Extended Notes

Note 1: Laboratory ecosystem

Development of a simple laboratory ecosystem: Complex ecosystems with many species and dynamic environments captivate the imagination: the Serengeti plain of Africa with its spectacular migrations, or the Amazon rainforest with its numerous species interacting in intricate food webs[1,2]. While the complexity of these systems is part of their appeal, it also presents challenges for experimental analysis and understanding. As an alternative approach to address fundamental questions about the function of ecosystems, we employed reductionist logic by conceiving of the simplest possible ecosystem. We reasoned that even the simplest ecosystem would embody basic principles of population dynamics that are relevant to complex ecosystems. Furthermore, a simple ecosystem offers powerful experimental advantages, since it can be analyzed, manipulated, and modeled. This logic leads to a question: what are the essential features of an ecosystem that must be included in a simple laboratory ecosystem? The formal definition of an ecosystem is that it is the unit of a community of organisms and their non living environment[3]. All ecosystems occupy a finite amount of space[4]. A simple ecosystem would have a small volume – this is quantitatively different from the vast Serengeti, but not qualitatively different. All ecosystems have a flux of nutrients to support organismal life [5]. A simple ecosystem would have a controlled influx of nutrients - this is different from a complex ecosystem based on sunlight that is converted to chemical energy by photosynthesis leading to trophic pyramids, but not qualitatively different. A simple ecosystem would have two species capable of indefinite reproduction - this is quantitatively different from the Amazon rainforest with thousands of species, but not qualitatively different[3]. Every species in the Amazon displays population dynamics: fluctuations in the size and age distribution of its populations. We propose that understanding the basic principles of population dynamics in a simple ecosystem will be relevant to complex ecosystems where the population dynamics of multiple species play out in parallel over time.

To generate such a simple ecosystem, we constructed an environment consisting of a plastic vial with 5 ml of water containing simple salts. To provide essential nutrients, we periodically added *E. coli* bacteria, the first species in the system. The *E. coli* do not replicate because the solution does not contain a carbon source to support bacterial cell division, and their controlled influx can be considered as migration. The replicating and main species in this environment is *Caenorhabditis elegans*, a terrestrial nematode. *C. elegans* is a good choice for this simple ecosystem for several reasons: (1) *C. elegans* is an important model organism for studies of basic biological processes, and there is a wealth of information about its genetics and biology. Procedures for measuring individual traits are well established, and this facilitates the creation of realistic models. (2) It has two sexual forms: self-fertile hermaphrodites and males. In liquid culture systems, essentially all animals are hermaphrodites, because males do not mate effectively in these conditions. A population of self-fertile hermaphrodites simplifies the analysis and modeling. (3) *C. elegans* has a rapid life cycle and a short adult lifespan –it develops from an egg to a fertile adult in about 3 days, and the adult lifespan is about 15 days. One of the most difficult challenges of studying population dynamics in an ecosystem is the time necessary to observe generational cycles, so this aspect of *C. elegans* biology makes it an ideal choice. A typical population dynamic experiment that lasts 100 days includes many generational cycles of *C. elegans*. In our experience, this system makes it possible to sustain and periodically measure *C. elegans* populations for months. We anticipate that the population could be sustained indefinitely with regular maintenance. Thus, we refer to this as a **laboratory ecosystem**, since it has the essential features of a simple ecosystem described above.

Environment of the laboratory ecosystem: The environment for the laboratory ecosystem can be considered in two time phases: initialization and maintenance. To **initialize** the environment, we add (1) 5 mL of a simple salt solution with cholesterol called S-Medium[6] to a 50 mL plastic culture bottle, and (2) 10 mg of *E. coli* bacteria that were grown overnight in nutrient

medium and then concentrated by centrifugation. To **maintain** the environment, we periodically remove 10% of the volume (0.5 mL) and replace it with an equal amount of S-Medium medium (0.5 mL) containing concentrated *E. coli*. We refer to the volume removal as culling, because it removes *C. elegans* and bacteria as well as the liquid medium. To **measure** the environment, we use a spectrophotometer to determine the optical density (OD600), which is proportional to the number of *E. coli.* We convert the OD600 value to a concentration of *E. coli* bacteria (mg/mL) using a standard curve (Fig. 1A, Suppl. Fig 1).

While the environment is initially S-Medium, we think that over time the presence of live *E. coli* and live *C. elegans* conditions the medium with complex chemicals, such as waste products and pheromones, as well as debris from dead worms. We think these chemicals gradually accumulate and eventually reach a steady state as a result of periodic culling and feeding. We have not experimentally determined the composition of the medium over time, so we do not know the number and nature of these chemicals or the time necessary for their concentrations to come to equilibrium.

Animals in the laboratory ecosystem: To **initialize** animals, we add about 250 *C. elegans* larvae to the culture medium. No other *C. elegans* are added during the experiment, so all additional *C. elegans* in the laboratory ecosystem are progeny of these hermaphrodites or their descendants. To **measure** animals, we remove a sample of the laboratory ecosystem by culling. Culling can be considered a form of extrinsic mortality, which in natural ecosystems results from predation, disease, accidents, or other causes. For example, in the Serenegeti a migrating wildebeest might accidentally drown crossing the Mara River, or it might be predated by crocodiles during the crossing. Culling can also be considered as a form of outmigration, which is also typical of natural ecosystems. An aliquot of the sample derived by culling is analyzed using a COPAS biosort designed for automated counting of *C. elegans,* or by visual inspection using a dissecting

microscope (Fig. 1A, Suppl. Fig. 1). The COPAS biosort instrument flows worms in liquid past a laser detection system.

As worms pass the laser, the animal's body blocks light transmission, and a detector measures the duration of the interruption, called the time of flight. Time of flight is proportional to the length of the worm, an indication of the developmental stage of the animal. This instrument allows high throughput analysis of the laboratory ecosystem, so that we can measure many different populations. However, the information from this system is limited. Time of flight does not accurately determine the stage of the animals, so while a positive signal indicates the presence of a worm, we do not know the precise developmental stage of that animal. In addition, the COPAS biosort does not reliably give a positive time of flight for eggs because of their small size. Thus, the COPAS biosort measures the number of larvae and adults in the culture. Visual inspection with a dissecting microscope provides detailed information about the number and stage of the animals, including the number of dauer larvae, since stages can be scored by a trained observer. However, visual inspection cannot be performed with high throughput. We used visual inspection to validate that the COPAS biosort provides reliable measurements of the number of larval and adult worms.

Data analysis and summary statistics in the laboratory ecosystem: The laboratory ecosystem displays two phases of population dynamics, which we named the **initialization** phase and the **culture** phase. (1) During the **initialization** phase, the number of worms in the population increases to a uniquely high peak. The initialization phase begins when the experiment begins, and we define the end of the initialization phase as the time when the population first declines to the average number of worms for the experiment. The initialization phase is characterized by three summary statistics: time to first peak, number of worms at first peak, and duration of initialization phase (Figure 1B). The initialization phase displays these unique characteristics because bacterial food accumulates during the first several days of the experiment due to the

small number of worms used to begin the culture; as the number of worms increases, the worms eventually ingest the accumulated food as they approach the high first peak. When the accumulated food is consumed, then the number of worms in the population begins to decline. (2) During the **culture** phase, the number of worms in the population stabilizes and oscillates around the average number of worms. The culture phase is characterized by three summary statistics: number of worms at highest peak (maximum), number of worms at lowest valley (minimum), and duration of culture phase (Figure 1B). The initialization phase and culture phase together represent the entire laboratory ecosystem experiment, which is typically 100 days. The entire experiment is characterized by one summary statistic: the average number of worms (Figure 1B). It is also possible to compute the average number of worms in the initialization phase and the culture phase separately.

The number of worms at highest peak and number of worms at lowest valley are informative summary statistics because they describe the resilience of a population. If a population displays a small number of worms at the lowest valley, then the population is at higher risk of extinction, which is defined as 0 worms at the lowest valley. The ratio of the number of worms at highest peak and number of worms at lowest valley is an important measure of dynamic volatility that may also have implications for the risk of extinction.

Note 2: Computational simulation

Development of an agent based model: To complement the laboratory ecosystem, we developed a computational simulation. Our goal was to develop a realistic simulation of the laboratory ecosystem. This task was facilitated by our decision to design the laboratory ecosystem to be well suited for simulation by an agent based model. Like all models of biological systems, this simulation is a simplification of reality. However, in designing the simulation, we attempted to

retain the essential elements of reality while simplifying when precision did not appear to be critical to the function of the system or when simplification was a practical approach to address a complex reality. The simulation has several advantages compared to the laboratory ecosystem, and together they form a powerful combination of experimental approaches to understand a complex system. The simulation offers three main advantages: (1) It provides detailed information about the life history of individual animals, which cannot be measured in the laboratory ecosystem. (2) The simulation makes it possible to model a wide range of different environments and worms with different traits. The laboratory ecosystem offers a limited ability to create different environments and a limited ability to investigate worm traits by analyzing mutant strains. (3) The simulation can be performed rapidly, in minutes, whereas the laboratory ecosystem takes 100 days and is laborious, requiring extensive sampling, maintenance and analysis. This is an incredible advantage for exploring the impact of different environments and worm traits. Although running the simulation can be rapid, analyzing the data from the simulation can be time consuming. Furthermore, our experience was that designing and coding the simulation was not rapid, since it required years.

We chose to use an agent based model to simulate the laboratory ecosystem[7]. Agent based models consist of an environment and agents. In our simulation the agents are worms. The behavior of agents is determined by a series of rules, which can be considered as if/then statements or decision trees. For worms, these rules specify behaviors such as feeding and egg laying, transitions between developmental stages, transition to death, etc. The computational simulation traces the life history of each individual worm by accounting for (1) biomass ingested as bacterial food, and (2) biomass spent as energy or converted into growth, eggs, or dauers. To calibrate parameters in the computational simulation, we measured single worm characteristics in the laboratory (Figure 2, Suppl Figs. 2-7, Suppl Table 4-7). Parameters of the model were fitted to these measurements where available. Some parameters could not be measured in the

laboratory; in these cases the parameter values were merely reasonable guesses (Note Table 2, 6).

The second aspect of an agent based model is the environment. The simulated physical environment is 5 mL of liquid medium. The medium contains bacteria at a concentration that can be calculated. The concentration of bacteria influences the behavior of the worms. Worms in the simulation do not interact directly, so we do not consider worms to be a part of the environment. However, worms do interact through the common bacterial food source, such that if worm #1 ingests bacterial food, that bacteria is not available for ingestion by worm #2. Time is quantized in 3-hour intervals, so that bacteria consumption, growth, and all life stage transitions are updated every 3-hour time period. We chose three hours because it is a relatively short time compared to the three day (72 hour) generation time, so it provides good granularity for the trajectory of each individual. Shorter time periods provide additional granularity but are more computationally demanding.

Environment of the computational simulation: The environment for the computational simulation can be considered in two phases: initialization and maintenance. To **initialize** the environment, the computational simulation adds a specified mass (mg) of *E. coli* bacteria to the virtual 5 ml volume (Note Table 3). To **maintain** the environment, the computational simulation periodically remove a specified percent of the *E. coli* bacteria (culling), and adds a specified mass (mg) of *E. coli* bacteria (feeding) (NoteTable 4). To **measure** the environment, the computational simulation calculates the mass of bacteria in the bacterial node at the beginning of each time step.

The simulation permits a variety of programmed schedules for feeding and culling. Feeding is specified by two variables: (1) The mass of bacteria added. (2) The frequency of addition. Culling is specified by three variables: (1) The fraction of animals/bacteria culled. (2) The stage of animals culled. (3) The frequency of culling. It is assumed that the population is well mixed; therefore, if all-stage culling is chosen, then an equal amount of each stage will be culled.

For example, if 10% all-stage culling is the user-programmable parameter for a simulation, then the model assumes that 10% of all eggs, 10% of all larvae, 10% of all dauers, 10% of all adults, and 10% of all parlads are removed, along with 10% of the uneaten bacterial food remaining from previous feedings. All-stage culling corresponds to the culling procedure performed in the laboratory ecosystem. The simulation also has the option of stage-specific culling, which cannot be achieved in the laboratory ecosystem. This option is useful for modeling single generations without contamination from offspring and for creating conditions in which old age as a cause of adult death can become significant.

In the computational simulation, bacteria exist in the bacterial node. Bacterial transitions (bt) can cause bacteria to enter or leave the bacterial node. There is only one way for bacteria to enter the bacterial node: **(1) A user-programmable input (i) for bacteria (b) named bt(i>b)**. There are three ways for bacteria to leave the bacterial node: (**1) Bacteria (b) can be ingested by a larva (l) in a transition named bt(b>l).** The amount of bacteria that a larva ingests is determined as described below. (**2) Bacteria (b) can be ingested by an adult (a) in a transition named bt(b>a).** The amount of bacteria that an adult ingests is determined as described below. (**3) Bacteria (b) can transition to death by culling (c) in a transition named bt(b>c).** The percent and frequency of bacteria culled is a user-programmable environmental parameter.

The major features of the laboratory ecosystem environment are modeled realistically in the simulation. The environments of the computational simulation and laboratory ecosystem have the identical volume, and the bacterial feeding and culling frequencies and amounts can also be programmed to be identical to the laboratory ecosystem. One difference between reality and simulation is that we think live *E coli* and live *C. elegans* condition the laboratory ecosystem medium with complex chemicals, such as waste products and pheromones, as well as debris from dead worms. Because we do not know the composition or consequence of these chemicals, we did not attempt to simulate this feature of the environment. Furthermore, the laboratory

ecosystem experiences small fluctuations in environmental conditions such as temperature and humidity, whereas the simulation is assumed to be constant.

Animals (agents) in the computational simulation: Worms were defined as being in one of five life stages: egg, larva, dauer, adult, or parlad (parent/larva/dauer). *C. elegans* has four larval stages during reproductive growth, L1, L2, L3, and L4, and one larval diapause stage called dauer. Worms transition between larval stages by molting. We chose to simplify the larval stages rather than specific every larval molt.

Egg: *C. elegans* hermaphrodites produce oocytes in the gonad that are fertilized by sperm in the spermatheca; the fertilized egg begins development as it passes through the uturus and is typically laid in the environment at the 32-128 cell stage. Eggs are provisioned with adequate nutrients to support development and do not ingest nutrients. The eggshell protects the developing embryo from the environment. The purpose of the life stage is to accomplish development from the fertilized single cell to the L1 larva. When development is complete, the L1 larva emerges and the egg shell is shed.

In the computational simulation, eggs are derived from one source: **(1) An adult (a) can lay an egg (e) in a transition named wt(a>e)**. All eggs have a mass of 65 ng; this value is based on laboratory measurements of L1 size, which were converted to mass (65.6 ng +/-11, n=4 N=166). Eggs do not eat bacterial food and do not use mass for maintenance; thus, the mass of an egg remains constant over time. Eggs can transition to two fates: (**1) An egg (e) can transition to a larva (l) in a transition named wt(e>l).** An egg hatches after 15 hours (5 time periods), at which time it transitions to a larva with a mass of 65ng. The time to hatching was based on laboratory measurements (15 hours +/-1, n=4). (**2) An egg (e) can transition to death by culling (c) in a transition named wt(e>c).** The percent and frequency of eggs culled is a user-programmable parameter.

The simulated eggs behave realistically, since the mass of an egg and the duration in the egg stage are the two main traits, and both were measured in the laboratory. The assumption that no mass is lost when an egg transitions to a larva is a simplification, since there is likely a small loss of mass when the egg shell is shed, and the efficiency of conversion between yolk and L1 larva is likely less than 100%. The assumption that all eggs have the identical mass and duration as eggs is a simplification, since there is some variability between individuals.

Larva: The larva is a feeding stage whose main biological imperatives are to ingest bacteria, grow, and transition to a sexually mature adult, all the while surviving periods of nutrient deprivation or other harsh conditions. When an L1 larva emerges from the egg, it is highly starvation resistant. As it begins to feed and grow, it becomes more vulnerable to starvation. With adequate nutrition, it molts to form an L2 larva. A L2 larva grows and molts into a L3 larva or a dauer larva, depending on environmental factors including the level of food, level of dauer pheromone, and temperature. The dauer larva is discussed below. With adequate nutrition, a L3 larva grows and molts into a L4 larva, the final larval stage. The L4 larva is characterized by growth and development of the reproductive system. With adequate nutrition, a L4 larva grows and molts into a sexually mature adult. The larval growth rate, and the size and mass of the larva at the L4 to adult transition, are highly dependent on the concentration of bacterial food in the environment.

In the computational simulation, larvae are derived from two sources: **(1) When it hatches, an egg (e) transitions to a larva (l) in a transition named wt(e>l).** The mass of the larva is 65 ng, and the age is zero time steps. **(2) A dauer (d) transitions to a larva (l) in response to high levels of bacterial food in a transition named wt(d>l).** The mass and age of the larva are equal to the mass and age of the larva that formerly transitioned into dauer, which can be different for each animal.

A larva (l) eats bacterial (b) food in a transition named bt(b>l). The amount of bacterial food that a larva eats depends on three variables – the mass of the larva, the concentration of

bacterial food in the culture medium, and the number of worms in the population. Food that a larva ingests is used in two ways: growth and maintenance. The growth rate of larvae in different concentrations of food was measured in the laboratory (Fig. 2E-H, Suppl Fig. 2B, 4). These measurements were used to determine how much simulated worms eat and grow, as described below. The amount of food used for maintenance is a reasonable guess.

Larvae can transition to four fates: **(1) A larva (l) can transition to an adult (a) in a transition named wt(l>a).** When bacterial food is abundant, a larva transitions to an adult after 20 time steps =2.5 days if it has achieved a minimum mass of 800 ng. If bacterial food is not abundant, then a larva grows more slowly and it can transition to an adult when it attains a minimum mass of 800 ng from time step 21-28 (3.5 days). The time of larval transition to adult and mass of the larva at the transition were based on laboratory measurements of growth and maturation (Fig. 2E-H, Suppl Fig. 2B, 4, Suppl Table 5). **(2) A larva (l) can transition to a dauer (d) in a transition named wt(l>d).** A larva has a specified probability of turning into a dauer if it experiences two successive time periods in which the bacterial food concentration is less than 0.05 mg/ml and the mass of the larva is between 0.6 and 2.0 times the standard dauer mass (equivalent to 153 – 456 ng). Standard dauer mass is fixed from worm parameters as the geometric mean between larva mass on hatching (65 ng) and minimum adult mass (800 ng). The geometric mean of 65 and 800 is 228 ng. The duration of low bacterial food that leads to a dauer transition and the mass range for larva that can transition to dauer are reasonable guesses. The actual probability of a larva transitioning to a dauer per unit time is computed by a formula shown in Note Table 2. A larva cannot transition to become a dauer a second time. **(3) A larva (l) can transition to death by starvation (s) in a transition named wt(l>s)**. A larva starves if it experiences two successive time periods in which bacterial food concentration is less than the dauer food threshold defined above and the mass of the larva is outside the range for dauer transitions (65-136 ng or 457-799 ng). A larva also transitions to starvation death if their age exceeds 28 time steps and they have not attained the minimum adult mass of 800 ng. The

duration of low bacterial food that leads to a starvation transition and the mass range for larva that can transition to starvation are reasonable guesses. **(4) A larva (l) can transition to death by culling (c) in a transition named wt(l>c).** The percent and frequency of larval culling is a user-programmable parameter.

The simulated larvae are fairly realistic. The growth rate of larvae in different concentrations of food and the transition to sexually mature adults were measured in the laboratory and are well modeled in the simulation. The environmental conditions that trigger starvation and transition to dauer are reasonable guesses that may be too stringent or lenient. The model does not include dauer pheromone, which is a simplification since it is established that pheromone influences the probability of larva transition to dauer [8]. The assumption that all larva that are too small or too large to transition to dauer have the same thresholds for starvation is a simplification, since newly hatched L1 larvae are known to be highly starvation resistant, and each larval stage may have a different ability to resist starvation. The assumption that larvae starve if they fail to achieve the minimum adult mass after some period of time is reasonable, but this time could not be measured and is a guess. The assumption that a certain amount of biomass is consumed for metabolic function is undoubtedly true, but the precise amount could not be measured and the simulated amount is a guess. Likewise, the assumption that ingested bacterial biomass is converted to worm mass during growth at less than 100% efficiency is undoubtedly true, but the precise efficiency could not be measured and the simulated efficiency is a guess. As described in Note 3, we guessed the value of these two parameters by calibrating the simulation with a training set of laboratory ecosystem data.

Dauer: The dauer is an alternative L3 larval stage considered a diapause state. L2 larvae molt to form dauer larvae under harsh environmental conditions, such as low levels of food, high temperature, and high levels of dauer pheromone that signal a high density of worms [8]. The dauer larvae do not feed, and they have a cuticle that is resistant to chemical insults. The dauer larvae

are metabolically active and monitor their environment, such that they will transition to the reproductive L4 larval form in response to high levels of food in the environment. They also engage in behaviors that promote dispersal. Thus, dauers must consume energy to sustain their metabolic activity, and although they are long lived, they cannot survive indefinitely. The biological imperative of the dauer is to survive periods of stress and deprivation and disperse, so that they can emerge when conditions improve and initiate a new population.

In the computational simulation, dauers are derived from two sources: **(1) A larva (l) transitions to a dauer (d) in response to low levels of bacterial food in a transition named wt(l>d).** The mass and age of the dauer are equal to the mass and age of the larva that made the transition. **(2) A parlad (p) transitions to a dauer (d) in a transition named wt(p>d).** The mass and age of a dauer derived from a parlad is equal to 228 ng and 5 time steps. Thus, dauer is a response to low levels of bacterial food. A dauer does not eat bacterial food and does not use mass for maintenance; thus, the mass of a dauer remains constant over time.

A dauer can transition to three fates: **(1) A dauer (d) can transition to a larva (l) in a transition named wt(d>l)**. A dauer will transition to a larva and resume growth if there is a sufficient concentration of bacterial food. The probability per time period for a dauer transitioning to a larva is determined by a formula shown in Note Table 2 independent of how long it has been a dauer. This is based on laboratory measurements of dauer to larva transitions in different concentrations of bacterial food (Fig. 2I-L, Suppl. Fig. 2C, 5, 6, Suppl Table 6). A dauer "remembers" the mass and age that it had when it transitioned from a larva or a parlad when it resumes life as a larva. The time spent as a dauer does not count toward the time limit to reach maturity. **(2) A dauer (d) can transition to death by starvation (s) in a transition named wt(d>s)**. Over time, dauers lose the ability to transition into a larva . The model accounts for this gradual loss of viability by including an increasing age-dependent probability that a dauer will die of starvation. Thus, a low percentage of dauer selected randomly will die of starvation in each

time-step (Note Table 2). **(3) A dauer (d) can transition to death by culling (c) in a transition named wt(d>c).** The percent and frequency of dauer culling is a user-programmable parameter.

The simulated dauer are fairly realistic. The bacterial concentrations that trigger dauer to larva transitions were measured in the laboratory and are accurately modeled in the simulation. The assumption that a dauer does not consume biomass for metabolic function is a simplification, but given the longevity of a dauer the rate of consumption must be small. The model does not include dauer pheromone, which is a simplification since it is established that pheromone influences the probability of a larva transitioning to a dauer [8].

Adult: The sexually mature adult hermaphrodite is the reproductive form. The hermaphrodite gonad first produces about 350 self sperm, which are stored in the spermatheca. Next the gonad begins to produce oocytes, and self sperm are used to fertilize oocytes. Adults with abundant bacterial food produce large numbers of progeny in the first several days, and then progeny production declines due to reproductive aging and sperm depletion. Adults must ingest bacteria to obtain the biomass to generate eggs. Thus, the biological imperative of the adult is to eat and produce eggs. If the sensory system of the adult determines that bacterial food levels are very low, then the adult will execute a behavioral program that causes it to cease egg laying [9]. After about 15 hours, the fertilized eggs will hatch inside the hermaphrodite, an event called matricidal hatching that leads to adult death. Adults display age-related degeneration, which leads to a mean lifespan of about 15 days.

In the computational simulation, adults are derived from one source: **(1) A larva (l) can transition to an adult (a), in a transition named wt(l>a).** As described above, a larva transitions to an adult if it grows to a mass of more than 800 ng between time steps 20-28. The mass of the adult at the transition is equal to the mass of the larva that made the transition. The adult age at the time of transition is 0 time steps.

An adult eats bacterial food in a transition named bt(b>a). The amount of bacterial food that an adult eats depends on four variables – the mass of the adult, the age of the adult,

the concentration of bacterial food in the culture medium, and the number of worms in the population. Food that an adult ingests is used in three ways: growth, maintenance, and generating eggs. The growth rate of adults in different concentrations of food was measured in the laboratory (Fig. 2E-H, Suppl Fig. 2B, 4, Suppl Table 5). These measurements were used to determine how much simulated worms eat and grow, as described below. The amount of food used for maintenance is a reasonable guess.

The rate of egg laying in different concentrations of food was measured in the laboratory (Fig. 2A-D, Suppl Fig. 2A, 3, Suppl Table 4). These measurements were used to determine how much simulated adult worms eat and lay eggs. A nine-parameter function controls the number of eggs laid as a function of adult age and bacterial food concentration. The function was fitted to data points measured daily in the laboratory at six different bacterial concentrations (Fig. 2A-D, Suppl. Fig. 2A, 3). Explicitly, the number of eggs laid in a 3-hour period is given by $M\ x^n \exp(-x)$, where x is adult age scaled by a suitable parameter. The three parameters M, n, and scale are all modeled as power law functions of adult age, of the form

$$M = A_M\ age^{B_M} + C_M$$

$$n = A_n\ age^{B_n} + C_n$$

$$scale = A_{scale}\ age^{B_{scale}} + C_{scale}$$

The nine parameters A, B and C are for best conformity with laboratory data on fertility. The values we computed via this process are shown in Note Table 1. Note Figure 1 shows one example of the close correspondence between the laboratory data for egg laying and this nine-parameter function for the bacterial food concentration 0.5 mg/ml.

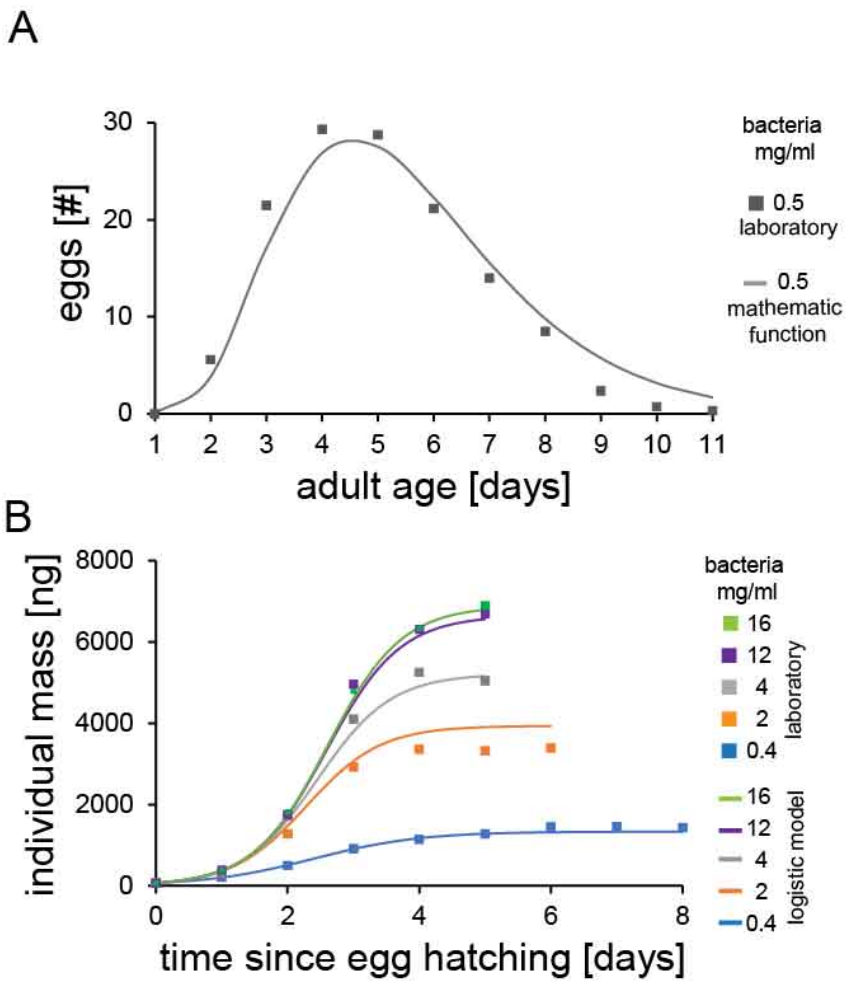
Note Table 1. Values of nine-parameter function that controls progeny production.

	Scale[1]	n[2]	M[3]
A	0.233	-1.867	-17.33
B	-0.762	-0.299	-0.166
C	0.581	5.908	30.08

[1] $scale = A_{scale}\, age^{B_{scale}} + C_{scale}$

[2] $n = A_n\, age^{B_n} + C_n$

[3] $M = A_M\, age^{B_M} + C_M$



Note Figure 1. Comparison of laboratory data and logistic models of daily progeny production and growth. Wild-type, self-fertile hermaphrodites were cultured in S-Medium with the indicated concentrations of *E. coli* (bacteria). (A) Average daily progeny production of individual adults measured in the laboratory is displayed as grey boxes. The same data are displayed in Fig. 2A-D, Suppl. Fig. 2A, 3. The gray line shows one example of the nine-parameter function that we calculated to describe these data for the bacterial food concentration 0.5 mg/ml. There is a close correspondence between the laboratory data for egg laying and the mathematical formula. (B) The average daily mass of individual animals measured in the laboratory is displayed as color boxes. The same data are displayed in Fig. 2E-H, Suppl. Fig 2B, 4. The color lines show examples of the logistic model for five different bacterial food concentrations. The lines correspond well with the laboratory data with the same concentration of *E. coli*.

An adult can transition to three fates: **(1) A adult (a) can transition to a parlad (p) in a transition named wt(a>p).** If total food in the environment falls below a threshold of 500 ng/ml for two successive time periods, then an adult transitions to a parlad. In response to food deprivation, self-fertile adults stop laying eggs and undergo matricidal hatching[9,10]. Thus, the transition to parlad is a form of starvation death. Eggs that hatch into larva inside the adult begin to feed on the adult biomass, ultimately generating dauer larva[9,10]. The parlad has the same mass as the adult. The level and duration of low bacterial food that leads to a parlad transition is a reasonable guess. **(2) An adult (a) can transition to death by old age (o) in a transition named wt(a>o)**. Death from old age is controlled by a Gompertz curve that was calibrated to laboratory measurements (Fig. 2M-P, Suppl. Fig. 2D, 7, Suppl Table 7). This transition is independent of bacterial food and fertility. Two user-programmable parameters control the timing and the steepness of the Gompertz curve. The probability of dying of old age per unit time is given by (exp(age/τ)-1) / A, where age is measured from the transition from larva to adult, in 3-hr periods $\tau = 28.6$ $A = 168 * (\exp(5) - 1) = 24{,}765$, where 5 is the number of Gompertz e-foldings in a lifespan. **(3) An adult (a) can transition to death by culling (c) in a transition named wt(a>c).** The percent and frequency of adult culling is a user-programmable parameter.

Simulated adults display realistic growth, egg laying, and adult lifespan, since these are based on laboratory measurements. The threshold and duration of low levels of bacteria that trigger the transition to parlad are reasonable guesses that may be too stringent or lenient. The assumption that a certain amount of biomass is consumed for metabolic function is undoubtedly true, but the precise amount could not be measured and the simulated amount is a guess. Likewise, the assumption that ingested bacterial biomass is converted to worm mass during growth or egg production at less than 100% efficiency is undoubtedly true, but the precise efficiency could not be measured and the simulated efficiency is a guess. As described in Note 3, we guessed the value of these two parameters by calibrating the simulation with a training set of laboratory ecosystem data.

Parlad: If the sensory system of the adult determines that bacterial food levels are very low, then the adult will execute a behavioral program that causes it to cease egg laying[9,10]. This behavioral response is presumed to be adaptive, since depositing eggs into an environment with low levels of food makes hatching larvae vulnerable to starvation. About 15 hours after the cessation of egg laying, the fertilized eggs in the uterus hatch inside the adult, resulting in matricidal hatching and adult death. Because larvae can ingest the hermaphrodite's soma as a source of nutrition, they can mature inside the adult until they reach the dauer stage[9,10]. The dead or dying adult hermaphrodite with live progeny inside is a common phenotype called egg-laying defective (Egl), also referred to as a "bag-of-worms". We chose the name parlad (parent/larva/dauer) for this form to emphasize that it is a colony with multiple individuals that are progeny of a single hermaphrodite.

In the computational simulation, parlads are derived from one source: **(1) An adult (a) can transition to a parlad (p) in a transition named wt(a>p).** An adult transitions to a parlad when it ceases egg laying in response to low levels of bacterial food. A parlad does not eat bacterial food and does not use mass for maintenance; thus, the mass of a parlad remains constant over time.

A parlad can transition to two fates: **(1) A parlad (p) can transition to starvation death (s) and generate dauers (d) in transitions named wt(p>s) and wt(p>d), respectively.** The parlad is dead and does not eat. The progeny are alive – the eggs transition to larvae inside the hermaphrodite, the larvae feed on the adult biomass and grow, and finally the larvae transition to dauer[9,10]. For the simulation, a parlad generates dauers with a mass of 228 ng after 30 hours (10 time periods), at which time the adult transitions to starvation death. The time to dauer generation was guessed and is independent of external conditions. The number of dauers generated is determined by dividing the adult mass by 228 ng and rounding to the highest whole number. The remaining mass transitions to death by starvation. **(2) A parlad (p) can transition to death by**

culling (c) in a transition named wt(p>c). The percent and frequency of parlad culling is a user-programmable parameter.

Growth of larvae and adults: Growth is a function of worm mass and bacterial food concentration in the medium. There is a single, four-parameter function that determines growth, and it is continuous between larval and adult life stages. The function was fitted to daily measurements of worm mass in the laboratory at five different bacterial concentrations (Fig. 2E-H, Suppl. Fig. 2B, 4, Suppl Table 5). Explicitly, the form of the function is the logistic equation, $\frac{dx}{dt} = Kx(1 - bx)$, where K is the exponential growth rate and b is the reciprocal of the terminal mass. Based on laboratory data, we find K is well represented by $K = 1.78 * \tanh(2.13 * food)$, when time is measured in days and bacterial food is measured in mg/ml. *b* is well represented by $b = 1.30 * 10^{-4} - \frac{2.49*10^{-4}}{food}$, where *b* is measured in reciprocal nanograms and bacterial food is measured in mg/mL. Note Figure 1B shows comparisons of laboratory measurements and this four-parameter function.

Bacterial food ingestion by larvae and adults: The quantity of food a worm eats in a time period is the most complicated function in the simulation. Food ingestion by individual worms is difficult to measure in the laboratory because of the small size of individuals. Therefore, in the simulation ingestion is computed from the growth and fertility curves defined above. We assume that in each time period an individual worm ingests an amount of bacterial food mass that is greater than the amount of mass it grows and/or generates in eggs. There is a user-programmable parameter that determines the efficiency of conversion of biomass. In each time step, a worm is determined to ingest a mass of *E. coli* equal to:

$$\frac{mass\ it\ grows + mass\ of\ eggs\ it\ lays}{effeciency\ of\ conversion}$$

For example, if the efficiency of conversion is 50%, and a larva is determined to grow by 5 ng, then the larva will ingest 10 ng of bacterial food. Food ingestion can be limited by any of three

things: (1) The worm's intrinsic capacity to ingest food at this stage of life. How much would it eat if food were plentiful? (2) The rate at which growth medium is pumped through the digestive tract is a limit when food concentration is low. (3) Competition with other worms may be a limit if the number of worms is large. In other words, food concentration may be substantially depleted by other worms during the course of the three-hour time period. The first and second are represented together in a function called “appetite” which computes the worm’s needs for growth, egg laying, and metabolism. Growth and egg laying are already functions of food concentration, so they incorporate the worm’s limited capacity to filter feed when food concentration is low. Appetite is adjusted by (1/efficiency) to account for metabolic losses in converting food to growth or eggs. The third is a function called “portion”, which is computed simply by dividing the worm’s appetite by the sum of all appetites of all worms, and multiplying this fraction by the total amount of food in the vial. Specifically, the larva’s appetite is computed as a “reduced quantity” based on (x) a fixed fraction of its mass and (y) the amount of food, at present concentrations, that it can filter in 3 hours. The “reduced quantity” is computed as $w = \frac{xy}{(x+y)}$. A further restriction on the amount eaten is the pro-rata portion of food available computed as the z=[total food] * [appetite of this worm] / [Σ appetites of all worms]. The final computation of the amount consumed by this larva is the reduced quantity $\frac{wz}{(w+z)}$. To validate our implementation of bacteria ingestion, we compared the concentration of bacteria in the laboratory ecosystem (Fig. 1C) to the simulation (Fig. 3C). Both showed qualitatively similar fluctuations with an early peak in the initialization phase and much lower levels during the culture phase.

Data analysis and summary statistics in the computational simulation: The simulation contains two types of data: (1) Detailed life histories of each individual, which are longitudinal data. (2) Detailed information about the behavior of the population, which are emergent properties determined by the traits of worms and the environment.

For each individual, the simulation records at each time step the stage, mass, bacteria mass ingested, and mass consumed for metabolic maintenance, growth, and egg laying. Thus for each individual it is possible to determine the growth rate as a larva and adult and calculate key summary statistics: mass at sexual maturity, sexual maturity growth span, maximum mass, and maximum mass growth span (Fig 2G , Suppl. Table 5). For each individual that lays eggs, it is possible to determine the egg-laying curve and calculate key summary statistics: peak progeny number, reproductive span to peak, total progeny number, and total reproductive span (Fig 2C, Suppl. Table 4). It is possible to determine the larval span, dauer span, adult span, age at death and cause of death. Each individual has a flux of mass that can be calculated. Mass flowing into the individual consists of (1) the starting mass of the egg or dauer, and (2) ingested bacterial mass. Mass flowing out of the individual consists of (1) mass consumed for metabolic maintenance, (2) mass converted to growth, (3) mass converted to eggs or dauer, (4) mass consumed in inefficient conversion of bacteria to growth or eggs, and (5) mass remaining at time of death. For each individual, the mass flowing in equals the mass flowing out, and it is possible to calculate the percent of mass used for each of the five categories.

For the behavior of the population, it is useful to consider the simulation as a flux system. It is possible to analyze each of the five worm nodes (egg, larva, dauer, adult, and parlad) and calculate the number of individuals in the node at each time and calculate key summary statistics: average worm number, maximum worm number and minimum worm number. Similarly, it is possible to analyze each of the transitions and determine the rate in worms per time. Different transition rates can be compared. For example, adults die of three causes - culling, starvation and old age - and it is possible to calculate the percent of each as a cause of death.

The computational simulation has mass accounting. Mass enters the simulation in two ways: (1) The majority of mass enters through periodic inputs of bacteria. (2) A small amount of mass enters in the form of worms that initialize the population. Mass in the simulation is converted

from bacteria to worms through worm feeding. Mass can transition between stages of worms. Mass leaves the simulation in four ways: (1) Worms utilize mass for metabolic maintenance. (2) Worms utilize mass when they grow or generate eggs, since these processes may be less than 100% efficient. (3) Worms transition to death, and their mass leaves the simulation. (4) Bacteria are removed by culling, and their mass leaves the simulation. Thus, the fate of all the mass that enters the simulation can be determined.

Note 3. Using a training data set to establish certain parameters.

To develop a realistic simulation of the laboratory ecosystem, we measured the traits of individual worms whenever it was feasible. However, some traits could not be measured, including “cost of living”, which we define as the percent of an individual’s mass that is consumed at each time step to maintain metabolism, and “metabolic efficiency”, which we define as the percent of ingested bacteria that is converted to growth mass or egg mass. To estimate these values, we used the data from one laboratory ecosystem experiment as a training set; we compared the data from replicate 2a of the laboratory ecosystem (Fig. 3A,B, 4B) to simulations with different values of “cost of living” and “metabolic efficiency.” When we used a value of 3.5%/3h for “cost of living” and 85% for “metabolic efficiency”, there was a good corresponsdance between the average number of worms in replicate 2a and the average number of worms in the simulation. We used these values for subsequent simulations, and replicate 2a was the only laboratory ecosystem data that was used as a training set.

Note Table 2. Equations and transition rules for the simulation.

worm behavior[1]	Equation[2]	Parameters[3]	Comment
larva and adult growth	$\frac{dm}{dt} = Km(1 - bm)$	$K = 1.78 * \tanh(2.13 * food)$ $b = 1.30 * 10^{-4} - 2.49 * 10^{-4} / food$	Logistic growth, continuous between larvae and adults. m is mass in ng. t is time in days. food is in mg/ml. b is in reciprocal ng.
adult egg laying	$\frac{eggs}{day} = Mx^n \exp(-x/x_o)$	$M = 17.33 * x\text{^}(-0.166) + 30.08$ $n = -1.867 * x\text{^}(-0.299) + 5.908$ $x_o = 0.233 * x\text{^}(-0.762) + 0.581$	x is adult age (days).
larva and adult feeding	$\frac{1}{food} = \frac{1}{appetite} + \frac{1}{portion}$		Adding the reciprocals assures that the food eaten will always be less than appetite and less than portion.
larva transition to dauer	$probability\ of\ dauering$ $= 0.5 \exp(-\frac{[avg\ food]}{2.5E5})$	Probability is for one-time period. Average food is the total amount of food in the medium averaged over the present time period and the start of the previous time period.	Larva is only eligible to dauer while its mass is between 137 ng and 456 ng.
dauer transition to larva	$probability = .0000324$ $[1 - \exp\left(-\frac{[avg\ food]}{2.5E5}\right)]$	Probability is for one-time period. Average food is the total amount of food in the medium averaged over the present time period and the start of the previous time period.	When a dauer transitions to a larva, the larva has the same mass and age as when it entered dauer.
dauer starvation	$probability = \frac{\exp\left(\frac{age}{800} - 1\right)}{\left(1 + \exp\left(\frac{age}{800} - 1\right)\right)}$	Probability is for one-time period. Age is number of periods that the dauer has been a dauer. 800 is the starvation time set in all experiments.	A low number of dauers starves every time step dependent on

			their age as a dauer.
parlad releases dauers	10 time periods (30h)	ε=0.66 dauer mass=228 ng	The whole of the parlad's biomass is converted to dauers with an efficiency ε.
adult mortality per unit time (die of old age)	$\frac{1}{\mathrm{A}}(\exp\left(\frac{x}{\tau}\right)-1)$	$A = 168(e^4 - 1)$ $\tau = 35.7$	Gompertz mortality Time measured in 3h periods. x is adult age.

[1]Worms feed on bacteria, grow, lay eggs, release dauers, transition to a new stage, or transition to death.

[2]Equations were created to describe worm behaviors.

[3]Equations include parameters whose values were deterimined to use these equations.

Note Table 3. Initialization of environment and agents in the simulation.

Component[1]	**Environment/agent[1]**	**unit**	**user programmable input values[2]**	**Description[3]**
bacteria	environment	mass in mg	5 or 10	
egg	agent	number	0, 100 or 250	65 ng; -1 to -5 time steps
larva	agent	number	0, 250, or 1000	228 ng; 0 to 5 time steps
dauer	agent	number	0, 100, 250, or 1000	228 ng; 5-15 time steps

[1]Bacteria are considered part of the environment, whereas egg, larva and dauer are agents.
[2]All of these inputs and no others were used in this study. Only one input value was used per simulation experiment.
[3]Mass of worms (ng), and age of the worms in time steps. Eggs are laid at time step -5, and eggs hatch at time step 0.

Note Table 4. Maintenance of environment and agents in the simulation.

Variable[1]	unit	user programmable input values[2]	Simulation duration[3]
bacterial addition interval	hours	24 or 48	100 days
bacterial addition amount	mg	5 or 10	100 days
fixed bacteria concentration[4]	mg/ml	0.04-16	one worm generation - only used to model a constant food environment
culling interval	hours	24 or 48	100 days
culling amount (all stages and bacteria)	percent	0, 5, or 10	100 days
culling larva (stage-specific)	percent	0-90	100 days
culling dauer (stage-specific)	percent	0-90	100 days
culling adult (stage-specific)	percent	0-40	100 days

[1]The amount and interval of bacteria addition and culling are specified for each simulation run.
[2]All of these inputs and no others were used in this study. Only one input value was used per simulation experiment.
[3]Most simulations had a duration of 100 days, but simulations with a constant concentration of bacteria had a duration of one worm generation.
[4]A fixed concentration of bacteria was used in simulations that compared virtual worms to live worms cultured in a fixed concentration of bacteria in the laboratory (Fig. 2, Suppl. Fig. 2-7).

Note Table 5. Agent traits with a user programmable input value in the simulation.

worm node[1]	Trait[2]	unit	user programmable input values[3]	Description	Determined[4]
adult	maximum adult life span	days	25, 40, or 60	This number scales the Gompertz function for senescent death.	laboratory measurement for wild type - 40 days.

[1]The worm stage affected by the user programmable input value.
[2]The property or trait of the agent specified by the user programmable input value, in this case maximum adult lifespan.
[3]All of these inputs and no others were used in this study. Only one input value was used per simulation experiment.
[4]User programmable input values were determined by laboratory measurement or estimated. The maximum adult lifespan of wild-type worms was measured to be ~40 days in the laboratory, and values of 25 and 60 days are theoretical maximum lifespans that do not correspond to wild type.

Note Table 6. Agent traits with a fixed input value in the simulation.

	worm node[1]	Trait[2]	unit	Fixed input value[3]	description	Transition[4]	Determined[5]
1	egg/adult	egg mass	ng	65	mass of every egg in the simulation	wt(a>e)	laboratory measurement
2	egg	duration of the egg stage	hours	15	Time before an egg hatches into a larva	wt(e>l)	laboratory measurement
3	larva	bacteria concentration triggering larva transition to starve or dauer	mg/ml	<0.25	if this condition is true larva are eligible to transition to starve or dauer	wt(l>s) wt(l>d)	estimate
4	larva	duration of low level of bacterial food that triggers larva transition to starve or dauer	hours	6	if this condition is true, larva are eligible to transition to starve or dauer	wt(l>s) wt(l>d)	estimate
5	larva	probability of larva transition to starvation	percent	50%	This trait ensures that not all larva starve at the same time and ensures variation if condition is true in rows 3 and 6. Stochastic.	wt(l>s)	estimate based on training set
6	larva	larva to dauer transition mass range	ng	137-456	larva can only transition to dauer within this mass range.	wt(l>d)	estimate
7	Larva/dauer	Mass of dauer derived from larva	ng	137-456	Dauer mass equals larva mass before transition, which must be in this range	wt(l>d)	estimate
8	larva/adult	Minimum age for larva to adult transition	hours	160	Larva cannot transition to adult before this age, irrespective of mass.	wt(l>a)	laboratory measurement
9	larva/adult	mass range for larva to adult transition	ng	800-3200	Larva cannot transition to adult unless they achieve this minimum mass.	wt(l>a)	laboratory measurement

10	larva/adult	metabolic efficiency	percent	85%	Percent of ingested bacteria mass converted into worm growth and eggs.		estimate based on training set
11	larva/adult	cost of living	percent/ hours	3.5%/3h	Mass consumed in metabolic processes (for example, locomotion).		estimate based on training set
12[6]	adult	Maximum life span	days	40	This number scales the Gompertz function for senescent death.	wt(a>o)	laboratory measurement (see Note Table 5)
13	adult	probabilty of dying of old age prior maximum adult lifespan	number	4	Another Gompertz parameter. Lower numbers correspond to an S-shaped survival curve, higher numbers to a square curve in which all adults die in a narrow window.	wt(a>o)	laboratory measurement
14	adult/parlad	duration of low level of bacterial food that triggers adult to parlad transition	hours	6	Number of time periods on average that it takes for an individual adult to transition to parlad when bacterial food concentration is below the parlad food threshold.	wt(a>p)	estimate
15	adult/parlad	bacteria concentration triggering adult transition to parlad	mg/ml	<0.0005	Bacteria concentration averaged over two time periods that induces adult transition to parlad.	wt(a>p)	estimate
16	parlad	duration of the parlad stage	hours	30	Time before a parlad bursts and releases dauers.	wt(p>d)	estimate

17	parlad/ dauer	Mass of dauer derived from parlad	ng	228	All dauers derived from parlads have this mass.	wt(p>d)	estimate
18	parlad/ dauer	number of dauers generated by parlad	number	mass parlad/mass dauer*ε	All parlad biomass is converted to dauers with an efficiency $\varepsilon=0.66$ rounded to the nearest whole number	wt(p>d)	estimate
19	dauer	bacteria concentration triggering dauer transition to larva	mg/ml	> 0.25	Bacteria concentration that scales individual propensity for dauer to larva transitions.	wt(d>l)	estimate
20	dauer	probability of dauer transition to larva	probability	3.24E-5	Probability per time step of a dauer transitioning to a larva when bacterial food concentration is above the dauer food threshold. Stochastic.	wt(d>l)	laboratory measurement

[1]The worm stage(s) affected by the fixed input value.
[2]The property or trait of the agent specified by the fixed input value.
[3]These input values and no others were used in this study, except for maximum adult lifespan as described in Note Table 5. Only one input value was used per simulation experiment.
[4]Worm transitions affected by the fixed input value.
[5]Fixed input values were determined by laboratory measurement or estimated. In some cases, estimates were based on a training data set (see Note 3).
[6]Maximum adult lifespan was measured to be 40 days. In some simulations, we analyzed virtual worms with 25 and 60 day maximum adult lifespans.

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