doi:10.1111/evo.14442

From microbes to mammals: The experimental evolution of aging and longevity across species

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Received July 16, 2021

Accepted December 1, 2021

Senescence, the functional deterioration of cells or organisms associated with increased age, is pervasive across the tree of life. Yet our understanding of the genetic and physiological basis underlying age-related declines in health and reproduction remains limited. Experimental evolution allows empirical examination of the question of why aging occurs; imposing selection for age-specific fitness traits shifts patterns of aging in experimental populations, enabling investigations of the variation underlying senescence and the mechanisms governing it. Whole-genome sequencing of experimentally evolved populations may reveal candidate genomic variants underlying particular aging patterns; unfortunately, most study systems suffer from limitations that weaken associations between genotypes and phenotypes. In this review, we provide a survey of experimental evolution studies that have altered population-level patterns of reproductive timing and senescence in a variety of species. We discuss the specific selection conditions that have increased longevity, the phenotypic responses and trade-offs that accompany these increases, and examine genomic data collected from these experiments. Additionally, we consider how selected field studies complement laboratory experiments on life-history evolution. Finally, we address the strengths and weaknesses of existing study systems, and evaluate which model organisms appear most promising for future genomic investigations of the evolutionary biology of aging.

KEY WORDS: **Evolutionary genomics, experimental evolution, life-history evolution, senescence, trade-offs.**

Nearly all multicellular organisms—as well as many unicellular organisms—experience senescence, or age-related declines in health and reproductive capacity. Senescence results from breakdowns in the biochemical, genetic, physiological, and evolutionary processes that shape and maintain fitness traits within organisms and populations. Each of these processes can be considered a different dimension of, and therefore a different lens through which to study, aging and senescence. Serious attempts to elucidate the causes of senescence or to control its phenotypic consequences are doomed to fail unless they can integrate research across these multiple dimensions of inquiry. Furthermore, integrating the results of these different types of aging studies should provide critical insight into the important questions of why aging came to be and how aging occurs. Enriching our understanding of these big questions has many applications in basic research, agriculture, and medicine. Ultimately, a more sophisticated grasp of the proximate (i.e., how aging occurs) and ultimate (i.e., why aging occurs) causes of aging in model organisms may lead to clinical interventions capable of prolonging health and longevity in humans.

The question of how aging occurs can be investigated through studies focused on different, but specific, dimensions of aging. For example, a genetic study might collect transcriptome data from an organism as it ages, to generate a list of candidate genes underlying senescence (e.g., Lund et al. 2002). A physiological study might test how factors such as caloric intake can alter biological processes in ways that lead to increased longevity (e.g., Wu et al. 2019), or common physiological characteristics shared by longer lived individuals in a population (e.g., Kulminski et al. 2008). By contrast, investigating the

question of why aging occurs necessarily must take place through an evolutionary lens. An evolutionary study might tackle why variation exists for genes and traits associated with longevity or how selection may act upon this variation (e.g., Reznick 1997). Evolution by natural selection should maintain traits within a population that are beneficial; and yet, aging and senescence persist almost universally despite the detrimental phenotypes they often bring about. Evolutionary theory reminds us that for selection to eliminate alleles that cause aging, these alleles also must reduce an individual's overall fitness. Any alleles that do not inhibit the reproductive success of an individual, and/or take effect after the age of reproduction, will not be selected against, and will accumulate in the genome. This "mutation accumulation" theory is widely accepted as one of the leading explanations for the evolution of aging (Haldane 1941; Medawar 1952). A distinct but complimentary explanation was worked out by Medawar (1946, 1952) and popularized by Williams (1957); because the force of natural selection will decrease with age due to decreased reproductive potential (which would later be demonstrated mathematically by Hamilton [1966]), any mutation that exhibits beneficial effects early in life, but detrimental effects in late life, will be selected for because the force of selection at younger ages is strong. This "antagonistic pleiotropy" theory suggests that aging may have emerged or been preserved as a trade-off for other improved early-life fitness traits. Combined, these ideas have come to form the foundation of an entire subfield: the evolutionary biology of aging (Rose 1994).

Multiple empirical strategies exist to study the evolutionary biology of aging in model organisms, with experimental evolution as the most powerful. In experimental evolution studies, only individuals that exhibit the phenotype of interest (e.g., long-term survival) are allowed to reproduce; thus, the subsequent generation is likely to inherit genes associated with the selected phenotype. This cycle is repeated over several generations, continually selecting progeny with combinations of genetic variants that promote the phenotype of interest and filtering out progeny lacking such combinations. These methods enable comparisons between selected and control populations, or selected populations and their ancestors, which reveal the phenotypic and genetic shifts produced by selection (reviewed by Kawecki et al. 2012). One example of such a study is M.R. Rose's classic experiment that demonstrated that selection for delayed reproduction leads to the evolution of increased longevity in *Drosophila melanogaster* (Rose 1984). This result was soon repeated by other groups (Luckinbill et al. 1984; Partridge and Fowler 1992), and evolved populations produced by these studies have provided essential models for studies of life-history evolution, and the evolutionary biology of aging, continuously through the decades since their creation. Earlier research focused primarily on characterizing the phenotypes that respond to this type of selection, including the correlations between life-history characters and potential trade-offs between age-specific phenotypes (reviewed by Burny et al. 2020). Within the past decade, investigators have begun to examine genomes and transcriptomes of evolved populations, allowing a clearer picture of the genetic architecture underling phenotypic shifts (e.g., Burke et al. 2010; Sarup et al. 2011; Remolina et al. 2012; Carnes et al. 2015; Graves et al. 2017; Fabian et al. 2018; Parker et al. 2020). Notably, aging has been manipulated with experimental evolution in many organisms beyond *Drosophila*, including bacteria (Ackermann et al. 2007b), *Caenorhabditis elegans* (Anderson et al. 2011), a variety of insects (e.g., Miyatake 1997; Reed and Bryant 2000; Hunt et al. 2006), and even mice (e.g., Nagai et al. 1995) and rabbits (e.g., Theilgaard et al. 2007). Additionally, a number of field studies have provided insight into how selection for longevity might occur in natural populations, as well as factors that may confound our predictions of evolutionary responses (e.g., Dudycha and Tessier 1999; Reznick et al. 2006; Carlson et al. 2007; Wit et al. 2013a). The synthesis of results across these studies should deepen our understanding of the unifying evolutionary principles governing aging and senescence across species. Such synthesis holds the tantalizing potential of unlocking the mysteries of how and why aging occurs, and of providing avenues for battling the specific mechanisms of aging in a medical context.

Empirical Studies Imposing Selection on Different Phases of Life History have Repeatedly Demonstrated Phenotypic Shifts in Correlated Traits

In the laboratory, four basic types of selection regime have been used to drive the evolution of longevity in experimental populations, including selection on (i) longevity; (ii) late-life reproduction; (iii) early-life reproduction; and (iv) increased reproductive lifespan. The experiments that have produced evolved increased longevity in experimental populations are listed in Table 1. The major aims of such studies are to investigate the phenotypic and genotypic responses to a selection regime, as well as to identify potential trade-offs or correlations between longevity and other life-history traits. The ability to probe genomic responses to selection has emerged only relatively recently; as a result, our current understanding of the responses to experimental evolution is based heavily on studies investigating phenotypic relationships between traits in evolved populations. These selection regimes have shown that selection on late-life survival and reproduction tend to produce correlated results that predictably oppose those that select for shorter life- spans and earlier reproduction, providing strong evidence that selection on length of life and

Table 1. Independent evolution experiments demonstrating that selection on life-history characteristics can increase longevity. Here, we list primary references for these studies, organized by taxonomic group. Often many publications follow up the primary reference by continuing to report on evolved lines (see main text for discussion), but for conciseness these are not listed. We also indicate the particular selection condition that led to increased longevity, as well as whether the reference is best classified as a laboratory experiment, a field study, or animal breeding.

Taxonomic group	Species	Primary reference	Study type	Selection condition
Bacteria	*Caulobacter crescentus*	Ackermann et al. 2007b	Laboratory	Early-life reproduction
Nematodes	*Caenorhabditis elegans*	Carvalho et al. 2014	Laboratory	Early-life reproduction
	Caenorhabditis remanei	Chen and Maklakov 2012	Laboratory	Condition-dependent extrinsic mortality
Noninsect arthropods	*Daphnia pulex-pulicaria*	Dudycha and Tessier 1999	Field	Reduced seasonal mortality
	Acanthoscelides obtectus	Tucic et al. 1996	Laboratory	Late-life reproduction
	Bactrocera cucurbitae	Miyatake 1997	Laboratory	Late-life reproduction
	Melanoplus sanguinipes-devastator	Tatar et al. 1997	Field and laboratory	Delayed seasonal mortality
Insects	*Teleogryllus commodus*	Hunt et al. 2006	Laboratory	Increased longevity
	Drosophila melanogaster	Zwaan et al. 1995	Laboratory	Increased longevity
		Rose 1984	Laboratory	Late-life reproduction
		Luckinbill et al. 1984	Laboratory	
		Partridge and Fowler 1992	Laboratory	
		Partridge et al. 1999	Laboratory	
		Remolina et al. 2012	Laboratory	
		Wit et al. 2013b[1]	Laboratory	
		Stearns et al. 2000	Laboratory	Low extrinsic mortality in adulthood
Fish	*Poecilia reticulata*	Reznick et al. 2004	Field and laboratory	High predation during adulthood
	Oncorhynchus nerka	Carlson et al. 2007	Field	High predation of senescent individuals
Marsupials	*Didelphis virginiana*	Austad 1993	Field	Low predation
Placental mammals	*Mus musculus*	Nagai et al. 1995[2]	Laboratory	Increased reproductive longevity
	Oryctolagus cuniculus	Sánchez et al. 2008[2]	Breeding	Increased reproductive longevity
		Larzul et al. 2014	Breeding	

[1] Evolved lines were originally described by Bubliy and Loeschcke (2005), but no change in longevity was observed in that study.
[2] Some evolved lines predate the cited study, but results from the reference cited here are more comprehensive.

reproductive timing can be used as complementary tools in the study of the evolution of aging. However, all the differences in resulting phenotypes under a given selection regime are rarely accounted for, suggesting that although each of these regimes provides valuable insight into the processes of aging and senescence, seemingly minor differences in experimental conditions may still influence the outcomes of selection in ways that have biological implications. To further complicate matters, some studies take advantage of wild populations, in which naturally occurring sources of extrinsic mortality have effectively selected for longer lived or later-reproducing individuals. These investigations may help us disentangle genetic from environmental influences on aging and longevity, but can also produce results at odds with their laboratory counterparts—these will be discussed in more detail in later sections.

DIRECT SELECTION ON LONGEVITY

Seemingly, the best way to investigate aging and senescence would be to select directly on longevity; however, studies on increased lifespan are limited in number, due to methodological difficulties in selecting for a condition that often cannot be identified until after the organism is no longer able to reproduce. Still, a few studies have found creative ways to circumvent this problem. Zwaan et al. (1995) selected on long- and short-lived female *D. melanogaster* by splitting offspring from each pairing into two groups: a longevity assay group and a selection group (two replicate lines, four to six generations of selection). The longevity assay group was maintained at a high temperature (29°C), which has been shown to reduce lifespan in *D. melanogaster*, whereas the selection group was maintained at a cooler temperature (15°C). Siblings of the longest lived flies from the longevity assay were used as founders for the next generation of the selection experiment for longevity. Selection for decreased longevity was conducted using similar methods. Lines selected for longevity exhibited longer lifespans than controls and short-lived lines, along with decreased overall reproductive output. Another evolution experiment selecting directly on longevity, performed by Hunt et al. (2006), turned to a cricket species (*Teleogryllus commodus*) with a useful life-history trait; nearly all male parents in this study died before their offspring had hatched, allowing for offspring to be sorted into longer and shorter lived categories for selection before hatching (five replicate lines, five generations of selection, 28°C). This study found increased longevity and reduced reproductive effort in lines of male crickets selected for increased longevity compared to those selected for reduced longevity.

Although selecting for longevity would be the most direct way to evaluate evolved differences in lifespan, the lack of existing studies reflects the methodological challenges they present. Such studies may be appropriate for certain study systems such as *T. commodus*, but in the majority of model systems the benefits of selecting directly on longevity are outweighed by practical limitations that lead to suboptimal experimental design (i.e., reduced population sizes and levels of replication).

SELECTION FOR DELAYED REPRODUCTION

Given the challenges inherent in selecting directly for longevity, investigators more frequently use selection on delayed reproduction to produce populations with increased longevity and delayed senescence. In accordance with evolutionary theory, the force of natural selection should be strongest before the age of first reproduction, at which point it decreases until approaching zero when an individual is no longer able to reproduce. By delaying the age of first reproduction, selection pressure against deleterious alleles remains stronger longer, effectively selecting for increased longevity.

Evolutionary studies have taken advantage of this principle, and selection for delayed reproduction has consistently produced populations with increased longevity (e.g., Luckinbill et al. 1984; Rose 1984; Partridge and Fowler 1992; Tucic et al. 1996; Miyatake 1997; Partridge et al. 1999; Remolina et al. 2012; Wit et al. 2013b; Carnes et al. 2015). These populations also frequently exhibit increased late-life fecundity (e.g., Luckinbill et al. 1984; Partridge and Fowler 1992; Miyatake 1997; Remolina et al. 2012; Carnes et al. 2015). Other age-specific fecundity phenotypes are less predictable across these studies; for example, selected lines in some experiments exhibited decreased early-life fecundity (e.g., Luckinbill et al. 1984; Rose 1984; Partridge et al. 1999; Remolina et al. 2012), whereas others were more fecund at all ages (Wit et al. 2013b; Carnes et al. 2015).

Although most of these trait correlations were evaluated in *D. melanogaster*, some have also been shown in selection experiments with house flies (Miyatake 1997) and bean weevils (Tucic et al. 1996). Additionally, several studies have also observed trade-offs and correlations between delayed reproduction and stress resistance phenotypes. Several studies in *D. melanogaster* observe correlations between delayed reproduction and increased starvation resistance (e.g., Service 1987; Rose et al. 1992; Bubliy and Loeschcke 2005, Wit et al. 2013b). A variety of other correlated stress responses have also been recorded (reviewed by Kirkwood and Austad 2000, also see Wit et al. 2013b); studies in *D. melanogaster* have shown that selection for longevity via delayed reproduction can result in decreased resistance to heat shock (Kuether and Arking 1999), increased resistance to heat stress (Service 1987), increased resistance to cold shock (Luckinbill 1998), increased starvation resistance and desiccation resistance (e.g., Service 1987), improved heart function (Shahrestani et al. 2017), and increased immune response (Fabian et al. 2018). Similarly, selection for desiccation resistance and starvation resistance has been correlated with increased evolved longevity

(Rose et al. 1992). Still, many of these correlations require further investigation; Norry and Loeschcke (2002) found that correlations between longevity and cold-shock resistance are highly dependent on several variables including sex and temperature of the culture environment, and Wit et al. (2013b) found reduced cold resistance in longer lived populations. Additionally, decreased longevity has been observed as a correlated response in studies selecting for increased fungal immune response (Shahrestani et al. 2021).

Furthermore, even in experiments where trade-offs have been reliably observed, in some cases correlations between delayed reproduction and early fecundity (Leroi et al. 1994) or desiccation and starvation resistance (Archer et al. 2003; Phelan et al. 2003) disappear after long-term selection. Similarly, Rose (1984) found flies selected for delayed reproduction showed reductions in early fecundity, but after hundreds of generations of continual selection, Carnes et al. (2015) reported the same lines had developed increased early fecundity. Explanations such as inbreeding effects, genotypic linkage, genetic drift, and genotype-by-environment interactions were deemed unable to explain the results of Phelan et al. (2003), Archer et al. (2003), or Rose (1984) and Carnes et al. (2015). Rather, Phelan et al. (2003) and Archer et al. (2003) argue that sustained selection may lead to changes in the genetic relationships and physiological interactions between traits over time.

SELECTION FOR EARLY-LIFE REPRODUCTION

Although selection for late-life reproduction is the most effective method for creating long-lived populations, and can produce remarkable delays in senescence over relatively short evolutionary time frames (dozens of generations), this strategy is still time-intensive as generation times will increase with each round of selection. For example, in the landmark study by Rose (1984), average longevity in *Drosophila* increased by 28.6% in females and 14.7% in males within 15 generations, but the time needed for each generation more than doubled (from 28 days to 70 days) within that time. Although manageable in *Drosophila*, such increases make this work more challenging in organisms with longer generation times, and investigators naturally began to explore if questions concerning the mechanisms underlying the evolution of longevity (and associated trade-offs) could be addressed with selection on early-life reproduction. If effective, this strategy would be more efficient and more practical to use with longer lived species.

Of course, there are limitations to this approach; directional selection on early-life reproduction may only be effective to a certain point, beyond which additional selection will no longer have the desired impact because of physiological constraints. For example, Chen et al. (2016) found that selection for accelerated juvenile development in *D. melanogaster* reached a threshold; in selected populations, juveniles never eclosed earlier than 7-8 days from egg. Although there are several reasons this may have occurred, one possible explanation is that the population had already reached the shortest viable average lifespan supported by the genetic variation present within the starting population.

Additionally, selection on early-life reproduction does not allow for investigation of specific alleles involved in mutation accumulation or antagonistic pleiotropy in the same way that studies on late-life reproduction can; although detrimental alleles may still accumulate and trade-offs can occur in late life, the specific alleles involved may differ from those found in aged populations. Despite this caveat, the majority of studies selecting on faster development and earlier reproduction reveal that resulting populations have shorter lifespans (e.g., Partridge and Fowler 1992; Reed and Bryant 2000; Burke et al. 2010).

However, not all associated phenotypes shift consistently across studies; early fecundity increases in some (Miyatake 1997; Anderson et al. 2011) and decreases in others (Reed and Bryant 2000). These discrepancies serve as a reminder that specific phenotypic outcomes resulting from a selection regime may vary among species and environments, and emphasize the importance of checking that selection exerts the expected influence on the trait of primary interest. Still, the emergence of some form of trade-off in response to selection for early-life fecundity appears practically unavoidable even if it is not manifest as a change in fecundity, and further exploration of the specific alleles underlying these trade-offs is warranted (reviewed by Austad and Hoffman 2018). Such investigations may further elucidate the relationship between aging and age-specific reproductive capacity.

SELECTION ON REPRODUCTIVE LONGEVITY

Another strategy for driving the evolution of aging in experimental populations involves selecting for increased reproductive longevity, which is defined as the average length of time between the first and final reproductive event of individuals within the population. This selection condition has primarily been used in breeding studies, with the goal of increasing livestock productivity. Some have discussed attempting such selection in livestock (e.g., Essl 1998; Serenius and Stalder 2006), and a few studies use historical records to retrospectively simulate selection in chickens and cattle (Ducrocq et al. 2000; Ducrocq 2005). To our knowledge, however, true experimental selection for longevity in mammals has only been successfully carried out in mice (Nagai et al. 1995; Farid et al. 2002) and rabbits (e.g., Theilgaard et al. 2007; Savietto et al. 2013; Larzul et al. 2014; Garreau et al. 2017). These studies frequently result in populations with increased reproductive lifespans (Nagai et al. 1995; Farid et al. 2002) and late-life fecundity (Theilgaard et al.

2007; Sánchez et al. 2008), as well as increased offspring survival rates (Nagai et al. 1995; Savietto et al. 2013; Larzul et al. 2014; Garreau et al. 2017) and increased number of litters (Nagai et al. 1995; Larzul et al. 2014). Additionally, populations selected for increased reproductive longevity often exhibit increases in average body fat (Theilgaard et al. 2007; Garreau et al. 2017) and differences in energy distribution (Savietto et al. 2013), which may suggest they have increased resistance to other stressors, although further studies would be needed to test this possibility.

APPLYING THE LESSONS OF LABORATORY EXPERIMENTS TO NATURAL POPULATIONS: SELECTION AS A RESULT OF AGE-SPECIFIC EXTRINSIC MORTALITY

One drawback of the selection conditions cataloged above is their limited applicability to natural populations. Laboratory populations generally experience selection related to domestication, and as a result may harbor significantly different genetic variation compared to their wild counterparts (Phillips et al. 2016). Additionally, investigator-imposed selection conditions vary substantially from those experienced in the wild, and involve environments lacking ecological complexity. For example, Wit et al. (2013a) found that laboratory-selected longevity did not carry over to natural environments; longevity-selected lines were less able to locate food, and thus had lower fitness in field conditions compared to controls. Experiments in oversimplified laboratory environments—which are necessary for imparting empirical power—limit our understanding of the forces that drive adaptation in complex natural environments. Although a handful of experimental evolution studies implicate the same adaptive loci as those observed in natural populations (Phillips and Burke 2021), the links between the lab and nature are especially tenuous when the phenotypes of interest involve senescence. Although lifespan is often closely linked to the onset of senescence in laboratory environments, mortality is much less predictable in nature, and generation times can be difficult to measure. External factors that prematurely truncate lifespan (e.g., predation, disease, climatic stressors, etc.) can obfuscate the relationship between senescence and longevity, and any distinction between physiological and reproductive senescence becomes even more difficult to uncouple. Still, studies that scrutinize natural populations with different levels of extrinsic mortality provide some of the best real-world evidence in support of evolutionary theories of aging (Table 1). These field studies can also help contextualize some of the discrepancies between empirical studies and classical interpretations of theory by providing direct insight into how selection shapes natural populations differently than laboratory populations (reviewed by Johnson et al. 2019).

Extrinsic mortality can be described as the combined effects of nongenetic causes of mortality, similar to the "hazard factor" described by Edney and Gill (1968). The stronger such effects are in a given population, the less likely it is that an individual will survive to old age; thus, populations with lower extrinsic mortality are more likely to see individuals survive to advanced age and experience selection for traits associated with improved late reproduction and survival. A number of studies provide evidence that natural populations experiencing high extrinsic mortality exhibit shorter lifespans and characteristics of earlier maturity across several species, including between two closely related species of *Daphnia* (Dudycha and Tessier 1999), and between populations of individual species of Garter snake (Bronikowski and Arnold 1999), guppy (Reznick et al. 2004), and opossum (Austad 1993). Some of these studies in natural populations have suggested potential longevity trade-offs as well; Austad (1993) found that island opossum populations experiencing reduced predation generally exhibited patterns of delayed senescence, but also had reduced litter sizes at all ages compared to mainland populations. Similar results have been observed in laboratory-reared populations of *D. melanogaster* (Stearns et al. 2000).

Still, differential rates of extrinsic mortality do not guarantee selection for longevity will occur, as age specificity of mortality is an important component of selection (e.g., Charlesworth 1994). Postponed longevity in natural populations can result as a by-product of environments where sources of extrinsic mortality favor the survival of longer lived individuals, and vice versa. For example, seasonal habitat loss (e.g., Dudycha and Tessier 1999) may truncate lifespan, creating an "upper limit" for longevity not experienced in populations that do not suffer such seasonal effects, and predators may preferentially target organisms with phenotypes (e.g., body size or condition) associated with different ages or developmental stages (e.g., Austad 1993; Reznick et al. 2001; Carlson et al. 2007).

This age specificity may also help to explain some of the more complex results from studies involving extrinsic mortality. Although populations of guppies experiencing increased predation pressure throughout life (as opposed to high predation only during early life) evolved earlier maturity, they also continued to reproduce at later ages, and had a higher rate of offspring production throughout their lives (Reznick et al. 2004, 2006). This remained true both in completely natural environments, and when predation rates were artificially manipulated by the introduction of predators. This result may indicate that increased fecundity throughout life and increased reproductive lifespan confer fitness benefits when more individuals survive to mid-life, even if fewer individuals survive to late ages.

Similarly, a study of sockeye salmon (*Oncorhynchus nerka*) experiencing differential levels of predation by bears found that

the rate of extrinsic mortality influenced the rate of senescence in a conditional manner. Populations where senescent salmon are preferentially killed by bears have evolved lower overall rates of senescence (estimated by cataloguing causes of death due to physiological decline vs. other factors) than populations where bears are more likely to kill healthier salmon (Carlson et al. 2007). Additionally, Chen and Maklakov (2012) found evidence that the circumstances surrounding high mortality rates may impact the evolution of longevity in laboratory populations of *Caenorhabditis remanei*. Although higher rates of random mortality resulted in the evolution of shorter lifespan, mortality imposed via heat shock resulted in populations with longer lifespans, likely because organisms experiencing physiological deterioration (i.e., due to senescence) were likely to be disproportionately affected by extrinsic stressors. Thus, studies in both laboratory and natural populations have suggested that when the source of extrinsic mortality acts unequally on individuals of different life stages or physiological condition, the phenotypes under selection may differ.

Although it is clear that field and laboratory studies can complement one another, a growing number of projects attempt to directly bridge the gap between them. In particular, field mesocosms provide opportunities to conduct evolution experiments in natural (or near-natural) environments, while still maintaining a greater level of control over external factors such as predation and migration compared to a typical field study (e.g., Rudman et al. 2019; Grainger et al. 2021). This framework could provide unique opportunities to directly parse the effects of age-specific extrinsic mortality on the evolution of longevity in *Drosophila,* and likely other systems. The idea that methods from the laboratory should be brought to the field, and vice versa, is being increasingly invoked as essential for deepening our understanding of ecoevolutionary dynamics in natural populations (cf. Bailey and Bataillon 2016). We expect that such combined approaches will be especially useful in efforts to identify natural genetic variation underlying longevity and age-specific reproduction, as these traits are notoriously difficult to measure in natural populations while controlling for sources of extrinsic mortality.

Genomic Evaluations of Experimentally Evolved Populations Promise to Reveal the Genetic Basis of Longevity, but in Practice Suffer Limitations Leading to Low Power

Although phenotypic surveys of experimentally evolved populations are informative, genomic surveys have become the new gold standard for pointing to potential genes or mechanisms underlying shifts in aging and related traits. Within the past decade, whole-genome population-level sequencing has become more readily available, such that experimental evolution studies routinely incorporate genomic data. Genomic evaluations of selection experiments for delayed reproduction have been performed in three distinct study systems of *D. melanogaster* (Table 2). Each of these genomic surveys implicates broad genomic regions harboring thousands of allelic variants in hundreds of potentially causative genes. Using expression profiling and functional annotation, Remolina et al. (2012), narrowed their list to 38 strong candidate genes underlying observed shifts in longevity, including genes previously implicated in reproduction, immunity, and proteolysis. Carnes et al. (2015) were similarly able to narrow their list to 98 genes in females and 175 in males as the strongest candidates underlying observed longevity increases in evolved populations. In a third set of populations, Fabian et al. (2018) found significant enrichment for genes involved in immune defense, among the hundreds of genes differentiated in long-lived populations. Fabian et al. (2018) further determined that 20 shared genes were implicated between all three studies, although these were not necessarily the same genes identified as strong candidates in their respective studies. Additionally, several of the genes identified through these experiments have since been functionally validated via RNAi knockdowns, and have been shown to influence life-history traits and mediate tradeoffs between longevity and reproduction (Huang et al. 2020; Parker et al. 2020).

Although these studies are promising, they are few in number, and several limitations in experimental design and scope restrict the conclusions that can be drawn from them. First, current best practices suggest that none are sufficiently powerful to comprehensively identify candidate genomic regions associated with phenotypic responses to selection. Simulation studies (e.g., Baldwin-Brown et al. 2014; Kofler and Schlötterer 2014) suggest that maximizing the number of independent replicate populations in an evolution experiment is essential for associating evolved phenotypes with genomic loci. Burke et al. (2014) empirically validated this point by showing that sequence data from 12 replicate populations in an experimental evolution study impart high statistical power to connect phenotypes to individual genotypes, but this power was completely eliminated when the dataset was reduced to only five replicate populations. None of the evolution experiments with genomic data selecting for delayed reproduction (Table 2) have replicates in excess of five; therefore, the results from these studies warrant critical interpretation in the context of our current knowledge; among existing genomic studies of the evolution of aging, it likely that the majority of candidate regions have not yet been identified. As a hopeful caveat, 10-fold replication has been employed in *Drosophila*

Table 2. Major *Drosophila melanogaster* study systems with experimentally evolved extended longevity, which feature publicly available genome data. The "primary source" cited in the table is the first publication that includes genomic analysis from each system. Generation times in the table refer to those estimated at the time of genome sequencing. Routine culture conditions were similar for all three studies (25°C, 12 h:12 h light:dark cycles, cornmeal medium), although the Rose populations were maintained on an ancestral banana-molasses diet, and in constant light; some methods of culture maintenance were changed when populations were shared, and it is not known if or how this may have influenced selection outcomes. The Hughes populations are named as such by us as Dr. Kimberly Hughes is listed as the corresponding author on the primary source manuscript. The Rose and Luckinbill populations are named as such as their creation is generally credited to Dr. Michael Rose and Dr. Leo Luckinbill, respectively, in the early 1980s.

	Hughes populations		Rose populations		Luckinbill populations	
Primary source	Remolina et al. (2012)		Carnes et al. (2015)		Fabian et al. (2018)	
	Selection treatment	Control treatment	Selection treatment	Control treatment	Selection treatment	Control treatment
Colloquial name	S "selected"	C"control"	O"old"	B"baseline"	R"random-bred"	L"long-lived"
Replicates	3	3	5	5	4	2
Generations	50	80	170	850	144	293
Age of reproduction	28-40 days (from egg) increased gradually	14 days (from egg)	70 days(from egg)	14 days(from egg)	20-30% longest-lived adults collected	4-30 days (adult age) chosen randomly
Population size	Offspring from 220-320 single-pair matings per replicate per generation		Census size >1000 adults per replicate per generation		Census size >1000 adults per replicate per generation	
DNA sequencing details	100 females pooled from each population		100 females pooled from each population		100 females pooled from each population	
Additional genomics methods	Microarrays		RNA sequencing[1]		RNA sequencing, RNAi knockdown	

[1] RNAi knockdown assays of these populations were conducted by Parker et al. (2020).

experiments where populations have been maintained on specific generational schedules that result in variation in lifespan (although these populations were not explicitly selected for longevity) and genomic data from these projects are beginning to be explored (Graves et al. 2017; Barter et al. 2019). Second, we cannot assume that similar experiments will necessarily select for the same genomic variants. Starting populations may contain different genomic variants, and studies have suggested that factors of experimental design such as temperature (Huang et al. 2020) and sexually antagonistic selection (Chen et al. 2016) can permit different genetic variants to persist in evolving populations based on minor differences in experimental conditions. Thus, we cannot conclude these results are broadly applicable across environments, and even seemingly minor differences in environment and laboratory protocols allow for potential confounding variables that may make extrapolating connections between existing studies problematic. Similarly, these studies have only been performed in one species, *D. melanogaster*, and it is unknown whether the identified genes will be conserved across species.

These limitations underscore the need for a more modern approach to incorporating genomics into experimental evolution studies of aging. Some of these limitations may be overcome by advances in genomic analysis tools. For example, Mueller et al. (2018) present a statistical learning tool called "FLAM," which can sift out differentiated but noncausal loci when inferring the genes that affect a particular phenotype; this tool is especially helpful when working with long-established experimental populations where it is known that multiple phenotypes have diverged, in addition to the trait under focal selection. Others have proposed "secondary Evolve-and-Resequencing" (Burny et al. 2020) as an experimental approach to follow an under-replicated primary evolution experiment. This involves backcrossing an evolved population to the ancestor, and tracking the frequencies of putatively selected alleles from this new baseline to confirm that they will respond to selection again. Clearly, these emergent approaches hold exciting potential for the continued study of existing populations with experimentally evolved longevity. On the other hand, it must be said that many of the *Drosophila* populations we discuss in this review were established in the 1980s, before high-throughput sequencing technologies could have been conceived of, let alone prepared for. Although these lines represent decades of research, and their value to the field cannot be overstated, in many ways they are fundamentally ill-equipped for strong-inference genomic approaches. Although post hoc genomic surveys of existing study systems are a natural and informative starting point, new experiments that have been carefully designed with genomic best practices in mind are the next logical step forward.

Experimental Evolution of Longevity in Different Organismal Systems Features Species-Specific Advantages for and Roadblocks to Advancing Knowledge

THE MOST INFORMATIVE SELECTION STUDIES ON THE EVOLUTION OF AGING TO DATE HAVE BEEN PERFORMED IN *D. melanogaster*

The majority of experimental evolution studies on aging have been and continue to be with *D. melanogaster*, and for good reason. Fruit flies can be easily maintained and manipulated in laboratory conditions, and large sample sizes can be studied over many generations relatively quickly and cheaply. A wealth of information is already available for *D. melanogaster*, including the sequenced and annotated genome, and a number of genes involved in health and development have also been shown to have orthologs in humans (e.g., Yamamoto et al. 2014). Furthermore, long-lived lines such as those established by Rose (1984) have been maintained over many generations, allowing for their continued study. Studies such as these not only set a precedent for laboratory evolution of aging, but they also provide many valuable insights into the phenotypic (e.g., Luckinbill et al. 1984; Rose 1984; Partridge and Fowler 1992) and genomic (e.g., Burke et al. 2010; Remolina et al. 2012; Carnes et al. 2015; Graves et al. 2017; Fabian et al. 2018) changes associated with selection on reproductive timing. As mentioned in the previous section, genomic analyses of experimentally evolved *D. melanogaster* (Table 2) have revealed a number of candidate genes likely to play a role in modulating longevity. Still, there is a clear need for continued investigation of the genetic variants underlying lifespan and senescence within and across populations. Although *D. melanogaster* is a promising model for continued study, experiments in additional model systems are needed. Given the incredible diversity of life, it follows that we may be unable to answer some questions about aging using fruit flies alone. By comparing results across different study systems, we will be better able to ascertain which genomic candidates are most likely to be conserved across species, and which therefore may be more useful in an applied context for human medical interventions. Thus, the question becomes: what other organisms are the most promising models for the experimental evolution of aging, and how can we choose?

MANY DIFFERENT INVERTEBRATE SYSTEMS HAVE INFORMED OUR CURRENT UNDERSTANDING OF THE EVOLUTION OF AGING

A minority of experiments studying the evolution of aging use invertebrates other than *D. melanogaster*, including houseflies, melon flies, bean weevils, crickets, and grasshoppers (see

Table 1). These systems have frequently shown increases in lifespan resulting from selection for increased longevity or late-life reproduction (Tucic et al. 1996; Miyatake 1997; Reed and Bryant 2000; Hunt et al. 2006) and decreased longevity due to selection for early reproduction (Miyatake 1997; Reed and Bryant 2000), as well as correlated changes in late-life fecundity.

Although model organisms with extensive genetic resources such as *D. melanogaster* are the most obvious choices for experimental evolution, investigators should not overlook the opportunities presented by less traditional systems that remain largely unexplored. Many natural populations exhibit life-history characteristics that may make them especially amenable to certain modes of selection (as was the case with Hunt et al. [2006] and *T. commodus*). Additionally, some invertebrate populations occupy environmental niches especially well-situated to select for differential life history traits. For example, Reznick (1993) argued that marine invertebrates such as fairy shrimp and copepods may be valuable systems to study the evolution of longevity in natural populations, as they frequently exist in ecological conditions in which precipitation or predation patterns may select for differential longevity between isolated populations in otherwise similar environments. Thus, unique features of invertebrate systems beyond *D. melanogaster* may provide opportunities to experimentally evaluate specific aspects of life-history evolution.

STUDIES IN FISH HAVE PROVIDED OPPORTUNITIES TO STUDY THE EVOLUTION OF AGING IN NATURAL ENVIRONMENTS

Along with marine invertebrates, Reznick (1993) also suggested studying the evolution of natural populations of guppies (*Poecilia reticulata*) experiencing differing predation patterns, an endeavor that has proved fruitful (reviewed in Reznick 1997; Reznick et al. 2004, 2006). As mentioned above, studies in guppies (e.g., Reznick et al. 2004) and salmon (Carlson et al. 2007) reveal that responses to selection on extrinsic mortality in natural environments often depend on the context of mortality and specific life-history traits in the population. For example, Reznick et al. (2004) suggest that despite increased mortality, the increased lifespans of guppies from high-predation locations may be a result of increased fecundity with age and decreased competition for resources, resulting in inadvertent selection for longevity. Although these possibilities illustrate some of the difficulties of studies conducted in natural environments, they also reveal the potential value of paired studies between laboratory-raised and natural populations. Although Reznick (1997) has made comparisons between populations with differing natural and artificially-altered predation rates in wild populations of guppies, no such studies have included laboratory-selected populations, and to our knowledge this work does not feature any genomic investigation. Although guppies (and other potential fish systems, such as zebrafish) have longer reproductive times than many other model organisms, they are short enough to conceivably be used for a laboratory selection study. Such a study could provide a useful system for comparison with natural populations, and for the collection of genomic data.

RELEVANT MAMMALIAN MODELS SUFFER FROM CLEAR LIMITATIONS TO EXPERIMENTAL DESIGN

In the context of experimentally evolved postponed longevity, mammalian systems present the ideal model for applications to human genetics and medicine, but they come with a number of complications. First, mammals typically have significantly longer lifespans than other model organisms. Given the nature of selection on postponed reproduction, the added time and expense of experiments in longer lived organisms quickly become compounded, as each successive generation will take even longer to reproduce than the previous generation. Another concern in mammalian studies is that due to space constraints, different selection treatments are not always replicated or maintained in the same location. At least one such study (Sánchez et al. 2008) found significant differences between responses in the same lines reared under the same selection regimes on different farms. Thus, existing experiments with mice and rabbits typically span few generations, employ minimal replication, and involve relatively smaller populations with some degree of inbreeding—all of these limitations weakening the ability to draw inference.

Despite these limitations, notable phenotypic trends have been observed following selection on reproductive longevity in mice (Nagai et al. 1990, 1995; Farid et al. 2002) and two sets of rabbit lines: (i) V (standard longevity) and LP (hyperlongevity) (Theilgaard et al. 2007; Sánchez et al. 2008; Savietto et al. 2013) and (ii) HL (high longevity) and LL (low longevity) (Larzul et al. 2014; Garreau et al. 2017). These similar responses include increased lifespan or reproductive lifespan (Nagai et al. 1990, 1995; Farid et al. 2002; Theilgaard et al. 2007; Sánchez et al. 2008; Larzul et al. 2014), increased offspring survival (Nagai et al. 1995; Savietto et al. 2013; Larzul et al. 2014; Garreau et al. 2017), and increased late-life fecundity (Theilgaard et al. 2007; Sánchez et al. 2008; Savietto et al. 2013).

It is not particularly surprising that evolution experiments involving reproductive longevity in mammals have focused solely on phenotypic analyses. To date, these studies selecting for longevity in mammals include only one or two replicates per treatment, which is expected to lead to unwanted noise in any attempt to scan the genomes of evolved populations for signatures of selection. However, Hillis et al. (2020) recently presented results from a long-term evolution experiment with house mice (*Mus domesticus*) that provide some optimism in this regard. After 61 generations (nearly 30 years) of selection for high voluntary wheel running, the genomes of mice from each of four

experimental and control replicates were sequenced, which led to the identification of 12 strong candidate regions associated with increased voluntary running. Thus, the challenges of executing an evolution experiment for postponed reproduction (with the goal of downstream genomic analysis) in mammals such as mice are not necessarily prohibitive, but the power of such experiments will never approach those performed in more tractable model systems. Of course, the increased generation time required in selection for longevity provides an additional complication, but one that is not insurmountable, given enough time—one unreplicated line of mice has undergone continual selection for longevity for at least 24 generations over a ∼20-year time period, with documented increases in longevity that exceed 17% (Nagai et al. 1995; Farid et al. 2002). Even if limited replication provides insufficient power to identify individual genes of interest, the combination of genome sequencing with other techniques in functional genomics may enable elucidation of chromosomal regions of interest underlying evolved longevity. In summary, although the massive investment of time and resources required to impose selection on longevity in mammals is a significant hurdle, the possibility of uncovering candidate genes with closely related human orthologs—genes with high potential value to tailored approaches in personalized medicine—would be worth the effort.

BACTERIA CAN PROVIDE MODELS FOR AGING RESEARCH, BUT KEY REPRODUCTIVE AND EVOLUTIONARY DIFFERENCES LIMIT GENERALIZATION TO OTHER TAXONOMIC GROUPS

Studies show that despite their simplicity, bacteria do in fact exhibit phenotypes of aging due to asymmetrical partitioning, where damaged cellular components are preferentially segregated into one daughter cell during division (Ackermann et al. 2007a; Rang et al. 2011). Stewart et al. (2005) showed experimentally in *Escherichia coli* that "aged" cells exhibit reduced growth rates, with the effect being compounded in successive divisions. Given that they can age, bacteria clearly have many potential advantages for use in laboratory experiments. They are easily maintained with large population sizes and fast generational turnover. However, bacteria lack many key features central to higher eukaryotes, including the ability to reproduce sexually. Because evolution in asexual organisms is driven by de novo mutations rather than standing genetic variation (Burke et al. 2010), unexpected phenotypic effects may be more likely to occur. For example, Ackermann et al. (2007b) found that although all populations of *Caulobacter crescentus* selected for early reproduction developed faster growth rates as expected, two out of their six replicates actually showed increased survival and reproduction at late ages, likely because of de novo mutations that may have also provided additional fitness benefits in early life. However, this study did not investigate specific genomic changes that occurred during their experiment, and to our knowledge, no other selection studies on longevity or reproductive timing have been performed in bacteria.

NEMATODES ARE UNDERUSED IN THE EXPERIMENTAL EVOLUTION OF AGING

Nematodes such as *C. elegans* represent a promising study system for the experimental evolution of aging and longevity. Nematodes have already proven to be valuable systems in experimental evolution generally (reviews by Gray and Cutter 2014; Teotónio et al. 2017). Like *D. melanogaster*, *C. elegans* is a versatile model system with short generations and practical culturing methods that in theory allow large population sizes and high-throughput experiments. A fully annotated genome holds the potential for in-depth genetic characterizations and functional genomics. In many ways, *C. elegans* is already a key model for studies of aging and life history (e.g., Johnson and Wood 1982; Johnson and Hutchinson 1993; Brooks et al. 1994).

However, it is important to distinguish between studies that have extended the *C. elegans* adult life compared to those that have extended the *dauer* stage. The *dauer* stage is a specialized larval stage in nematodes, cued by environmental stimuli, that alters metabolism and confers increased stress resistance. Mutant screens and RNAi knockdowns in *C. elegans* have identified many longevity-conferring mutations that are *dauer* related (e.g., Muñoz and Riddle 2003), but have also revealed several mutations that may act on adult lifespan independently of (or in some cases, in addition to) this *dauer* stage (e.g., De Castro et al. 2004; Yanos et al. 2012, also see review by Ewald et al. 2017). By focusing on these alleles that increase adult longevity, either independently of or in conjunction with the *dauer* stage, studies in *C. elegans* and other nematodes have the potential to provide insight into the evolution of aging and longevity that may be more broadly applicable across taxa.

Although some studies have described the fitness costs and benefits of identified longevity mutants (Walker et al. 1991; Jenkins et al. 2004), surprisingly few have investigated aging and life history in *C. elegans* through experimental evolution. Anderson et al. (2011) showed that selection for increased early-life reproduction led to evolved changes in age-specific fecundity without an effect on lifespan. Interestingly, a later study of these evolved lines reported increased longevity when the rates of outcrossing were increased (Carvalho et al. 2014). Taken together, these observations suggest strong phenotypic connections between aging and mating system in nematodes.

Caenorhabditis species other than *C. elegans* may be even better suited for the experimental evolution of aging and longevity. *Caenorhabditis remanei* in particular stands out as attractive in this regard; it is dioecious rather than androdiecious, which enables experiments with discrete generations and better

control over the experimental manipulation of mating and age-specific reproduction. In addition, *C. remanei* has been shown to harbor increased genetic variation and reduced linkage disequilibrium in comparison to *C. elegans* (Reynolds and Phillips 2013). Chen and Maklakov (2012) showed that selection on extrinsic mortality can exert divergent selection for lifespan in populations of *C. remanei*, depending on the source of mortality. Still, although a number of evolution experiments in *Caenorhabditis* species lead to longevity changes when the trait under direct selection is not directly related (e.g., selection for sexual conflict in Palopoli et al. [2015]), Anderson et al. (2011) is the only evolution experiment we are aware of that selects directly on age-specific reproduction in nematodes.

Overall, nematodes provide promising opportunities for the experimental evolution of aging, and in some ways, it is surprising they have not been used more for this type of work, but their complex life history may lessen the potential for particular candidate genes discovered in them being relevant across taxonomic groups.

BUDDING YEASTS HOLD PROMISE FOR THE EXPERIMENTAL EVOLUTION OF AGING

Another promising system for the experimental evolution of aging are budding yeasts such as *Saccharomyces cerevisiae*. Yeast strains are diverse and can be hybridized, allowing for the creation of recombinant populations harboring a great deal of genetic variation for use in selection experiments. *Saccharomyces cerevisiae* is well-suited to a variety of molecular methods, and is a popular model for medical research due to its versatility. Despite being separated by a billion years of evolution, there are thousands of recognizable orthologs between *S. cerevisiae* and humans (Skrzypek et al. 2018). Yeasts are unicellular, and simpler than other eukaryotic systems, but this could be viewed as a feature rather than a bug. With fast generation times, ease of culture maintenance, and readily accessible genomic data, yeasts have repeatedly proven valuable systems for experimental evolution (reviewed by Zeyl 2006), and are also ubiquitous in aging research (reviewed by Kaeberlein et al. 2007). Although yeast life history is perhaps more complex than that of most higher eukaryotes (i.e., they can reproduce sexually as well as asexually), this also increases their empirical utility for pursuing answers to a broad range of evolutionary questions.

Aging in yeast can be measured via two primary metrics; replicative lifespan (RLS) measures the number of times the cell is capable of reproducing via asexual division (Mortimer and Johnston 1959), whereas chronological lifespan (CLS) measures the total time that the cell remains viable without dividing. Genetic screens can identify individual genes associated with the regulation of CLS, as well as determine the effects of various environmental conditions on CLS (reviewed by Longo et al. 2012). However, actively replicating cells (such as germ cells) acquire additional damage each time they divide; studies on CLS do not account for this acquired damage, and may therefore be more applicable for cell types that divide only infrequently. By contrast, investigations of RLS may be more suitable for testing questions about the longevity of metabolically active, dividing cells. Assays of RLS are often performed through manual separation of individual cells, making them incredibly precise, but also time-intensive and low throughput. Methods using microfluidics, biotin-labelling, and fluorescence-based sorting offer possible solutions for isolation of aged cells with increased experimental throughput. However, the continued proliferation of daughter cells in these populations greatly reduces the average age of cells (and proportion of aged cells) within the culture. To deal with this, techniques have been developed to conditionally arrest daughter cell division and reduce the invasion of aged cultures by young cells (Jarolim et al. 2004; Lindstrom and Gottschling 2009). One such method, the Mother Enrichment Program (Lindstrom and Gottschling 2009), is particularly promising for the isolation of aged cultures for phenotypic or genotypic comparison. This program causes cell cycle arrest of daughter cells without reducing a mother cell's lifespan via the insertion of an estradiol-dependent genetic element. Because this arrest is conditional, it could still be used in the context of a selection experiment, where daughter cells must be frequently permitted to reproduce to allow for evolutionary change to occur throughout generations. However, large population sizes may allow for mutations that allow daughter cells to circumvent cell cycle arrest, and there is an added technical challenge of introducing the Mother Enrichment Program into the genome (Lindstrom and Gottschling 2009). Despite these challenges, each of these methods offer valuable opportunities to probe varying aspects of longevity. Additionally, genetic screens have identified many genes that appear to be involved in regulation of both RLS and CLS (Longo et al. 2012), and there is evidence that RLS and CLS are interdependent, with chronologically older cells displaying reduced RLS (Ashrafi et al. 1999), suggesting further studies investigating the relationship between these two aspects of lifespan may be warranted.

Another useful aspect of *S. cerevisiae* biology is that cells can be manipulated to reproduce either sexually (through sporulation) or asexually (by budding). The physiological consequences of these two reproductive schemes lend themselves to selection regimes for increased RLS. Yeast cells typically undergo asexual reproduction via budding, and each time a new bud is formed a chitinous scar is formed on both the mother and daughter cells at the location of division. These scars can be counted using the methods mentioned previously, allowing for staging and enrichment. Sporulation, then, can be induced in aged cells; mother cells will produce four haploid spores that will either mate with

each other or be released into the environment. These spores will have their lifespan "reset" (Ünal et al. 2011) and do not appear to exhibit the age-related phenotypes of their mother cells, but will be able to mate and outcross.

Because evolution in sexual populations primarily acts on standing variation within the population rather than *de novo* mutations (cf. Burke 2012), experimental evolution of outcrossing yeast populations (e.g., Burke et al. 2014) may be especially helpful in identifying specific genes underlying life-history traits such as longevity compared to other single-celled systems that do not reproduce sexually. Selection on standing variation is more likely to be repeatable across replicate populations, compared to asexual populations such as bacteria. Thus, inconsistencies such as those observed in Ackermann et al. (2007b) may be less frequently observed in sexually reproducing populations. Additionally, sexual populations provide opportunities for beneficial haplotypes to recombine, increasing the efficacy of selection and the investigator's ability to detect genomic evidence of adaptation. In short, we expect evolution experiments in sexually reproducing populations to be more powerful and generalizable than those conducted in asexually reproducing microbes.

Despite their potential, to our knowledge no experimental evolution studies on longevity or delayed reproduction have been conducted in yeast. However, at least one study has found that older cells are less efficient at sexual reproduction in strains of *S. cerevisiae* (Boselli et al. 2009), suggesting that by isolating cells with increased replicative age and forcing them to produce the next generation of cells via sporulation, we can effectively impose selection for late-life sexual reproduction in yeast populations. This would allow for the design of a selection experiment similar to those discussed earlier in other organisms, providing a valuable new system for the experimental evolution of aging.

Concluding Remarks

Selection on life-history traits in organisms ranging in complexity from microbes to mammals have revealed that aging and longevity can be manipulated through experimental evolution in both natural and laboratory-reared populations. These studies have identified the occurrence of several consistent phenotypic trade-offs associated with increased lifespan. Furthermore, advances in whole-genome sequencing have allowed genomic studies to begin to identify genes and genomic regions that may underlie these phenotypic shifts. Still, these early genomic studies have been isolated to experiments performed in a single species, *D. melanogaster*, and lack the replication necessary for conclusive association. Additional highly-replicated studies, perhaps involving underused model organisms such as nematodes or budding yeasts, have the potential to provide key insight into the genes and alleles that influence the evolution of longevity and correlated life-history traits.

AUTHOR CONTRIBUTIONS

MKB conceived the idea for the manuscript, and KMM and MKB wrote the manuscript.

ACKNOWLEDGMENTS

We thank M. R. Rose for providing comments on a previous version of this manuscript, and Burke Lab members for general discussion during the writing of this piece. This work was supported by startup funds to MKB provided by the College of Science at Oregon State University, as well as by a seed grant awarded to MKB by the Collins Medical Trust.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

LITERATURE CITED

Ackermann, M., L. Chao, C. T. Bergstrom, and M. Doebeli. 2007a. On the evolutionary origin of aging. Aging Cell 6:235–244.

Ackermann, M., A. Schauerte, S. C. Stearns, and U. Jenal. 2007b. Experimental evolution of aging in a bacterium. BMC Evol. Biol. 7:126.

Anderson, J. L., R. M. Reynolds, L. T. Morran, J. Tolman-Thompson, and P. C. Phillips. 2011. Experimental evolution reveals antagonistic pleiotropy in reproductive timing but not life span in C*aenorhabditis elegans*. J. Gerontol. A Biol. Sci. Med. Sci. 66:1300–1308.

Archer, M. A., J. P. Phelan, K. A. Beckman, and M. R. Rose. 2003. Breakdown in correlations during laboratory evolution. II. Selection on stress resistance in *Drosophila* populations. Evolution 57:536–543.

Ashrafi, K., D. Sinclair, M. Gordon, and L. Guarente. 1999. Passage through stationary phase advances replicative aging in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 96:9100–9105.

Austad, S. N. 1993. Retarded senescence in an insular population of Virginia opossums (*Didelphis virginiana*). J Zool. 229:695–708.

Austad, S. N., and J. M. Hoffman. 2018. Is antagonistic pleiotropy ubiquitous in aging biology? Evol. Med. Public Heal. 1:287–294.

Bailey, S. F., and T. Bataillon. 2016. Can the experimental evolution programme help us elucidate the genetic basis of adaptation in nature? Mol. Ecol. 25:203–218.

Baldwin-Brown, J. G., A. D. Long, and K. R. Thornton. 2014. The power to detect quantitative trait loci using resequenced, experimentally evolved populations of diploid, sexual organisms. Mol. Biol. Evol. 31:1040–1055.

Barter, T. T., Z. S. Greenspan, M. A. Phillips, L. D. Muller, M. R. Rose, and J. M. Ranz. 2019. *Drosophila* transcriptomics with and without ageing. Biogerontology 20:699–710.

Boselli, M., J. Rock, E. Ünal, S. S. Levine, and A. Amon. 2009. Effects of age on meiosis in budding yeast. Dev. Cell. 16:844–855.

Bronikowski, A. M., and S. J. Arnold. 1999. The evolutionary ecology of life history variation in the garter snake *Thamnophis elegans*. Ecology 80:2314–2325.

Brooks, A., G. J. Lithgow, T. E. Johnson, and G. J. Lithgow. 1994. Mortality rates in a genetically heterogeneous population of *Caenorhabditis elegans*. Science 263:668–671.

Bubliy, O. A., and V. Loeschcke. 2005. Correlated responses to selection for stress resistance and longevity in a laboratory population of *Drosophila melanogaster*. Journal of Evolutionary Biology 18:789–803.

Burke, M. K. 2012. How does adaptation sweep through the genome? Insights from long-term selection experiments. Proc. R. Soc. B Biol. Sci. 179:5029–5038.

Burke, M. K., J. P. Dunham, P. Shahrestani, K. R. Thornton, M. R. Rose, and A. D. Long. 2010. Genome-wide analysis of a long-term evolution experiment with *Drosophila*. Nature 467:587–590.

Burke, M. K., G. Liti, and A. D. Long. 2014. Standing genetic variation drives repeatable experimental evolution in outcrossing populations of *Saccharomyces cerevisiae*. Mol. Biol. Evol. 31:3228–3239.

Burny, C., V. Nolte, P. Nouhaud, M. Dolezal, and C. Schlötterer. 2020. Secondary evolve and resequencing: an experimental confirmation of putative selection targets without phenotyping. Genome Biology and Evolution 12:151–159.

Burke, M. K. & M. R. Rose. 2009. Experimental evolution with *Drosophila*. American Journal of Physiology: Regulatory, Integrative and Comparative Physiology 296:R1847–R1884.

Carlson, S. M., R. Hilborn, A. P. Hendry, and T. P. Quinn. 2007. Predation by bears drives senescence in natural populations of salmon. PLoS ONE 2:e1286.

Carnes, M. U., T. Campbell, W. Huang, D. G. Butler, M. A. Carbone, L. H. Duncan, S. V. Harbajan, E. M. King, K. R. Peterson, A. Weitzel, et al. 2015. The genomic basis of postponed senescence in *Drosophila melanogaster*. PLoS ONE 10:e0138569.

Carvalho, S., P. C. Phillips, and H. Teotonio. 2014. Hermaphrodite life history and the maintenance of partial selfing in experimental populations of *Caenorhabditis elegans*. BMC Evol. 14:117.

Charlesworth, B. 1994. Evolution in age-structured populations. 2nd ed. Cambridge Univ. Press, Cambridge, U.K.

Chen, H. Y., and A. A. Maklakov. 2012. Longer life span evolves under high rates of condition-dependent mortality. Curr. Biol. 22:2140–2143.

Chen, H. Y., F. Spagopoulou, and A. A. Maklakov. 2016. Evolution of male age-specific reproduction under differential risks and causes of death: males pay the cost of high female fitness. J. Evol. Biol. 29:848–856.

Chippindale, A. K., J. A. Alipaz, H. - W. Chen, and M. R. Rose. 1997. Experimental evolution of accelerated development in *Drosophila*. 1. Developmental speed and larval survival. Evolution 51:1536–1551.

De Castro, E., S. H. De Castro, and T. E. Johnson. 2004. Isolation of long-lived mutants in *Caenorhabditis elegans* using selection for resistance to juglone. Free Radic. Biol. Med. 37:139–145.

Ducrocq, V. 2005. An improved model for the French genetic evaluation of dairy bulls on length of productive life of their daughters. Anim. Sci. 80:249–256.

Ducrocq, V., B. Besbes, and M. Protais. 2000. Genetic improvement of laying hens viability using survival analysis. Genetics Selection Evolution 32:23-40.

Dudycha, J. L., and A. J. Tessier. 1999. Natural genetic variation of life span, reproduction, and juvenile growth in *Daphnia*. Evolution 53:1744–1756.

Edney, E. B., and R. B. Gill. 1968. Evolution of senescence and specific longevity. Nature 220:281-282.

Essl, A. 1998. Longevity in dairy cattle breeding: a review. Livest. Prod. Sci. 57:79–89.

Ewald, C. Y., J. I. Castillo-Quan, and T. K. Blackwell. 2017. Untangling longevity, Dauer, and healthspan in *Caenorhabditis elegans* insulin/IGF-1-signalling. Gerontology 64:96–104.

Fabian, D. K., K. Garschall, P. Klepsatel, G. Santos-Matos, É. Sucena, M. Kapun, B. Lemaitre, C. Schlötterer, R. Arking, and T. Flatt. 2018. Evolution of longevity improves immunity in *Drosophila*. Evol. Lett. 2:567–579.

Farid, A., D. C. Crober, H. Van Der Steen, D. L. Patterson, and M. P. Sabour. 2002. Reproductive performance of mice selected for reproductive longevity. *In* 7th World Congress on Genetics Applied to Livestock Production 08–13.

Garreau, H., C. Larzul, F. Tudela, J. Ruesche, V. Ducrocq, and L. Fortun-Lamothe. 2017. Energy balance and body reserves in rabbit females selected for longevity. World Rabbit Sci. 25:205–213.

Grainger, T. N., S. M. Rudman, P. Schmidt, and J. M. Levine. 2021. Competitive history shapes rapid evolution in a seasonal climate. Proc. Natl. Acad. Sci. USA 118:e2015772118.

Graves, J. L., K. L. Hertweck, M. A. Phillips, M. V. Han, L. G. Cabral, T. T. Barter, L. F. Greer, M. K. Burke, L. D. Mueller, M. R. Rose, et al. 2017. Genomics of parallel experimental evolution in *Drosophila*. Mol. Biol. Evol. 34:831–842.

Gray, J. C., and A. D. Cutter. 2014. Mainstreaming *Caenorhabditis elegans* in experimental evolution. Proc R Soc B. 281:20133055.

Haldane, J. 1941. New paths in genetics. George Allen and Unwin, Lond.

Hamilton, W. D. 1966. The moulding of senescence by natural selection. J. Theor. Biol. 12:12–45.

Hillis, D. A., L. Yadgary, G. M. Weinstock, D. P. Pardo-Manuel de Villena, A. S. Fowler, S. Xu, F. Chan, and T. Garland. 2020. Genetic basis of aerobically supported voluntary exercise: results from a selection experiment with house mice. Genetics 216:781–804.

Huang, W., T. Campbell, M. A. Carbone, W. E. Jones, D. Unselt, R. R. H. Anholt, and T. F. C. Mackay. 2020. Context-dependent genetic architecture of *Drosophila* life span. PLoS Biol. 18:e3000645.

Hunt, J., M. D. Jennions, N. Spyrou, and R. Brooks. 2006. Artificial selection on male longevity influences age-dependent reproductive effort in the black field cricket *Teleogryllus commodus*. Am. Nat. 168:72–86.

Jarolim, S., J. Millen, G. Heeren, P. Laun, D. S. Goldfarb, and M. Breitenbach. 2004. A novel assay for replicative lifespan in *Saccharomyces cerevisiae*. FEMS Yeast Res. 5:169–177.

Jenkins, N. L., G. McColl, and G. J. Lithgow. 2004. Fitness cost of extended lifespan in *Caenorhabditis elegans*. Proc. R. Soc. B Biol. Sci. 271:2523–2526.

Johnson, A. A., M. N. Shokhirev, and B. Shoshitaishvili. 2019. Revamping the evolutionary theories of aging. Ageing Res. Rev. 55:100947.

Johnson, T. E., and E. W. Hutchinson. 1993. Absence of strong heterosis for life span and other life history traits in *Caenorhabditis elegans*. Genetics 134:465–474.

Johnson, T. E., and W. B. Wood. 1982. Genetic analysis of life-span in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. U. S. A. 79:6603–6607.

Kaeberlein, M., C. R. Burtner, and B. K. Kennedy. 2007. Recent developments in yeast aging. PLoS Genet. 3:e84.

Kawecki, T. J., R. E. Lenski, D. Ebert, B. Hollis, I. Olivieri, and M. C. Whitlock. 2012. Experimental evolution. Cell Press. 27:547–560.

Kirkwood, T. B. L., and S. N. Austad. 2000. Why do we age? Nature 408:233–238.

Kofler, R., and C. Schlötterer. 2014. A guide for the design of evolve and resequencing studies. Mol. Biol. Evol. 31:474–483.

Kuether, K., and R. Arking. 1999. *Drosophila* selected for extended longevity are more sensitive to heat shock. Age 22:175–180.

Kulminski, A. M., S. V. Ukraintseva, I. V. Culminskaya, K. G. Arbeev, K. C. Land, L. Akushevich, and A. I. Yashin. 2008. Cumulative deficits and physiological indices as predictors of mortality and long life. J Gerontol A Biol Sci Med Sci. 63:1053–1059.

Larzul, C., V. Ducrocq, F. Tudela, H. Juin, and H. Garreau. 2014. The length of productive life can be modified through selection: an experimental demonstration in the rabbit. J. Anim. Sci 92:2395–2401.

Leroi, A. M., A. K. Chippindale, and M. R. Rose. 1994. Long-term laboratory evolution of a genetic life-history trade-off in *Drosophila melanogaster*. 1. The role of genotype-by-environment interaction. Evolution 48:1244-1257.

Lindstrom, D. L., and D. E. Gottschling. 2009. The mother enrichment program: a genetic system for facile replicative life span analysis in *Saccharomyces cerevisiae*. Genetics 183:413–422.

Longo, V. D., G. S. Shadel, M. Kaeberlein, and B. Kennedy. 2012. Replicative and chronological aging in *Saccharomyces cerevisiae*. Cell Metab. 16:18-31.

Luckinbill, L. S. 1998. Selection for longevity confers resistance to low-temperature stress in *Drosophila melanogaster*. Journals Gerontol. Ser. A Biol. Sci. Med. Sci. 53:47–53.

Luckinbill, L. S., R. Arking, M. J. Clare, W. C. Cirocco, and S. A. Buck. 1984. Selection for delayed senescence in *Drosophila melanogaster*. Evolution 38:996-1003.

Lund, J., P. Tedesco, K. Duke, J. Wang, S. K. Kim, and T. E. Johnson. 2002. Transcriptional profile of aging in *C. elegans*. Current Biology 12:1566–1573.

Medawar, P. B. 1946. Old age and natural death. Modern Q 2:30–49.

Medawar, P. B. 1952. An unsolved problem of biology. Lewis, Lond.

Miyatake, T. 1997. Genetic trade-off between early fecundity and longevity in *Bactrocera cucurbitae* (Diptera: Tephritidae). Heredity 78:93–100.

Mortimer, R. K., and J. R. Johnston. 1959. Life span of individual yeast cells. Nature 183:1751–1752.

Mueller, L. D., M. A. Phillips, T. T. Barter, Z. S. Greenspan, and M. R. Rose. 2018. Genome-wide mapping of gene-phenotype relationships in experimentally evolved populations. Mol. Biol. Evol. 35:2085–2095.

Muñoz, M. J., and D. L. Riddle. 2003. Positive selection of *Caenorhabditis elegans* mutants with increased stress resistance and longevity. Genetics 163:171–180.

Nagai, J., C. Y. Lin, and M. P. Sabour. 1995. Lines of mice selected for reproductive longevity. Growth, Dev. Aging. 59:79–91.

Nagai, J., C. Y. Lin, and H. Sasada. 1990. Selection for increased length of reproductive life in mice. Theor. Appl. Genet. 79:268–272.

Norry, F. M., and V. Loeschcke. 2002. Temperature-induced shifts in associations of longevity with body size in *Drosophila melanogaster*. Evolution 56:299–306.

Palopoli, M. F., C. Peden, C. Woo, K. Akiha, M. Ary, L. Cruze, J. L. Anderson, and P. C. Phillips. 2015. Natural and experimental evolution of sexual conflict within *Caenorhabditis* nematodes. BMC Evol. Biol. 15:1–13.

Parker, G. A., N. Kohn, A. Spirina, A. McMillen, W. Huang, and T. F. C. Mackay. 2020. Genetic basis of increased lifespan and postponed senescence in *Drosophila melanogaster*. G3 Genes, Genomes, Genet 10:1087–1098.

Partridge, L., and K. Fowler. 1992. Direct and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. Evolution 46:76–91.

Partridge, L., N. Prowse, and P. Pignatelli. 1999. Another set of responses and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. Proc. R. Soc. B Biol. Sci. 266:255–261.

Phelan, J. P., M. A. Archer, K. A. Beckman, A. K. Chippindale, T. J. Nusbaum, and M. R. Rose. 2003. Breakdown in correlations during laboratory evolution. I. Comparative analyses of *Drosophila* populations. Evolution 57:527–535.

Phillips, M. A., and M. K. Burke. 2021. Can laboratory evolution experiments teach us about natural populations? Mol Ecol. 30:877–879.

Phillips, M. A., A. D. Long, Z. S. Greenspan, L. F. Greer, M. K. Burke, B. Villeponteau, K. C. Matsagas, C. L. Rizza, L. D. Mueller, and M. R. Rose. 2016. Genome-wide analysis of long-term evolutionary domestication in *Drosophila melanogaster*. Sci. Rep. 6:39281.

Rang, C. U., A. Y. Peng, and L. Chao. 2011. Temporal dynamics of bacterial aging and rejuvenation. Curr. Biol. 21:1813–1816.

Reed, D. H., and E. H. Bryant. 2000. The evolution of senescence under curtailed life span in laboratory populations of *Musca domestica* (the housefly). Heredity 85:115–121.

Remolina, S. C., P. L. Chang, J. Leips, S. V. Nuzhdin, and K. A. Hughes. 2012. Genomic basis of aging and life-history evolution in *Drosophila Melanogaster*. Evolution 66:3390–3403.

Reynolds, R. M., and P. C. Phillips. 2013. Natural variation for lifespan and stress response in the nematode *Caenorhabditis remanei*. PLoS One 8:E58212.

Reznick, D. 1993. New model systems for studying the evolutionary biology of aging: Crustacea. Genetica 91:79–88.

Reznick, D., M. Bryant, and D. Holmes. 2006. The evolution of senescence and post-reproductive lifespan in guppies (*Poecilia reticulata*). PLoS Biol. 4:0136–0143.

Reznick, D., G. Buckwalter, J. Groff, and D. Elder. 2001. The evolution of senescence in natural populations of guppies (*Poecilia reticulata*): a comparative approach. Exp. Gerontol. 36:791–812.

Reznick, D. N. 1997. Life history evolution in guppies (*Poecilia reticulat*a): guppies as a model for studying the evolutionary biology of aging. Exp. Gerontol 32:245-258.

Reznick, D. N., M. J. Bryant, D. Roff, C. K. Ghalambor, and D. E. Ghalambor. 2004. Effect of extrinsic mortality on the evolution of senescence in guppies. Nature 431:1095–1099.

Rose, M. R. 1994. Evolutionary biology of aging. Oxford Univ. Press, New York.

Rose, M. R. 1984. Laboratory evolution of postponed senescence in *Drosophila melanogaster*. Evolution 38:1004-1010.

Rose, M. R., L. N. Vu, S. U. Park, and J. L. Graves. 1992. Selection on stress resistance increases longevity in *Drosophila melanogaster*. Exp. Gerontol 27:241–250.

Rudman, S. M., S. Greenblum, R. C. Hughes, S. Rajpurohit, O. Kiratli, D. B. Lowder, S. G. Lemmon, D. A. Petrov, J. M. Chaston, and P. Schmidt. 2019. Microbiome composition shapes rapid genomic adaptation of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA. 116:20025–20032.

Sánchez, J. P., P. Theilgaard, C. Mínguez, and M. Baselga. 2008. Constitution and evaluation of a long-lived productive rabbit line. J. Anim. Sci. 86:515–525.

Sarup, P., P. Sørensen, and V. Loeschcke. 2011. Flies selected for longevity retain a young gene expression profile. Age 33:69–80.

Savietto, D., C. Cervera, E. Blas, M. Baselga, T. Larsen, N. C. Friggens, and J. J. Pascual. 2013. Environmental sensitivity differs between rabbit lines selected for reproductive intensity and longevity. Animal 7: 1969–1977.

Serenius, T., and K. J. Stalder. 2006. Selection for sow longevity. J Anim Sci. 84:E166-E171.

Service, P. M. 1987. Physiological mechanisms of increased stress resistance in *Drosophila melanogaster* selected for postponed senescence. Physiol. Zool. 60:321–326.

Shahrestani, P., M. K. Burke, R. Birse, J. N. Kezos, K. Ocorr, L. D. Mueller, M. R. Rose, and R. Bodmer. 2017. Experimental evolution and heart function in *Drosophila*. Physiol. Biochem. Zool. 90: 281–293.

Shahrestani, P., E. King, R. Ramezan, M. Phillips, M. Riddle, M. Thornburg, Z. Greenspan, Y. Estrella, K. Garcia, P. Chowdhury, et al. 2021. The genetic basis of Drosophila melanogaster defense against Beauveria bassiana explored through evolve and resequence and quantitative trait locus mapping. G3 11:jkab324.

Skrzypek, M. S., R. S. Nash, E. D. Wong, K. A. Macpherson, S. T. Hellerstedt, S. R. Engel, K. Karra, S. Weng, T. K. Sheppard, G. Binkley,

et al. 2018. *Saccharomyces* genome database informs human biology. Nucleic Acids Res. 46:D736–D742.

Stearns, S. C., M. Ackermann, M. Doebeli, and M. Kaiser. 2000. Experimental evolution of aging, growth, and reproduction in fruitflies. Proc. Natl. Acad. Sci 97:3309–3313.

Stewart, E. J., R. Madden, G. Paul & F. Taddei. 2005. Aging and death in an organism that reproduces by morphologically symmetric division. PLoS Biol. 3:0295–0300.

Tatar, M., D. W. Grey, and J. R. Carey. 1997. Altitudinal variation for senescence in *Melanoplus* grasshoppers. Oecologia 111:357–364.

Teotónio, H., S. Estes, P. C. Phillips, and C. F. Baer. 2017. Experimental evolution with *Caenorhabditis* nematodes. Genetics 206:691–716.

Theilgaard, P., J. P. Sánchez, J. J. Pascual, P. Berg, N. C. Friggens, and M. Basegla. 2007. Late reproductive senescence in a rabbit line hyper selected for reproductive longevity, and its association with body reserves. Genet. Sel. Evol. 39:207–223.

Tucic, N., I. Gliksman, D. Seslija, D. Milanovic, S. Mikuljanac, and O. Stojkovic. 1996. Laboratory evolution of longevity in the bean weevil (*Acanthoscelides obtectus*). J. Evol. Biol. 9:485–503.

Ünal, E., B. Kinde, and A. Amon. 2011. Gametogenesis eliminates age-induced cellular damage and resets life span in yeast. Science 332: 1554–1557.

Walker, D. W., G. McColl, N. L. Jenkins, J. Harris, and G. J. Lithgow. 1991. Evolution of lifespan in *C. elegans*. Nature 405:296–297.

Williams, G. C. 1957. Pleiotropy, natural selection, and the evolution of senescence. Evolution 11:398–411.

Wit, J., T. N. Kristensen, P. Sarup, J. Frydenberg, and V. Loeschcke. 2013a. Laboratory selection for increased longevity in *Drosophila melanogaster* reduces field performance. Exp. Gerontol. 48: 1189–1195.

Wit, J., P. Sarup, N. Lupsa, H. Malte, J. Frydenberg, and V. Loeschcke. 2013b. Longevity for free? Increased reproduction with limited trade-offs in *Drosophila melanogaster* selected for increased life span. Exp. Gerontol. 48:349–357.

Wu, Z., M. Isik, N. Moroz, M. J. Steinbaugh, P. Zhang, and T. K. Blackwell. 2019. Dietary restriction extends lifespan through metabolic regulation of innate immunity. Cell Metab. 29:1192.e8–1205.e8.

Yamamoto, S., M. Jaiswal, W. L. Charng, T. Gambin, E. Karaca, G. Mirzaa, W. Wiszniewski, H. Sandoval, N. A. Haelterman, B. Xiong, et al. 2014. A drosophila genetic resource of mutants to study mechanisms underlying human genetic diseases. Cell 159:200–214.

Yanos, E., F. Melana, C. Bennett, and M. Kaeberlein. 2012. Genome-wide RNAi longevity screens in *Caenorhabditis elegans*. Curr. Genomics. 13:508–518.

Zeyl, C. 2006. Experimental evolution with yeast. FEMS Yeast Res. 6:685–691.

Zwaan, B., R. Bijlsma, and R. F. Hoekstra. 1995. Direct selection on life span in *Drosophila melanogaster*. Evolution 49:649–659.

Associate Editor: N. G. Prasad
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