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Opinion

Immunity and lifespan: answering long-standing questions with comparative genomics

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Long life requires individuals to defend themselves against pathogens over prolonged periods of time whilst minimising damage to themselves. In vertebrates, pathogen defence is provided by two integrated systems, innate and adaptive immunity. Innate immunity is relatively nonspecific, resulting in collateral damage to hosts, and does not involve canonical immunological memory. In contrast, adaptive immunity is highly specific and confers long-lasting memory, which are features that are predicted to facilitate long life. However, there is long-standing debate over the general importance of adaptive immunity for the evolution of extended lifespans, partly because this is difficult to test. We highlight how recent improvements in whole genome assemblies open the door to immunogenomic comparative analyses that enable the coevolution of longevity and specific immune traits to be disentangled.

Highlights

Comparative genomics offers an opportunity to answer unresolved questions about the importance of different immune traits for the evolution of longevity.

Long-read sequencing is enabling the assembly of high-quality genomes across a broad range of non-model species. This is solving the problem of the notoriously poor representation of many immune genes in short-read whole genome assemblies.

High-quality immune gene data from species across a range of lifespans opens the door to testing whether genetic innovations in the immune system are key to the evolution of long life.

The enduring question of whether adaptive immunity facilitated the evolution of long life across vertebrates can now be empirically tested.

The evolution of long life in a world with pathogens

The question of how some species achieve lifespans of hundreds of years whilst others live just days has long fascinated biologists [1–5]. Long life requires individuals to maintain a healthy body and defend themselves against pathogens over extended time periods. This is challenging, as immunity against pathogens often comes at a cost to self-maintenance. How have long-lived species solved this problem and can we understand its molecular basis?

There are two main systems that have evolved to fight pathogens that differ in their **immunological specificity**, **immunological memory** (see Glossary), and level of host damage (Table 1). All multicellular organisms have some form of an innate immune system that provides a relatively nonspecific, broad-spectrum defence, but can also result in collateral damage to the host [6–10]. Vertebrates have evolved a second integrated system, commonly referred to as the adaptive immune system [11,12]. Adaptive immunity is highly pathogen-specific, reducing damage to host tissue and conferring long-lasting immune memory, such that immune responses to previously encountered pathogens become faster and stronger [6,10,12]. These characteristics, combined with the fact that vertebrates are relatively long-lived, has led to idea that adaptive immunity plays a pivotal role in longevity [13–19] (Figure 1).

There are, however, numerous examples of extremely long-lived plants and invertebrates that fuel debate over the importance of adaptive immunity for the evolution of long life (Box 1) [20–22]. This is exacerbated by it being difficult to test whether selection on innate and adaptive immunity are linked to lifespan evolution. Whilst studies on single species help characterise immune systems and their influence on patterns of ageing, examining adaptations that have promoted longevity over evolutionary timescales can only be addressed using comparative analyses. This requires

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Table 1. Summary of the key differences between innate and adaptive immune responses in vertebrates

	Innate	Adaptive
Speed	Rapid (immediate to minutes)	Slow (hours to days)
Receptors	Germline encoded pattern recognition receptors (PRRs)	Antigen-specific receptors generated through the somatic rearrangement of gene segments
Specificity	Limited to groups of pathogen-associated or damage-associated molecular patterns (PAMPs/DAMPs)	Highly specific to the level of epitopes within antigens
Typical effector mechanisms	Inflammation, complement activation, phagocytosis, and destruction of the pathogen/infected cells	Antigen-specific T and B cells proliferate and differentiate into clones of effector cells that target the pathogen/infected cells
Memory	Short-term *de facto* memory through trained immunity	Long-term antigen-specific memory mediated by T and B cells
Overlap	Innate sensor cells involved in triggering adaptive responses	Adaptive responses recruit innate effector molecules

comparable estimates of past selection on innate versus adaptive immunity across species that vary in their lifespans, which, up until now has not been feasible for many species.

We propose that comparative immunogenomics offers a solution to the problem of estimating past selection on immune genes. Specifically, **long-read sequencing (LRseq)** technology offers the opportunity to study the evolution of innate and adaptive **immune genes** in ways that were previously not possible. Broad-scale comparative immunogenomic analyses have been hindered by the poor representation of immune genes in the fragmented whole genome (WG) assemblies produced by **short-read sequencing (SRseq)** [23–25]. The more contiguous WG assemblies produced by LRseq will make it possible to extract accurate information on immune genes across a broad range of non-model species with different lifespans.

In this opinion article, we first outline the theory of why adaptive immunity is important for the evolution of longevity. The dichotomous view of innate versus adaptive immunity is reductive, as there are complex bidirectional interactions between the two systems and their functional roles can overlap (Box 2) [12]. However, this terminology is widely used and practical, especially given that long-term **antigen**-specific immunological memory mediated through lymphocytes is unique to the vertebrate adaptive immune system. As a result, we use the conceptual division between innate and adaptive immunity as a way of describing suites of immune traits with distinct features. Second, we describe how information on immune genes from LRseq can be used to study the relationship between different aspects of immunity and lifespan where we also draw on knowledge from studies of ageing. Third, we provide a road map for combining immune gene data with modern phylogenetic comparative methods (PCMs) to test how immune traits and lifespan coevolve. Finally, we examine future research directions that are created by the availability of high-quality immune gene data from species across a range of lifespans.

Immune system adaptations predicted to influence lifespan

Beneficial immune traits for long-lived species

Immunological specificity and immunological memory are expected to be especially beneficial to long-lived species. These attributes are the hallmarks of adaptive immunity. Recognising an expansive range of specific antigens is thought to enable long-lived vertebrates to keep pace with rapidly evolving pathogens with short generation times, whilst reducing damage to host cells [6,10,26,27]. **Somatic recombination** of the genes that encode antigen receptors during the development of T and B cells allows an almost limitless repertoire of antigens to be recognised.

Glossary

Antigen: a molecule capable of stimulating an immune response by activating lymphocytes (primarily T and B cells).
Contig: a contiguous length of genomic sequence in which the order of bases is known to a high degree of confidence based on a set of overlapping DNA segments that represent a consensus region of DNA.
Epigenomics: the study of all epigenetic changes that modify the expression and function of the genetic material of an organism without changing the DNA sequence. For example, the addition of methyl groups to the promoter regions of genes that suppresses gene transcription.
Gene isoform: different possible messenger RNAs from the same gene due to alternative splicing, resulting in multiple variants of a protein.
Genome scaffolding: linking together noncontiguous sequence information to create an ordered and orientated set of **contigs** and gaps of known (or close to known) length.
Hi-C: a chromosome conformation capture method used to identify sequences in close 3D space at the genome-wide scale.
Immune genes: genes that encode proteins involved in immune pathways. In practice, the genes classed as immune genes may be identified through the use of reference databases such as gene ontology (GO) terms that categorise genes by their predicted function (e.g., 'Immune System Process: GO:0002376') or the biological pathways the gene product has been linked to, such as KEGG pathways (e.g., 'Immune System' pathways).
Immunological memory: the ability to mount a faster and stronger immune response to a pathogen after a previous encounter. Immunological memory was originally believed to be exclusive to adaptive immunity, enacted through antigen-specific T and B cells. However, alternative forms of immune memory are being increasingly recognised in the innate immune system.
Immunological specificity: the ability to mount a tailored immune response to a specific pathogen. The highest degree of immunological specificity is the antigen-specific responses of adaptive immunity. Specificity exists to a lesser degree in the innate immune system through the recognition of molecular patterns associated with groups of pathogens or cellular damage.

Specific immune responses can then be tailored to virtually any pathogen. T and B cells also exhibit specialised memory enabling a stronger, more rapid, immune response with subsequent exposures to the same pathogens. As the probability of repeated encounters with the same pathogen increases over time, selection for immunological memory is predicted to increase with lifespan [21].

Additional support for adaptive immunity promoting longevity comes from studies of ageing, particularly in humans. Whilst ageing is different to lifespan evolution, involving selection for age-specific reproduction and somatic maintenance according to rates of extrinsic mortality [28], it does offer insight into the mechanisms that facilitate extended lifespans. Studies of centenarian and supercentenarians suggest that people who reach extreme ages maintain more 'youthful' T cell profiles or may even possess unique T cell profile characteristics that help maintain healthy immune responses [29,30]. Furthermore, particular variants of adaptive immune genes [major histocompatibility complex MHC genes] have been associated with extreme longevity in humans [31]. These lines of evidence suggest that adaptive immunity is linked to longevity in humans.

A form of *de facto* immunological memory also exists within the innate immune systems of vertebrates, referred to as 'trained immunity', whereby innate immune responses become faster and stronger over subsequent encounters with pathogens [32]. Trained immunity is, generally short-term and reversible and its lack of specificity is believed to contribute to 'inflammageing' [32,33]: a chronic, sterile, low-grade inflammation associated with many age-related diseases across a broad range of vertebrates [34–36]. Consequently, the memory capacity of the innate immune systems is quite distinct from that of adaptive immunity.

Costly immune traits for long-lived species

Selection on immunity in the context of lifespan not only depends on the benefits to short- and long-lived species, but also the costs. The running costs of adaptive immunity are lower than innate immunity, but the development costs are higher [10,16]. Therefore, long life may be required for the benefits of adaptive immunity to offset the early-life costs [10,16]. Additionally, innate immunity imposes higher long-term costs. Innate responses are triggered by cells that recognise conserved pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) produced by damaged cells (Box 2) [7,13]. The recognition of PAMPs or DAMPs triggers a signalling cascade that results in a general inflammatory response [7,13]. Many innate immune receptors are also activated by certain dietary compounds and metabolic products from the gut microbiota that can lead to further inflammation [35].

Inflammation causes considerable damage to self-tissue that accumulates over time, which is likely to be particularly costly for long-lived species [6–8]. The cumulative damage caused by the nonspecific nature of innate immunity is evident from inflammageing [34–36]. In humans, inflammageing strongly predicts death and chronic disease [37,38] and genome-wide association studies have found that chronic inflammation is linked to ageing rates [39]. Chronic inflammation has also been implicated in ageing across a range of non-model organisms, including mice, nematode worms (*Caenorhabditis elegans*), and primates [40–42].

The damage caused by the ageing adaptive and innate immune systems gives us insights into how these different arms of the immune system may influence longevity. In general, adaptive immune function diminishes with age, whereas innate immune function is maintained [34,43–46]. Whilst this may initially suggest that the innate immune system withstands the test of time better than the adaptive immune system, a chronic stimulation of innate immunity underpins this pattern [35]. Innate immune cells become increasingly proinflammatory with age [46,47] and trained

Long-read sequencing (LRseq): a technology that produces continuous sequence reads ranging from 10 kilobases to several megabases in length, directly from a single molecule of native DNA.
Metabolomics: the study of all the metabolites in an organism or system.
Optical mapping: a light microscope-based technique used to physically locate specific sequence motifs by imaging fluorescently labelled DNA molecules. This can provide positional information for motifs to facilitate genome scaffolding.
Proteomics: the study of all the proteins produced or modified by an organism or system.
Short-read sequencing (SRseq): a technology that produces sequence reads up to a few hundred base pairs (bp) in length. The dominant provider of SRseq is the Illumina platform, which uses sequencing by synthesis to generate reads from 50 to 300 bp. PCR is often required in preparing samples for sequencing.
Somatic recombination: a process of genetic rearrangement that occurs within a somatic cell and is passed to daughter cells. In developing T and B cells somatic recombination reorganises the gene segments that encode the T cell receptor (TCR) and B cell receptor (BCR).
Transcriptomics: the study of all the RNA molecules expressed in a given entity, such as a cell, tissue, or organism.

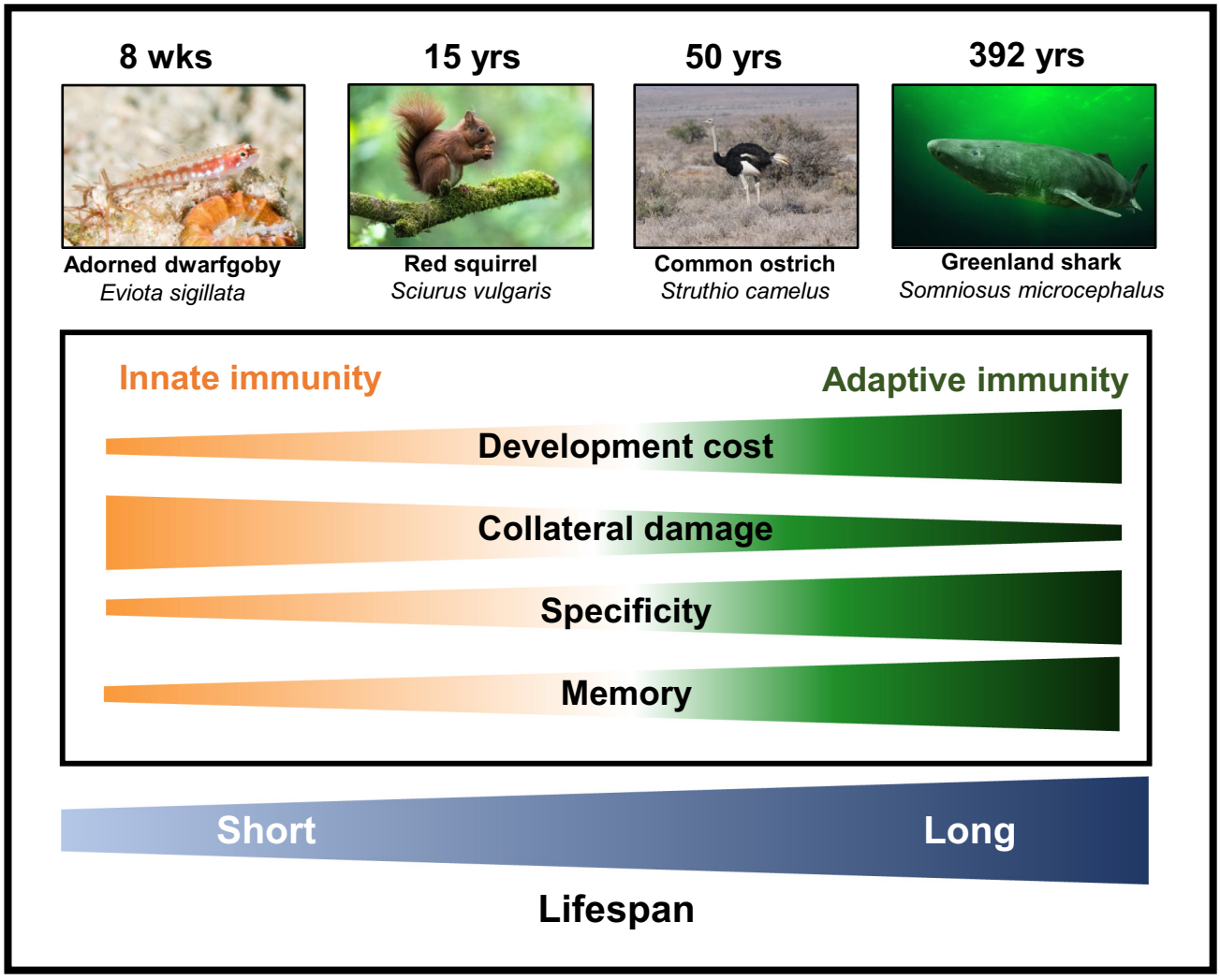

Figure 1. Lifespan varies dramatically across vertebrate species, placing different demands on immunity. The vertebrate immune system is characterised by both innate and adaptive immunity, with different relative costs and benefits that may play a role in lifespan variation. Photographs courtesy of Mark Rosenstein (*E. sigillata*), Koen Frantzen (*S. vulgaris*), Charlie Cornwallis (*S. camelus*), and dotted zebra/Alamy Stock Photos (*S. microcephaus*).

Box 1. Avoiding disease over the course of a long life

The canonical characteristics of the adaptive immune system (i.e., long-term immunological memory and specificity) are expected to facilitate the evolution of long life. However, some long-lived species may inhabit environments that reduce the need for such immune system adaptations, such as environments with few pathogens. It is notable that several examples of extreme longevity are found in species that inhabit the deep ocean, where pathogens may be relatively rare [112]: Greenland shark (*Somniosus microcephalus*, >390 years) [113]; rougheye rock-fish (*Sebastes aleutianus*, >200 years) [65]; and vestimentiferan tubeworm (*Escarpia laminata*, >200 years) [114]. Whilst there are likely to be many factors driving an association between living in the deep ocean and longevity, it is hard to ignore the influence of depauperate pathogen communities.

Other mechanisms by which long-lived species may avoid disease, without relying on adaptive immunity, include mitigating the impact of infections by isolating or discarding infected tissue. Examples of this include gall formation and dropping diseased leaves in plants [27].

The relationship between lifespan and immunity is therefore likely to be modulated by environmental and life-history factors.

Box 2. Genetic markers of immunity

Immune responses involve complex interactions between receptors and a multitude of signalling and effector molecules produced by intricate networks of genes. There is extensive crosstalk between innate and adaptive responses in vertebrates [12]. For example, innate effector molecules are recruited as part of the adaptive immune response and adaptive immunity plays a role in modulating innate immune responses [11,12]. Given such links, using immune genes to disentangle selection on adaptive versus innate immunity poses a challenge.

Focusing on the genes that encode receptors, which are responsible for initiating either innate or adaptive immune pathways offers a solution. Innate immune responses are initiated by cells that recognise evolutionarily conserved pathogen- or danger-associated molecular patterns (PAMPS or DAMPS) via pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-like receptors (RLR), and C-type lectin receptors (CLR). Adaptive immune responses, however, are triggered by the presentation of foreign antigens to T cells by major histocompatibility complex (MHC) molecules. MHC molecules are encoded by a large family of highly polymorphic genes (MHC genes).

The direct link between pathogen recognition and the activation of immune responses make the genes that encode PRRs and MHC targets for pathogen-mediated selection [115,116]. Accordingly, genes involved in pathogen detection generally exhibit stronger signals of positive selection than genes encoding downstream features of the immune response, such as signalling or effector molecules [117,118]. The distinct roles of PRRs and MHC genes in innate and adaptive immunity, respectively, make these genes excellent candidates for examining selection on innate versus adaptive immunity across vertebrates.

immunity can become hyperactivated, exacerbating the age-related damage caused by innate immune responses [33]. The risk of collateral damage by the adaptive immune system also potentially increases with age via autoimmunity factors, but this is believed to be counteracted by a parallel rise in self-protective mechanisms [42]. Overall, the collateral damage inflicted by the innate immune system over the course of a long life is likely to be greater than that caused by adaptive immunity.

Long-lived species that lack adaptive immunity

Long-lived species that possess only an innate immune system challenge the idea that adaptive immunity is generally important for the evolution of longevity. One explanation for how such species live a long time without the need for such sophisticated defences is that they live in environments with relatively few pathogens (Box 1). Alternatively, it is possible that the innate immune system of some species has evolved enhanced pathogen specificity and immunological memory, blurring the functional distinctions between innate and adaptive immunity.

There is increasing recognition of alternative versions of immunological memory and mechanisms that enhance the specificity of innate immune responses [48,49]. For example, in both long-lived tree species and an exceptionally long-lived invertebrate, (the purple sea urchin, *Strongylocentrotus purpuratus*), dramatic expansions have been observed in genes involved in specific pathogen recognition [26,50]. This highlights how functionally similar immune traits may be linked to longevity across different taxa via different genetic pathways. Species that possess only an innate immune system offer the potential to test whether there are universal features of immunity that are linked to longevity.

Using immune genes to study the coevolution between immunity and longevity

Examining the evolution of immunity across species where long life has repeatedly evolved requires the comparison of analogous immune traits. It is inherently difficult to quantify immune responses across species, as the physiological pathways involved are complex resulting in 'noisy' data with high interindividual variation. Additionally, accurately characterising immune responses can require experimental infections that make gaining sufficient sample sizes challenging, raise ethical concerns and are difficult to conduct across species due to host specificity. One solution is to mine the information in immune genes that carry signatures of past natural selection (Box 2)

[51–53]. Immune genes are often highly conserved, allowing comparisons across species, and the molecules they encode provide insights into immune functions [53–55].

A major hurdle in using immune genes to identify which immune traits coevolve with long life is their poor representation in SRseq WG assemblies. Immune genes often fail to be fully represented in SRseq WG assemblies because of extensive gene duplication that is characteristic of many immune gene families [24,49,56,57]. It is challenging to accurately assemble duplicated gene copies from SRseq data due to their high sequence similarity, especially in the case of tandem duplications [58–61]. Further complications in assembling immune genes often arise through atypical GC contents, high rates of recombination, intergenic gene conversion and repetitive regions [25,59,62]. As the majority of published WGs from non-model organisms have been produced from SRseq, thorough genomic analyses of immunity in relation to lifespan across species is currently lacking, with comparative genetic studies being limited to a handful of immune genes [63].

LRseq can help resolve the problems associated with assembling immune genes, opening up possibilities for comparative immunogenomics [23,58–60]. Recent developments in techniques that facilitate **genome scaffolding** such as **Hi-C** and **optical mapping** can be combined with LRseq to make chromosome-level genome assemblies increasingly feasible [64–67]. As more genomes are sequenced using LRseq, the opportunities for collecting accurate data on both innate and adaptive immune genes across diverse species is rapidly increasing.

Improved genome assemblies have already been shown to lead to significant improvements in our understanding of immune genes that are typically challenging to characterise. For example, the notoriously difficult to assemble MHC gene region has now been described in several non-model species, including the water buffalo (*Bubalus bubalis*) [67], wild Bactrian camel (*Camelus ferus*) [66], the paradise crow (*Lycocorax pyrrhopterus*) [61], and the common pheasant (*Phasianus colchicus*) [68]. As MHC genes represent one of the key gene families underpinning adaptive immunity, such studies enhance the potential for comparative immunogenomics.

Although comparative immunogenomic analyses clearly benefit from highly quality WG assemblies, costs may still prevent large-scale analyses involving many species. Nevertheless, the availability of high-quality reference genomes for select species within target clades can enable much more accurate assembly and annotation of other species using SRseq, providing a way forward whilst minimising costs (e.g., [65], Figure 2, Key figure).

Measuring genomic signals of selection on immune genes

The strength and type of past selection (neutral, negative/purifying, or positive) on immune genes can be estimated from the protein coding sequences of orthologous genes [51,53]. The more contiguous WG assemblies enabled by LRseq greatly aid the identification of orthologous immune genes and quantification of their entire protein coding sequence. Once obtained, estimates of past selection on innate and adaptive immune genes can be paired with information on longevity across species to indicate the importance of particular immune traits with evolutionary shifts in lifespan [69].

A further advantage of LRseq is that it makes it possible to accurately quantify structural genetic variation, such as gene copy number variation, inversions, gene fusions and gene translocations [24,70–72]. The presence or absence of particular immune genes, as well as the expansion or contraction of whole immune gene families, can be indicative of their functional relevance in different species [57,73]. For example, a recent comparative genomic study across rockfish (genus

Key figure

The combination of high-quality whole genome (WG) information for species across a range of lifespans and modern phylogenetic comparative methods (PCMs) provide a powerful toolkit to address questions about the role of different immune traits in the evolution of extended lifespans

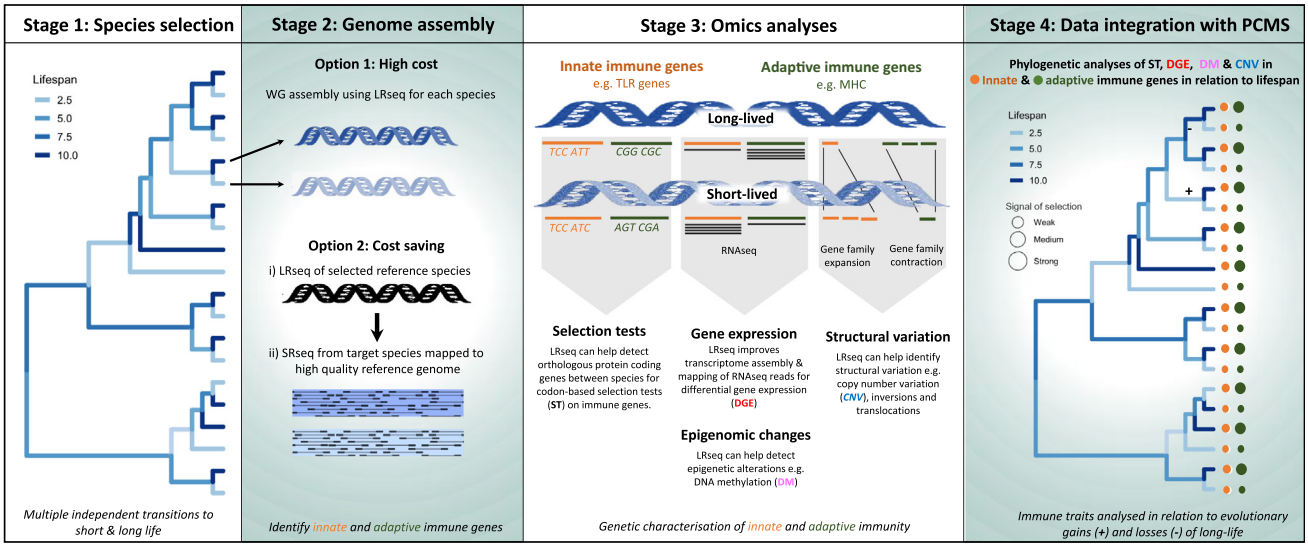

Figure 2. Abbreviations: LRseq, long-read sequencing; MHC, major histocompatibility complex; SRseq, short-read sequencing, TLR, Toll-like receptor.

Sebastes) made use of chromosome-level WG assemblies and found that particularly long-lived species have more copies of butyrophilin genes, which have an immunosuppressive function [65]. This analysis was only possible with the aid of highly contiguous LRseq WG data that meant the association between the segmental duplication of butyrophilin genes and lifespan could be resolved.

Gene losses and gains occur frequently within the immune system and can lead to novel functions [73–77]. For example, gadiform fish have lost MHC class II genes, which seems to have been compensated for by a subsequent expansion of MHC class I genes [78]. Naked mole rats (*Heterocephalus glaber*) lack natural killer (NK) cells, innate immune cells that mediate antitumor and antiviral responses, and show restricted diversity in the genes regulating NK cell function [81]. Bats have lost several genes linked to NF-κB signalling, which regulate responses to infection [82]. The idea that gene losses can lead to functional innovations is being increasingly recognised [83,84]. Quantifying genomic rearrangements in innate and adaptive immune genes across short- and long-lived species is therefore likely to provide new insights into the mechanisms of immunity that influence longevity (Figure 2). LRseq WGs are likely to be essential for such studies.

LRseq is improving other 'omics' technologies, including **transcriptomics** and **epigenomics**. LRseq of RNA (or complementary DNA) improves *de novo* transcriptome assembly, enables the accurate detection of **gene isoforms**, and enhances the annotation of WG assemblies [65,85,86]. Additionally, LRseq in differential gene expression studies can remove ambiguity during read mapping, which is a common drawback of SRseq [86]. LRseq WGs also aid epigenomic studies as more complete genome information improves our ability to predict the impact of epigenetic modifications in

given locations of the genome [87]. LRseq technology offers the promise of measuring base modifications in native DNA or RNA in epigenetic studies without the current need for chemical pretreatment of nucleic acids (e.g., bisulfite conversion of unmethylated cytosines to uracils) that makes library preparation more complex and can introduce errors [88].

The advantages of LRseq increase the potential for epigenomic and transcriptomic studies to be used in comparative frameworks to address the relationship between immunity and longevity (Figure 2). A recent epigenomics study on bats demonstrated longevity-related changes in DNA methylation of immune genes [87]. Additionally, comparative transcriptomic analyses across mammals suggest that long-lived bats do not show the typical pattern of upregulated expression of chronic inflammation genes with age [89]. The extraordinarily long lives of some bat species may therefore be associated with augmented immune responses. Furthermore, a comparison between transcriptomes from the long-lived gray whale (*Eschrichtius robustus*) and similar data from other mammals highlight that long-lived mammals may share common expression patterns in genes involved in immune responses compared with shorter-lived mammals [90]. An interesting avenue of future research will be to investigate whether immune gene regulation differs between innate and adaptive immune genes in short- versus long-lived species (Figure 2).

The rapid advance of omics technologies, more generally, opens up a wealth of research avenues. For example, single-cell transcriptomics is being increasingly used to profile circulating immune cells in the context of ageing [30]. **Proteomics** has recently been used in combination with machine learning to show that plasma proteins that predict age are predominantly associated with immunity [91]. State-of-the-art **metabolomics** approaches are also now allowing age-related changes in metabolite profiles to be studied, which provide new insights into the physiological mechanisms of ageing [92,93]. The integration of multiple datasets generated from genomes, epigenomes, transcriptomes, proteomes, and metabolomes, an approach termed 'multi-omics', offers great promise for teasing apart the complex processes linking immunity, ageing, and lifespan evolution [38,92].

Integrating PCMs with genomic data

The term 'PhyloG2P' has recently been coined to refer to the growing field of research that uses modern PCMs to understand the relationship between genotypic and phenotypic traits [94]. Extensive species phylogenies are now available for many clades, including amphibians, birds, mammals, sharks, and squamates (see the VertLife.org tool for information on dated phylogenies[i]). There are also resources that provide data-driven estimates of lifespan for many taxa. For example, at the time of writing, AnAge contains ageing, longevity, and life history data for more than 4200 species[ii]. The combination of phylogenies, life-history information, and high-quality genome data from species across a range of lifespans provides a powerful toolkit to explore the links between immunity and evolutionary shifts in longevity (Figure 2).

PCMs have long been used to account for shared ancestry when examining the relationships between traits across species, which remains important for 'PhyloG2P' analyses [95]. Developments in PCMs, such as phylogenetic mixed models [96–98], now also allow for more nuanced evolutionary questions to be addressed and greater flexibility in controlling for potentially confounding variables. For example, pathogens can be more abundant in certain environments, such as the tropics, where conditions also favour higher survival [99,100]. Not including such variables in analyses may lead to spurious relationships between selection on immune genes and lifespan. Additionally, such methods can be extended to deal with variation in data quality across species [101], such as the quality of genome assemblies used to gain information on immune genes, and sample sizes used to estimate lifespan.

Deeper insights into the way immunity and lifespan coevolve can be gained by using PCMs to infer evolutionary history. For example, PCMs can be used to reconstruct the ancestral states of lifespan across taxa and locate areas of phylogenetic trees where evolutionary transitions in lifespan have occurred [65,102]. It is crucial to identify multiple independent evolutionary transitions to understand if immune adaptations associated with long life are lineage specific or have been repeatedly associated with long life ('parallel evolution') [65].

The causality behind phylogenetic correlations between traits can be inferred where traits have repeatedly evolved independently using transition rate analyses [96,97,103]. If immune gene changes consistently precede shifts in lifespans, then this suggests that preadaptations in the immune system are important in promoting the evolution of longevity. Conversely, if immune gene changes follow shifts in longevity, then extended lifespans may have generated selection for certain immune adaptations.

Inferences about the order of evolutionary events have historically been constrained to binary traits [103,104]. For example, transitions from short to long life can be analysed in relation to transitions from weak to strong selection on immune genes. However, novel comparative methods are now emerging that allow causality in coevolutionary relationships to also be tested between continuous traits [105]. Together with increases in high-quality immune gene data, modern PCMs enable us to move beyond purely correlative approaches and unravel the relationship between immunity and lifespan in ways that were previously not possible.

Concluding remarks

The improved representation of immune genes in WGs assembled from LRseq combined with PCMs will allow us to test whether adaptive immunity is a key adaptation promoting the evolution of long life in vertebrates. The hallmarks of adaptive immunity, namely canonical immunological memory and specificity, are predicted to be important for long life in vertebrates. In long-lived species that lack adaptive immunity, it is possible that adaptations in the innate immune system may facilitate longevity via similar mechanisms: specificity and memory. Consequently, it might not be adaptive immunity *per se* that is required for long life, but rather certain immune traits that allow hosts to meet the challenges posed by pathogens whilst minimising the costs and risk of collateral damage.

Whilst the more contiguous WGs achieved through LRseq has special relevance to studying the genomic signatures of immunity in the context of lifespan, there are many related areas in the biological and medical sciences that will also be advanced by improved comparative immunogenetics. For example, studies of the way long-lived species suppress cancer have relied heavily on comparative genomic approaches [106,107]. The patchy representation of immune genes in SRseq WG assemblies makes it possible that there are yet undiscovered immunological processes involved in tumour suppression across long-lived species. Similarly, sex differences in immune responses and ageing have been observed in many species, but systematic comparative data to help us explain the molecular mechanisms underlying such differences is lacking [73,108,109].

The scale of the current increase in genomic resources is exemplified by initiatives such as the vertebrate genome project, which is in the process of producing near error-free genome assemblies for all extant vertebrate species [110]. LRseq greatly facilitates such ambitious agendas and the capacity for this to advance all areas of comparative genomics is clear [60,82,111]. The influx of high-quality WGs will be particularly important for understanding immunity and, together with continuing advances in PCMs, will help reveal the role of immune adaptations in the radiation of lifespans across the tree of life (see Outstanding questions).

Outstanding questions

Has adaptive immunity played a fundamental role in the evolution of prolonged lifespans across vertebrates?

What are the contributions of changes in the protein coding sequence, structural variation, and gene expression in innate versus adaptive immune genes to the evolution of long-life?

Do long-lived species that lack adaptive immunity have innate immune traits that promote immunological specificity and memory?

Has the evolution of long life been promoted by immune system adaptations or do immune system adaptations occur after evolutionary transitions to prolonged lifespans?

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Resources

[i]http://vertlife.org/phylosubsets

[ii]https://genomics.senescence.info/species/index.html

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