Proceedings of the Nutrition Society (2014), **73**, 532–542 doi:10.1017/S0029665114001141
 First published online 15 September 2014

The Nutrition Society Annual Summer Meeting was held at Newcastle University on 15–18 July 2013

Conference on 'Nutrition and healthy ageing'
Plenary Lecture 3

Plasticity of lifespan: a reaction norm perspective

Thomas Flatt
Department of Ecology and Evolution, University of Lausanne, UNIL Sorge, Biophore, CH-1015 Lausanne, Switzerland

It is a well-appreciated fact that in many organisms the process of ageing reacts highly plastically, so that lifespan increases or decreases when the environment changes. The perhaps best-known example of such lifespan plasticity is dietary restriction (DR), a phenomenon whereby reduced food intake without malnutrition extends lifespan (typically at the expense of reduced fecundity) and which has been documented in numerous species, from invertebrates to mammals. For the evolutionary biologist, DR and other cases of lifespan plasticity are examples of a more general phenomenon called phenotypic plasticity, the ability of a single genotype to produce different phenotypes (e.g. lifespan) in response to changes in the environment (e.g. changes in diet). To analyse phenotypic plasticity, evolutionary biologists (and epidemiologists) often use a conceptual and statistical framework based on reaction norms (genotype-specific response curves) and genotype × environment interactions ($G \times E$; differences in the plastic response among genotypes), concepts that biologists who are working on molecular aspects of ageing are usually not familiar with. Here I briefly discuss what has been learned about lifespan plasticity or, more generally, about plasticity of somatic maintenance and survival ability. In particular, I argue that adopting the conceptual framework of reaction norms and $G \times E$ interactions, as used by evolutionary biologists, is crucially important for our understanding of the mechanisms underlying DR and other forms of lifespan or survival plasticity.

Lifespan: Ageing: Phenotypic plasticity: Reaction norms: Genotype × environment interactions: Dietary restriction: Nutritional geometry

Lifespan, survival ability and phenotypic plasticity

Lifespan, and the underlying processes of ageing, are tremendously variable among species, populations and among individuals within populations[1–5]. For example, some tree species live thousands of years and some tortoises of the order of 150 years, whereas on the more short-lived end of the spectrum we have species such as fruit flies, which live on average 40–50 d, and some mayflies which only live for about 30 min[1–5]. We know that much of this variation, even among individuals within a single population, is ultimately due to genetic differences. Consequently, most of the work on the mechanisms of ageing has focused on the genetic factors that influence longevity in yeast, nematode worms, fruit flies, mice and even human subjects, resulting in the identification of hundreds of genes that influence lifespan and ageing[6,7]. In particular, this research programme has led to the discovery of genes that have evolutionarily conserved effects on lifespan and ageing, for example in the insulin/insulin-like growth factor signalling pathway[6,7]. However, this focus on genetic factors distracts from the important but often neglected fact that ageing and lifespan can also be strongly influenced and modified by environmental factors[1,2,4–6]. Importantly, such environmental effects can interact with genetic determinants to affect ageing and lifespan in ways that cannot be understood from considering the genetic (or environmental) factors alone.

Abbreviations: AL, *ad libitum*; DR, dietary restriction; $G \times E$ interactions, genotype × environment interactions.

Corresponding author: T. Flatt, fax +41 21 692 4165, email thomas.flatt@unil.ch

Indeed, in many organisms survival and lifespan react highly sensitively (plastically) to changes in the environment, for example to changes in diet, temperature etc.[1,2,4–6,8]. In small poikilotherms such as insects, for instance, reduced temperature tends to increase lifespan within the range of sustainable temperatures[9,10]. Such phenotypic responses of lifespan (or, more generally, of somatic maintenance and survival ability) to environmental changes are specific examples of a more general phenomenon which evolutionary biologists call phenotypic plasticity[3,5,11,12]. Phenotypic plasticity refers to the ability of a single genotype (genome) to produce and exhibit different phenotypes in response to changes in the environment. More generally, we can define phenotypic plasticity as that part of phenotypic variation in a trait that is purely elicited by changes in the environment, as opposed to the part of variation in a trait that is caused by genetic differences. To consider a fairly trivial example: a given genotype may grow to relatively large body size when dietary conditions for growth are optimal but may stay relatively small when conditions are suboptimal. Such phenotypic plasticity is extremely widespread; it can be observed for numerous traits in many different organisms exposed to environmental heterogeneity.

Although many researchers working on the molecular mechanisms of ageing and lifespan may not be intimately familiar with the concept of phenotypic plasticity as used by evolutionary biologists, they are certainly well aware of the phenomenon itself: in many species, changes in the environment can lead to dramatic changes in lifespan or survival ability. The probably best-known example of such lifespan plasticity is dietary restriction (DR), a physiological state of lifespan extension (and typically reduced reproduction) caused by reduced food intake without malnutrition. Remarkably, this DR response of lifespan has been observed, with a few exceptions, in almost every invertebrate and vertebrate species examined so far[6,7,13].

Here, I discuss plasticity of lifespan from a reaction norm perspective[3,5,11,12]. I first give a few illustrative examples of lifespan or survival plasticity, provide some arguments for the adaptive significance of such plasticity, and introduce the general concepts that evolutionary biologists use to analyse phenotypic plasticity, in particular reaction norms (genotype-specific response curves) and genotype × environment interactions ($G \times E$; differences in the plastic response among genotypes). I then apply these concepts to the problem of DR; similar to two recent papers by Tatar[14,15], I emphasise that a better mechanistic understanding of the DR process will require studying this phenomenon from a proper reaction norm and $G \times E$ perspective, something still rarely done among molecular biogerontologists interested in DR.

Survival and lifespan are often remarkably plastic

As mentioned earlier, DR is perhaps the most widely known example of plasticity of lifespan or survival ability (sometimes also called senescence plasticity[16]), even though most people working on ageing may not be familiar with the term plasticity and therefore not use it when referring to DR. However, numerous other examples of plastic changes in survival ability and lifespan in response to environmental change exist, as is well appreciated among organismal biologists[1,2,5,6,8,12,16].

The probably most extreme case of lifespan plasticity is found in social insects[8,17,18]. In ants and bees, distinct castes develop from an identical genome but differ tremendously in the length of lifespan, often by several orders of magnitude. For example, in ants, reproductive queens can live 500 times (!) longer than males and ten times longer than sterile workers. Similarly, lifespan plasticity can be observed within castes, for example between long-lived worker honeybees, which perform nest tasks, and short-lived workers, which perform for ageing tasks.

Another quite famous example is dauer formation in the worm *Caenorhabditis elegans*, a widely used genetic model system, and related nematode species[8,19–21]. If environmental conditions become stressful during larval development, for instance due to crowding, starvation, or high temperature, these nematodes can bypass normal larval development by expressing an alternative, very long-lived and stress-resistant larval stage, the so-called dauer larva or simply dauer (from the German word for enduring). The dauer larval stage represents a form of diapause, a programmed state of dormancy which is triggered by adverse conditions and which ensures somatic persistence and survival until conditions have improved. Diapause states are also found in other animals, for instance in adult insects such as butterflies, grasshoppers and fruit flies[8,16,21]. In such insects, low temperatures and/or short day length, for example, can trigger reproductive diapause, a state of arrested reproduction accompanied by increased stress resistance and greatly improved survival ability. Similar manifestations of dormancy also occur in mammals. Some mammalian species survive adverse environmental conditions (cold temperature, shortage of food and water) by undergoing torpor, a state of reduced energy expenditure which is often triggered by fasting and which lasts for a few hours or days[21]. The well-known phenomenon of hibernation in mammals is a prolonged state of such torpor that lasts throughout winter[21].

Likewise, seasonal changes are also known to lead to quite dramatic plastic differences in survival ability and somatic maintenance in a tropical African butterfly, *Bicyclus anynana*[22]. In this species, warm temperature and relatively high humidity cause the expression of a 'wet' season form, characterised by short lifespan, rapid reproduction, low-fat content, high behavioural activity, and beautiful, conspicuous wing patterns consisting of so-called eye spots. In contrast, in response to cooler temperatures and low humidity, the 'dry' season form exhibits the opposite traits: long lifespan, delayed reproduction, low activity, high-fat content and a cryptic wing pattern.

Water fleas of the genus *Daphnia*, small freshwater crustaceans, provide however another, morphologically quite conspicuous, example of plasticity of survival ability. In response to the presence of predators such as

dragonfly larvae (or chemical cues released by predators, so-called kairomones), water fleas react by forming protective 'helmets' on the head or similar protective structures such as neck teeth or tail spines[12].

Together, these examples illustrate that survival and lifespan are traits whose phenotypic expression can be highly contingent upon the environment. For a more detailed discussion and for more examples of survival, lifespan or senescence plasticity, see references[1,8,16,21].

The adaptive significance of survival and lifespan plasticity

Generally, plasticity is often thought or assumed to be adaptive: plastic genotypes produce, depending on the specific environment, an optimal phenotype that is optimally matched to that given environment, where 'optimal' stands for maximising Darwinian fitness (reproductive success, i.e. some function of both survival and reproduction)[3,5,8,12,23,24]. In many cases, however, plasticity might in fact be non-adaptive or even maladaptive (deleterious), especially when organisms are exposed to unusual or novel environments[12]. Measuring the costs and benefits of plasticity, and empirically demonstrating that plasticity is adaptive, is not a trivial undertaking.

Although it seems obvious that in some cases plastic changes in survival and lifespan might be physiologically inevitable or even detrimental, the specific examples discussed earlier strongly suggest that in some cases organisms might have evolved a specific adaptive ability to plastically adjust their somatic maintenance, survival and lifespan phenotypes in a functionally optimal way when the environment changes. In such cases, the potential adaptive significance of survival or lifespan plasticity seems clear[8,16]. Changing environments often impose major challenges or even threats to organismal survival and reproduction, with reproductive success being conditional upon successful survival. Under such circumstances, and especially if environmental challenges are recurrent and predictable, it might pay off to evolve a plastic strategy which allows organisms to maintain physiological homoeostasis and somatic function and which optimises fitness given, and despite the environmental change or constraint.

An evolved plastic ability to adjust maintenance and survival in response to environmental change might thus enable organisms to endure and survive temporarily stressful conditions until favourable conditions have returned[6,8,25,26]. Since fitness requires both successful survival and reproduction, and since successful reproduction is contingent upon successful survival, organisms exposed to stressful environments might prioritise survival over reproduction; they are expected to switch to increased investment into maintenance and survival until conditions for reproduction have improved[6,8,25,26]. In contrast, under optimal conditions, organisms should invest more into reproduction, even if this possibly comes at the expense of diminished future survival or reproduction, for the future may be highly uncertain[6,8,25,26].

DR might be an example of such an adaptive plastic survival strategy[26]. Since DR typically extends lifespan at the cost of decreased fecundity or fertility, DR might enable organisms to survive and to tolerate nutritionally poor conditions by plastically and reversibly reallocating energy resources normally invested into reproduction into somatic maintenance and survival until optimal nutritional conditions for successful reproduction have returned. Although there is still debate on the question whether DR represents a case of adaptive plasticity, theoretical work suggests that it may likely be an adaptive strategy[6,26]. Moreover, the fact that the DR response is evolutionarily conserved, to a large extent[13], supports an adaptive interpretation. However, despite much research on the molecular mechanisms of DR, our understanding of how and why it has evolved, and of the fitness costs and benefits it entails, remains rudimentary.

Things may be a bit clearer when we consider cases of diapause or dormancy[8,16,19–21]. Given that dauer larvae in *C. elegans* are highly resistant to a variety of environmental insults and that they can survive for a long time in a state of dormancy under harsh environmental conditions (e.g. crowding or starvation), the fitness advantage of being able to undergo dauer development seems obvious: under adverse conditions it might be best to invest into survival while postponing reproduction until better times have returned. In support of an adaptive interpretation, it has been found that *C. elegans* strains that have evolved in the laboratory under conditions of constantly high population density have lost their ability to undergo dauer development[27]. If high-density conditions elicit a non-reproductive dauer state, and if high-density conditions persist over time, the dauer strategy would be an evolutionary dead-end: non-reproducing dauer animals are clearly at a disadvantage relative to individuals that are able to grow, develop and reproduce under such conditions. This implies that dauer diapause, a plastic ability that seems to be maintained under normal circumstances, might indeed be adaptive in temporally heterogeneous, stressful environments but might be too costly to express when conditions are permanently favourable or when a poor environment persists indefinitely.

Similar considerations also apply to the adaptive nature of insect diapause. In the fruit fly (*Drosophila melanogaster*), for example, certain genotypes (e.g. in northern, high-latitude populations) are often well able to enter reproductive diapause under conditions of low temperature and short photoperiod, whereas others (e.g. in southern, low-latitude populations) may not be able to do so[8,16,28]. Since reproductive diapause involves a state of reproductive arrest, it is obvious that it cannot represent a good adaptive long-term strategy; however, under temporarily harsh conditions it might be appropriate to transiently shut down reproduction and to prioritise maintenance and survival. If the environments are sufficiently harsh, the realised evolutionary cost of temporarily not being able to reproduce is far outweighed by the potential cost of not surviving at all. Indeed, when high-diapause genotypes are competed against low- or no-diapause genotypes in population cages, the

former win out under stressful conditions (e.g. alternating bouts of cold and starvation stress), whereas the latter win out under non-stressful standard conditions[28]. New theoretical work also suggests that seasonally varying, temperate environments strongly favour the evolution and maintenance of insect diapause states that enable survival over winter[29].

In some cases, therefore, we have good, albeit preliminary, evidence that survival plasticity can be adaptive, which makes intuitive sense.

The phenotypic plasticity and reaction norm framework

Before discussing lifespan plasticity in more depth, we need to say more about the concept of plasticity. The concept of phenotypic plasticity, as is commonly used by evolutionary biologists, is rooted in quantitative and statistical genetics[3,5,11,12]. In quantitative genetics (and epidemiology), we think of phenotypic differences among individuals in a population (denoted V_P, for phenotypic variation) as being caused by underlying genetic (V_G) plus environmental (V_E) sources (components) of phenotypic variation (so that $V_P = V_G + V_E +$ some unexplained variation). Technically, phenotypic plasticity refers to V_E, i.e. phenotypic variation that is entirely due to environmental heterogeneity, not due to genetic differences among individuals. Another way of saying this is that phenotypic plasticity is the ability of a single genotype (a single individual genome or clone) to produce different phenotypes across a range of different environments (where by environment we mean different qualitative or quantitative levels of the same environmental factor, e.g. different concentrations of protein in the diet, or DR *v. ad libitum* (AL) food, or different temperatures etc.)[3,11].

To analyse plasticity, it is useful to invoke the concept of a reaction norm (or norm of reaction, as it is called in the older literature)[3,11]. A reaction norm maps a specific genotype onto its phenotype as a function of the environment; it represents the set of all phenotypes that a given genotype is able to express across the range of all environments considered. Such a reaction norm can be visualised as a line or curve (i.e. a defined mathematical function) in a *x*–*y* plot, with the *x*-axis representing the environment (most easily imagined for continuous environmental variables such as varying temperatures) and the *y*-axis representing the phenotype (again, most easily imagined for continuously varying phenotypes such as body height; Fig. 1). The line or curve (the reaction norm) then represents the genotype, and the different points along the curve (the different *x, y* coordinates) give the different phenotypes (*x*) in the different environments (*y*). Thus, a reaction norm is a genotype-specific response curve that measures how a specific genotype reacts phenotypically to different environments (Fig. 1A). For two environmental states (e.g. two distinct temperatures) on the *x*-axis, we deal with maximally two phenotypes along the *y*-axis (i.e. the number of environmental states sets an upper bound to the number of potentially distinct phenotypes), so that the reaction norm is constrained to be linear (Fig. 1B). Correspondingly, for more than two environmental states (e.g. a whole range of temperatures), we might deal with more than two distinct phenotypes, and the reaction norm may thus either be linear or non-linear (i.e. a more or less complicated curve; Fig. 1C). If the slope of the reaction norm is very shallow or zero, then a change in the environment translates into little or no change in phenotype, so there is either very little or no plasticity (Fig. 1D). In contrast, when the slope of the reaction norm is either positive, negative or if the slope changes a lot, then we have plasticity, i.e. the genotype produces different phenotypes across the different environments (Fig. 1E and F)[3,11].

As mentioned earlier, the concept of a reaction norm is most easily used for cases of continuous plasticity

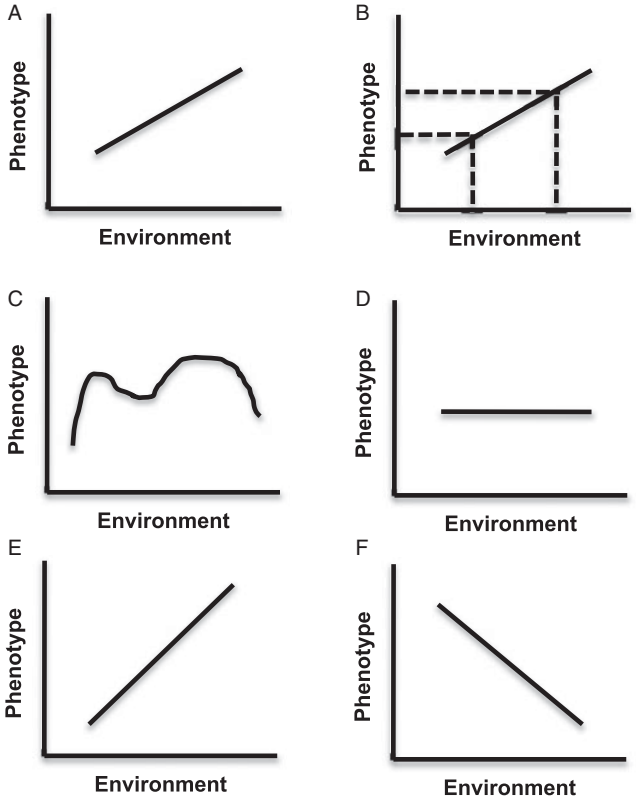

Fig. 1. Reaction norms. Simplified, schematic reaction norms, with the phenotype/trait being measured on the *y*-axis and the environmental factor being represented on the *x*-axis. (A) Linear reaction norm with a positive slope, with line representing a continuous function translating the values of a continuously varying environmental factor (e.g. temperature) into phenotypic values of a continuously varying trait (e.g. body size). The non-zero slope of the reaction norm implies that the genotype is phenotypically plastic. (B) Two environmental values (e.g. two temperatures) translate into maximally two distinct phenotypes, forcing the reaction norm to be linear. (C) An example of a non-linear reaction norm. (D) A flat reaction norm with slope zero. In this case, the genotype is not phenotypically plastic. (E) A plastic reaction norm with positive slope. (F) A plastic reaction norm with negative slope. For further details see text.

(continuous changes in quantitative/continuous phenotypes across a continuous range of environments); however, in principle it can also be applied to cases of discrete plasticity (so-called polyphenisms)[8], where an environmental change induces a switch from one phenotypic state (e.g. non-diapause) to another (e.g. diapause), by using step-like functions which relate dichotomous (binary) phenotypes to continuously varying environments, with such functions exhibiting a sharp (vertical) transition between the two phenotypic states at a specific threshold value of the environment. An alternative to using reaction norms for quantifying plasticity is the so-called character state approach, but this shall not concern us here[11].

Things get quite a bit more interesting if we consider not one but several reaction norms and thus several genotypes, say two (Fig. 2). If the two reaction norms are congruent and thus indistinguishable from each other, the two genotypes are absolutely identical in the way they phenotypically react when they are exposed to the same range of environments (Fig. 2A). When the reaction norms differ but have identical slope (and shape), i.e. if they are just shifted along the *y*-axis in parallel, the two genotypes differ on average in their phenotypes in an additive fashion but, again, their phenotypic response to the environment is the same. Both genotypes differ from each other but show the same kind of plastic response (Fig. 2B). Similarly, if the reaction norms have identical slope (and shape) and are shifted along the *x*-axis, the genotypes differ in the range of environments across which they show plasticity, but their plastic reactions are qualitatively identical (Fig. 2C). More interesting is what happens when the reaction norms are non-parallel, i.e. if their slopes differ among genotypes. In this case, the two genotypes differ in their phenotypic response to the environment; thus, there is genetic variation for phenotypic plasticity for a particular trait across a given range of environments (Fig. 2D–F). This is $G \times E$: different genotypes exhibit different response curves[3,11]. The model that explains the sources of phenotypic variation V_P then becomes: $V_P = V_G + V_E + V_{G\times E}$ + some unexplained variation. Here V_E measures the amount of phenotypic variation that is due to the average main effect of environmental heterogeneity (i.e. plasticity), averaged across all genotypes, and $V_{G\times E}$ measures the amount of phenotypic variation that is due to the fact that different genotypes differ in plasticity, i.e. in their reaction norms[3,11]. Reaction norms that cross each other represent a particularly strong kind of $G \times E$ interaction since they change (i.e. revert) the phenotypic rank order of the genotypes across environments[3,11]: for example, genotype A might exhibit a higher body weight than genotype B in environment 1, whereas in environment 2 genotype B might be heavier than genotype A (Fig. 2F). Note that, when reaction norms have identical slope (and shape) and are shifted along the *x*-axis, as discussed earlier, they might intersect each other; even though this can result in a statistically significant $G \times E$ interaction, this pattern merely means that the genotypes differ in the range of environments across which they show plasticity

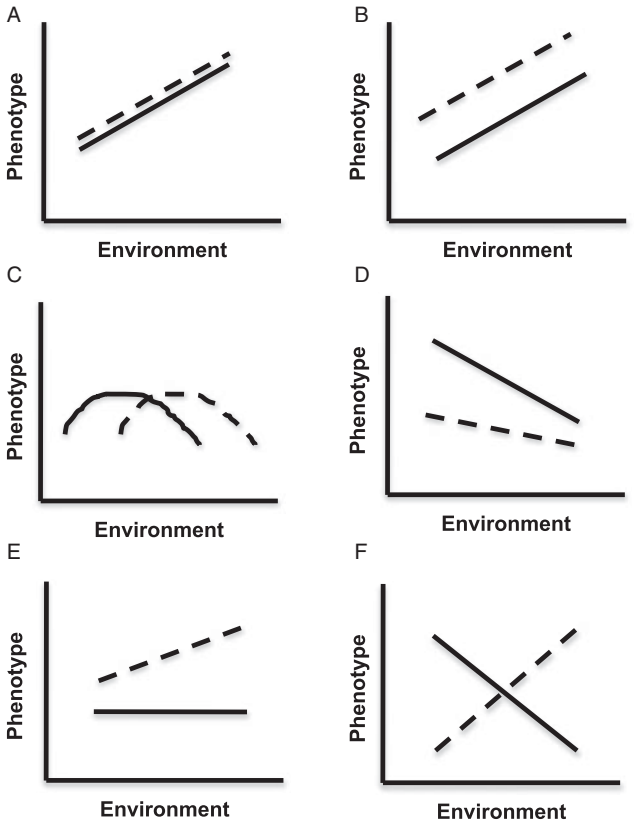

Fig. 2. Genotype × environment ($G \times E$) interactions. Hypothetical reaction norms for two genotypes (solid *v.* dashed line), with the phenotype/trait on the *y*-axis and the environmental factor on the *x*-axis. (A) Both genotypes exhibit plastic reaction norms of identical positive slope; but the two reaction norms are congruent and thus indistinguishable. Thus, there is neither any additive genetic difference between the two genotypes for the phenotype, nor any $G \times E$ interaction since the reaction norm slopes are identical. The absence of a $G \times E$ interaction implies that the genotypes do not differ in their plastic response. (B) Similar to (A), both genotypes show plastic reaction norms of identical positive slope, but now the genotypes differ additively in their phenotype. Again, since the slopes of the reaction norms do not differ, there is no $G \times E$ interaction, implying that the genotypes have an identical plastic response to the environment. (C) The two genotypes exhibit non-linear, single-humped reaction norms; since the reaction norms intersect, we have evidence for variation among genotypes in reaction norm slope (i.e. $G \times E$), yet the reaction norms have identical shape and seem to be merely shifted across the *x*-axis. (D), (E) Clear-cut cases of $G \times E$ interactions with substantial differences in reaction norm slope between the two genotypes, indicating that they differ genetically in their plastic response to the environment. (F) The two reaction norms cross each other, representing a particularly strong form of $G \times E$ interaction: crossing reaction norms imply that rank order of the phenotypes is inversed in the extreme environments on the left and on the right of the *x*-axis. Also note the point where the two reaction norms intersect: in this environment the two genotypes exhibit an identical phenotype, so that the genotypes could not be distinguished phenotypically. For further details see text.

(Fig. 2C). Despite the fact that this qualifies as a proper $G \times E$ interaction, the plastic reactions of the genotypes are qualitatively identical. Thus, while such a form of

$G \times E$ interaction implies that the genotype modulates the plastic reaction, the genotypes do not really differ qualitatively and meaningfully in their basic pattern of plasticity.

Stearns[3] provides an excellent introduction into 'reaction norm thinking' and phenotypic plasticity; for technical details of the reaction norm framework in evolutionary quantitative genetics I refer the reader to the book by Roff[11]. In the following, we shall apply this reaction norm perspective to the problem of DR.

Dietary restriction, reaction norms and nutritional geometry

Many studies have shown that lifespan is maximised at a relatively low concentration of nutrients, with any further decrease or increase in nutrient concentration away from this optimum producing a decrease in lifespan either due to malnutrition or overfeeding[9,13–15]. Thus, strictly speaking, DR refers only to the specific restricted diet or nutrient level/concentration that maximises lifespan; it describes the fact that lifespan is maximised on such a restricted diet. Since this DR effect is relative to the (lower) lifespan values observed on other diets (e.g. on a control diet), it would actually be more correct to refer to such DR experiments as dietary manipulation experiments; however, this is rarely done, and we shall therefore, for the sake of simplicity, refer to such as experiments as DR experiments.

The way lifespan changes in response to changes in diet provides an excellent example of a plastic response that can be analysed using a reaction norm approach, something that is especially relevant for studies of the genetic mechanisms underlying DR. We shall discuss this by focusing mainly on experiments in the fruit fly (*D. melanogaster*)[14,15], but the implications of our discussion are more general.

In DR experiments with *Drosophila*, flies are usually kept on a diet that consists of agar (and often also maize meal), sugar, yeast (the main dietary source of protein) and water. In most studies, diets are manipulated either by simultaneously diluting all food components (i.e. sugar and yeast = SY; whole-food dilution) or by altering the concentration of yeast (Y) while keeping sugar constant[14,15]. Lifespan is then measured on both a DR diet (e.g. 2–5 % SY; or low yeast, e.g. 2 % Y) and on a control (AL) diet (e.g. 10 % SY; or high yeast, e.g. 15 %Y)[14,15]. The resulting average lifespan data can be plotted for each diet level (*y*-axis, lifespan; *x*-axis, diet, e.g. DR *v.* AL or 2 %Y *v.* 15 %Y) and the two lifespan estimates connected with a straight line; this produces (actually forces) a linear reaction norm for lifespan as a function of diet. Strictly speaking, the procedure of connecting the two lifespan estimates with a straight line is only justified when we have continuous values on the *y*-axis (e.g. a continuum of yeast concentrations, i.e. not discrete environmental states); moreover, it implies that, with only two dietary levels, we are interpolating the values of lifespan for any concentrations/levels laying in-between the DR and AL diets; this may be acceptable in many cases but it is not always justified, especially when diet reaction norms are non-linear. Moreover, by only examining two diets we are restricting the inference we can make to those two specific diets[14,15].

A much better and complete design is to examine the lifespan response across a range of diet concentrations, typically yielding a nonlinear reaction norm with a single hump (peak) at the optimal diet that maximises lifespan or with a narrow plateau around the optimal concentration[14,15]. This is a much more informative approach since using only two diet levels might lead us to miss the lifespan maximum. Nowadays, quite a few studies, at least in *Drosophila*, have gone on to investigate lifespan across a more continuous range of concentrations of a single nutrient (e.g. a continuous range of different yeast concentrations) rather than looking at just two levels (e.g. DR *v.* AL levels of yeast)[14,15].

Experiments on the effects of dietary change on lifespan can also be extended to multiple diets or nutrients, for example by examining lifespan at different concentrations of both yeast and sugar in the fly food[14,15,30]. In this case, we are dealing with two environmental axes (e.g. x = sugar and z = yeast) and one phenotypic (y-) axis, with the lifespan estimates across all combinations of sugar and yeast levels defining a response (or reaction norm) surface (rather than the reaction norm being represented by a line). Such a multidimensional approach to investigating the effects of multiple nutrients has been called nutritional geometry, a framework developed by Simpson *et al.*[31–34]. Thus, the crux of this method is to analyse organismal responses (e.g. changes in lifespan, fecundity or physiological parameters) to dietary change by analysing a 'nutrient space', whose axes are defined by the different food components (e.g. levels of carbohydrates (C) *v.* amounts of protein (P) ingested) rather than just recording the response to changes in either carbohydrates or proteins. This allows us to examine, for instance, at which specific ratio of nutrients (e.g. C:P) lifespan is maximised[31–34]. Overall, such nutritional geometry studies suggest that lifespan is typically maximised by a specific balance of dietary components (e.g. P:C = 1:16 for lifespan in *Drosophila*)[31], not by restriction of energies themselves (suggesting that the term energetic restriction, as often used in the ageing field, might be misleading)[35]. This geometric framework can therefore clearly be viewed as representing a higher-dimensional extension of the reaction norm concept, even though the proponents and practitioners of nutritional geometry do not usually think of it in terms of plasticity or reaction norms.

The advantages of adopting a reaction norm perspective when analysing lifespan data in studies of DR (or similar instances of lifespan plasticity) are quite clear: it offers a simple and very effective way of visualising and interpreting the data. In particular, it helps us to identify those diets (or combinations of nutrients) that maximise or minimise lifespan, and it gives us a general idea of how sensitively lifespan reacts to changes in diet levels. When coupled with statistical analysis (e.g. with linear or non-linear statistical models, the details of

which are not discussed here), this approach gives us a powerful and robust tool for qualitatively and quantitatively analysing the effects of DR.

An example from the recent literature illustrates the importance of carefully considering the potential, plastic responses of lifespan across diet levels and of adopting a reaction norm perspective when studying lifespan. In 2004 a team of researchers found that overexpression of the transcription factor *foxo* downstream of insulin/insulin-like growth factor signalling extends *Drosophila* lifespan on a standard diet when the *foxo* transgene is constitutively activated in a fat layer situated above the brain (the so-called head fat body), but not when *foxo* is overexpressed in fat tissue in the thorax or abdomen[36]. Subsequently, another team which was independently working on the same question reported that in their experiments the opposite is true: overexpressing *foxo* in thoracic and abdominal fat body extended lifespan but overexpression in head fat body did not[37]. These contradictory findings were ultimately resolved when the first team decided to investigate both *foxo* transgenic genotypes (the head fat body construct *v.* the thoracic/abdominal fat body construct) across a continuum of yeast concentrations in the diet[38]. They found that, intriguingly, *foxo* overexpression in head fat body extends lifespan at 8 and 12 % yeast, whereas overexpression in thoracic and abdominal fat body extends lifespan only very slightly at 4, 8 and 12 % yeast but very strongly at a yeast concentration of 2 %[38]. Thus, the original findings of both research teams were correct and the discrepancies in their results simply due to the fact that they had performed their experiments on their respective standard diets which happened to differ in yeast content. Thus, the two *foxo* transgenic constructs differ significantly in their lifespan reaction norms across yeast levels, underscoring the importance of taking plasticity into account and adopting a reaction norm perspective when dealing with environmentally sensitive traits such as lifespan.

As this example already hints at, the most important point about adopting a reaction norm and $G \times E$ point of view is that we can employ it as a conceptual and quantitative tool for uncovering the genetic mechanisms underlying DR, namely by explicitly studying the nature of specific genotype × diet interactions.

Dietary restriction and 'genotype by environment' interactions: why reaction norms matter

Many studies dealing with the genetics of ageing, particularly those using *C. elegans* and *Drosophila*, are devoted to uncovering the mechanisms underlying DR, an important goal in molecular research on ageing[7,13–15]. Here I argue that the reaction norm framework is of particular importance when attempting to identify the genetic factors that underlie the DR response. The same point has already been discussed very clearly in two recent papers by Tatar[14,15], but to my mind it is worth reiterating these important arguments, especially given the fact that many molecular biologists who are working on ageing may not yet be familiar with the subtleties of the reaction norm framework. While I closely follow Tatar[14,15] in my discussion, I differ from him in some subtle points.

How can we identify candidate genes that are functionally required for a 'normal' DR response? This is usually done by comparing the lifespan of a mutant allele (or of a transgene misexpressing the candidate gene) to the wild-type allele of the candidate gene across two or more diet levels (in the simplest case just comparing DR *v.* AL, although this is clearly not an optimal design). What one hopes to find is that mutation or misexpression of a specific candidate gene reduces the DR-induced lifespan extension seen in the wild-type control; if so, one might have found evidence that the candidate gene is functionally somehow involved in, or maybe even required for, the DR response. More specifically, following Tatar, we might say that a candidate gene is involved in the DR response if its loss or reduction of function (or its misexpression) leads to a change of the slope of the reaction norm relative to the wild-type variant of the candidate gene[14,15]. Hence, what we are looking for is a significant candidate gene × diet interaction. But, as we shall see, if we want to claim that a candidate gene is functionally required for the DR response, this criterion may actually not be sufficiently specific.

Let us consider a few hypothetical examples of reaction norms for two genotypes at the candidate locus (wild-type *v.* mutation/misexpression) across a range of diet levels (from DR to AL). This is illustrated in Fig. 3, which represents a modified version of a figure by Tatar[14].

In Fig. 3(A), both reaction norms are parallel and exhibit a lifespan maximum on the DR diet; they have a negative slope with lifespan decreasing along the diet gradient towards the AL diet. In this scenario, both mutant and wild-type exhibit plasticity (non-zero slopes of the reaction norms), i.e. the typical DR response, but since the reaction norms are parallel there is no $G \times E$ interaction. The two genotypes do not differ in their DR response. The top reaction norm simply represents the genotype with higher lifespan across all diet levels; whether this reaction norm represents the wild-type or mutant does not matter here. What we would say in this case is that we are dealing with a normal DR response, that there is an additive genotypic difference in lifespan, but that the candidate gene in not functionally involved in the DR response.

In Fig. 3(B), the top reaction norm with zero slope represents the mutant, whereas the reaction norm with negative slope represents the wild-type. Both genotypes have maximal and identical lifespan on DR. In this case, the reaction norms are non-parallel and we have a significant $G \times E$ interaction. Although the wild-type shows the expected and typical DR response, the mutant does not. The zero slope of the mutant reaction norm implies that it shows no lifespan plasticity across the diet range at all. Since in the mutant the normal wild-type response is completely abolished, we might say that we have a candidate gene that is functionally

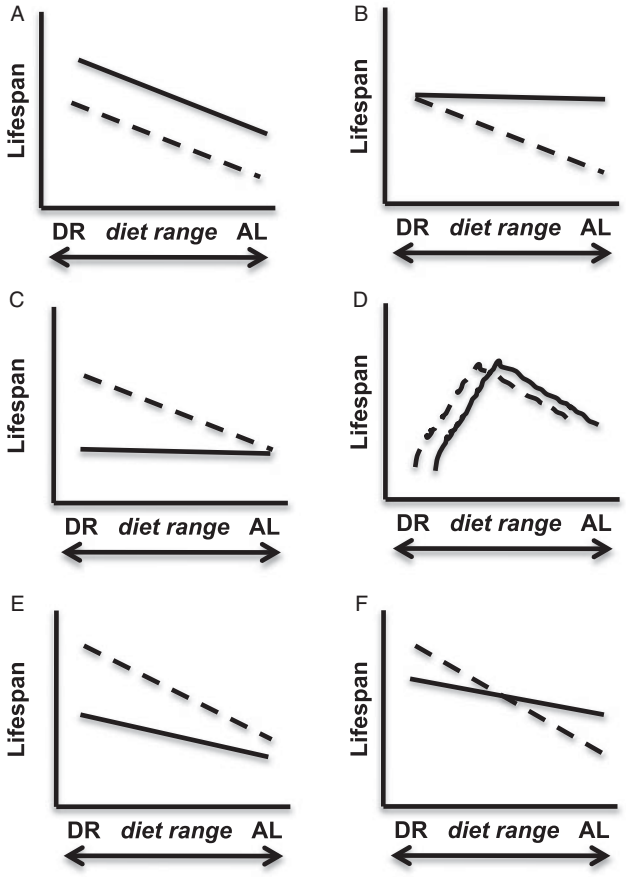

Fig. 3. Reaction norms and genotype × environment ($G \times E$) interactions for dietary restriction (DR). The figure represents a modified version of a figure by Marc Tatar[14]. Hypothetical DR reaction norms for a wild-type genotype (dashed line) and a mutant genotype (solid line) for a candidate gene; lifespan is shown on the *y*-axis, whereas the *x*-axis represents a continuous diet gradient (e.g. yeast concentration), ranging from dietary restriction (DR, e.g. a yeast level that is low but that does not cause malnutrition or starvation) to *ad libitum* (AL). Lifespan is maximised at the DR level of the diet. (A) Example of DR plasticity for both wild-type and mutant but no gene × diet interaction. (B), (C) Two extreme cases of gene × diet interaction in which the wild-type shows the normal DR response but the mutant reaction norm is completely flat, i.e. invariant lifespan across the whole range of diet levels. In (B) the mutant has constitutively high and in (C) constitutively low lifespan relative to wild-type. (D) Schematic example of non-linear, single-humped DR reaction norms similar to what has been observed in a study of the *Drosophila* gene *chico*. Here the mutant reaction norm (solid curve) seems to be simply shifted relative to wild-type but has otherwise identical shape and slope. (E), (F) Two clear cases of the specific kind of gene × diet interaction pattern we are looking for when attempting to identify a candidate gene that is functionally required for the normal DR response. In both cases, the mutant reaction norm shows an increase in lifespan from AL to DR but the slope is shallower than that of the wild-type reaction norm. On the DR diet, the wild-type exhibits a higher lifespan than the mutant. Thus, DR plasticity is clearly impaired in the mutant as compared with the normal wild-type response, indicating that the examined locus represents a solid candidate gene that is required for DR. For further details see text.

implied in the DR response. However, to my mind, this example, discussed by Tatar[14], represents a slightly odd scenario. What we expect to see under normal DR is an increase in lifespan from the AL diet to the DR diet (with even stronger food limitation decreasing lifespan again); hence, what we would expect to see if the candidate gene is functionally required for a normal DR response is that the mutant fails to increase lifespan from AL to DR relative to wild-type. In our example, however, the mutant is constitutively more long-lived that the wild-type across all diet levels. Thus, the mutant already has extended lifespan on AL diet, can therefore not possibly show an increase in lifespan from AL to DR, and seems to affect the entire response across all diets including AL and DR. Although we clearly have a $G \times E$ interaction in this case, it is difficult to interpret. It seems as if the constitutively long-lived mutant overrides the normal DR response; to my mind this pattern does not really imply that the candidate gene is functionally required for a normal DR response, even though it can be said to somehow modulate the DR response.

The situation is similar in Fig. 3(C). Again, the wild-type shows the normal DR response but the mutant exhibits a flat reaction norm with constitutively decreased lifespan across all diet levels. Again, the DR response is abolished in the mutant, and we have a significant $G \times E$ interaction. This scenario is more interesting because the mutant, just like the wild-type, has low lifespan on AL and clearly fails to increase lifespan from AL to DR, as expected if the gene is involved in DR plasticity. However, the pattern is pathological in the sense that the mutant is constitutively short-lived relative to wild-type across all diets. Again, we might say that the candidate gene is somehow involved in DR plasticity, but we cannot confidently claim that it is functionally required for the normal DR response as seen in the wild-type. In my opinion, the cases shown in Fig. 3(B) and (C) are rather extreme, somewhat ambiguous and thus functionally difficult to interpret.

In Fig. 3(D), we have a schematic representation of data from a real experiment using long-lived mutants of the gene encoding the insulin receptor substrate *chico*[14,15,39]. In the wild-type we see the typical non-linear, single-humped reaction norm usually seen in *Drosophila* DR experiments. The reaction norm of the *chico*1 mutant seems to have the same overall shape as that of the wild-type; it seems to be shifted in parallel in the *x*–*y* plane relative to that of the wild-type. Thus, as discussed by Tatar[14,15], the *chico*1 mutation shifts the entire dietary reaction norm across all diets but overall the two response curves are remarkably similar in shape and slope. In such cases, we might say that a parallel shift of the reaction norms implies that the genotypes differ in the dietary range across which they show the DR response, however, the responses of both genotypes are qualitatively identical. Hence, although the reaction norms intersect and we have a clear case of $G \times E$ interaction, I would say that this is not really what we are looking for. Genes that show such a behaviour can be said to modulate

the DR response[40], but we cannot say with confidence that such genes are functionally required for the normal DR response.

I would argue that if we want to claim that a candidate gene is functionally required or necessary for a normal DR response (rather than merely modulating the DR response) the following requirements should be met, as illustrated in Fig. 3(E) and (F).

(1) Both the wild-type and the mutant should exhibit lower lifespan (and higher fecundity) on the AL end of the dietary continuum as compared with the DR end of the nutritional range.
(2) When moving from right to left, i.e. from AL to DR, we should see an increase in lifespan towards a lifespan maximum in the DR region of the dietary continuum for both wild-type and mutant (the lifespan maxima may or may not coincide). Similarly, since DR is normally defined in terms of the restricted diet that maximises lifespan while simultaneously reducing fecundity, we expect to see a reduction of fecundity from AL to DR in both wild-type and mutant.
(3) Importantly, the rate of lifespan increase (and fecundity decrease) from AL towards DR should be more slow, i.e. the slope of the reaction norm should be more shallow, for the mutant as compared with wild-type. This implies that the mutant is impaired relative to wild-type in terms of increasing lifespan (and decreasing fecundity) in the range from AL to DR. Together with criterion 2 earlier, this excludes likely pathological and difficult-to-interpret cases for which the mutant reaction norm is completely flat. This is perhaps an overly strict requirement but in terms of causality and inference it might help us to exclude potentially confounded interpretations of the data. Moreover, even though perhaps not strictly necessary, we might want to add the further requirement that the wild-type should exhibit a higher lifespan on DR than the mutant, for if the mutant has a higher lifespan than the wild-type across a range of diet levels (including DR), then other 'longevity things' might be going on in the mutant that are superimposed on and/or unrelated to the DR process (see Fig. 3F for such a case).
(4) To the left of the DR region (not shown in the figure panels), i.e. to the left of the DR/lifespan maximum, we expect that lifespan and fecundity are reduced due to malnutrition and starvation in both wild-type and mutant, albeit this reduction might happen at different rates for the two genotypes.

These requirements ensure that we are dealing with a DR response that is Fig. 3(A) functionally impaired in the mutant relative to wild-type, yet Fig. 3(B) physiologically normal (albeit clearly genotypically different and distinct) and non-pathological for both wild-type and mutant. In my opinion, finding evidence for a gene × diet interaction is therefore insufficient for claiming that a candidate gene is functionally required for a normal DR response; instead, what we require is a special kind of gene by diet interaction as defined by criteria 1–4 earlier. To see whether such a specific candidate gene × diet interaction is present and statistically significant in the data, and to determine whether criteria 1–4 are fulfilled, we should do two things. First, we should make a reaction norm plot, as in Fig. 3. Second, we should analyse the mortality data with a statistical model which seek to explain variation in mortality (or age at death) as a function of two main effects (genotype and diet) and one interaction (genotype × diet). Such regression models for mortality data are called failure time models and include: (i) Cox regression (proportional hazards) models or (ii) accelerated failure time models[14–15].

As discussed by Tatar, several genes have been tested for their functional involvement in the DR response[14–15]. The majority of such studies use only two diets, AL *v.* DR, which is problematic since inferences based on only two diets are limited and the interpretation of the observed patterns may be confounded. This is especially true since dietary reaction norms may be non-linear across a continuous range of diets, the DR/lifespan maximum may differ between genotypes and lay outside the diet range examined, and/or the relevant $G \times E$ might be found beyond the range considered. Only few studies exist that have used an appropriate continuous range of diet levels. When carefully reviewing and reanalysing the best data available to date, as Tatar has done[14–15], and when considering these data from a proper reaction norm and $G \times E$ perspective, it becomes clear that there is practically no evidence to date for any gene to be strictly functionally required for a normal DR response. Practically all published examples essentially involve remarkably (even though not perfectly) parallel shifts of the wild-type and mutant reaction norms in the *x*–*y* plane, with very little evidence for strong and clear-cut $G \times E$ and even less evidence for the specific kind of genotype × diet interaction we have defined earlier[14–15]. While in some of these cases we might say that the candidate gene modulates the DR response, practically none of the published studies provide convincing prima facie evidence for a functional requirement of a candidate gene in the DR process[14–15,40]. This even holds for genes involved in the insulin/insulin-like growth factor signalling and target of rapamycin signalling pathways, which, because of their involvement in nutrient signalling, energy metabolism and lifespan regulation, have been hypothesised to be major candidates for 'DR genes'[7,41].

In conclusion, what we need in future studies of DR genetics are well-replicated experiments which use an appropriate, continuous range of diets (rather than simply comparing DR *v.* AL) and which employ a proper, statistically sound reaction norm and $G \times E$ approach[14,15], as it is often used in evolutionary biology and statistical epidemiology. Such a framework can not only be applied to single candidate genes (i.e. mutant *v.* wild-type), but could also be used for systematically screening entire collections of mutants or transgenes (e.g. RNAi constructs) in order to discover the genetic factors that are functionally required for the process of DR. The prospects for such an approach are good, I think: preliminary data

based on a set of forty-one mouse recombinant inbred lines suggest that large panels of genotypes or lines might indeed harbour substantial genetic variation for the DR response[42]. The method we have described earlier for detecting genotype × diet interactions can in principle also be extended beyond one dietary axis or dimension. Similar criteria to the ones we have defined can be readily formulated for nutrient space-like response surfaces, for instance in terms of surface curvature, etc. Although the nutritional geometry method has been successfully used to study DR in several species including *Drosophila*[31–34], it has not yet been applied to the analysis of genotype × diet interactions. Thus, combining nutritional geometry with genetic manipulations might hold great promise for identifying genes that are functionally required for DR. In a similar vein, the reaction norm and $G \times E$ framework outlined here could also be used to determine whether and how genotypes interact with diet to influence gene expression (e.g. using whole-transcriptome RNA sequencing)[43,44] or key metabolites (e.g. using metabolomics). Such future studies will greatly improve our understanding of the mechanisms underlying DR.

What are the implications for dietary restriction in human subjects?

As far as we know today, DR likely represents the most universal method of extending lifespan and might also have beneficial effects in human subjects[45]. Indeed, although in human subjects no data on the impact of DR on mortality are available, a few studies have examined how DR affects human health and age-related diseases, and some of the preliminary results, for example from the CALERIE study (http://calerie.dcri.duke.edu/), are encouraging. Similarly, data from two major studies in non-human primates (Rhesus macaques) are tentatively promising[45–47]: DR reduces body weight, fat mass, and the concentration of TAG; improves insulin sensitivity; and delays diabetes, CVD, and cancer. However, there were also major differences in the results of these studies, including the fact that one found that DR reduces mortality, whereas the other did not[46,47]. Interestingly, a recent report[48] now suggests that in the National Institute on Aging study[47] control animals might in fact have been undergoing DR which would explain why DR did not have a greater impact in that study. Some of these discrepancies might stem from differences in experimental design, including differences in food composition and the genetic origin of the animals. Given the importance of genotype × diet interactions in modulating the effects of DR, as for example seen in rodents[42,49,50], it will be of major importance to control and/or quantify such interaction effects in future studies of DR in non-human primates and human subjects.

Acknowledgements

I thank Professor John Mathers (Newcastle University), the organiser of the 2013 Nutrition Society Summer Meeting on 'Nutrition and Healthy Ageing', for kindly having invited me to speak and to write the present paper. I also thank Professor Tom Kirkwood (Newcastle University) for discussion and support and two reviewers for helpful suggestions on a previous version of the present paper. My research was funded by a grant from the Swiss National Science Foundation (SNF Professorship Grant no. PP00P3_133641).

Financial Support

Source of Funding: Swiss National Science Foundation, Grant PP00P3_133641 to T. F. The Swiss National Science Foundation had no role in the design, analysis or writing of the present paper.

Conflicts of Interest

None.

Authorship

T. F. conceived and wrote the paper.

References

1. Finch CE (1990) *Longevity, Senescence, and the Genome*. Chicago and London: The University of Chicago Press.
2. Rose MR (1991) *Evolutionary Biology of Aging*. New York: Oxford University Press.
3. Stearns SC (1992) *The Evolution of Life Histories*. Oxford: Oxford University Press.
4. Fabian D & Flatt T (2011) The evolution of aging. *Nature Edu Knowl* **3**, 9.
5. Fabian D & Flatt T (2012) Life history evolution. *Nature Edu Knowl* **3**, 24.
6. Flatt T & Schmidt PS (2009) Integrating evolutionary and molecular genetics of aging. *Biochim Biophys Acta* **1790**, 951–962.
7. Kenyon CJ (2010) The genetics of ageing. *Nature* **464**, 504–512.
8. Flatt T, Amdam GV, Kirkwood TBL *et al.* (2013) Life-history evolution and phe Polyphenic regulation of somatic maintenance and survival. *Q Rev Biol* **88**, 185–218.
9. Mair W, Goymer P, Pletcher SD *et al.* (2003) Demography of dietary restriction and death in *Drosophila*. *Science* **301**, 1731–1733.
10. Maynard Smith J (1958) The effects of temperature and of egg-laying on the longevity of *Drosophila subobscura*. *J Exp Biol* **35**, 832–842.
11. Roff DA (1997) *Evolutionary Quantitative Genetics*. New York: Chapman and Hall.
12. Stearns SC (1989) The evolutionary significance of phenotypic plasticity. *BioScience* **39**, 436–445.
13. Mair W & Dillin A (2008) Aging and survival: the genetics of life span extension by dietary restriction. *Annu Rev Biochem* **77**, 727–754.
14. Tatar M (2007) Diet restriction in *Drosophila melanogaster*. Design and analysis. *Interdiscip Top Gerontol* **35**, 115–136.

15. Tatar M (2011) The plate half-full: Status of research on the mechanisms of dietary restriction in *Drosophila melanogaster*. *Exp Gerontol* **46**, 363–368.
16. Tatar M & Yin CM (2001) Slow aging during insect reproductive diapause: why butterflies, grasshoppers and flies are like worms. *Exp Gerontol* **36**, 723–738.
17. Keller L & Genoud M (1997) Extraordinary lifespans in ants: a test of evolutionary theories of ageing. *Nature* **389**, 958–960.
18. Keller L & Jemielity S (2006) Social insects as a model to study the molecular basis of ageing. *Exp Gerontol* **41**, 553–556.
19. Braendle C, Milloz J & Felix MA (2008) Mechanisms and evolution of environmental responses in *Caenorhabditis elegans*. *Curr Top Dev Biol* **80**, 171–207.
20. Felix MA & Braendle C (2010) The natural history of *Caenorhabditis elegans*. *Curr Biol* **20**, R965–R969.
21. Gerisch B & Antebi A (2011) Molecular basis of life history regulation in *C. elegans* and other organisms. In *Mechanisms of Life History Evolution*, pp. 284–298 [T Flatt and A Heyland, editors]. Oxford: Oxford University Press.
22. Brakefield PM & Zwaan BJ (2011) Seasonal polyphenisms and environmentally induced plasticity in the Lepidoptera: the coordinated evolution of many traits at many levels. In *Mechanisms of Life History Evolution*, pp. 243–252 [T Flatt and A Heyland, editors]. Oxford: Oxford University Press.
23. Beldade P, Mateus ARA & Keller RA (2011) Evolution and molecular mechanisms of adaptive developmental plasticity. *Mol Ecol* **20**, 1347–1363.
24. Nylin S & Gotthard K (1998) Plasticity in life-history traits. *Annu Rev Entomol* **43**, 63–83.
25. Kirkwood TBL (1977) Evolution of ageing. *Nature* **270**, 301–304.
26. Shanley DP & Kirkwood TBL (2000) Calorie restriction and aging: a life-history analysis. *Evolution* **54**, 740–750.
27. McGrath PT, Xu Y, Ailion M *et al*. (2011) Parallel evolution of domesticated *Caenorhabditis* species targets pheromone receptor genes. *Nature* **477**, 321–325.
28. Schmidt PS & Conde DR (2006) Environmental heterogeneity and the maintenance of genetic variation for reproductive diapause in *Drosophila melanogaster*. *Evolution* **60**, 1602–1611.
29. Kivelä SM, Välimäki P & Gotthard K (2013) Seasonality maintains alternative life-history phenotypes. *Evolution* **67**, 3145–3160.
30. Skorupa DA, Dervisefendic A, Zwiener J *et al*. (2008) Dietary composition specifies consumption, obesity, and lifespan in *Drosophila melanogaster*. *Aging Cell* **7**, 478–490.
31. Lee KP, Simpson SJ, Clissold FJ *et al*. (2008) Lifespan and reproduction in *Drosophila*: new insights from nutritional geometry. *Proc Natl Acad Sci USA* **105**, 2498–2503.
32. Simpson SJ & Raubenheimer D (2007) Caloric restriction and aging revisited: the need for a geometric analysis of the nutritional bases of aging. *J Gerontol A* **62**, 707–713.
33. Simpson SJ & Raubenheimer D (2009) Macronutrient balance and lifespan. *Aging (Albany NY)* **1**, 875–880.
34. Piper MDW, Partridge L, Raubenheimer D *et al*. (2011) Dietary restriction and aging: a unifying perspective. *Cell Metab* **14**, 154–160.
35. Mair W, Piper MDW & Partridge L (2005) Calories do not explain extension of life span by dietary restriction in *Drosophila*. *PLoS Biol* **3**, e223.
36. Hwangbo DS, Gersham B, Tu MP *et al*. (2004) Drosophila dFOXO controls lifespan and regulates insulin signalling in brain and fat body. *Nature* **429**, 562–566.
37. Giannakou ME, Goss M, Junger MA *et al*. (2004) Long-lived *Drosophila* with overexpressed dFOXO in adult fat body. *Science* **305**, 361.
38. Min KJ, Yamamoto R, Buch S *et al*. (2008) Drosophila lifespan control by dietary restriction independent of insulin-like signaling. *Aging Cell* **7**, 199–206.
39. Clancy DJ, Gems D, Hafen E *et al*. (2002) Dietary restriction in long-lived dwarf flies. *Science* **296**, 319.
40. Giannakou ME, Goss M & Partridge L (2008) Role of dFOXO in lifespan extension by dietary restriction in *Drosophila melanogaster*: not required, but its activity modulates the response. *Aging Cell* **7**, 187–198.
41. Partridge L, Alic N, Bjedov I *et al*. (2011) Ageing in *Drosophila*: the role of the insulin/Igf and TOR signalling network. *Exp Gerontol* **46**, 376–381.
42. Liao CY, Rikke BA, Johnson TE *et al*. (2010) Genetic variation in the murine lifespan response to dietary restriction: from life extension to life shortening. *Aging Cell* **9**, 92–95.
43. Pletcher SD, Macdonald SJ, Marguerie R *et al*. (2002) Genome-wide transcript profiles in aging and calorically restricted *Drosophila melanogaster*. *Curr Biol* **12**, 721–723.
44. Zhou S, Campbell TG, Stone EA *et al*. (2012) Phenotypic plasticity of the *Drosophila* transcriptome. *PLoS Genet* **8**, e1002593.
45. Anderson RM & Weindruch R (2012) The caloric restriction paradigm: implications for healthy human aging. *Am J Hum Biol* **24**, 101–106.
46. Colman RJ, Anderson RM, Johnson SC *et al*. (2009) Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science* **325**, 201–204.
47. Mattison JA, Roth GS, Beasley TM *et al*. (2012) Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. *Nature* **489**, 318–321.
48. Colman RJ, Beasley TM, Kemnitz JW *et al*. (2014) Caloric restriction reduces age-related and all-cause mortality in rhesus monkeys. *Nat Commun* **5**:3557.
49. Gelegen C, Collier DA, Campbell IC *et al*. (2006) Behavioral, physiological, and molecular differences in response to dietary restriction in three inbred mouse strains. *Am J Physiol Endocrinol Metab* **291**, E574–581.
50. Liao CY, Johnson TE & Nelson JF (2013) Genetic variation in responses to dietary restriction – an unbiased tool for hypothesis testing. *Exp Gerontol* **48**, 1025–1029.