Published in final edited form as:

Dev Dyn. 2010 May ; 239(5): 1306–1314. doi:10.1002/dvdy.22224.

Developmental biomarkers of aging in *C. elegans*

Zachary Pincus and Frank J. Slack*

Department of Molecular, Cellular and Developmental Biology, PO Box 208103, New Haven, CT 06520

Abstract

The developmental process of the nematode *Caenorhabditis elegans* is famously invariant; however these animals have surprisingly variable lifespans, even in extremely homogenous environments. Inter-individual differences in muscle-function decline, accumulation of lipofuscin in the gut, internal growth of food bacteria, and ability to mobilize heat-shock responses all appear to be predictive of a nematode's remaining lifespan; whether these are causal, or mere correlates of individual decline and death, has yet to be determined. Moreover, few “upstream” causes of inter-individual variability have been identified. It may be the case that variability in lifespan is entirely due to stochastic damage accumulation; alternately, perhaps such variability has a developmental origin and/or genes involved in developmental canalization also act to buffer phenotypic heterogeneity later in life. We review these two hypotheses with an eye toward whether they can be experimentally differentiated.

Keywords

aging biomarker; longevity; inter-individual variability

Introduction

The tremendous variability in lifespan between species, and the existence of mutations and conditions that can drastically alter lifespan in model organisms (up to a 10-fold extension *Caenorhabditis elegans*: Arantes-Oliveira et al., 2003; Ayyadevara et al., 2008) may at times obscure the smaller but still quite significant — and of course, intensely relevant to a given individual — differences in lifespan between members of the same species, even in very similar environments. Indeed, in the extreme case of fully homozygous, genetically identical (isogenic) *C. elegans* raised in the homogenous environment of a completely defined liquid culture at 20°C, the mean reported lifespan was 30 days, with a 20-day standard deviation and a 93-day maximum (Szewczyk et al., 2006)! (Lifespan on standard NGM plates with bacterial OP50 food in that study also showed significant variability about the mean: 16±6 days, with a 30-day maximum.) What, then, underlies the width of these distributions? Is epigenetic variation in gene expression or chromatin state a root cause of this phenotypic heterogeneity? Or perhaps microenvironmental fluctuations or stochastic damage accumulation dominates the equation?

If measures can be found that predict which animals are likely to live longer than others, they may suggest genetic pathways or physiological responses that underlie the inter-individual variability in longevity. Often called “biomarkers of aging” (Baker and Sprott, 1988; Ingram, 1988; Miller, 2001), such measures must, at most basic, predict the remaining

*(corresponding author) zachary.pincus@yale.edu; frank.slack@yale.edu.

lifespan or health-span (the length of time before senescent decline) of a given individual, and do so better than chronological age alone. Figure 1 illustrates the levels of putative biomarkers in three different nematodes: a short-lived “frail” animal, an average worm, and a long-lived “robust” individual. The first row shows an uninformative marker that changes strictly with the passage of time: at a given time (dashed vertical line), the level of the marker does not differ between the different individuals, and thus cannot provide any information about which animal is likely to live the longest. The second row illustrates what has been conventionally understood to be a “biomarker of age” (Miller, 2001): a parameter that varies with respect to the remaining lifespan of an individual. Here, the robust individual has a lower level of the biomarker than others at the same age, indicating that it is “physiologically younger” – that is, a smaller portion of its life has elapsed. Finally, the third row serves to remind that processes that do not change through time may still vary between individuals in a manner predictive of overall longevity.

Much is known about the physiology and genetics of aging in *C. elegans*, though it remains an open question as to whether lifespan variability is driven by the same pathways that are known to influence longevity when experimentally manipulated (for recent reviews of genetic determinants of lifespan, see e.g. Antebi, 2007; Braeckman and Vanfleteren, 2007; Daitoku and Fukamizu, 2007; Houthoofd and Vanfleteren, 2007; Kennedy, 2008; Piper et al., 2008; Miller, 2009; Salminen and Kaarniranta, 2009). Overall, the extensive knowledge of the biology of *C. elegans*, the ability to control the culture conditions to a very high degree, and the large inter-individual differences in longevity, make this nematode an ideal system for elucidating the root causes of lifespan variability.

Descriptive Biology of Lifespan Variability

In a pioneering study, Michael Klass provided the initial description of many now-well-known features of nematode longevity, including the dependence of lifespan on temperature and dietary intake, and the gradual increase in autofluorescent intestinal granules through time (Klass, 1977). In addition, this work reported slight but significant effects of parental age (anti-correlated with progeny lifespan) and parental lifespan (correlated with progeny lifespan). In the same year, an age-related decline in muscle function (assayed by locomotory ability, pharyngeal pumping, and defecation rates) was described (Croll et al., 1977). Much of the subsequent work on variability in nematode aging has focused on muscle function and gut autofluorescence, which correlate with chronological age and vary greatly between same-aged individuals.

Shortly after this initial work, a direct analysis of heterogeneity in the decline of nematode locomotory function through aging was performed by grouping individual animals into one of three categories: those with smooth, sinusoidal, and spontaneous movement; those with irregular yet spontaneous movement; and finally, those with movement only after physical stimulus (Hosono et al., 1980). Individuals proceed through these classes at different rates, and remaining lifespan (after 8 days of life) was shown to depend dramatically on an individual's locomotory class. Moreover, various other markers that changed with chronological age — loss of pharyngeal pumping and loss of resistance to Nile blue staining — also correlated with these locomotory classes in same-aged populations in this study. This type of analysis was later revisited by the Driscoll lab, which classified individual animals based on response to physical stimulus (smooth movement, irregular movement, or head movement only) (Herndon et al., 2002), finding that the “remaining longevity” left to worms in each class was different between the classes and moreover not strongly dependent on the age of the animals. That is, motor function was more predictive of remaining lifespan than chronological age alone (see also the re-analysis of this data in Golden et al., 2008). Moreover, via electron microscopy, this study demonstrated that the nervous system

remained relatively intact with age, while muscle cells succumbed to degeneration over time — though in a stochastic manner, such that a particular cell might be dysfunctional though its neighbors remain unimpaired. Overall, therefore, motor and muscle-function decline, which not only varies between different individuals but also between cells in a given individual, appears to be predictive of lifespan. (See also Wolkow, 2007 for an excellent review of muscle-function aging in *C. elegans*.)

Does the correlation between movement and lifespan hold quantitatively, however? That is, moving beyond broad functional categories, can quantitative measures of movement rate predict with any reliability a given individual animal's lifespan? An early study measured movement rate after mechanical stimulation (in body waves/minute) and concluded that while movement declines through time, the rate of decline in movement rates was not correlated with overall lifespan (Bolanowski et al., 1981). (These authors obtained similar results with defecation rates, also reported elsewhere to slow through time (Croll et al., 1977; Thomas, 1990).) However, recent work using quantitative machine-vision approaches to measure un-stimulated movement rates (in mm/sec) demonstrated a strong correlation between decline in movement rates (between days of adulthood 3 and 9) and lifespan (Hsu et al., 2009). Moreover, this latter work showed weaker but non-trivial anti-correlation between longevity and movement rate at day 3 (faster-moving 3-day-olds die sooner; perhaps a “live fast die young” effect), and correlation between longevity and movement rate at day 9 (faster-moving 9-day-olds die later). Work by Tom Johnson in a slightly different experimental context — recombinant inbred lines constructed by an out-cross followed by multiple generations of self-fertilization — had also demonstrated that differences in the rate of decline in spontaneous movement rates was correlated with different longevities in the various lines (Johnson, 1987; Johnson et al., 1988). Strains with specific pro-longevity mutations were also shown to have slower declines in movement rates over time (Duhon and Johnson, 1995).

In a different vein, a recent study applied machine-vision and machine-learning techniques to devise an “age score” based on high resolution light micrographs of the muscular nematode pharynx, which visibly deteriorates through time (Johnston et al., 2008). Previously, qualitative analysis of head tissues imaged with light microscopy had shown that visible changes in tissue “texture” correlated in a stereotyped manner with the time and other age-related phenotypes (like gut autofluorescence accumulation), and could be used to differentiate progeric short-lived mutants (those with accelerated aging *per se*) from other short-lived mutants with specific pathologies (Garigan et al., 2002). Another study documented in more detail the natural history of pharyngeal muscle decline through time (via measures of both pumping rate and visual scores of muscle decrepitude), and determined, using slow-pumping mutants, that pumping rate was correlated with muscle decline (Chow et al., 2006). Though no explicit inter-individual comparisons were made, it remains an interesting hypothesis that differences in pumping rates within isogenic animals might correlate with differential rates of muscle decline. In each study, significant heterogeneity in pharyngeal texture in same-age animals was found, and the quantitative age scores generated by Johnston et al. were predictive of future pharyngeal pumping ability. (These investigators did not attempt to predict remaining longevity from the age score.) Another work took a different approach to quantitatively integrating information about pharyngeal pumping rate, movement, and lifespan, by defining measures of health that included the length of time before movement speed or pumping rates fell below a certain threshold (Huang et al., 2004). This study found that lifespan was indeed correlated with “fast movement span” (length of time before an individual no longer made smooth, spontaneous movements of a certain speed, which is roughly equivalent to the initial classes of movement phenotypes in the previous studies of locomotory class: Hosono et al., 1980;

Herndon et al., 2002) and two different “pumping spans” (length of time until pharyngeal pumping rates fell below two different thresholds).

Given that in most studies, death is defined as cessation of muscle function, there is some circularity to the logic of predicting time-of-death by measures of muscle function. This could be remedied by the use of more direct indicators of widespread loss of cellular homeostasis (that is, organismal death), such as dyes that are excluded from intact cell membranes but stain “dead” cells (Gill et al., 2003; Moy et al., 2009). Alternately, if other age-related processes correlate with muscle decline better than with time alone, that might suggest that there is a continuum process of organismal aging in general. Indeed, there is one such process: the accumulation autofluorescent gut granules, which, as above, occurs through time (Klass, 1977; Davis et al., 1982; Garigan et al., 2002). Autofluorescent “age pigment”, or lipofuscin, is composed of non-degradable, highly oxidized material that cannot be exocytosed, and thus accumulates over time in lysosomes of post-mitotic cells (across taxa, from nematodes to humans: for reviews, see Yin, 1996; Terman and Brunk, 2004; Terman and Brunk, 2006). Earlier work in the Russell laboratory also demonstrated an age-dependent increase in per-animal lysosomal hydrolase activity (Bolanowski et al., 1983), which may be related to lipofuscin accumulation. (This work also demonstrated the technical feasibility of single-animal assays of hydrolase activity, though no attempt was made to correlate individual animals' health-states with enzyme function.)

Following the work of Herndon et al., Gerstbrein and colleagues measured *in vivo* fluorescent spectra from same-age animals that had been segregated into the previously defined three classes of locomotory function, and found that poor motor function correlated with larger amounts of age pigment accumulation: animals in the decrepit, high-immobile class had four times the lipofuscin accumulation of animals in the healthiest class (Gerstbrein et al., 2005). Overall, the relationship suggests that some coherent aging process — beyond the simple effects of time, which are controlled for by examining same-aged animals — causes both fluorescent macromolecular aggregation and muscle decline to occur together. Alternately, one may be a direct or indirect cause of the other.

Homeostatic Mechanisms

What could be the basis of such a “coherent aging process”? Both lipofuscin accumulation and muscle decline may be end results of a gradual diminution in macromolecular homeostasis: the loss of the ability to degrade (in the former case) and/or maintain through degradation and re-synthesis (in the latter) large protein structures (for a review of this perspective on the mechanistic basis of aging see Rattan, 2008). The hypothesis that aging is a coordinated decline in various functions across multiple tissues appears to be unable to explain the striking stochasticity in muscle-cell decline observed, in which muscle tissues in single animals contained both visibly sarcopenic and intact cells (according to both light and electron microscopy Herndon et al., 2002). By definition homeostatic processes buffer an internal state from change; however, once this buffering capacity is exhausted, the change in cellular state can often be dramatic and rapid. This threshold effect can thus magnify small inter-cell differences, producing a situation where one cell is intact (buffering ability not quite exhausted) while a neighbor is decrepit (buffering ability only recently exhausted), despite both cells experiencing largely the same diminution in homeostatic capacity. (For example, the ability of buffering processes to lead to bimodal responses to environmental stresses has been studied in some detail in bacteria: for a review see e.g. Avery, 2006).

Are inter-individual differences in longevity explicable by declines in specific homeostatic processes? It seems broadly clear that long-lived mutants are generally resistant to various stressors (for reviews see e.g. Johnson et al., 2002; Kenyon, 2005; Wolff and Dillin, 2006;

Miller, 2009), and that overexpression of stress-response genes can prolong longevity (Hsu et al., 2003; Walker and Lithgow, 2003; Morley and Morimoto, 2004). Might differences in the capacity to buffer stressors determine inter-individual differences in longevity? A series of studies in the Johnson lab and others has clearly demonstrated that, in a process called “hormesis”, exposure to mild stressors can extend nematode lifespan (see e.g. Butov et al., 2001; Cypser and Johnson, 2002; Cypser et al., 2006). For example, mild heat shock can cause *C. elegans* to live 15% longer than un-heated, genetically identical controls (Lithgow et al., 1995), perhaps by providing a pulse of chaperone and protein turnover activity that gives cells a one-time “clean out” without altering the long-term rate of damage accumulation (in demographic terms, single heat shock decreases initial mortality rates, without changing the rate of increase in mortality with time: Wu et al., 2008). Moreover, within the heat-shocked population, Rea and colleagues demonstrated that nematodes that mobilize stronger heat shock responses (assayed via expression of a *hsp16.2::GFP* reporter) have significantly longer post-stress lifespans (Rea et al., 2005). This work represents perhaps the clearest case to date of a single gene's expression providing a biomarker for ultimate longevity – albeit in an experimentally perturbed population.

It is not clear whether inter-individual variability in baseline, unperturbed longevity is mechanistically related to these reported inter-individual differences in hormetic responses to heat shock. Certainly, heat-shock proteins do act in non-stressed conditions to buffer macromolecular heterogeneity by ensuring (as in heat-shock conditions) that proteins are folded correctly (Roberts and Feder, 1999; Frydman, 2001; Rutherford, 2003). Moreover, HSP90 appears to also act in developmental canalization, buffering phenotype against environmental and even genetic variability, at least in other species (Milton et al., 2003; Debat et al., 2006; Milton et al., 2006). Further, in *C. elegans*, other heat-shock factors also appear to play a role in normal development (Walker et al., 2003) and in protein homeostasis during infection (Mohri-Shiomi and Garsin, 2008). Finally, various heat-shock proteins, in interaction with insulin-like signaling, have been shown to change longevity when manipulated (Walker and Lithgow, 2003; Morley and Morimoto, 2004). As such, it is tempting to speculate that individual variability in these factors may determine individual variability in basal (unperturbed) longevity.

Other genes involved in macromolecular homeostasis may also underlie inter-individual differences. One such additional connection may lie in the autophagy pathway: the ability of cells to turn over their protein contents decreases over time (resulting in and/or resulting from an increase in autofluorescent lipofuscin). Moreover, many genes involved in autophagy have lifespan phenotypes when mutated (Terman and Brunk, 2006; Hansen et al., 2008; Eskelinen and Saftig, 2009; Rajawat et al., 2009; Salminen and Kaarniranta, 2009). No studies on inter-individual variability in autophagocytic ability in nematodes have yet been performed, however.

Aging hermaphrodite nematodes also appear to lose homeostatic control of germ line DNA proliferation. After the end of the reproductive period, large masses of DNA accumulate in the body cavity of hermaphrodite worms (Golden et al., 2007). The degree of this accumulation is extremely variable between individuals, as measured by genome copy number; however different degrees of accumulation of genetic material has not yet been shown to predict other measures of individual longevity or health. (In addition, pharmacological prevention of DNA duplication in adults nematodes does not extend life (Gandhi et al., 1980), suggesting that the accumulation *per se* does not cause dysfunction; nevertheless it may report on loss of at least one homeostatic mechanism.)

Transcriptional Biomarkers

In and of itself, the fact that a gene changes expression over time is not sufficient for that gene to be a useful biomarker of longevity (see e.g. the first row of Figure 1; note also that the third row illustrates that change through time is not necessary for a biomarker either.) Nevertheless, genes that change expression over time — especially those that have longevity phenotypes with knockout or over-expression — may have a role in inducing or limiting senescence, and may provide clues about the origins of lifespan variability.

Early work in the Johnson lab demonstrated that there were indeed genes with expression changes between young adult and aged populations (Fabian and Johnson, 1995). With the advent of microarray technology, the Johnson and Kim labs were able to systematically identify many more genes with temporally variable expression patterns. In particular, many of these genes, such as those involved in heat-shock response and insulin-like signaling, had known longevity phenotypes (Lund et al., 2002). More recently, these groups mined expression-profiling data to identify an age-correlated upward “drift” in the levels of the embryonically active GATA transcription factors ELT-5 and ELT-6. This drift was demonstrated to be responsible age-related changes in expression levels of many other genes (Budovskaya et al., 2008), and moreover, knockdown of ELT-5 or ELT-6 extended lifespan. Together, this indicates a causal role in lifespan determination for this drift in expression, at least at the population level.

Ultimately, however, measurements must be made on different individuals to determine if gene-expression variability at a given time correlates with lifespan or health states. Toward this end, Golden and Melov have devised protocols to profile gene expression in individual nematodes (a technical as well as statistical challenge, as such experiments require many individual animals in order to achieve significant results (Golden et al., 2006)). An early foray into this realm compared gene expression in many individuals at different ages in wild-type and a long-lived mutant background, but did not detect any systematic increase in inter-individual gene expression variance with longevity; moreover only one gene (a transcription initiation factor) was found to have a variance that changed significantly between time points in wild-type animals (Golden and Melov, 2004). A further study correlated individual gene expression patterns with chronological age or locomotory class (a reasonable proxy for remaining longevity, as total RNA isolation is an invariably fatal procedure) (Golden et al., 2008). This work found that gene expression can indeed quantitatively predict chronological age, and can predict locomotory class with high probability. While it is not possible to determine from such studies whether the levels of certain genes will predict future longevity better than chronological age alone, many of the genes shown to change with time, or between animals in different locomotory classes, may be candidate biomarkers. (It would also be of great interest to compare gene expression profiles between *same-aged* animals of different locomotory classes, in order to tease apart genes that change generally with time from those that report directly on health state.) These studies identified many genes and gene classes of interest that change with time, including decreases in genes related to protein, DNA, and lipid metabolism, and increases in cell-death related genes and various transcription factors (Golden et al., 2008). Overall, this work makes it clear that statistical analysis of gene expression profiles can predict age and health states — that is, expression profiles in aggregate can be used as age biomarkers — and also highlights particular mechanisms of age-related deterioration that may be worth detailed examination for inter-individual variability.

Bacterial Accumulation

Bacterial accumulation in the pharynx and intestine has long been observed in aged *C. elegans* (Vanfleteren et al., 1998), and feeding nematodes with dead or growth-arrested bacteria as their food source significantly increases lifespan over the standard culture conditions of the freely-proliferating (if uracil auxotrophic) *E. coli* strain OP50 (Gems and Riddle, 2000; Garigan et al., 2002). Further, the decline in pharyngeal pumping rates with time is diminished on non-proliferating bacteria (Chow et al., 2006). Finally, long-lived *daf-2* mutant worms are also more resistant to infection with pathogenic bacteria, and the relative lifespan extension compared to wild-type is diminished on nonpathogenic strains (Garsin et al., 2003). It thus appears that bacterial proliferation is a cause of death in *C. elegans*, and that in some long-lived mutant strains, lifespan extension is effected through increased pathogen resistance.

Might inter-individual differences in bacterial accumulation account for longevity differences? Feeding UV-killed bacteria has been shown to decrease inter-individual variability in male survival (Gems and Riddle, 2000), and more recently, intestinal bacterial load (measured using OP50 expressing YFP) at adult day 3 was shown to anti-correlate with individual longevity (Baeriswyl et al., 2009). The precise mechanism of killing of worms by proliferating bacteria is not well understood (and it is unlikely related to decreased nutrient uptake *per se*, as that promotes longevity: see e.g. Masoro, 2005). It has been speculated that the shortened lifespan is due to host responses: perhaps there is an adaptive shift in reproductive schedule toward increased early fecundity (at the cost of reproductive and overall lifespan) in the presence of pathogenic bacteria (Baeriswyl et al., 2009), or perhaps there is collateral tissue damage from the nematode innate immune responses, which involve production of reactive oxygen species (Chávez et al., 2007; Mohri-Shiomi and Garsin, 2008). These species, which damage the intestine, also promote the formation of lipofuscin deposits (Chávez et al., 2007); as such, one might imagine that muscle decline could lead to decreased bacterial grinding and disruption in the pharynx, promoting intestinal accumulation of living bacteria, which induces damaging host responses (visible via lipofuscin accumulation) that may also further impair muscle function. As above, decline in pharyngeal muscle function is diminished (but not eliminated) in the absence of proliferating bacteria (Chow et al., 2006), which absence also decreases the rate of lipofuscin accumulation (Chávez et al., 2007; Baeriswyl et al., 2009); however, as of yet there has been no direct demonstration that muscle decline *per se* permits bacterial accumulation. Note also that while bacterial load predicts differences in longevity (Baeriswyl et al., 2009), in at least one study, nematodes reared in a liquid, bacteria-free, chemically-defined medium had much larger variability in longevity (relative to the mean lifespan) than did those raised on bacterial food on solid media (Szewczyk et al., 2006), though firm conclusions are difficult to draw because of the liquid-versus-solid culture confound. In general, though, it appears that killing by bacterial crowding is one but not the only driver of inter-individual differences in life and health spans in worms.

Perspective

The fundamental question to be asked about inter-individual differences — in lifespan or any other phenotype — is simply *where does the variability originate?* In the case of longevity, are different animals born with different degrees of “frailty” or do genetically identical individuals diverge as a result of stochastic insults (like damage from reactive oxygen species) of different number or of different severity? In the former case, inter-individual differences should be detectable early, while in the latter, individual phenotypes would become more divergent through time. Thus, the ability of events early in life (such as degree of bacterial accumulation at the third day of adult life) to predict in a reasonably

linear fashion eventual longevity (in the ten to fifteen day range) (Baeriswyl et al., 2009) would appear to augur for the former hypothesis. However, perhaps even at that early time, the relevant damage has already been accumulated? On the other hand, findings of stochastic divergence between individuals only late in life do not necessarily falsify a hypothesis of differential rates of basal “frailty”, which may be obscured by homeostatic buffering until later in life when these mechanisms finally become overwhelmed. Of course, it is entirely likely that both mechanisms are at play, as they are in no sense mutually exclusive.

Biomarkers of either mechanism might be expected to exist: a marker could correlate with the state of damage accumulation in particular cells or tissues, or it could correlate with (or in fact determine!) that individual's fundamental “frailty”. It will likely be very difficult, for the reasons outlined above, to perfectly distinguish between these two cases, but the possibility to do so does exist. If damage accumulation is “cell-autonomous” or “tissue-autonomous”, it would be reasonable to expect that different cells and tissues would age stochastically and independent of one another; as such, correlations between disparate biomarkers would be surprising. For example, suppose the decline in pharyngeal pumping rate were shown to correlate with gut autofluorescence accumulation. If it could be shown that decreasing the pumping rate does not cause an increase in lipofuscin (or vice versa), then these two effects must be correlated due to some common upstream cause. *A priori*, stochastic damage at the single-cell level would appear to be an unlikely cause of coordinated decline in disparate tissues; thus, declines in function that correlate better than expected due to time alone would hint that there are mechanisms that determine over-all frailty at the organismal level. Nevertheless, if inter-individual differences in frailty are to be invoked, their causes must be positively identified. Moreover, it is important to publicize negative results of studies designed to look for such frailty mechanisms: while repeated failure to find something does of course not confirm its non-existence, it would be suggestive.

Some demographic analyses also provide suggestive evidence that frailty differences do exist. In particular, throughout most of life, an individual's probability of death per unit time increases approximately exponentially; however at the end of life, this probability begins to plateau. This “mortality deceleration” has been observed in nematodes and across diverse taxa (Vaupel et al., 1998; Johnson et al., 2001), and may indicate that the original population was made up of individuals with different frailties: as the frailer die off, the more robust, with a lower probability of death over time, remain (Vaupel et al., 1979). Typically, this posited heterogeneity cannot be observed directly; and indeed, mortality deceleration can be explained equally well by demographic models that account only for stochastic damage accumulation (Yashin et al., 1994). However, in the case of an intervention such as hormetic heat-shock, it may be possible to make inferences about the origins of post-intervention variability through demographic analysis. Examination of mortality curves, which plot probability of death versus time, shows that in heat-pulsed populations mortality increases through time at the same rate as in untreated animals, but the treated animals start from a position of lower initial risk (Wu et al., 2008). Thus, it appears that heat treatment increases some basal fortitude. This hypothesis is further buttressed by results from statistically modeling the overall population as a mixture of animals with different degrees of frailty, which indicate that heat shock somehow shifts individuals from more frail to more robust (Yashin et al., 2002; Wu et al., 2006; Wu et al., 2008). On the other hand, following multiple heat pulses, treated animals experience both lower initial mortality and a slower increase in the daily probability of death (Wu et al., 2009); thus in these conditions, an increase in initial health is joined by a lower rate of damage accumulation through time.

Overall, therefore, individual frailty does appear to be modulable by heat shock. As Waddington pointed out in his work introducing the notion of developmental canalization, traits that are defined by stereotyped responses to environmental conditions can be easily co-opted by developmental processes (which may then drive the response from an endogenous as opposed to environmental stimulus) (Waddington, 1942). Thus, it may not be folly to expect that findings that frailty can be manipulated by heat shock might also indicate that frailty can be manipulated developmentally. Indeed, as above, heat-shock genes are important in normal development in nematodes (Walker et al., 2003) and other species; in particular these genes participate in buffering phenotype from environmental fluctuation (Debat et al., 2006; Milton et al., 2006; Salathia and Queitsch, 2007).

In general, if different individuals do have different, innate, degrees of frailty or robustness, then to some extent the question of longevity becomes a matter of developmental biology. Potential biomarkers of lifespan may then be found early in life, perhaps among genes involved in buffering developmental processes from environmental perturbation. This sort of canalization may exist beyond heat shock proteins: it has been predicted to be a generic feature of developmental gene-regulatory networks (Siegal and Bergman, 2002; Bergman and Siegal, 2003), and yeast genes with “increased inter-individual variability” knockout phenotypes are over-represented among highly-connected gene and protein interaction network “hubs” (Levy and Siegal, 2008). Studies of synthetic gene interactions in *C. elegans* also identify such hubs, some of which may have phenotypic buffering abilities (Lehner et al., 2006). It is of interest that these latter nematode hub genes are primarily chromatin regulators, which are also identified in the yeast studies. In addition, transcriptional profiles of individual aging nematodes show increases in transcription factor and RNA metabolism genes (Golden et al., 2008), as might be expected from a homeostatic response to decreased gene expression due to age-related aberrations in nuclear architecture (Haithcock et al., 2005; Golden et al., 2007). Perhaps, then, some of these hub genes involved in chromatin homeostasis may buffer against environmental fluctuations, and thus may play a role in determining inter-individual frailties.

Finally, it is worth noting that specific mechanisms to generate inter-individual differences in phenotype may be selectively advantageous: within an isogenic population, maintaining a distribution of phenotypes, including some that are non-optimal for the current environment, can allow the genotype to survive environmental changes (see e.g. Martin, 2009). (Observe that isolated, self-fertilizing nematodes are driven to homozygosity at every locus (Brenner, 1974); thus near-isogeny may be a natural feature of *C. elegans*.) For example, individuals with faster reproductive schedules (though lower overall fertility) may be at an advantage in certain harsh environments (see e.g. Williams, 1957), though not in others (Ratcliff et al., 2009). As such a distribution of reproductive schedules may be adaptive in uncertain conditions (Wilbur and Rudolf, 2006). If, as a consequence of accelerated reproduction, some individuals are more “frail” than others (and there appears to be a trade off between longevity and fecundity: Chen et al., 2007; Mukhopadhyay and Tissenbaum, 2007), then inter-individual frailty differences may be due to “epigenetic bet-hedging” in the distribution of reproductive schedules. Thus, genes involved in developmental timing (which have been shown to be involved in longevity in nematodes: Boehm and Slack, 2005) and reproductive rates may also be good candidates for biomarkers of longevity.

In conclusion, though *C. elegans* have extremely homogenous developmental dynamics, adult life histories of genetically identical individuals can be extremely variable. The eventual longevity of each animal can be predicted in advance from a handful of known biomarkers, including body muscle function, rate of decline in pharyngeal pumping rates, bacterial accumulation, and post-heat HSP-16 upregulation. However, all of these markers, with the possible exception of the heat-shock responses, appear to be relatively

“downstream”: what are the upstream causes of this variability? Perhaps only the slings and arrows of each worm's individual fortune determine life and health span. Alternately, there is suggestive evidence that different animals may begin life with different internal fortitudes; potential mechanisms of which have been briefly sketched above.

Acknowledgments

This work was supported by grants to FJS from the Ellison Medical Foundation and the NIA (AG033921). ZP was supported by a postdoctoral fellowship from the Jane Coffin Childs Fund.

References

- Antebi A. Genetics of aging in *Caenorhabditis elegans*. PLoS Genet. 2007; 3:1565–1571. [PubMed: 17907808]
- Arantes-Oliveira N, Berman JR, Kenyon C. Healthy animals with extreme longevity. Science. 2003; 302:611. [PubMed: 14576426]
- Avery SV. Microbial cell individuality and the underlying sources of heterogeneity. Nat Rev Microbiol. 2006; 4:577–587. [PubMed: 16845428]
- Ayyadevara S, Alla R, Thaden JJ, Shmookler Reis RJ. Remarkable longevity and stress resistance of nematode PI3K-null mutants. Aging Cell. 2008; 7:13–22. [PubMed: 17996009]
- Baeriswyl S, Diard M, Mosser T, Leroy M, Manière X, Taddei F, Matic I. Modulation of aging profiles in isogenic populations of *Caenorhabditis elegans* by bacteria causing different extrinsic mortality rates. Biogerontology. 2009
- Baker GT, Sprott RL. Biomarkers of aging. Exp Gerontol. 1988; 23:223–239. [PubMed: 3058488]
- Bergman A, Siegal ML. Evolutionary capacitance as a general feature of complex gene networks. Nature. 2003; 424:549–552. [PubMed: 12891357]
- Boehm M, Slack FJ. A developmental timing microRNA and its target regulate life span in *C. elegans*. Science. 2005; 310:1954–1957. [PubMed: 16373574]
- Bolanowski MA, Jacobson LA, Russell RL. Quantitative measures of aging in the nematode *Caenorhabditis elegans*: II. Lysosomal hydrolases as markers of senescence. Mech Ageing Dev. 1983; 21:295–319. [PubMed: 6412000]
- Bolanowski MA, Russell RL, Jacobson LA. Quantitative measures of aging in the nematode *Caenorhabditis elegans*. I. Population and longitudinal studies of two behavioral parameters. Mech Ageing Dev. 1981; 15:279–295. [PubMed: 7253717]
- Braeckman BP, Vanfleteren JR. Genetic control of longevity in *C. elegans*. Exp Gerontol. 2007; 42:90–98. [PubMed: 16829009]
- Brenner S. The genetics of *Caenorhabditis elegans*. Genetics. 1974; 77:71–94. [PubMed: 4366476]
- Budovskaya YV, Wu K, Southworth LK, Jiang M, Tedesco P, Johnson TE, Kim SK. An *elt-3/elt-5/elt-6* GATA transcription circuit guides aging in *C. elegans*. Cell. 2008; 134:291–303. [PubMed: 18662544]
- Butov A, Johnson T, Cypser J, Sannikov I, Volkov M, Sehl M, Yashin A. Hormesis and debilitation effects in stress experiments using the nematode worm *Caenorhabditis elegans*: the model of balance between cell damage and HSP levels. Exp Gerontol. 2001; 37:57–66. [PubMed: 11738147]
- Chávez V, Mohri-Shiomi A, Maadani A, Vega LA, Garsin DA. Oxidative stress enzymes are required for DAF-16-mediated immunity due to generation of reactive oxygen species by *Caenorhabditis elegans*. Genetics. 2007; 176:1567–1577. [PubMed: 17483415]
- Chen J, Senturk D, Wang J-L, Müller H-G, Carey JR, Caswell H, Caswell-Chen EP. A demographic analysis of the fitness cost of extended longevity in *Caenorhabditis elegans*. J Gerontol A Biol Sci Med Sci. 2007; 62:126–135. [PubMed: 17339638]
- Chow DK, Glenn CF, Johnston JL, Goldberg IG, Wolkow CA. Sarcopenia in the *Caenorhabditis elegans* pharynx correlates with muscle contraction rate over lifespan. Exp Gerontol. 2006; 41:252–260. [PubMed: 16446070]

- Croll NA, Smith JM, Zuckerman BM. The aging process of the nematode *Caenorhabditis elegans* in bacterial and axenic culture. *Experimental aging research*. 1977; 3:175–189. [PubMed: 334555]
- Cypser JR, Johnson TE. Multiple stressors in *Caenorhabditis elegans* induce stress hormesis and extended longevity. *J Gerontol A Biol Sci Med Sci*. 2002; 57:B109–114. [PubMed: 11867647]
- Cypser JR, Tedesco P, Johnson TE. Hormesis and aging in *Caenorhabditis elegans*. *Exp Gerontol*. 2006; 41:935–939. [PubMed: 17067771]
- Daitoku H, Fukamizu A. FOXO transcription factors in the regulatory networks of longevity. *J Biochem*. 2007; 141:769–774. [PubMed: 17569704]
- Davis BO, Anderson GL, Dusenbery DB. Total luminescence spectroscopy of fluorescence changes during aging in *Caenorhabditis elegans*. *Biochemistry*. 1982; 21:4089–4095. [PubMed: 7126532]
- Debat V, Milton CC, Rutherford S, Klingenberg CP, Hoffmann AA. Hsp90 and the quantitative variation of wing shape in *Drosophila melanogaster*. *Evolution*. 2006; 60:2529–2538. [PubMed: 17263114]
- Duhon SA, Johnson TE. Movement as an index of vitality: comparing wild type and the *age-1* mutant of *Caenorhabditis elegans*. *J Gerontol A Biol Sci Med Sci*. 1995; 50:B254–261. [PubMed: 7671016]
- Eskelinen E-L, Saftig P. Autophagy: a lysosomal degradation pathway with a central role in health and disease. *Biochim Biophys Acta*. 2009; 1793:664–673. [PubMed: 18706940]
- Fabian TJ, Johnson TE. Identification genes that are differentially expressed during aging in *Caenorhabditis elegans*. *J Gerontol A Biol Sci Med Sci*. 1995; 50:B245–253. [PubMed: 7671015]
- Frydman J. Folding of newly translated proteins *in vivo*: the role of molecular chaperones. *Annu Rev Biochem*. 2001; 70:603–647. [PubMed: 11395418]
- Gandhi S, Santelli J, Mitchell DH, Stiles JW, Sanadi DR. A simple method for maintaining large, aging populations of *Caenorhabditis elegans*. *Mech Ageing Dev*. 1980; 12:137–150. [PubMed: 6445025]
- Garigan D, Hsu A-L, Fraser AG, Kamath RS, Ahringer J, Kenyon C. Genetic analysis of tissue aging in *Caenorhabditis elegans*: a role for heat-shock factor and bacterial proliferation. *Genetics*. 2002; 161:1101–1112. [PubMed: 12136014]
- Garsin DA, Villanueva JM, Begun J, Kim DH, Sifri CD, Calderwood SB, Ruvkun G, Ausubel FM. Long-lived *C. elegans daf-2* mutants are resistant to bacterial pathogens. *Science*. 2003; 300:1921. [PubMed: 12817143]
- Gems D, Riddle DL. Genetic, behavioral and environmental determinants of male longevity in *Caenorhabditis elegans*. *Genetics*. 2000; 154:1597–1610. [PubMed: 10747056]
- Gerstbrein B, Stamatas G, Kollias N, Driscoll M. *In vivo* spectrofluorimetry reveals endogenous biomarkers that report healthspan and dietary restriction in *Caenorhabditis elegans*. *Aging Cell*. 2005; 4:127–137. [PubMed: 15924569]
- Gill MS, Olsen A, Sampayo JN, Lithgow GJ. An automated high-throughput assay for survival of the nematode *Caenorhabditis elegans*. *Free Radic Biol Med*. 2003; 35:558–565. [PubMed: 12957648]
- Golden TR, Beckman KB, Lee AHJ, Dudek N, Hubbard A, Samper E, Melov S. Dramatic age-related changes in nuclear and genome copy number in the nematode *Caenorhabditis elegans*. *Aging Cell*. 2007; 6:179–188. [PubMed: 17286610]
- Golden TR, Hubbard A, Dando C, Herren M, Melov S. Age-related behaviors have distinct transcriptional profiles in *C. elegans*. *Aging Cell*. 2008
- Golden TR, Hubbard A, Melov S. Microarray analysis of variation in individual aging *C. elegans*: approaches and challenges. *Exp Gerontol*. 2006; 41:1040–1045. [PubMed: 16876364]
- Golden TR, Melov S. Microarray analysis of gene expression with age in individual nematodes. *Aging Cell*. 2004; 3:111–124. [PubMed: 15153179]
- Haithcock E, Dayani Y, Neufeld E, Zahand AJ, Feinstein N, Mattout A, Gruenbaum Y, Liu J. Age-related changes of nuclear architecture in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA*. 2005; 102:16690–16695. [PubMed: 16269543]
- Hansen M, Chandra A, Mitic LL, Onken B, Driscoll M, Kenyon C. A role for autophagy in the extension of lifespan by dietary restriction in *C. elegans*. *PLoS Genet*. 2008; 4:e24. [PubMed: 18282106]

- Herndon LA, Schmeissner PJ, Dudaronek JM, Brown PA, Listner KM, Sakano Y, Paupard MC, Hall DH, Driscoll M. Stochastic and genetic factors influence tissue-specific decline in ageing *C. elegans*. *Nature*. 2002; 419:808–814. [PubMed: 12397350]
- Hosono R, Sato Y, Aizawa SI, Mitsui Y. Age-dependent changes in mobility and separation of the nematode *Caenorhabditis elegans*. *Exp Gerontol*. 1980; 15:285–289. [PubMed: 7409025]
- Houthoofd K, Vanfleteren JR. Public and private mechanisms of life extension in *Caenorhabditis elegans*. *Mol Genet Genomics*. 2007; 277:601–617. [PubMed: 17364197]
- Hsu A-L, Feng Z, Hsieh M-Y, Xu XZS. Identification by machine vision of the rate of motor activity decline as a lifespan predictor in *C. elegans*. *Neurobiol Aging*. 2009; 30:1498–1503. [PubMed: 18255194]
- Hsu A-L, Murphy CT, Kenyon C. Regulation of aging and age-related disease by DAF-16 and heat-shock factor. *Science*. 2003; 300:1142–1145. [PubMed: 12750521]
- Huang C, Xiong C, Kornfeld K. Measurements of age-related changes of physiological processes that predict lifespan of *Caenorhabditis elegans*. *Proc Natl Acad Sci USA*. 2004; 101:8084–8089. [PubMed: 15141086]
- Ingram DK. Key questions in developing biomarkers of aging. *Exp Gerontol*. 1988; 23:429–434. [PubMed: 3058496]
- Johnson TE. Aging can be genetically dissected into component processes using long-lived lines of *Caenorhabditis elegans*. *Proc Natl Acad Sci USA*. 1987; 84:3777–3781. [PubMed: 3473482]
- Johnson TE, Conley WL, Keller ML. Long-lived lines of *Caenorhabditis elegans* can be used to establish predictive biomarkers of aging. *Exp Gerontol*. 1988; 23:281–295. [PubMed: 3197780]
- Johnson TE, Henderson ST, Murakami S, de Castro E, de Castro SH, Cypser J, Rikke B, Tedesco P, Link C. Longevity genes in the nematode *Caenorhabditis elegans* also mediate increased resistance to stress and prevent disease. *J Inher Metab Dis*. 2002; 25:197–206. [PubMed: 12137228]
- Johnson TE, Wu D, Tedesco P, Dames S, Vaupel JW. Age-specific demographic profiles of longevity mutants in *Caenorhabditis elegans* show segmental effects. *J Gerontol A Biol Sci Med Sci*. 2001; 56:B331–339. [PubMed: 11487591]
- Johnston J, Iser WB, Chow DK, Goldberg IG, Wolkow CA. Quantitative image analysis reveals distinct structural transitions during aging in *Caenorhabditis elegans* tissues. *PLoS ONE*. 2008; 3:e2821. [PubMed: 18665238]
- Kennedy BK. The genetics of ageing: insight from genome-wide approaches in invertebrate model organisms. *J Intern Med*. 2008; 263:142–152. [PubMed: 18226092]
- Kenyon C. The plasticity of aging: insights from long-lived mutants. *Cell*. 2005; 120:449–460. [PubMed: 15734678]
- Klass MR. Aging in the nematode *Caenorhabditis elegans*: major biological and environmental factors influencing life span. *Mech Ageing Dev*. 1977; 6:413–429. [PubMed: 926867]
- Lehner B, Crombie C, Tischler J, Fortunato A, Fraser AG. Systematic mapping of genetic interactions in *Caenorhabditis elegans* identifies common modifiers of diverse signaling pathways. *Nat Genet*. 2006; 38:896–903. [PubMed: 16845399]
- Levy SF, Siegal ML. Network hubs buffer environmental variation in *Saccharomyces cerevisiae*. *PLoS Biol*. 2008; 6:e264. [PubMed: 18986213]
- Lithgow GJ, White TM, Melov S, Johnson TE. Thermotolerance and extended lifespan conferred by single-gene mutations and induced by thermal stress. *Proc Natl Acad Sci USA*. 1995; 92:7540–7544. [PubMed: 7638227]
- Lund J, Tedesco P, Duke K, Wang J, Kim SK, Johnson TE. Transcriptional profile of aging in *C. elegans*. *Curr Biol*. 2002; 12:1566–1573. [PubMed: 12372248]
- Martin G. Epigenetic gambling and epigenetic drift as an antagonistic pleiotropic mechanism of aging. *Aging Cell*. 2009
- Masoro EJ. Overview of caloric restriction and ageing. *Mech Ageing Dev*. 2005; 126:913–922. [PubMed: 15885745]
- Miller RA. Biomarkers of aging. *Science of aging knowledge environment*. SAGE KE. 2001; 2001:pe2. [PubMed: 14602951]

- Miller RA. Cell stress and aging: new emphasis on multiplex resistance mechanisms. *J Gerontol A Biol Sci Med Sci*. 2009; 64:179–182. [PubMed: 19225033]
- Milton CC, Huynh B, Batterham P, Rutherford SL, Hoffmann AA. Quantitative trait symmetry independent of Hsp90 buffering: distinct modes of genetic canalization and developmental stability. *Proc Natl Acad Sci USA*. 2003; 100:13396–13401. [PubMed: 14595030]
- Milton CC, Ulane CM, Rutherford S. Control of canalization and evolvability by Hsp90. *PLoS ONE*. 2006; 1:e75. [PubMed: 17183707]
- Mohri-Shiomi A, Garsin DA. Insulin signaling and the heat shock response modulate protein homeostasis in the *Caenorhabditis elegans* intestine during infection. *J Biol Chem*. 2008; 283:194–201. [PubMed: 17951251]
- Morley JF, Morimoto RI. Regulation of longevity in *Caenorhabditis elegans* by heat shock factor and molecular chaperones. *Mol Biol Cell*. 2004; 15:657–664. [PubMed: 14668486]
- Moy T, Conery A, Larkins-Ford J, Wu G, Mazitschek R, Casadei G, Lewis K, Carpenter A, Ausubel FM. High-Throughput Screen for Novel Antimicrobials using a Whole Animal Infection Model. *ACS Chem Biol*. 2009
- Mukhopadhyay A, Tissenbaum HA. Reproduction and longevity: secrets revealed by *C. elegans*. *Trends Cell Biol*. 2007; 17:65–71. [PubMed: 17187981]
- Piper MDW, Selman C, McElwee JJ, Partridge L. Separating cause from effect: how does insulin/IGF signalling control lifespan in worms, flies and mice? *J Intern Med*. 2008; 263:179–191. [PubMed: 18226095]
- Rajawat YS, Hilioti Z, Bossis I. Aging: central role for autophagy and the lysosomal degradative system. *Ageing Res Rev*. 2009; 8:199–213. [PubMed: 19427410]
- Ratcliff WC, Hawthorne P, Travisano M, Denison RF. When stress predicts a shrinking gene pool, trading early reproduction for longevity can increase fitness, even with lower fecundity. *PLoS ONE*. 2009; 4:e6055. [PubMed: 19557134]
- Rattan SIS. Increased molecular damage and heterogeneity as the basis of aging. *Biol Chem*. 2008; 389:267–272. [PubMed: 18208348]
- Rea SL, Wu D, Cypser JR, Vaupel JW, Johnson TE. A stress-sensitive reporter predicts longevity in isogenic populations of *Caenorhabditis elegans*. *Nat Genet*. 2005; 37:894–898. [PubMed: 16041374]
- Roberts S, Feder M. Natural hyperthermia and expression of the heat shock protein Hsp70 affect developmental abnormalities in *Drosophila melanogaster*. *Oecologia*. 1999; 121:323–329.
- Rutherford SL. Between genotype and phenotype: protein chaperones and evolvability. *Nat Rev Genet*. 2003; 4:263–274. [PubMed: 12671657]
- Salathia N, Queitsch C. Molecular mechanisms of canalization: Hsp90 and beyond. *J Biosci*. 2007; 32:457–463. [PubMed: 17536165]
- Salminen A, Kaarniranta K. Regulation of the aging process by autophagy. *Trends in molecular medicine*. 2009; 15:217–224. [PubMed: 19380253]
- Siegal ML, Bergman A. Waddington's canalization revisited: developmental stability and evolution. *Proc Natl Acad Sci USA*. 2002; 99:10528–10532. [PubMed: 12082173]
- Szewczyk NJ, Udransky IA, Kozak E, Sunga J, Kim SK, Jacobson LA, Conley CA. Delayed development and lifespan extension as features of metabolic lifestyle alteration in *C. elegans* under dietary restriction. *J Exp Biol*. 2006; 209:4129–4139. [PubMed: 17023606]
- Terman A, Brunk UT. Lipofuscin. *Int J Biochem Cell Biol*. 2004; 36:1400–1404. [PubMed: 15147719]
- Terman A, Brunk UT. Oxidative stress, accumulation of biological 'garbage', and aging. *Antioxid Redox Signal*. 2006; 8:197–204. [PubMed: 16487053]
- Thomas JH. Genetic analysis of defecation in *Caenorhabditis elegans*. *Genetics*. 1990; 124:855–872. [PubMed: 2323555]
- Vanfleteren JR, De Vreese A, Braeckman BP. Two-parameter logistic and Weibull equations provide better fits to survival data from isogenic populations of *Caenorhabditis elegans* in axenic culture than does the Gompertz model. *J Gerontol A Biol Sci Med Sci*. 1998; 53:B393–403. discussion B404–398. [PubMed: 9823735]

- Vaupel JW, Carey JR, Christensen K, Johnson TE, Yashin AI, Holm NV, Iachine IA, Kannisto V, Khazaeli AA, Liedo P, Longo VD, Zeng Y, Manton KG, Curtsinger JW. Biodemographic trajectories of longevity. *Science*. 1998; 280:855–860. [PubMed: 9599158]
- Vaupel JW, Manton KG, Stallard E. The impact of heterogeneity in individual frailty on the dynamics of mortality. *Demography*. 1979; 16:439–454. [PubMed: 510638]
- Waddington CH. Canalization of Development and the Inheritance of Acquired Characters. *Nature*. 1942; 50:563–565.
- Walker GA, Lithgow GJ. Lifespan extension in *C. elegans* by a molecular chaperone dependent upon insulin-like signals. *Aging Cell*. 2003; 2:131–139. [PubMed: 12882326]
- Walker GA, Thompson FJ, Brawley A, Scanlon T, Devaney E. Heat shock factor functions at the convergence of the stress response and developmental pathways in *Caenorhabditis elegans*. *FASEB J*. 2003; 17:1960–1962. [PubMed: 12897069]
- Wilbur HM, Rudolf VHW. Life-history evolution in uncertain environments: bet hedging in time. *The American Naturalist*. 2006; 168:398–411.
- Williams G. Pleiotropy, natural selection, and the evolution of senescence. *Evolution*. 1957; 11:398–411.
- Wolff S, Dillin A. The trifecta of aging in *Caenorhabditis elegans*. *Exp Gerontol*. 2006; 41:894–903. [PubMed: 16919905]
- Wu D, Cypser J, Yashin A, Johnson T. Multiple Mild Heat-Shocks Decrease The Gompertz Component of Mortality in *C. elegans*. *Exp Gerontol*. 2009; 44:607–612. [PubMed: 19580861]
- Wu D, Cypser JR, Yashin AI, Johnson TE. The U-shaped response of initial mortality in *Caenorhabditis elegans* to mild heat shock: does it explain recent trends in human mortality? *J Gerontol A Biol Sci Med Sci*. 2008; 63:660–668. [PubMed: 18693219]
- Wu D, Rea SL, Yashin AI, Johnson TE. Visualizing hidden heterogeneity in isogenic populations of *C. elegans*. *Exp Gerontol*. 2006; 41:261–270. [PubMed: 16480844]
- Yashin AI, Cypser JW, Johnson TE, Michalski AI, Boyko SI, Novoseltsev VN. Heat shock changes the heterogeneity distribution in populations of *Caenorhabditis elegans*: does it tell us anything about the biological mechanism of stress response? *J Gerontol A Biol Sci Med Sci*. 2002; 57:B83–92. [PubMed: 11867644]
- Yashin AI, Vaupel JW, Iachine IA. A duality in aging: the equivalence of mortality models based on radically different concepts. *Mech Ageing Dev*. 1994; 74:1–14. [PubMed: 7934200]
- Yin D. Biochemical basis of lipofuscin, ceroid, and age pigment-like fluorophores. *Free Radic Biol Med*. 1996; 21:871–888. [PubMed: 8902532]

Figure 1.

Each graph illustrates changes in the levels of a putative “biomarker of longevity” — that is, a parameter that can predict age at death — in three nematodes with different lifespans. The first row illustrates that simple change through time does not a biomarker make: at any given time (vertical dashed line), each worm has the same biomarker level and thus the marker cannot differentiate the animals. However, as in the second row, a marker that correlates directly with the fraction of each animal's lifespan that is elapsed (or remains) is of distinct interest. Finally, as in the third row, a marker need not change through time to be informative regarding eventual lifespan.