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Delayed development and lifespan extension as features of metabolic lifestyle alteration in *C. elegans* under dietary restriction

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Summary

Studies of the model organism *Caenorhabditis elegans* have almost exclusively utilized growth on a bacterial diet. Such culturing presents a challenge to automation of experimentation and introduces bacterial metabolism as a secondary concern in drug and environmental toxicology studies. Axenic cultivation of *C. elegans* can avoid these problems, yet past work suggests that axenic growth is unhealthy for *C. elegans*. Here we employ a chemically defined liquid medium to culture *C. elegans* and find development slows, fecundity declines, lifespan increases, lipid and protein stores decrease, and gene expression changes relative to that on a bacterial diet. These changes do not appear to be random pathologies associated with malnutrition, as there are no developmental delays associated with starvation, such as L1 or dauer diapause. Additionally, development and reproductive period are fixed percentages of lifespan regardless of diet, suggesting that these alterations are adaptive. We propose that *C. elegans* can exist as a healthy animal with at least two distinct adult life histories. One life history maximizes the intrinsic rate of population increase, the other maximizes the efficiency of exploitation of the carrying capacity of the environment. Microarray analysis reveals increased transcript levels of *daf-16* and downstream targets and past experiments demonstrate that DAF-16 (FOXO) acting on downstream targets can influence all of the phenotypes we see altered in maintenance medium. Thus, life history alteration in response to diet may be modulated by DAF-16. Our observations introduce a powerful system for automation of experimentation on healthy *C. elegans* and for systematic analysis of the profound impact of diet on animal physiology.

Supplementary material available online at
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Introduction

The soil nematode *Caenorhabditis elegans* has become a much favored model organism since it was proposed as a multi-cellular organism in which genetics could be used for studies of physiology (Dougherty and Calhoun, 1948). Relatively little is known of the natural history of *C. elegans*, as distinct from that of nematodes in general, but it is generally accepted that *C. elegans* is an R-strategist (Hodgkin and Barnes, 1991). It has been noted that the nearly exclusive use of one standard culture system for growing *C. elegans* (using *Escherichia coli* as a food source) has brought immense success, but has also potentially biased our understanding of *C. elegans* biology (Hodgkin and Doniach, 1997). For example, when grown on a bacterial diet, *C. elegans* displays essentially invariant development with a predictable cell lineage (Sulston et al., 1983), cell number and nervous system (White et al., 1986); we do not know if these properties remain invariant under other growth conditions.

Following the pioneering work of Dougherty (Dougherty, 1959), a chemically defined axenic medium, *C. elegans* maintenance medium (CeMM), has been established for liquid culturing of *C. elegans* (Lu and Goetsch, 1993; Szewczyk et al., 2003). The use of liquid culture techniques allows automation, an important factor for spaceflight and high throughput experimentation and analysis. Additionally, the chemically defined nature of CeMM is advantageous to the use of *C. elegans* as a biological environmental monitor (Custodia et al., 2001), in pharmaceutical discovery (Kaletta and Hengartner, 2006), and/or in toxicity testing (Dengg and van Meel, 2004) where secondary effects of *E. coli* metabolism can be a confounding variable (Dougherty, 1959). Past liquid culturing of *C. elegans* employing undefined axenic diets have

found increased lifespan, decreased body mass, reduced fecundity and increased metabolic rate per unit mass, relative to worms cultivated on bacteria (Houthoofd et al., 2002). The similarity of these results to *C. elegans* grown on reduced amounts of *E. coli* (Klass, 1977), suggests that axenic growth may be 'unhealthy' for *C. elegans*. Alternatively, axenic cultivation of *C. elegans* may simply result in an altered life history in response to altered diet.

Theoretically, a species entering a new environment has a choice in how best to attempt to thrive (MacArthur and Wilson, 1967). On the one hand, the intrinsic rate of population growth could be maximized, at the expense of environmental resource depletion, by adopting a life history of rapid development and large number of progeny as is seen with cultivation of *C. elegans* on a bacterial diet. On the other hand, the organism could maximize the efficiency of exploitation of the carrying capacity of the environment resulting in delayed development and a prolonged reproductive period.

In order to test directly if *C. elegans* undergo a life history alteration in response to diet, we have compared life history on two diets. We show that worms grown in chemically defined medium (CeMM) do indeed undergo a life history alteration. Animals in CeMM take longer to develop and exhibit a prolonged reproductive period. Additionally, animals have decreased fecundity, increased lifespan, reduced metabolic stores, and an increased lifespan. As development, reproductive period, and lifespan are all prolonged in CeMM, we find that developmental stages and reproductive period remain fixed percentages of the 90th percentile of maximum lifespan regardless of diet. This conservation of life stages as a percentage of lifespan suggests that the alterations seen in CeMM are adaptive to the diet present and not simply random pathological alterations (i.e. CeMM-grown animals have a decreased 'rate of living') (Finkel and Holbrook, 2000; Pearl, 1928). Our findings imply that *C. elegans* can adopt at least two adult life histories that are each normal and healthy for a specific diet. As 'rate of living', broodsize and metabolic stores are decreased in CeMM-grown animals, we suspect that metabolic resources available, per unit time, for various biochemical reactions are decreased as the result of animals adopting a life history of more efficient exploitation of the carrying capacity of the environment. Prior studies have shown that the forkhead-class transcription factor DAF-16 (McElwee et al., 2003) can control all the adult phenotypes we observe are altered in CeMM. Microarray analysis indicates increased levels of *daf-16* transcript in CeMM grown animals, suggesting that DAF-16, whose activity is regulated by insulin signaling (Ogg and Ruvkun, 1998), modulates the metabolic life history alteration we observe in response to altered diet. Our observations demonstrate that animals grown in CeMM are 'healthy but different' from animals grown on nematode growth medium (NGM) and introduce a powerful system for automation of experimentation on healthy *C. elegans* and for systematic analysis of the profound impact of diet on animal physiology.

Materials and methods

Wild-type strains of *Caenorhabditis elegans* (Maupas 1900, Dougherty 1955) used in this study were N2 from the Caenorhabditis Genetics Center, and CC1 (Szewczyk et al., 2003). To control for potential maternal effects of temperature, N2 and CC1 were allowed to grow for three generations at 15°, 20° or 25°C prior to the onset of these studies. Growth of animals in CeMM at 15°C results in approximately a 10% frequency of arrest at the L4 stage or development into sterile adults and about 2% male development. These animals were not excluded from the longevity or broodsize assessments.

To assess the growth of individual animals we allowed five wild-type adults to lay eggs for 2 h on NGM plates or for 24 h in 200 μl of CeMM (Fisher) in a well of a 24-well tissue culture plate. Each assessment was completed in triplicate and averaged.

To assess brood size, 100 individual animals were allowed to develop separately on NGM plates or in 200 μl of CeMM in a well of a 24-well tissue culture plate at 15°, 20° or 25°C. Animals were transferred to new plates or wells as needed to distinguish the parent from the progeny (approximately every day or two for NGM and every 3–5 days for CeMM). The total number of L1 stage progeny of each animal was scored and the total number of progeny for each 100 animals was averaged. These animals were also scored once a day for death. Death was determined by a lack of pharyngeal pumping and a lack of movement in response to physical stimulation.

Acute transfer of animals from NGM to CeMM was accomplished by picking early adults off an NGM plate, into a small Petri dish containing 3 ml of BU buffer (70 mmol l^{-1} potassium phosphate, 70 mmol l^{-1} NaCl, pH 7.0) with added streptomycin (200 μg ml^{-1}) and kanamycin (20 μg ml^{-1}). After a few minutes of swimming in the BU, individual animals were pipetted into a well of a 24-well tissue culture dish containing 200 μl of CeMM with added streptomycin (200 μg ml^{-1}) and kanamycin (20 μg ml^{-1}) and broodsizes determined as above. We also confirmed that CeMM growth curves, as described above, are unaltered by the addition of streptomycin (200 μg ml^{-1}), kanamycin (20 μg ml^{-1}), or nystatin (500 i.u. ml^{-1}), either individually or together in any combination at 20°C (not shown).

Total RNA for microarray analysis was isolated using TRI Reagent (Molecular Research Center, Cincinnati, OH, USA) following the supplied protocol. mRNA isolation was accomplished using the MACS mRNA isolation kit and protocol (Miltenyi Biotec, Cologne). Isolated RNA was labeled (DeRisi et al., 1997) as previously described for hybridization to near full genome *C. elegans* DNA microarrays (Jiang et al., 2001). Microarray processing was preformed as previously described (Wang and Kim, 2003) and the data are available from the Stanford Microarray Database (Gollub et al., 2003).

Reliable data (r>0.6 across the spot, mean value of normalized intensity above background) was obtained for 10204 cDNAs on at least one array, 6484 on two, and 1202 on all three. This represents at least one reliable data point for a little over half the genome and three reliable data points for a little over

5% of the genome. As displayed here, data from three reliable data points were averaged with the mountains as previously assigned (Kim et al., 2001).

Results

A general slowing of C. elegans development in defined medium

Like animals grown in complex axenic media (DeCuyper and Vanfleteren, 1982), animals grown in defined CeMM at 20°C display a developmental delay (Szewczyk et al., 2003). To assess the nature of this developmental delay we created a developmental profile for CeMM-grown animals.

Because the rate of *C. elegans* development is temperature dependent (Byerly et al., 1976), we examined the growth rate at three common temperatures of culture. Animals grown on either an NGM or CeMM diet displayed temperature dependency of growth and reached early adulthood in times consistent with those previously published for NGM or complex axenic media (Fig. 1) (Byerly et al., 1976; DeCuyper and Vanfleteren, 1982). Although growth in liquid CeMM may be different in many respects from growth on CeMM solidified with agar, it has previously been reported that the physical form of CeMM does not influence the growth rate at 20°C (Szewczyk et al., 2003).

We next examined larval moulting, measuring and plotting the shed cuticular sheath lengths in 2-week-old cultures, as previously done for the related species *C. briggsae* (Jantunen, 1964). The lengths of CeMM liquid-grown animals at moults (Fig. 2) differ from the calculated lengths of *C. elegans* on NGM plates (Byerly et al., 1976). It may simply be that fewer resources are available for cuticle formation in CeMM. However, unlike any other trait we have measured, length at time of moult is relatively temperature insensitive for both NGM and CeMM grown animals. The simplest explanation for this is that length dictates moulting, with some unknown factor setting an appropriate length in response to diet. This would explain why the distribution patterns of expression of moulting genes (Frand et al., 2005) and length at moults (Fig. 2) look strikingly similar.

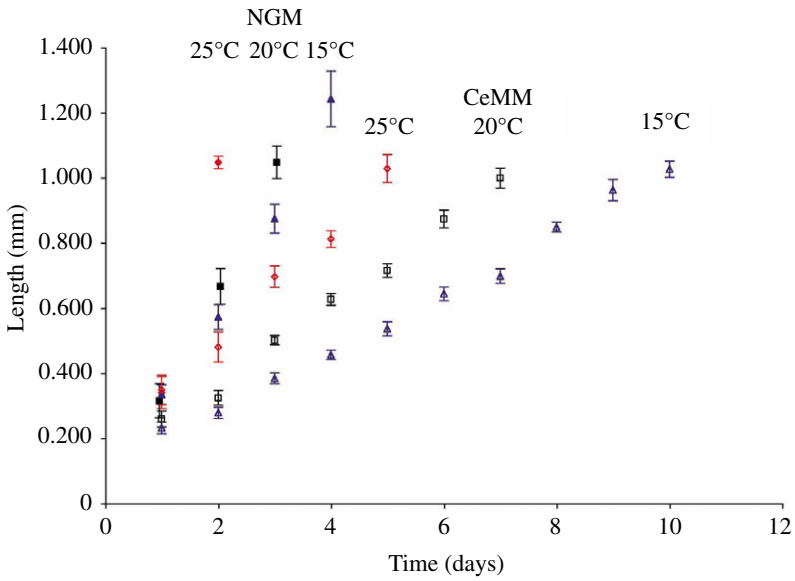

Fig. 1. Development is temperature and diet dependent. Animals grown on NGM (filled symbols) develop faster than animals grown in CeMM (open symbols) at the same temperature. Animals also grow faster as temperature is increased from 15°C (blue) to 20°C (black) or 25°C (red) regardless of diet. Each point represents the mean ± s.d. of three trials of ten animals each.

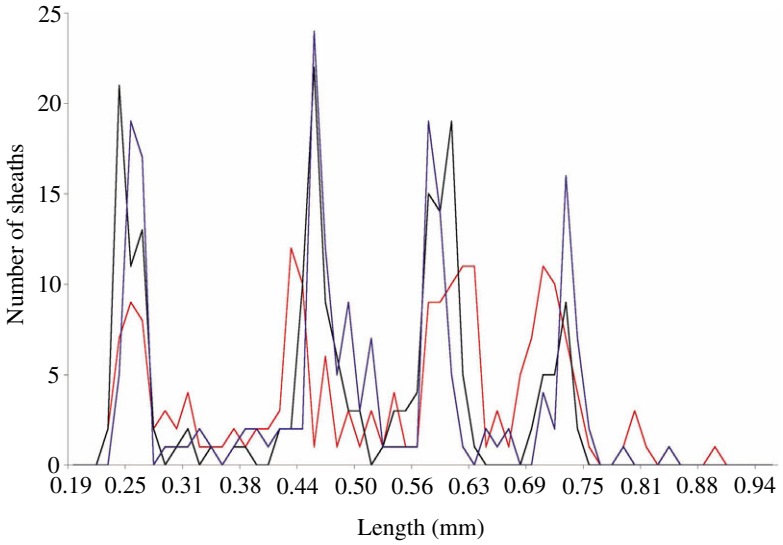

Fig. 2. Moulting profile in CeMM. Animals grown in CeMM appear to moult at approximately the same length regardless of temperature. 200 moulted sheaths were measured following random selection from 3-week-old cultures grown at 15°C (blue), 20°C (black), or 25°C (red). Each of the four peaks represents one of the four larval stage transitions (L1–L2, L2–L3, L3–L4, L4–adult).

Combining the growth and moulting data, we generated developmental growth curves and determined the approximate amount of time spent in each developmental stage as a percentage of the total time to adulthood (Table 1). There were no stage-specific developmental delays, such as the L1 or dauer diapause associated with starvation. NGM-grown animals have a temperature-dependent increase in the time spent in the L1 stage. By contrast, the amount of time spend in each developmental stage was more evenly distributed in CeMM-cultivated animals. Thus, CeMM-grown animals have a general slowing of developmental processes and do not display any abnormal developmental pauses associated with malnutrition.

A lengthening of the reproductive period in defined medium

The general slowing of development in response to culturing in CeMM is consistent with the developmental delay predicted

Table 1. *Developmental profile of C. elegans*

	NGM			CeMM		
	15°C	20°C	25°C	15°C	20°C	25°C
Total to adulthood						
Days	0–4	0–3	0–2	0–8	0–6	0–4
Percent of time to adulthood	100	100	100	100	100	100
Percent of lifespan*	11±1	11±1	13±2	10±1	10±1	10±1
L1						
Days	0–1	0–1	0–1	0–2	0–1	0–1
Percent of time to adulthood	~31	~40	~52	~25	~20	~18
Percent of lifespan*	3±1	4±1	6±2	3±1	2±1	2±1
L2						
Days	1–2	1–2	1–2	2–4	1–3	1–2
Percent of time to adulthood	~20	~20	~13	~30	~38	~33
Percent of lifespan*	2±1	2±1	2±1	3±1	4±1	3±1
L3						
Days	2–3	3	1–2	4–6	3–4	2–3
Percent of time to adulthood	~18	~12	~11	~19	~25	~33
Percent of lifespan*	2±1	1±1	2±2	2±1	3±1	3±1
L4						
Days	3–4	3	1–2	6–8	5–6	3–4
Percent of time to adulthood	~31	~28	~23	~24	~16	~16
Percent of lifespan*	3±1	3±1	2±2	2±1	2±1	2±1

*Larval stages are expressed as a percentage of the 90th percentile of maximum lifespan (*N*=3 trials, 10 animals per trial, means ± half maximal error).

from a life history alteration toward maximization of the exploitation of the carrying capacity of the environment (MacArthur and Wilson, 1967). We therefore examined the reproductive period of CeMM grown animals and found it was likewise lengthened (Table 2), consistent with a life history alteration. Broodsize is an inverse function of temperature but is not a strong diagnostic of temperature of cultivation (Fig. 3; Table 2). The same curve describes the relationship between broodsize and temperature for both diets (not shown), indicating conservation of at least some of the processes underlying reproduction. However, the lengthening of the reproductive period did not allow CeMM-grown animals to produce as many eggs as NGM-grown counterparts. This may suggest that, for *C. elegans*, there is a 'reproductive cost' to shifting toward a life history that maximizes exploitation of the carrying capacity of the environment.

Table 2. *Reproductive profile of C. elegans*

	Number of eggs*	Egg laying period	Egg laying period as percentage of 90th percentile of maximum lifespan
NGM			
15°C	254±52	Days 4–8	14±3†
20°C	240±59	Days 3–5	9±3†
25°C	155±42	Days 2–4	13±4†
CeMM			
15°C	107±58	Days 9–19	13±1†
20°C	81±31	Days 7–14	13±1†
25°C	42±18	Days 5–9	11±3†

N=100 for each treatment (mean ± s.d.).
*Values are means ± s.d.
†Deviation was calculated assuming an error of 1 day in measurement (half maximal).

Broodsize and lifespan are independently responsive to the environment

Growth of *C. elegans* in undefined medium has previously been shown to result in decreased fecundity, and these animals have been shown to have an increased lifespan (DeCuyper and Vanfleteren, 1982). Animals of various species with increased lifespan as the result of dietary restriction or reduction of function mutations in an insulin signaling pathway have led to the hypothesis that broodsize may directly influence lifespan as the result of a 'cost of reproduction' (Partridge et al., 2005). To test directly the hypothesis that *C. elegans* broodsize influences lifespan, we compared broodsize and lifespan of animals grown on NGM and CeMM. Consistent with prior reports, we found that *C. elegans* exhibits both reduced fecundity and increased lifespan when on an axenic diet (Fig. 3). Because *C. elegans* broodsize and lifespan are temperature-dependent traits (Byerly et al., 1976; Klass, 1977) we chose to apply a standard

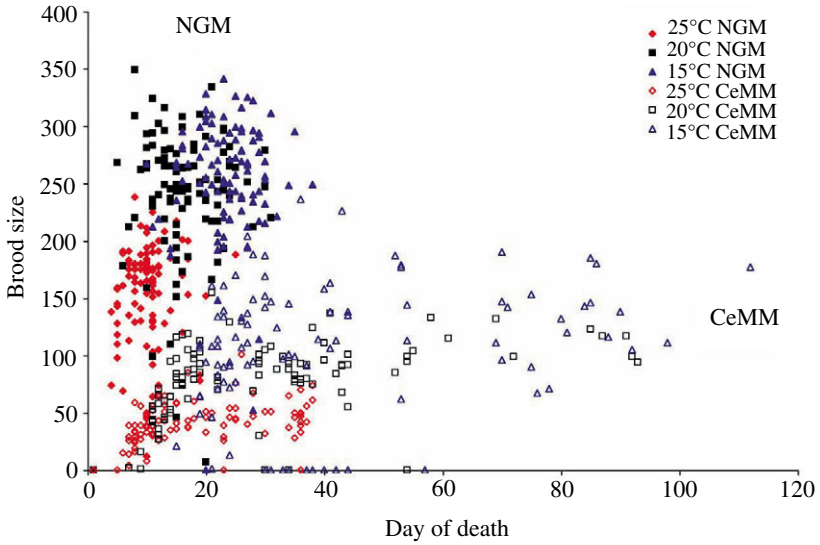

Fig. 3. Life history alteration in response to diet. Animals grown on NGM have larger broodsizes whereas animals grown in CeMM live longer. Each point represents a single animal. Growth at 25°C is represented by diamonds, 20°C by squares and 15°C by triangles. Growth on NGM is represented by filled symbols and growth on CeMM by open symbols. Data on these same cohorts (N=100 each) also appear in Fig. 4 and Tables 1–3.

systems biology approach of altering a second variable (Sachs et al., 2005), temperature, to understand the relationship between fecundity and lifespan. Broodsize and lifespan covary for both temperature and diet but there is a direct correlation for temperature and an inverse correlation for diet (Fig. 3). Thus, there is not a clear relationship between broodsize and lifespan. It may be that the covariance results from simple coregulation or that a feedback system maintains a balance between the two.

Does survival come at the cost of a shorter lifespan?

Like animals grown in other, chemically undefined, axenic media (DeCuyper and Vanfleteren, 1982), animals grown in defined CeMM have a lengthened lifespan compared to those grown on NGM (Fig. 4A). As is also true for animals grown in complex axenic media (Vanfleteren et al., 1998), CeMM survival curves are best fit using a three-parameter logistic equation. A question was posed (Vanfleteren et al., 1998) as to whether or not such curves were only appropriate for axenic culture. We find our NGM survival curves are also best fit using this method, although the actual values of the parameters are quite different. This point can easily be seen when survival is plotted against the median lifespan (Fig. 4B).

The observation that many animals die sooner as temperature increases has, in part, led to the hypothesis that there is a ‘rate of living’ (Finkel and Holbrook, 2000; Pearl, 1928). Consistent with this hypothesis, we find that temperature dependence of population death curves holds regardless of diet (Fig. 4A).

In contrast to the temperature dependence of population death curves, the temperature dependence of population survival curves is quite different for animals grown on NGM *versus* in CeMM (Fig. 4C). Animals always die sooner as temperature increases (Fig. 4A) but the fraction of animals dying at a given age (i.e. the steepness of the curve) increases with temperature for NGM- but not CeMM-grown animals (Fig. 4C). Since the cause of the temperature dependence of population survival curves is unknown for NGM-grown animals, we can only speculate as to why temperature dependence is lost in CeMM-grown animals. The simplest explanation is that *E. coli* toxicity (Walker et al., 2005) is temperature dependent. This possibility could be tested by utilizing UV-killed *E. coli*, which are already known to increase lifespan at 20°C (Gems and Riddle, 2000). An alternative explanation is that failure of critical biochemical processes, which are inherently temperature dependent, underlies death in *C. elegans*. Theoretically, increased efficiency in biochemical processes as the result of increased efficiency in exploitation of the carrying capacity of the environment in CeMM could mask apparent temperature sensitive failure of these processes (i.e. CeMM growth shifts the temperature tolerance of survival). This latter explanation could explain why CeMM-grown animals die sooner at 25°C than predicted from the 15°C and 20°C data.

Taken together, the data in Fig. 4 add weight to the suggestion (Kirkwood et al., 2005) that *C. elegans* population survival curves describe a stochastic process where individual deaths occur randomly across the adult lifespan. The data in Fig. 4B suggest that this process can be altered by diet, so as to affect the likelihood that time of death has a large deviation from the median. Fig. 4C further suggests that a bacterial diet prolongs survival, making deaths of individuals appear less randomly distributed over the population lifespan, whereas an axenic diet prolongs lifespan. An ironic interpretation of these data is that the cost of reduced susceptibility to stochastic death is a shortened lifespan.

Reproduction comes with a higher risk of death in defined medium

When grown in defined CeMM, a small percentage of animals appear to live substantially longer than others (Fig. 4A), as reported for animals grown in other, chemically undefined, axenic medium (DeCuyper and Vanfleteren, 1982). To determine why some animals appear to live longer, we examined the cause(s) of death. We found that animals grown in CeMM are twice as likely to die from prolapsed or ruptured vulva and internal hatching than are NGM grown animals (Table 3). When these deaths are omitted from death curves such as in Fig. 4A, the curves still depict a stochastic process but the extent to which a small percentage of animals appear to live much longer than others is greatly reduced (not shown). This suggests that apparent tails in death curves of axenically grown *C. elegans* are observed, in part, because reproduction

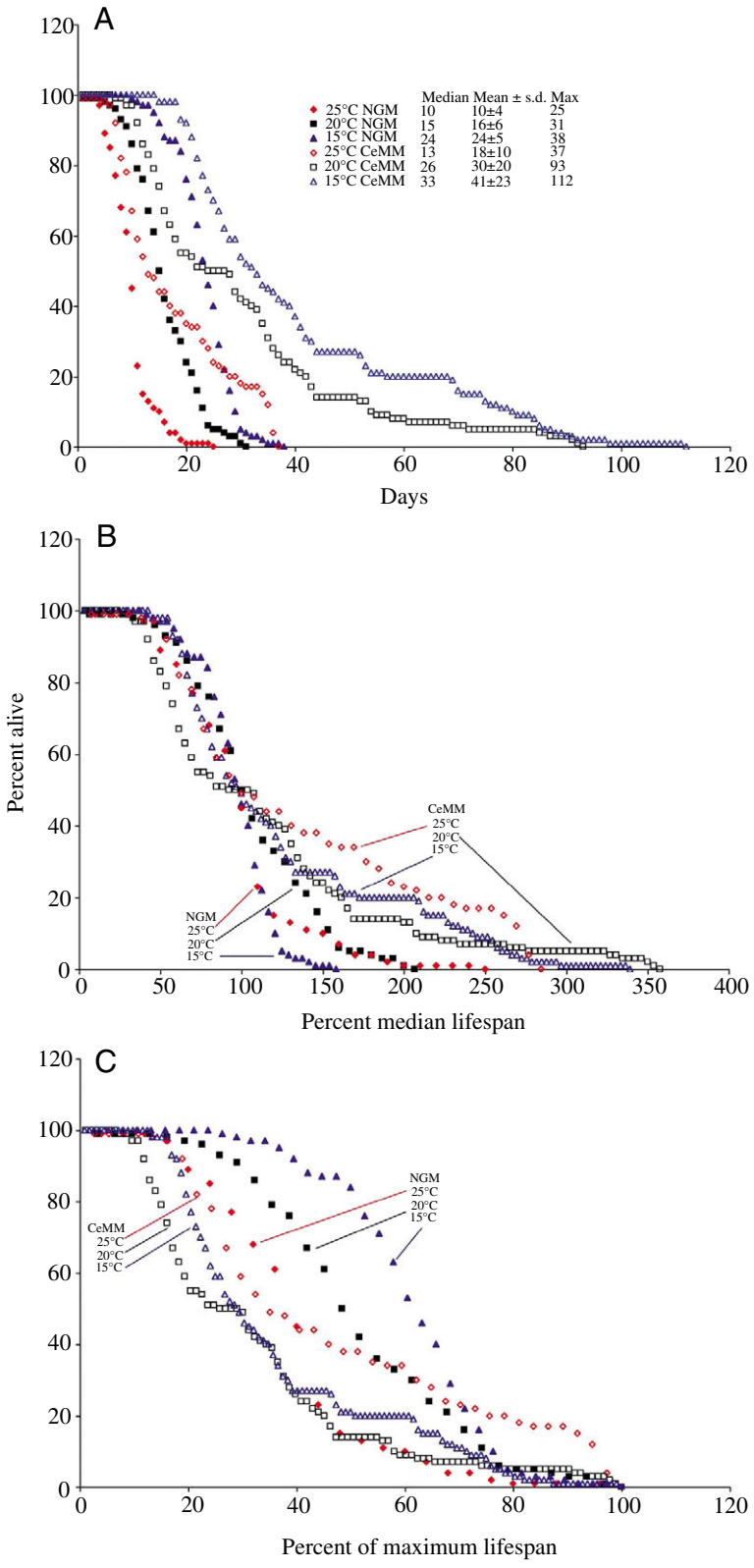

Fig. 4. Effects of diet and temperature on survival. Lifespan of *C. elegans* at 25°C (diamonds), 20°C (squares) and 15°C (triangles) on NGM (filled symbols) or in CeMM (open symbols). (A) A typical death curve. Inset is a table of median, mean ± s.d. and maximum life span. (B) Data expressed as percentage of median lifespan. (C) Data expressed as a percentage of maximum lifespan. This format is also known as a survival curve.

comes at large cost (i.e. risk of death) to a population in axenic medium.

Whereas reproduction may come at a huge 'cost' to an individual animal, there is not an obvious 'cost' that can account for the decreased broodsizes of CeMM-grown animals as even those animals that do not die as the result of reproducing do not produce broodsizes comparable to those of NGM grown animals (Fig. 3). The general slowing of development, and lengthening of the reproductive period suggest that mobilization of the resources needed to proceed through each developmental stage and to produce a zygote takes longer. The hypothesis that CeMM-grown animals have fewer resources available, per unit time, for developmental processes and zygote production predicts that stored resources are decreased.

Decrease metabolic storage in defined medium

Like other axenically grown animals (Houthoofd et al., 2002), those grown in CeMM are thinner than counterparts on NGM. To test the idea that CeMM-grown animals place fewer metabolic resources in storage, we examined lipid and protein stores.

Using Nile Red to visualize lipid stores (Ashrafi et al., 2003), we find CeMM-grown animals have decreased lipid stores in the intestine (Fig. 5A). Ninety percent of animals fed on CeMM had lipid stores at or below the level observed in animals starved for 24 h after having been fed on NGM. The remaining ten percent had higher lipid stores but not as high as those of animals fed on NGM.

Transgenic proteins can be used to follow the expression and degradation of endogenous cytosolic or myofibrillar proteins (Fostel et al., 2003). We employed *unc-54::lacZ* transgenic animals to examine a muscle-expressed transgenic β-galactosidase fusion protein that remains stable in fed animals on NGM, and which is catabolised upon starvation (Zdinak et al., 1997). Nearly all animals fed on NGM displayed normal X-gal staining in the body wall muscles and complete loss of staining following 48 h of starvation (Fig. 5B). By contrast, only 20% of animals fed on CeMM displayed normal staining in the body wall muscles, with the remaining 80% displaying no staining in the body wall muscles. Microarray analysis (see below), suggests that decreased synthesis of the reporter protein is sufficient to account for the decreased staining. These results are consistent with CeMM-grown animals having decreased protein stores in muscle.

The lipid and protein staining patterns do not appear to represent the effects of continued starvation, as staining does not decrease with age throughout the reproductive period. The reduced stores could simply represent decreased commitment to storage, or a more

Table 3. *Deaths associated with reproduction*

Cause of death	% Died	
	Cultured on NGM	Cultured on CeMM
25°C		
Ruptured vulva	2	11
Prolapsed vulva	1	17
Internal hatch	21	18
Combined	24	46
Post egg laying	100	76
20°C		
Ruptured vulva	3	4
Prolapsed vulva	1	19
Internal hatch	21	18
Combined	24	41
Post egg laying	97	63
15°C		
Ruptured vulva	4	2
Prolapsed vulva	6	11
Internal hatch	5	37
Combined	15	50
Post egg laying	100	88

N=100 for each treatment.

complex situation. In the case of the proteins, microarray analysis (see below) suggests decreased expression of muscle proteins, which is consistent with decreased commitment to storage. The reduced lipid stores however, might themselves alter endocrine signaling (Dowell et al., 2005). Manipulation of *C. elegans* diet by changing the composition of chemically defined media should provide more insight into the mechanism(s) of resource allocation to various biological processes (e.g. reproduction, storage, movement, etc.) than single diet based studies.

Development and reproductive period are a relatively constant fraction of lifespan

The slowed development, extended reproductive period, conserved relationship between broodsize and temperature, extended lifespan, and the decreased metabolic stores are consistent with adjustment of a "rate of living" that is determined by metabolism (Finkel and Holbrook, 2000; Pearl, 1928). If such an adjustment has occurred, then the reproductive period should be a scaled percentage of lifespan regardless of diet. We examined reproductive period as a percentage of the 90th percentile of maximum lifespan to avoid any statistical error introduced by fluctuation in the day of death of the last survivor. Surprisingly, both developmental stages and reproductive period are constant percentages, within experimental error, of the 90th percentile of maximum lifespan regardless of diet (Table 1, 2). Thus, the amount of time *C. elegans* spends in any developmental stage or the reproductive period can be used to predict accurately the maximum population (though not necessarily individual) lifespan, when temperature and diet are held constant. Intriguingly, the temperature-dependent increase in time spent in the L1 stage observed for NGM-grown animals remains even when development is viewed in the context of lifespan (Table 1). This may be consistent with L1 diapause, like dauer diapause, being regulated by both temperature and diet.

Short term plasticity of life history traits?

While we have shown that *C. elegans* can adopt two alternative adult life histories as a long-term response to two different diets, an interesting and important question remains: Are life history traits, individually and/or collectively, also plastic as a short-term response to abrupt changes in diet and/or temperature? *C. elegans* lifespan and rates of behavior appear to be set early in life (Dillin et al., 2002), suggesting that once selected, life history is committed. However, prior studies using complex axenic media suggest life history has some level of plasticity. Acute diet shift results in lifespan alteration (Houthoofd et al., 2005b) and acute temperature shift results in broodsize alteration (Houthoofd et al., 2005a); in both cases the alteration is toward the predicted value for animals raised exclusively on the new diet or temperature and away from the value for animals raised on the original diet or temperature. In keeping with these past results, we find acute shift from NGM to CeMM at 20°C, prior to egg laying, results in broodsizes that are much smaller than on NGM. These broodsizes are also smaller than those from animals grown exclusively in CeMM, but this difference is not statistically significant (41±15, *N*=10; compare with Table 2). An appropriate increase in the reproductive period is also observed (7.5 days; compare with Table 2). Systematic alteration of diet and temperature throughout the lifespan should clarify the nature and extent of such lifestyle plasticity.

Transcript levels of daf-16 are increased in defined medium

The question of how the phenotypes observed in response to diet are tied to the molecular mechanism of metabolic alteration led us to probe gene expression. Roughly 10% of the genome, 1881 cDNAs, had a reliable and significant (at least a fourfold) expression difference between NGM and CeMM grown animals in at least one of three trials. Given the large-scale changes in gene expression in response to diet, any discussion of the results is necessarily limited by our incomplete understanding of the function of each gene in the *C. elegans* genome and our current limited ability to appreciate how simultaneous changes in 2000 genes translate into organism scale changes. Therefore, the full microarray dataset is available as supplementary material or from the Stanford Microarray Database or upon request from the authors.

The overlapping set of genes that is reliably, significantly, and reproducibly altered on all three trials is displayed in Table 4. Consistent with the reduced lipid and protein stores, 8 of the 26 genes identified as downregulated have a putative role in metabolism based upon sequence identity. Experimental evidence confirms this for three: *daf-18* (Ogg and Ruvkun, 1998) and F25D1.1 and K05C4.5 (Wang and Kim, 2003).

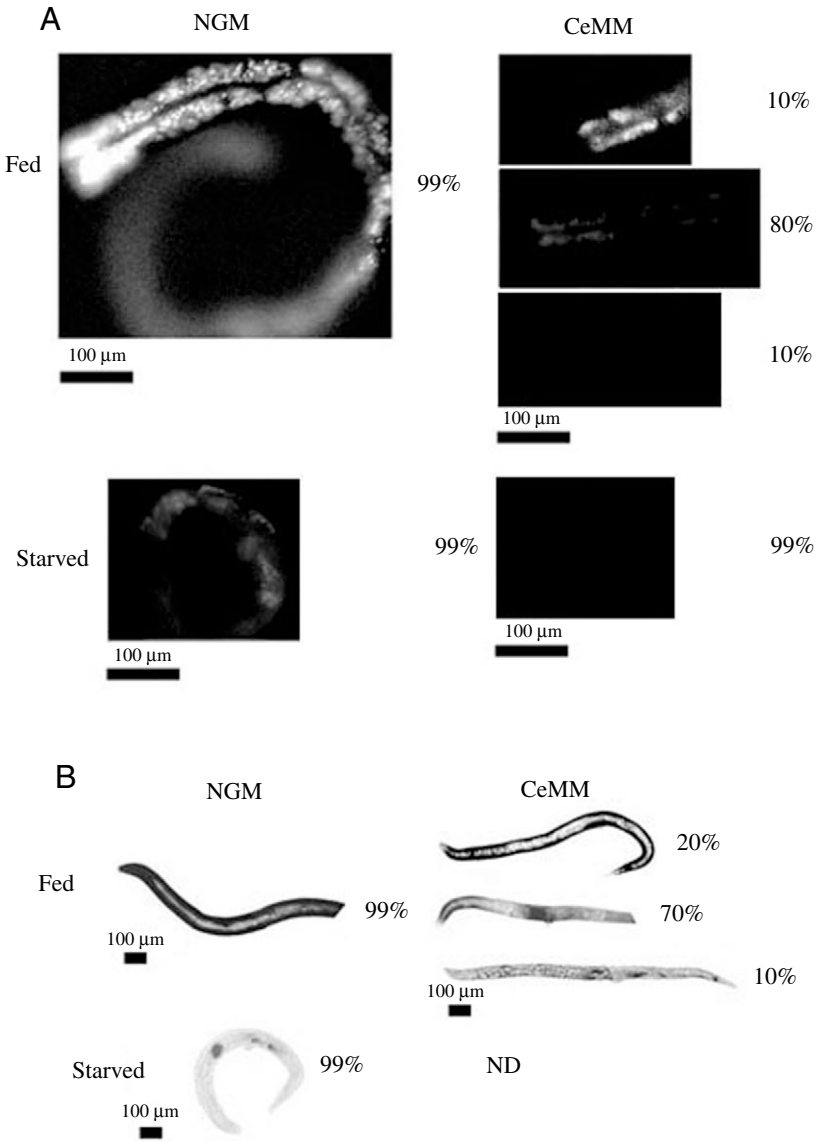

Fig. 5. CeMM culture results in decreased lipid and protein stores. (A) Nile Red staining of lipid deposits in the intestinal cells reveals decreased lipid stores. Almost all animals display strong staining when fed on NGM and decreased staining when starved for 24 h or fed CeMM. All images had the same exposure time. The percentage of 100 animals per condition displaying similar fluorescence intensity is shown on the right of each image. Images of animals fed CeMM are only of the gut immediately posterior to the pharynx. This is the most fluorescent portion of the gut as seen in the image of the NGM fed animal. The CeMM fed group fall into three categories: normal fluorescence (top), weak fluorescence (middle) and very weak fluorescence (bottom). Assignment to these three groups reflects the relative exposure time (RE) needed to produce an image with normal fluorescence top (RE=1), middle (RE=2) bottom (RE=4). Starved NGM have an RE=2 whereas starved CeMM have an RE=4. Note the fed NGM image is saturated at RE=1. (B) Staining for β-galactosidase activity in *unc-54::lacZ* transgenic animals reveals decreased protein stores. The percentage of 100 animals per condition displaying the staining pattern is shown on the right of each image. Almost all animals display full body wall muscle staining when fed on NGM and no staining when starved for 48 h (stain near the vulva is embryo staining). Most animals in CeMM display no body wall muscle staining but head and vulval muscle staining, as associated with 24 h starvation (Zdinak et al., 1997). A smaller percentage of animals display body wall muscle staining when fed CeMM and an even smaller percentage no staining. In contrast to the degradation of this reporter seen in starved animals, microarray analysis suggests the decreased staining is due to decreased synthesis alone.

Similarly, 8 of the 22 genes identified as upregulated in the overlapping dataset are important for recovery from the metabolically altered dauer state and/or are downstream targets of the transcription factor DAF-16, which is known to regulate metabolic alterations (McElwee et al., 2003; Murphy et al., 2003; Wang and Kim, 2003). The predicted effects of the genes identified by the overlapping dataset are consistent with our life history and metabolic storage data.

The overlapping dataset identifies *daf-16* and three known downstream targets as being upregulated. Previous studies of *daf-16* have suggested a role in regulation of lifespan and metabolism (Ogg and Ruvkun, 1998) and transgenic overexpression yields thinner animals with delayed development, increased reproductive period and decreased broodsize (Henderson and Johnson, 2001). Therefore upregulation of *daf-16* is consistent with the morphological and physiological changes observed in CeMM-grown animals.

Discussion

Using stringent criteria for microarray analysis

We took a relatively novel approach in choosing criteria for deciding which microarray data to display in Table 4. We chose to display the overlapping data set for which genes were identified as quantitatively reproducibly measured (less than 10% error on the mean) and significantly altered (greater than fourfold change). Of this overlapping dataset, the genes identified as down- or upregulated are consistent with the observed biological changes. Thus it appears that relatively stringent selection criteria can be used in reporting the results of microarray analysis. However, it is clear that setting stringent criteria for microarray analysis may cause one to overlook genes that contribute significantly to the biological changes observed. For example, *daf-16* and three known downstream targets were identified but a number of other predicted downstream targets of *daf-16* did not pass our criteria even thought they displayed significantly altered level, because

Table 4. *Altered gene expression in CeMM*

Fold decrease*	Genepairs name	Mountain	Encodes or predicted to encode
(a) Genes downregulated in CeMM versus NGM			
2.12±0.16	F59A1.10	1	Diacylglycerol acyltransferase 2-like
2.09±0.17	C18C4.5	1	Myosin/CLIP-190 like
2.06±0.07	T21B10.3	2	Nuclear protein
2.23±0.16	F42H10.7	2	DGCR14/ES2 like
2.31±0.22	K05C4.5	2	UTP4 like
2.23±0.06	ZK856.9	2	Zinc finger
2.58±0.15	ZC395.8	2	C2H2 zinc finger
2.82±0.23	T10F2.3	2	*ulp-1* Ubiquitin like protease
2.96±0.29	ZK593.8	5	*dnc-1* dynactin
2.36±0.15	F22B7.5	5	*dnj-10* protein with DNA J domain
2.07±0.12	K12D12.1	5	DNA topoisomerase
3.21±0.32	T07A9.6	7	*daf-18* PTEN
2.70±0.15	Y39A1A.12	7	Origin recognition complex 1
2.27±0.11	F49E8.2	7	Glutaredoxin
2.39±0.16	ZC317.7	7	Dynactin interacting protein like
2.78±0.17	T02B5.1	8	Carboxylesterase
2.44±0.22	ZK1320.9	8	Acetyl-CoA hydrolase/transferase
3.90±0.28	F25D1.1	11	Protein phosphatase
3.88±0.35	F56A6.1	11	eIF-2C, Piwi/argonaute family
2.46±0.18	C55B7.1	11	*glh-2* germ line helicase
2.41±0.20	C10H11.1	11	Supervillin
2.74±0.15	K07D8.1	16	*mup-4* transmembrane protein required for hypodermis and muscle attachment
4.90±0.20	T22B7.7	22	Long-chain acyl-CoA thioester hydrolase
3.82±0.38	F59D8.C	22	*vit-3* vitellogenin
3.01±0.24	C47B2.6	None	UDP-galactose-4-epimerase
3.63±0.32	F02A9.4	None	Methylcrotonoyl-CoA carboxylase
(b) Genes upregulated in CeMM versus NGM			
2.11±0.11	R13H8.1a	1	*daf-16* transcription factor
2.33±0.19	T20D3.6	2	Transmembrane protein
2.27±0.16	F31E3.6	2	Transmembrane protein
3.95±0.29	F35A5.2	6	Transmembrane protein with signal peptide
3.58±0.10	C18A11.2	6	Transmembrane protein with signal peptide
2.46±0.17	C39D10.2	6	Cytochrome *c* binding protein
2.31±0.19	M03E7.4	7	Protein with chitin binding peritrophin-A, and LD-lipoprotein receptor class A domains
3.92±0.36	T08G5.10	8	*mtl-2* calcium binding, heavy metal responsive
3.80±0.21	T10B10.6	8	Heavy metal binding with signal peptide
3.15±0.09	F32H5.3	8	Transmembrane protein with signal peptide
2.15±0.14	C25E10.8	8	Protein with trypsin inhibitor like cysteine rich domain
2.85±0.27	K11G9.6	15	*mtl-1* calcium binding, heavy metal and heat shock responsive
2.14±0.18	F18E9.2	15	*nlp-7* neuro-peptide like
7.64±0.18	F36D3.9	15	*cpr-2* Cathepsin
2.37±0.20	F54E7.2	23	*rps-12* 40S ribosomal protein S12
2.28±0.16	F37C12.9	23	*rps-14* 40S ribosomal protein S14
2.60±0.20	F37C12.11	23	*rps-21* 40S ribosomal protein S21
2.10±0.15	F39B2.6	23	*rps-26* 40S ribosomal protein S26
2.47±0.16	B0412.4	23	*rps-29* 40S ribosomal protein 29
2.49±0.12	Y48B6A.2	23	*rpl-43* 60S ribosomal protein L37A
2.53±0.13	F28F8.3	None	*lsm-5* U6 snRNA-associated Sm-like
2.84±0.11	Y46G5.36	None	Unknown

*Values are means ± s.d. of $\log_2$ (N=3).

the data were not also quantitatively reproducible. RNAi knockdown of each of three of the genes identified as significantly downregulated, *mrp-5*, *vit-5* and *rbr-2*, results in increased lifespan (Lee et al., 2003a; Murphy et al., 2003), suggesting that these genes may be involved in the lifespan extension seen in CeMM-grown animals.

DAF-16 (FOXO) as a modulator of three C. elegans life histories

As discussed above, DAF-16 is known to control all the adult phenotypes we have reported as being altered in CeMM. The microarray analysis demonstrates that *daf-16* is upregulated and that known downstream targets of *daf-16* are both up and downregulated (note that *daf-16* is known to both up- and downregulate various downstream targets) (Lee et al., 2003a; Lee et al., 2003b; McElwee et al., 2003; Murphy et al., 2003). In other words, upregulation of *daf-16* may be sufficient to account for our observations and alteration of *daf-16* and its downstream targets correlate with the alteration of diet. Thus, it is reasonable to conjecture that *daf-16* and its downstream targets are involved in the responses to growth on CeMM.

Here, we have compared *C. elegans* development, metabolism, gene expression and physiology in two different systems of culture and found two life histories for *C. elegans*. On NGM, *C. elegans* adopts a life history of rapid development and a large number of progeny. On CeMM, *C. elegans* adopts a life history of efficient exploitation of the carrying capacity of the environment. The modulation of these two extremes of life history strategies appears to involve DAF-16. This may not be surprising given the appreciated role of insulin-like signaling in response to levels of food. What is potentially surprising is that DAF-16 may modulate three alternative life histories: the two distinct developmental courses represented by normal and dauer larva development (Wolkow et al., 2000), and third, the choice between the life histories displayed during growth on NGM or CeMM. An attractive model is that under conditions of limited resources, such as in CeMM, DAF-16 levels, not just activity, are elevated across the developmental stages of *C. elegans*, and are poised to push animals to an L1 or dauer arrest should conditions further deteriorate. In the future, it may be of interest to examine the life histories of *C. elegans* expressing different, quantifiable, levels of DAF-16.

Implications of life history alteration on studying the genetic control of lifespan

Years of studying the genetic basis of lifespan control in *C. elegans* have identified an insulin-like signaling pathway acting, *via* the action of *daf-16* upon metabolism, as a principal mechanism controlling *C. elegans* lifespan (Hansen et al., 2005). The details of the link between levels and activities of metabolic enzymes and lifespan remain to be studied in detail. However, our finding that *C. elegans* can adopt two distinct adult life histories suggests that many of the genes identified as regulating *C. elegans* lifespan actually act *via* global life history alteration rather than solely upon lifespan. It should prove a matter of much interest to determine to what extent the various life history parameters (developmental timing, reproductive period, brood size, metabolic stores and lifespan) are coordinately controlled. Our observations suggest that genes newly identified as controlling lifespan should be examined for effects on all life history parameters and in animals exhibiting both life histories, so as to distinguish if genes that extend lifespan are invariably associated with global life history alteration.

C. elegans 'health' is defined by diet

During the development of axenic media for nematode cultivation, the field was driven by the urge to identify missing nutrients. Perhaps because of this, the observation that measured parameters of animals grown in axenic media resemble those of animals grown on reduced amounts of *E. coli* is often interpreted as indicating that axenic cultivation is unhealthy for *C. elegans*. Indeed, a cursory look at Tables 1, 2 and 3 gives the impression that animals grown in CeMM are less healthy than their NGM-grown counterparts. Development takes longer, broodsize is decreased, and deaths associated with reproduction are increased in CeMM-grown animals relative to NGM-grown ones. However, if development is represented as a function of maximum lifespan rather than days, it is striking that both the length of developmental stages (Table 1) and the reproductive period (Table 2) remain constant percentages of maximum lifespan regardless of NGM or CeMM diet. These observations suggest that both CeMM- and NGM-grown animals are normal and 'healthy'. In other words, *C. elegans* is capable of exhibiting two very different life histories each of which is adaptive for the diet present. Thus, 'health' is conditional upon environment such that what is healthy in one environment is not in another. This idea has been suggested to be true of all animals (Geist, 1979). Perhaps what is most remarkable of our observations is that *C. elegans* can change between these states of 'health' or 'fitness'. As previously mentioned, it should prove a matter of much interest to clarify the nature and extent of lifestyle plasticity.

In addition to providing a mechanism for exploring lifestyle plasticity, the combination of *C. elegans* genetics and dietary manipulations offers a powerful platform on which to study the profound impact of diet on animal physiology, as originally suggested by Dougherty (Dougherty and Calhoun, 1948). When making dietary manipulations, chemically defined medium or *E. coli* variants such as strains deleting specific genes of interest (for example coenzyme Q) (Larsen and Clarke, 2002), appear to be attractive approaches. In order to decide whether an *E. coli* variant or CeMM is a more appropriate tool for a particular manipulation, it is important to learn how much of what is known of *C. elegans* biology on *E. coli* is applicable to *C. elegans* biology in CeMM. Our data provide a starting point to guide such decisions.

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