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# What accounts for the wide variation in life span of genetically identical organisms reared in a constant environment?

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**Abstract**

Individual organisms show marked variability in life span, even when they are of the same genotype and are raised in a common environment protected from extrinsic hazards. This intrinsic variability of life span is thought to arise from the stochastic nature of the cellular and molecular mechanisms controlling development and ageing. In this article we review what is currently understood about the factors underlying the variability of life span and consider the implications for research that aims to improve the predictability of health in old age.


*Keywords:* Ageing; Longevity; Variation; Genes; Environment; Errors; Development; Stochastic

## 1. Introduction

Life span varies greatly within populations. In free-living, out-bred populations this variation is usually attributed to the combined effects of individual genetic and environmental factors and to causes such as infection, accident, starvation, predation and cold. However, even when a population comprises genetically uniform individuals, reared in a constant environment, and protected from extrinsic mortality, the individuals display very different life spans.

Isogenic populations of the nematode *Caenorhabditis elegans* represent a striking instance of intrinsic variability of life span (Kirkwood and Finch, 2002). The great majority of any *C. elegans* population is made up of self-fertilising hermaphrodites, facilitating the production and maintenance of genetically uniform cultures. In addition, *C. elegans* has a highly reproducible developmental process, adult worms have precisely 959 somatic cells, and standard laboratory culture procedures provide exceptionally constant environments. It might be expected that such close control of genes, development and environment would result in a narrow distribution of age at death within the population. The truth, however, does not match the prediction. Individual

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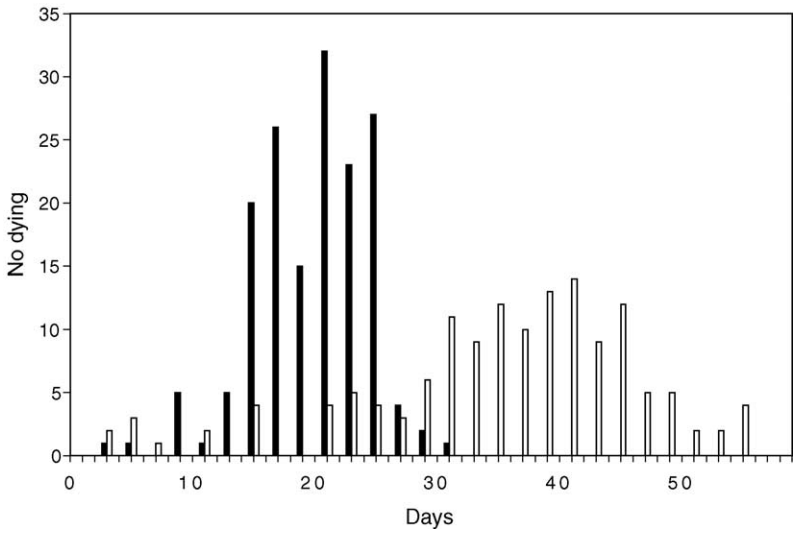


Fig. 1. Life span distributions for individual *Caenorhabditis elegans* nematodes in isogenic populations of wild-type (filled bars) and *age-1* (open bars) strains. Redrawn from Kirkwood and Finch, 2002; original data from Johnson (1990).

nematode life spans vary as much as three-fold within the population (Fig. 1). Furthermore, this variability persists in populations with average life spans that have been enhanced by genetic mutations, such as *age-1* and *daf-2*.

Although it may be argued that there is no such thing as a completely genetically identical population or a fully constant environment, it seems highly unlikely the significant variation in the life spans of *C. elegans* individuals can be explained by these small genetic and environmental disparities. Furthermore, similar non-genetic and non-environmental variation is a major component of the intrinsic life span variability in free-living, out-bred populations of this and other species (Finch and Kirkwood, 2000). So what causes the observed variation in life span?

## 2. Is life span variation truly random?

We distinguish between variation whose cause is unknown and that due to chance, and in this article we focus exclusively on the latter. At least some, and perhaps much, of the non-genetic, non-environmental variation in life span is indeed stochastic. By 'stochastic' we mean that the underlying process contains an element of chance, but not that the outcome is entirely random. For example, mutational processes are intrinsically stochastic and arise from random damage to, or error in the replication of, a particular DNA sequence. Although the *individual* stochastic event is random, the *distribution* of events in space and time is modulated by genetic factors such as the efficiency of DNA repair and/or by environmental factors such as the presence of a mutagen. It is known that species display different DNA repair rates and rates of mutation accumulation through time, while genomes contain mutational hotspots that result from flanking sequences or chromatin configuration.

Although variation tends to be negative or at best neutral, some biological processes have evolved to exploit stochastic variability, and in certain contexts this uncertainty in outcomes is beneficial. For example, the hypermutability of the immune system serves to protect the organism from infections and cancer. And noise, by which we mean the presence of a certain level of stochastic variation, is an intrinsic feature of complex systems. The field of engineering has long noted that to introduce background noise into the control elements of complex machinery (such as the control surfaces of rockets and high-performance aircraft) means more rapid and precise response; this so-called 'stochastic resonance' may also have evolved in living systems (Bezrukov and Vodyanoy, 1997).

## 3. How far need variation be constrained?

A striking feature of most developmental processes is that, in spite of the many things that can and do go wrong (see above), the overall process of morphogenesis is remarkably reliable with many developmental processes behaving as if 'canalised'. Nevertheless, small early perturbations can result in large effects, and there is evidence of significant organ size variations among genetically identical organisms (Finch and Kirkwood, 2000), or even between the two sides of a single individual (known as 'fluctuating asymmetry'; Moller and Swaddle, 1997). In addition to variations in the endpoints of development, such as adult organ size, there can be significant variations in the timing of key developmental events, such as puberty (Vom Saal et al., 1990). On the other hand, there is plenty of evidence that where it really matters, such as in the specification of the body plan during embryogenesis, developmental processes include diverse

mechanisms to ensure developmental precision. The key question is whether the variation has deleterious effects on fitness. If it does, then stabilising selection will tend to keep variation within tight bounds. In terms of variations that contribute to the pathophysiology of ageing, gerontologists already recognise that the force of natural selection declines progressively with age during the adult portion of the life history (Williams, 1957; Hamilton, 1966; Kirkwood and Austad, 2000). Thus, the idea that intrinsic variation is associated with the ageing process, resulting in significant non-genetic and non-environmental variation in life span is entirely unsurprising.

## 4. Sources of intrinsic variability

A number of well-characterized sources of intrinsic variability within organisms might explain the life span variation observed in genetically identical organisms reared in a constant environment (Table 1). At the molecular level, subcellular processes are subject to diffusion, which generates variation associated with the random Brownian motion of molecules that will be superimposed on any directional trafficking. Transcription in eukaryotes is generally controlled by multiple transcription factors, each of which may be present only in very small numbers within the cell. Many genes are transcribed to produce only a few mRNA's per cell, which can cause large statistical fluctuations in biosynthesis. There is growing evidence of the intrinsic stochastic nature of gene expression and macromolecular biosynthesis (McAdams and Arkin, 1999; Zlokarnik et al., 1998).

Genome instability, which results in somatic mutations and chromosomal abnormalities, is an important source of intrinsic variation. In ageing mice, mutation frequencies as high as $10^{-4}$ per gene per cell have been reported (Dollé et al., 1997; Odagiri et al., 1998). Epimutations may also occur through loss or disruption of DNA methylation patterns, affecting gene expression (Wolffe and Matzke, 1999). Most of these changes will be deleterious, but the extent of the impact on the cell and its host tissue is highly variable. Even in the immune system, where chance variations in DNA recombination are essential to produce the immunological capacity to deal with unfamiliar antigens, a proportion of the resultant clones are self-reactive and may, if not deleted, contribute to autoimmune diseases. Genome instability also manifests as mitochondrial DNA point mutations and deletions. These aberrations arise at a ten-fold greater rate than nuclear mutations and accumulate in certain tissues throughout life (Zhang et al., 1998; Kraytsberg et al., 2003), presumably leading to impaired bioenergenesis. Exactly how genome instability, in its various forms, contributes to the phenotype of aged organisms is still unclear; as organisms age they will accumulate a wide array of mutations and some,

Table 1
Some sources of intrinsic variation arising during development and adulthood, that may explain variation in life span of genetically identical organisms reared in a constant environment

| Level of effect | Timing of effect | |
|---|---|---|
| | During development | During adulthood |
| Molecular | Mutation | Mutation |
| | Epimutation | Epimutation |
| | Errors in biosynthesis | Errors in biosynthesis |
| | Modification (e.g. glycosylation, cross-linkage) | Modification (e.g. glycosylation, cross-linkage) |
| | Damage (e.g. oxidation) | Damage (e.g. oxidation) |
| | Diffusion (Brownian motion) | Diffusion (Brownian motion) |
| | Molecular interactions | Molecular interactions |
| | Combinatorial assembly of molecular complexes (e.g. transcription factors) | Combinatorial assembly of molecular complexes (e.g. transcription factors) |
| Cellular | Cell partitioning at division | Cell partitioning at division |
| | Configuration of organelles, etc | Configuration of organelles, etc |
| | Differentiation (cell fate) | Differentiation (cell fate) |
| | Cell-cell contacts | Cell–cell contacts |
| | Cell death | Cell death |
| | Cell migration | Cell migration |
| | | Accumulation of metabolic wastes |
| Organ/system | Cell number variation | Remodelling |
| | Size variation | Cell loss |
| | Internal organization | Injury due to toxins, infection, trauma |
| | Asymmetry | Homeostatic imbalance |
| | | Allostasis (multiple 'attractors') |
| Community (social species) | Specification of role | Modification of role |

The sources are grouped according to the level at which they arise (molecule, cell, organ, community) and whether they contribute during development or adulthood. In general the effects of variation tend to propagate both down the table (i.e. from bottom up in terms of biological level) and from left to right (i.e. through time).

such as mutations in regulatory switches, are likely to have the greatest effects.

Abnormal proteins, which may resist proteolysis and accumulate over time, regularly arise as a result of damage, misfolding, denaturation, post-translational modification, or errors in biosynthesis (Rosenberger, 1991). Synthesis errors occur at rates high enough that a sizeable fraction of newly synthesised proteins (particularly larger proteins) are predicted to contain at least one sequence error. Because high-throughput techniques for sequencing individual protein molecules (the equivalent of PCR for nucleic acids) are lacking, we know little about the sequence heterogeneity of proteins in vivo and its possible biological consequences.

A fundamental aspect of multicellular life is the unfolding of cell lineages during morphogenesis and the homeostatic regulation of cell populations during adulthood. Individuals begin life as a single cell but become, effectively, a large, variegated cell clone comprising a mosaic of innumerable sub-clones. During the extensive proliferation of cells in developing embryos, the number and type of individual cells formed are influenced by intrinsic variations. Asymmetric cell divisions give rise to specialised cells, daughter cells in a given lineage having characteristic probabilities for their future fates. In the developing mammalian central nervous system, for example, neuroblasts proliferate in a sheet of epithelial cells, which has an apical-basal polarity in which the key signalling protein Notch is basally located. How Notch is apportioned during cell division determines the fate of the daughter cell (Chenn and McConnell, 1995; Lin and Schagat, 1997). In culture, clones of single neurons from vertebrate embryos give rise to mixtures of cell types in the resulting colony, with large variation in the numbers of each type of cell produced (Kilpatrick and Bartlett, 1993).

Intrinsic variability may also arise during cell migration, which in early development of most animals involves complex movements of cells from one part of the embryo to another. During these migrations clones are dispersed (Walsh and Cepko, 1992), with some cells failing to reach their correct locations and dying. Migrations of cell bodies or processes are dependent on molecular signals and gradients, and thus are subject to intrinsic variability in the surrounding molecules and cells.

The cumulative effects of intrinsic variations at the cell level during development are seen in the variability of organ size and cell number when morphogenesis is complete. In genetically identical individuals, such as inbred mice or monozygotic human twins, the variation in organ size can be large (Finch and Kirkwood, 2000). For example, the size of the ovary in newborn mice of the same strain may vary threefold (Jones and Krohn, 1961; Gosden et al., 1982), and there can be differences of 10–20% in the sizes of the hippocampi in human twin pairs (Plassman et al., 1997).

During adulthood a variety of processes can impact on the maintenance of organ size and structure. These range from the effects of toxins or trauma in organs such as liver, to the gradual remodeling that appears to take place in the ageing immune system. In the latter example, the overall maintenance of T-cell number often masks the fact that the population is changing from one characterized by large numbers of relatively small T-cell clones, to one that contains fewer but larger clones (Franceschi et al., 2000). In tissues that are continually renewed from stem cell pools, such as the immune system or intestinal epithelium, age-related changes can affect the functional properties of the remaining stem cell pools and hinder the regenerative capacity of the organ when stressed. For organs concerned with maintenance of the hypothalamus–pituitary–adrenal axis, repeated stresses over long periods of time can result in a state of 'allostasis' (McEwen, 2002), a permanently perturbed balance point. This finding suggests that, in the terminology of systems biology, regulation of the organ system possesses multiple, quasi-stable 'attractors' (that is, stable states).

For social organisms, particularly bees and ants, an additional level at which variability can arise involves the specification or modification of the individual's role within the community. Role transitions, such as from a hive worker to a forager in honey bees, follow a well-ordered sequence, but a number of factors can perturb how roles are specified.

## 5. How can we use these insights?

Randomness in the pathophysiology of ageing and, hence, variation in the life spans of genetically identical organisms reared in constant environments are consistent with numerous observations on human senescence. Not all people with extensive atherosclerotic lesions die of them, and many with widespread amyloid plaques and neurofibrillary tangles in their brains show no signs of dementia. On the other hand, death 'from old age' can result in a person who has no obvious life-critical pathology in their major organs but in whom it appears that everything 'just fell apart'.

During ageing, multicellular organisms develop somatic mosaicism. For example, as a result of the somatic mutations that arise during DNA replication and cell division, it is unlikely that any two cells within an adult human being contain exactly the same DNA sequence. This heterogeneity is in principle detectable, but to do so in practice would be both labour-intensive and costly. Nevertheless, the unavoidable conclusion is that many of the cells in our bodies carry their own repertoire of 'acquired errors of metabolism', and this is merely one aspect of the intrinsic variation that limits the predictability of individual longevity.

Despite the development of personalised diagnosis and prognosis of current and future ill health using genomics, proteomics, metabolomics and other techniques, at least part of the intrinsic variability of life span and its causes may remain inexplicable. Further work aimed at understanding the underlying causes of the intrinsic variation in life span,

and its roots in development, should help quantify this perhaps irreducible uncertainty.

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