.

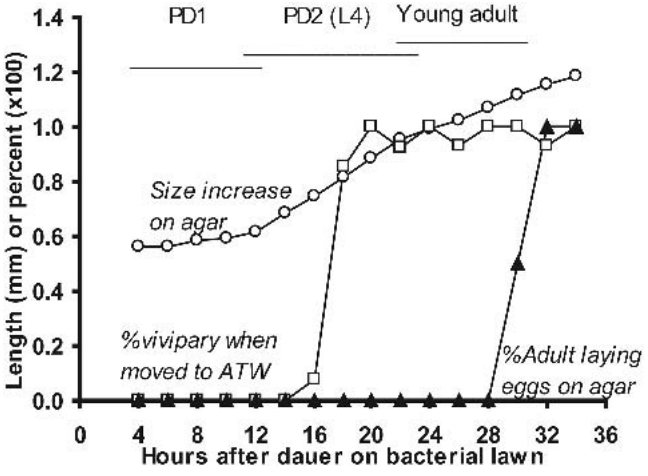

FIG. 3. Induction of bagging in *Caenorhabditis elegans*. Dauer larvae were individually placed onto NGM dishes with OP50 at time 0 and transferred to ATW at the times indicated. The length of worms (circles are the mean of 10 worms, 2 trials) and percent of adults laying eggs (triangles are the mean of 10 worms, 2 trials) and of adults bagging (squares are the mean of 15 worms, 2 trials) were monitored every 2 hours. PD1 = Post-dauer stage prior PD1-PD2 molt. PD2 = 4th-stage larval development.

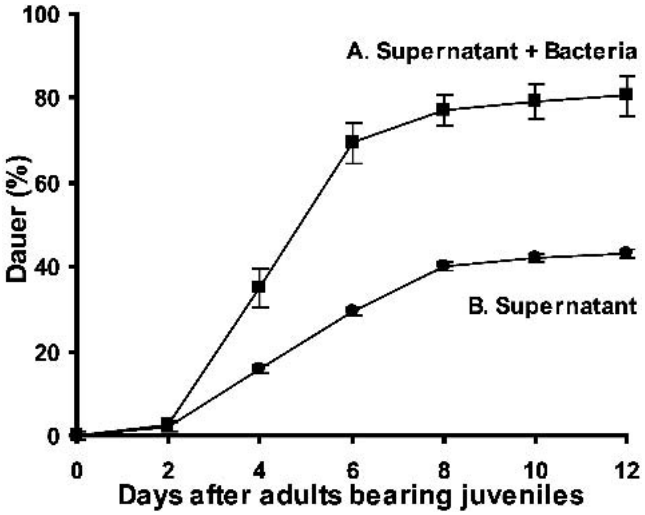

FIG. 5. The proportions of *Caenorhabditis elegans* larvae from bagging that achieve the dauer stage. A) The percentage of larvae that became dauers from 50 bagging adults (bearing internal larvae) placed in 10-µl bacteria (8×10^7) plus 490-µl supernatant from an exhausted NGM culture containing bagging adults. B) The percentage of larvae that became dauers from individual bagging adults placed in 50-µl supernatant. All experiments were repeated (error bar is the standard deviation).

Dauer formation under starvation: When exposed to the environmental conditions that induce L1 from laid eggs to become dauers, larvae arising from bagging also became dauers. To assess this, adults containing larvae were placed into ATW augmented with aqueous rinsate from an exhausted NGM-OP50 *C. elegans* culture (condition of food depletion and high dauer pheromone concentration) and a suspension of OP50 bacterial cells (ca. 8×10^7 cells); 81% of the L1 from bagging became dauer larvae (Fig. 5). If bagging hermaphrodites were placed in ATW that contained an aqueous rinsate from an exhausted *C. elegans*-OP50 culture, 43% of the resulting larvae became dauers.

Use of parent body contents for dauer formation: The hypothesis that larvae from bagging become dauers under complete starvation by consuming the parent body to obtain nutrition to reach the dauer stage was tested under three conditions. In one experiment, individual adults ($n = 20$) bearing larvae were placed into ATW. In another experiment, energetic gravid adults were given short-time surface sterilization (15 minutes to 4 hours) in ReNu and then placed in ATW (Chen and Caswell-Chen, 2003). Both treatments resulted in ca. 4% or less of larvae becoming dauers. To more critically assess whether larvae from bagging reached the dauer stage without any nutrition beyond that provided by the parental body, experiments were conducted with worms that were surface-sterilized for longer times (15 to 20 hours), in addition to 18 hours at 25 °C reported previously (Chen and Caswell-Chen, 2003). Of larvae surfaced-sterilized 18 hours at 20 °C, 32% became dauers after 13 days at 20 °C (Fig. 6), and 14% to 21% of larvae surface- sterilized 15, 17, and 19 hours at 20 °C or 25 °C became dauers after 5 days at 20 °C or 25 °C (Table 3). Of the larvae surface-sterilized 18 and 20 hours at 20 °C, 22% to 25% reached dauer stage after 7 days at 25 °C (Fig. 7). On the average, about 50 progeny per adult were produced after short-time surface sterilization, and about 8 to 15 larvae per adult were produced after 15 to 19 hours of surface sterilization (Table 3).

The bacteria available in the surface-sterilization treatments were insufficient for larvae to reach the dauer (Chen and Caswell-Chen, 2003), suggesting that the parent body was the primary food source used to support dauer formation, consistent with the empty parent cuticles observed after the progeny from bagging exited the body.

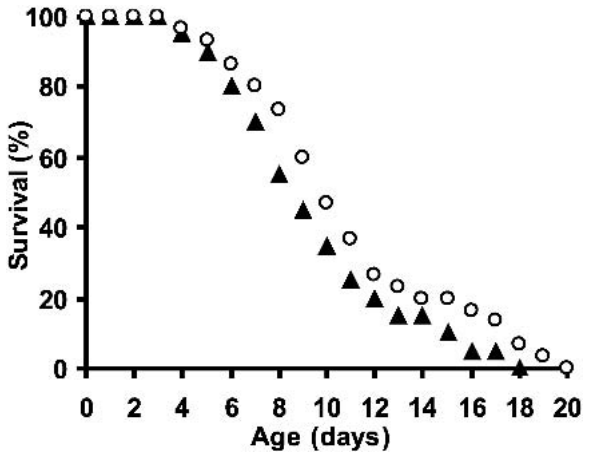

FIG. 4. Comparison of life span of larvae arising from bagging or from laid eggs. The cohort started with eggs laid by adults (circles) or larvae that emerged from adults (triangles).

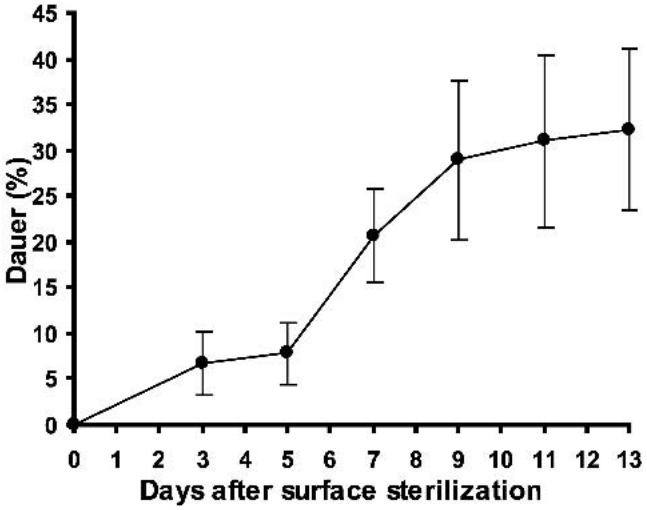

FIG. 6. The percentage of *Caenorhabditis elegans* larvae that became dauers under 13 days of starvation. Energetic gravid adults from NGM with OP50 were placed into ReNu contact lens cleaning solution for 18 hours at 20 °C and then moved into ATW at 20 °C. The experiment was repeated with similar results. Means and standard errors are shown.

FIG. 7. The percentage of *Caenorhabditis elegans* larvae from starved, surface-sterilized adults that achieved the dauer stage after 18 or 20 hours exposure to ReNu. Energetic gravid adults were immersed in ReNu for 18 or 20 hours and then moved into ATW. Dauer larvae were identified by either morphological characteristics (solid lines), or by resistance to a 30-minute exposure to 1% SDS (dotted lines).

DISCUSSION

Bagging has been observed in *C. elegans* under many different conditions, including in egg-laying defective mutants of *C. elegans*, despite the presence of food (Trent et al., 1983) and also, obviously, in vulvaless mutants (Horvitz and Sulston, 1980). We are not aware of any research that has discussed or considered bagging as a life-history trait in *C. elegans*. We have reported that body contents of bagging adults are consumed by progeny as nutrition to reach the long-lived, resistant dauer stage (Chen and Caswell-Chen, 2003). The experiments described herein document bagging under a range of stressful conditions; however, bagging is apparently reversible if the stress is removed before the adult has sustained lethal internal damage due to larval movement. Induction of bagging is indicative of signaling or a decision process in the adult hermaphrodite relative to environmental stress, particularly lack of food, as egg laying ceases shortly after such tress begins. Previous suggestions that bagging represents defective reproduction in older worms (Hirschmann, 1960; Mitchell et al., 1979) were not strictly supported because we observed that "bagging" was not restricted to older gravid adults. Most importantly, bagging is a means for adults to provide nutrition to progeny in food-limited environments. The presumption that such altruistic behavior might be an important part of *C. elegans* survival strategy has not been experimentally tested. Our results lead us to infer that bagging is a response to stress, and we suggest that bagging is a change in reproduction from ovipary to vivipary.

TABLE 3. Early dauer formation using parent body contents in *Caenorhabditis elegans*.

Temperature/time				
ReNu	ATW	Progeny/adult	Dauer/adult	Dauer percent
20 °C (hours)	25 °C (days)			
15	5	9.5	1.1	20.8
17	5	8.6	0.6	12.3
19	5	13.1	0.6	16.4
25 °C	25 °C			
15	5	14.8	2.1	17.9
17	5	8.9	1.3	19.6
19	5	8.3	1.3	14.1

Energetic gravid adults from NGM with OP50 were placed in ReNu contact lens cleaning solution for 15, 17, or 19 hours at 20 °C or 25 °C. They were then moved into ATW and incubated for 5 days at 20 °C or 25 °C.

The dauer pathway is a well-understood survival strategy, and it is inducible and reversible and numerous genes are involved (Cherkasova et al., 2000; Kenyon et al., 1993; Riddle and Albert, 1997). It is possible that genes that influence dauer development might also influence bagging, but genes from dauer-defective phenotypes did not yield bagging-defective phenotypes. The *clk-1* allele that is involved in the timing of developmental processes did appear to influence the timing of bagging, an observation consistent with the *clk-1* phenotype (Felkai et al., 1999; Lakowski and Hekimi, 1996, 1998).

Although the *C. elegans* life cycle has not been studied in nature, we would expect that their rapid rate of reproduction would often lead to populations causing their food source to crash. Given the high organic matter substrates that *C. elegans* is likely to dwell in, it seems likely that it is routinely exposed to environments that contain many different compounds that result from decomposition of organic matter. Experiments with surface sterilization of adults resulted in different numbers of progeny being produced with variable numbers of those progeny becoming dauers (Chen and Caswell-

Chen, 2003). Adults in the sterilizing solution continued to pump, and therefore ingestion of such solution imposes a stress beyond that of simple food reduction. Starvation, as observed under the first two different experimental conditions, yielded some dauer progeny, albeit at low frequency (ca. ≤ 4.2%), while the combination of starvation and a longer exposure to chemical stress resulted in fewer progeny and a higher proportion of larvae reaching the dauer stage, with the average body length of dauer larvae after 18 hours of surface sterilization 12% to 38% longer than that reported for dauers by Riddle (1988) and Cassada and Russell (1975).

In this regard, reproduction is the production of a new individual whether or not the original parent survives (Blackwelder and Shepherd, 1981). According to current definitions, vivipary is defined as occurring when young are nourished from parental sources, including provisioning from the mother's own soma, before live birth (Clutton-Brock, 1991), or when females retain developing eggs inside their reproductive tracts or body cavity and give birth to living offspring (Blackburn, 1998). Among invertebrates, vivipary is commonly associated with harsh environments (Clutton-Brock, 1991), and our data suggest that facultative vivipary also represents a survival-enhancing pathway in the *C. elegans* life cycle (Fig. 8).

Vivipary is not unheard of among nematodes and is known to occur in the oviparous parasitic nematode *Haemonchus contortus* (Ayalew and Murphy, 1986), in other bacterial-feeding rhabditid nematodes (Belogurov et al., 1977; Kampfe et al., 1993; Sudhaus, 1974), and in other nematode species (Ivashkin and Babaeva, 1973). Intra-uterine birth with resulting death of the parent is relatively common in rhabditid nematodes and is typical in *Heterorhabditis* (Johnigk and Ehlers, 1999) and has also been observed in *Mehdinema allii*, *Metacrobeles amblyurus*, and *C. elegans* (Chiu et al., 2002; Johnigk and Ehlers, 1999; Luong et al., 1999). Viviparity and matrotrophy are phenomena of considerable biological interest, and some animal lineages have evolved a form of matrotrophy in which embryos ingest maternal tissues (Blackburn, 1998). In the case described here, the phenotypic plasticity of *C. elegans* associated with stress is remarkable. The body contents of the parent seem to allow the resulting progeny to achieve a large body size for dauers, and the maternal body may also protect progeny against environmental stresses (Blackburn, 1998).

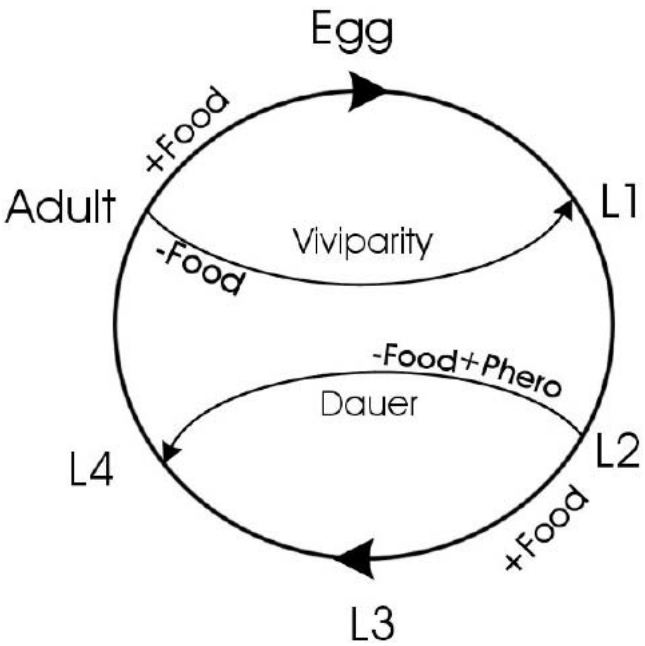

FIG. 8. A depiction of the *Caenorhabditis elegans* life cycle with facultative vivipary indicated. Vivipary is the development of first-stage larvae (L1) within the adult body before live birth, with maternal body contents consumed by L1. The two inside loops that include vivipary and the dauer stage are complementary stress-survival paths in the life cycle of *C. elegans*. Starvation and environmental stress induce facultative vivipary. Low food and elevated concentration of nematode pheromone induce *C. elegans* development to the dauer stage. L = Larva. Number indicates the stage of development.

Most significantly, facultative vivipary results in a reduction of *C. elegans* adult life span (Chen and Caswell-Chen, 2003). Evolutionary theories of aging and life span in different genders stipulate that the longer-lived gender will be the gender that experiences the lower mortality in nature. The evolution of longer life span in male *C. elegans* as compared to hermaphrodites may be a result of the greater mortality in hermaphrodites because of bagging (McCulloch and Gems, 2003).

We have shown that vivipary is an altruistic adult behavior and an inducible pathway that allows some larvae to reach the dauer stage under starvation and stress. Our observations are consistent with the dauer and vivipary being complementary life-history traits as fitness-enhancing adaptations relative to ephemeral resources and harsh environments. *C. elegans* serves as a model system to explore functional and evolutionary questions on longevity, developmental plasticity, parental investment in progeny, and colonizer life-history traits that have intrigued reproductive and evolutionary biologists (Blackburn, 1998).

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