A population dynamics tipping point for aging as a cause of adult death

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Abstract

Populations are a fundamental level of biological organization that poses major challenges for analysis. Individual traits that influence development, diapause, reproduction, aging, and lifespan interact in complex ways to determine birth and death. Birth and death drive population dynamics and determine whether a population survives or is doomed for extinction. However, we lack a deep understanding of the relationships between individual traits and population dynamics, a major challenge in the emerging field of ecology-development (eco-devo). Here we establish a laboratory ecosystem using the model organism *C. elegans* and a computational simulation that realistically models the laboratory ecosystem. We used these platforms to investigate the conditions that permit animals in the population to die of old age, a critical step in understanding the role of aging in population dynamics. Old age as a cause of death was influenced by three conditions: maximum lifespan, rate of adult culling, and progeny number/food stability. Remarkably, populations displayed a tipping point for aging as the primary cause of adult death. With high numbers of progeny, almost all adults in a population died young, whereas a slight decrease in progeny number caused a dramatic shift in the population, and almost all adults died of old age. The conditions defined here establish a conceptual framework for understanding why certain animals die of old age in the wild, such as mayflies and elephants.

Introduction

Animals maintained in laboratories or captivity, where conditions are gentle and consistent, display age-related degenerative changes that cause progressive frailty; this frailty eventually becomes so severe that it results in death, which is referred to as dying of old age. By observing animals in these conditions, it is possible to determine the maximum adult lifespan, a life history trait that is characteristic of a species. Maximum adult lifespan varies widely between species, ranging from ~ 1 day in mayflies, ~ 40 days in *C. elegans*, ~ 80 years in Asian elephants, and ~ 120 years in humans. A major goal of aging biology research is to understand the mechanisms that control age-related degenerative changes and establish these characteristic maximum lifespans. However, animals evolved in the wild, where conditions are neither gentle nor consistent, leading to an important question: do animals living in the wild also display age-related degenerative changes that result in frailty and lead to death? Alternatively, animals in the wild may consistently succumb to other causes of extrinsic mortality such as disease, predation, accident, or starvation before the onset of age-related frailty. In a foundational paper that has influenced the field for decades, Medawar suggested that the answer to this question is important for developing a theory of the evolutionary biology of aging[1]. Furthermore, based on the studies of field scientist of that time, Medawar thought that few senescent-related deaths would ever occur in wild populations because individuals typically succumbed to extrinsic mortality. This understanding is deeply imbedded in the theory he proposed. However, starting in the 1990s, extensive field studies have been conducted to carefully examine this question, and the results are clear. Field studies have documented senescence in wild animals from insects to birds and mammals [2–4]. For many different species, animals living in the wild do display age-related degenerative changes that are likely to contribute to mortality, although they do not

typically display the extreme frailty that can be observed in animals aged in captivity. Today we know that senescence patterns display a broad diversity among the tree of life [5]. Despite these advances, a conceptual framework for understanding how aging and limited lifespan influence population dynamics has not been established.

To gain a deeper insight into how individual traits such as lifespan impact on population dynamics, we used *C. elegans*, a nematode worm that has been a major model system to investigate the molecular and cellular control of aging. *C. elegans* is well suited for life history trait experiments with its average lifespan of 15 days, ability to build clonal populations due to its hermaphroditism, and well defined lifecycle [6]. When individual *C. elegans* are cultured in the laboratory they display age-related degenerative changes that ultimately result in frailty and death, similar to other animals in captivity. However, culture of isolated individuals in a constant environment is very different from the wild, where animals live in populations and environmental conditions fluctuate. To begin to understand the role of aging in a population, we established a laboratory ecosystem comprised of *C. elegans* and the bacteria *E. coli* that serves as its food source. Laboratory ecosystems, also referred to as experimental microcosms, have been used to investigate a variety of important questions in ecology. These ecosystems have been developed for *Didinium nasutum* and its prey *Colpidium campylum*, as well as *Daphnia* and its prey phytoplankton, revealing important aspects of population dynamics and predator-prey interactions [7–9]. Agent-based models have been used in a variety of applications from economics to ecology. In this modeling approach, the system consists of agents that operate in an environment. At each time step, the model updates the environment and each agent. The characteristics of the agents are specified by a series of rules, and the model computes agent behaviors at each time interval. These models are powerful because the behavior of individuals considered in aggregate results in emergent properties of the population, which are not directly specified. This approach is well suited for determining how the traits of individuals influence the properties of the population.

Here we describe the development of a laboratory ecosystem in which a population of *C. elegans* with an *E. coli* food source can be propagated indefinitely. By measuring the number of worms and the amount of bacteria, we can monitor the population as it fluctuates over time. One important feature of this laboratory ecosystem is that it was designed to be well suited for simulation, and the coordinated development of the laboratory ecosystem and the agent-based model is a distinctive feature of this study[10]. We describe the development of an agent-based model that is informed by measurements of individual worms in a variety of food environments. The model specifies how worms feed on bacteria, grow, transition between stages, lay eggs and die from old age, starvation or culling. The model is based on conceptualizing the *C. elegans* life cycle as a flux system, and the outputs include intuitive graphical representations of life cycle dynamics during a simulation. Thus, the model links the development and biochemistry of individual worms to the emergent properties of the population. The behavior of individual worms in the model closely resembles individuals in the lab, and the emergent property of population dynamics in the model closely resembles population dynamics in the laboratory ecosystem. We used these approaches to determine how environmental conditions and intrinsic traits of worms influence

whether animals in a population die of old age. We show that large numbers of progeny destabilize food availability, resulting in adult death from starvation. Controlling progeny number by culling stabilizes the bacterial food supply and permits adults to die of old age. The transition between these states displayed tipping point behavior. Whereas culling the larval stages promotes adults dying of old age, culling the adult stage has the opposite effect and diminishes the chance that an adult will die of old age. Finally, we examined maximum adult lifespans. Short adult lifespans promoted death from old age, whereas long adult lifespans diminished it. These results define conditions that make it possible for animals in a population to die of old age, and suggest that populations may alternate between periods when conditions permit animals to die of old age and conditions where this is a rare event.

Results

Development of a laboratory ecosystem for C. elegans and E. coli

C. elegans is well suited for analyzing population dynamics because of their brief, well-defined lifecycle of ~ 3 days and brief mean lifespan of ~ 15 days [6]. In addition, worms can be cultured in liquid medium and counted using an automated system, enabling frequent monitoring of large populations. Furthermore, individual properties such as fecundity or lifespan can be accurately measured, which is critical for developing a realistic computational model. To establish a laboratory ecosystem, we introduced 250 larvae into 5 mL of liquid S-Medium in a 50 mL culture bottle and cultured at 20°C (Fig. 1A). To analyze the ecosystem, we removed 500 µL (10% volume) samples at regular intervals. We refer to removing volume as culling, since it mimics extrinsic mortality caused by predation, disease or accidents. However, culling is randomly distributed over the population, whereas extrinsic mortality in the wild may be influenced by properties of individuals. To maintain a constant volume and provide a source of food, we added 10 mg live *E. coli* in 500 µL S-Medium immediately after culling. Culling and feeding were performed every 24 hours for 100 days. With regular feeding and culling, these populations can be maintained indefinitely (Note 1).

Samples were analyzed using (1) a COPAS Biosort to count the number of worms, and (2) a spectrophotometer to measure OD600, which was converted to bacterial concentration (mg/mL) (Figure S1). These populations consistently displayed two phases: (1) an initialization phase that extends from the beginning of the culture until the population peaks and returns to the average size, and (2) the culture phase that extends from the end of the initialization phase until the end of the experiment on day 100 (Fig. 1B). The initialization phase displayed a steady increase to a maximum of ~ 127,000 worms on day 29, and then declined to ~ 74,000 worms on day 40 (Table S1). This pattern reflects the concentration of bacteria, which accumulated during days 1–5, since bacteria are added daily and there are few worms at the beginning (Fig. 1C). As the number of worms increases, they consume the excess food and settle into a pattern in which the daily feeding is largely consumed in ~ 5 hours (Fig. 1C'). During the culture phase (day 41 to 100), the worm population number oscillated with a maximum of ~ 112,000, minimum of ~ 58,000 and average of ~ 81,000. To address reproducibility, we analyzed biological replicates of

laboratory ecosystems conducted in parallel or years apart. While every culture displayed a unique pattern of fluctuations of the worm number, the overall features are consistent (Fig. 1D,E, Table S1).

Development of a realistic computational simulation informed by measurements of individual animals

The laboratory ecosystem does not reveal important features of the system, such as the developmental stages of individuals or longitudinal information about individual life histories. To complement the laboratory ecosystem and address these issues, we created an agent-based computational model where the agents are *C. elegans* [11–13]. The environment of the simulation consists of bacteria in a 5 mL volume. We conceptualized the *C. elegans* lifecycle as a flux system that accounts for individuals and mass flow (Fig. 1F,G). This system contains five nodes corresponding to developmental stages of worms: egg, larva, dauer, adult, and parlad (parent/larva/dauer). Dauer is an alternative L3 larval form that is stress resistant; parlad, also called “bag of worms”, is the result of matricidal hatching, which occurs when hermaphrodites stop laying eggs and self-fertilized eggs hatch into larvae and mature into dauers inside the hermaphrodite. Each node is characterized by two values: the number of individual worms in that developmental stage, and the total mass of those worms. The nodes are connected by arrows labeled worm transition (wt) that represent rates in the units worms/time or mass/time. An egg transitions to a larva when it hatches. A larva eats bacteria and grows; it transitions to an adult when food is plentiful and to a dauer when food is limiting. A dauer transitions back to a larva when food is plentiful. An adult eats bacteria, grows, and generates eggs when food is plentiful, thereby transferring germline parental biomass to progeny. An adult transitions to a parlad when food is limiting. A parlad generates dauers, thereby transferring somatic parental biomass to larval progeny. A worm transitions out of the system when it dies from one of three possible causes: all stages can die from culling, larvae and adults can die of starvation, and adults can die of old age.

The system contains one bacteria node that has a value equal to the mass of bacteria in the system. The bacteria node is connected by arrows labeled bacterial transition (bt) that represent rates in the units mass/time (Fig. 1G). Bacterial mass enters the node by periodic feeding and can exit the system by culling. Bacterial mass transitions to *C. elegans* larval and adult mass as a result of feeding. The system is grounded by conservation of mass – worms must consume bacterial mass to grow and produce progeny. The model uses discrete time steps of 3 hours. At each time step, the environment and every virtual worm is evaluated and updated based on a defined set of decision trees described in detail in Note 2. The frequency and amount of bacteria input, and the frequency and percent culling rate are user-programmable parameters specified for each run. The model compiles the complete trajectory of each individual, including rates of growth, time of transitions, production of progeny and cause and time of death (Table S2,S3). The individual data of one simulated population can be combined to create the emergent property of population dynamics.

To create a realistic model, we measured the properties of individuals cultured in conditions similar to the laboratory ecosystem: growth of larvae and adults, egg-laying by adults, transition of dauer to

reproductive growth, and adult lifespan. We varied the concentration of bacteria to establish how this key environmental factor affects these properties and define the extremes of individual worm performance. Growth, egg-laying, and dauer transition to reproductive growth were highly sensitive to the concentration of bacteria, whereas adult lifespan was relatively insensitive (Fig. 2A-P). These measured data were coded into the decision trees so that virtual worms mimic the behavior of real worms. Simulated individual worms and worms measured in the laboratory displayed similar behavior, providing a first level of validation (Fig. 2A-P, S2-7, Table S4-7).

Comparisons of population dynamics in the laboratory ecosystem and the computational simulation

Having calibrated individual *in silico* worms to laboratory measurements, we next compared population dynamics *in silico* to population dynamics in the laboratory ecosystem. Three biological replicates of the laboratory ecosystem and three simulation replicates were performed using 10% culling and 10 mg bacterial feeding every 24 hours for 100 days (Fig. 3A,B, Figure S8, Table S8). The simulation initialization phase displayed a steady increase to a maximum of ~ 165,000 on day 11, and then declined to ~ 60,000 on day 15. This pattern reflects the concentration of bacteria, which accumulated for 6 days before declining into a daily oscillation (Fig. 3C,D”,D‴). The laboratory ecosystem initialization phase displayed a steady increase to a maximum of ~ 120,000 on day 26, and then declined to ~ 36,000 on day 29. During the culture phase (day 29 to 100), the simulated population oscillated with an average of ~ 62,000, a maximum of ~ 136,000, and a minimum of ~ 19,000; the laboratory ecosystem population oscillated with an average of ~ 32,000, a maximum of ~ 71,000, and a minimum of ~ 3,000. Although the simulated population displayed larger average, minimum and maximum numbers of worms, the overall patterns of initialization and culture phases were similar.

The simulation data includes longitudinal measurements of every individual, allowing a detailed understanding of population dynamics. By graphing the number of animals in each node, we can observe the progression of population peaks and valleys (Fig. 3D-E). For example, the population reached a minimum size of ~ 17,000 animals on day 41, including ~ 3,000 adults. With food available, these adults produced a burst of eggs that peaked on day 43 with ~ 60,000 eggs. Eggs hatched into larvae that peaked at ~ 85,000 on day 45. The large population depleted the bacterial food, triggering starvation as adults transitioned to parlads on day 44 and larvae transitioned to dauers that peak on day 46. As the population declines and food becomes more available, adults begin to appear on day 47 and a new cycle begins. The average behavior of the system can be displayed graphically, which reveals that most worms are eggs, larvae, and dauer, with few adults and parlads. Adults primarily generate progeny by forming eggs, and primarily die of starvation and culling; very few die of old age. Most larvae starve or form dauer; relatively few transition to adults (Fig. 3E). These results resemble *C. elegans* populations isolated from nature that sometimes consist of just larvae and adults and sometimes consist of mostly dauers [14].

Population dynamics in the laboratory ecosystem and the computational simulation display similar responses to changes in feeding and culling

We predicted that decreasing the amount of bacterial feeding would decrease the average worm number, whereas decreasing the culling percent would increase the average worm number. To test these predictions and the correspondence between the laboratory ecosystem and the simulation, we reduced bacterial feeding from 10 mg/24h to 5 mg/24h. (Fig. 4B,C, 5B,C, S9A,D, Table S8). Using 5 mg/24h feeding, we reduced culling from 10%/24h to 5%/24h. (Fig. 4C,D, 5C,D, S9B,E, Table S8). Finally, we changed both culling and feeding simultaneously by comparing 10 mg bacteria and 10% culling every 24 hours to every 48 hours (Fig. 4A,B, 5A,B, S9C,F Table S8). The trends in the laboratory ecosystem and the simulation were similar: the average number of worms positively correlated with feeding amount and inversely correlated with culling percent (Fig. 4E,F). In the feeding and culling every 48-hour regime, the number of worms in the laboratory ecosystem and simulation are very similar, whereas with feeding and culling every 24 hours the simulation tended to have higher numbers of worms (Fig. 4G, Table S8). It is important to note that the simulation parameters (see Note 3) were fixed according to the training set (Fig. 4B) and never changed afterwards to fit laboratory data.

Progeny number affects the frequency of old age as a cause of adult death

The simulation of the laboratory ecosystem indicates adults typically die of starvation and culling, but rarely die of old age. These results resemble *C. elegans* populations isolated from nature that lack senescent individuals [15]; however, senescent individuals may exist in the wild under specific conditions and be difficult to isolate due to their fragility [16]. We reasoned that culling only dauer and larva stages would (1) decrease competition for food, thereby reducing starvation as a cause of adult death and (2) by definition eliminate adult culling as a cause of death. When dauer and larva culling was varied from 0–85%/24h, the average number of worms decreased from ~ 66,000 to ~ 13,000 (Fig. 6A). The fraction of eggs and adults increased progressively, whereas the fraction of larva, dauer, and parlads decreased progressively (Fig. 6B,C, Table S9). The amount of bacteria in the system increased progressively, indicating overall consumption decreases as dauer and larva culling increases (Fig. 6D). Aging as a cause of adult death displayed tipping point behavior (Fig. 6E). With 75%/24h dauer and larva culling, periodic episodes of food deprivation caused ~ 99% of adults to die of starvation, whereas only ~ 1% died of old age (Fig. 7A). Slightly increasing dauer and larva culling to 80%/24h reduced food deprivation to just 3 episodes at the transition to the culture phase, and ~ 52% of adults died of starvation whereas ~ 48% died of old age (Fig. 7B). Slightly increasing dauer and larva culling to 85%/24h eliminated episodes of food deprivation, and 100% of adults died of old age (Fig. 7C). The simulation makes it possible to examine the behavior of each node in these different environments (Figure S10-19). The egg laying behavior of individual adults revealed that dauer and larva culling of 10%/24h results in a low average total progeny number of 25, because adults frequently die of starvation. By contrast, dauer and larva culling of 85%/24h results in a maximum average total progeny number of 106, since adults live their entire lives with adequate bacterial food (Fig. 6F).

Intrinsic lifespan affects the frequency of old age as a cause of adult death

Having established conditions where all adults die of old age, we investigated the effects of intrinsic adult lifespan and adult culling. Maximum adult lifespan is a user-programmable parameter that was initially set to 40 days based on laboratory measurements [17]. To explore this variable, we analyzed virtual worms that were short or long-lived (maximum lifespan 25 or 60 days). All three cases displayed a tipping point, but the percent dauer and larva culling necessary to cause 50% of adults to die of old age shifts from 77–80% to 85% as maximum adult lifespan increased (Fig. 8A-B, Table S9-11). Thus, if maximum adult lifespan is longer, then juvenile culling must be more stringent to allow adults to die of old age.

Adult culling affects the frequency of old age as cause of adult death

Starting with conditions that cause 50% of adults to die of old age and 50% to die of starvation, we analyzed the effect of adult culling. When adult culling was varied from 0–40%/24h, aging and starvation as causes of adult death decreased rapidly, replaced by culling (Fig. 8C-E, Table S12-14). Long-lived worms were the most sensitive to adult culling, with no animals dying of old age at an adult cull rate of ~ 20%/24h. By contrast, short-lived worms maintained some adults dying of old age until 40%/24h adult culling (Fig. 8F). Thus, these observations define three factors that influence aging as a cause of adult death: (1) Large numbers of juveniles create food instability, increasing starvation as a cause of adult death and thereby decreasing old age as a cause of adult death. (2) Adult culling, or extrinsic adult death, decreases old age as a cause of adult death. (3) Intrinsic adult lifespan plays an important role, with short life increasing old age as a cause of adult death and long lifespan doing the opposite (Fig. 8G, H).

Discussion

Establishment of a laboratory ecosystem and computational simulation for C. elegans **and** E. coli **– a new platform to investigate the relationships between individual traits and the emergent property of population dynamics.** Biological systems are characterized by levels of organization that proceed from microscopically small to immense, and every level displays emergent properties. Atoms combine to form simple molecules, such as H_2O, and properties such as polarity emerge that are not displayed by atoms alone. Simple molecules combine to form complex macromolecules, such as DNA, which displays the fascinating emergent property of self-replication that is the essence of life. Macromolecules assemble to form organelles and cells, which display emergent properties such as ion gradients. Cells assemble to form organs and organisms, which display emergent properties such as blood pressure. These levels of organization encompass the fields of biochemistry, cell biology, physiology and developmental biology. In the next level of biological organization, organisms assemble to form populations, which display the emergent property of population dynamics. Whereas individual organisms are born and die, when these

individuals assemble in populations, the age-structure and total number of organisms fluctuates over time. Finally, populations of different species assemble to form complex ecosystems, a level of organization encompassed by the field of ecology [18,19]. Populations with their emergent property of population dynamics are an interdisciplinary level of organization, because it bridges the traits of individual organisms, the domain of physiology and developmental biology, with the behavior of populations of organisms, the domain of ecology. A key objective in biology is to understand how the properties of the assembled parts determine the nature of emergent properties at the next level.

Population dynamics is of fundamental importance, because when the number of organisms in the population fluctuates to zero, the population is extinct. Extinction is a crisis in the modern world due to human activity. More fundamentally, extinction of populations and species is a driving force in evolution. Thus, it is of considerable value to develop approaches to study extinction. There are two basic approaches to experimentally address population dynamics and extinction: field studies of wild ecosystems and laboratory ecosystems. While field studies are by definition relevant to natural conditions, they suffer from practical limitations. For example, many species are impossible to reliably track because they are too small or hard to observe, wild populations exist in complex ecosystems affected by many variables, and manipulation of these ecosystems may not be possible or ethical. Laboratory ecosystems represent a reductionist approach to the problem of the ecosystem complexity rooted in the idea that fundamental aspects of population dynamics will apply to small populations in a laboratory. Because they can be readily manipulated and exhaustively analyzed, laboratory ecosystems overcome the major limitations of field studies [20–23]. Of course, laboratory ecosystems have their own limitations. They lack the complexity of the natural world, and laboratory conditions can be highly artificial.

The continuity of populations depends on the replacement of the ancestor generations by future generations. In principle, population dynamics is a straightforward function of birth and death, which has led to extensive modeling based on equations. However, modeling birth and death is far from straightforward, since these outcomes depend on complex interactions between individual organisms and the environment. Commonly used matrix models simulate populations as birth and death rates and neglect the adaptive behavior of the individual. To address the complexity of modeling birth and death, we developed an agent based model. This approach is ideal for this purpose, since the rules that govern the behavior of individuals can include complex interactions between the stage of the animals and environmental conditions, which is not possible with mathematical equations. The behavior of the individual worms is based on measured traits of individual *C. elegans* in the laboratory. Although all worms operate by the same rules, each displays a unique life trajectory including growth rates, time in the dauer stage, and reproductive output, etc., depending on the fluctuations in the environment during its life. This allows a realistic simulation of *in silico* worms and their population dynamics. Furthermore, this is a sturdy platform to investigate *in silico* mutant worms that have properties distinct from wild-type worms.

We reasoned that specific traits of individual organisms determine population dynamic behavior when these organisms assemble, and that rules that govern the interface between the level of individual

organisms and the level of population dynamics could be elucidated by combining a simple laboratory ecosystem and computational simulation. To bridge the gap between laboratory experiments of isolated individuals and complex natural ecosystems, we developed a laboratory ecosystem with just two species: *C. elegans* and its food source *E. coli*. A complementary computational model that simulates *C. elegans* population dynamics as a flux system based on measured individual traits adds data depth and predictive power. Controlled laboratory ecosystem have been previously established, mainly with plankton-algae ecosystems in large water tanks [24]. These have been used to investigate multiple topics such as prey evolution [22], steady state biomass levels [8], or toxic effects of heavy metals [24]. Although the zooplankton species *Daphnia magna* is used as a model organism [25], it is rarely used in aging studies. By contrast, *C. elegans* is a premier model organism for studies of development, physiology and aging [26]. It can be reliably measured in different environmental conditions such as variable food concentrations. In addition, the COPAS biosort is an automated counting machine developed specifically for *C. elegans* that makes it possible to perform high throughput monitoring of population dynamics. The experimental system described here is distinct from previous laboratory ecosystems in several respects. (1) The *C. elegans* laboratory ecosystem was designed with the goal of creating a complementary agent-based model, so it well suited for this purpose. (2) The simulation outputs include intuitive graphical representations of the *C. elegans* life cycle, conceptualized as a flux system. Thus, the simulation outputs integrate the development and physiology of individuals with the properties of the population. (3) The simulation was designed to make it convenient to analyze *in silico* mutant worms, creating a platform that complements the large collections of *C. elegans* mutants that can be analyzed in the laboratory ecosystem.

C. elegans is an example of a species that is difficult to analyze in a natural ecosystem because of its small size and subterranean lifestyle. *C. elegans* can be recovered from nature, but the process is time consuming and does not support direct measurements of population dynamics. It is hypothesized that wild *C. elegans* populations undergo boom-bust cycles [14]. A cycle begins when a dauer enters a new food patch, such as a rotten apple or wood. The dauer transitions into a larva, matures, and reproduces to initiate a new population. This population proliferates until the food source is exhausted, leading to the generation of many dauers. These dauers must disperse to find a new food patch to restart the cycle. Galimov and Gems (2020) used a computational approach to test the hypothesis that programmed death is an adaptive strategy for *C. elegans* to secure food for clonal progeny [27]. This computational simulation models single boom-bust cycles on a predefined single food patch. The authors concluded that adult death has fitness advantages defined as amount of dauers produced in a single boom-bust cycle. The laboratory ecosystem described here is a liquid culture that involves regular addition of *E. coli* as a food source. During the initialization phase, the population expands rapidly since food is abundant, similar to what is hypothesized to occur when a dauer disperses to a new food patch. This phase is characterized by adults, eggs, and larval stages. When bacterial food is depleted, the stage composition changes and is characterized by parlads and dauers, similar to what is hypothesized to occur when a food patch is exhausted. Thus, the laboratory ecosystem appears to model key features of the boom-bust cycle that is proposed to occur in the wild. In addition, the laboratory ecosystem and simulation could be adapted to

specifically model the episodic food cycle proposed to occur in the wild. The current system cannot model dispersal of dauers to new food sources, since the simulation is a single food environment. However, the agent based model could be adapted to have multiple food sources separated in space, so in principle dauer dispersal could be incorporated into an expanded model.

For animals living in a population, dying of old age depends on conditions. To begin to elucidate how aging and lifespan influence population dynamics, we identified environmental and intrinsic factors that influence whether animals in a population die of old age. In a controlled laboratory setting, individual *C. elegans* become frail and die of old age. While it is not possible to directly determine if this occurs in the wild, it has been suggested to be unlikely [28–31]. In the laboratory ecosystem that we analyzed, our simulation modeling indicates that adults typically die of starvation and culling rather than old age. We used the simulation to identify conditions where adults do die of old age. One key factor is progeny number, which we manipulated by stage specific culling. Interestingly, old age as a cause of adult death displays tipping point behavior – it rarely occurs with high levels of progeny but can become frequent when progeny levels are reduced to a critical level. The tipping point suggests the populations can exist in two states. State 1 is characterized by frequent episodes of starvation and an abundance of dauers; state 2 is characterized by a stable food supply and an absence of dauers. This result may be related to observations in the wild - abrupt shifts of ecosystems from one state to another state have been observed and described [32,33]. A second key factor is adult culling. As expected, when adult culling increases, fewer adults die of old age. This factor did not display tipping point behavior but was relatively continuous. The third key factor was maximum adult lifespan. *In silico* worms with a 25-day maximum lifespan died of old age more frequently, whereas *in silico* worms with a maximum adult lifespan of 60 days died of old age less frequently. Thus, conditions that promote adults dying of old age include, (1) reproductive restraint, which leads to food stability and minimizes death from starvation, (2) infrequent adult culling, and (3) a short maximum adult lifespan. By contrast, conditions that inhibit adults from dying of old age include (1) abundant reproduction, which leads to food instability and death from starvation, (2) frequent adult culling, and (3) a long maximum adult lifespan.

We speculate that these results could be relevant to the natural world, and shifting environmental conditions might cause populations to alternate between time periods when few or no adults die of old age and time periods when many adults die of old age. The factors defined here provide a framework that can explain diverse animals that die of old age in the wild (Table 1). For example, elephants are intrinsically long-lived animals that have been observed to have aging as a cause of adult death in nature. Our model predicts that elephants must have a low level of adult culling and a small number of juvenile animals. Indeed, elephants make very few progeny, and their large size makes them essentially immune to predation[34–36]. Mayflies have a very short intrinsic lifespan and have been observed to have aging as a cause of adult death in nature. These adults do not feed, so they are immune to starvation, and even though they are subject to high levels of adult culling, the lifespan is so short they can still frequently die of old age [37,38]. Our future goal is to combine this powerful experimental platform with the

advanced tools of *C. elegans* genetics to bridge the gap between individual traits and the behavior of populations and expand our understanding of “eco-devo” [39].

Material And Methods

Experimental methods:

All experiments were conducted at 20°C with *E. coli* OP50 and the *C. elegans* wild-type strain N2. Eggs were isolated by bleach treating gravid adults (2 mL NaOH, 4 mL NaClO, 4 mL H_2O) and incubated in M9 for 15–18 hours on a shaker to allow L1 larvae to hatch and arrest development.

Measurements of individual worms

Egg-laying

Hatched larvae were cultured in 1 mg/mL *E. coli*/S-Medium for 72 hours until the L4 larval stage, washed 3x with S-medium, and single animals were placed into 96 well plates. The final volume was 150 µL per well with *E. coli* concentrations of 4, 0.5, 0.25, 0.125, or 0.061 mg/mL. Worms were transferred to new wells every 24 hours, and hatched progeny were counted.

Growth

Hatched larvae were cultured in 25 mL S-Medium with 16, 12, 4, 2 and 0.4 mg/mL *E. coli*. Worms were imaged every 24 hours with a Leica M80 microscope equipped with a camera, and images were analyzed with Image J and the worm sizer plugin [40]. Worms were scored as adults when they displayed eggs, and measurements were continued until the first progeny matured to adults. Worm mass was calculated using the measured volume and reported mass densities [41].

Lifespan

Lifespan assays were conducted as described[42]. Hatched larvae were cultured in 96 well plates with approximately 5–10 larvae per well. Each well contained 100 µl S-Medium and 16, 12, 4, 2, or 0.4 mg/mL *E. coli*. After 48 hours, 0.15 mM 5-fluorodeoxyuridin was added to prevent progeny development. Adults were scored as alive or dead based on movement and body tension.

Dauer transition to larva

To obtain dauer larvae, we cultured a population in liquid medium, starved the animals for 10 days or 2 months, and isolated dauers by treatment with 1% SDS for 30 min [43]. 5–10 dauers were placed in 96 well plates with 16, 12, 4, 2, 0.4, or 0 mg/mL *E. coli*. The transition to larvae was scored after 12 hours and every 24 hours thereafter by visual inspection. After 120 hours, we added 4 mg/mL *E. coli* to the control with no *E. coli* and measured transition to larvae.

Laboratory ecosystem

The population in the laboratory ecosystem was initialized with 250 larvae and 5 or 10 mg live *E. coli* in 5 mL of liquid S-Medium [42] in 50 mL cell culture bottle. To analyze the worm number and/or *E. coli* concentration, we removed 5–10% of the volume every 24 or 48 hours. To maintain a constant volume and provide a source of food, we immediately added 5 or 10 mg live *E. coli* in 250 or 500 µL S-Medium. Samples were analyzed using (1) a COPAS Biosort to count the number of worms in a 10–50 µL sample, which was used to calculate the total number of worms in the population, and (2) a spectrophotometer to measure OD600, which was converted to bacterial concentration (mg/mL) using a standard curve (Figure S1).

Statistics

Statistical analysis of egg-laying behavior of simulated worms in populations with different dauer & adult culling was done with R using a one-way ANOVA with $F = 197.3$, $Df = 3$, and $p > 0.001$ followed by a Tukey Post-hoc test (see Fig. 6F). All error bars show standard deviations.

Computational Simulation

The description of the agent-based model wormPOP follows the Overview, Design concepts, and Details (ODD) protocol[44,45]. More details are described in the extended Notes in the Supplemental Material.

Purpose

The purpose of the agent-based model wormPOP is to simulate the population dynamics of the nematode worm *C. elegans* cultured in a simple laboratory ecosystem. The laboratory ecosystem and wormPOP simulation include only two species – worms are the agents, and *E. coli* bacteria are the food source. wormPOP is designed to facilitate the investigation of how environmental factors - bacterial food and culling - and the life history traits of individuals impact population dynamics. The analysis of the laboratory ecosystem is limited to population level measurements, since the behavior of individuals cannot be tracked. wormPOP is designed to provide a more detailed examination of individual worms based on explicit laboratory measurements of these behaviors, and aggregates the behaviors to predict population dynamics and demography. Specifically, wormPOP (1) tracks the life history of every individual worm (2) outputs data for the average behavior of individuals, and (3) combines individual behaviors to generate the emergent properties of population dynamics.

wormPOP employs strict conservation of mass, since worms can only grow and reproduce by consuming the *E. coli* food source. A purpose of the model is to investigate how fluctuations in food supply influence population dynamics. wormPOP uses culling to model extrinsic mortality. A purpose of the model is to investigate how the extent and stage-specificity of culling influence population dynamics. wormPOP allows the life history traits of the simulated (*in silico*) worms to be modified to create mutant worms. A purpose of the model is to investigate how specific life history traits of an individual affects the emergent property of population dynamics. Thousands of *C. elegans* mutants exist in extensive collections of laboratory strains, and the model is designed to make it possible to simulate the population dynamics of mutant worms in the laboratory ecosystem. wormPOP is based on conceptualizing the life cycle of *C.*

elegans as a flux system, and it was designed to output the life cycle data in an intuitive graphical form. A purpose of the model is to investigate how environmental factors and life history traits of individuals influence the life cycle flux system. The overall purpose of wormPOP is to be a tool that connects two levels of biological organization – the life history traits of individual animals that are generated during development and the emergent property of population dynamics in an ecosystem.

Entities, state variables, and scales

wormPOP contains two entities: (1) The agents are individual *C. elegans* hermaphrodites (worms), the first species in the simple ecosystem, and (2) the environment includes one entity: *E. coli* bacteria that is the second species in the simple ecosystem.

The state variables or attributes that characterize each agent worm are as follows: (1) identity number – each worm is monitored as a unique individual. (2) Life cycle stage. The lifecycle contains five developmental stages of worms: egg, larva, dauer, adult, and parlad (parent/larva/dauer). Each live worm is in only one of these five stages at a time, and worms progress through stages as they mature. (3) Previous life cycle stages the worm has occupied. This can affect behavior; for example, a worm can only enter the dauer stage once. (4) Age (hours) spent in each developmental stage, which can be used to determine age (hours) since it entered the simulation, (5) Mass (ng) of worm soma at each time step, (6) Mass (ng) of unlaid eggs in adult stage animals at each time step, and (7) Feeding history for the most recent two time steps. This information affects the probability of a larva transitioning to dauer or the probability of an adult transitioning to parlad.

The environment volume is 5 ml of liquid, which is based on the laboratory ecosystem. The environment contains the bacteria, the second species in the ecosystem and food source for worms. The volume of the environment is only relevant in determining the bacteria concentration. The environment is characterized by the state variable of bacteria concentration (ng/ml).

wormPOP operates in discrete time steps that represent 3 hours. This time resolution is well suited for this simulation based on the duration of the life cycle stages and egg laying rates of *C. elegans*. By contrast to the 24-hour time resolution of the laboratory ecosystem, 3 hour time steps allows more detailed data collection. wormPOP was typically run for 100 days (800 time steps).

Process overview and scheduling

In each time-step of the simulation, the environment and the worms proceed through the following processes.

(1) The user-programmable feeding and culling schedule is consulted. If applicable, feeding/culling is performed at the beginning of the time step.

> a. If the culling schedule calls for it, then worms are culled (removed from the simulation by extrinsic mortality) and/or bacteria are culled (removed from the simulation). Worms are randomly selected for culling based on their stage and according to a specified percentage of the

group. This is implemented in code as a separate random probability that each worm at whatever stage will be removed from the system. Individual life histories of worms are updated, specifying cull as the cause of death. The state variable of bacteria concentration in the environment is updated.

b. If the feeding schedule calls for it, then a specified amount of bacteria (ng) is added, and the state variable of bacteria concentration in the environment (ng/ml) is updated.

(2) The “appetite” of every worm is computed. This is the amount of food it would eat if food were plentiful. The sum of appetites is used later to assure approximately uniform access to food, independent of computational ordering of the worms’ eating behavior.

(3) The worms’ behaviors are considered one-by-one, in random order, depending on the life stage and history which each worm retains.

a. Eggs stage progress: eggs can remain eggs or transition (hatch) into larva. Individual life histories of worms are updated, specifying developmental stage.

b. Larva:

i Larva stage progress to dauer or starve: If larva don’t get enough food, they transition to dauer or die of starvation. Individual life histories of worms are updated, specifying developmental stage or starvation as cause of death.

ii. Larva ingest bacteria: Worms in the larva stage ingest bacteria based on age, mass, the bacteria concentration in the environment and the appetite of other worms. Individual life histories of worms are updated, specifying bacteria ingested at time step.

iii. Larva use ingested mass for maintenance and growth. Individual life histories of worms are updated, specifying mass used for maintenance, growth, and growth efficiency loss.

iv. Larva stage progress to adult or starvation: larva can transition into adults or die of starvation. Individual life histories of worms are updated, specifying developmental stage.

c. Dauer

i. Dauers do not eat or grow or lay eggs or starve to death. They only test the environment for available food and if it is greater than the (user-programmable) threshold, the dauer returns to the same larval stage and picks up just where it left off before it was a larva, with the same history and the same mass. (Note that the probability of this dauer transition to larva is dependent on the food available at the moment that this worm’s turn comes up in the random ordering, so to this extent it is stochastic.)

ii. Dauers stage progress to larva or starve: dauers can transition into larva based on the bacteria concentration. If dauers don’t sense food for too long, they die of starvation.

Individual life histories of worms are updated, specifying developmental stage or starvation as cause of death.

d. Adult:

i. Adults ingest bacteria: Worms in the adult stage ingest bacteria based on age, mass, the bacteria concentration in the environment and the appetite of other worms. Individual life histories of worms are updated, specifying bacteria ingested at time step.

ii. A fraction of the mass is consumed in metabolism. The consumed food is apportioned between growth and egg mass.

iii. Adults use ingested mass for maintenance, growth and egg-laying: Individual life histories of worms are updated, specifying mass used for maintenance, growth, growth efficiency loss, eggs, and reproductive efficiency loss, and new eggs are added to the simulation as new agents.

iv. Adults die of starvation and transition to parlad: If adults don't get enough food, they die of starvation. Individual life histories of worms are updated, specifying starvation as the cause of death and transition to parlad.

v. Adults die of old age: If adults reach a specified age, they die from intrinsic causes called old age. Individual life histories of worms are updated, specifying old age as the cause of death.

e. Parlads stage progress: parlads can remain parlads or transition (burst) into dauers. Individual life histories of worms are updated, specifying developmental stage or death by starvation when the parlad bursts. When parlads burst, new dauers are added to the simulation as new agents.

(4) Various bookkeeping functions are performed, including removal of dead worms from the roster.

Design concepts

Basic principles

To exploit the power of agent-based models, we designed a simple laboratory ecosystem to be amenable for simulation. Several features make the ecosystem well suited for modeling: (1) The ecosystem includes only two species – each individual nematode is an agent, and the *E. coli* bacteria considered as a whole is one entity. (2) We can measure the population dynamics of worms and bacteria in the laboratory ecosystem, resulting in quantitative benchmarks for the behavior of the simulation. (3) We can measure the properties of individuals in conditions very similar to the laboratory ecosystem, which allows realistic simulations of individual behavior. The agent-based model complements the laboratory ecosystem by simulating the behavior of each individual worm in the population and allowing detailed

analyses of the population with 3-hour resolution. Furthermore, we conceptualized the *C. elegans* life cycle as a flux system that links individuals to population phenomenon, and generated an intuitive graphical output that shows the flux of individuals through the life cycle. We implemented mass conservation, which is critical for long term studies of population dynamics. By using laboratory measurements of growth and reproduction at different environmental bacterial concentrations, the model incorporates the plasticity of individual worm responses to variable food environments. Details are in Notes 2 and 3.

Emergence

For bacteria, the input frequency and amount are determined by a user programmable input, and culling frequency and percent are determined by a user programmable input. The emergent properties of bacteria include (1) the concentration at each time step (size of bacterial node), (2) amount ingested by larvae at each time step: bt(b > l), (3) the amount ingested by adults at each time step: bt(b > a), and (4) the amount culled at a relevant time step: bt(b > c).

For individual worms, many traits such as growth and reproduction depend on the food environment. Thus, the detailed life history of each individual worm is an emergent property based on the fluctuating food environment during its lifetime and stochastic events such as entry into dauer or culling. Similarly, the average behavior of worms in the simulation, such as the average egg laying curve of all adults, is an emergent property, based on analyzing many different individuals, each of which has a life history with emergent properties.

All of the population dynamic values are emergent properties, including the size of each worm node at each time step and the worm transition (wt) rates at each time step, which can be calculated as worm number or worm mass.

Adaptation

Larvae and adult worms display bacteria ingestion, growth and egg laying plasticity that depends on the bacteria concentration. Larvae display transition into adult, dauer or starvation based on bacteria concentration. Adults display transition to parlad based on bacteria concentration. Dauer display transition into larvae or starvation based on bacteria concentration.

Sensing

All worm agents are assumed to sense the bacterial concentration. Individuals are simply assumed to know this state variable.

Interaction

Worm agents interact directly with bacteria, since worms ingest bacteria as food. Worms do not interact directly with other worms; however, worms interact indirectly with other worms through the shared bacterial food source. For example, if one worm ingests bacteria, it is no longer available as a food resource for other worms.

Stochasticity

Stochasticity in the model is limited to:

(1) Culling, in which each individual is subjected individually to a constant probability of culling at programmed intervals.
(2) Life stage transitions: larva to and from dauer and adults die of old age are controlled by probability functions.
(3) At each time step, the order of analyzing the worms is randomly determined using stochastic probability.

Observation

In each simulation, detailed information regarding the bacteria in the environment, the individual agents, and the population of agents are collected and saved. (A) For the environment, the simulation collects and saves the bacteria concentration at the beginning of each time step and the bacteria transition (bt) rates. (B) For the worm agents, the simulation collects and saves age, developmental stage, mass, and laid and unlaid egg mass at each time step, as well as cause of death. The fate of all ingested mass of each individual is collected and saved, including mass that was used by larvae and adults for growth, maintenance and efficiency loss. (C) For the population of worm agents, the simulation collects and saves the size of each node and the worm transition (wt) rates at each time step, measured in both number and mass.

Initialization

The initial state of the model world is specified by the initial amount of bacteria and the initial number and stage of worms. These are user programmable inputs that can be varied. Simulations were initialized with 5 or 10 mg bacteria. Simulations were initialized with 250 or 1000 eggs, larva, or dauer. The state variables of eggs are a mass of 65 ng, they have been 1 to 5 time steps in the egg stage and hatch after 5 time steps. The state variables of larvae are 228 ng and they are 0–5 time steps in the larva stage. The state variables of dauer are a mass of 228 ng and they 5–15 time steps in the dauer stage. The initial variables were chosen to match the laboratory ecosystem.

Input data

Input data for the wormPOP model is periodic addition of bacteria and culling, and the frequency and amount is user programmable. Bacteria were added and culling was performed every 24 or 48 hours to mimic the laboratory ecosystem.

Submodels

All submodels and parameters (traits) are described in detail in Note 2 and in Note Table 2, 5, 6. Submodels of the environment are “culling” and “adding bacteria”. Submodels of the worm agents are specific to worm stages and describe worm behavior or worm transitions: (1) Eggs behave according to

the submodels “culling” and “hatching”; (2) Larva behave according the submodels “culling”, “feeding”, ”starvation”, “cost of living”, “growth”, “larva transition into dauer”, and “larva transition into adults”; (3) Dauers behave according the submodels “culling”, “dauer transition into larva”, and “starvation”; (4) Adults behave according to the submodels “culling”, “feeding”, “cost of living”, “growth”, “egg-laying”, “adult transition into parlad”, and “die of old age”; (5) Parlads behave according to the submodels “culling” and “parlad transition into dauers”.

Data&Code availability

Datasets are included in the Supplementary Tables. The code used for all simulations will be freely available for download at https://github.com/.

Declarations

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Author Contributions

A.S. and K.K conceived and designed the experiments. A.S., B.A., D.S., F.S., B.B., N.R., G.D. performed experiments, A.S., H.J., and K.K analyzed data, A.S, Z.K., C.T., A.M. and K.K. provided scientific input, A.S., J.M., and K.K contributed reagents/materials/analysis tools. A.S, J.M., and K.K. wrote the paper.

Declaration of Interest

The authors declare no competing interests.

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Table 1

Table 1. Conditions that permit animals to die of old age.

			food stability			
	max lifespan	adult culling	progeny number	progeny culling	adult starvation episodes	die of old age
Asian elephant	80 years[35]	low	2.62 +/- 1.76 (range 1-11)[35]	low	low	yes[35]
C. elegans	40 days	low	70-130	high	low	yes
Mayfly	1 hour to 14 days[37]	high[38]	500-3000[37]	high[38]	never (adults don't feed) [37]	yes

Figures

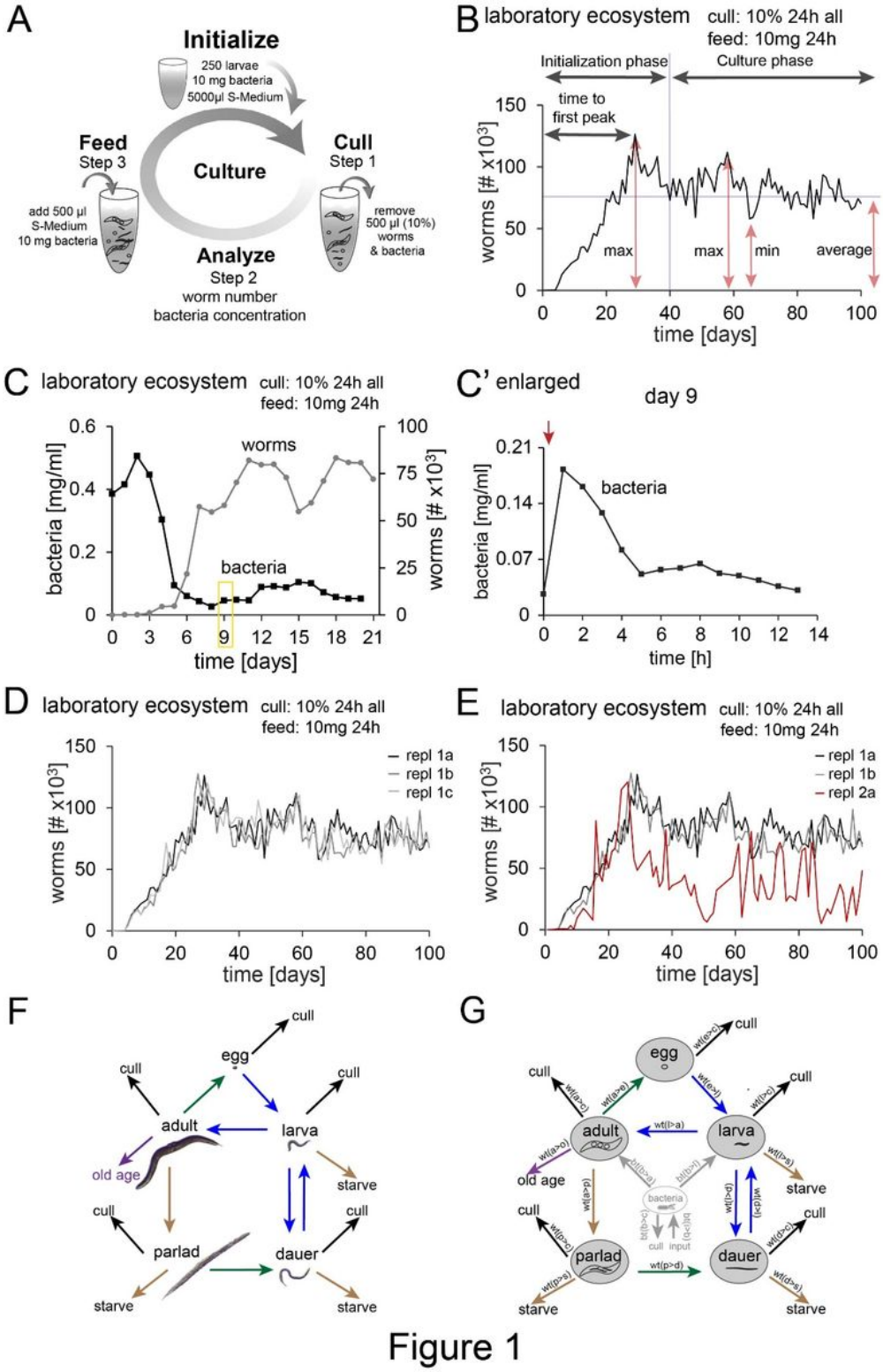

Figure 1

Figure 1

A laboratory ecosystem of C. elegans and E. coli. (A) Schematic of the laboratory ecosystem: one-time initialization followed by periodic culling, analyzing and feeding. (B,C) Data from wild-type worms in the laboratory ecosystem. Culling values indicate percent culled (10%), culling interval (24h), and stage of worms culled (all stages). Feeding values indicate amount of bacteria (10mg) and feeding interval (24h). (B) Analysis of summary statistics: time spans (black double arrows) and worm numbers (red double

arrows) (C) Bacteria (black squares) and worms (gray circle) were analyzed daily. Yellow box indicates region enlarged in C'. (C') Bacteria were analyzed hourly on day 9; bacteria were added between 0 and 1 hour (indicated by red arrow). (D) Three worm populations were initiated on the same day with larvae from the same group of synchronized worms and bacteria from the same concentrated solution (replicate 1a-1c). For the next 100 days, these laboratory ecosystems were maintained separately and never mixed. We designate 1a-1c as biological replicates conducted in parallel. Replicate 1a is shown in panel B. (E) Replicate 2a was initiated on a different day with larvae from a different group of synchronized worms and bacteria from a different concentrated solution. We designate replicate 2a and replicate 1a/1b as biological replicates conducted at different times. Replicate 2a is shown in Figure 3A. (F) Lifecycle of C. elegans in the laboratory ecosystem. Adult, egg, larva, dauer and parlad are the five life stages. Birth transition arrows are green: adult to egg, and parlad to dauer. Developmental transition arrows are blue. Death transition arrows are orange (starve), black (cull), and purple (old age). (G) The lifecycle forms the foundation of the computational simulation. The five life stages are the worm nodes. Each node has three to six arrows that depict the worm transitions (wt). The bacteria node has four arrows that depict bacteria transitions (bt).

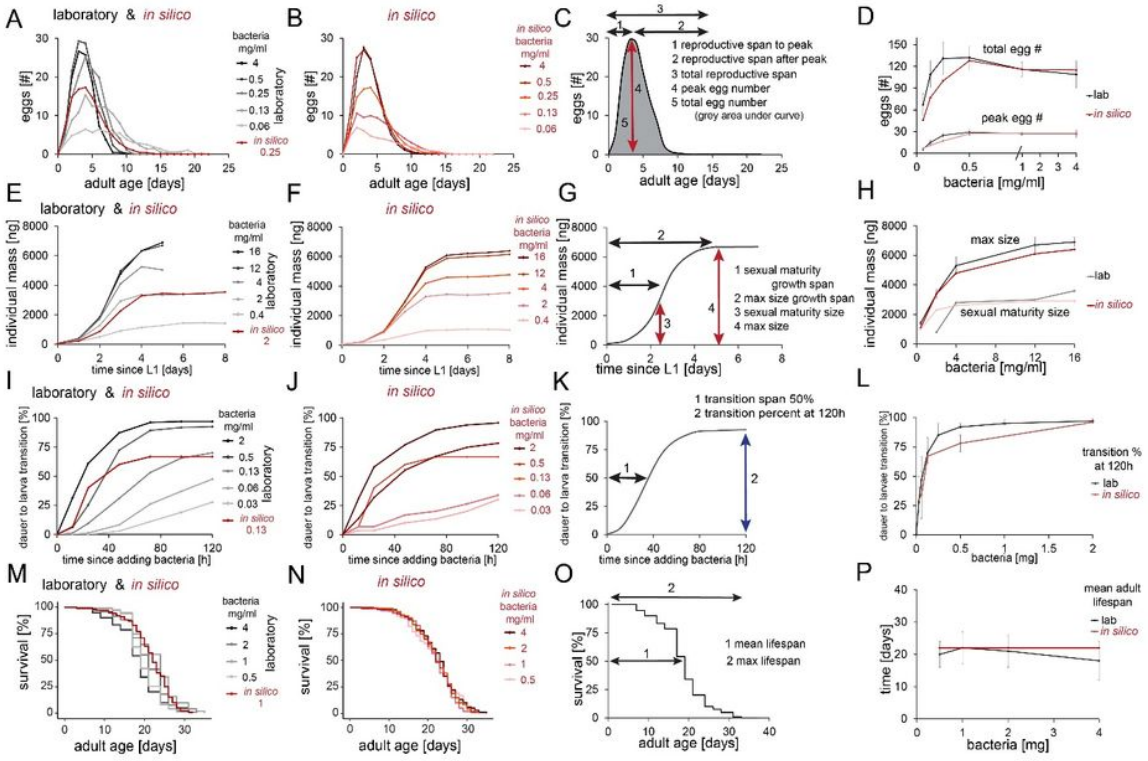

Figure 2

Figure 2

. A realistic computational simulation based on the C. elegans lifecycle and measurements of individual animals (A-P) Wild-type, self-fertile hermaphrodites were cultured in S-Medium with the indicated concentrations of E. coli bacteria in the laboratory (gray lines). in silico worms were computationally simulated in bacteria concentrations that correspond to the laboratory conditions (red lines). (A) Average daily progeny production of individual adults in the laboratory (black) and in silico worms (red). The

single red curve corresponds well with the grey laboratory data with the same concentration of E. coli. (B) Average daily progeny production of in silico worms. (C) Analysis of summary statistics for daily egg production: reproductive span to the peak, reproductive span after the peak, and total reproductive span (black arrows); peak egg number (red arrow); total egg number (grey area under curve). (D) Comparison of peak egg number and total egg number from laboratory conditions and computational simulations. (E) Average daily mass of individuals in the laboratory (black) and in silico worms (red). The single red curve corresponds well with the grey laboratory data with the same concentration of E. coli. (F) Average daily mass of in silico worms. (G) Analysis of summary statistics for daily mass of individuals: sexual maturity growth span and maximum size growth span (black arrows); size at sexual maturity and maximum size (red arrows). (H) Comparison of size at sexual maturity and maximum size from laboratory conditions and computational simulations. (I) A population of dauers were cultured with bacteria starting at time 0 - data shows average percent of larvae in the population. Animals were in the dauer stage for as many as 10 days. The black curves correspond to laboratory conditions and the red curve to in silico worms. The single red curve corresponds well with the grey laboratory data with the same concentration of E. coli. (J) Average percent of larvae in the population of in silico worms in bacteria concentrations that correspond to the laboratory conditions. (K) Analysis of summary statistics for daily dauer to larva transition: time until 50% of dauers transition to larvae (black arrow) and percent of dauers that transition after 120 hours (blue arrow). (L) Comparison of percent of dauers that transition after 120 hours from laboratory conditions and computational simulation. (M) Survival curves for populations of individuals cultured with the bacterial concentration beginning at the L1 stage under laboratory conditions (black) and computationally simulated (red). The single red curve corresponds well with the grey laboratory data with the same concentration of E. coli. Lower concentrations of bacteria did not cause a substantial extension of adult lifespan, as might have been expected based on studies of caloric restriction. Notably, we initiated exposure to the bacterial concentration at the L1 stage and continued this same concentration throughout the adult life, whereas caloric restriction experiments often involve transferring young adults to the restricted food environment. (N) Survival curves for populations based on the analysis of in silico worms in bacteria concentrations that correspond to the laboratory conditions. (O) Analysis of summary statistics for survival of individuals: mean lifespan and maximum lifespan (black arrows). (P) Comparison of mean adult lifespan from laboratory conditions and computational simulations.

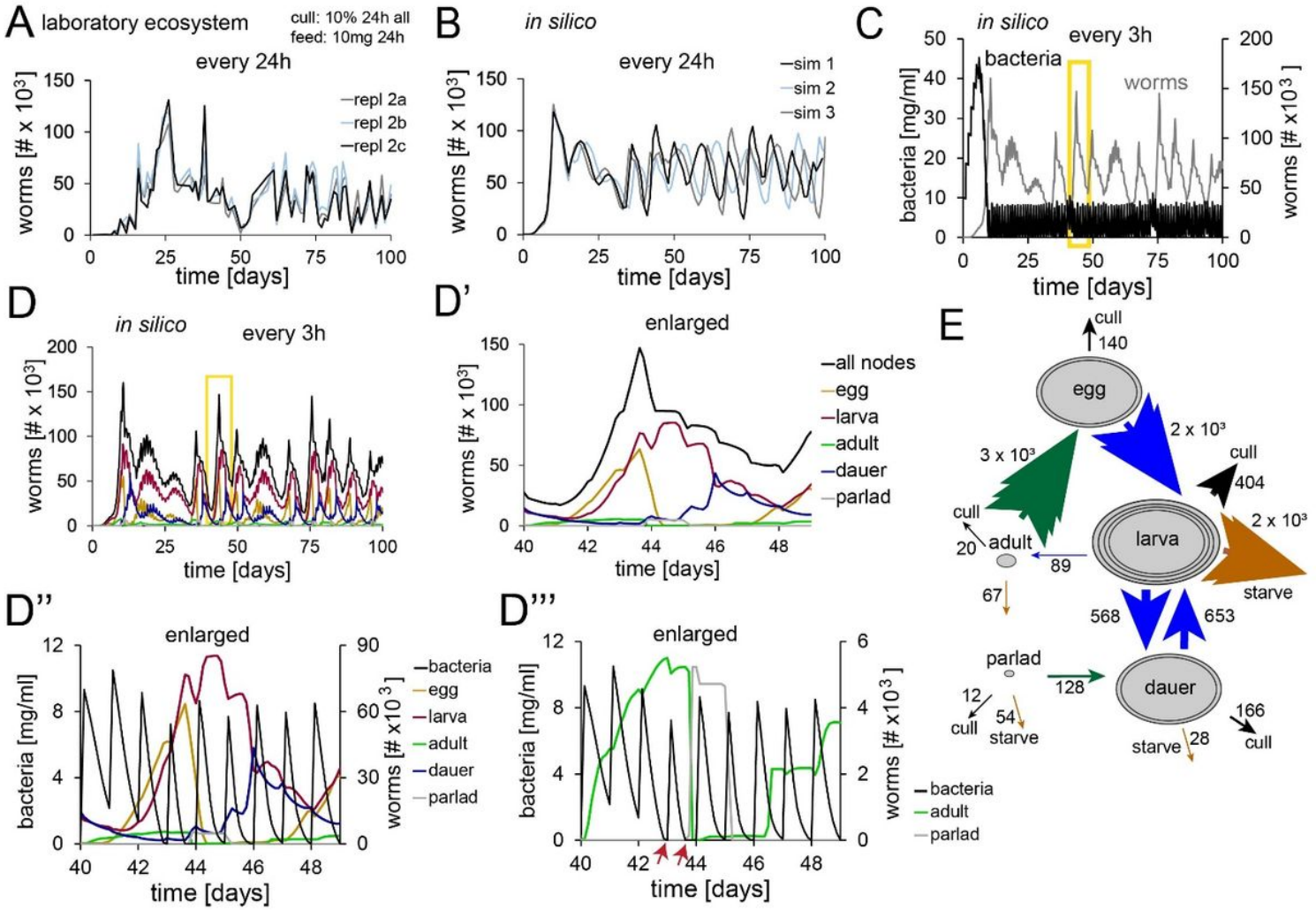

Figure 3

Figure 3

Population dynamics in the computational simulation. (A) Data from three biological replicates conducted in parallel of wild-type worms in the laboratory ecosystem with culling and feeding schedules shown. These data were used as the training set for the computational simulation (Note 3). (B) Data from three computational simulations corresponding to the laboratory ecosystem population shown in panel A; 1 value/24 h is graphed as in the laboratory ecosystem. (C-D‴) Stages are shown separately and combined; enlargement shows days 40-49. The lines represent 8 values/24 hours (every three hours) (C) Gray line displays the total number of worms (all stages combined), and the black line displays the concentration of bacteria. The lines represent 8 values/24 hours (every three hours). The yellow box indicates the interval enlarged in panels D'-D‴. (D) Stages are shown separately and combined. (D',D" D‴) Enlargements show days 40-49, corresponding to the yellow boxes in panels C and D. The number of worms in each node (egg, larva, dauer, adult, and parlad) is shown separately and combined in panel D'; panel D" only shows stages separately and bacteria. Panel D‴ shows only bacteria, adults and parlads. The worm scale was adjusted to visualize the dynamics of adults in each D panel. Note that the bacteria

level drops to zero on day 43 and day 44 (red arrow), triggering adults to starve and transition to parlads. (E) Flow diagram shows average number of worms in a node by the size of the circle and average number of worms transitioning per 3-hour time step by the size of the arrow; numbers specify the size of larger arrows. See Figure 4 for scale bars.

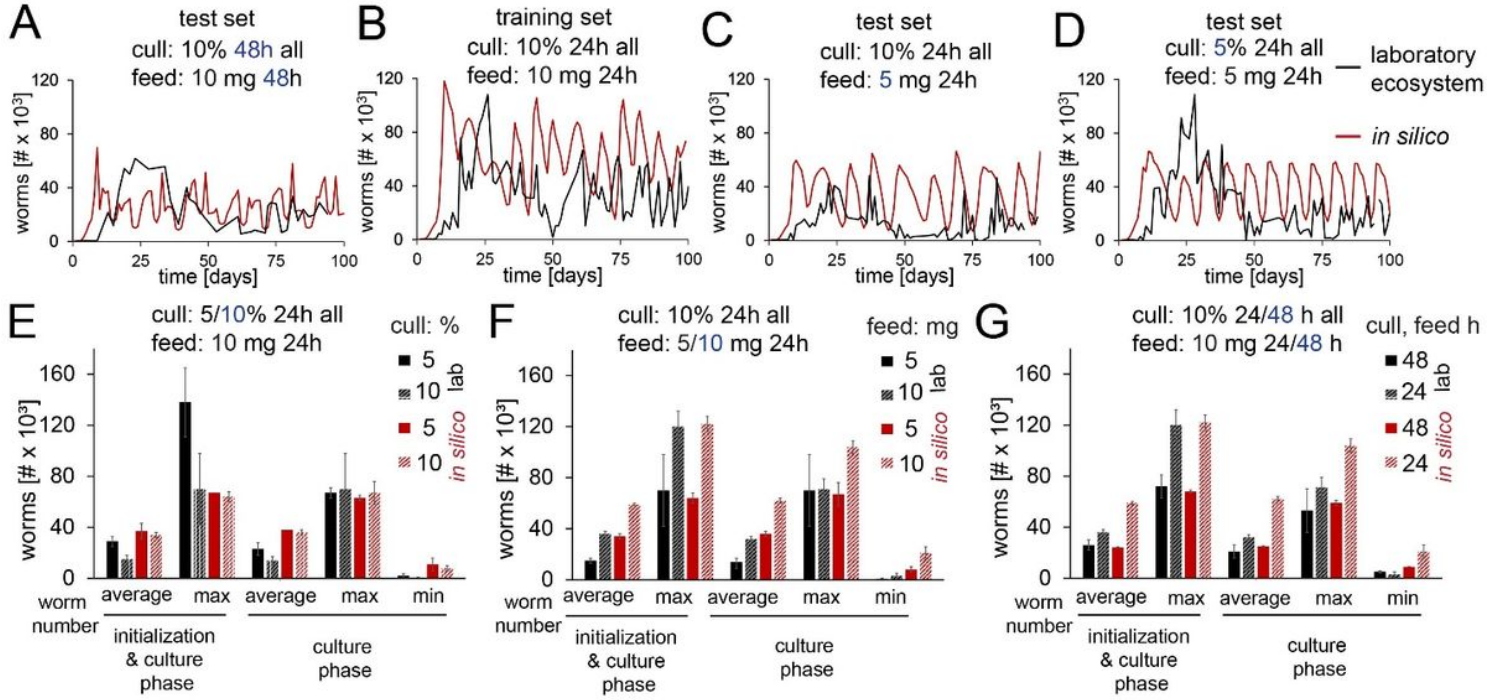

Figure 4

Figure 4

Direct comparisons of population dynamics in the laboratory ecosystem and computational simulation in four conditions. (A-D) Data from worms in the laboratory ecosystem (black) and corresponding simulations (red); culling and feeding schedules show the parameter that was varied in blue. The laboratory ecosystem data in panel B (10% culling all stages and 10 mg feeding every 24 hours) was used as the training set to determine the value of the following parameters: 1) cost of living and 2) metabolic efficiency (see Note 3). (E-G) Comparisons of population summary statistics from the laboratory ecosystem (black) and corresponding simulations (red): Average and maximum worm number in initialization and culture phase; average, maximum, and minimum worm number in the culture phase (see Fig 1B). Culling and feeding schedules show the parameter that was varied in blue. The red simulated data show similar patterns as the black laboratory data with changing culling and feeding conditions.

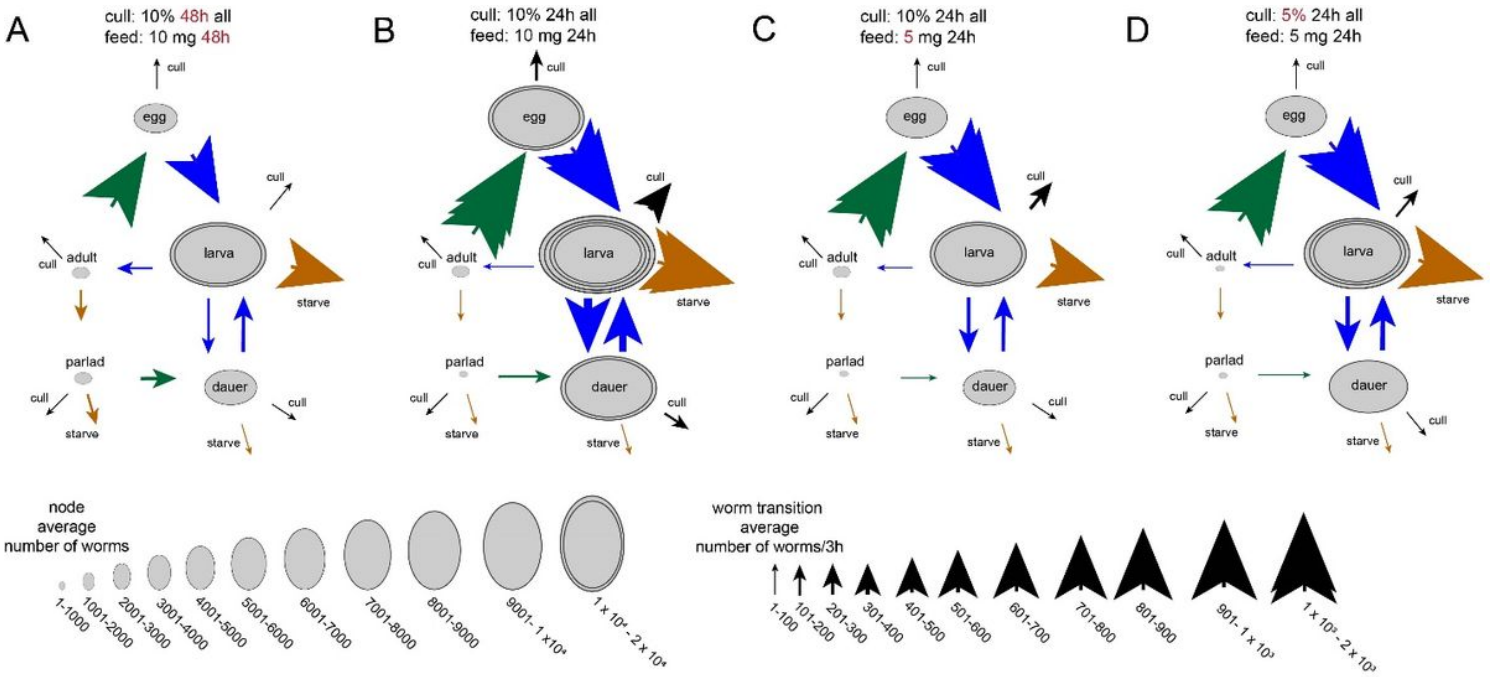

Figure 5

Figure 5

Population dynamics in the computational simulation in four conditions. (A-D) Flow diagrams of simulated populations with indicated feeding and culling schedules. Panel B is the same as Figure 3F. The key shows the relationship between node size and average number of worms in that node during the 100-day simulation. Similarly, the key shows the relationship between arrow size and the average number of worms making the transition during a 3-hour time period.

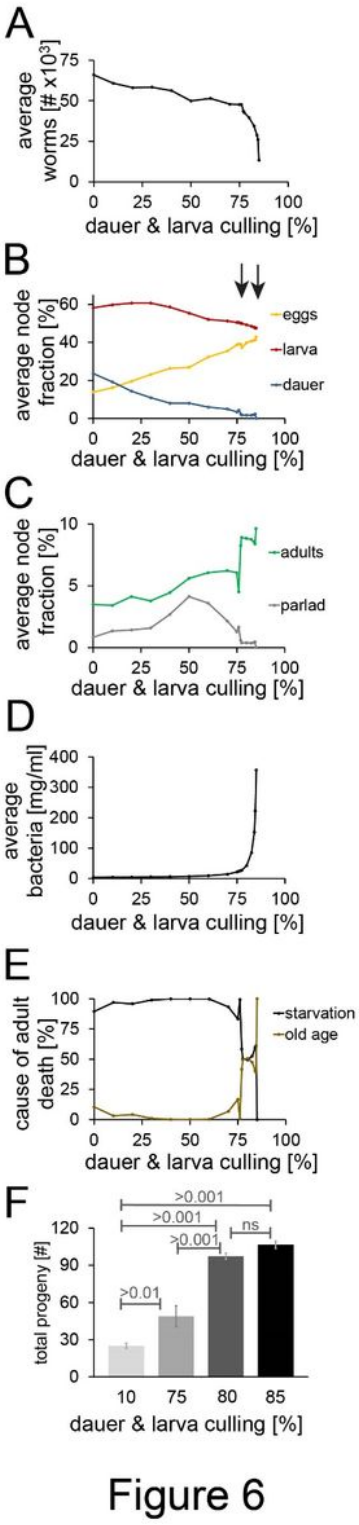

Figure 6

Figure 6

Progeny culling reveals a tipping point for old age as a cause of adult death. (A-D) Summary statistics for simulated populations with a variable percentage of dauer & larva culling: (A) Average number of all worms. (B) Average percent of eggs, larva, and dauer among all worms. The black arrows show the tipping point for 50% and 100% old age as a cause of adult death. (C) Average percent of adults and parlad among all worms (D) Average amount of bacteria in the bacterial node (D) Cause of death for

adults; with no adult culling, adults only die of starvation or old age. At each point on the horizontal axis, the values sum to 100%. (E) Total progeny number of individual adults in populations with 10, 75, 80, and 85% dauer & larva culling. Values are the average of three independent experiments with n=153, 258, 83 and 52 worms, Tukey's post-hoc HSD.

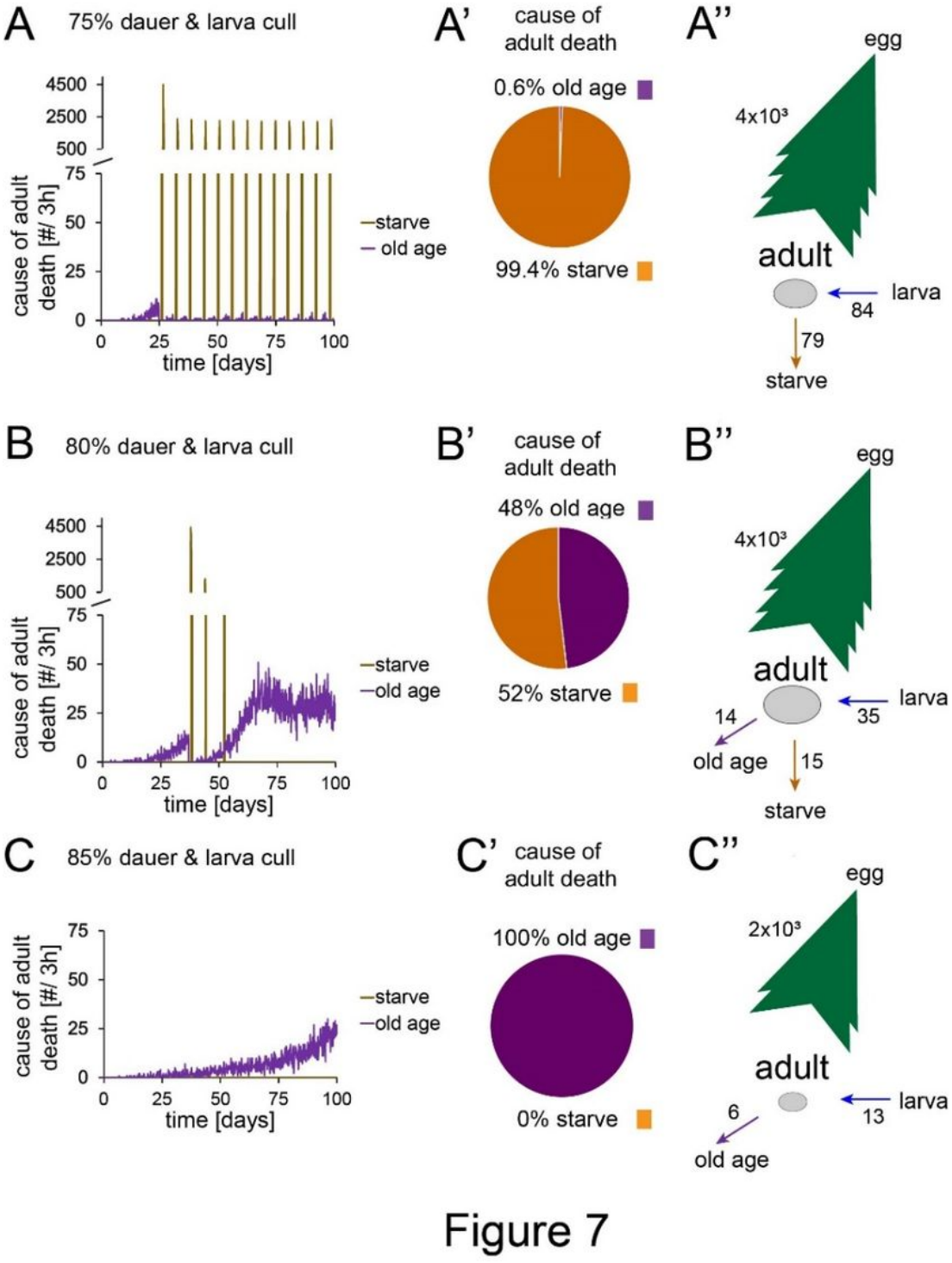

Figure 7

Figure 7

Population states where adults die of starvation or old age. (A-C) The death transitions of the adult node, starve (wt(a>p)) and old age (wt(a>o)), are displayed as number of worms/3 hours. One representative simulated population is depicted for dauer & larva culling of 75% (A), 80% (B), and 85% (C). (A', B', C') Pie charts display the cause of adult death for 75, 80, and 85% dauer & larva culling. (A", B", C") Flow diagrams of the adult node displaying all worm transition rates for 75, 80, and 85% dauer & larva culling.

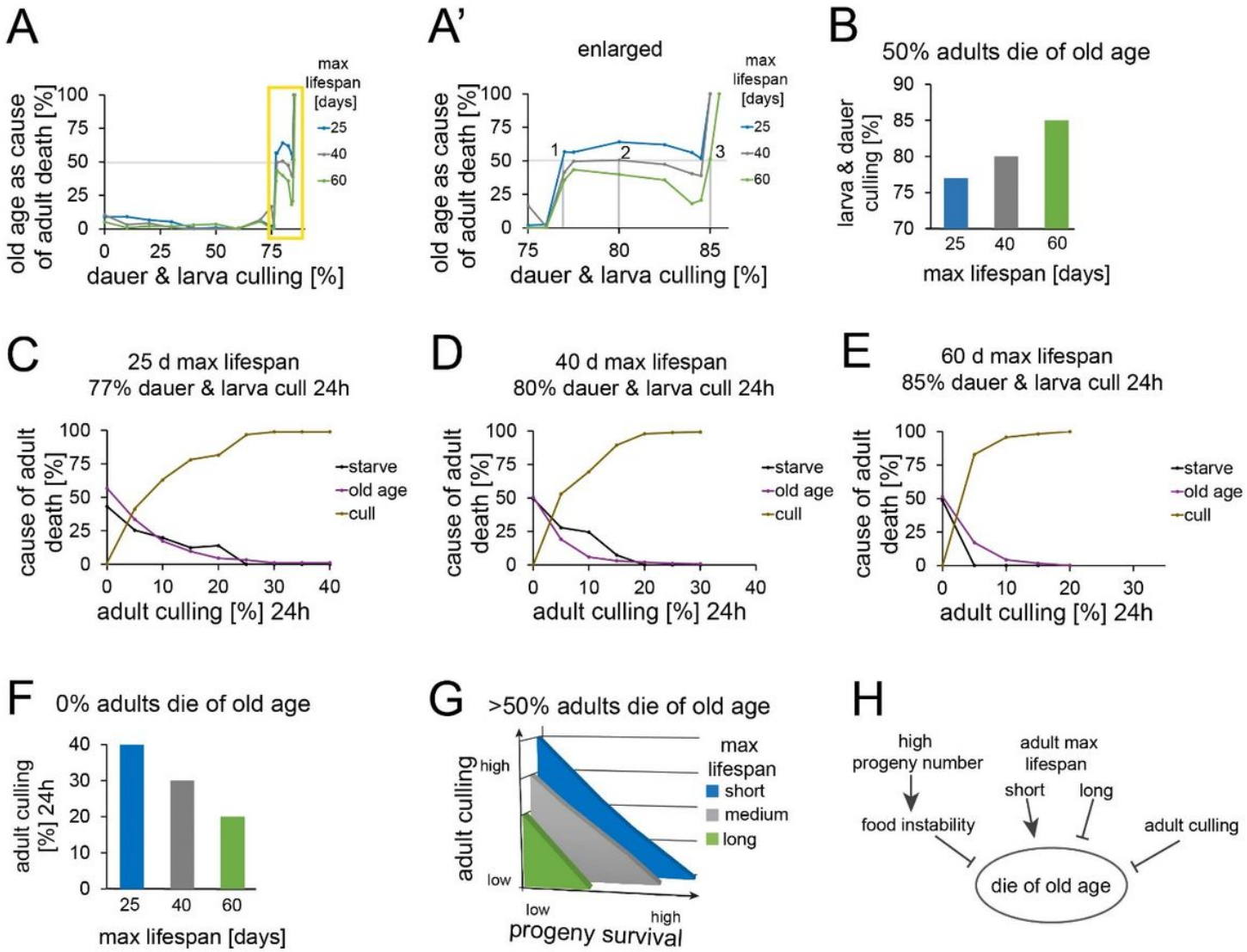

Figure 8

Figure 8

Maximum lifespan and adult culling influence old age as a cause of adult death. (A, A') Summary statistics for simulated populations with a variable percentage of dauer & larva culling: average percent of adults that die of old age. Maximum lifespan was 25, 40, or 60 days. (A') Gray lines and numbers depict the lowest percent of dauer & larva culling that causes 50% of adults to die of old age: 25 days (1), 40 days (2), 60 days (3). (B) Bars depict the lowest percent of dauer & larva culling that causes 50% of adults to die of old age based on the data in panel A'. (C-E) Summary statistics for simulated populations

with a variable percentage of adult culling: average percent of adults that die of starvation, old age or culling. At each point on the horizontal axis, the values sum to 100%. We used the dauer & larval culling value that causes 50% of adults to die of old age with 0% adult culling: 77% for the 25-day maximum lifespan (C), 80% for the 40-day maximum lifespan (D), and 85% for the 60 day maximum lifespan (E). (F) Bars depict the lowest percent of adult culling that causes 0% of adults to die of old age based on the data in panels C-E. (G) Summary of the relationship between maximum lifespan, food security (progeny survival), and extrinsic adult death (culling). Triangles indicate conditions in which more than 50% of adults die of old age. (H) Multiple factors influence the number of adults in the population that die of old age.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementalmaterial.pdf
- flatScharfepc.pdf
- flatScharfrs.pdf
- flatScharfspc.pdf
- SupplementalTable3.xlsx