Chapter 37

The Potential of Comparative Biology to Reveal Mechanisms of Aging in Rotifers

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INTRODUCTION

The considerable progress over the last 20 years in our understanding of the biology of aging has largely been achieved through elucidation of genetic pathways affected by single mutations that drastically extend life span. What these mutations and the genetic pathways they modify reveal about the aging process is less clear. In particular, there may not be a straightforward connection between extending life span and increasing health span, the period of relative stability that is followed by an accelerated age-associated decline in function. Many mutations that extend life span do so by extending the postreproductive period rather than the reproductive period, or do not change the ratio of the two, and the postreproductive period is generally associated with decreased health (Van Voorhies et al., 2006; Bansal et al., 2015). Most life span-extending mutations also have a negative effect on fecundity, as shown in a variety of insects (Lee et al., 2008; Maklakov et al., 2008; Le Rohellec and Le Bourg, 2009; Carey et al., 2008), *Caenorhabditis elegans* (Chen et al., 2008; Jenkins et al., 2004), and mice (Solon-Biet et al., 2015). While many studies of mutant strains show a correlation between life span and health span metrics under some conditions (Kenyon, 2005; Goddeeris et al., 2003; Wessells et al., 2004; Jones et al., 2009; Martin and Grotewiel, 2006), others show mixed results or no correlation (Cook-Wiens and Grotewiel, 2002; Herndon et al., 2002; Kerr et al., 2011; Martin et al., 2009; Broughton et al., 2005), or a negative correlation (Bansal et al., 2015; Bhandari et al., 2007; Tatar et al., 2001). This suggestion that health span and life span are uncoupled at the genetic level is supported by studies of genetic variance (Van Voorhies et al., 2006; Burger et al., 2007; Burger and Promislow, 2006; Ismail et al., 2015).

Uncoupling changes in life span from changes in other aspects of aging will be necessary to understand and optimize interventions that maximize different functions of health span. As illustrated in Fig. 37.1, aging functions have different times of onset and rates of decline. Each is modulated by a balance of stimulatory and inhibitory signals tuned by evolution. A limitation of traditional model systems used in aging studies is that exploring the phenotype of life span extension through mutation screens in single, laboratory-adapted isogenic strains provides only a limited understanding of how genetic systems in adapted optima behave (Austad, 2009; Nussey et al., 2012; Jones et al., 2014). An alternative strategy is to examine natural variants that differ in their responses to aging interventions. Comparing natural variation in phenotypes and genotypes between well-adapted products of evolution, rather than single or a small number of mutations in components of specific pathways, allows dissection of the overlap, distinction, and trade-offs between genetic systems involved in life span and those involved in health span. Rotifers, which have recently reemerged as a valuable tool in aging studies, present an attractive system for this approach (Snell et al., 2015).

Rotifers are microscopic, aquatic, basally branching triploblast animals similar in complexity to nematodes. Unlike nematodes and arthropods, rotifers undergo direct development without larval stages. Monogononts, the largest group of rotifers, are facultatively sexual, generally reproducing asexually with a diploid female producing diploid eggs by mitosis. These eggs hatch into asexual (amictic) females, giving rise to a clonal population. In response to a quorum-sensing mechanism (Snell et al., 2006; Stelzer and Snell, 2003, 2006), sexual (mictic) females are produced that generate haploid eggs through meiosis. If unfertilized, these haploid eggs hatch into males that can fertilize other haploid gametes to produce diploid, diapausing (resting) eggs. The best-studied monogonont rotifers are those of the *Brachionus plicatilis* Mueller, 1786 species complex. Because of their biology, *Brachionus* rotifers have a number of advantages as an animal system for the study of aging (Austad, 2009; Snell et al., 2015; Snell, 2014). Their eutelic development, with little or no cell division after birth (Wallace and Snell, 2001), virtually eliminates telomere attrition and stem cell exhaustion as explanations of aging in this system, allowing focus on nutrient and environmental sensing, intercellular communication, and epigenetic phenomena. Direct development without a larval stage facilitates the study of interventions on life span and relevant function of

Conn's Handbook of Models for Human Aging. https://doi.org/10.1016/B978-0-12-811353-0.00037-3

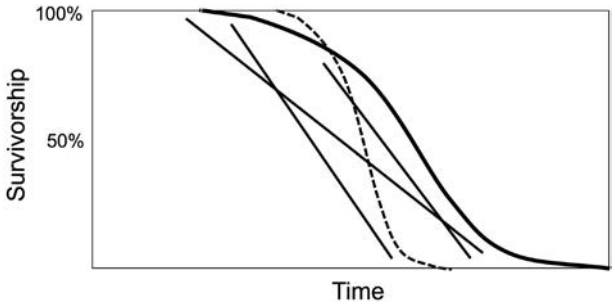

FIGURE 37.1 Model of life span and other functions of the aging process. The *dark curve* represents relative mortality (life span) while the *dashed curve* represents the transition from reproductive to postreproductive periods. *Straight lines* represent the decline of different health span functions, which may be linked or independent. Some individuals die before reaching the postreproductive period (which is suboptimal), and the onset of decline of different health span functions may occur before or after reproductive senescence, at different rates.

health span. Clonal reproduction preserves allelic relationships across generations without relying on inbreeding, greatly simplifies examination of epigenetic inheritance, and obviates consideration of the cost of mating on health span (Fowler and Partridge, 1989; Chapman et al., 1995; Wigby and Chapman, 2005; Papanastasiou et al., 2013). Females have a life span of 2–3 weeks, with clearly defined pre- and postreproductive periods, and display distinct age-specific changes in fecundity, morphology, and swimming speed that relate health span to life span. They can be cultured in liquid media with a variety of algae that mimic their natural diet, avoiding many of the problems of using artificial diets in aging studies (Cerqueira and Kowaltowski, 2010).

The history of rotifers as models for the biology of aging and recent advances in interventions that extend life span in rotifers are thoroughly reviewed in Chapter 36. Here I focus on the potential of comparative biology within clones, within species, and within the *B. plicatilis* species group as specific strengths of the model.

COMPARATIVE BIOLOGY OF AGING WITHIN A GENETIC CLONE

The reproductive cycle of *Brachionus* provides an unusual opportunity to study phenotypic difference in aging and the response to aging interventions within a single genotype differing only as a result of epigenetic or other maternal effects. As described in Fig. 36.1, reproduction in the *Brachionus* life cycle is dominated by an asexual cycle of amictic (asexual) females reproducing by mitotic division of oocytes to generate amictic eggs that hatch as amictic females (AE-amictic females). In response to a mixis induction cue a fraction of amictic eggs hatch as mictic females (AE-mictic females) that produce gametes by meiosis. Unfertilized gametes develop into haploid males and fertilized gametes become resting eggs that hatch as amictic females (RE-amictic females), reinstating the asexual reproductive cycle.

As shown in Fig. 37.2, in *Brachionus manjavacas* RUS the three types of females have similar minimum, mean, and median life spans under standard laboratory conditions with ad libitum feeding (mictic females have a relatively shorter prereproductive period and longer postreproductive period than amictic females). However, they respond differently to two standard aging interventions: chronic caloric restriction (CCR) and intermittent fasting (IF) (Gribble and Mark Welch, 2013). When exposed to IF (alternating food supply at ad libitum or zero every 24 h), all three types respond with an increase in median life span of ~50%. The proportion of life span spent in the reproductive period, as a proxy for health span, does not change in mictic females but increases significantly in amictic females. The increase in reproductive period is accompanied by a nearly 30% decrease in fecundity in amictic females hatched from amictic eggs (AE-amictic females), consistent with a classic trade-off between investing in somatic maintenance versus reproduction. However, amictic females hatched from resting eggs (RE-amictic females) experience a 25% increase in fecundity. This suggests that the evolutionarily optimal conditions for *B. manjavacas* RUS females hatching from resting eggs do not include a continuous ad libitum food supply. Deciphering the differences in resource allocation between these two genetically identical types of female will lead to a more sophisticated understanding of strengths and limits of the disposable soma theory of aging (Kirkwood, 1977; Holliday, 1989).

The three types of female respond very differently when food availability is reduced to 10% of ad libitum levels (Gribble and Mark Welch, 2013). CCR has little effect on mictic females hatched from amictic eggs (AE-mictic females) beyond extending the postreproductive life span of a small number of individuals. The same food reduction extends median life span by 25% in AE-amictic females. This is largely due to a decrease in mortality at early ages shifting the survival curve to the right, and the primary effect is a significant increase in postreproductive life span rather than an increase in health span. In contrast, CCR extends median life span by 50% in females hatched from resting eggs (RE-amictic) by decreasing the hazard rate across most ages, which results in an increase in both reproductive and postreproductive life span. The different responses of the three reproductive modes are consistent with their different ecological roles, suggesting that each response has been driven by natural selection to maximize reproductive output. The fact that the three types of females are genetically identical (or extremely isogenic in the case of inbred RE-amictic females) indicates that the biological differences

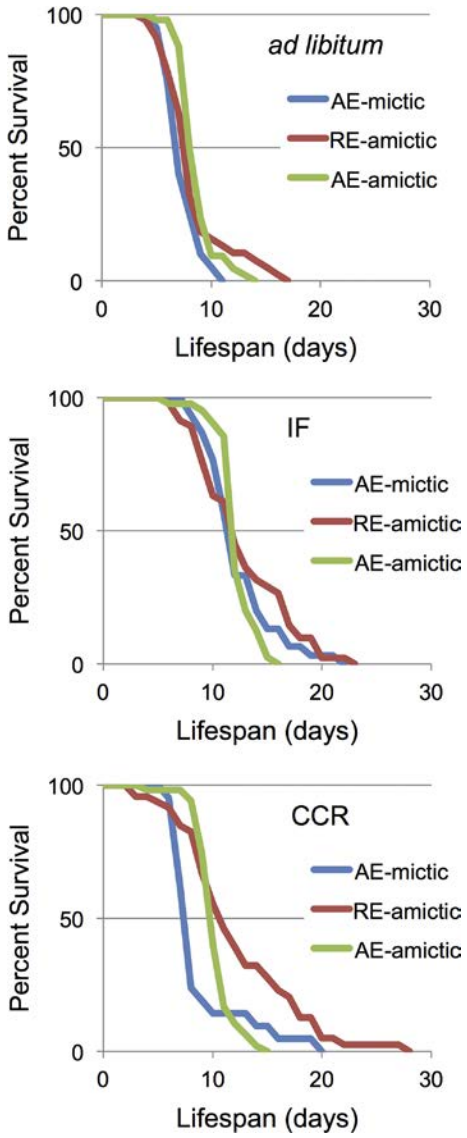

FIGURE 37.2 Kaplan–Meir survival curves for three types of *Brachionus manjavacas* RUS females under ad libitum (top), intermittent fasting (IF) (middle), and chronic caloric restriction (CCR) (bottom) conditions. *(Data from Gribble, K.E., Mark Welch, D.B., 2013. Life-Span extension by caloric restriction is determined by type and level of food reduction and by reproductive mode in* Brachionus manjavacas *(Rotifera). J Gerontol A Biol Sci Med Sci 68 (4), 349–358.)*

between them must be due to epigenetic or other maternal effects. Examination of differences in chromatin modification, maternal contributions leading to transcription cascades, as well as biochemical and physiological differences between the three types would reveal how life span and health span can be differentially modified through identical interventions in the same genetic background.

COMPARATIVE BIOLOGY OF AGING WITHIN AND BETWEEN SPECIES

A unique strength of the *Brachionus* system for aging studies is the large amount of work that has gone into understanding the biology of the species complex. The eponymous species, *B. plicatilis*, was described in 1786 and has been actively studied for more than 100 years. Long considered a cosmopolitan generalist of coastal marine and athalassohaline inland environments, the past 40 years have seen the accumulation of evidence supporting the concept that *B. plicatilis* is a complex of species. In 1995, Segers used morphometric (Fu et al., 1991a), genetic (Fu et al., 1991b), cytological (Rumengan et al., 1991), and behavioral (Snell and Hawkinson, 1983; Gómez and Serra, 1995) data accumulated over 20 years to define the "L" and "SS"

morphotypes—long used informally—as *B. plicatilis sensu stricto* and *Brachionus rotundiformis* Tschugunoff, 1921, respectively. This initiated the goal of formally defining the species that make up the complex. Six year later, Ciros-Perez et al. (2001a) defined the "SM" clade and described *Brachionus ibericus*. More recently genetic and phenotypic data described *B. manjavacas* (Fontaneto et al., 2007) and *Brachionus koreanus* (Hwang et al., 2013), and identified the "L" type "Austria" strain as *Brachionus asplanchnoidis* Charin, 1947 (Michaloudi et al., 2016).

Genetic data indicate that the *B. plicatilis* species complex is comprised of at least nine additional genetically distinguishable species that are as yet not formally named (Fig. 37.3) (Mills et al., 2016). Observed nucleotide polymorphism at the noncoding ITS region ranges from 0.3% to 1.9% within species and 3% to 10% within clades (Mills et al., 2016). Nonsynonymous polymorphism in protein coding genes is much lower, in the range of 2%–5% across the complex

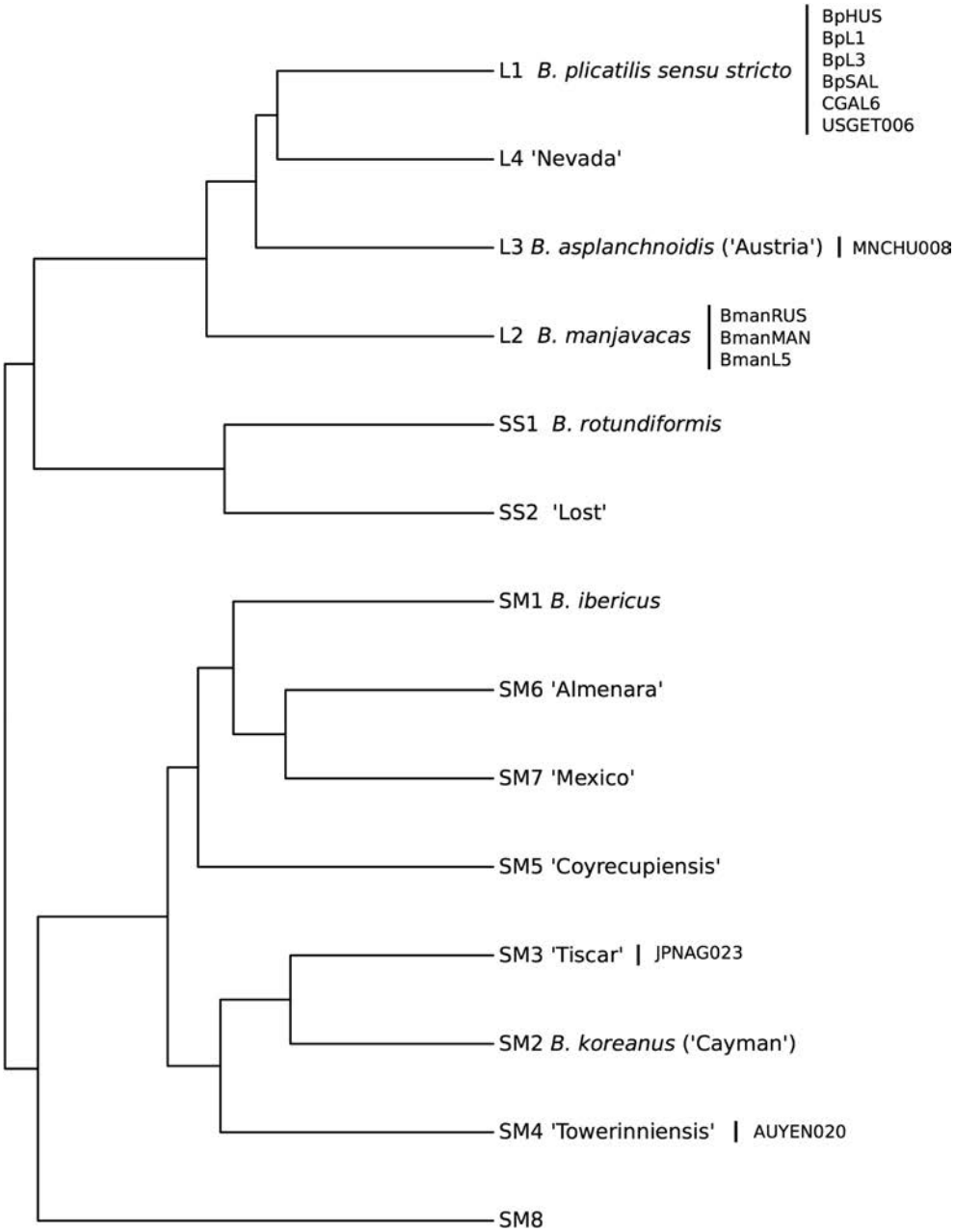

FIGURE 37.3 Phylogenetic relationship among 14 species in the *Brachionus plicatilis* species complex, based on RAxML analysis of combined COI and ITS1 data. Species are labeled as L ("large"), Small-Medium (SM), and S ("small") with clade numbers after Mills et al. (2016) with official names in italics when they exist and unofficial names in single quotes when they exist. The affiliation of strains described in Figs. 37.4 and 37.5 are shown for reference.

(Gribble et al., 2011). Species have adapted to particular environmental conditions such as nutrient availability and type, salinity, and temperature (Gómez and Serra, 1995; Gómez et al., 1997; Ciros-Perez et al., 2001b; Serra et al., 1998; Mills et al., 2007). Their widespread distribution and ease of culturing make this group a useful model system for studies of population dynamics, adaptation, speciation, the evolution of sexual reproduction, and other evolutionary processes (Stelzer and Snell, 2003; Gómez et al., 2002; Yoshinaga et al., 2003; Gomez, 2005; Papakostas et al., 2005; Suatoni et al., 2006; Snell et al., 2009; Campillo et al., 2009; Alcántara-Rodríguez et al., 2012; Stelzer et al., 2011; Tang et al., 2012).

Natural variation in the species complex, both between and within species, offers an opportunity to understand the evolution of aging and of responses to interventions that modulate life span and health span. Under ad libitum conditions across examined species, mean life span varies by a factor of more than 2.5, from 4.5 (sd 0.2) to 11.8 (sd 0.4) days, and the postreproductive portion of life span ranges over an order of magnitude, from 3.1% to 35% (Fig. 37.4). Within the six strains of *B. plicatilis sensu stricto* that have been reared under identical conditions, total life span varies by 30% and the postreproductive portion of life span ranges from 20% to 33% (Gribble et al., 2014). Responses to caloric restriction regimens reveal additional differences between and within species. CCR and IF each significantly extends life span in some but not all strains; IF significantly reduces life span in some other strains. Among the three strains of *B. manjavacas* tested, one responds to CCR but not IF with increased mean life span, one responds to IF but not CCR, and one responds to both interventions. There is similar variation between strains in the effect of CCR and IF on the absolute and proportional length of the reproductive period (Gribble et al., 2014).

The two caloric restriction regimes also reveal differences between strains in the relationship between life span and fecundity (Gribble et al., 2014). Fig. 37.5 shows the fold change in life span against the fold change in fecundity between intervention and control in Cartesian coordinates, such that quadrant I represents an increase in both life span and fecundity under treatment conditions and quadrant IV represents the classic trade-off between increased life span and decreased fecundity. Under CCR conditions, most strains increase life span with little or no change in fecundity, while some increase both life span and fecundity. In contrast, under IF conditions most strains decrease both life span and fecundity or show the classic trade-off between increased life span and decreased fecundity. Among those that show a trade-off under IF conditions, there is an increase in mortality during the reproductive period compared to ad libitum or CCR-fed individuals.

There is no clear correlation between changes in health span, life span, and fecundity under CCR and IF that would unite the responses of different strains of the *B. plicatilis* species complex. This is consistent with the phenotype of each strain being the product of a unique evolutionary trajectory, whether in response to selection or genetic drift. That such significant differences can evolve over relatively short evolutionary timescales suggests that minor genetic differences can play a major role in modulating health span and life span. Lineages within the species complex are also capable of rapid changes in genome size, which could have dramatic effects on gene dosage, general physiology, and fitness, and lead to accelerated evolution (Stelzer et al., 2011; Stelzer, 2010; Riss et al., 2016). Studies ongoing in several laboratories of the genomic and transcriptomic differences among *Brachionus* strains will help elucidate differences between strains and suggest new avenues of research. Another powerful approach will be crossing strains within species, which are generally capable of producing hybrid offspring (Mills et al., 2016; Suatoni et al., 2006; Riss et al., 2016), and observing the response of F1 hybrids, which can be maintained indefinitely as clonal lines.

SUMMARY

Variability within a species in the response to interventions such as caloric restriction is often considered a confounding variable in attempts to understand the biology of aging (Swindell, 2012; Nakagawa et al., 2012; Harper et al., 2006; Liao et al., 2010). Differences among strains within the *B. plicatilis* species complex contribute to the evidence of intraspecies variation in intervention responses and emphasize the danger of using a single isolate to draw conclusions about how a species responds to interventions. However, these differences can also be a powerful tool to understand subtle genetic and epigenetic controls over life span and health span. Even absent genetic dissection, the varied responses in life span, health span, and fecundity to CCR and IF in the *B. plicatilis* species complex support other evidence that these two common types of caloric restriction have different mechanistic bases (Greer and Brunet, 2009; Anson et al., 2005; Dogan et al., 2011). Variation in the relationship of life span and fecundity between CCR and IF interventions suggests that the relative balance between reproduction and somatic maintenance is under selection in natural populations (Kirkwood, 1977; Holliday, 1989). This could be tested by crosses between strains of a species or through selection experiments, which can be conducted rapidly and easily in *Brachionus* (Smith and Snell, 2012).

While the results discussed above suggest that total fecundity and the relative length of the postreproductive period can be uncoupled from life span, these demographic metrics are only one function of aging that can be measured in *Brachionus*. Other measures of health span include physiological metrics such as grazing rate, swimming speed, phototropism, body

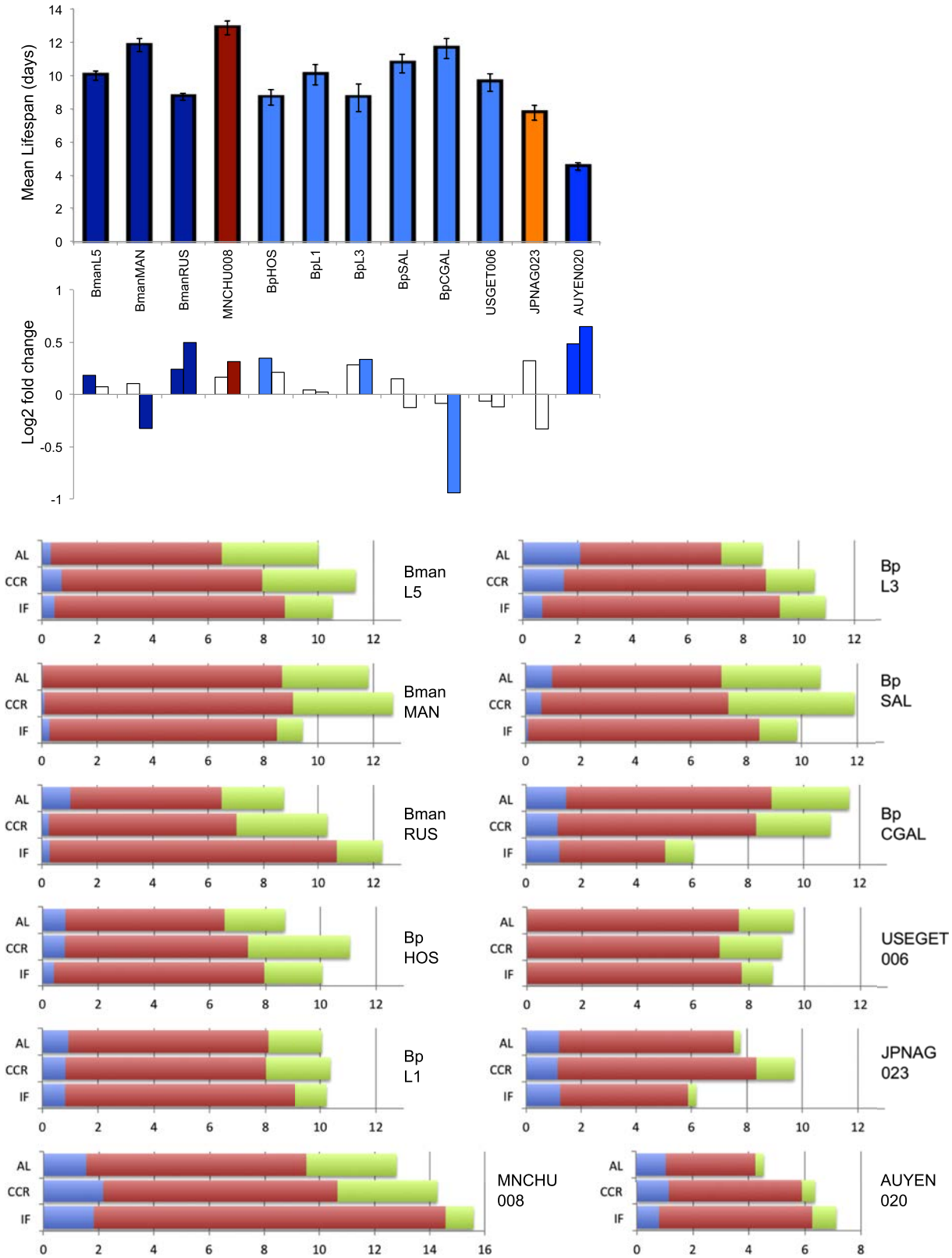

FIGURE 37.4 Differences in life span within the *Brachionus plicatilis* species complex. Top: Mean life span with standard deviation for 12 isolates indicated in Fig. 37.3; colors unite species. Middle: $\log_2$ fold change between intervention and ad libitum conditions for chronic caloric restriction (CCR) (first bar) and intermittent fasting (IF) (second bar); *solid bars* indicate significant difference from ad libitum conditions at $P < .05$ by Student's t-test. Bottom: Mean number of days (x axis) spent in prereproductive, reproductive, or postreproductive stage for indicated strain under ad libitum (AL), CCR, or IF conditions. *(Data from Gribble, K.E., Kaido, O., Jarvis, G., Mark Welch, D.B., 2014. Patterns of intraspecific variability in the response to caloric restriction. Exp Gerontol 51, 28–37.)*

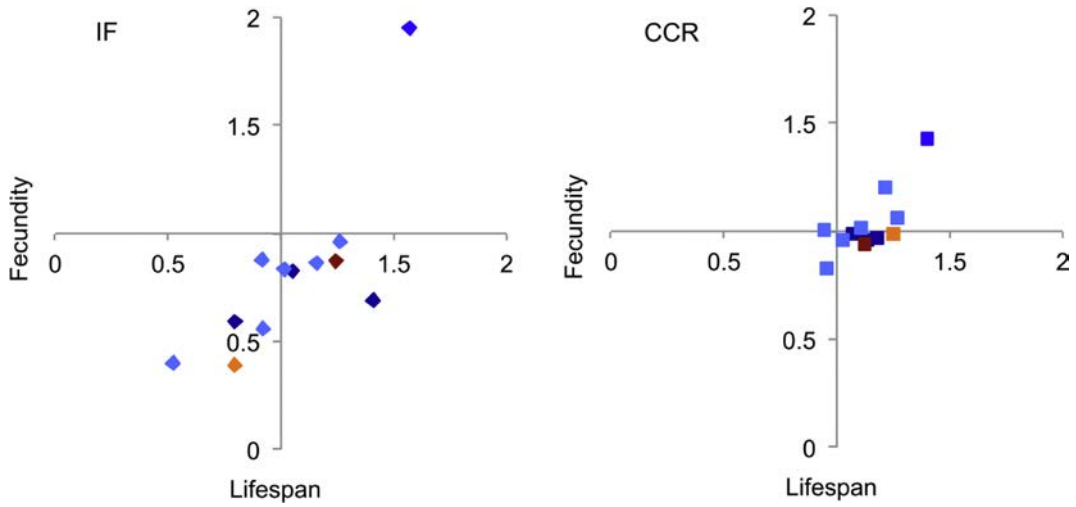

FIGURE 37.5 Relationship between change in mean life span (x axis) and mean total fecundity (y axis) between intermittent fasting (IF) (left) or chronic caloric restriction (CCR) (right) and ad libitum conditions. Colors denote species following Fig. 37.4.

size, and resistance to external stressors, and cellular metrics such as mitochondrial and lysosome activity, redox maintenance, and the accumulation of lipid, protein, and DNA damage. Because of the tractability of the *Brachionus* system, the effect(s) of interventions of interest—including those that have already been shown to extend life span in at least one *Brachionus* strain (Chapter 36)—on any of these health span functions can be measured across multiple strains relatively quickly and inexpensively. Strains displaying phenotypic differences of informative magnitude can be investigated in greater detail at the biochemical, genomic, or transcriptomic level. Comparing the response of well-tuned genetic systems to interventions that modulate aging, without the confounding disruption caused by mutation, can be a powerful tool to identify genetic pathways modulating health span and lead to a great understanding of the biology of aging.

ACKNOWLEDGMENTS

I thank the National Institute on Aging and the G. Unger Vetlesen Foundation for support and Kristin Gribble, our coauthors, and collaborators for helpful conversation.

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