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Review

Nothobranchius furzeri: A Model for Aging Research and More

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The short-lived killifish *Nothobranchius furzeri* inhabits ephemeral ponds in southeastern Africa and is characterized by rapid growth and early sexual maturation. With respect to the molecular, cellular, and integrative traits of aging, *N. furzeri* shows significant similarities to mammals, including humans. Recently, reference sequences for the *N. furzeri* genome have been published. Also, methods for transgenesis and genomic engineering have been established. In this review we discuss why the killifish is a valuable model for aging research and what we have learned from the genome sequence. The respective insights are not limited to the biology of aging but are also relevant for developmental biology and the evolution of sex determination.

Nothobranchius as an Emerging Model System

Nothobranchius furzeri, the turquoise killifish, is the shortest-lived vertebrate that can be kept in captivity [1]. It is an annual fish that inhabits seasonal freshwater ponds in the southeast of Africa and is characterized by rapid growth and early sexual maturation [2] (Figure 1, Key Figure). The short median lifespan of between 3 and 7 months reflects an adaptation to the ephemeral nature of the habitat. In 2003, when the extremely short lifespan of a particular strain, namely GRZ, was described it was suggested for the first time to use *N. furzeri* as a model for aging research [1]. Now, 13 years later, several laboratory strains exist that differ in lifespan [3] and many 'hallmarks of aging' in *N. furzeri* have been studied and characterized. In addition, transgenesis has been established [4–6], single genes have been modified using genome editing tools [7], and recently **reference sequences** (see Glossary) for the *N. furzeri* genome have been published [8,9]. Thus, while still a 'newcomer' to the field, *N. furzeri* could join the group of well-established aging models including yeast, *Caenorhabditis elegans*, *Drosophila*, and mouse in the future. In this review we summarize recent achievements that make *N. furzeri* a valuable aging model, with an emphasis on genetics and genomics. For further information on phylogeny, ecology, distribution, and population structure in the wild, the reader is referred to Cellerino *et al.* [10].

N. furzeri Aging Resembles Mammalian Aging

The concept of aging, defined as the continuous and irreversible physiological decline affecting most organisms, has recently been deconstructed into nine categories that have been named 'hallmarks of aging' [11]. These comprise telomere attrition, mitochondrial dysfunction, cellular senescence, loss of proteostasis, epigenetic alterations, altered intercellular communication, stem cell exhaustion, genomic instability, and deregulated nutrient sensing. In the past years many of these hallmarks have been shown to be relevant for aging in *N. furzeri*. Telomere shortening has been shown to accompany aging in muscle and skin. In this case, tissue samples from 5- and 21-week-old animals were compared [12]. Importantly, at 5–7 kb the length of killifish telomeres resembles that of human telomeres (5–10 kb [13]) more closely than that of mouse telomeres (50–150 kb [14]). Mitochondrial dysfunction occurs during *N. furzeri* aging.

Trends

The turquoise killifish *Nothobranchius furzeri* is the shortest-lived vertebrate that can be kept in captivity. Its aging shows many of the characteristics of mammalian aging.

The short lifespan of *N. furzeri* presents a unique opportunity to perform longitudinal studies in a vertebrate.

The availability of natural strains with different lifespans, the possibility of engineering the genome, and its published genome have established *N. furzeri* as a novel model in aging research.

The genome sequence of *N. furzeri* provides new insights into the genetic architecture and evolution of aging, like clustering of aging-related genes in specific genomic regions and positive selection of lifespan determinants.

N. furzeri not only serves as a platform for rapid exploration of aging and disease but also allows insights into development, like embryonic arrest (diapause), and early sex chromosome evolution.

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Key Figure

Life Cycle of *Nothobranchius furzeri* and Selected Key Questions Addressed by Current Research

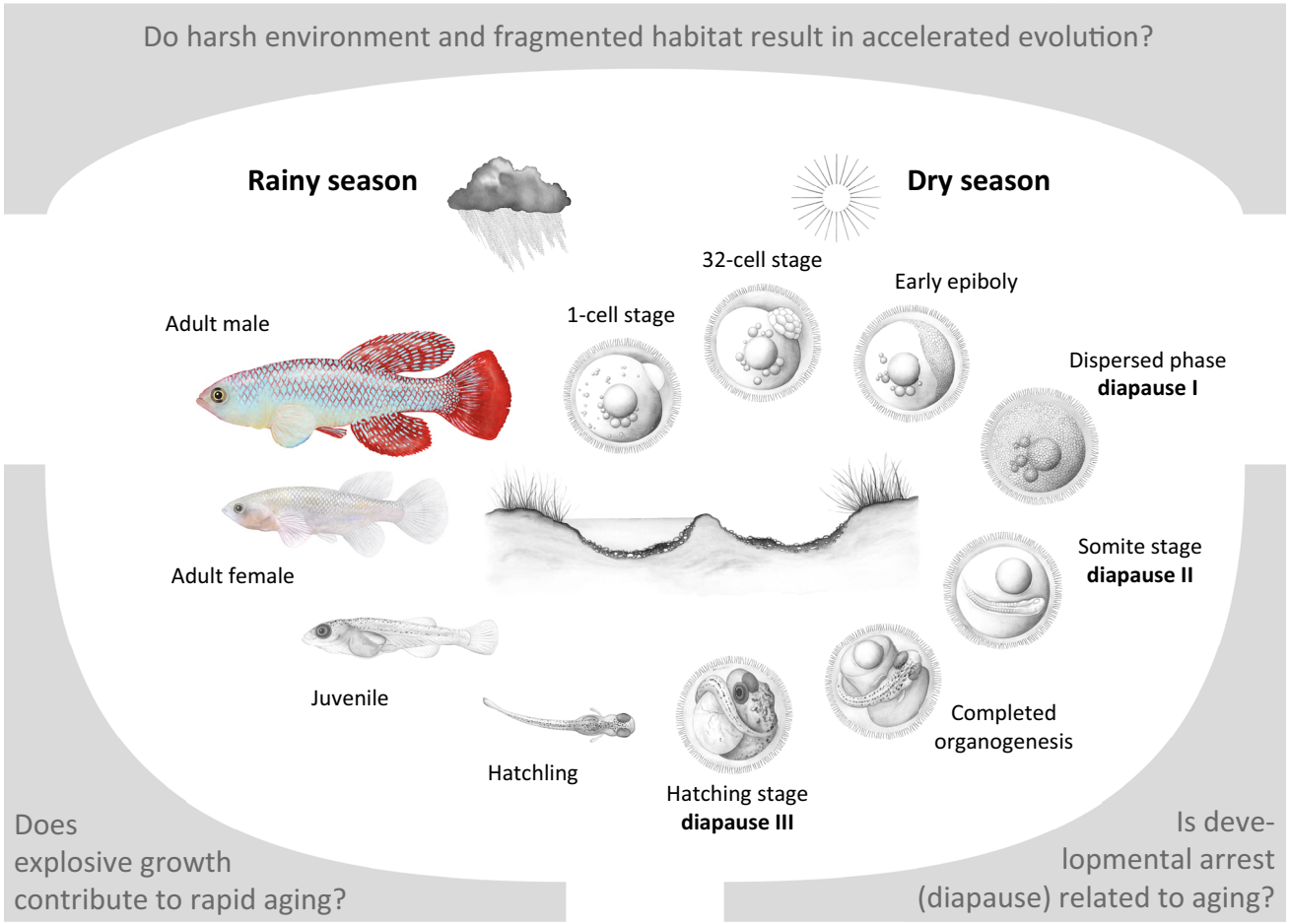

Figure 1. Center: With the start of the rainy season, *N. furzeri* larvae hatch and develop rapidly. After 4–5 weeks juveniles reach sexual maturity and begin laying eggs. Depending on environmental conditions (i.e., the availability of water), a fraction of the embryos complete development and hatch in the same season. Most of the embryos, however, will enter diapause at any of the three stages indicated (dispersed phase, somite stage, hatching stage). The arrest in diapause can last for more than 1 year, until the next rainy season begins. Source: FLI/© Alexander Schmidt, Atelier Symbiota.

While there was no evidence for age-related deletions of mitochondrial DNA (mtDNA), as typically observed in mammals [15], a decline in mtDNA copy number, downregulation of mtDNA-associated genes, and impairment of mitochondrial function could be observed [16]. Cellular senescence as well as an increase in aging markers has been observed in old compared with young *N. furzeri*. This is true for senescence-associated β-galactosidase and lipofuscin as well as the cell cycle inhibitors p21 and p16 [17,18].

Although direct studies on loss of proteostasis remain lacking for *N. furzeri*, the aging-associated upregulation of translation and ribosomal processes as well as the increased expression of genes encoding lysosomal proteins [8,19,20] can be regarded as indicators of this hallmark of

Trends in Genetics

Figure 2. Hallmarks of Aging in *Nothobranchius furzeri*. Phenotypic changes on aging of *N. furzeri*. Individual male fish from a strain with a maximum lifespan of 1 year have been recorded at the indicated time points. Typical signs of aging comprise weight loss, spinal curvature, discoloration, and craniofacial malformation. Reproduced, with permission, from Heiß, W. *Altermedizin aktuell*, 21. Erg. Lfg. 10/2011 © ecomed-Storck, Landsberg/Lech, Germany.

aging in the fish. The same is true for epigenetic alterations, as demonstrated by increased expression of genes encoding members of the polycomb complex and upregulation of H3K27me3 with age [19]. The upregulation of genes encoding components of various signaling pathways including cytokine–cytokine receptor interaction and Jak–Stat signaling [20] suggests that altered intercellular communication occurs on aging in *N. furzeri*.

Integrative aging-associated markers and phenotypes have also been observed in *N. furzeri*. Phenotypic changes include reduced coloration in males, malformations of the spine and face, and weight loss (Figure 2). Significant differences between old and young animals have also been seen in locomotor activity, open-field exploration, and learning and memory function [17,21,22]. In association with these studies, age-dependent decay in adult neurogenesis was described for *N. furzeri* [23]. This indicates aging-associated exhaustion of neuronal stem cells, which is another important hallmark of aging. Along these lines, regenerative capacity reduction has recently been shown in the *N. furzeri* caudal fin [24]. Over past decades, fish have emerged as ideal models to study the regeneration of various organs, including the fin, heart, kidney, and brain [25–28]. Given the short lifespan of *N. furzeri* the demonstration that regenerative capacity is significantly influenced by age makes killifish a strong candidate to study the aging-associated decline in regenerative capacity, a phenomenon that is central for mammalian aging, including in humans.

Finally, *N. furzeri* also shows a high incidence of age-dependent neoplasias in liver and kidney [29]. Given the killifish's short lifespan this is rather surprising and makes *N. furzeri* a potential model for studying genes and pathways underlying age-dependent tumorigenesis. Thus, there are indications that the aging of *N. furzeri* encompasses many hallmarks that have been postulated to contribute to the aging process. Therefore, *N. furzeri* resembles a true and valuable model for aging research.

Genomic Engineering in *N. furzeri*

An animal model in biomedicine is viable when it is amenable to genomic manipulation. In the case of *N. furzeri*, three groups independently established transgenesis, each using a Tol2 transposon-based fluorescence reporter driven by a ubiquitous or inducible promoter [4–6]. Founder fish harbored multiple integration sites of the respective transgenes, which were transmitted through the germline and have given rise to stable transgenic lines.

Additionally, **clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated 9 (Cas9)** technology has been established in *N. furzeri* [7]. In this report, 13 genes related to aging were mutated. For example, various deletions were introduced into the *telomerase reverse transcriptase* (*tert*) gene. In humans, mutations in the telomerase genes lead to syndromes such as dyskeratosis congenita, aplastic anemia, and idiopathic pulmonary fibrosis [30–32]. These syndromes are characterized by telomere shortening and defects in

Glossary

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated 9 (Cas9): this system is found in many bacteria and archaea and is used to modify the genome of many organisms ranging from plants to humans.

Convergent evolution: independent evolution of the same/similar features in species of different lineages.

Diapause: phase of developmental arrest or dormancy. A mechanism to survive phases of unfavorable environmental conditions; in the case of *Nothobranchius furzeri*, a state to survive dryness.

Genome reference sequence: major result of a genome sequencing effort. It does not necessarily represent the entire genome. Where the genomic sequence is resolved, it shows one allele, not necessarily the major allele of a species/population. Normally, and depending on the number, ploidy, and heterogeneity of the sequenced individual genomes, it represents an artificial/chimeric haplotype.

Male-specific region of the Y chromosome (MSY): the region of the male-specific chromosome that differentiates the sexes genetically in an organism with an XY sex-determining system, rendering males the heterogametic sex.

Positional gene-enrichment (PGE) analysis: statistical test of whether an observed accumulation of genes/loci with specific features (e.g., functional annotation 'aging related') in a chromosomal region is likely to occur by chance.

Positive selection (directional selection): mode of natural selection in which an (not necessarily newly evolved) advantageous allele increases its frequency and eventually might become fixed in the species as the consequence of differences in survival and reproduction among different phenotypes.

Quantitative trait locus (QTL): chromosomal region where allele ratios correlate with variation in a quantitative phenotype (e.g., lifespan).

Restriction-site-associated DNA (RAD) linkage map: genetic linkage map based on small nucleotide variations in the vicinity of restriction endonuclease recognition sites, determined by high-throughput

tissue homeostasis. In *N. furzeri*, male homozygous *tert* mutants showed a dramatic reduction in fertility accompanied by testicular atrophy and germ cell loss. In addition, first-generation homozygous fish displayed defects in other highly proliferative tissues including blood and intestine. These phenotypic abnormalities became significantly worse in the second generation, most probably due to further shortening of telomeres. This is different from the situation in mice, where phenotypic alterations in telomerase knockout animals become apparent only after three generations [33]. Thus, with regard to phenotypic alterations associated with telomere shortening, *N. furzeri* closely resembles the situation in humans and allows fast identification of pathologies caused by telomere attrition.

Lessons from the Genome

As in the case of genomic manipulation, in the genomic era the availability of a genome reference sequence is usually considered a prerequisite for an organism to be broadly recognized as a new model organism by the scientific community. Extensive efforts have been undertaken over the past 10 years to reach this goal. Toward this end, the first insights were provided by cytogenetics and genome survey Sanger sequencing [34]. The *N. furzeri* genome contains 19 chromosomes ($2n = 38$) and was estimated to be ~1.5 Gb in size and extremely repeat rich, particular in satellite sequences. The shortest-lived strain (GRZ), collected in 1969 in the game reserve Gona Re Zhou in Zimbabwe [35], proved to be highly inbred. Longer-lived strains, established only several years ago, are genetically heterogeneous. Crosses of these strains provided the first genetic maps of the *N. furzeri* genome [36] and lifespan-controlling **quantitative trait loci (QTLs)** [37], showing that lifespan determination is polygenic. Both studies identified males as the heterogametic sex, concordant with an XY sex-determining (SD) system.

Advances in sequencing technologies made it possible for two independent *N. furzeri* genome assemblies to be constructed and published in parallel [8,9]. Different strategies led to considerable differences in assembly metrics (Table 1). In the first study, to reach chromosome-scale long-range contiguity, a five-step strategy was used comprising sequence assembly, **scaffold**/gap filling, integration of optical and genetic linkage maps, and, finally, comparative **synteny** mapping in two closely related fish species. The second study used RNA-seq and a high-density **restriction-site-associated DNA (RAD) linkage map** to improve contiguity and to assign sequence scaffolds to chromosome-scale linkage groups (Figure 3).

sequencing (RAD-seq). Presence/absence of a restriction site can also be regarded as a RAD marker.
Scaffolding: an approach to assembling regions of contiguously resolved genomic sequences (contigs) into larger units (scaffolds) when the order and orientation of the contigs are known by other means (e.g., large-insert clone end sequences, optical mapping). The sequence gaps between the contigs are usually filled with Ns, resembling the distance of the contigs if this is known.
Synteny: colocalization of genes/loci on the same chromosome regardless of their order, orientation, and distance.

Table 1. Metrics of the Recently Published *Nothobranchius furzeri* Genome Assemblies

Metric	Reichwald *et al.*		Valenzano *et al.*	
	Scaffolds[a]	Synteny Groups	Scaffolds[b]	Linkage Groups
Number	6012	19	42 796	19
Total bases (kb)	1 230 899	1 078 720	1 079 977	379 581
Largest unit (kb)	44 272	98 476	27 998	27 998
N50[c] (kb)	15 858	63 667	247	21 055
N[d] (%)	30.4	33.6	7.7	7.8
Protein-coding genes	26 141		28 494	
High confidence	20 299[e]		22 521[f]	

[a]After optical mapping integration.
[b]After paired-end RNA-seq data and linkage map integration.
[c]N50, 50% of assembly is of equal or longer length.
[d]Unresolved nucleotide position; N stands for A, C, G, or T.
[e]Supported by at least two of the three methods for gene prediction (protein similarity, RNA-seq, *in silico* model).
[f]Tiers 1 and 2.

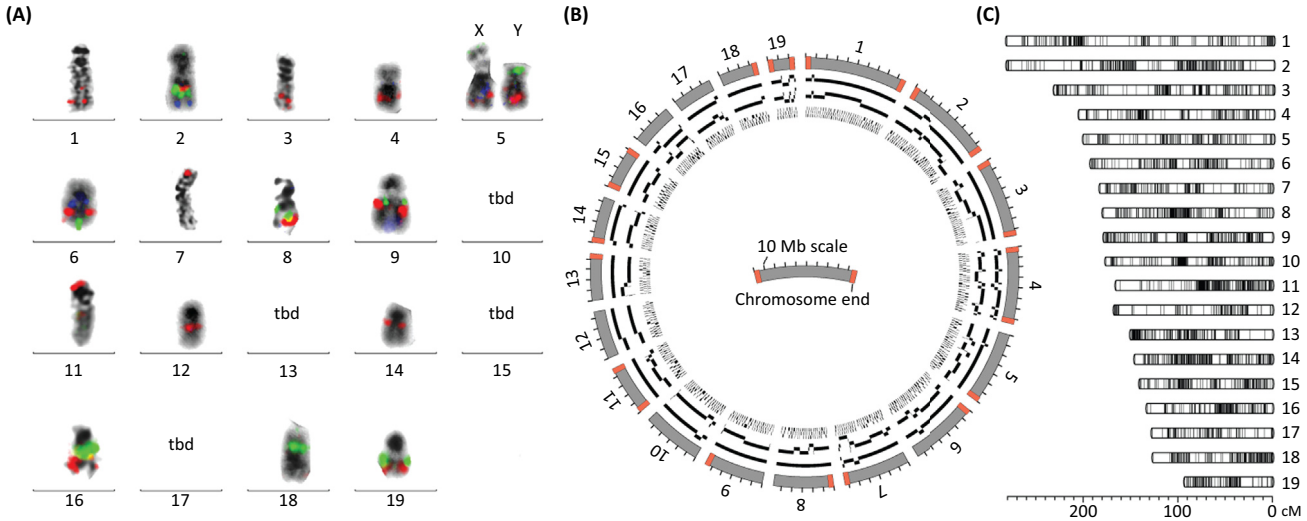

Figure 3. The Genome of *Nothobranchius furzeri*. (A) Composite male GRZ karyotype where synteny groups (sgrs) are assigned to chromosomes by hybridization with up to three fluorescently labeled BAC probes per sgr [8]. Assignment was successful for 15 of 19 sgrs (tbd, to be done). The order of chromosomes given is based on the assembled sequence length of respective sgrs, starting with the longest. Both sex chromosomes are shown (sgr05). (B) Stepwise assembly of the 19 sgrs of the reference [8]: inner circle – scaffolds obtained by sequence assembly; second circle – super scaffolds built on integration of optical map; third circle – genetic scaffolds generated by linkage map integration; outer circle – sgrs defined on analyses of synteny in medaka and stickleback. Center: Legend for sgr display. (C) High-density restriction-site-associated DNA (RAD) map of 19 linkage groups comprising 5736 RAD markers [9].

Despite using very different approaches, the two studies identified genomic regions enriched in aging-related genes. The first took advantage of the long-range contiguity of their *N. furzeri* reference sequence and performed a genome-wide **positional gene-enrichment (PGE) analysis** for differentially expressed genes (DEGs) in aging short- and long-lived *N. furzeri* strains. In total, they detected seven PGE regions. One of these regions was detected based on DEGs in skin aging, which is consistent with the well-accepted aging-related phenotype of decreased regenerative capacity. The second study, by contrast, performed QTL mapping of lifespan by crossing short- and long-lived stains. They identified one genome-wide significant lifespan QTL, located on the sex chromosomes. This was consistent with earlier findings [37] that were not able to resolve this region from the SD region. This region was found to be enriched for known aging-related genes[1]. Based on the above information, the authors speculated that a haplotype block containing a cluster of genes, rather than a single gene, might be involved in the observed lifespan difference. Remarkably, the QTL enriched in aging-related genes identified by Valenzano *et al.* is contained within two overlapping regions detected by the PGE analysis in the first study. Taken together, these findings are in line with increasing data suggesting that eukaryotic genes located in physical proximity may be coregulated and/or have similar functions. In this context, it is noteworthy that 3D chromatin structure couples nuclear compartmentalization of chromatin domains with the control of gene activity [38] and that cellular senescence is associated with modifications of the global chromatin interaction network [39]. This may provide a mechanistic link between nonrandom genomic distribution of genes related to aging and their potential coregulation in the process of *N. furzeri* organismal aging.

Both studies also searched their protein-coding gene annotations for signs of **positive selection** – a common approach in genome analysis – to identify candidates for driving the species' short lifespan. At first glance, the results of these analyses differ. While Valenzano *et al.* identified

[1]http://genomics.senescence.info/genes

497 *N. furzeri* genes with at least one site under positive selection, Reichwald *et al.* found only seven in *N. furzeri* and one in *N. pienaari*, a sympatric species showing **convergent evolution** of very short lifespan [3]. A closer look at the respective analyses reveals the causes of the discrepancy: the two studies focused on substitutions observed along phylogenetic branches of very different lengths. By choosing platyfish as the closest related outgroup, Valenzano *et al.* searched for sites of positive selection along an evolutionary branch of 50–70 million years [40] (although annualism evolved along this branch only ~20 million years ago [41]), while Reichwald *et al.* used as an outgroup *Aphyosemion*, the nonannual sister genus of *Nothobranchius*, and focused their analysis on the terminal branch of <1 million years leading to very short lifespan. Respectively, most of the substitutions detected by the approach of Valenzano *et al.* predate the evolution of annual life cycle (for more details see Sahm *et al.* in this issue). Interestingly, the results of this follow-up study suggest convergent evolution toward very short lifespan among *Nothobranchius* species.

Currently, the reference sequences of the *N. furzeri* genome are provided online by two dedicated browsers (http://www.nothobranchius.info/NFINgb and http://africanturquoisekillifishbrowser.org). Efforts are under way to integrate these resources and maintain an improved genome assembly (e.g., by gap filling using long PacBio reads) at the established Ensembl and UCSC genome browsers in the future.

Insights into *N. furzeri* Sex Determination

As often occurs in genome science, mining of a novel reference sequence guides research into rather unexpected directions. The finding of a surprisingly large **male-specific region of the Y chromosome (MSY)** in the inbred GRZ strain allowed the identification of an unprecedented intraspecies Y chromosome polymorphism [8]. This polymorphism represents different stages of sex chromosome evolution 'in action' that display features of early mammalian XY formation. To date, intraspecies sex chromosome polymorphisms have been observed in only exceptional cases and only by using cytogenetic methods (e.g., in the guppy [42]). Moreover, analysis of the minimal MSY suggests that a TGF-β family growth factor, growth differentiation factor 6 (Gdf6), is the master regulator in *N. furzeri* sex determination [8]. Although other members of the TGF-β family and their receptors are important factors in vertebrate sexual development and function as master sex determinants in several fish species [43], Gdf6 has not been described in the context of gonad development so far, warranting further investigation. Comparative variation analyses of the *N. furzeri* Y chromosome polymorphism indicate a three-step scenario for its evolution (Figure 4). First, deletion of a miRNA-binding site in the emerging *gdf6Y* allele led to prolonged gene expression in the developing male gonads. Second, a newly accumulating Y-specific 35-kb tandem-repeat cluster prevented recombination in a 200-kb region. Third, inversions encompassing larger regions (7–37 Mb) occurred independently in three strains as the secondary crossover barrier. Also, the sex chromosomes of the flatfish *Cynoglossus semilaevis*, estimated to be ~30 million years old, have most likely diverged due to recombination by a large inversion [44]. In mammals, the XY evolution has shaped these chromosomes by four consecutive inversions into evolutionary strata over 320 million years [45]. For *N. furzeri* it was estimated that primary and secondary recombination suppression (the second/third steps of the above scenario) occurred <1 million and 70 000–38 000 years ago [8]. Thus, these events are very young in evolutionary terms compared with previously studied SD systems. The findings indicate that during early sex chromosome evolution multiple Ys can emerge and subsequently the most successful Y might make a sweep through the species. From both the evolutionary and the population genetic viewpoint, it is of great interest whether the deep geographic structuring [46], short lifespan, and/or other adaptations of *N. furzeri* facilitated the recent emergence of a novel SD system and the intraspecies Y chromosome polymorphism. The intriguing possibility of coevolution between sex chromosomes and lifespan is indicated by the recent finding that the strongest lifespan QTL signal is closely linked but distinct from the SD region [9]. Moreover,

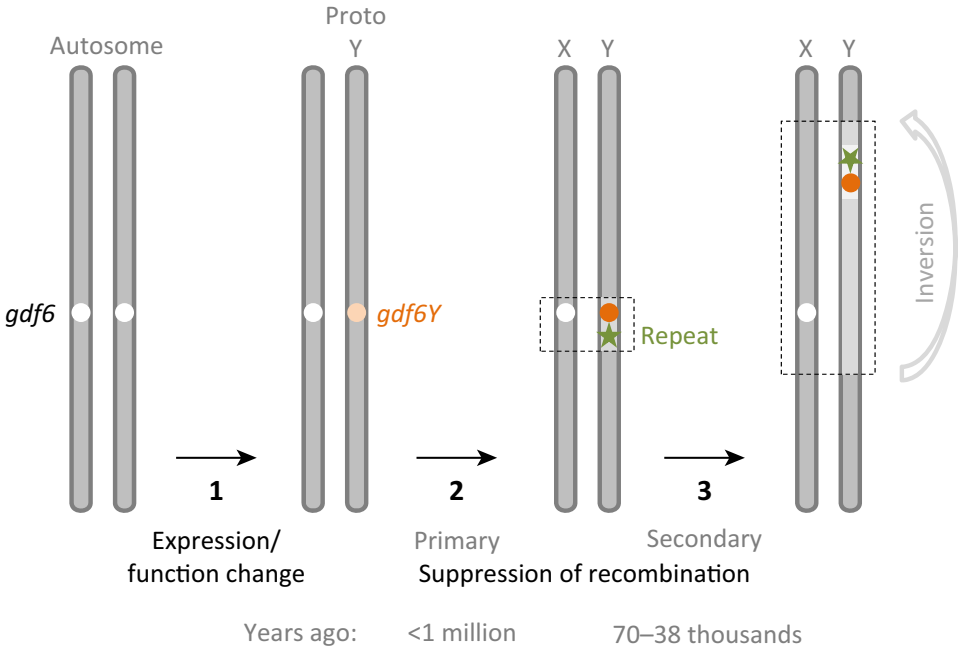

Figure 4. Three-Step Hypothesis of *Nothobranchius furzeri* Sex Chromosome Evolution. First step: Emergence of a proto-Y chromosome from a pair of autosomes by an expression- and/or function-changing mutation in one of the *gdf6* autosomal alleles (white circles) resulting in a sex-determining *gdf6Y* allele (light orange; e.g., the observed deletion of a mir-430-binding site) [8]. Second step: Primary/local recombination suppression by Y-specific repeat accumulation in close vicinity to the sex-determining (SD) gene (e.g., the identified 35-kb tandem-repeat cluster) resulting in accretion of further *gdf6Y*-specific variations (dark orange). Third step: Secondary crossover barrier by large inversion. Dotted rectangles, region of suppressed recombination; shades of lighter gray, gradual accumulation of variations in the male-specific region of the Y chromosome (MSY).

the extreme *N. furzeri* lifespan [1,3], the shortest among *Nothobranchius* spp., and its SD system evolved in the same time frame [8].

Diapause and Aging: Is There a Connection?

One measure how *N. furzeri* survives in its ephemeral habitat is a state of developmental arrest termed **diapause**. This arrest can occur at three distinct developmental stages and can last for more than 1 year (Figure 1). It is interesting to note that in *N. furzeri* a connection has been observed between the rate of embryonic development and post-hatching life-history traits [47]. In *C. elegans*, one of the classical models of aging research, a similar state of developmental arrest termed dauer larvae, exists. This arrested form can be induced by crowding and limited food supply. With *daf-2* encoding the insulin/insulin-like growth factor 1 receptor, a gene has been identified that regulates the formation of dauer larvae and leads to a dramatic lifespan extension when mutated [48]. In addition, similar gene expression signatures have been identified in dauer larvae as well as in *daf-2*-mutant adults [49]. This suggests the existence of genes that are involved in regulation of the dauer larval state and lifespan. This has prompted an examination of whether the *N. furzeri* genome also has gene signatures that are similar in diapause and aging, and such common signatures could indeed be found. Genes that were downregulated both in developmentally arrested embryos and on aging of the brain were involved in cell-cycle regulation and chromosome segregation [8]. This was not unexpected, since in diapause proliferation is halted. It had also been shown earlier that mitotic activity declines in the aging *N. furzeri* brain [23]. Surprisingly, genes upregulated in diapause as well as during *N. furzeri* brain and skin aging could almost exclusively be attributed to translational

elongation and ribosome biogenesis. While aging-related upregulation of genes encoding components of the translational machinery has been reported for human tissues [50] and their expression has been negatively correlated with individual lifespan in *N. furzeri* (see below), their upregulation in *N. furzeri* diapause is unexpected. One explanation could be that embryos in diapause prepare themselves to be able to immediately execute gene expression when environmental conditions become favorable again. These data suggest that the study of *N. furzeri* diapause can reveal genes and pathways that are associated with aging and lifespan determination. Upregulation of genes encoding components of the translational machinery has also been recently described in aging of the rat brain [51] as well as during replicative aging in yeast [52]. While it is unclear whether this is a cause or consequence of the loss of proteostasis during aging, the observation that 'translational' and 'ribosomal' genes are significantly activated in older animals implies that loss of proteostasis, another hallmark of aging (see above), also accompanies aging in *N. furzeri*.

Toward Predicting Lifespan

The short lifespan of *N. furzeri* together with the possibility of repeatedly taking samples from the same tissue (blood, fin) presents a unique opportunity to perform longitudinal studies in a vertebrate during manageable timeframes. Such studies can be employed to address the questions of how far exogenous or endogenous conditions during early life influence aging and lifespan and allow us to ask how much of lifespan is predictable. So far, similar studies have been performed in *C. elegans*, where it was shown that the amount of heat-shock proteins as well as of miRNA miR-71 is a predictor of lifespan [53,54]. In a first longitudinal study, *N. furzeri* gene expression during early life (i.e., at 15% and 30% of maximum lifespan) was analyzed and correlated with individual lifespan [20]. Two interesting results were reported. First, the longest-lived animals showed the smallest differences in overall gene expression between the two time points, suggesting that aging rate and lifespan are influenced and can even be predicted by the magnitude of gene expression changes during early adulthood. Second, the expression levels of several gene sets, including oxidative phosphorylation and ribosome and other biosynthetic pathways, showed lower expression levels early in the life of long-lived individuals. Network analysis identified nuclear genes encoding complex I of the respiratory chain as a central hub in a module of genes whose expression is negatively correlated with individual lifespan. It was shown that treatment of both *N. furzeri* and zebrafish with rotenone, an inhibitor of complex I, had a rejuvenating effect on the transcriptome; that is, it reverted gene expression signatures to those of younger age. In addition, treatment with a low dose of rotenone led to extension of lifespan in *N. furzeri* by 15%. These data are consistent with the extension of lifespan in *C. elegans* on knockdown of genes related to mitochondrial activity [55] and suggest that early-life gene expression patterns are critical predictors of lifespan.

Concluding Remarks and Future Perspectives

Several studies in the past decade have shown that, in many aspects, aging in *N. furzeri* resembles mammalian aging. In addition, the existence of several natural strains with different lifespans and the possibility of engineering the genome as well as the recent availability of its genome sequence have helped *N. furzeri* become an accepted model for aging research. With CRISPR/Cas9 technology *N. furzeri* should be an ideal model to explore – in short time – genes and pathways that control aging in vertebrates, including humans. This will also include areas like epigenetics, metabolism, and behavior as well as address the role of the microbiome in aging (see Outstanding Questions). However, the genome also taught us that the killifish is more than a model for aging research, but also allows insights into development, ecology, and evolutionary biology. Eventually, *N. furzeri* could also serve as a platform to perform high-throughput genetic and pharmacological screens for genes and substances affecting aging and longevity as well as aging-associated diseases.

Outstanding Questions

What are the genetic determinants of the short lifespan of *Nothobranchius furzeri*?

How far do gene expression patterns in early life predict lifespan?

Did the short lifespan coevolve with sex determination and did annualism, deep geographic structuring, and/or other adaptations of *N. furzeri* facilitate the intraspecies Y chromosome polymorphism?

Are there genes and pathways that regulate both diapause and aging and what are the signals that trigger entering into diapause and exit from it?

Can *N. furzeri* be developed as a model to perform high-throughput genetic, pharmacological, and environmental (e.g., microbiome) screens for aging and lifespan?

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