

Perspective

Nutritional Geometry Provides Food for Thought

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Dietary Restriction extends lifespan in a diverse range of animals, but this often comes at a cost to reproduction. While a number of molecular pathways integral to these relationships have been characterised, we still do not fully understand whether restriction of specific nutrients or calories is responsible. Two recent studies on insects have offered novel insights into this longstanding issue via the application of Nutritional Geometry. This technique promises to significantly advance our understanding of how nutrition influences reproduction and longevity.

Key Words: Nutritional geometry—Reproduction—Longevity.

FOOD, SEX AND DEATH

Animals consuming a diet restricted in some, as of yet, unidentified component but with adequate nutrients so as to avoid malnutrition, typically show extended lifespan (1–3), improved health (4) and delayed aging (5). This treatment, termed Dietary Restriction (DR), has been shown to increase longevity across a broad range of species (6). However, despite over seventy years of research, the mechanisms underlying this process are poorly understood. Whilst DR's life extending properties have often been attributed to Caloric Restriction (CR) (7–9), accumulating research shows that, in some species, a limited intake of specific nutrients is necessary and sufficient to increase lifespan (3,10–12). For example, diets restricted in either yeast or sugar increased longevity and decreased mortality rate in *Drosophila melanogaster*, despite these effects being unrelated to the caloric content of the diet (3). Protein restriction has been reported to both extend (13) and reduce (5) lifespan in rodents. The magnitude of the response of longevity, and/or of oxidative stress to methionine restriction varies across species relative to the responses induced by CR (10,14), whether this extreme dietary manipulation operates via the same mechanisms as more mild DR treatments is unclear. Furthermore, there is debate regarding whether DR slows the rate of ageing or only delays its onset, with support for either hypothesis derived from studies of a number of different species (reviewed in (15)). Whilst DR protocols should be flexible, devised in each laboratory on the basis of the diet optimal for fecundity and lifespan of the species or specific population being examined (16), it will remain unclear whether divergent responses reflect species specific idiosyncrasies or differences in methodologies until a robust practical framework can be applied that allows across species comparisons.

In contrast to the positive effects of DR on lifespan, reproductive output is often greatly reduced by DR. Life-history

theory predicts that if reproduction is costly, it should be traded against longevity (17). Thus, a decrease in reproductive effort with DR may occur if longevity is prioritised over reproduction (18,19). However, the effect of DR may differ between the sexes due to different nutritional requirements (20) and variation in the nature of any trade-offs between reproduction and longevity. Females require greater protein for egg production, whilst males need more carbohydrates to fuel sexual display (21). If these macronutrients differentially affect longevity, restriction of either will influence reproduction and/or lifespan differently in each sex and set the stage for intersexual conflict over optimal diet (22).

TECHNICAL CHALLENGES AND KNOWLEDGE GAPS

Many studies have documented associations between reproductive effort, nutrition and senescence, yet no general consensus has been reached as to the mechanisms underlying the interactions between these factors (23). There are a number of possible reasons for this. One of the main issues concerns whether the effects of DR on longevity are primarily artefacts of laboratory environments. Foremost, given that there is little data available on food consumption patterns of animals in the wild, the beneficial effects of DR may simply reflect the adverse effects of overfeeding in the lab (24,25). Methodological limitations also pose problems. Empirically uncoupling the effects of calories from those of the nutrients from which they are derived remains a major challenge. Perhaps as a consequence of this, DR studies often apply too few dietary treatments and therefore, only explore a limited nutritional landscape (25). In addition, it is difficult to accurately quantify food consumption in lower organisms, especially where liquid diets are used and restriction is accomplished via nutrient dilution. Without precise measurements of dietary consumption it may not be possible to detect compensatory feeding (24,26); where

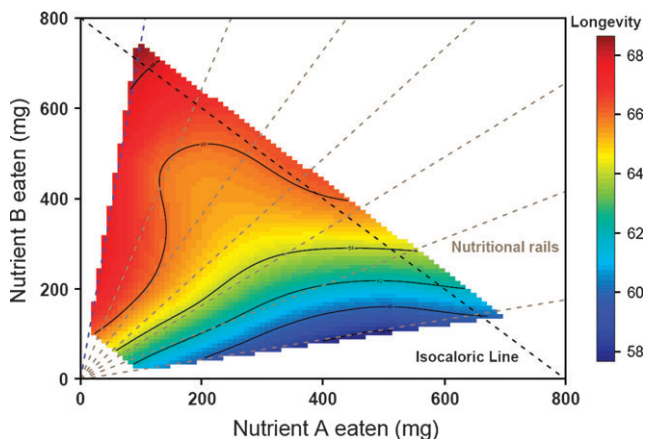


Figure 1. A Hypothetical Example of a Nutritional Surface. Diets that vary in the ratio and concentration of nutrients A and B have been provided along nutritional rails (beige lines). Both intake and longevity have been accurately measured as animals feed along these rails. Full, black lines are fitness contours and colours illustrate how longevity decreases with distance from optimal lifespan (red peak) and the diet that optimises longevity (blue line). In this case fitness costs are approximately linear and the optimal strategy is to consume high levels of nutrient B relative to nutrient A. The effect of diet on longevity is independent of calories, as illustrated by there being no relationship between fitness contours and the isocaloric line.

animals consume greater quantities of diluted nutrients so as to regulate intake (25) and inadvertently mitigate the effects of DR. Studies seldom address assimilation efficiency, which may not be constant across an organism's lifetime. Moreover, animals are often housed in mixed sex groups where sexual activity and reproductive investment are not controlled (26,27). Even in studies where reproductive effort has been measured, it is often measured with greater precision (and more frequently) in females than in males (28–32). This is possibly due to the relative ease with which female reproductive effort can be quantified in laboratory model organisms (counting eggs) compared with measuring lifetime investment by males in sexual signalling.

Two recent studies on insects have overcome many of these limitations via the application of Nutritional Geometry (NG). The progress made with this technique not only advances our understanding of the link between DR and ageing but also provides a practical framework that promises to further progress research on this topic.

NUTRITIONAL GEOMETRY

The NG protocol is detailed elsewhere (33,34) and the benefits of this technique specific to ageing research are comprehensively reviewed by Simpson and Raubenheimer (35). Using NG performance and behaviour can be investigated in a multidimensional nutritional framework, which aims to differentiate between nutrient intake and utilisation. Essentially, NG entails varying the concentrations and ratios of nutrients in the diet and then accurately measuring their intake in a series of feeding trials. Quantifying the intake for each unique nutrient combination allows the

construction of a fine-scale nutritional surface with as many dimensions as there are nutrients being examined. On this surface, performance traits (such as lifespan or rates of ageing) can be mapped (see Figure 1). This enables powerful statistical analysis using response surface methodologies to examine both the independent effect of and the interaction between the different dietary components on the performance trait to be examined. Feeding trials where individuals are given the choice between two diets can then be used to examine the behavioural rules that govern dietary intake and to identify the “intake target” or nutritional point to which an animal feeds. This intake target may be “static”, i.e. a point integrated over time, or “dynamic”, in which case it is possible to track its trajectory as an animal grows, develops, reproduces or senesces. With respect to ageing, this means that the effects of DR can be examined relative to these intake targets rather than laboratory standards.

Lee et al. (25) employed NG in an elegant study of *D. melanogaster*. Liquid diet of varying protein and carbohydrate ratio and concentration was provided in microcapillary tubes and consumption measured according to a scale bar. Longevity was greatest at a low Protein - Carbohydrate ratio (P:C) of 1:16, in both their empirical study and associated meta-analysis. The long life observed under low protein consumption was attributed to a reduction in initial mortality and retarded acceleration of age-dependent mortality. Other studies of *Drosophila* have found only initial mortality rate to be affected by DR (20). This discrepancy may be because yeast, which features in dietary treatments used in this paper as well as many others examining *Drosophila*, has uncharacterised odorants known to affect longevity (36). In *Drosophila*, restoration of chemosensory signals completely reverses the reduction of initial mortality rate seen in DR animals but not that attributable to a reduced rate of age dependent acceleration (36). Yen and Mobbs (36) propose that the relative roles of reduced initial mortality versus retardation of the subsequent trajectory of mortality depend on the relative contributions of chemosensory and metabolic mechanisms. Given these findings, it is clear that future studies should place greater emphasis on partitioning the effects of chemosensory stimuli from consumption.

Lee et al. (25) found that the fecundity of mated females was optimized under higher protein: the rate of egg production peaked at a P:C of 1:2 and lifetime egg production at a P:C of 1:4. The difference in optimal P:C for longevity and fecundity suggests a trade-off, however, it appears that the interplay between reproduction and lifespan is not integral to determining how the latter responds to DR, as suggested by past research (37,38). Since the P:C contour lines ran almost perpendicularly to the isocaloric line on the nutrient surface that traverses diets composed of equal caloric value, it reveals that calories *per se* did not have a significant influence on any of the investigated life history attributes. There was evidence of compensatory feeding and active regulation of nutrient intake, with flies consuming a P:C ratio

corresponding to that which maximises lifetime reproductive success across a range of nutrient concentrations.

Maklakov et al. (21) used similar methods to investigate senescence in the Australian field cricket, *Teleogryllus commodus*. Male *T. commodus* call to attract females; this energetic sexual advertisement is associated with a four-fold increase in metabolic rate (39) and is under strong selection through female choice (40). In a no-choice test, 24 powdered diets were provided in excess and intake measured by weighing the dried food every 3 days throughout the cricket's lifetimes. Male calling effort was monitored using an acoustic call chamber and the fecundity of mated females quantified by counting the number of eggs produced. Two choice experiments were also conducted: the first provided isocaloric diets that differed in their P:C ratio and the second a choice between three pairs of foods that varied in both their P:C ratio and energetic content.

As was the case for *D. melanogaster*, lifespan was maximised on a low P:C ratio. However, in *T. commodus* male longevity declined with high carbohydrate consumption, whilst, in either sex, as the P:C intake ratio increased, longevity decreased. This study provides the first, clear evidence that there is potential for sexual conflict over optimal diet. Male calling effort is greatest at the P:C ratio that maximises lifespan. This contradicts past work suggesting that male reproductive effort in *T. commodus* trades-off with longevity (19). Conversely, in females, greater dietary protein increases fecundity but decreases longevity (21). This trade-off between female reproductive fitness and long life does not manifest itself in a female's choice of diet: whilst there is evidence of active regulation of diet influenced, in part, by total nutrient value, and females consume slightly more protein than males, there is little sexual dimorphism in diet. Whilst neither sex apparently chooses the diet that maximises lifetime reproductive success in the laboratory conditions in which they were tested, female fecundity is highly compromised by their choice of diet.

CONCLUSIONS AND FUTURE DIRECTIONS

These studies add to a growing body of evidence that protein restriction, not CR *per se*, is primarily responsible for longevity extension under DR regimes in insect species. Whilst there is evidence for a reproduction-longevity trade-off, with sex and species specific differences in its magnitude, this is not the sole influence over longevity extension under DR. The study by Maklakov et al. (21), which highlights the potential for intersexual conflict over diet choice, provides scope for research examining the genetic basis of such conflict, its prevalence and resolution. NG may also facilitate investigation of the mechanistic basis of ageing. For example, the free radical theory (41), which attempts to explain the mechanistic basis of ageing, proposes that accumulation of oxidative damage induced by ROS causes the functional decline that characterises senescence. Mapping ROS-induced

oxidative damage onto the geometric framework may provide a further, and potentially more powerful, test of this theory.

NG requires a large number of experimental animals and is therefore both costly and labour intensive to implement. This has, so far, limited its application to insects. There is strong evidence that DR operates differently in mammals and insects (reviewed in 15), and so the application of this conceptual framework across taxa is likely to prove informative. Methionine restriction has been shown to increase longevity in some species (11). It has been postulated that this is due to disruption of either carbohydrate (35) or protein (15) metabolism. Simpson and Raubenheimer (35) suggest that NG may enable the interaction between these nutrients to be assessed and these alternate hypotheses addressed. Moreover, they propose this may be feasible in mammals were the nutritional surface investigated first restricted on the basis of preliminary trials.

Given that other research has drawn similar conclusions regarding the role of specific nutrients in influencing longevity (e.g. 3) to those of Lee and colleagues (25) and Maklakov et al. (21) is the added expense justified? Obviously where only one nutrient is being investigated, application of this technique is excessive. However, while other approaches have made qualitatively similar conclusions, NG has more accurately quantified the relationship between calories, specific nutrients, longevity and sex. Protocols previously employed for investigating the contributions of specific nutrients to longevity extension under DR, have commonly varied the concentration of one nutrient at a time, whilst holding all others constant (e.g. 10), this does not allow interactions between nutrients to be assessed. Authors have recently stressed the importance of a multidisciplinary approach to ageing research (22, 42, 43). NG aims to integrate physiology, behaviour and life-history parameters within a comprehensive nutritional framework and, in doing so, provides a robust platform on which to build such a research approach.

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