Patterns of telomere length change with age in aquatic vertebrates and the phylogenetic distribution of the pattern among jawed vertebrates

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"A gentle way to age"

…Carina Dennis (2006)

Declaration

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Izzo, C., Bertozzi, T., Donnellan, C. E. & Gillanders, B. M. (*In prep*). Telomere length varies independently of age in the Port Jackson shark, *Heterodontus portusjacksoni*: with a commentary on telomere length methods.

Izzo, C., Bertozzi, T, Gillanders, B. M. & Donnellan, C. E. (*In prep*). Variation in telomere length of the common carp, *Cyprinus carpio* (Cyprinidae) in relation to age and tissue type.

Izzo, C., Hamer, D. J., Bertozzi, T., Donnellan, C. E. & Gillanders, B. M. (*In prep*). Telomere length analysis for rapidly determining age in pinnipeds: the endangered Australian sea lion as a case study.

Izzo, C., Gillanders, B. M. & Donnellan, C. E. (*In prep*). Telomere length of fishes correlates with variation in longevity.

Izzo, C., Donnellan, C. E. & Gillanders, B. M. (*In prep*). Recent evolution of telomere length change with age in gnathostomes.

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"Bad times have a scientific value.
These are occasions a good learner
would not miss"

…Ralph Waldo Emerson (1837)

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Thesis Abstract

Telomeres, the protective caps at the ends of all vertebrate chromosomes, naturally undergo changes in length. These changes in telomere lengths may be a "molecular clock" by providing a counting mechanism of DNA replication events. In populations of jawed vertebrates (gnathostomes), telomere length has been shown to change with age; and thus measurements of telomere lengths may provide a novel means of determining the ages of free-living animals. Determinations of the age structure of populations of aquatic vertebrates (teleosts, chondrichthyans and marine mammals) are vital for sustainable management and conservation efforts. Yet, the commonly applied increment based ageing techniques are limited by the subjectivity of increment patterning and destructive sampling. I aimed to assess the application of telomere length as an age determinate for populations of aquatic vertebrates and to evaluate the biological implications and evolutionary origins of this trait amongst the gnathostomes.

Telomere length change with age was investigated in an exemplar chondrichthyan, teleost and marine mammal species, to determine whether aquatic gnathostomes share the general pattern of declining telomeres with age, as found in terrestrial mammals. Chapter Two provides the first assessment of telomere length change with age in a chondrichthyan species, the Port Jackson shark. Four types of tissues from Port Jackson sharks, ranging in age from 0 to 17 years, were sampled and telomere length were estimated using three measurement methods: (i) relative quantitative PCR (qPCR); (ii) absolute qPCR; and (iii) the terminal restriction fragment (TRF) analysis. No relationship between telomere length and age was found for any of the tissues, using any method.

In Chapter Three, telomere length was measured in specimens of the common carp from two tissues using the absolute qPCR method. Telomere length measurements were then correlated with ages estimated from otolith increment counts and length-at-age calculations. Measurements of telomere length were highly variable in both muscle biopsies and fin clips; however, telomeres from muscle biopsies significantly increased in length – in contrast to the more generalised pattern of telomere length attrition and marking the second reported case of an increase in telomere length with age in vertebrates.

In terrestrial mammals telomere shortening is negatively correlated with donor age. In Chapter Four, I tested whether this pattern of declining telomere lengths was found in a pinniped species, the Australian sea lion. Telomere lengths were measured in flipper clips from specimens by absolute qPCR and compared between three age classes: pups, juveniles, and adults. Mean telomere lengths of the adults were significantly smaller than the juvenile and pup classes confirming that the Australian sea lion shares the general mammalian pattern of telomere length attrition.

Relationships between the rate of telomere length change with age and species longevity have been observed in birds and mammals, suggesting that the rate of telomere length change is an informative measure of ageing. In Chapter Five, using a data set of 20 teleost and chondrichthyan species, I tested whether fishes showed a similar pattern. I found that the rate of telomere length change with age is significantly different between species of fishes and that these rates of change are inversely correlated with longevity.

The findings of Chapters Two, Three & Four indicated that telomeres do not provide a suitable means of determining the ages of individuals and at best are limited to assigning broad age classes. This is largely due to the high degree of variability of telomere lengths between individuals within all age classes. In addition, these Chapters (2, 3 & 4) also highlight that patterns of telomere length change with age are highly variable within the gnathostomes; and thus, telomere length change cannot be characterised by a single pattern for all lineages. In fact, three patterns of telomere length change with age in the gnathostomes were found: (i) declining telomere lengths; (ii) increasing telomere lengths; and (iii) no significant change in telomere length with age. However, identifying the selective factors responsible for the assignment of patterns of telomere length change is hampered by a lack of the understanding of the evolutionary origins of these patterns.

Therefore, in Chapter Six I sought to outline the phylogenetic distribution of patterns of telomere length change with age in the gnathostomes to determine the evolutionary origin(s) of this trait. Two alternative hypotheses for the evolution of telomere length change were tested by ancestral state reconstruction in a set of 40 gnathostomes, for which I have significantly expanded the sampling of chondrichthyans and teleosts. The most likely/parsimonious pattern of telomere length change in the common gnathostome ancestor suggested that telomere length change with age was not present ancestrally and has since evolved independently. I was also able to elucidate the evolutionary history of transitions to and between the three patterns of telomere length change within the available gnathostome lineages, with the birds and teleosts displaying the highest rates of evolutionary lability of patterns of telomere length change with age.

The macro-evolutionary analysis (Chapter 6) identified relatively rapid evolutionary patterns of telomere length change with age in two gnathostome clades. However, as highlighted by the variability of telomere lengths among individuals within all age classes, furthering an interpretation of the causes and consequences of variable patterns of telomere length change will require a focus at the species level and a shift to following individuals through out their lifetime.

Table of Contents

Thesis Preface

Note on Chapter Style

This thesis begins with a brief General Introduction (Chapter One) to telomeres and the factors that affect telomere length dynamics. The introduction also highlights the variable nature of telomere length change with age in the jawed vertebrates (gnathostomes) and the paucity of telomeric research in the teleostean and chondrichthyan lineages. Here the broad research objectives of the thesis will be established. The General Introduction is followed by five research chapters (Chapters Two to Six) addressing the outlined research objectives. Finally, the biological and evolutionary implications of the findings presented herein are addressed in the General Discussion (Chapter Seven).

The research chapters of this thesis have been written in a style suitable for publication in a scientific journal and can be read as separate studies, therefore there is some repetition in each introduction and in the methodological descriptions. Furthermore, as these chapters have co-authors they have been written in plural.

Each research chapter is preceded by a preface, which includes the chapter abstracts, to summarise the contents and guide the reader, as well as a brief preamble that presents information on the publication status of the chapter at the time of thesis submission, and describes the contributions of all co-authors to the research therein. Finally, the chapter acknowledgements are also provided.

All tables and figures appear embedded within the text and the numbering of figures and tables begins at one for each chapter to simplify referral to the results. All literature cited in the thesis chapter‟s have been compiled at the end of the thesis and not at the end of each chapter. Additional appendices provided are referred to in the text as appropriate.

“Picture an aglet…”

…the author

ag·let (ag ’lit) *n*. A tag or sheath, as of plastic, on the end of a lace, cord, or ribbon to facilitate its passing through eyelet holes and maintain end integrity