Contents lists available at ScienceDirect

Ageing Research Reviews

journal homepage: www.elsevier.com/locate/arr

Review article

High-throughput screening for ageing and age-related disease drug discovery: Advances and challenges

Xingkun Ji [a,1], Yan Pan [b,1], Jiajun Lei [a,1], Lisha Liang [a], Changquan Wang [c,d], Xinyi Zeng [e], Danyang Yu [e], Fangyi Chen [c], Jiongming Lu [f], Jian-Kang Zhu [a,g,*], Alessandro Cellerino [h,i,**], Judith Bossen [j,k,l,***], Bo Xian [b,****], Yu-Xuan Lyu [a,e,*]

[a] Institute of Advanced Biotechnology, Institute of Homeostatic Medicine, and School of Medicine, Southern University of Science and Technology, Shenzhen, China
[b] Laboratory of Aging Research, School of Medicine, University of Electronic Science and Technology of China, Chengdu, China
[c] Department of Biomedical Engineering, Southern University of Science and Technology, Shenzhen, China
[d] Changsha Normal University, Changsha, China
[e] Sirio Institute on Ageing (SIA), Sirio Life Technology Co., Ltd, Shanghai, China
[f] Shanghai Institute of Nutrition and Health, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Shanghai, China
[g] School of Medicine, Macau University of Science and Technology, Macao Special Administrative Region of China
[h] BIO@SNS, Scuola Normale Superiore, Pisa, Italy
[i] Leibniz Institute on Aging–Fritz Lipmann Institute (FLI), Jena, Germany
[j] Zoological Institute, Department of Molecular Physiology, Kiel University, Kiel, Germany
[k] Airway Research Center North (ARCN), Member of the German Center for Lung Research (DZL), Kiel, Germany
[l] Max Planck Institute for Evolutionary Biology, Plön, Germany

ARTICLE INFO

Keywords:
Ageing
Age-related diseases
Model organisms
High-throughput screening
Artificial intelligence

ABSTRACT

Ageing is the primary risk factor for many chronic, degenerative, and life-threatening disorders, yet the translational pipeline for geroprotective interventions remains comparatively sparse. Short-lived, experimentally tractable models with conserved ageing pathways, particularly *Caenorhabditis elegans*, *Drosophila melanogaster*, and the African turquoise killifish (*Nothobranchius furzeri*), have expanded discovery beyond traditionally mammalian-centric pipelines. By leveraging advances in automation, high-content imaging, and artificial intelligence (AI), these models have shifted the field from low-throughput, reductionist assays to scalable, mechanistically informed *in vivo* phenotypic discovery. Here, we review recent advances in middle- to high-throughput screening (HTS) technologies across these models, review key phenotypic and molecular biomarkers, such as motility, cognition and memory, intestinal integrity, mitochondrial function, and immune response, and discuss their strengths and limitations. We further evaluate the expanding role of AI from *in silico* screening, automated and high-content phenotyping, to integrative multi-layer mechanistic inference. Key challenges, including data standardisation, reproducibility across laboratories, limited cross-species pharmacokinetic comparability, AI model interpretability, and the translational gap between invertebrate hits and vertebrate or mammalian efficacy, are also discussed. By highlighting recent developments in *in vivo* disease models, HTS methodologies, and AI integration, this review provides a comprehensive resource for developing effective models and screening strategies to accelerate therapeutics for ageing and age-related diseases.

* Corresponding authors at: Institute of Advanced Biotechnology, Institute of Homeostatic Medicine, and School of Medicine, Southern University of Science and Technology, Shenzhen, China.
** Corresponding author at: BIO@SNS, Scuola Normale Superiore, Pisa, Italy.
*** Corresponding author at: Zoological Institute, Department of Molecular Physiology, Kiel University, Kiel, Germany.
**** Corresponding author at: Laboratory of Aging Research, School of Medicine, University of Electronic Science and Technology of China, Chengdu, China.
E-mail addresses: zhujk@sustech.edu.cn (J.-K. Zhu), alessandro.cellerino@leibniz-fli.de (A. Cellerino), jbossen@zoologie.uni-kiel.de (J. Bossen), xianbo@uestc.edu.cn (B. Xian), lvyx@sustech.edu.cn (Y.-X. Lyu).
[1] These authors contributed equally

https://doi.org/10.1016/j.arr.2026.103124
Received 18 December 2025; Received in revised form 16 March 2026; Accepted 1 April 2026

1. Introduction

Ageing is characterised by the progressive accumulation of molecular and cellular damage that impairs tissue and organ function, increasing vulnerability to chronic and life-threatening diseases such as cancer, cardiovascular disease, metabolic dysfunctions and neurodegenerative disorders (Cortes-Canteli and Iadecola, 2020; Ni et al., 2020). Over recent decades, significant advances have elucidated conserved hallmarks of ageing, including primary hallmarks (genomic instability, telomere attrition, epigenetic alternations, loss of proteostasis, and impaired macroautophagy), antagonistic hallmarks (mitochondrial dysfunction, altered nutrient sensing, and cellular senescence), and integrative hallmarks (stem cell exhaustion, altered intercellular communication, chronic inflammation, dysbiosis and changed extra cellular matrix). Notably, these same hallmarks are also involved in the pathophysiology of multiple age-related diseases (ARDs) (Kroemer et al., 2025).

Beyond its biological complexity, ageing poses a pressing global health and socioeconomic challenge. Population ageing is accelerating worldwide due to increased life expectancy coupled with declining birth rates, resulting in a growing proportion of older adults (World Health Organization, 2025). Although lifespan has increased substantially, healthspan, the period of life spent in good health free from chronic disease, has not kept pace (Garmany and Terzic, 2024). This widening gap means many individuals spend their later years suffering from multiple, often debilitating ARDs, reducing quality of life and increasing healthcare costs.

Efforts to target the fundamental mechanisms of ageing to prevent, delay, or even partially reverse age-related decline, termed 'geroprotection', are expanding rapidly (Dos Santos and Cochemé, 2024). Lifestyle interventions, such as exercise and dietary modification, provide measurable benefits (Partridge et al., 2005; Piazza et al., 2009), but are insufficient to fully counteract the complex biology of ageing. Consequently, there is growing interest in nutraceutical and pharmacological approaches. Candidate geroprotectors include NAD+ precursors (Yaku et al., 2018), α-ketoglutarate (AKG) (Asadi Shahmirzadi et al., 2020; Chin et al., 2014), spermidine (Eisenberg et al., 2009), urolithin A (Ryu et al., 2016), ergothioneine (Katsube et al., 2024) and bile acid signalling molecules such as lithocholic acid (Qu et al., 2025), alongside small molecules and drugs like metformin (Martin-Montalvo et al., 2013) and rapamycin (Bjedov et al., 2010), and emerging biologic approaches involving factors like Klotho (Roig-Soriano et al., 2025) or FGF21 (Gliniak et al., 2025). These agents modulate conserved ageing pathways and hallmarks, thereby promoting healthier ageing outcomes in model organisms.

Despite these advances, the discovery of novel geroprotectors with translational potential remains limited. The largest publicly available resource, DrugAge, catalogues only a little over 1000 distinct nutraceutical and pharmacological interventions tested across 27 model organisms (Barardo et al., 2017). The National Institute on Aging's Interventions Testing Program (ITP) is one of the few large-scale systematic efforts to identify new longevity-promoting compounds. Since 2004, the ITP has evaluated more than 55 compounds and combinations in genetically heterogeneous UM-HET3 mice of both sexes, yielding a small number of reproducibly successful candidates, including rapamycin, acarbose, and canagliflozin (Nadon et al., 2017; NIA Interventions Testing Program, 2021). However, such studies are time-consuming, costly, and labour-intensive, which limits scalability and integration with emerging AI-driven predictive models and automation technologies.

To broaden and accelerate discovery, a diverse set of shorter-lived model organisms complements traditional mouse studies, thereby enhancing the understanding of complex biological processes. Invertebrates such as *Caenorhabditis elegans* and *Drosophila melanogaster* are exquisite genetic models enabling fine dissection of pathways and high-throughput screening (and increasingly high-content) *in vivo* phenotypic screens. Their short lifespans, extensive genetic toolkits, and compatibility with automated handling allow rapid identification of interventions that ameliorate age-related functional and anatomical traits (Pitt et al., 2019; Ségalat, 2007; Seong et al., 2020). The short-lived vertebrate African turquoise killifish (*Nothobranchius furzeri*) further complements these invertebrate systems by offering closer physiological relevance to mammalian genetics and physiology (e.g. conserved ApoE, adaptive immunity, tissue complexity) while retaining experimental tractability and a lifespan amenable to accelerated intervention testing.

Ageing and age-associated traits have a polygenic architecture and arise from network-level dysregulation across multiple, interacting cellular processes. Single-target discovery paradigms often fail to capture the most effective interventions. Thus, unbiased phenotypic screening approaches, especially high-throughput or high-content assays, have become central when molecular targets are incomplete, pleiotropic, redundant, or context dependent (Moffat et al., 2017; Vincent et al., 2022). Recent advances in artificial intelligence (AI) and multi-omics integration further empower these efforts. For example, deep learning techniques are now routinely used to identify compounds that modulate cellular senescence, predict senolytic activity, and prioritise hits from large chemical libraries, even with limited or noisy data (Kusumoto et al., 2021; Wong et al., 2023). These AI-enabled pipelines enhance both screening efficiency (through improved hit triage) and downstream validation (through standardised, high-resolution, quantitative phenotyping), accelerating the progression of promising candidates into more complex preclinical models.

This review examines recent progress in high-throughput screening (HTS) for ageing and ARD drug discovery, with a focus on model organisms such as *C. elegans*, *Drosophila*, and the killifish. It highlights how advances in AI and automation, in combination with these models, are enabling more efficient and effective screening workflows. We also discuss the strengths and limitations of these systems and outline prospects and challenges for translating preclinical discoveries into interventions that promote healthy ageing in humans. Notably, this review aims to provide a practical, model-informed framework for researchers and developers designing scalable geroprotector screening pipelines, rather than serving as a catalogue of individual compounds.

2. Model organisms to accelerate geroprotector discovery

The selection of appropriate model systems is fundamental to elucidating ageing mechanisms and accelerating the discovery of geroprotectors. Among the diverse *in vitro* and *in vivo* models available, *C. elegans*, *Drosophila*, and killifish represent a well-balanced combination of throughput, speed, cost, regulatory burden, and translatability, which are especially valuable for geroprotective drug screening (Fig. 1). Each model offers distinct strengths and limitations, making them complementary tools in the ageing research and the development of effective geroprotective interventions.

2.1. *C. elegans*

C. elegans, a small, transparent nematode with a short lifespan of 15–25 days and a fully mapped cell lineage, is particularly amenable to genetic and pharmacological screening. Furthermore, its near-complete isogenicity enables rigorous interpretation of genetic interactions and epistasis without confounding effects from genetic variation. The ease of gene knockdown via RNAi feeding, simply by feeding worms bacteria expressing double-stranded RNA, provides a scalable and cost-effective method for functional genomics, further solidifying its position as a cornerstone of high-throughput genetic and drug screening. Its genome shares substantial homology in stress response, metabolic, proteostatic, and nutrient-sensing pathways relevant to mammalian ageing (Kuwabara and O'Neil, 2001). The rapid lifecycle, ease of maintenance, and suitability for automated imaging have established *C. elegans* as a

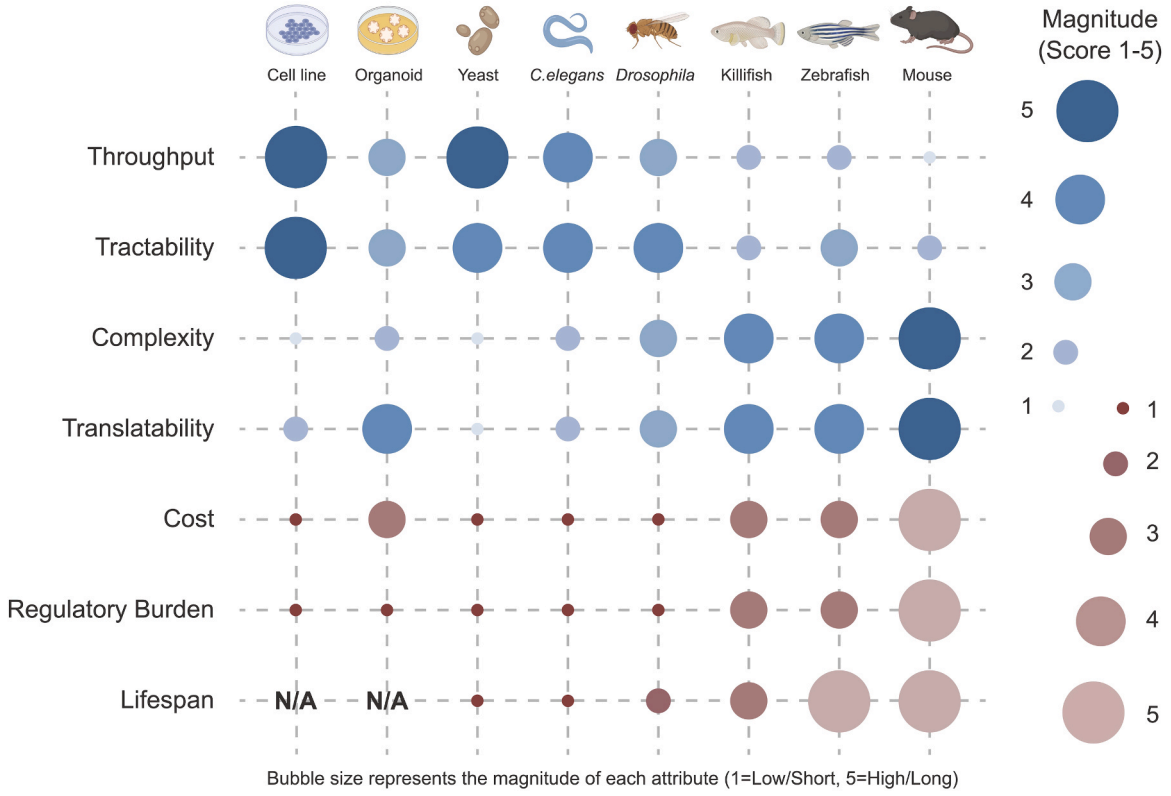

Fig. 1. Common models for drug discovery. Comparative attributes of drug discovery models, including throughput tier, tractability, tissue complexity, translation potential to humans, cost, regulation burden, and lifespan (*in vitro* models are not applicable).

cornerstone of functional genomics and screening in ageing research.

Foundational longevity genes and pathways, including the insulin/IGF-1 signalling pathway, key nutrient sensors like mTOR and AMPK, along with the role of mitochondrial and ribosomal proteins in regulating lifespan, were first elucidated in *C. elegans* (Kenyon, 2011; Uno and Nishida, 2016). Since then, a number of ARD models, particularly for neurodegenerative diseases, have been generated in *C. elegans*. These include, most prominently, models for neurodegenerative diseases such as Alzheimer's and Parkinson's, using humanised transgenes such as Aβ or phospho-Tau to induce behavioural defects and reduced lifespan (Alvarez et al., 2022; Caldwell et al., 2020; Kim et al., 2016a; Link, 2006). Models for other conditions, such as muscular dystrophy, have also been developed, further expanding the platform's utility for disease research (Chamberlain and Benian, 2000).

The phenotypic changes (motility decline, paralysis onset, stress resistance deficits) in these models can be used in large-scale phenotypic HTS aimed at delay or rescue. Large compound screenings targeting different areas have identified a number of candidates with anti-ageing, neuroprotective, anti-tumoral and metabolic modulatory activity across diverse pharmacological classes (Carretero et al., 2015; Giunti et al., 2021), and have also contributed to deciphering the mechanism of action (Luo et al., 2009). Recent AI-guided, target-based pipelines now nominate lifespan-extending molecules, which are subsequently validated in nematode assays (Avchaciov et al., 2025).

2.2. *Drosophila*

Drosophila, the fruit fly, has a short adult lifespan of 40–60 days with greater tissue complexity (central nervous system organisation, gut compartmentalisation, heart tube physiology) and approximately three-quarters homology to human disease genes (Pandey and Nichols, 2011). Powerful genetic toolkits (GAL4/UAS, CRISPR, inducible silencing/activation) enable spatial and temporal control of gene function over age (Atoki et al., 2025), and studies in flies have uncovered several conserved longevity-regulating pathways, including insulin/insulin-like signalling (IIS) (Clancy et al., 2001; Hwangbo et al., 2004), mTOR/S6 kinase signalling (Kapahi et al., 2004), and stress-response pathways such as Nrf2/JNK signalling (Wang et al., 2003). Natural sexual dimorphism, adult stem cell niches, and quantifiable behavioural and physiological outputs (locomotion, sleep, cognition, cardiac rhythm, gut barrier integrity) support multifaceted phenotyping at scale, facilitating sophisticated investigations into the biology of ageing.

Reduced genetic redundancy facilitates mechanistic dissection of complex diseases (Azuma et al., 2018; Verheyen, 2022). Established ARD-relevant models encompass cancer (Bangi et al., 2019; Mirzoyan et al., 2019; Tipping and Perrimon, 2013; Villegas et al., 2019), neurodegeneration, including Parkinson's and Tauopathies (Feany and Bender, 2000; Iijima-Ando and Iijima, 2010; Wittmann et al., 2001), metabolic dysfunction (Lee et al., 2024; Musselman and Kühnlein., 2018) and cardiac ageing (Zhao et al., 2023), with emerging machine learning pipelines automating cardiac parameter extraction (Melkani et al., 2024).

Drosophila has a strong track record in *in vivo* phenotypic HTS and translational bridging: repurposing screens of FDA-approved drugs (Bossen, 2019; Levine et al., 2016; Markstein, 2014), informing approvals or lead optimisation (Dar et al., 2012; Das and Cagan, 2017; Sonoshita et al., 2018; Vidal et al., 2005), and enabling personalised "fly avatar" oncology (Bangi et al., 2019, 2021). In addition to these studies focusing on cancer and other ARDs, a number of studies have investigated ageing and the discovery of novel geroprotectors in invertebrates such as *Drosophila* with the aim of finding drugs that delay ageing and prolong healthspan in general (Jafari, 2010; Lee and Min, 2019; Lujan et al., 2024).

2.3. Killifish

The African turquoise killifish represents a newer addition to the repertoire of ageing models. It is the shortest-lived vertebrate that can be bred in laboratory conditions (median lifespan 4–6 months), has a very small size (1–3 g), and offers the advantage of vertebrate-specific genes (e.g. ApoE), vertebrate body plan and physiology, including adaptive immunity, enabling interrogation of processes that cannot be modelled in invertebrates.

Canonical vertebrate ageing phenotypes were chatacterized in both peripheral organs (Di Cicco et al., 2011; Ma et al., 2025; Ruparelia et al., 2024), including neuroinflammation, protein aggregation, selective neuronal loss and cognitive decline (Bagnoli et al., 2022; Bergmans, 2023; De Bakker and Valenzano, 2023; Kelmer Sacramento et al., 2020; Valenzano et al., 2006a). At a systemic level, immunosenescence with pronounced remodelling of immune compartments (Morabito et al., 2024) and age-related microbiome alterations feature reduced microbial diversity and an overrepresentation of pathogenic species (Smith et al., 2017). Notably, age-related phenotypes and lifespan can be modulated by dietary and pharmacological interventions (Baumgart et al., 2016; Kothmayer et al., 2025; McKay et al., 2022; Terzibasi et al., 2009; Valenzano et al., 2006b), specific senotherapeutic interventions (Van Houcke et al., 2023), as well as by microbiome transplantation (Smith et al., 2017) illustrate its growing potential as a vertebrate platform for drug screening. In addition, a sequenced genome and advancing CRISPR/Cas genome engineering (Bedbrook et al., 2023; Oginuma et al., 2022) enabled the identification of novel pathways regulating ageing (Astre et al., 2023; Ripa et al., 2017, 2023), Genetic interventions targeting key regulators of metabolism and development, including genes such as *dnd1* (Moses et al., 2024), *AMPKγ1* (Ripa et al., 2023), *APRT* (Astre et al., 2023), and *C/EBPα* (Müller et al., 2025), have further demonstrated that lifespan in killifish can be experimentally extended through targeted genome manipulation. However, generating stable lines remains slower than in invertebrate models.

2.4. Conservation of hallmarks and the strategic continuum

The majority of the 14 hallmarks of ageing, such as genomic

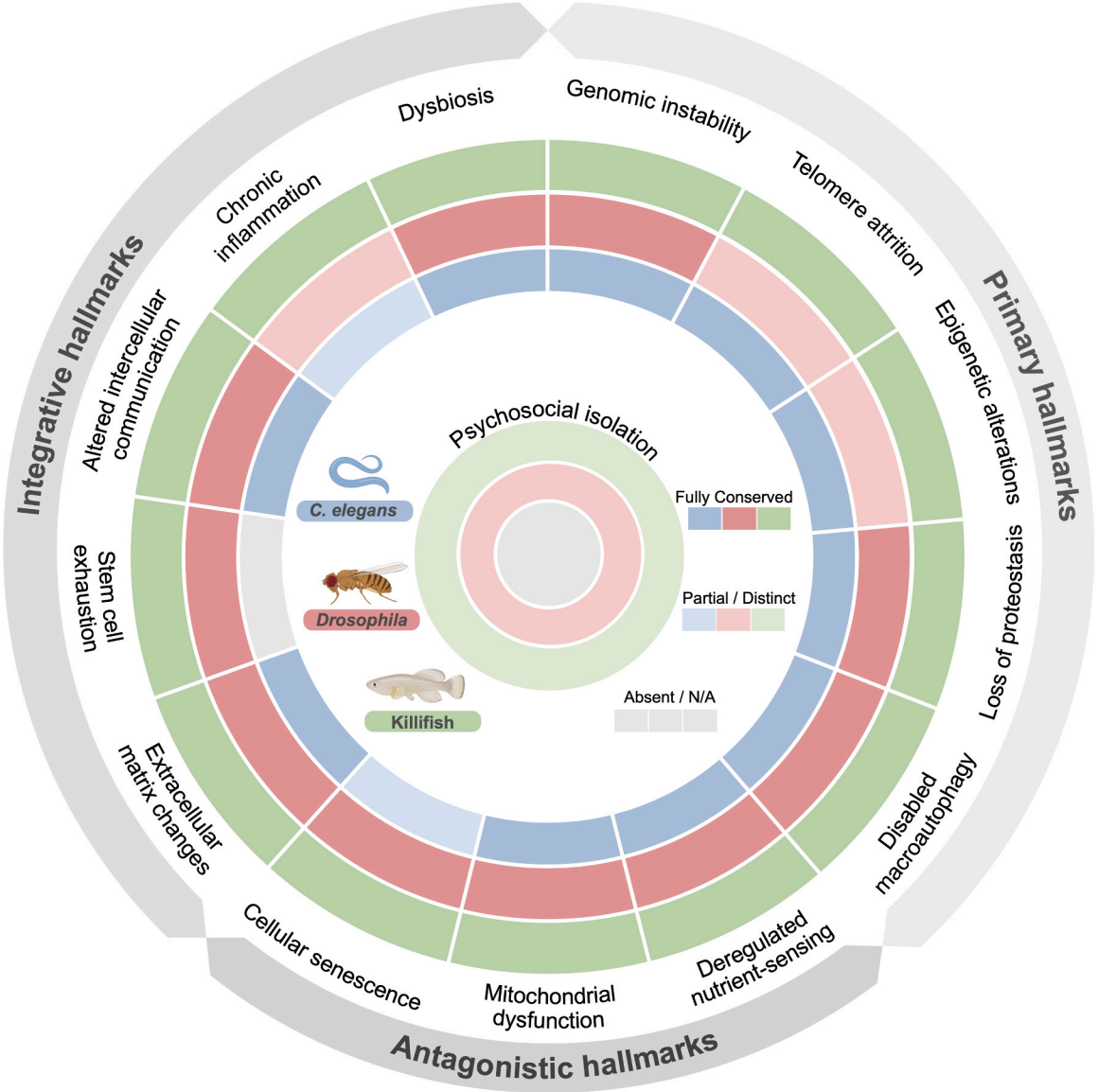

Fig. 2. Conservation of hallmarks of ageing across *C.elegans*, *Drosophila*, and Killifish. These three model organisms: *C. elegans* (nematode), *Drosophila* (fruit fly), and the African turquoise killifish, recapitulate fundamental ageing mechanisms, including the majority of hallmarks, facilitating the study of conserved pathways relevant to human ageing and geroprotector discovery.

instability, telomere attrition, epigenetic alterations, loss of proteostasis, disabled macroautophagy, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, extracellular matrix changes, stem cell exhaustion, altered intercellular communication, chronic inflammation, dysbiosis, and psychosocial isolation, can be observed in these organisms (Biga et al., 2025; Kroemer et al., 2025; López-Otín et al., 2023). This conservation suggests they serve as useful models not only for studying phenotypic and functional decline but also for elucidating the underlying conserved biological mechanisms that enhance our understanding of human ageing (Fig. 2).

Together, they represent a strategic continuum: *C. elegans* offers maximal genetic manipulability and ultra-high screening throughput; *Drosophila* contributes multi-organ functional assays and enhanced translational phenotyping, including sex-specific differences; while the African turquoise killifish provides vertebrate physiology, adaptive immunity, and conserved neural and systemic ageing within a compressed lifespan. While other models like yeast, rodents, dogs, and non-human primates also contribute significantly to understanding ageing mechanisms and drug discovery, these three species uniquely balance physiological relevance, experimental tractability, and throughput, shaping different priorities in the discovery process. In the following sections, we review recent advances in high-throughput screening technologies and platforms for each model.

3. High-throughput screening in *C. elegans*

Leveraging its genetic tractability, optical transparency, and small size, *C. elegans* has become the earliest and most extensively industrialised metazoan for organism-level high-throughput screening (HTS) in ageing research (Del Carmen-Fabregat et al., 2024; Pitt et al., 2019). Over the past decade, *C. elegans* HTS technologies have evolved from manual, low-density lifespan or motility assays to automated, longitudinal, high-content platforms capable of extracting multivariate phenotypes (Table 1). These technologies broadly fall into two strategic categories: 1) microfluidic and other custom-developed platforms that maximise environmental control, temporal resolution, and imaging precision; 2) traditional Petri dish or multi-well plate methods enhanced by automation, computer vision, and artificial intelligence (AI), which emphasize accessibility, scalability, and widespread adoption. In this section, we explore their strengths, limitations, and implications for scalability and adoption, and discuss key biomarkers used in *C. elegans* HTS for ageing research.

3.1. Custom-developed platforms: microfluidic chips and beyond

Custom-developed platforms, particularly those based on microfluidic technology, have revolutionized *C. elegans* HTS by offering precise control and advanced imaging capabilities. Representative platforms demonstrate varying balances between precision and scalability. For example, WormFarm is a microfluidic system with chambers for culturing and monitoring groups of worms, supporting automated imaging over their lifespan. Its reliance on complex fabrication restricts its accessibility (Xian et al., 2013). WormSpa is a microfluidic device designed for high-resolution imaging and immobilising worms for detailed phenotypic analysis. However, its complex setup and limited scalability restrict its widespread use (Lee and Levine, 2018). WorMotel integrates a microfabricated device into multi-well plates for tracking individual worms, offering a balance of scalability and precision (~240 animals) (Churgin et al., 2017). NemaLife Chip provides controlled culturing and high-content imaging on a chip-based system, though it requires specialised equipment, posing barriers to adoption(Rahman et al., 2020).

Recent innovations have further expanded these capabilities. For example, HeALTH is an automated, microfluidic-based system for robust longitudinal behavioural monitoring, providing long-term spatiotemporal environmental control (60 animals per run)(Le et al., 2020). Moreover, this platform supports precise environmental control and high-content imaging for up to 1000 animals, allowing quantification of phenotypes such as reproductive output, pharyngeal pumping, stress resistance, and age-related biomarkers like lipofuscin or protein aggregates.

Collectively, these systems excel in precise and dynamic environmental perturbation, high spatial and temporal resolution of behaviour and fluorescence, and mitigation of confounds such as plate crowding or uneven bacterial lawns. However, their adoption is constrained by capital costs (cleanroom fabrication, pressure and flow controllers, high-end imaging), engineering expertise requirements, substantial prototyping and validation time, heterogeneity of designs limiting standardisation, and generally lower absolute animal counts per run compared with the highest-density plate-based platforms. Notably, microfluidic systems also commonly suffer from insufficient aeration, risk of contamination, and difficulties in cleaning, which further limit their broader application. These factors make microfluidic platforms less feasible for large-scale or resource-limited settings.

3.2. Traditional plate-based platforms with automation and AI

Standard Petri dishes (5.5–6.0 cm) and multi-well plates (12-, 96-, 384-well formats) remain the practical backbone of large-scale *C. elegans* HTS because they are inexpensive, globally available, easily parallelized with liquid handling and robotic automation, and compatible with scanner- or camera-based imaging integrated into computer vision and deep learning pipelines.

Petri dish–centerd systems illustrate the spectrum of simplicity. For example, WormScan utilises 5.5–6.0 cm petri dishes and a flatbed scanner to monitor survival and movement. Its simplicity ensures repeatability with minimal setup (Mathew et al., 2012; Puckering et al., 2017). The Lifespan Machine (LM) employs scanners to monitor 16 Petri dishes per scanner, capturing 24 images per dish daily for automated lifespan scoring. While precise, it requires custom software and is assay-specific (Del Carmen-Fabregat et al., 2024; Stroustrup et al., 2013). The Observatory uses an automated system for group-housed *C. elegans*, using custom imaging hardware and software to cycle through trays of 6 cm Petri dishes. It runs four assays daily on up to 576 plates per incubator, capturing behavioural metrics like movement speed and stimulus-induced turning (Kerr et al., 2022).

Multi-well–oriented systems emphasise scaling and computational phenotyping. WormBot, an open-source system using 12-well plates and a digital camera to track behaviour and lifespan of up to 1800 animals (Pitt et al., 2019). Megapixel Camera Array images 96-well plates with square wells to process up to 23,000 animals in parallel, assessing lifespan, motility, and stress responses (Barlow et al., 2022). WormCNN integrates automated bright-field and fluorescence imaging of 384-well plates with convolutional neural networks for health classification (Pan et al., 2024). Emerging algorithmic platforms such as WormSwim (Deserno and Bozek, 2023) and WormYOLO (Dong and Chen, 2025) focus on extracting posture, swim or thrash dynamics, and early ageing signatures.

Earlier locomotion feature-extraction frameworks (Javer et al., 2018) form the foundation for the architecture of newer deep learning classifiers. Plate-based systems provide high absolute throughput, low barriers to replication across laboratories, and rapid integration of updated AI models for pose estimation, activity-state segmentation, and healthspan scoring. They also enable straightforward incorporation of multiplex fluorescent biosensors without requiring hardware redesign. However, lifespan assays using plate-based systems often rely on FuDR or infertile strains to prevent progeny production, which can introduce confounding effects in drug discovery. These systems also trade off fine environmental control, such as microgradients and precise thermal modulation, and are subject to variability in agar quality and bacterial lawn morphology. Historically, they offered coarser temporal resolution, although advances like dense camera arrays and scheduler-based

Table 1
Screening platforms in *C. elegans*.

Strategic categories	Tool	Healthspan	Lifespan	Capacity (throughput)	Limitations	References	
Custom-Developed Platforms	WormFarm	Movement related health parameters, e.g. body size, motility	Yes, can be assessed by movement	320 (Medium)	1. Automated scoring limited to small groups due to worm clustering 2. Potential stress from confinement in microfluidic environment	Xian et al. Aging Cell 2013	https://onlinelibrary.wiley.com/doi/10.1111/acel.12063
	WormSpa	Excellent for longitudinal healthspan: motility decline, pumping rate, body posture, fluorescence reporters, egg-laying dynamics	Yes, possible for multi-day lifespan tracking, but not designed for full lifespan (typically 7–14 days max reported)	32 (Medium-low)	1. Chamber size fixed for young adults – older worms or certain mutants can become trapped or stressed	Kopito et al. Lab Chip. 2014	https://pubs.rsc.org/en/content/articlelanding/2014/lc/c3lc51061a
	WorMotel	Movement related health parameters, e.g. unstimulated-and blue light stimulated-locomotion, speed	Yes, can be assessed by movement	240 (Medium)	1. Need to use special plate for experiments. 2. The mechanical design of the system admitted longer-lasting vibration that reduce the reliability to detect tap-induced behavior such as reversals.	Churgin et al. eLife. 2017	https://elifesciences.org/articles/26652
	NemaLife	Movement related health parameters, e.g. unstimulated-and shake stimulated-locomotion, speed	Yes, can be assessed by movement	100–200 (Medium)	1. Not providing direct measures of individual worm 2. The microfluidic enviorment create challenges to compare it results to tranditional agar-plated based expriments 3. The worm scoring has to be done mannually	Rahman et al. Sci Rep. 2020	https://www.nature.com/articles/s41598–020–73002–6
Traditional Plate-Based Platforms with Automation and AI (Petri dishes)	WormScan	Phenotypic parameters, e.g. size, fecundity, viability	Yes, can be assessed by movement detection	300–1000 + (High)	1. Requires FUdR for progeny elimination, potentially affecting lifespan 2. Risk of starvation at high worm densities unless adjusted	Mathew et al. PLoS One 2012	https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0033483
	Lifespan Machine (LSM)	Movement related health parameters, e.g. unstimulated-locomotion, speed	Yes, can be assessed by movement	640 (Medium-high)	Use FudR to maintain progeny-free populations, which might affect lifespan	Carmen-Fabregat et al. J Vis Exp. 2024	https://app.jove.com/t/65462/high-throughput-behavioral-aging-lifespan-assays-using-lifespan
	Observatory	Movement related health parameters, e.g. unstimulated-and shake stimulated-locomotion, speed	Yes, can be assessed by movement	~50000 (High)	The mechanical design of the system admitted longer-lasting vibration that reduce the reliability to detect tap-induced behavior such as reversals.	Kerr et al. Front. Aging 2022	https://www.frontiersin.org/journals/aging/articles/10.3389/fragi.2022.932656/full
Traditional Plate-Based Platforms with Automation and AI (Multi-well plates)	Wormbot	Movement related health parameters, e.g. unstimulated-locomotion, speed	Yes, can be assessed by movement	1800 (High)	Use FudR to maintain progeny-free populations, which might affect lifespan	Pitt et al. Geroscience 2018	https://link.springer.com/article/10.1007/s11357–019–00124–9
	Megapixel camera array	Movement related health parameters, e.g. unstimulated-and blue light stimulated-locomotion, speed	Yes, can be assessed by movement	23000 (High)	The mechanical design of the system admitted longer-lasting vibration that reduce the reliability to detect tap-induced behavior such as reversals.	Barlow et al. Commun Biol. 2022	https://www.nature.com/articles/s42003–022–03206–1

(*continued on next page*)

Table 1 (*continued*)

Strategic categories	Tool	Healthspan	Lifespan	Capacity (throughput)	Limitations	References	
	WormCNN	Age classification based on morphology (e.g., young/old categories via CNN)	Yes, age prediction supports lifespan monitoring	384 (Medium)	1. Binary young/old classification limits nuance 2. Requires high-quality, preprocessed images; no real-time tracking 3. Not directly integrated with hardware	Pan et al. Int J Mol Sci 2024	https://www.mdpi.com/1422–0067/25/17/9675
	WormSwim	Specialized for swimming/thrashing healthspan: stroke frequency, amplitude, speed, body-wave propagation, asymmetry, curling; extremely sensitive early frailty biomarker	No, not designed for lifespan (short-term assays, minutes to hours)	1000 + (High)	1. Liquid-culture only (swimming, not crawling) 2. Short-term assay – no longitudinal individual tracking 3. Sensitive to bacterial density and buffer depth 4. Cannot score death (movement-based only) 5. Requires video, not still images	Deserno et al., 2023	https://www.nature.com/articles/s41598–023–38213–7
	WormYOLO	Excellent for crawling and mixed healthspan: real-time posture, speed, curvature, eccentricity, omega turns, reversals; used as high-resolution frailty index	No, primarily short- to medium-term behavioral assays (hours to days)	1000 + (High)	1. Requires GPU for real-time; CPU mode slow 2. Performance drops in very dense or clumped populations without retraining 3. No built-in fluorescence support yet 4. Not optimized for very long videos (>1–2 h) without re-tracking 5. Still primarily crawling-focused (swimming version in development)	Dong et al., 2025	https://www.nature.com/articles/s41598–025–93533–0

imaging are narrowing this gap. AI-driven segmentation, multi-object tracking, posture manifold learning, and temporal modelling now enable recovery of subtle micro-behavioural decline, stress-response dynamics, and longitudinal fluorescence trajectories that previously required custom microfluidic platforms. This positions plate-based systems combined with AI to dominate primary discovery and medium-depth phenotyping.

3.3. Biomarkers in C. elegans HTS

Biomarkers are essential in *C. elegans* high-throughput screening (HTS), providing measurable indicators of health, ageing progression, and the efficacy of geroprotectors. These biomarkers fall into two main categories: traditional health indicators assessing general vitality and stress responses, and disease-specific markers targeting age-related conditions such as neurodegeneration, metabolic dysfunction, and muscle decline. The integration of automated imaging and artificial intelligence (AI) has transformed HTS, enabling rapid, scalable, and precise phenotypic evaluation. Despite some limitations, *C. elegans* remains a powerful model for uncovering conserved ageing pathways and identifying geroprotective agents.

Traditional biomarkers focus on easily quantifiable traits that reflect overall health and ageing, including motility and fluorescence signals. Movement assays, such as thrashing (swimming) or crawling speed, serve as robust indicators of neuromuscular function and vitality, with age-related declines serving as reliable proxies for healthspan (Herndon et al., 2002). Platforms such as WormScan and WormBot automate locomotion tracking across thousands of worms, facilitating large-scale screens for compounds that maintain motor function (Mathew et al., 2012; Pitt et al., 2019). Fluorescent signals, including lipofuscin, an age-associated pigment accumulating in the intestine, are quantified by microscopy, offering a direct measure of cellular ageing and oxidative damage (Núñez et al., 2025). GFP reporters linked to stress response genes (e.g., hsp-16.2) enable real-time monitoring of cellular stress and resilience, commonly used to identify compounds enhancing stress resistance, a hallmark of longevity (Strayer et al., 2003).

Disease-specific biomarkers model age-related pathologies and support therapeutic discovery for neurodegeneration, metabolic disorders, and sarcopenia. Neurodegeneration is monitored through protein aggregation (e.g., α-synuclein) and chemotaxis deficits, reflecting neuronal health (Van Ham et al., 2008). Metabolic dysfunction is assessed via lipid storage visualised with Oil Red O, which has been validated as a reliable marker in contrast to Nile red (O'Rourke et al., 2009), and mitochondrial function is evaluated with probes like MitoTracker, aiding investigations into obesity, diabetes, and oxidative stress (Yee et al., 2014). Muscle health is assessed by pharyngeal pumping rates and body wall muscle integrity, with automated analyses detecting age-related decline or compound effects (Chow et al., 2006; Herndon et al., 2002). These biomarkers enable efficient and targeted screening.

3.4. Limitations and challenges

Despite these advances, several limitations remain. *C. elegans* is a simple invertebrate lacking many organs found in mammals, as well as an adaptive immune system. Its innate immune system, while sophisticated, is highly diverged from that of vertebrates, limiting direct translational comparisons (Tran and Luallen, 2024). Regarding stem cell biology, although *C. elegans* possesses well-characterized adult germline stem cells capable of exhibiting cellular senescence-like stage (Nonninger et al., 2025), it lacks the diverse somatic stem cell populations found in mammalian tissues. Moreover, *C. elegans* is primarily hermaphroditic, with males being rare and no distinct female sex, which limits the study of sex-specific effects (Burkhardt et al., 2023). Its nervous and immune systems are comparatively simple, and drug absorption, metabolism, and excretion can differ substantially from mammals,

potentially restricting the direct translation of findings. Nevertheless, *C. elegans* HTS platforms have proven invaluable for the rapid identification of compounds that modulate longevity and ameliorate disease-relevant phenotypes. They have revealed conserved pathways such as insulin/IGF-1 signaling and dietary restriction mimetics, and continue to serve as a cornerstone for the initial screening and characterization of candidate geroprotectors (Sohrabi et al., 2023; Zavagno et al., 2023).

4. High-Throughput Screening in *Drosophila*

Drosophila offers distinctive and complementary advantages for HTS in ageing research and geroprotector discovery. It combines sophisticated, versatile genetics with the capacity to model relatively complex behaviours, organ-level physiology, and male-female dimorphisms compared to *C. elegans* in these aspects. Its short lifecycle and experimental tractability enable rapid generation of disease and signal pathway-specific models. In addition, the rich genetic variation strains, such as the *Drosophila* Genetic References Panel, allow systematic evaluation of gene-environment and gene-drug interactions, providing insights that are more relevant to human population ageing (Dos Santos and Cochemé, 2024; Harrison et al., 2024).

Recent advances have produced automated HTS platforms primarily focused on behavioural and healthspan phenotyping, with a few also encompassing lifespan assessment (Table 2). These systems integrate robotics for fly handling and sorting, automated imaging for high-content behavioural and physiological readouts, and AI-driven analytics for precise, scalable phenotype extraction. Together, they facilitate systematic screening of large chemical libraries, RNAi collections, and transgenic resources for modifiers of ageing and age-associated pathology. Specialised auxiliary tools, such as The Ultimate Reader of Dung (T.U.R.D.), which quantifies excreta to assess intestinal function and host–microbiome interactions (Wayland et al., 2014), expand the repertoire of accessible healthspan proxies beyond survival and gross locomotion.

The well-characterised developmental biology and nervous system of *Drosophila* support faithful modelling of cancers and neurodegenerative disorders (e.g., Alzheimer's and Parkinson's diseases), enabling evaluation of interventions that mitigate dysregulated proliferation, dysplasia, protein aggregation, neuronal loss, and behavioural deficits. Similarly, the accessible and experimentally tractable gut serves as a robust platform for probing age-associated declines in barrier integrity, stem cell homeostasis, and microbiota dynamics (Martins et al., 2018; Regan et al., 2022). Advances in AI-driven video tracking and robotic automation now allow simultaneous measurement of complex, system-level phenotypes, such as sleep architecture, circadian rhythms, locomotion, and social behaviours, which are increasingly recognised as integral to healthy ageing (Flores-Valle, 2025; Keleş and Mehmet, 2025). The relative simplicity of the fly microbiome and innate immune system facilitates controlled dissection of host-microbe-drug interactions.

4.1. Behavioural analysis of Drosophila using multi-well platforms

Among recent innovations, the DIAMonDS platform enables parallel lifespan (up to 288 adults) and stress survival (up to 1152) assays in standard 96- or 384-well plates and solid media food. Using the DIAMonDS platform, the *methuselah* (*mth*) gene was validated to extend lifespan and confer resistance to various stressors, including paraquat. Flies of the genotype *R29H01-GAL4>UAS-TeTxLC*, in which the *R29H01-GAL4* neuronal driver line directs expression of the tetanus toxin light chain (TeTxLC) to inhibit synaptic transmission, displayed a developmental delay. (Seong et al., 2020). It supports individual tracking and is amenable to sleep behaviour measurement, but its confined arenas limit complex behavioural assessment and exclude social interaction due to single-fly occupancy. The *Drosophila* Activity Monitoring (DAM) system remains widely used for automated quantification of locomotor activity and sleep, typically employing 32 individual tubes (Pfeiffenberger et al., 2010). Multi-beam successors, such as the MB5 incorporate 17 infrared beams spanning each tube's length (16 tubes per unit), enabling detection of short local displacements and positional changes that single-beam devices miss. This higher spatial resolution improves quantification of sleep fragmentation, micro-arousals, and locomotor structure. Using DAM, researchers have identified multiple drugs that significantly alter activity rhythms or sleep structure. For example, the wake-promoting stimulant caffeine (Wu et al., 2009) and sleep-enhancing GABA receptor agonists (e.g., gaboxadol) (Berry et al., 2015), making it a central tool in *Drosophila* behavioural pharmacology. The Ethoscope platform, which combines a Raspberry Pi 3 microcomputer, an infrared camera, and a 3D-printed behavioural chamber, enables modular, distributed, real-time video tracking within 24-well configurations. In practice, the system has successfully differentiated and validated 40 compounds with distinct modes of action (such as DDT, dieldrin, flubendiamide, and rotenone) and sensitively detected behavioural differences caused by key resistance-related gene mutations, including L1029F in the voltage-gated sodium channel gene *paralytic (Para)* and A301S in the GABA receptor gene *Resistance to dieldrin (Rdl)*, demonstrating its strong utility in drug screening and functional genetic analysis. (Jones et al., 2023). Integration of advanced tracking with large language model–assisted analytics (GPT-3.5 architecture) recently enabled a genome-wide screen identifying 758 genes regulating sleep (e.g., *mre11*, *NELF-B*) and circadian locomotor activity (e.g., *AstC*, *Atx2*, *Cdk5*, *Cdk5α*). (Peng et al., 2024). Collectively, these platforms extend traditional survival and coarse activity monitoring toward richer, high-content behavioural phenotyping, including micro-movement classification, sleep consolidation metrics, and stimulus-responsive behaviours.

4.2. Biomarkers in Drosophila HTS

In recent years, the development of HTS platforms using *Drosophila* has enabled systematic identification of compounds and genetic regulators that modulate ageing and associated pathologies. Central to these advances is the use of ageing biomarkers, like quantifiable phenotypic, physiological, or molecular features that reflect biological age, organismal health, and the efficacy of interventions. These biomarkers broadly fall into two categories: (1) general health indicators, such as lifespan trajectories, locomotor/vigour decline, stress resistance, and fecundity; and (2) tissue- or disease-specific readouts that capture functional deterioration in muscle, nervous system, gut, heart, or in models of neurodegeneration, metabolic, and immune dysfunction. This rich and sophisticated biomarker repertoire informs age-related disorders and healthspan, allowing finer discrimination of intervention quality beyond lifespan extension alone.

Among general health indicators, lifespan remains the most direct and widely used biomarker in *Drosophila* ageing studies, reflecting cumulative systemic health influenced by genetic, environmental, and pharmacological factors (Bushey, 2010; Gaitanidis, 2019). However, lifespan alone provides limited insight into quality of life or specific ageing mechanisms. Consequently, healthspan, the period during which the organism remains functionally competent, has gained prominence.

Healthspan is assessed through behavioural and physiological measures, including locomotion, stress resistance, fecundity, cognitive performance, and intestinal homeostasis. For example, locomotor decline is a robust and quantifiable biomarker of ageing and frailty. Negative geotaxis, the innate climbing response to gravity, evaluates neuromuscular coordination. The rapid iterative negative geotaxis (RING) assay quantifies climbing ability across hundreds of flies using automated imaging (Gargano et al., 2005). Declines in climbing speed and height correlate strongly with ageing and mortality. Motor decline is also measured via flight ability or walking speed on flat arenas, serving as

Table 2
AI Tools for Drug Discovery for *Drosophila*.

Strategic categories	Tool	Healthspan	Lifespan	Capacity (Throughput)	Limitations	References	
Cultured and recorded in multi-well plates with AI-based data analysis	MAPLE	Locomotor, Social Behavior	N.A.	96, 182 (medium)	1. Throughput remains lower than that of highly specialized commercial systems 2. Slower than trained experimentalist	Alisch T et al. Elife. 2018	https://elifesciences.org/articles/37166
	MARGO	Locomotor,e.g. centroid position, speed, movement bouts	N.A.	960, 5000 (Medium-high)	1. Cannot maintain identity through collisions 2. No limb/posture tracking	Werkhoven Z et al. PLoS One. 2019	https://journals.plos.org/plosone/article?id=10.1371%2Fjournal.pone.0224243
	DIAMonDS	Pausible for sleep bahavior measurement	Yes, can be assessed by movement	288, 1152 (Medium-high)	1. Fly transfer weekly 2. Insufficient space for behavior	Seong et al. eLife. 2020	https://elifesciences.org/articles/58630
	Whole Animal Feeding FLat (WAFFL)	Food intake and locomotor,e.g.feeding behavior, gastrointestinal tract food content, distance traveled	N.A.	96 (low)	1. Liquid media evaporation 2. Expensive 3. Time-consuming	Jaime MDLA et al. G3 (Bethesda). 2023	https://academic.oup.com/g3journal/article/13/3/jkad012/6989864
	RoboCam	eggs laying	N.A.	144 (medium)	1. Daily media replacement requirement 2. Inter-well variability 3. Chemical aversive effects	Gomez A et al. Toxics. 2024	https://www.mdpi.com/2305-6304/12/9/658
	FlyBox	Locomotor and sleep, e.g. sleep, circadian activity patterns, bimodal activity, optogenetic neuronal excitation and inhibition	N.A.	96 (low)	1. Not sufficiently light-tight 2. Tended to overheat (fan issue) 3. Variability between the intensity output of each FlyBox	Jasmine Quynh Le et al. bioRxiv. 2024	https://www.biorxiv.org/content/10.1101/2024.05.15.594443v1
	DrosoVAM	Locomotor and food intake, e.g. post-mating activity changes, position analysis, displacement activity, food preference assay, feeding behaviours	N.A.	32 (low)	1. Noisiness of individual data. 2. Trade-off between number of chambers and tracking precision. 3. Occasional misdetection by DeepLabCut. 4. Food drying after 10 days.	Revel M, Nagoshi E, Maeda R. R Soc Open Sci. 2025	https://royalsocietypublishing.org/rsos/article/12/9/250764/235360
Cultured and recorded in vials with AI-based data analysis	DAM	Locomotor, Sleep	N.A.	1(low)	1. Special code numbers associated with being offline. 2. Rhythmic component obscured by high baseline activity. 3. Trends interfere with the assessment of 24 hour periodicities	Hendricks JC et al. Neuron. 2000	https://www.cell.com/neuron/fulltext/S0896-6273(00)80877-6
	DAM	Locomotor, Sleep	N.A.	1, 32 (low)	1. Percentage of sleep tend to be overestimated 2. Low throughput	Chiu JC et al. J Vis Exp. 2010	https://pmc.ncbi.nlm.nih.gov/articles/PMC3229366/
	ShinyR-DAM	Locomotor, Sleep, Circadian rhythms	N.A.	32 (low)	low throughput	Cichewicz K et al. Commun Biol. 2018	https://www.nature.com/articles/s42003-018-0031-9
	Ethoscope	Locomotor and sleep, e.g. immobile, micro-movement, walking,	N.A.	1400 (high)	1. Limited computational power	Jones H et al. Elife. 2023	https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.2003026

(*continued on next page*)

Table 2 (*continued*)

Strategic categories	Tool	Healthspan	Lifespan	Capacity (Throughput)	Limitations	References	
		spatial distribution, sleep rebound, activity rhythm, event triggers			2. low temporal resolution		
	FlyVISTA	Locomotor and sleep, e.g. sleep and wake-associated microbehaviors, postural relaxation, antennal drooping, proboscis extension, haltere switch, leg adjustment, grooming	N.A.	1 (low)	1. Low throughput 2. Large data size 3. Haltere switch misclassification 4. Subtle postural relaxation undetectable.	Keleş MF et al. bioRxiv. 2024	https://www.science.org/doi/10.1126/sciadv.adq8131
Culturing and imaging are performed independently with AI-based data analysis	DeepFly3D	Locomotor, e.g. walking, backward walking, forward walking andgrooming.	N.A.	1 (low)	1. Expensive, structurally complex 2. Low-throughput 3. It difficult to apply to free or group behaviors.	Semih Günel, et al. eLife.2019	https://elifesciences.org/articles/48571
	DVT	Locomotor and social behavior, e.g. acquaintance, average number of crowded *Drosophila*, motion explosiveness, endurance	N.A.	4-8 (low)	1. Low recognition accuracy 2. Experimental environment dependency 3. Unsuitable for free flight or non-planar behaviors 4. Large data volume	Mi K et al. Cell Biosci. 2023	https://cellandbioscience.biomedcentral.com/articles/10.1186/s13578-023-01125-0
	FlyMAX	Locomotor and physical characteristics, e.g. turning index, pitch, roll, turning index, distance traveled	N.A.	1,000+ (high)	1. Geotactic behavior-dependent 2. Open collection: escape risk	Je Woo S et al. bioRxiv 2024	https://www.biorxiv.org/content/10.1101/2024.08.21.607451v1
AI-based data analysis	Ultimate Reader of Dung (T.U. R.D.)	Intestinal health and gut microbiota	N.A.	N.A.	1. Resolution-sensitive 2. High false positives 3. Many manual parameters 4. Limited statistics	Wayland et al. Journal of Insect Physiology. 2014	https://www.sciencedirect.com/science/article/pii/S0022191014001048

sensitive indicators of neurodegeneration, sarcopenia, or mitochondrial dysfunction.

Resistance to stressors such as heat shock, oxidative agents (e.g., paraquat, H_2O_2), starvation, and hypoxia declines with age, making stress assays valuable health biomarkers. Long-lived or healthspan-extending interventions typically enhance stress tolerance, which is linked to cellular defence pathways including FOXO, JNK, and Nrf2 (Sykiotis and Bohmann, 2008). Antioxidant response element (ARE) transgenic flies report *in vivo* antioxidant levels via fluorescence intensity(Chatterjee and Bohmann, 2012).

Fecundity declines with age in female flies, making it a sensitive indicator of physiological ageing. Egg-laying assays, especially in early and mid-life, reflect endocrine and reproductive system integrity. Automated egg-counting platforms enable large-scale, time-course studies (Toivonen and Partridge, 2009). Although not always central to HTS, fertility assays assess trade-offs between lifespan extension and reproductive investment, a key evolutionary ageing concept (Takeshita, 2024). Sleep patterns also change with age. Aged flies show reduced total sleep and frequent nighttime interruptions, manifesting as fragmented, intermittent sleep. Lifespan-extending flies display more distinct sleep cycles, with shorter daytime but prolonged nighttime sleep, reflecting more regular, rhythmic patterns (Metaxakis et al., 2014).

Tissue- or disease-specific readouts that capture functional deterioration. Mitochondrial dysfunction and oxidative damage are key hallmarks of ageing. Relevant biomarkers include lipid peroxidation, measured via malondialdehyde levels, protein carbonylation, and mitochondrial membrane potential assessed by JC-1 or TMRE staining in *Drosophila* tissues. Mitochondrial morphology and dynamics, specifically the balance between fusion and fission, are visualised in flight muscles or neurons using mito-GFP and confocal microscopy. Aged flies typically exhibit fragmented mitochondria alongside reduced respiratory efficiency (Brandt et al., 2017). Chronic low-grade inflammation, often termed "inflammaging," is primarily mediated by the innate immune IMD and Toll pathways. Overactivation of antimicrobial peptide (AMP) genes such as *Diptericin* and *Drosomycin* in fat body and gut tissues can be quantified by qPCR or reporter constructs (Perna et al., 2024). These markers reflect immune dysregulation and serve as targets for longevity-promoting interventions aimed at suppressing sterile inflammation.

Cognitive decline is modelled using learning and memory assays, including olfactory conditioning, courtship suppression, and place learning. Age-related deficits in these assays parallel synaptic dysfunction and brain ageing (Harel et al., 2016; Melnattur et al., 2021; Tamura et al., 2003). Neuropathological markers, including aggregates of Aβ42, Tau, or α-synuclein introduced via transgenic models, are quantified through immunostaining or fluorescent reporters. These markers serve as established biomarkers for neurodegenerative diseases such as Alzheimer's and Parkinson's (Feany and Bender, 2000).

Gut ageing is characterised by intestinal dysplasia, stem cell hyperproliferation, and microbiota imbalance, all dampening gut homeostasis and contributing to systemic decline. The "Smurf assay," which detects dye leakage from the gut into the hemolymph, indicating barrier failure, is a widely used biomarker for gut integrity (Rera et al., 2011). This

assay can be further combined with faecal imaging tools (e.g., T.U.R.D.) (Wayland et al., 2014) to systematically assess gut health and dysbiosis.

4.3. Limitations and challenges

Despite recent advances, several limitations constrain the use of *Drosophila* in high-throughput ageing research. As an invertebrate, it occupies an intermediate position between *C. elegans* and vertebrates, more complex in organ physiology (brain regions, gut compartments, trachea, fat body), yet lacking adaptive immunity and some mammalian metabolic features. Translational gaps arise from differences in xenobiotic absorption, pharmacokinetics, lipid transport, and endocrine regulation, complicating dose extrapolation and mechanistic interpretation (Dos Santos and Cochemé, 2024).

While greater behavioural and physiological complexity enhances biological relevance, it comes at the cost of reduced throughput. Single-fly housing improves precision and longitudinal tracking but removes social and microbial influences. Moreover, batch effects from genetic background, *Wolbachia* status, and diet further complicate cross-study comparisons. Nonetheless, conservation of key longevity and stress pathways, insulin/IGF, TOR, JNK, NF-κB/IMD, and Nrf2/CncC, combined with powerful genetic tools (GAL4/UAS, CRISPR, RNAi), short generation time, and scalable assays, offers *Drosophila* as a versatile platform for geroprotective discovery and mechanistic ageing studies. Continued improvements in assay standardisation, environmental control, and open data analytics, alongside balancing the complexity of different health data collection with cost, time, and biological relevance considerations, will further enhance its translational relevance.

5. High-throughput screening in killifish

The African turquoise killifish has rapidly emerged as a valuable vertebrate model for ageing research, uniquely combining a short lifespan of 4–6 months with vertebrate complexity (Cellerino et al., 2016; Kim et al., 2016b). While its application in middle to high-throughput screening (HTS) is still nascent compared to invertebrate models, it holds great promise for bridging the translational gap between simple organisms and mammals. The killifish recapitulates key hallmarks of vertebrate ageing, including cognitive decline (Valenzano et al., 2006b), muscle atrophy (Ruparelia et al., 2024), and age-associated pathologies (Di Cicco et al., 2011; Ma et al., 2025), within a few months. Recent technological advances, such as the automated feeding platform (McKay et al., 2022), are beginning to enable large-scale drug screening.

Importantly, killifish models have revealed age-dependent protein aggregation in the brain (De Bakker et al., 2024; Kelmer Sacramento et al., 2020) and selective neuronal loss (Bagnoli et al., 2022), supporting their use for screening therapeutics for age-dependent cognitive decline. Their amenability to genetic manipulation, including CRISPR/Cas9 genome editing, further enhances their utility for dissecting ageing mechanisms and validating candidate interventions identified in invertebrate screens (Harel et al., 2016). The model offers a practical solution for drug delivery; for example, compounds can be efficiently incorporated into food pellets for long-term administration (Valenzano et al., 2006b). This platform also allows assessment of drug effects on vertebrate-specific processes like adaptive immunity (Bradshaw et al., 2022) and tissue regeneration (Van Houcke et al., 2023). As screening technologies mature and standardisation improves, the killifish is poised to become a standard model in geroscience, enabling rapid vertebrate-based evaluation of geroprotective agents.

5.1. Biomarkers in killifish HTS

Age-related morphological and behavioural changes provide practical, scalable biomarkers for killifish ageing studies. One prominent phenotypic marker is pigmentation loss: body colouration, especially in fins and scales, gradually fades with age. This change is readily quantifiable via image analysis and serves as a reliable, non-invasive indicator of biological age (Nikiforov-Nikishin et al., 2022).

Motor patterns represent an integrative biomarker with high translational value, given the association of walking speed and muscle strength with mortality in human cohorts (López-Bueno et al., 2022; Studenski, 2011) that can be easily and scalably assessed with video-recordings and automated analysis. Spontaneous swimming activity, startle response, and exploratory behaviour decrease significantly in older fish, reflecting neuromuscular and cognitive deterioration (Mariën et al., 2024; Ruparelia et al., 2024). Locomotor readouts have been successfully used to screen anti-ageing compounds (Valenzano et al., 2006b). Cognitive decline is demonstrated through learning and memory tasks: visual cue-based associative learning, including colour discrimination and spatial learning in T-mazes, shows significant deficits in aged fish (Valenzano et al., 2006b). Although less amenable to large-scale automation, these cognitive assays provide high-content behavioural biomarkers for neuroprotective screening, particularly relevant to cognitive decline models.

Reproductive senescence follows a stereotyped pattern, with marked reductions in fecundity, mating frequency, and gonadal morphology. Female killifish exhibit ovarian atresia and reduced oocyte production, while males show decreased spermatogenesis and testicular degeneration with age (Cattelan and Valenzano, 2025; Di Cicco et al., 2011; Žák and Reichard, 2021). These traits serve both as ageing biomarkers and endpoints for evaluating sex-specific responses to anti-ageing interventions. Finally, omics ageing signatures in the killifish are very similar to those detected in humans (Aramillo Irizar et al., 2018; Giannuzzi et al., 2024). Therefore, high-throughput molecular profiling combined with AI offers the possibility of holistic translational biomarkers.

5.2. Limitations and challenges

Despite its promise, several challenges limit the use of killifish in HTS. Lifespan studies in males require housing fish individually to avoid aggression, increasing space and resource demands compared to models like *C. elegans* and *Drosophila*, which can be housed in large groups. There is a strong need for standardization of diets and husbandry practices. A species-specific genetic toolkit is missing, and in particular, no genetic disease models are currently available. Husbandry is labour-intensive, requiring daily maintenance and strict water quality control. Moreover, in worms and flies, drugs can be added directly to the medium and will be consumed. In killifish, inexpensive and stable compounds can be added to the water (Baumgart et al., 2016), but otherwise they would need to be incorporated into food pellets (Valenzano et al., 2006b) or injected intraperitoneally (Kelmer Sacramento et al., 2020),

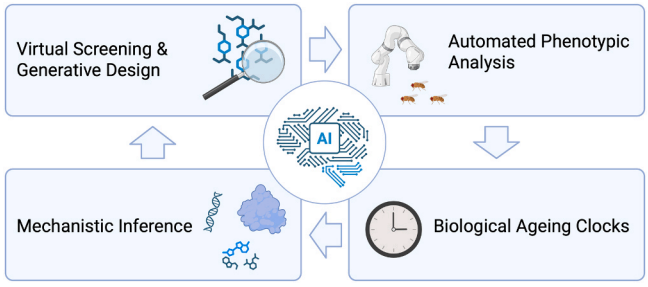

Fig. 3. AI Integration in High-Throughput Screening for Ageing Research and Geroprotector Discovery. AI enhances every stage of high-throughput screening (HTS) in ageing research and drug discovery by enabling: virtual and generative compound design to explore vast chemical spaces; automated phenotypic screening using computer vision in model organisms for quantitative analysis of complex behaviours, morphology, and functional decline; and biological ageing clocks that serve as surrogate endpoints and provide mechanistic insights, facilitating target discovery and mode-of-action elucidation.

complicating drug administration methods. These factors hinder the possibility of performing interventions at scale.

6. The integration of artificial intelligence in high-throughput screening

Artificial intelligence (AI) has become a transformative force in drug discovery and ageing research by enabling the analysis of complex, high-dimensional datasets and accelerating the identification of geroprotective compounds. AI techniques, from machine learning and deep learning to generative models and computer vision, are revolutionizing every stage of HTS, from virtual compound design, phenotypic profiling and multi-omics integration to biological ageing clocks and mechanistic inference (Fig. 3).

6.1. Virtual & generative design

Traditional HTS is constrained by the physical size of compound libraries, limiting chemical space exploration. AI-powered virtual screening and generative models address this limitation by exploring extensive chemical spaces, enabling the identification of novel senolytic ot anti-ageing candidates with targeted properties (Smer-Barreto et al., 2023; Wong et al., 2023). For example, AgeXtend is a platform to discover anti-ageing compounds. It uses machine learning models to screen over 1 billion compounds, including small molecules and natural metabolites. The platform predicts the biological activity, toxicity, and mechanisms of potential anti-ageing agents, identifying promising candidates for aging-related diseases (Arora et al., 2024). Similarly, ElixirSeeker employs machine learning to fuse molecular fingerprints from external databases, enabling the identification of promising anti-ageing drug candidates through integrative chemical similarity and activity prediction (Pan et al., 2025).

These AI-driven approaches significantly accelerate hit identification and improve precision by focusing on compounds with the highest predicted geroprotective efficacy (Table 3). AI can efficiently explore vast chemical spaces, identifying novel compounds with high potential for geroprotective effects.

6.2. Phenotypic & computer vision approaches

AI-powered computer vision has transformed phenotypic screening in ageing research by automating the quantitative analysis of complex behaviours, morphology and functional decline in organisms with high accuracy and throughput (Table 4). In *C. elegans*, deep learning methods now perform tasks that previously required labour-intensive manual scoring, including automated live worm counting (García-Garví et al. 2023) and life stage classification using object detection architectures such as YOLOv3 (Song et al., 2021). WormCNN analyses body morphology features to predict biological age, enabling rapid, objective assessment of ageing phenotypes and compound efficacy (Pan et al., 2024). In *Drosophila*, AI systems have successfully tracked locomotion, feeding behaviour, and morphology, generating high-content behavioural profiles linked to healthspan modulatio (Gálvez Salido et al., 2024; McKenzie-Smith et al., 2025).

Collectively, these approaches reduce reliance on subjective manual scoring, improve reproducibility, increase throughput, and accelerate the validation and prioritisation of candidate geroprotective compounds by linking morphological and behavioural signatures to ageing outcomes. When needed, these pipelines can also be integrated with multi-omics readouts to further enhance mechanistic insights.

6.3. Biological ageing clocks and mechanistic inference

AI-driven ageing clocks are increasingly employed as HTS-ready surrogate endpoints, target-discovery tools, and phenotypic analysis readouts. Trained on multi-omics and digital phenotypes (e.g., imaging and gait), these clocks enable phenotypic screening by quantifying compound-induced shifts in biological age alongside morphological or functional readouts (Srour et al., 2025). In addition, the recent advanced human facial and retinal image-based ageing clocks (Bobrov et al., 2018; Yu et al., 2024) could, in principle, be adapted for high-throughput screening by serving as rapid, quantitative endpoints for evaluating compound effects on cellular or tissue-level ageing phenotypes across large imaging datasets.

Meanwhile, clock-informative genes and pathways help nominate dual-purpose targets relevant to ageing and age-related diseases (Chen et al., 2025). This “from clock to clock” workflow aligns discovery and validation: clocks guide hit triage in cell- or organism-based assays and serve as pharmacodynamic readouts to confirm biological-age deceleration, thereby improving throughput and decision quality.

Mechanistically, integrating ageing clocks with interpretable AI and network inference facilitates the generation of causal hypotheses. Feature attribution methods such as SHAP (SHapley Additive exPlanations), a game theory-based approach that quantifies each feature's contribution to model predictions, along with integrated gradients and network enrichment localize clock signals to conserved longevity pathways such as insulin/IGF, mTOR, inflammatory, stress-responsive, mitochondrial, and proteostasis modules, supporting target triage and mode-of-action elucidation. Coupling clocks with perturbational datasets (CRISPR, RNAi, small molecules) and longitudinal study designs helps distinguish correlation from causation. Additionally, cross-species transfer learning enhances translational fidelity from models to humans,

Table 3
AI Tools for Drug Discovery in Ageing Research.

Tool	Description	Key Features	Reference	
ElixirSeeker	Machine learning tool for fusing molecular fingerprints	Identifies anti-aging drugs from external databases	Pan et al. Aging Cell. 2025	https://onlinelibrary.wiley.com/doi/10.1111/acel.70116
AgeXtend	Explainable AI platform for discovering geroprotectors	Screens over 1 billion compounds, evaluates geroprotective potential, toxicity risks, and protein targets; modular design for transparency	Arora et al. Nat Aging. 2025	https://www.nature.com/articles/s43587–024–00763–4#citeas
PandaOmics	AI-enabled biological target discovery platform	Identifies dual-purpose targets for aging and age-associated diseases; integrates omics, text, finance, and KOL scores	Kamya et al. J Chem Inf Model. 2024	https://pubs.acs.org/doi/10.1021/acs.jcim.3c01619
Deep Neural Network	Multi-pathway targeting AI for anti-aging drug identification	Targets dopamine, serotonin, and histamine receptors; > 70% of identified drugs extend C. elegans lifespan	Avchaciov et al. Nat Commun. 2022	https://www.nature.com/articles/s41467–022–34051–9
Machine Learning Model	Machine learning model for senolytic drug discovery	Trained on > 2500 chemical structures; screens > 4000 chemicals to identify 21 candidates; identifies ginkgetin, periplocin, oleandrin	Smer-Barreto et al. Nat Commun. 2023	https://www.nature.com/articles/s41467–023–39120–1
Deep Neural Network	AI-guided screening for selective senolytic compounds	Screens > 800,000 compounds; discovers three potent senolytics with superior medicinal chemistry properties	Wong et al. Nat Aging. 2023	https://www.nature.com/articles/s43587–023–00415-z

Table 4
AI Tools for Computer Vision in Ageing Research.

Tool	Model Organism	Application	Key Features	Reference	
WormCNN	*C. elegans*	Biological age prediction	Analyzes body morphology to predict age	Pan et al. Int J Mol Sci. 2024	https://www.mdpi.com/1422–0067/25/17/9675
Unspecified	*Drosophila*	Tracking locomotion, feeding, and morphology	Quantifies phenotypic traits linked to healthspan	Wang et al. Nature. 2020	https://www.nature.com/articles/s41586–020–2055–9
Deep Lifespan Predictor	*C. elegans*	Lifespan and pathology prediction	ML model analyzes microscopy images of pharynx/intestine pathology; predicts > 70% lifespan variation	Kern et al. Biorxiv. 2024	https://www.biorxiv.org/content/10.1101/2024.03.20.585803v1
Bimodal Neural Network	*C. elegans*	Lifespan stage estimation	Combines image sequences and live counts for curve termination prediction; bimodal inputs for uncertainty estimation	García-Garví et al. Comput Struct Biotechnol J. 2022	https://www.csbj.org/article/S2001-0370(22)00590-6/
Multidimensional Phenotyper	*C. elegans*	Healthspan and lifespan prediction	Extracts 100 + morphological/postural/behavioural features from videos; predicts healthspan as fraction above 50% initial prognosis	Martineau et al. PLoS Comput Biol. 2020	https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1008002
Movement Monitor	*C. elegans*	Healthspan quantification	Tracks population movement in plates; AUC over days 2–7 correlates with full lifespan; detects extensions in 7 days	Zavagno et al. Geroscience. 2024	https://link.springer.com/article/10.1007/s11357–023–00998-w
Visual Frailty Index	Mice	Frailty and age prediction	Machine vision on open-field videos extracts morphometric/behavioural features; predicts frailty score and age (RSE=8.5–15.9)	Hession et al. Nat Aging. 2022	https://www.nature.com/articles/s43587–022–00266–0
Machine Learning Fall Detector	*Drosophila*	Fall detection in locomotion	Identifies falls in 2D video trajectories; reveals increased falls near death in aged flies	Mattins et al. J Gerontol A Biol Sci Med Sci. 2025	https://academic.oup.com/biomedgerontology/article/doi/10.1093/gerona/glaf029/8016084
Posture-based Behaviour Classifier	*Drosophila*	Long-term behavioural monitoring	Extracts fine-grained behaviours (grooming, locomotion speed, proboscis extension) from high-resolution postural videos; tracks age-related declines in walking and circadian patterns	McKenzie-Smith et al. PLoS Comput Biol. 2025	https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1012753
MAFDA	*Drosophila*	Group behaviour annotation	Machine learning for tracking and classifying complex social behaviours (courtship, feeding) in groups using camera feeds	Sun et al. Sci Adv. 2023	https://www.science.org/doi/10.1126/sciadv.adf6254
DVT Pipeline	*Drosophila*	Locomotion and social interaction analysis	High-throughput video tracking quantifying 74 spatiotemporal metrics; assesses effects of density, light on behaviour	Mi et al. Cell Biosci. 2023	https://link.springer.com/article/10.1186/s13578–023–01125–0
Deep Learning Larval Tracker	*Drosophila*	Larval locomotion analysis	Tracks larval movement in videos; quantifies mobility decline due to parental aging and microplastic exposure	Wang et al. J Environ Manage. 2025	https://www.sciencedirect.com/science/article/abs/pii/S0301479725004785
CNN Morph Classifier	*Drosophila*	Morphology-based gender identification	Deep learning on images for automatic sex sorting; analyzes subtle morphological traits in real-time	Genaev et al. Mathematics. 2022	https://www.mdpi.com/2227-7390/10/3/295
flyGrAM	*Drosophila*	Group locomotor activity quantification	Video-based real-time tracking of multiple flies; measures ethanol-induced activity changes	Scaplen et al. Sci Rep. 2019	https://www.nature.com/articles/s41598–019–40952–5

supporting preclinical-to-clinical bridging within HTS pipelines (Chen et al., 2025).

6.4. Limitations and challenges

Despite their promise, AI applications in ageing research face significant challenges. Bias and limited generalizability arise when models are trained on restricted or unrepresentative datasets, potentially reducing applicability across diverse biological contexts or species and increasing the risk of false positives or negatives. Interpretability remains a critical hurdle, as many deep learning models function as "black boxes," complicating biological insight and hypothesis generation (Wu et al., 2023). Rigorous experimental validation is essential to confirm predicted hits across diverse models and conditions, ensuring both efficacy and safety. Moreover, heterogeneity in experimental protocols, data formats, and annotation standards hampers reproducibility and benchmarking of AI models (Schaduangrat et al., 2020).

7. Translational priorities and future directions

HTS with automation and AI has significantly accelerated the discovery of geroprotectors, yet substantial translational challenges remain. Invertebrate models such as *C. elegans* and *Drosophila* enable rapid, cost-effective, and genetically tractable interrogation of conserved longevity pathways (Kenyon, 2010; Piper and Partridge, 2018). However, physiological differences, such as the lack of key genes, absence of adaptive immunity, dissimilar body plans, and divergent pharmacokinetics, limit their direct extrapolation to mammals. Crucially, drug-target interactions are not always conserved across species, meaning that compounds identified in model organisms may not engage homologous molecular targets in humans due to structural divergence. Furthermore, short-lived organisms often exhibit greater physiological plasticity and accelerated life-history dynamics compared with longer-lived mammals, which may alter responses to metabolic or pharmacological interventions.

The African turquoise killifish serves as a short-lived vertebrate model bridging this gap, offering vertebrate-like tissue complexity and emerging genomic tools (Harel et al., 2015; Reichard et al., 2015). Still, its laborious husbandry, higher resource demands, immature disease and genetic panels, and lack of harmonised protocols constrain scalability. Moreover, transitioning into vertebrate studies also raises ethical oversight complexity and costs, while attrition often arises from interspecies differences in absorption, distribution, metabolism, excretion, and toxicity (ADMET) and context-dependent pathways (Waring et al.,

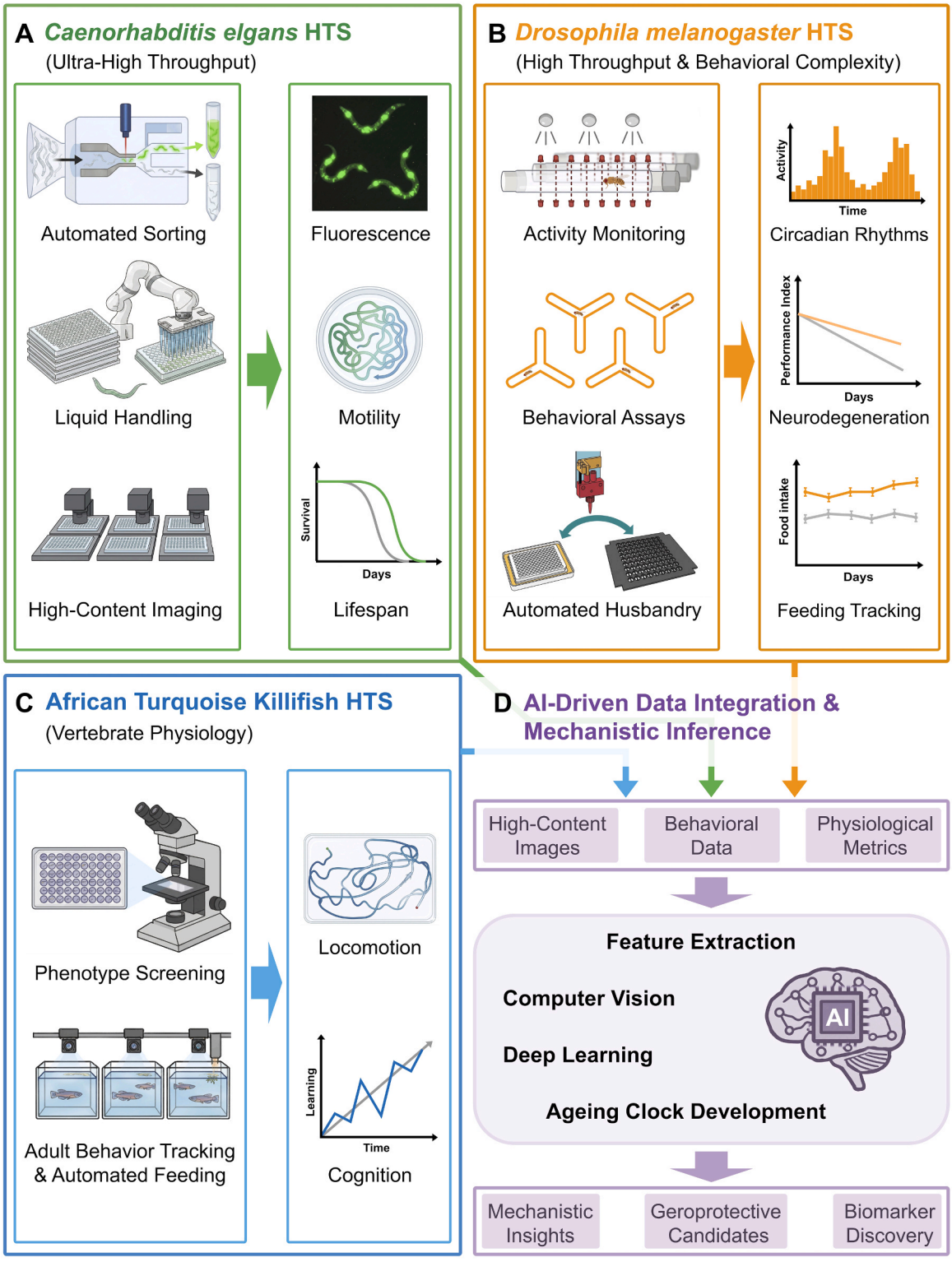

Fig. 4. High-throughput screening platforms in short-lived model organisms and AI-enabled integration of multimodal data for geroprotector discovery. **A.** *C. elegans* enables ultra-high-throughput screening through automated sorting, liquid handling, and high-content imaging, with common outputs including fluorescence, motility, and lifespan. **B.** *Drosophila* supports high-throughput screening with greater behavioural and physiological complexity, allowing assessment of activity, circadian rhythms, neurodegeneration-related decline, and feeding behaviour. **C.** The African turquoise killifish serves as a short-lived vertebrate model for medium- to high-throughput phenotypic screening, with representative outputs including locomotor and cognition-related traits. **D.** AI-assisted integration of imaging, behavioural, and physiological data across model systems enables feature extraction, deep learning-based analysis, ageing clock development, mechanistic inference, candidate prioritisation, and biomarker discovery.

2015).

Despite these limitations, convergence between model systems and human data has begun to yield results. For example, metformin has demonstrated robust lifespan extension in model organisms, paralleling epidemiological evidence of reduced age-related disease risk in humans (Barzilai et al., 2016). In parallel, an alternative discovery route called "reproposing" has emerged from human epidemiology and clinical medicine, where drugs first developed for metabolic disorders, such as SGLT2 inhibitors and GLP-1 receptor agonists, have demonstrated systemic benefits, including improved metabolic health, reduced cardiovascular risk (Gajjar et al., 2025), and potential geroprotective effects (Santulli et al., 2025), an area significantly accelerated by comparative studies in short-lived models. Together, these complementary strategies highlight the importance of integrating model organism discovery pipelines with human clinical and population-level data to advance translational geroscience.

These strengths and limitations underscore the need to strategically choose and/or integrate screening platforms according to the primary aims of geroprotective drug discovery, whether prioritising lifespan, healthspan and disease-related functional endpoints, conserved signaling pathways or vertebrate-relevant physiological processes, while considering cost, speed, scalability and regulatory burdens across different countries. Practically, this supports a stepwise and adaptive screening framework in which candidate interventions are identified rapidly in invertebrate models and then iteratively evaluated in complementary systems to validate phenotypic effects, improve mechanistic resolution, and assess translational potential, rather than following a rigid, linear progression based solely on increasing organismal complexity. As highlighted in Fig. 4, integrating outputs from distinct platforms, including high-content imaging, behavioural measurements, and physiological metrics, can further strengthen cross-model comparisons and decision-making. Moreover, earlier mechanistic anchoring and pharmacology-aware profiling, together with AI-assisted multimodal data integration, are likely to improve the prioritization and translational success of geroprotective candidates.

A critical bottleneck is linking early lifespan or healthspan phenotypes to mechanistic causal understanding. AI and machine learning models trained on heterogeneous, partially annotated datasets risk overfitting correlative signatures unless grounded in structured causal hypotheses. Emerging causal inference frameworks, such as structural causal models, interventional analyses of perturbation datasets (CRISPR, RNAi, small molecules), time-resolved multi-omics integration, and counterfactual simulation, are increasingly essential to distinguish mechanism from association and prioritise targets with translational potential (Pearl, 2009; Peters et al., 2017). Integrating these approaches can improve the ranking of compounds that act on upstream regulatory cascades (nutrient sensing, proteostasis, mitochondrial dynamics) rather than downstream correlates.

Achieving human relevance will depend on hybrid, feedback-rich pipelines: rapid discovery in invertebrates; iterative cross-species "cross-checks"; integration of perturbational multi-omics to refine causal structure; early deployment of organoid and organ-on-chip platforms to uncover human-specific liabilities (Ingber, 2022; Lancaster et al., 2013); and targeted mammalian validation guided by conserved mechanistic signatures. Iterative back-propagation of unexpected mammalian findings (e.g., off-target toxicity, sex-specific divergence) into earlier model selection and feature engineering should progressively reduce late-stage attrition (Bou Sleiman et al., 2022; Harrison et al., 2009).

Looking forward, continued advances in model systems, screening modalities, and computational methods promise to accelerate the development of interventions that promote healthy ageing. The convergence of standardised data infrastructures, multi-site replication, domain-adaptive and causally informed AI, ethically governed interpretability, and cost-aware platform deployment is poised to enhance reliability and translational yield. Institutionalising cross-model cross-checks and embedding causal inference within core analytic layers can reduce false positives, deepen mechanistic insight, and expedite progress toward interventions that robustly extend healthspan while meeting ethical and economic benchmarks (Bou Sleiman et al., 2022; Wilkinson et al., 2016). As AI and automation mature, their tighter integration with high-throughput biological screening is likely to unlock new frontiers in geroscience, enabling interventions that extend healthspan and mitigate the burden of age-related disease.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used DeepSeek and OpenAI ChatGPT in order to spell check and improve readability. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

XJ, JL, LL, JKZ, and YXL are supported by Southern University of Science and Technology, the Guangdong Science and Technology Program (2024B1111130001), and the Shenzhen Science and Technology Program (KJZD20240903102703005). YXL also acknowledges support from the Shenzhen Science and Technology Program (JCYJ20240813094515020) and the TimePie Longevity Research Grant Program. XZ and DY are supported by SIRIO Pharma Co., Ltd. CF is supported by grants from the National Key R&D Program of China (No.2024YFC2417900) and Shenzhen-Hong Kong Institute of Brain Science-Shenzhen Fundamental Research Institutions (No.2024SHIBS000). JL is supported by the National Natural Science Foundation of China (32371219), the Shanghai Pujiang Program (23PJ1415500), and the Shanghai Institute of Nutrition and Health. JB acknowledges the financial support from Kiel University. PY and BX are grateful for support from the 2021 Research Start-up Fund Fresh Wave (Central Finance Special, Y030212059003033). Figures were created with Biorender.com.

References

Alvarez, Javier, et al., 2022. Modeling Alzheimer's disease in caenorhabditis elegans. Biomedicines 10 (2), 288.

Aramillo, Irizar, Peer, et al., 2018. Transcriptomic alterations during ageing reflect the shift from cancer to degenerative diseases in the elderly'. Nat. Commun. 9 (1), 327.

Arora, Sakshi, et al., 2024. Discovering geroprotectors through the explainable artificial intelligence-based platform ageXtend. Nat. Aging 5 (1), 144–161.

Asadi Shahmirzadi, Azar, et al., 2020. Alpha-ketoglutarate, an endogenous metabolite, extends lifespan and compresses morbidity in aging mice. Cell Metab. 32 (3), 447–456.e6.

Astre, Gwendoline, et al., 2023. Genetic Perturbation of AMP Biosynthesis Extends Lifespan and Restores Metabolic Health in a Naturally Short-Lived Vertebrate. Dev. Cell 58 (15), 1350–1364.e10.

Atoki, Ayomide V., et al., 2025. Exploring the versatility of *Drosophila melanogaster* as a model organism in biomedical research: a comprehensive review. Fly (Austin) 19 (1), 2420453. https://doi.org/10.1080/19336934.2024.2420453.

Avchaciov, Konstantin, et al., 2025. AI -driven identification of exceptionally efficacious polypharmacological compounds that extend the lifespan of *Caenorhabditis elegans*. Aging Cell 24 (7), e70060.

Azuma, Yumiko, et al., 2018. Amyotrophic Lateral Sclerosis Model. In: Yamaguchi, Masamitsu (Ed.), Drosophila Models for Human Diseases. Springer, Singapore, pp. 79–95. https://doi.org/10.1007/978-981-13-0529-0_6.

Bagnoli, Sara, et al., 2022. Quantification of noradrenergic-, dopaminergic-, and tectal-neurons during aging in the short-lived killifish *Nothobranchius furzeri*. Aging Cell 21 (9), e13689.

Bangi, Erdem, et al., 2019. A personalized platform identifies trametinib plus zoledronate for a patient with kras-mutant metastatic colorectal cancer. Sci. Adv. 5 (5), eaav6528.
Bangi, Erdem, et al., 2021. A Drosophila platform identifies a novel, personalized therapy for a patient with adenoid cystic carcinoma. iScience 24 (3), 102212.
Barardo, Diogo, et al., 2017. The drugage database of aging-related drugs. Aging Cell 16 (3), 594–597.
Barlow, Ida L., et al., 2022. Megapixel camera arrays enable high-resolution animal tracking in multiwell plates. Commun. Biol. 5 (1), 253.
Barzilai, Nir, et al., 2016. Metformin as a tool to target aging. Cell Metab. 23 (6), 1060–1065.
Baumgart, Mario, 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 (2), 122–132.
Bedbrook, Claire N., et al., 2023. Rapid and precise genome engineering in a naturally short-lived vertebrate. eLife 12, e80639.
Bergmans, Steven, et al., 2023. A review on neurodegeneration in the fast-ageing killifish, the first animal model to study the natural occurrence of neuronal cell loss. Ageing Res. Rev.
Berry, Jacob A., et al., 2015. Sleep facilitates memory by blocking dopamine neuron mediated forgetting. Cell 161 (7), 1656–1667.
Biga, Peggy R., et al., 2025. Hallmarks of aging: a user's guide for comparative biologists. Ageing Res. Rev. 104, 102616.
Bjedov, Ivana, et al., 2010. Mechanisms of life span extension by rapamycin in the fruit fly drosophila melanogaster. Cell Metab. 11 (1), 35–46.
Bobrov, Eugene, et al., 2018. Photoageclock: deep learning algorithms for development of non-invasive visual biomarkers of aging. Aging 10 (11), 3249–3259.
Bossen, Judith, et al., 2019. An EGFR-induced drosophila lung tumor model identifies alternative combination treatments'. Mol. Cancer Ther. 18 (9), 1659–1668.
Bou Sleiman, Maroun, et al., 2022. Sex- and age-dependent genetics of longevity in a heterogeneous mouse population. Science 377 (6614), eabo3191.
Bradshaw, William John, et al., 2022. Extensive age-dependent loss of antibody diversity in naturally short-lived turquoise killifish'. eLife 11, e65117.
Brandt, Tobias, et al., 2017. Changes of mitochondrial ultrastructure and function during ageing in mice and drosophila. eLife 6, e24662.
Burkhardt, Russell N., et al., 2023. Sex-specificity of the C elegans metabolome. Nat. Commun. 14 (1), 320.
Bushey, Daniel, et al., 2010. Sleep, Aging, and Lifespan in Drosophila. BMC Neuroscience 11, 56. https://doi.org/10.1186/1471-2202-11-56.
Caldwell, Kim A., et al., 2020. Modeling neurodegeneration in *Caenorhabditis elegans*. Dis. Models & Mech. 13 (10), dmm046110.
Carretero, Maria, et al., 2015. Pharmacological classes that extend lifespan of caenorhabditis elegans. Front. Genet. 6.
Cattelan, Silvia, Valenzano, Dario Riccardo, 2025. Age-dependent decline in sperm quality and function in a naturally short-lived vertebrate. BMC Ecol. Evol. 25 (1), 2.
Cellerino, Alessandro, et al., 2016. From the bush to the bench: the annual *Nothobranchius* fishes as a new model system in biology. Biol. Rev. 91 (2), 511–533.
Chamberlain, Jeffrey S., Benian, Guy M., 2000. Muscular Dystrophy: the worm turns to genetic disease. Curr. Biol. 10 (21), R795–R797.
Chatterjee, Nirmalya, Bohmann, Dirk, 2012. A Versatile ΦC31 based reporter system for measuring ap-1 and nrf2 signaling in drosophila and in tissue culture. In: Jennings, B. (Ed.), PLoS ONE, 7, e34063.
Chen, Jianjiu, et al., 2025. From Clock to clock: therapeutic target discovery for aging and age-related diseases. Ageing Res. Rev. 112, 102871.
Chin, Randall M., et al., 2014. The metabolite α-ketoglutarate extends lifespan by inhibiting ATP synthase and TOR. Nature 510 (7505), 397–401.
Chow, D., et al., 2006. Sarcopenia in the caenorhabditis elegans pharynx correlates with muscle contraction rate over lifespan. Exp. Gerontol. 41 (3), 252–260.
Churgin, Matthew A., et al., 2017. Longitudinal imaging of caenorhabditis elegans in a microfabricated device reveals variation in behavioral decline during aging. eLife 6, e26652.
Clancy, David J., et al., 2001. Extension of life-span by loss of CHICO, a drosophila insulin receptor substrate protein. Science 292 (5514), 104–106.
Cortes-Canteli, Marta, Iadecola, Costantino, 2020. Alzheimer's disease and vascular aging. J. Am. Coll. Cardiol. 75 (8), 942–951.
Dar, Arvin C., et al., 2012. Chemical genetic discovery of targets and anti-targets for cancer polypharmacology. Nature 486 (7401), 80–84.
Das, Tirtha Kamal, Ross, Leigh Cagan, 2017. KIF5B-RET oncoprotein signals through a multi-kinase signaling hub. Cell Rep. 20 (10), 2368–2383.
De Bakker, Dennis E.M., et al., 2024. Amyloid-beta precursor protein contributes to brain aging and learning decline. BioRxiv. https://doi.org/10.1101/2024.10.11.617841.
De Bakker, Dennis E.M., Valenzano, Dario R., 2023. Turquoise killifish: a natural model of age-dependent brain degeneration. Ageing Res. Rev. 90, 102019.
Del Carmen-Fabregat, Andrea, et al., 2024. High-throughput behavioral aging and lifespan assays using the lifespan machine. J. Vis. Exp. (203), 65462.
Deserno, Maurice, Bozek, Katarzyna, 2023. WormSwin: instance segmentation of c elegans using vision transformer. Sci. Rep. 13 (1), 11021.
Di Cicco, Emiliano, et al., 2011. The short-lived annual fish nothobranchius furzeri shows a typical teleost aging process reinforced by high incidence of age-dependent neoplasias. Exp. Gerontol. 46 (4), 249–256.
Dong, Bingyue, Chen, Weiyang, 2025. A high precision method of segmenting complex postures in caenorhabditis elegans and deep phenotyping to analyze lifespan. Sci. Rep. 15 (1), 8870.
Dos Santos, Eliano, Cochemé, Helena M., 2024. Pharmacology of Aging: *Drosophila* as a tool to validate drug targets for healthy lifespan. Aging Biol. 2 (1), 20240034.
Eisenberg, Tobias, et al., 2009. Induction of autophagy by spermidine promotes longevity. Nat. Cell Biol. 11 (11), 1305–1314.
Feany, Mel B., Bender, Welcome W., 2000. A drosophila model of Parkinson's Disease. Nature 404 (6776), 394–398.
Flores-Valle, Andres, et al., 2025. Dynamics of glia and neurons regulate homeostatic rest, sleep and feeding behavior in drosophila. Nat. Neurosci. 28 (6), 1226–1240.
Gaitanidis, Alexandros, et al., 2019. Longitudinal Assessment of Health-Span and Pre-Death Morbidity in Wild Type Drosophila. Aging 11 (6), 1850–1873.
Gajjar, Aryan, et al., 2025. SGLT2 Inhibitors and GLP-1 Receptor Agonists in Cardiovascular-Kidney-Metabolic Syndrome. Biomedicines 13 (8), 1924.
Gálvez Salido, Aaron, et al., 2024. Automatic counting and identification of two *Drosophila melanogaster* (diptera: drosophilidae) morphs with image-recognition artificial intelligence. Can. Èntomol. 156, e40.
García-Garví, Antonio, et al., 2023. Automation of caenorhabditis elegans lifespan assay using a simplified domain synthetic image-based neural network training strategy. Comput. Struct. Biotechnol. J. 21, 5049–5065.
Gargano, J., et al., 2005. Rapid Iterative Negative Geotaxis (RING): a new method for assessing age-related locomotor decline In. Exp. Gerontol. 40 (5), 386–395.
Garmany, Armin, Terzic, Andre, 2024. Global healthspan-lifespan gaps among 183 world health organization member states. JAMA Netw. Open 7 (12), e2450241.
Giannuzzi, Chiara et al. 2024. Epigenetic Clock and Lifespan Prediction in the Short-Lived Killifish Nothobranchius furzeri'. BioRxiv. ⟨https://www.biorxiv.org/content/10.1101/2024.08.07.606986v1⟩.
Giunti, Sebastián, et al., 2021. Drug discovery: insights from the invertebrate *Caenorhabditis elegans*. Pharmacol. Res. & Perspect. 9 (2), e00721.
Gliniak, Christy M., et al., 2025. FGF21 promotes longevity in diet-induced obesity through metabolic benefits independent of growth suppression. Cell Metab. 37 (7), 1547–1567.e6.
Harel, Itamar, et al., 2015. A platform for rapid exploration of aging and diseases in a naturally short-lived vertebrate. Cell 160 (5), 1013–1026.
Harel, Itamar, et al., 2016. Efficient genome engineering approaches for the short-lived African turquoise killifish. Nat. Protoc. 11 (10), 2010–2028. https://doi.org/10.1038/nprot.2016.103.
Harrison, David E., et al., 2009. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 460 (7253), 392–395.
Harrison, Benjamin R., et al., 2024. Wide-ranging genetic variation in sensitivity to rapamycin in *Drosophila melanogaster*. Aging Cell 23 (11), e14292.
Herndon, Laura A., et al., 2002. Stochastic and genetic factors influence tissue-specific decline in ageing C Elegans. Nature 419 (6909), 808–814.
Hwangbo, Dae Sung, et al., 2004. Drosophila dFOXO Controls Lifespan and Regulates Insulin Signalling in Brain and Fat Body. Nature 429 (6991), 562–566.
Iijima-Ando, Kanae, Iijima, Koichi, 2010. Transgenic drosophila models of Alzheimer's disease and tauopathies. Brain Struct. Funct. 214 (2–3), 245–262.
Ingber, Donald E., 2022. Human Organs-on-chips for disease modelling, drug development and personalized medicine. Nat. Rev. Genet. 23 (8), 467–491.
Jafari, Mahtab, 2010. *Drosophila melanogaster* as a model system for the evaluation of anti-aging compounds'. Fly 4 (3), 253–257.
Javer, Avelino, et al., 2018. An open-source platform for analyzing and sharing worm-behavior data. Nat. Methods 15 (9), 645–646.
Jones, Hannah, et al., 2023. A reductionist paradigm for high- throughput behavioural fingerprinting in *Drosophila melanogaster*. eLife 12, RP86695. https://doi.org/10.7554/eLife.86695.3.
Kapahi, P., et al., 2004. Regulation of lifespan in drosophila by modulation of genes in the TOR Signaling Pathway. Curr. Biol. CB 14 (10), 885–890. https://doi.org/10.1016/j.cub.2004.03.059.
Katsube, Makoto, et al., 2024. Ergothioneine promotes longevity and healthy aging in male mice. GeroScience 46 (4), 3889–3909.
Keleş, Mehmet F., et al., 2025. FlyVISTA, an integrated machine learning platform for deep phenotyping of sleep in *Drosophila*. Sci. Adv. 11, eadq8131.
Kelmer Sacramento, Erika, et al., 2020. Reduced proteasome activity in the aging brain results in ribosome stoichiometry loss and aggregation. Mol. Syst. Biol. 16 (6), e9596.
Kenyon, Cynthia J., 2010. The genetics of ageing. Nature 464 (7288), 504–512.
Kenyon, Cynthia, 2011. The first long-lived mutants: discovery of the insulin/igf-1 pathway for ageing. Philos. Trans. R. Soc. B Biol. Sci. 366 (1561), 9–16.
Kerr, Rex A., et al., 2022. The C elegans observatory: high-throughput exploration of behavioral Aging. Front. Aging 3, 932656.
Kim, Dong-Kyu, et al., 2016a. Mechanisms of aging-related proteinopathies in caenorhabditis elegans. Exp. & Mol. Med. 48 (10) e263–e263.
Kim, Yumi, et al., 2016b. The Short-Lived African turquoise killifish: an emerging experimental model for ageing. Dis. Models & Mech. 9 (2), 115–129.
Kothmayer, Michael, et al., 2025. Aging reduces intestinal stem cell activity in killifish and intermittent fasting reverses intestinal gene expression patterns. Aging Cell 24 (11), e70229.
Kroemer, Guido, et al., 2025. From geroscience to precision geromedicine: understanding and managing aging. Cell 188 (8), 2043–2062.
Kusumoto, Dai, et al., 2021. Anti-senescent drug screening by deep learning-based morphology senescence scoring. Nat. Commun. 12 (1), 257.
Kuwabara, P.E., O'Neil, N., 2001. The use of functional genomics in c elegans for studying human development and disease. J. Inherit. Metab. Dis. 24 (2), 127–138.
Lancaster, Madeline A., et al., 2013. Cerebral organoids model human brain development and microcephaly. Nature 501 (7467), 373–379.
Le, Kim N., et al., 2020. An automated platform to monitor long-term behavior and healthspan in caenorhabditis elegans under precise environmental control'. Commun. Biol. 3 (1), 297.

Lee, Daewon, et al., 2024. Diabetic sensory neuropathy and insulin resistance are induced by loss of UCHL1 in drosophila. Nat. Commun. 15 (1), 468.
Lee, Kyung Suk, Levine, Erel, 2018. A microfluidic platform for longitudinal imaging in caenorhabditis elegans. J. Vis. Exp. 57348 (135).
Lee, Shin-Hae, Min, Kyung-Jin, 2019. Drosophila melanogaster as a model system in the study of pharmacological interventions in aging. Transl. Med. Aging 3, 98–103.
Levine, Benjamin D., Cagan, Ross L., 2016. Drosophila lung cancer models identify trametinib plus statin as candidate therapeutic. Cell Reports 14 (6), 1477–1487. https://doi.org/10.1016/j.celrep.2015.12.105.
Link, Christopher D., 2006. C elegans models of age-associated neurodegenerative diseases: lessons from transgenic worm models of Alzheimer's Disease. Exp. Gerontol. 41 (10), 1007–1013.
López-Bueno, Rubén, et al., 2022. Thresholds of handgrip strength for all-cause, cancer, and cardiovascular mortality: a systematic review with dose-response meta-analysis. Ageing Res. Rev. 82, 101778.
López-Otín, Carlos, et al., 2023. Hallmarks of aging: an expanding universe. Cell 186 (2), 243–278.
Lujan, Celia, et al., 2024. An expedited screening platform for the discovery of anti-ageing compounds in vitro and in vivo. Genome Med. 16 (1), 85.
Luo, Yuan et al. 2009. 'Caenorhabditis elegans Model for Initial Screening and Mechanistic Evaluation of Potential New Drugs for Aging and Alzheimer's Disease', Methods of Behavior Analysis in Neuroscience. 2nd Edition. CRC Press/Taylor & Francis. ⟨https://www.ncbi.nlm.nih.gov/books/NBK5226/⟩.
Ma, Xueling, et al., 2025. Nothobranchius furzeri: A Vertebrate Model for Studying Cardiac Aging and Cellular Senescence. Npj Aging 11 (1), 63.
Mariën, Valerie, et al., 2024. Age-related alterations in the behavioral response to a novel environment in the african turquoise killifish (Nothobranchius furzeri). Front. Behav. Neurosci. 17, 1326674.
Markstein, Michele, et al., 2014. Systematic screen of chemotherapeutics in drosophila stem cell tumors. Proc. Natl. Acad. Sci. 111 (12), 4530–4535.
Martin-Montalvo, Alejandro, et al., 2013. Metformin improves healthspan and lifespan in mice. Nat. Commun. 4 (1), 2192.
Martins, Raquel, et al., 2018. How to catch a smurf? – ageing and beyond…in vivo assessment of intestinal permeability in multiple model organisms. ' BIO-Protoc. 8 (3).
Mathew, Mark D., et al., 2012. WormScan: A Technique for High-Throughput Phenotypic Analysis of Caenorhabditis elegans, (B. Lehner, ed.). PLoS ONE 7 (3), e33483.
McKay, Andrew, et al., 2022. An automated feeding system for the african killifish reveals the impact of diet on lifespan and allows scalable assessment of associative learning'. eLife 11, e69008.
McKenzie-Smith, Grace C., et al., 2025. Capturing continuous, long timescale behavioral changes in drosophila melanogaster postural data. PLOS Comput. Biol. 21 (2), e1012753.
Melkani, Yash, et al., 2024. Automated assessment of cardiac dynamics in aging and dilated cardiomyopathy drosophila models using machine learning. Commun. Biol. 7 (1), 702.
Melnattur, Krishna, et al., 2021. A conserved role for sleep in supporting spatial learning in *Drosophila*. Sleep 44 (3), zsaa197.
Metaxakis, Athanasios, et al., 2014. Lowered Insulin Signalling Ameliorates Age-Related Sleep Fragmentation in Drosophila. In: Mignot, E. (Ed.), PLoS Biology, 12, e1001824.
Mirzoyan, Zhasmine, et al., 2019. Drosophila melanogaster: a model organism to study cancer. Front. Genet. 10, 51.
Moffat, John G., et al., 2017. Opportunities and challenges in phenotypic drug discovery: an industry perspective. Nat. Rev. Drug Discov. 16 (8), 531–543.
Morabito, Gabriele, et al., 2024. Immune aging in annual killifish. Immun. & Ageing 21 (1), 18.
Moses, Eitan, et al., 2024. The Killifish Germline Regulates Longevity and Somatic Repair in a Sex-Specific Manner. Nat. Aging 4 (6), 791–813.
Müller, Christine, et al., 2025. Enhanced C/EBPα function extends healthspan and lifespan in the African turquoise killifish. Aging Cell 24 (10), e70211.
Musselman, Laura Palanker, Kühnlein, Ronald P., 2018. *Drosophila* as a Model to Study Obesity and Metabolic Disease. In: Suarez, R.K., Hoppeler, H.H. (Eds.), Journal of Experimental Biology, 221, p. jeb163881.
Nadon, Nancy L., et al., 2017. NIA interventions testing program: investigating putative aging intervention agents in a genetically heterogeneous mouse model. EBioMedicine 21, 3–4.
Ni, Yu-Qing, et al., 2020. Roles and Mechanisms of MFG-E8 in vascular aging-related diseases. Ageing Res. Rev. 64, 101176.
NIA Interventions Testing Program, 2021. A Collaborative Approach for Investigating Interventions to Promote Healthy Aging'. Handbook of the Biology of Aging. Academic Press, pp. 219–235. ⟨https://www.sciencedirect.com:5037/science/chapter/edited-volume/abs/pii/B978012815962000010X⟩.
Nikiforov-Nikishin, Alexei, et al., 2022. Effects of three feed additives on the culturable microbiota composition and histology of the anterior and posterior intestines of Zebrafish (Danio rerio). Animals 12 (18), 2424.
Nonninger, Tim J., et al., 2025. A TFEB–TGFβ axis systemically regulates diapause, stem cell resilience and protects against a senescence-like state. Nat. Aging 5 (7), 1340–1357.
Núñez, Sonia, et al., 2025. Black Mulberry (Morus nigra L) prevents deleterious effects of excess glucose in obese c elegans decreasing lipofuscin accumulation and ROS Production. Heliyon 11 (2), e41898.
O'Rourke, Eyleen J., et al., 2009. C elegans major fats are stored in vesicles distinct from lysosome-related organelles. Cell Metab. 10 (5), 430–435. https://doi.org/10.1016/j.cmet.2009.10.002.
Oginuma, Masayuki, et al., 2022. Rapid reverse genetics systems for nothobranchius furzeri, a suitable model organism to study vertebrate aging. Sci. Rep. 12 (1), 11628.
Pan, Yan, et al., 2024. WormCNN-assisted establishment and analysis of glycation stress models in c elegans: insights into disease and healthy aging. Int. J. Mol. Sci. 25 (17), 9675.
Pan, Yan, et al., 2025. ElixirSeeker: a machine learning framework utilizing fusion molecular fingerprints for the discovery of lifespan-extending compounds. Aging Cell 24 (8), e70116. https://doi.org/10.1111/acel.70116.
Pandey, Udai Bhan, Nichols, Charles D., 2011. Human Disease Models in *Drosophila melanogaster* and the Role of the Fly in Therapeutic Drug Discovery. In: Barker, E.L. (Ed.), Pharmacological Reviews, 63, pp. 411–436.
Partridge, Linda, et al., 2005. Dietary restriction in drosophila. Mech. Ageing Dev. 126 (9), 938–950.
Pearl, Judea, 2009. Causal inference in statistics: an overview. Stat. Surv. 3 (e).
Peng, Di, et al., 2024. Large-Language Models Facilitate Discovery of the Molecular Signatures Regulating Sleep and Activity. Nat. Commun. 15 (1), 3685.
Perna, Sabrina et al. 2024, April 3. 'Shared Transcriptomic Signatures of Inflammaging Among Diverse Strains of Drosophila melanogaster'. ⟨https://www.researchsquare.com/article/rs-4146509/v1⟩.
Peters, Jonas et al. 2017. Elements of Causal Inference: Foundations and Learning Algorithms, Adaptive Computation and Machine Learning Series. Cambridge, MA, USA: MIT Press. ⟨https://mitpress.mit.edu/9780262037310/elements-of-causal-inference/⟩.
Pfeiffenberger, Cory, et al., 2010. Processing circadian data collected from the drosophila activity monitoring (DAM) System: Fig. 1'. Cold Spring Harb. Protoc. 2010 (11), pdb.prot5519.
Piazza, Nicole, et al., 2009. Exercise-training in young drosophila melanogaster reduces age-related decline in mobility and cardiac performance, (M.V. Blagosklonny, ed.). PLoS ONE 4 (6), e5886.
Piper, Matthew D.W., Partridge, Linda, 2018. Drosophila as a Model for Ageing. Biochim. Et. Biophys. Acta (BBA) - Mol. Basis Dis. 1864 (9), 2707–2717.
Pitt, Jason N., et al., 2019. Wormbot, an open-source robotics platform for survival and behavior analysis in C Elegans. GeroScience 41 (6), 961–973.
Puckering, Timothy, et al., 2017. Automated Wormscan'. F1000Research 6, 192.
Qu, Qi, et al., 2025. Lithocholic Acid Phenocopies Anti-Ageing Effects of Calorie Restriction. Nature 643 (8070), 192–200. https://doi.org/10.1038/s41586-024-08329-5.
Rahman, Mizanur, et al., 2020. NemaLife chip: a micropillar-based microfluidic culture device optimized for aging studies in crawling C Elegans'. Sci. Rep. 10 (1), 16190.
Regan, Jennifer C., et al., 2022. Sexual identity of enterocytes regulates autophagy to determine intestinal health, lifespan and responses to rapamycin. Nat. Aging 2 (12), 1145–1158.
Reichard, Martin, et al., 2015. Turquoise Killifish. Curr. Biol. 25 (17), R741–R742.
Rera, Michael, et al., 2011. Modulation of longevity and tissue homeostasis by the drosophila PGC-1 Homolog. Cell Metab. 14 (5), 623–634.
Ripa, Roberto, et al., 2017. MicroRNA miR-29 controls a compensatory response to limit neuronal iron accumulation during adult life and aging. BMC Biol. 15 (1), 9.
Ripa, Roberto, et al., 2023. Refeeding-associated AMPKγ1 complex activity is a hallmark of health and longevity. Nat. Aging 3 (12), 1544–1560. https://doi.org/10.1038/s43587-023-00521-y.
Roig-Soriano, Joan, et al., 2025. Long-Term Effects of s-KL treatment in wild-type mice: enhancing longevity, physical well-being, and neurological resilience. Mol. Ther. 33 (4), 1449–1465.
Ruparelia, Avnika A., et al., 2024. The African killifish: a short-lived vertebrate model to study the biology of sarcopenia and longevity. Aging Cell 23 (1), e13862.
Ryu, Dongryeol, et al., 2016. Urolithin A Induces Mitophagy and Prolongs Lifespan in C Elegans and Increases Muscle Function in Rodents. Nat. Med. 22 (8), 879–888.
Santulli, Gaetano, et al., 2025. GLP-1 Receptor Agonists and SGLT2 inhibitors: new anti-aging tools? Future Cardiol. 21 (1), 5–8.
Schaduangrat, Nalini, et al., 2020. Towards reproducible computational drug discovery. J. Chemin.-. 12 (1), 9.
Ségalat, Laurent, 2007. Invertebrate animal models of diseases as screening tools in drug discovery. ACS Chem. Biol. 2 (4), 231–236.
Seong, Ki-Hyeon, et al., 2020. The drosophila individual activity monitoring and detection system (DIAMonDS). eLife 9, e58630.
Smer-Barreto, Vanessa, et al., 2023. Discovery of senolytics using machine learning. Nat. Commun. 14 (1), 3445.
Smith, Patrick, et al., 2017. Regulation of life span by the gut microbiota in the short-lived African turquoise killifish. eLife 6, e27014.
Sohrabi, Salman, et al., 2023. *Ce* Lab, a microfluidic platform for the study of life history traits, reveals metformin and SGK-1 regulation of longevity and reproductive span'. Lab a Chip 23 (12), 2738–2757.
Song, Yao et al. 2021. 'Predict the Caenorhabditis elegans Life Stage through Hybrid Deep Neural Network', 2021 IEEE International Conference on Bioinformatics and Biomedicine (BIBM). Presented at the 2021 IEEE International Conference on Bioinformatics and Biomedicine (BIBM).
Sonoshita, Masahiro, et al., 2018. A Whole-Animal Platform to Advance a Clinical Kinase Inhibitor into New Disease Space. Nat. Chem. Biol. 14 (3), 291–298.
Srour, Luma, et al., 2025. Deep Aging Clocks: AI-Powered Strategies for Biological Age Estimation. Ageing Res. Rev. 112, 102889.
Strayer, Amy, et al., 2003. Expression of the small heat-shock protein Hsp-16-2 in *Caenorhabditis elegans* Is Suppressed by *Ginkgo biloba* Extract EGb 761. FASEB J. 17 (15), 2305–2307.
Stroustrup, Nicholas, et al., 2013. The Caenorhabditis elegans Lifespan Machine. Nat. Methods 10 (7), 665–670.
Studenski, Stephanie, 2011. Gait speed and survival in older adults. JAMA 305 (1), 50.

Sykiotis, Gerasimos P., Bohmann, Dirk, 2008. Keap1/Nrf2 signaling regulates oxidative stress tolerance and lifespan in drosophila. Dev. Cell 14 (1), 76–85.
Takeshita, Rafaela S.C., 2024. A Life for a (Shorter) Life: The Reproduction–Longevity Trade-Off. Proc. Natl. Acad. Sci. 121 (17), e2405089121.
Tamura, Takuya, et al., 2003. Aging specifically impairs amnesiac-dependent memory in drosophila. Neuron 40 (5), 1003–1011.
Terzibasi, Eva, et al., 2009. Effects of dietary restriction on mortality and age-related phenotypes in the short-lived fish *Nothobranchius furzeri*. Aging Cell 8 (2), 88–99.
Tipping, Marla, Perrimon, Norbert, 2013. *Drosophila* as a model for context-dependent tumorigenesis: context-dependent tumorigenesis model. J. Cell. Physiol. n/a–n/a.
Toivonen, Janne M., Partridge, Linda, 2009. Endocrine regulation of aging and reproduction in drosophila. Mol. Cell. Endocrinol. 299 (1), 39–50.
Tran, Tuan D., Luallen, Robert J., 2024. An organismal understanding of C elegans innate immune responses, from pathogen recognition to multigenerational resistance. Semin. Cell & Dev. Biol. 154, 77–84.
Uno, Masaharu, Nishida, Eisuke, 2016. Lifespan-regulating genes in C Elegans. Npj Aging Mech. Dis. 2 (1), 16010.
Valenzano, Dario R., et al., 2006a. Temperature Affects Longevity and Age-related Locomotor and Cognitive Decay in the Short-lived Fish *Nothobranchius furzeri*. Aging Cell 5 (3), 275–278.
Valenzano, Dario R., et al., 2006b. Resveratrol prolongs lifespan and retards the onset of age-related markers in a short-lived vertebrate. Curr. Biol. 16 (3), 296–300. https://doi.org/10.1016/j.cub.2005.12.038.
Van Ham, Tjakko J., et al., 2008. C elegans model identifies genetic modifiers of α-synuclein inclusion formation during aging. In: Kim, S.K. (Ed.), PLoS Genetics, 4, e1000027.
Van Houcke, Jolien, et al., 2023. A short dasatinib and quercetin treatment is sufficient to reinstate potent adult neuroregenesis in the aged killifish'. Npj Regen. Med. 8 (1), 31.
Verheyen, Esther M., 2022. The Power of *Drosophila* in modeling human disease mechanisms. Dis. Models & Mech. 15(3) dmm0 49549.
Vidal, Marcos, et al., 2005. ZD6474 Suppresses Oncogenic RET Isoforms in a *Drosophila* Model for type 2 multiple endocrine neoplasia syndromes and papillary thyroid carcinoma. Cancer Res. 65 (9), 3538–3541.
Villegas, Santiago Nahuel, et al., 2019. Using drosophila models and tools to understand the mechanisms of novel human cancer driver gene function. In: Deng, Wu-Min (Ed.), The Drosophila Model in Cancer. Springer International Publishing, Cham, pp. 15–35. https://doi.org/10.1007/978-3-030-23629-8_2.
Vincent, Fabien, et al., 2022. Phenotypic drug discovery: recent successes, lessons learned and new directions. Nat. Rev. Drug Discov. 21 (12), 899–914.
Wang, Meng C., et al., 2003. JNK signaling confers tolerance to oxidative stress and extends lifespan in *Drosophila*. Dev. Cell 5 (5), 811–816.
Waring, Michael J., et al., 2015. An analysis of the attrition of drug candidates from four major pharmaceutical companies. Nat. Rev. Drug Discov. 14 (7), 475–486.
Wayland, Matthew T., et al., 2014. Spotting the differences: probing host/microbiota interactions with a dedicated software tool for the analysis of faecal outputs in drosophila. J. Insect Physiol. 69, 126–135.
Wilkinson, Mark D., et al., 2016. The FAIR guiding principles for scientific data management and stewardship. Sci. Data 3 (1), 160018.
Wittmann, Curtis W., et al., 2001. Tauopathy in *Drosophila*: Neurodegeneration without neurofibrillary tangles. Science 293 (5530), 711–714.
Wong, Felix, et al., 2023. Discovering small-molecule senolytics with deep neural networks. Nat. Aging 3 (6), 734–750.
World Health Organization, 2025'Ageing and Health'. 2025, October 1. Retrieved 26 November 2025 from ⟨https://www.who.int/news-room/fact-sheets/detail/ageing-and-health⟩.
Wu, Mark N., et al., 2009. The effects of caffeine on sleep in drosophila require PKA activity, but not the adenosine receptor. J. Neurosci. 29 (35), 11029.
Wu, Zhenxing, et al., 2023. From black boxes to actionable insights: a perspective on explainable artificial intelligence for scientific discovery. J. Chem. Inf. Model. 63 (24), 7617–7627.
Xian, Bo, et al., 2013. WormFarm: a quantitative control and measurement device toward automated *Caenorhabditis elegans* Aging Analysis. Aging Cell 12 (3), 398–409.
Yaku, Keisuke, et al., 2018. NAD Metabolism: implications in aging and longevity. Ageing Res. Rev. 47, 1–17.
Yee, Callista, et al., 2014. The intrinsic apoptosis pathway mediates the pro-longevity response to mitochondrial ROS in C Elegans. Cell 157 (4), 897–909.
Yu, Zhengqing, et al., 2024. Thermal facial image analyses reveal quantitative hallmarks of aging and metabolic diseases. Cell Metab. 36 (7), 1482–1493.e7.
Žák, Jakub, Reichard, Martin, 2021. Reproductive Senescence in a Short-lived Fish. In: Gaillard, J. (Ed.), Journal of Animal Ecology, 90, pp. 492–502.
Zavagno, Giulia, et al., 2023. Rapid measurement of ageing by automated monitoring of movement of c elegans populations. GeroScience 46 (2), 2281–2293.
Zhao, Yunpo, et al., 2023. The opportunities and challenges of using drosophila to model human cardiac diseases. Front. Physiol. 14, 1182610.