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REVIEW

# The turquoise killifish: a genetically tractable model for the study of aging

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## ABSTRACT

Lifespan is a remarkably diverse trait in nature, ranging from just hours in adult mayflies to hundreds of years in the Greenland shark and quahog clam. Great disparities in lifespan are often observed even among somewhat closely related species; for example, in the laboratory, wild-derived strains of the common house mouse have a maximum observed lifespan of approximately 6 years, while a similarly sized rodent, the naked mole rat, can live for over 30 years. Comparative biology of aging across the tree of life provides a tremendous opportunity for understanding the molecular and genetic basis underlying lifespan and aging. However, a lack of molecular and laboratory tools has limited the ability of researchers to take full advantage of the incredible diversity of aging phenotypes in nature. Recent developments in genomic technology have made it increasingly possible to study non-canonical model organisms for aging. One promising new genetic model organism amenable to a range of experimental interventions is the turquoise killifish (*Nothobranchius furzeri*). This fish species has a naturally short lifespan and undergoes a wide range of aging-related transformations. These fish have a fully sequenced genome and transcriptome, and killifish embryos are accessible to transgenesis and genome editing. Furthermore, different killifish species and populations show striking differences in lifespan, providing the opportunity for comparative analysis of aging. This Review introduces the natural life history of the turquoise killifish, its emerging applicability as an aging model system, the genetic tools that have been developed to study aging for this species and a summary of recent studies facilitated by these new tools.


**KEY WORDS: Aging, Evolution, *Nothobranchius furzeri*, Lifespan, Model organism**

## Killifish as a laboratory model for the biology of aging

Aging consists of progressive functional decline and increased risk of mortality over an organism's life, and is the leading risk factor for numerous lethal diseases, motivating significant effort in recent decades to understand its molecular and genetic basis (López-Otín et al., 2013). A great deal of progress to understand the molecular basis of aging has been made by studying canonical laboratory model organisms including yeast, nematodes, fruit flies and mice. Studying this group of organisms has helped in the discovery of shared aging regulatory mechanisms relying on the insulin/IGF-1 pathway, mTOR signaling, telomere and mitochondrial functions, and processes involved in DNA and protein homeostasis (Baker et al., 2016; Gems and Partridge, 2013; Kauppila et al., 2017).

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Nematodes and fruit flies have short lifespans and are experimentally tractable; however, they cannot recapitulate many aspects of vertebrate aging, including processes involving stem cell dynamics and cancer. Adult worms and flies are largely post-mitotic, lacking adult stem cells in many organs, and rely exclusively on innate immunity to fight off parasitic infections, establish commensal microbial communities, and regulate tissue repair and regeneration. Furthermore, worms and flies entirely lack a lymphocyte-based adaptive immune system, which provides vertebrates with a sophisticated system to precisely respond to a vast diversity of antigens, with increasing speed and robustness across multiple encounters through immune memory (Flajnik and Kasahara, 2010; Müller et al., 2018). Additionally, small invertebrates like nematodes and flies have simple commensal microbial communities, unlike vertebrates, which normally have large and diverse microbial communities that modulate several aspects of host life (Seidel and Valenzano, 2018; Smith et al., 2017). In contrast, common vertebrate model organisms such as mice and zebrafish have long median lifespans of over three years, making it difficult to experimentally follow the entire course of aging as a continuous longitudinal process (Gerhard, 2003; Yuan et al., 2009). Thus, classical experimental aging models consist on the one hand of short-lived invertebrates, and on the other hand of longer-lived vertebrates.

Thus, there is a need for new aging models, not only to bridge the gap between short-lived invertebrates and long-lived vertebrates, but also to explore more of the rich diversity of aging phenotypes observed in nature. The turquoise killifish is a particularly interesting candidate, as it has an invertebrate-like lifespan and yet maintains conserved features of vertebrate aging.

## Annual killifish life cycle

Killifishes of the genus *Nothobranchius* are distributed throughout Central and Eastern Africa in freshwater environments characterized by pronounced seasonal differences in water availability resulting from alternating dry and rainy seasons. In the driest regions, the typical rainy season lasts from December to February, with a dry season from March to November (Tozzini et al., 2013). These fish are small (usually <7 cm long), and sexually dimorphic in size and coloration. Killifish inhabit seasonal water pools that fill during the short rainy season and dry up completely during the subsequent and longer dry season. To survive and reproduce in this transient environment, killifish have evolved a unique annual life cycle, characterized by a prolonged period of embryonic stasis called diapause, followed by rapid growth and sexual maturation (Fig. 1). Following sexual maturation, annual killifish display a short adult lifespan and a wide range of aging-related transformations (Cellerino et al., 2016). An annual life cycle is unusual among vertebrates, and has been reported only in annual killifish and in the Labord's chameleon (*Furcifer labordi*), a semelparous chameleon species from Madagascar (Furness, 2016; Karsten et al., 2008; Polačik et al., 2014; Wourms, 1972). With a median lifespan of

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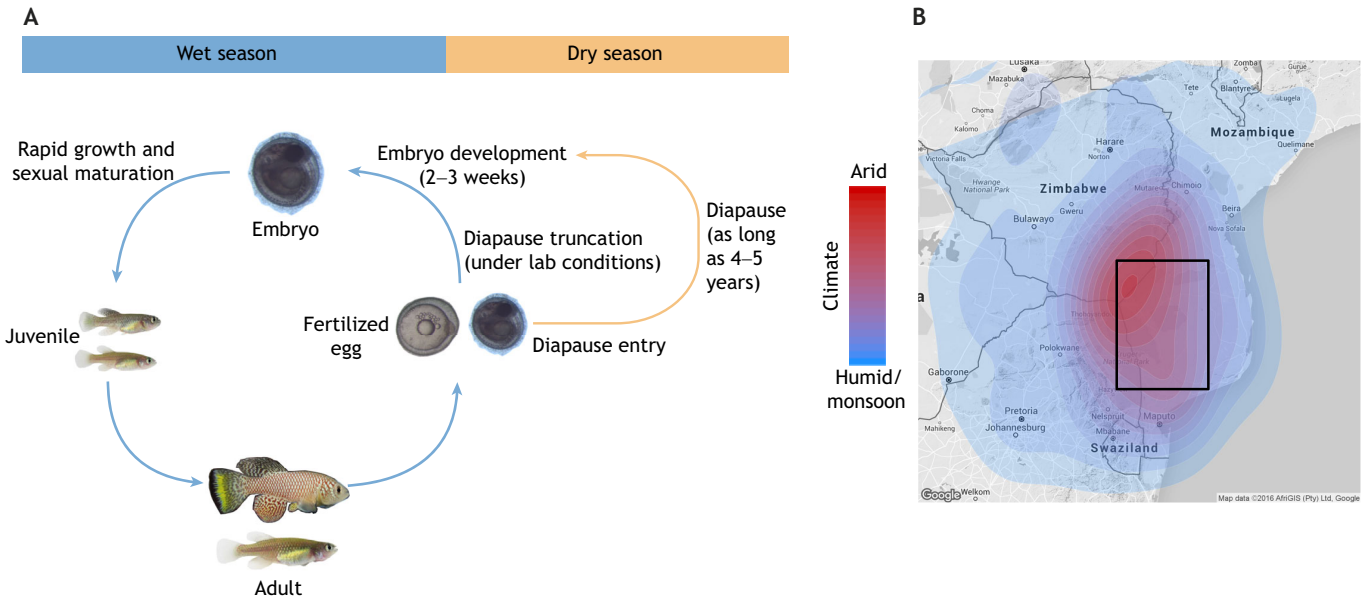


**Fig. 1. Life cycle and habitat of the turquoise killifish *Nothobranchius furzeri*.** (A) The turquoise killifish life cycle. After hatching, killifish mature rapidly and begin breeding within a few weeks. After sexual maturation, killifish continue growing throughout their lifespan. Fertilized eggs can developmentally arrest in diapause at various stages of development and can persist in diapause for several years. Upon diapause escape, embryos mature in 2–3 weeks before hatching. (B) Turquoise killifish inhabit seasonal ponds in Zimbabwe and Mozambique.

4 months in the shortest-lived strain, the turquoise killifish (*Nothobranchius furzeri*) is the shortest-lived vertebrate to date raised in captivity, making it well-suited for longitudinal aging studies (Smith et al., 2017; Valenzano et al., 2017). Both the exceptionally compressed lifespan and the wide range of conserved aging phenotypes, have made annual killifish a powerful model system for the study of vertebrate aging (Hu and Brunet, 2018).

In nature, once the dry season ends and natural pools are replenished with rainwater, embryos hatch and rapidly grow to sexual maturity. Male and female turquoise killifish have been observed in the wild to achieve sexual maturity within 14–15 days, growing 10-fold in length over this period, from 5 mm up to >50 mm (Vrtílek et al., 2018b). Upon reaching maturity, killifish breed and lay eggs daily (Cellerino et al., 2016). Annual killifish populations survive by producing desiccation-resistant embryos that have entered a developmental arrest stage called diapause, which remain sequestered in pond sediment. Killifish diapause consists of three stages occurring before gastrulation (diapause I), during somitogenesis (diapause II) and in late development just prior to hatching, upon completion of organogenesis (diapause III). All three stages are obligatory, but can last different lengths of time, depending on external stimuli. Diapause entry and escape have been linked in annual American killifishes to regulation via environmental temperature, as well as maternally transmitted cues (Podrabsky et al., 2010; Romney and Podrabsky, 2017) and have recently been found to depend on a vitamin D signaling pathway (Romney et al., 2018).

Overall, annual killifishes have adapted to their unique environment by evolving a life cycle characterized by a prolonged embryonic developmental arrest during the dry seasons and explosive growth and sexual maturation during wet seasons. Among annual species, different selective and demographic constraints have shaped adult lifespan. In extreme cases, severe population bottlenecks occurring in annual environments can cause a decrease of effective population size, a weakening of purifying selection, and may lead to the genome-wide accumulation of deleterious mutations responsible for a vast range of age-related dysfunctions (Cui et al., 2019). Together, embryonic diapause, short lifespan and age-related dysfunctions contribute to making the killifish an ideal model organism for aging research.

Several *Nothobranchius* species have been raised in the lab and their lifespan has been studied. Maximum lifespans for captivity-raised *Nothobranchius* can reach 18 months (Genade et al., 2005; Herrera and Jagadeeswaran, 2004; Lucas-Sanchez et al., 2014; Zhou et al., 2019). The turquoise killifish (*Nothobranchius furzeri*), which is the shortest-lived vertebrate raised in captivity, has been proposed as an ideal model organism to bridge the gap between long-lived vertebrate and short-lived invertebrate models for aging (Kim et al., 2016; Valdesalici and Cellerino, 2003). The original laboratory strain of turquoise killifish was isolated in Gonarezhou National Park in 1968 and maintained as a pure-breeding line known as ‘GRZ’ since then. This strain has a median lifespan ranging between 12 and 16 weeks, depending on husbandry and laboratory setup (Kirschner et al., 2012; Valenzano et al., 2015). Captive lifespan in turquoise killifish is consistent with natural aging observed in the wild, where adult populations have been observed to decline in size and individual fitness and eventually disappear before pools have dried out, indicating that their rapid aging is not an experimental artifact due to laboratory conditions (Vrtílek et al., 2018a). Importantly, the turquoise killifish retains common signatures of vertebrate aging despite its compressed lifespan. At the physiological level, turquoise killifish display observable indications of aging that include loss of pigmentation, spinal kyphosis, sarcopenia, a decline in fecundity, and vision deterioration (Kim et al., 2016). As killifish age, they spend less time actively swimming and swim more slowly, exploring new environments less vigorously (Genade et al., 2005; Smith et al., 2017; Valenzano et al., 2006b). Furthermore, aged killifish perform worse on a test of active avoidance (Valenzano et al., 2006b), indicating that a global decline in overall health occurs during aging.

Turquoise killifish also demonstrate molecular signatures of vertebrate aging (López-Otín et al., 2013), including a decrease in

Journal of Experimental Biology (2020) 223, jeb209296. doi:10.1242/jeb.209296

mitochondrial copy number and function (Hartmann et al., 2011), an increase in senescence-associated β-galactosidase staining (Ahuja et al., 2019; Baker et al., 2016; Cristofalo, 2005; Dimri et al., 1995; Valenzano et al., 2006a), shortened telomeres (Hartmann et al., 2009), loss of regenerative capacity in the fin (Wendler et al., 2015), as well as an increased risk of cancer, especially in the liver (Baumgart et al., 2015).

Similarly to other teleosts, adult neurogenesis occurs in killifish neurogenic niches throughout the brain (Tozzini et al., 2012). However, during aging, neurogenic niches in the turquoise killifish are less active, and the overall patterns of increased age-dependent gliosis and global changes in brain transcriptional regulation are conserved between the killifish and humans (Baumgart et al., 2014; Tozzini et al., 2012). Specific non-coding microRNAs display consistently higher or lower expression with age in the killifish brain, and miRNA-29 has been identified as a potential factor that counteracts age-related damage due to iron accumulation, implicating miRNAs as regulators of neural aging (Ripa et al., 2017; Terzibasi Tozzini et al., 2014). During aging, turquoise killifish remarkably display hallmarks of Parkinson's disease, including deterioration of dopaminergic and noradrenergic neurons and an accumulation of α-synuclein-containing inclusion bodies (Matsui et al., 2019).

Notably, turquoise killifish aging has been repeatedly demonstrated to be responsive to interventions. Established aging interventions such as dietary restriction and resveratrol feeding have prolonged lifespan and improved cognitive health in laboratory-raised killifish, indicating that the underlying aging processes are similar to those in other species and can be modulated (Terzibasi et al., 2009; Valenzano et al., 2006b). Reducing habitat water temperature increased turquoise killifish lifespan, delaying the onset of age-related behavioral deficits and slowing the rate of accumulation of age-related damage (Valenzano et al., 2006a). Mitochondrial complex I inhibition with rotenone in middle-aged fish extended the lifespan and reset the transcriptomic signatures of aging (Baumgart et al., 2016). Most recently, transfer of gut microbiota from young fish into middle-aged fish after antibiotic treatment prolonged lifespan and improved functional health in old age, as measured by their exploratory behavior (Smith et al., 2017). Along with demonstrating the modulability of the underlying aging mechanisms in fish, the responsiveness of killifish aging and lifespan to interventions also suggests its suitability as a candidate for testing potential anti-aging therapies.

The broad aging spectrum of aging-related phenotypes and its experimental accessibility have motivated the development of the killifish as a widely usable model for the study of aging, inspiring the establishment of standardized, easily implementable protocols for turquoise killifish husbandry and breeding (Dodzian et al., 2018; Polačik et al., 2016). Owing to its origins in small, subtropical pools, turquoise killifish tolerate a large range of husbandry conditions, including tank space, water temperature and salinity parameters (Polačik et al., 2016). Turquoise killifish facilities can be readily set up using available systems developed for zebrafish (Dodzian et al., 2018). Male and female turquoise killifish are easily distinguishable as adults, can be co-housed and breed constantly, without special requirements to facilitate reproduction. Prior to reproduction, turquoise killifish do not require that breeders are kept isolated; nor do they require seasonal simulation of the variations in light–dark cycles and temperatures, as occurs in other species, such as zebrafish, sticklebacks and salmon. Individual adult breeding killifish pairs lay around 100 embryos per week, which require 2–3 weeks to complete development and be ready to hatch (Dodzian et al., 2018; Reichard and Polačik, 2019).

## Genetic tools in the killifish

To make the turquoise killifish an experimentally tractable model for aging, genetic and genomic tools have been developed, including the transposon-based Tol2 transgenesis method, CRISPR/Cas9-based protocols for genome editing (Fig. 2), and a completely sequenced and annotated genome. In this section, we will summarize the tools that have been developed and important resulting findings.

### Transgenesis

The killifish's extremely short maturation time (20–30 days from hatching to sexual maturity in captivity) makes it especially attractive as a system for transgenesis, as stable transgenic lines can be generated within 2–3 months, which is unprecedented among vertebrates (Harel et al., 2016). Transgenesis in the killifish is based on microinjection into fertilized eggs at the one- and two-cell stage. Knock-in systems based on Tol2, an active transposable DNA element initially identified in medaka fish, allow efficient random genomic integration of a genetic construct in the turquoise

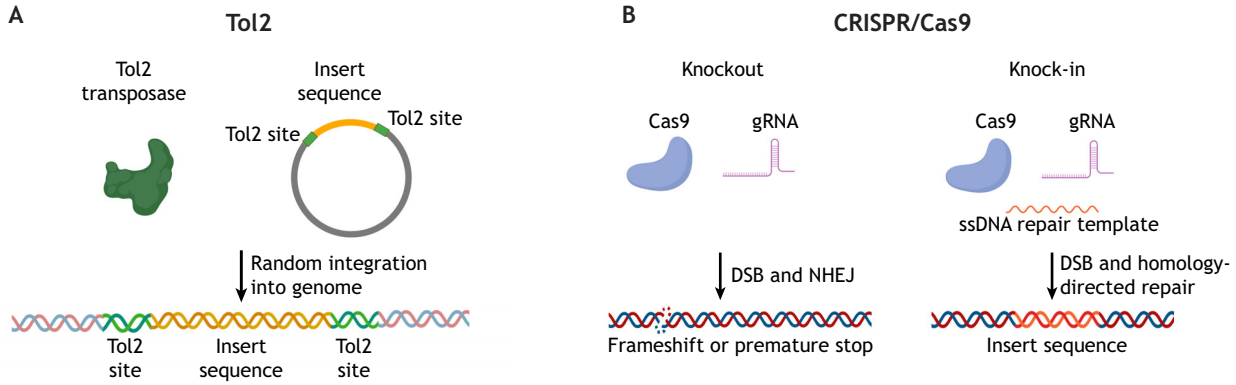


**Fig. 2. Transgenesis tools developed for use in *N. furzeri*.** Two methods for transgenesis have been developed and implemented for the turquoise killifish, both based on injection into the fertilized embryo at the one- or two-cell stage. (A) For Tol2-based transgenesis, Tol2 transposase mRNA is injected with a construct containing the desired DNA sequence flanked by two Tol2 recognition sequences, which then randomly integrates into the genome. (B) For CRISPR/Cas9 gene knockout, Cas9 mRNA or protein is injected along with a guide RNA target sequence. For editing via homology-directed repair, a single-stranded DNA template is also injected (Harel et al., 2015, 2016). This figure was created using BioRender.

killifish genome (Fig. 2) (Hartmann and Englert, 2012; Valenzano et al., 2011). The Tol2 transposon system for transgenesis requires injection of an RNA molecule encoding the transposase enzyme, and a DNA construct containing the gene of interest surrounded by two Tol2 recognition sequences. The transposase is then translated by host cells and recognizes the target sequence, which it then randomly integrates into the host genome (Kawakami, 2007).

The Tol2 system has been used recently to generate FUCCI (fluorescent ubiquitin-based cell cycle indicator) transgenic *Nothobranchius furzeri* strains, which express different fluorescent reporter proteins at either the G1 or S/G2 phases of the cell cycle and a double-transgenic strain that expresses both fluorescent reporters (Dolfi et al., 2019). By observing the relative intensities of each fluorescent protein over multiple days in the developing embryo using time-lapse microscopy, the authors were able to study cell cycle dynamics during the embryonic diapause process of the turquoise killifish in unprecedented detail, showing that exits from diapauses I and II are characterized at the cellular level by synchronized release from cell-cycle arrest and rapid bursts of cell division with consequent changes in the developing embryo's morphology (Dolfi et al., 2019). These transgenic reporter lines represent an excellent resource to continue uncovering the signaling pathways and the cellular transitions involved in killifish diapause. Given that some of the genetic pathways involved in diapause in *C. elegans* also regulate longevity, elucidating the mechanisms of diapause in the killifish has the potential to shed light on mechanisms involved in vertebrate aging (Kimura et al., 1997).

CRISPR/Cas9-based transgenesis systems have recently been developed in killifish and used to target specific genetic loci in order to study aging (Harel et al., 2015). This tool was first used to model telomere-related pathology in the killifish. Telomeres are DNA sequence repeats which protect the ends of eukaryotic chromosomes and are important for maintaining genomic stability (Carneiro et al., 2016). Average telomere length declines with age, and mutations in telomerase are associated with a variety of disorders, some of which resemble premature aging and result in an increased risk of cancer (Armanios and Blackburn, 2012). The role of telomere attrition in these processes has been extensively studied using telomerase-deficient mice; however, laboratory mice have much longer telomeres than humans and can tolerate the complete loss of telomerase for several generations before an effect is observed (Wright and Shay, 2000). In contrast, teleosts including zebrafish and killifish have shorter, human-like telomeres that progressively decline with age, potentially making them a good model to study telomere attrition in humans (Carneiro et al., 2016; Hartmann et al., 2009). Harel et al. (2015) used CRISPR/Cas9 to knock out TERT, the protein component of telomerase. Illustrating the advantages of the turquoise killifish's short life cycle for transgenesis, it took only a few months to generate a stable TERT-deficient line. TERT-deficient killifish showed defects in highly proliferative tissues including blood, intestine and germline, where a significant reduction in fertility was observed. TERT-deficient killifish also recapitulated the feature observed in some human telomeropathies of 'genetic anticipation', in which subsequent generations show increasingly severe phenotypes (including increased telomere shortening) due to cumulative germline damage. Thus, TERT-deficient turquoise killifish are a suitable short-lived model to recapitulate human telomerase deficiency. These authors (Harel et al., 2015) also generated several additional mutants in genes involved in other aging pathways, demonstrating that both targeted base-pair substitutions and knock-ins are possible in the killifish. CRISPR/Cas9 has also been used to knockdown the α-synuclein gene in *N. furzeri*, demonstrating that depletion of α-synuclein rescued the observed degeneration in dopaminergic and noradrenergic neurons in senescent turquoise killifish, supporting its use as a new model for idiopathic Parkinson's disease (Matsui et al., 2019).

### Mapping the turquoise killifish genome

The genome engineering approaches described above have been greatly facilitated by efforts to map the killifish genome. An initial partial shotgun sequencing effort characterized the size and structure of the killifish genome, revealing that the killifish genome is composed of 19 chromosomes, and between 1.6 and 1.9 billion nucleotide base pairs, although recent work has revised this estimate to ∼1.5 billion base pairs (Valenzano et al., 2015). The genome of the turquoise killifish has been shown to carry a high proportion of tandem repeat expansions (Reichwald et al., 2009; Willemsen et al., 2019 preprint). Early shotgun genome sequencing, together with an effort to genetically map the turquoise killifish genome, also confirmed that the short-lived GRZ strain is highly inbred, and led to the identification of a large number of genome-wide microsatellite repeat sequences that were used as genotypic markers to generate initial linkage maps based on classical analysis of recombination frequency between the microsatellites (Valenzano et al., 2009). Genetic mapping showed that the male tail yellow–red color polymorphism mapped to a single locus, revealed the turquoise killifish XX/XY sex-determination system and also identified the linkage group containing the sex-determination locus. Additionally, genetic mapping of the sex chromosome showed suppressed recombination in proximity to the sex-determination locus, but not in more telomeric regions of the male-specific sex-determining chromosome, which are pseudo-autosomal (Valenzano et al., 2009). Further characterization of the sex-determining locus in turquoise killifish showed that gdf6, a member of the TGF-β family, is the putative sex-determining gene in this species (Reichwald et al., 2015). A microsatellite-based linkage map of the turquoise killifish genome paved the way to further characterization, via quantitative trait locus (QTL) mapping, of the genetic factors involved in lifespan variation among killifish populations. Crossing the short-lived GRZ strain to the longer-lived MZM-0403 strain and measuring lifespan in the $F_2$ generation, Kirschner et al. (2012) generated a single-nucleotide variant-based linkage map and identified four loci on different linkage groups affecting lifespan, which together accounted for 27.4% of the observed variance in lifespan in the $F_2$ cross. Although a transcriptome catalogue was published in 2013 (Petzold et al., 2013), the lack of a complete genome sequence in the killifish made determining the specific genetic elements driving the observed differences in lifespan difficult, demonstrating the need for a full genome assembly.

In 2015, two independent groups assembled and annotated the turquoise killifish genome *de novo* (Reichwald et al., 2015; Valenzano et al., 2015). With one approach, 497 genes under positive selection were identified, some of which have been linked to aging in other species, and QTL mapping was conducted showing that differences in survival among turquoise killifish populations have a complex genetic architecture and that the major QTL associated with lifespan is located on the sex chromosome close to the sex-determination region and contains multiple aging-related genes known from studies of mice and humans (Valenzano et al., 2015). The second assembly approach involved RNA-seq, which found seven genomic regions with differential gene expression upon aging, and identified many genes that display similar differential expression patterns in both diapause and aging, suggesting links in mechanistic pathways between these two processes (Reichwald et al., 2015).

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### Comparative evolutionary genomics in killifishes

The declining cost of sequencing has made it possible to analyze genomic data in ever-increasing throughput and resolution, which has enabled several recent comparative genomics studies in the killifish. Interestingly, different strains of turquoise killifish show marked differences in lifespan and rate of aging, both in the laboratory and in the wild (Blažek et al., 2017; Valenzano et al., 2015). Furthermore, even closely geographically located populations show distinct genetic structuring, making them suitable for comparative genomics (Bartáková et al., 2013, 2015). The existence of closely related

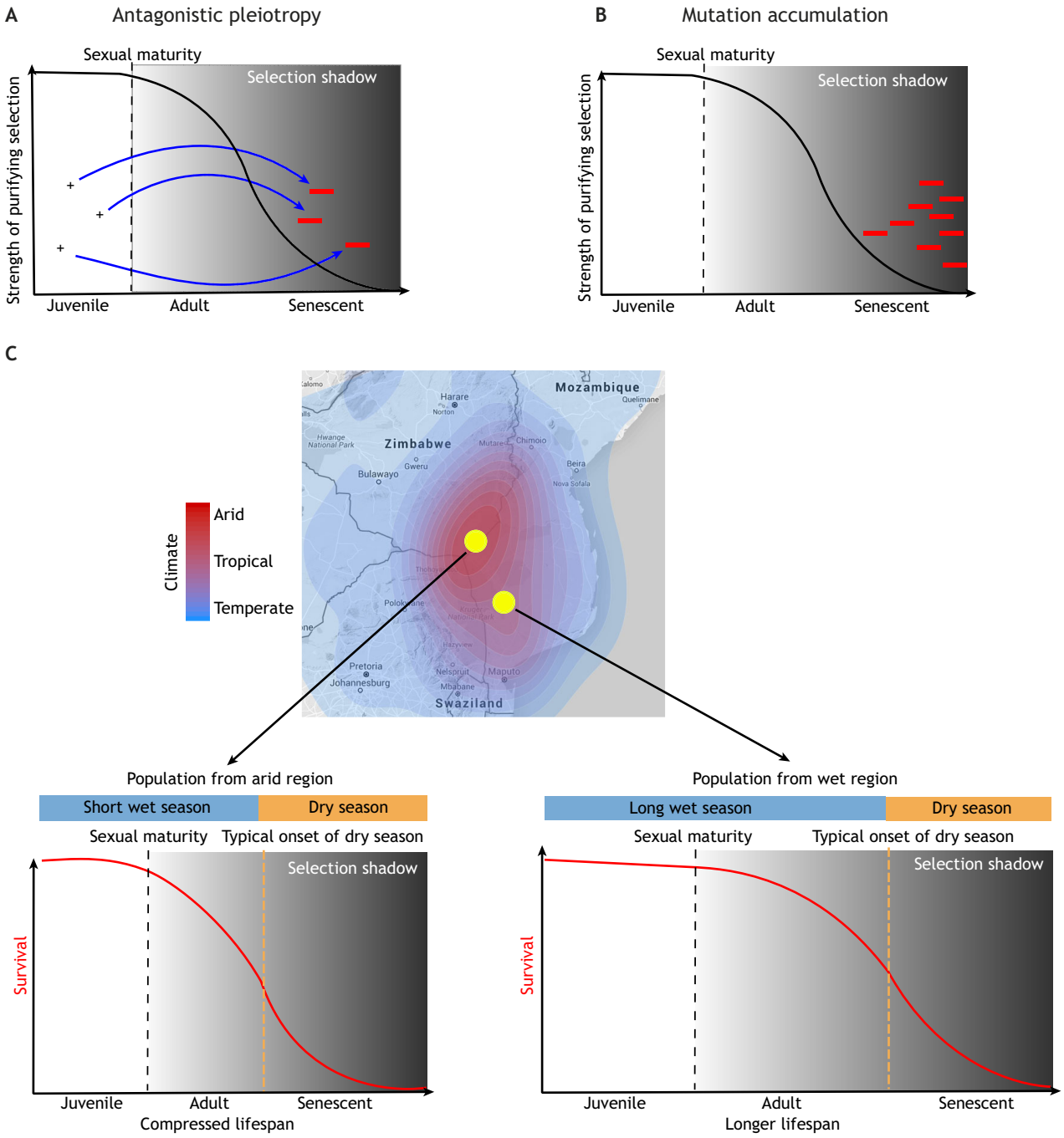


**Fig. 3. Testing evolutionary theories of aging in the killifish.** Classical evolutionary theories of aging are based on the conjecture that the strength of selection for a trait is proportional to the probability that an individual survives to that age, resulting in weaker selection pressure at higher age classes. This is referred to as the 'selection shadow'. (A) The antagonistic pleiotropy (AP) hypothesis proposes that genes that have detrimental effects later in life can increase in frequency and even be positively selected if they improve reproduction early in life and their overall contribution to fitness is positive. The '+' indicates variants with positive effects on fitness, and red '–' indicates gene variants that negatively impact fitness. Under AP, these are the same variants. (B) The mutation accumulation hypothesis states that late-acting deleterious variants will not be efficiently selected against and will persist in the population, cumulatively causing aging. (C) Turquoise killifish habitats vary in aridity. Populations experiencing a shorter rainy season and continuous bottlenecks are subject to a more rapid decrease in selection pressure due to drift, and are thus predicted to evolve more rapid aging.

comparable populations differing in lifespan and environment presents a unique natural experiment to test evolutionary theories of aging and life history.

Evolutionary theories of aging explain the evolution of genes affecting survival and aging, providing scenarios that justify the emergence of gene variants that negatively affect individual survival and reproduction at specific ages. Genes affecting age-dependent functional decline have been expected to evolve under two main scenarios (Fig. 3A,B). One scenario proposes that if a gene variant is positively selected, its frequency increases in the population, even if its phenotypic effect in late life is negative, contributing to disease and aging. However, as long as its overall fitness contribution is positive, its frequency will increase in the population (Williams, 1957). Another scenario shows that genes having phenotypic effects occurring after sexual maturation are under weaker selection than those expressed before sexual maturation, hence genes with deleterious effect in late life are less likely to be removed by purifying selection compared with those that have a deleterious effect before sexual maturation (Charlesworth, 2000; Medawar, 1952). Populations experiencing high extrinsic mortality are further expected to evolve more rapid aging, attributable to greater fitness gains from investment in early reproduction than in somatic maintenance (Stearns, 2000). However, there is no evidence that extrinsic mortality shapes age-specific mortality and aging patterns in nature (Reznick et al., 2004). The seasonal desiccation of the killifish habitat constitutes a strong source of extrinsic mortality, and may be partly responsible for the evolution of the remarkably short lifespan of annual killifish (Fig. 3C). Indeed, short lifespan in different turquoise killifish populations is correlated with greater aridity (and therefore harshness) of the habitats from which these populations are derived (Blažek et al., 2017; Valenzano et al., 2015). Population genetic inference on different turquoise killifish populations from a gradient of dry to wet environments has been recently used to test under which evolutionary scenario killifish populations have evolved longer or shorter lifespans.

Whole-genome sequencing of 120 fish collected from four natural populations of turquoise killifish in both dry and wet regions, showed that populations evolving in drier regions – which display shorter lifespan and accelerated aging – accumulate large numbers of deleterious mutations (Willemsen et al., 2019 preprint). Additionally, populations from dry regions had overall a smaller historical effective population size. Indeed, smaller effective population size limits the efficiency of natural selection and is compatible with the accumulation of deleterious mutations (Ohta, 1973), which may ultimately underlie the evolution of rapid aging and short lifespan (Willemsen et al., 2019 preprint; Cui et al., 2019).

By studying closely related annual and non-annual species of African killifishes using comparative genomics and population genetics, Cui et al. (2019) recently characterized the genomic basis underlying the evolution of short lifespan and rapid aging in African killifishes. Combining *de novo* genome assembly in four killifish species, and re-sequencing 41 additional African killifish species, the authors found that annual life cycle is associated with genome (both nuclear and mitochondrial) expansion, genome-wide relaxation of selection, and with widespread accumulation of deleterious mutations, including in genes already known to be important for longevity. Cui et al. (2019) also conducted population resequencing of 235 individuals from both dry and wet regions in two species, the bluefin notho (*Nothobranchius rachovii*) and the spotted killifish (*Nothobranchius orthonotus*), showing that individuals from dry habitats carry a higher mutational load in their genomes, in conjunction with fish from dry environments having smaller population sizes. These show that intermittent water availability is an extrinsic hazard underlying relaxation of selection and ultimately life history trait evolution. Together, comparative genomics and population genetics in killifish demonstrate an important role for relaxation of purifying selection as a force shaping the evolution of lifespan, both within and between killifish species, and provide an extended list of potential aging-related gene targets for further functional studies.

## Gene expression analysis in the killifish

Concurrent advances in gene expression analysis have recently enabled global interrogation of transcriptomic differences across lifespan in the turquoise killifish. RNA sequencing analysis of turquoise killifish brains at five distinct post-maturation time points identified gene pathways that appear to be upregulated or downregulated with age (Baumgart et al., 2014). Broadly, ribosome- and lysosome-related genes were found to be upregulated during aging, while mitochondrial, proteasomal and spliceosomal genes were downregulated, consistent with results from human brain aging studies (Baumgart et al., 2014). Comparing life-long transcriptomic changes in individual turquoise killifish from the same strain led to the identification of significant differences in gene expression between the shortest- and longest-lived fish, namely higher expression of mitochondrial complex I genes (Baumgart et al., 2016). Notably, this result was functionally validated, as administration of the complex I inhibitor rotenone to both killifish and zebrafish extended lifespan (Baumgart et al., 2016).

Transcriptome analysis was also used to identify host gene expression changes in the gut environment after microbial transfer to aged fish that may underlie observed differences in lifespan (Smith et al., 2017) and to validate transcriptional changes observed in immune response pathways in mice with age (Benayoun et al., 2019). Taken together, relying on the killifish genome assembly and advances in sequencing technology, killifish transcriptomics has shed light on genes and gene pathways that are relevant to vertebrate aging.

## Conclusions

Aging manifests in highly diverse ways across the tree of life, yet much of what has been learned about this fundamental process has come from the study of just a few organisms. Recent advances in genomic technology have made it possible for non-canonical organisms, including African killifishes, to be used for the study of aging. The assembly of high-quality genomic reference sequences in the killifish has enabled recent work that has begun to uncover the genetic basis of turquoise killifish aging, as well as the underlying evolutionary mechanisms. Furthermore, the development of readily implementable transgenesis tools has made it possible to quickly test potential aging mechanisms and has facilitated studies of killifish embryonic development. Looking forward, genomics and transgenesis have great potential to continue uncovering the evolutionary and molecular mechanisms underlying the unique biology of killifish, as well as helping to test potential conserved mechanisms of vertebrate aging, making killifish a powerful and broadly accessible model that fulfils the need for a short-lived vertebrate in aging research.

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#### Competing interests

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## References


**Ahuja, G., Bartsch, D., Yao, W., Geissen, S., Frank, S., Aguirre, A., Russ, N., Messling, J. E., Dodzian, J., Lagerborg, K. A. et al.** (2019). Loss of genomic integrity induced by lysosphingolipid imbalance drives ageing in the heart. *EMBO Rep.* **20**, e47407. doi:10.15252/embr.201847407

**Armanios, M. and Blackburn, E. H.** (2012). The telomere syndromes. *Nat. Rev. Genet.* **13**, 693-704. doi:10.1038/nrg3246

**Baker, D. J., Childs, B. G., Durik, M., Wijers, M. E., Sieben, C. J., Zhong, J., Saltness, R. A., Jeganathan, K. B., Verzosa, G. C., Pezeshki, A. et al.** (2016). Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. *Nature* **530**, 184-189. doi:10.1038/nature16932

**Bartáková, V., Reichard, M., Janko, K., Polačik, M., Blažek, R., Reichwald, K., Cellerino, A. and Bryja, J.** (2013). Strong population genetic structuring in an annual fish, *Nothobranchius furzeri*, suggests multiple savannah refugia in southern Mozambique. *BMC Evol. Biol.* **13**, 196. doi:10.1186/1471-2148-13-196

**Bartáková, V., Reichard, M., Blažek, R., Polačik, M. and Bryja, J.** (2015). Terrestrial fishes: rivers are barriers to gene flow in annual fishes from the African savanna. *J. Biogeogr* **42**, 1832-1844. doi:10.1111/jbi.12567

**Baumgart, M., Groth, M., Priebe, S., Savino, A., Testa, G., Dix, A., Ripa, R., Spallotta, F., Gaetano, C., Ori, M. et al.** (2014). RNA-seq of the aging brain in the short-lived fish *N. furzeri* - conserved pathways and novel genes associated with neurogenesis. *Aging Cell* **13**, 965-974. doi:10.1111/acel.12257

**Baumgart, M., Di Cicco, E., Rossi, G., Cellerino, A. and Tozzini, E. T.** (2015). Comparison of captive lifespan, age-associated liver neoplasias and age-dependent gene expression between two annual fish species: *Nothobranchius furzeri* and *Nothobranchius korthause*. *Biogerontology* **16**, 63-69. doi:10.1007/s10522-014-9535-y

**Baumgart, M., Priebe, S., Groth, M., Hartmann, N., Menzel, U., Pandolfini, L., Koch, P., Felder, M., Ristow, M., Englert, C. et al.** (2016). Longitudinal RNA-Seq analysis of vertebrate aging identifies mitochondrial complex i as a small-molecule-sensitive modifier of Lifespan. *Cell Syst.* **2**, 122-132. doi:10.1016/j.cels.2016.01.014

**Benayoun, B. A., Pollina, E. A., Singh, P. P., Mahmoudi, S., Harel, I., Casey, K. M., Dulken, B. W., Kundaje, A. and Brunet, A.** (2019). Remodeling of epigenome and transcriptome landscapes with aging in mice reveals widespread induction of inflammatory responses. *Genome Res.* **29**, 697-709. doi:10.1101/gr.240093.118

**Blažek, R., Polačik, M., Kačer, P., Cellerino, A., Řežucha, R., Methling, C., Tomášek, O., Syslová, K., Terzibasi Tozzini, E., Albrecht, T. et al.** (2017). Repeated intraspecific divergence in life span and aging of African annual fishes along an aridity gradient. *Evolution* **71**, 386-402. doi:10.1111/evo.13127

**Carneiro, M. C., Henriques, C. M., Nabais, J., Ferreira, T., Carvalho, T. and Ferreira, M. G.** (2016). Short telomeres in key tissues initiate local and systemic aging in zebrafish. *PLoS Genet.* **12**, e1005798. doi:10.1371/journal.pgen.1005798

**Cellerino, A., Valenzano, D. R. and Reichard, M.** (2016). From the bush to the bench: the annual *Nothobranchius* fishes as a new model system in biology. *Biol. Rev. Camb. Philos. Soc.* **91**, 511-533. doi:10.1111/brv.12183

**Charlesworth, B.** (2000). Fisher, Medawar, Hamilton and the evolution of aging. *Genetics* **156**, 927-931.

**Cristofalo, V. J.** (2005). SA beta Gal staining: biomarker or delusion. *Exp. Gerontol.* **40**, 836-838. doi:10.1016/j.exger.2005.08.005

**Cui, R., Medeiros, T., Willemsen, D., Iasi, L. N. M., Collier, G. E., Graef, M., Reichard, M. and Valenzano, D. R.** (2019). Relaxed selection limits lifespan by increasing mutation load. *Cell* **178**, 385-399.e20. doi:10.1016/j.cell.2019.06.004

**Dimri, G. P., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., Medrano, E. E., Linskens, M., Rubelj, I., Pereira-Smith, O. et al.** (1995). A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc. Natl. Acad. Sci. USA* **92**, 9363-9367. doi:10.1073/pnas.92.20.9363

**Dodzian, J., Kean, S., Seidel, J. and Valenzano, D. R.** (2018). A protocol for laboratory housing of turquoise killifish (*Nothobranchius furzeri*). *J Vis Exp.* **134**, e57073. doi:10.3791/57073

**Dolfi, L., Ripa, R., Antebi, A., Valenzano, D. R. and Cellerino, A.** (2019). Cell cycle dynamics during diapause entry and exit in an annual killifish revealed by FUCCI technology. *EvoDevo* **10**, 1-22.

**Flajnik, M. F. and Kasahara, M.** (2010). Origin and evolution of the adaptive immune system: genetic events and selective pressures. *Nat. Rev. Genet.* **11**, 47-59. doi:10.1038/nrg2703

**Furness, A. I.** (2016). The evolution of an annual life cycle in killifish: adaptation to ephemeral aquatic environments through embryonic diapause. *Biol. Rev. Camb. Philos. Soc.* **91**, 796-812. doi:10.1111/brv.12194

**Gems, D. and Partridge, L.** (2013). Genetics of longevity in model organisms: debates and paradigm shifts. *Annu. Rev. Physiol.* **75**, 621-644. doi:10.1146/annurev-physiol-030212-183712

**Genade, T., Benedetti, M., Terzibasi, E., Roncaglia, P., Valenzano, D. R., Cattaneo, A. and Cellerino, A.** (2005). Annual fishes of the genus *Nothobranchius* as a model system for aging research. *Aging Cell* **4**, 223-233. doi:10.1111/j.1474-9726.2005.00165.x

**Gerhard, G. S.** (2003). Comparative aspects of zebrafish (*Danio rerio*) as a model for aging research. *Exp. Gerontol.* **38**, 1333-1341. doi:10.1016/j.exger.2003.10.022

**Harel, I., Benayoun, B. A., Machado, B., Singh, P. P., Hu, C.-K., Pech, M. F., Valenzano, D. R., Zhang, E., Sharp, S. C., Artandi, S. E. et al.** (2015). A platform for rapid exploration of aging and diseases in a naturally short-lived vertebrate. *Cell* **160**, 1013-1026. doi:10.1016/j.cell.2015.01.038

**Harel, I., Valenzano, D. R. and Brunet, A.** (2016). Efficient genome engineering approaches for the short-lived African turquoise killifish. *Nat. Protoc.* **11**, 2010-2028. doi:10.1038/nprot.2016.103

**Hartmann, N. and Englert, C.** (2012). A microinjection protocol for the generation of transgenic killifish (Species: *Nothobranchius furzeri*). *Dev. Dyn.* **241**, 1133-1141. doi:10.1002/dvdy.23789

**Hartmann, N., Reichwald, K., Lechel, A., Graf, M., Kirschner, J., Dorn, A., Terzibasi, E., Wellner, J., Platzer, M., Rudolph, K. L. et al.** (2009). Telomeres shorten while Tert expression increases during ageing of the short-lived fish *Nothobranchius furzeri*. *Mech. Ageing Dev.* **130**, 290-296. doi:10.1016/j.mad.2009.01.003

**Hartmann, N., Reichwald, K., Wittig, I., Dröse, S., Schmeisser, S., Lück, C., Hahn, C., Graf, M., Gausmann, U., Terzibasi, E. et al.** (2011). Mitochondrial DNA copy number and function decrease with age in the short-lived fish Nothobranchius furzeri. *Aging Cell* **10**, 824-831. doi:10.1111/j.1474-9726.2011.00723.x

**Herrera, M. and Jagadeeswaran, P.** (2004). Annual fish as a genetic model for aging. *J. Gerontol. A Biol. Sci. Med. Sci.* **59**, 101-107. doi:10.1093/gerona/59.2.B101

**Hu, C.-K. and Brunet, A.** (2018). The African turquoise killifish: a research organism to study vertebrate aging and diapause. *Aging Cell* **17**, e12757. doi:10.1111/acel.12757

**Karsten, K. B., Andriamandimbiarisoa, L. N., Fox, S. F. and Raxworthy, C. J.** (2008). A unique life history among tetrapods: an annual chameleon living mostly as an egg. *Proc. Natl. Acad. Sci. USA* **105**, 8980-8984. doi:10.1073/pnas.0802468105

**Kauppila, T. E. S., Kauppila, J. H. K. and Larsson, N.-G.** (2017). Mammalian mitochondria and aging: an update. *Cell Metab.* **25**, 57-71. doi:10.1016/j.cmet.2016.09.017

**Kawakami, K.** (2007). Tol2: a versatile gene transfer vector in vertebrates. *Genome Biol.* **8** Suppl. 1, S7. doi:10.1186/gb-2007-8-s1-s7

**Kim, Y., Nam, H. G. and Valenzano, D. R.** (2016). The short-lived African turquoise killifish: an emerging experimental model for ageing. *Dis. Model. Mech.* **9**, 115-129. doi:10.1242/dmm.023226

**Kimura, K. D., Tissenbaum, H. A., Liu, Y. and Ruvkun, G.** (1997). daf-2, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science* **277**, 942-946. doi:10.1126/science.277.5328.942

**Kirschner, J., Weber, D., Neuschl, C., Franke, A., Bottger, M., Zielke, L., Powalsky, E., Groth, M., Shagin, D., Petzold, A. et al.** (2012). Mapping of quantitative trait loci controlling lifespan in the short-lived fish *Nothobranchius furzeri*–a new vertebrate model for age research. *Aging Cell* **11**, 252-261. doi:10.1111/j.1474-9726.2011.00780.x

**López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M. and Kroemer, G.** (2013). The hallmarks of aging. *Cell* **153**, 1194-1217. doi:10.1016/j.cell.2013.05.039

**Lucas-Sanchez, A., Almaida-Pagan, P. F., Mendiola, P. and de Costa, J.** (2014). Nothobranchius as a model for aging studies. A review. *Aging Dis.* **5**, 281-291. doi:10.14336/AD.2014.0500281

**Matsui, H., Kenmochi, N. and Namikawa, K.** (2019). Age- and alpha-synuclein-dependent degeneration of dopamine and noradrenaline neurons in the annual killifish *Nothobranchius furzeri*. *Cell Rep.* **26**, 1727-1733.e6. doi:10.1016/j.celrep.2019.01.015

**Medawar, P.** (1952). *An Unsolved Problem of Biology*. London: H.K. Lewis.

**Müller, V., de Boer, R. J., Bonhoeffer, S. and Szathmáry, E.** (2018). An evolutionary perspective on the systems of adaptive immunity. *Biol. Rev. Camb. Philos. Soc.* **93**, 505-528. doi:10.1111/brv.12355

**Ohta, T.** (1973). Slightly deleterious mutant substitutions in evolution. *Nature* **246**, 96-98. doi:10.1038/246096a0

**Petzold, A., Reichwald, K., Groth, M., Taudien, S., Hartmann, N., Priebe, S., Shagin, D., Englert, C. and Platzer, M.** (2013). The transcript catalogue of the short-lived fish Nothobranchius furzeri provides insights into age-dependent changes of mRNA levels. *BMC Genomics* **14**, 185. doi:10.1186/1471-2164-14-185

**Podrabsky, J. E., Garrett, I. D. F. and Kohl, Z. F.** (2010). Alternative developmental pathways associated with diapause regulated by temperature and maternal influences in embryos of the annual killifish *Austrofundulus limnaeus*. *J. Exp. Biol.* **213**, 3280-3288. doi:10.1242/jeb.045906

**Polačik, M., Blažek, R., Řežucha, R., Vrtílek, M., Terzibasi Tozzini, E. and Reichard, M.** (2014). Alternative intrapopulation life-history strategies and their trade-offs in an African annual fish. *J. Evol. Biol.* **27**, 854-865. doi:10.1111/jeb.12359

**Polačik, M., Blazek, R. and Reichard, M.** (2016). Laboratory breeding of the short-lived annual killifish *Nothobranchius furzeri*. *Nat. Protoc.* **11**, 1396-1413. doi:10.1038/nprot.2016.080

**Reichard, M. and Polačik, M.** (2019). *Nothobranchius furzeri*, an 'instant' fish from an ephemeral habitat. *Elife* **8**, e41548. doi:10.7554/eLife.41548

**Reichwald, K., Lauber, C., Nanda, I., Kirschner, J., Hartmann, N., Schories, S., Gausmann, U., Taudien, S., Schilhabel, M. B., Szafranski, K. et al.** (2009). High tandem repeat content in the genome of the short-lived annual fish

*Nothobranchius furzeri*: a new vertebrate model for aging research. *Genome Biol.* **10**, R16. doi:10.1186/gb-2009-10-2-r16

**Reichwald, K., Petzold, A., Koch, P., Downie, B. R., Hartmann, N., Pietsch, S., Baumgart, M., Chalopin, D., Felder, M., Bens, M. et al.** (2015). Insights into sex chromosome evolution and aging from the genome of a short-lived fish. *Cell* **163**, 1527-1538. doi:10.1016/j.cell.2015.10.071

**Reznick, D. N., Bryant, M. J., Roff, D., Ghalambor, C. K. and Ghalambor, D. E.** (2004). Effect of extrinsic mortality on the evolution of senescence in guppies. *Nature* **431**, 1095-1099. doi:10.1038/nature02936

**Ripa, R., Dolfi, L., Terrigno, M., Pandolfini, L., Savino, A., Arcucci, V., Groth, M., Terzibasi Tozzini, E., Baumgart, M. and Cellerino, A.** (2017). MicroRNA miR-29 controls a compensatory response to limit neuronal iron accumulation during adult life and aging. *BMC Biol.* **15**, 9. doi:10.1186/s12915-017-0354-x

**Romney, A. L. and Podrabsky, J. E.** (2017). Transcriptomic analysis of maternally provisioned cues for phenotypic plasticity in the annual killifish, *Austrofundulus limnaeus*. *Evodevo* **8**, 6. doi:10.1186/s13227-017-0069-7

**Romney, A. L. T., Davis, E. M., Corona, M. M., Wagner, J. T. and Podrabsky, J. E.** (2018). Temperature-dependent vitamin D signaling regulates developmental trajectory associated with diapause in an annual killifish. *Proc. Natl. Acad. Sci. USA* **115**, 12763-12768. doi:10.1073/pnas.1804590115

**Seidel, J. and Valenzano, D. R.** (2018). The role of the gut microbiome during host ageing. *F1000Res* **7**, F1000. doi:10.12688/f1000research.15121.1

**Smith, P., Willemsen, D., Popkes, M., Metge, F., Gandiwa, E., Reichard, M. and Valenzano, D. R.** (2017). Regulation of life span by the gut microbiota in the short-lived African turquoise killifish. *eLife* **6**, e27014. doi:10.7554/eLife.27014

**Stearns, S. C.** (2000). Life history evolution: successes, limitations, and prospects. *Naturwissenschaften* **87**, 476-486. doi:10.1007/s001140050763

**Terzibasi, E., Lefrancois, C., Domenici, P., Hartmann, N., Graf, M. and Cellerino, A.** (2009). Effects of dietary restriction on mortality and age-related phenotypes in the short-lived fish *Nothobranchius furzeri*. *Aging Cell* **8**, 88-99. doi:10.1111/j.1474-9726.2009.00455.x

**Terzibasi Tozzini, E., Savino, A., Ripa, R., Battistoni, G., Baumgart, M. and Cellerino, A.** (2014). Regulation of microRNA expression in the neuronal stem cell niches during aging of the short-lived annual fish *Nothobranchius furzeri*. *Front. Cell Neurosci.* **8**, 51. doi:10.3389/fncel.2014.00051

**Tozzini, E. T., Baumgart, M., Battistoni, G. and Cellerino, A.** (2012). Adult neurogenesis in the short-lived teleost *Nothobranchius furzeri*: localization of neurogenic niches, molecular characterization and effects of aging. *Aging Cell* **11**, 241-251. doi:10.1111/j.1474-9726.2011.00781.x

**Tozzini, E. T., Dorn, A., Ng'oma, E., Polačik, M., Blažek, R., Reichwald, K., Petzold, A., Watters, B., Reichard, M. and Cellerino, A.** (2013). Parallel evolution of senescence in annual fishes in response to extrinsic mortality. *BMC Evol. Biol.* **13**, 77. doi:10.1186/1471-2148-13-77

**Valdesalici, S. and Cellerino, A.** (2003). Extremely short lifespan in the annual fish *Nothobranchius furzeri*. *Proc. Biol. Sci.* **270** Suppl. 2, S189-S191. doi:10.1098/rsbl.2003.0048

**Valenzano, D. R., Terzibasi, E., Cattaneo, A., Domenici, L. and Cellerino, A.** (2006a). Temperature affects longevity and age-related locomotor and cognitive decay in the short-lived fish *Nothobranchius furzeri*. *Aging Cell* **5**, 275-278. doi:10.1111/j.1474-9726.2006.00212.x

**Valenzano, D. R., Terzibasi, E., Genade, T., Cattaneo, A., Domenici, L. and Cellerino, A.** (2006b). Resveratrol prolongs lifespan and retards the onset of age-related markers in a short-lived vertebrate. *Curr. Biol.* **16**, 296-300. doi:10.1016/j.cub.2005.12.038

**Valenzano, D. R., Kirschner, J., Kamber, R. A., Zhang, E., Weber, D., Cellerino, A., Englert, C., Platzer, M., Reichwald, K. and Brunet, A.** (2009). Mapping loci associated with tail color and sex determination in the short-lived fish *Nothobranchius furzeri*. *Genetics* **183**, 1385-1395. doi:10.1534/genetics.109.108670

**Valenzano, D. R., Sharp, S. and Brunet, A.** (2011). Transposon-mediated transgenesis in the short-lived African killifish *Nothobranchius furzeri*, a vertebrate model for aging. *G3 (Bethesda)* **1**, 531-538. doi:10.1534/g3.111.001271

**Valenzano, D. R., Benayoun, B. A., Singh, P. P., Zhang, E., Etter, P. D., Hu, C.-K., Clément-Ziza, M., Willemsen, D., Cui, R., Harel, I. et al.** (2015). The African turquoise killifish genome provides insights into evolution and genetic architecture of lifespan. *Cell* **163**, 1539-1554. doi:10.1016/j.cell.2015.11.008

**Valenzano, D. R., Aboobaker, A., Seluanov, A. and Gorbunova, V.** (2017). Non-canonical aging model systems and why we need them. *EMBO J.* **36**, 959-963. doi:10.15252/embj.201796837

**Vrtílek, M., Zak, J., Polacik, M., Blazek, R. and Reichard, M.** (2018a). Longitudinal demographic study of wild populations of African annual killifish. *Sci. Rep.* **8**, 4774. doi:10.1038/s41598-018-22878-6

**Vrtílek, M., Žák, J., Pšenička, M. and Reichard, M.** (2018b). Extremely rapid maturation of a wild African annual fish. *Curr. Biol.* **28**, R822-R824. doi:10.1016/j.cub.2018.06.031

**Wendler, S., Hartmann, N., Hoppe, B. and Englert, C.** (2015). Age-dependent decline in fin regenerative capacity in the short-lived fish *Nothobranchius furzeri*. *Aging Cell* **14**, 857-866. doi:10.1111/acel.12367

**Willemsen, D., Cui, R., Reichard, M. and Valenzano, D. R.** (2019). Intra-species differences in population size shape life history and genome evolution. *bioRxiv* 852368. doi:10.1101/852368

**Williams, G. C.** (1957). Pleiotropy, natural selection, and the evolution of senescence. *Evolution* **11**, 398-411. doi:10.1111/j.1558-5646.1957.tb02911.x

**Wourms, J. P.** (1972). The developmental biology of annual fishes. 3. Pre-embryonic and embryonic diapause of variable duration in the eggs of annual fishes. *J. Exp. Zool.* **182**, 389-414. doi:10.1002/jez.1401820310

**Wright, W. E. and Shay, J. W.** (2000). Telomere dynamics in cancer progression and prevention: fundamental differences in human and mouse telomere biology. *Nat. Med.* **6**, 849-851. doi:10.1038/78592

**Yuan, R., Tsaih, S.-W., Petkova, S. B., Marin de Evsikova, C., Xing, S., Marion, M. A., Bogue, M. A., Mills, K. D., Peters, L. L., Bult, C. J. et al.** (2009). Aging in inbred strains of mice: study design and interim report on median lifespans and circulating IGF1 levels. *Aging Cell* **8**, 277-287. doi:10.1111/j.1474-9726.2009.00478.x

**Zhou, Y., Ni, S., Song, L., Wang, X., Zhang, Y. and Zhang, S.** (2019). Late-onset administration of GDF11 extends life span and delays development of age-related markers in the annual fish Nothobranchius guentheri. *Biogerontology* **20**, 225-239. doi:10.1007/s10522-018-09789-9