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# Commentary: A gerontological perspective on Klaus Gärtner's discovery that phenotypic variability of mammals is driven by stochastic events

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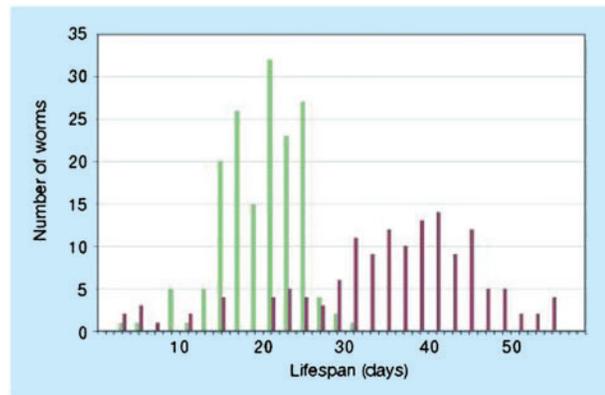
About 40 years ago, an exceptionally dedicated investigator began a 20-year series of remarkable studies in an effort to understand why, despite every effort to control the genotypes and the environments of his rodent colonies, there was a frustrating degree of phenotypic variability. Many of his papers were published in German and therefore perhaps not widely available to the international community. This situation began to change when Klaus Gärtner published his seminal review of that 20-year odyssey in 1990.<sup>1</sup> I say 'began' because there was a lag period before one began to see a crescendo of growth of citations to this paper; of the 71 citations to that review listed in PubMed as I write this review, some 19 were published in 2010 and the still incomplete record for 2011. Possibly because I am a gerontologist recently interested in stochastic variation underlying intra-specific variations in health span and life span, I must admit that I too have come very late to the party celebrating Gärtner's contributions. I am grateful to George Davey Smith, co-editor of this journal, for having introduced me to this classic paper and to his splendid summary of the previous history of publications of relevance to this field of scholarship.<sup>2</sup>

Gärtner's review cited research comparing parameters of phenotypic variability of rats at two age groups: 81 and 181 days. A typical gerontological experiment with rats or mice would choose the latter cohort as the baseline of young mature animals and compare them with middle-aged animals about twice that age and old animals at about four times that age. If Professor Gärtner had embraced that experimental design, he would have found a much greater degree of phenotypic variability, even for body and organ weights, especially given the impacts of the many diseases of ageing that are driven by intrinsic mechanisms of biological ageing. (He also would have annihilated his research budget and the patience of his co-workers!) But what is the evidence for the statement that variability increases during the course of ageing, and why do I believe that this phenomenon is driven by stochastic events? Moreover, what categories of stochastic events may be primarily responsible for such variegation?

The best model system to address the variability of the penultimate gerontological phenotype, life span, is the humble roundworm, *Caenorhabditis elegans*. As these nematodes are hermaphrodites, every diploid

locus is driven to homozygosity; they are therefore identical twins. Moreover, it is possible to grow the worms in media free of bacteria (their usual food) under highly reproducible environmental conditions. Nevertheless, there is extraordinary variability for life-span. A graphic demonstration of this variability is given in Figure 1.<sup>3</sup> Despite the marked increase in mean life span of a mutant strain, the distributions of life spans for mutant and wild-type strains overlap and both exhibit a wide range of life spans. Strong evidence that these differences are driven by stochastic events has been provided by experiments in the laboratory of Thomas E. Johnson at the University of Colorado.<sup>5</sup> Somatic mutations do not appear to have sufficiently high frequencies to account for such results (at least in ageing mammalian cells, where they can reach levels of  $\sim 10^{-3}$ – $10^{-5}$ ), although they could contribute to varying degrees in different cell types and in different species. Errors in transcription and translation could also contribute and could theoretically lead to an error catastrophe of protein synthesis; that theory, however, was even questioned by its originator.<sup>6</sup> I suspect that the most likely mechanism is epigenetic drift—that is to say, a gradual increase in the variegation of gene expression during ageing that is not related to alterations in the sequence of DNA. Such drift has been documented at the molecular level in ageing human identical twins and has been interpreted as being driven by environmental factors.<sup>7</sup> There is little doubt that environmental perturbations can alter gene expression. A more interesting explanation, perhaps, comes from considerations of evolutionary biology. There is a large literature in this field that deals with ‘bet hedging’—genetically determined variegations in phenotypic expressions that are adaptive.<sup>8</sup> I prefer the term ‘epigenetic gambling’ on the basis of the hypothesis that the selected gene actions operate via modulations of the degree of epigenetic variegation.<sup>9</sup> One can imagine that the degrees of such epigenetic variegations are driven by the ecologies in which a species evolves. Given a highly stable, ‘boring’ environment, it would not be an energetically wise investment to evolve too much epigenetic gambling. It may pay off, however, in highly unpredictable environments. Readers are once again directed to Professor Davey Smith’s review for a more thorough history of some of these concepts.<sup>2</sup>

My view is that, whereas the degree of evolved epigenetic gambling may be adaptive during the reproductive life of an organism, once it has been established, it could have ‘a life of its own’, continuing and enlarging unabated into the post-reproductive portions of the life course.<sup>9</sup> As such, it could be responsible for many different types of geriatric pathology. I have referred to such pathologies as ‘quasi-stochastic’; whereas there is a propensity to attack a specific organ or a specific tissue and cell type within an organ, their distributions within the target tissues



**Figure 1** Stochastic events dominate intra-specific variations in life span. Overlap between the distributions of life spans of wild-type *C. elegans* and the age-1 long-lived mutant strain. Source: Kirkwood & Finch, 2002<sup>3</sup>, from original data of Johnson, 1990<sup>4</sup>. Reproduced from Kirkwood & Finch<sup>3</sup> with permission

appear to be largely random.<sup>10</sup> This is the case for major diseases such as dementias of the Alzheimer type, atherosclerosis and neoplasia.<sup>10</sup> Epigenetic drift involving the expression of genes involved in the maintenance of proliferative homeostasis would be expected to lead to both atrophy and hyperplasia, features that are found in many organs of ageing mammals. Hyperplasia may, in fact, be the first step in oncogenesis. Evidence consistent with that hypothesis comes from the discovery of clonal expansions surrounding adenocarcinomas of the colon.<sup>11</sup>

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## Commentary: Gärtner's 'third component': still an open question

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In this paper, published in 1990, Klaus Gärtner<sup>1</sup> provides evidence that there is more to phenotype than simply genotype and environment. He presents data, collected over many years, that suggest that genotype and environment, alone, cannot explain the random variability seen in quantitative traits in mammals and suggests the existence of something else, which he calls a 'third component'. He compares this to Falconer's 'intangible variation', a term coined much earlier to explain much the same phenomenon.<sup>2</sup> This manuscript is rarely discussed and has been cited less than 100 times, despite its potential significance. Over the 20 years since publication, our understanding of genetics has progressed considerably. Do Gärtner's ideas survive the test of time?

Gärtner had, for many years, been puzzled that despite inbreeding, many laboratory animals displayed considerable variability in most measurable traits and he reasoned that a reduction in variability would make research easier; smaller numbers of animals would be required to detect differences between control and experimental groups. He spent much of

his scientific career trying to standardize the phenotypes of laboratory animals. In this particular paper, he presents two main findings. First, he shows that standardization of the environment, such as standardizing the food, cage conditions, group size, etc. has little effect on the range of kidney weights in inbred rats. In most inbred strains this ranged from 80 to 120% of the mean. He also found that the range did not change significantly even when the rats were housed in 'wild' conditions, where temperature changes were extreme. Similar findings were made using other phenotypic measures, and he concludes that the postnatal physical environment has no major role in random variability.

Secondly, he carried out twinning experiments in which he divided eight-cell embryos in half and transferred each half separately to one foster mother and found that the phenotypic variation, in this case body weight, was reduced. In these experiments, he used inbred mice and some of this data had appeared in a previous publication.<sup>3</sup> He argues that if the random variability of body weight is