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Telomeric attrition with age and temperature in Eastern mosquitofish (Gambusia holbrooki)

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Abstract

Telomeric attrition has repeatedly been found to correlate with the ageing of organisms; however, recent research is increasingly showing that the determinants of attrition dynamics are not well understood. This study examined the relative telomere lengths in Eastern mosquitofish, Gambusia holbrooki, kept at different temperatures and at different ages. Newly born fry were randomly selected for one of four treatment groups: 20, 30, 20-30, and 30-20 C, where the third and fourth treatment groups were gradually changed from their starting temperature to their final temperature between days 10 and 14. Telomere length was measured, and it was found that length decreased with age and that fish exposed to the 20 C treatment had significantly shorter telomeres than those that received the 30-20 C treatment. Telomeric attrition with age agrees with results previously found in studies of telomeres; however, the variation in attrition with temperature was not simply predictable and may be the synergistic effects of temperature and some other factor. 2014 Springer-Verlag Berlin Heidelberg.

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Telomeric attrition with age and temperature in Eastern mosquitofish (*Gambusia holbrooki*)

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Telomeric attrition has repeatedly been found to correlate with the ageing of organisms, however recent research is increasingly showing that the determinants of attrition dynamics are not well understood. This study examined the relative telomere lengths in Eastern mosquitofish, *Gambusia holbrooki*, acclimated to different temperatures and at different ages. Newly born fry were randomly selected for one of four treatment groups: 20°C, 30°C, 20-30°C, and 30-20°C, where the third and fourth treatment groups were gradually changed from their starting temperature to their final temperature between days 10 and 14. Telomere length was measured and it was found that length decreased with age and that fish exposed to the 20°C treatment had significantly shorter telomeres than those that received the 30-20°C treatment. Telomeric attrition with age agrees with results previously found in studies of telomeres, however the variation in attrition with temperature was less expected and could potentially be due to changes in antioxidant gene expression.

Key words: *Gambusia holbrooki*, life-history, telomeres, growth

Introduction

Telomeres are repetitive sequences of DNA found at the ends of eukaryotic chromosomes (Shalev 2012). They function to protect coding DNA from being lost during the DNA synthesis that precedes cell division (Shalev 2012). Telomeres may also be damaged by reactive oxygen species (ROS) produced as a result of metabolism and, in particular, by an upregulation of metabolism (Monaghan et al. 2009). As a result, telomeres typically become shorter over time, and this shortening has been implicated in the ageing process (Navarro et al. 2004; Selman et al. 2012).

The life-history of an organism is typically defined along the fast-slow growth continuum (Selman et al. 2012). In an organism that has a short lifespan it can be expected that more resources are being placed into reproduction rather than other cellular processes, which might prolong life (Monaghan et al. 2009). As telomere attrition can lead to senescence of cells and a reduced capacity for healing it is possible that higher rates of telomere attrition will be seen in organisms that reproduce rapidly and have shorter lifespan (Bize et al. 2009).

This study seeks to assess telomere attrition in juvenile Eastern mosquitofish (*Gambusia holbrooki*), a species with a life-history of growing fast and dying young, and assess whether attrition varies under different temperature treatments (Pyke 2008). Fish of the genus *Gambusia* display great phenotypic plasticity and are able to tolerate changes in environmental conditions, however survivorship is typically higher if the changes occur gradually (Pyke 2005). Given an adequate chance to acclimate they can tolerate temperatures between 0 and 45°C (Pyke 2008; Hubbs 2000). While the species as a whole exhibits a short lifespan and rapid reproduction, different temperatures have the capacity to alter the life-history of individuals. Fish acclimated to higher temperatures have been found to mature more quickly and at a smaller size (Meffe 1992). Growth is rapid in mosquitofish as they generally only reproduce for one season and they need to be of sufficient size to reproduce effectively (Meffe 1992). The species is highly fecund and females give birth to live young, making it simple to monitor the age of the juveniles (Plath et al. 2007; Pyke 2005).

Since growth and reproduction is favoured over survival in this species, it is predicted that telomeric attrition will be observed as the fish ages. Temperature has been established as an environmental factor which can alter the growth of the fish and therefore it may also affect telomeric attrition. In the current study we seek to assess whether telomeric attrition does occur in mosquitofish and whether the attrition is affected by environmental temperature.

Materials and Methods

The mosquitofish used in this study were obtained using hand nets from Lake Northam in Victoria Park, Sydney (33.8855°S, 151.1934°E) between April and June 2011. Animals were transported to the University of Sydney and housed in colonies in opaque plastic tanks (800 × 600 × 450 mm) at 25°C. The fish were housed with a 12 h light: 12 h dark photoperiod and fed *ad libitum* on fish flakes (Total Tropical, Wardley, USA). The fish were allowed to habituate to their new conditions for at least one week before any experimental work commenced.

Pregnant females were identified within the captive colonies and positioned in individual brooding chambers (130 × 75 × 75 mm). These chambers were placed together at the bottom of a large plastic tank (800 × 600 × 450 mm) at 25°C. The chambers were placed on their side so that the largest holes (normally located on the top of the brooding chamber) were more easily swum through by fry in order to reduce predation by the mother. Any offspring produced within 24 hours were randomly selected for one of four treatment groups: 20°C, 30°C, 20-30°C, and 30-20°C, where the third and fourth treatment groups were gradually changed from their starting temperature to their final temperature between days 10 and 14.

They were placed in opaque plastic tanks (300 × 250 × 190 mm) at 25°C and the temperature was either increased to 30°C or decreased 20°C over a period of 24 hours.

Quantifying Relative Telomere Length

To assess relative telomere length, randomly selected fish were euthanised using MS 222 (0.4 g/L, pH 7.0, Sigma, Sydney, NSW, Australia) from the four treatments between 26 and 72 days old (for logistical reasons). Fish were measured from the snout to the base of the tail, using manual calipers, to the nearest millimetre and stored in 80% ethanol (EtOH) until required. DNA was extracted from tail muscle using a GentraPuregene tissue extraction kit (Qiagen, Australia). Tail muscle was weighed using an electronic balance (CP224S, Sartorius, Dandenong South, VIC, Australia) to 0.0001 g and the protocol adjusted accordingly in relation to muscle mass. Briefly, the sample was placed in a tube containing lysis solution and proteinase K, and incubated at 55°C overnight to allow the tissue to digest. The following day, the DNA was precipitated, washed with 70% EtOH, dried and reconstituted with DNA hydration solution. The DNA concentration (ng/µL) of each sample was measured using a PHERAstar*FS* (BMG Labtech, Germany), allowing each sample to be diluted to a working concentration of 20 ng/µL using Milli-Q water. The samples were stored at -20°C.

Telomere length was measured using real-time quantitative PCR (qPCR) using SensiMix SYBR No-ROX Kit (Bioline, Sydney, Australia). The control single copy gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified using primers GAPDH-F and GAPDH-R (Criscuolo et al. 2009). The telomere primers used were: Tel1b and Tel2b (Criscuolo et al. 2009). The qPCR for both GAPDH and telomeres was performed using 20 ng of DNA per reaction and the primers were used at a concentration of 200 mM. Briefly, 1 µL of DNA was added to 11.25 µL SensiMix, 2.4 µL Milli-Q water, 0.675 µL $MgCl_2$ (50 mM), 2 µL forward primer and 2 µL reverse primer and reactions were run in duplicate for each sample.

Amplifications were carried out in a Rotor-Gene 6000 thermocycler (Corbett Research/Qiagen, Australia) using an initial Taq activation step at 95°C for 10 min, a total of 40 cycles of 95°C for 15 s, 60°C for 15 s and 72°C for 15s. A melt curve was created after each run over the temperature range of 60 to 95°C, to ensure no non-specific product amplification. No-template control reactions were run in duplicate for each primer set during every qPCR run to ensure no contamination. Standard curves were created for both telomere and GAPDH amplification using three-fold serial dilutions of DNA, yielding amplification efficiencies of 0.96 and 1.08, respectively. Relative telomere length was compared between treatments by averaging the crossing threshold (Ct) values for each sample and using an equation developed by Pfaffl (2001), which is suitable when qPCR efficiencies vary more than 10%.

$$ratio = \frac{(E_{Telomere})^{\Delta CP_{Telomere}(control-sample)}}{(E_{GAPDH})^{\Delta CP_{GAPDH}(control-sample)}}$$

Where E is the amplification efficiency and ΔCP is the difference between the negative control Ct value and the sample Ct. This ratio is the telomere expression relative to the GAPDH gene.

Statistical Analyses

We analysed our data with a mixed model analysis with temperature treatment as a fixed factor and tank as a random factor with age as a covariate. Our temperature treatments were 20˚C, 30˚C, a shift from 20˚C to 30˚C after 10 days, and a change from 30 to 20˚C after 10 days. The rationale for this design was our interest in whether a shift in temperature may increase (or decrease) a fish gene expression profile and, hence, production of antioxidants, which may affect telomere attrition. Standard length, mass and skeletal growth (length [mm] per day) were all included in a first model but none of these showed any significant effects (P > 0.40) on telomere length and are therefore not further reported on.

Results

Our temperature treatment showed only an overall global effect (P = 0.066; Table 1, Fig. 1) with the only significant treatment difference between treatments being 20 ˚C differing significantly from the 30-20 ˚C (P = 0.044; Table 1, Fig. 1) and with 20 ˚C fish having shorter telomeres than 30-20 ˚C fish. Age affected telomere length significantly and negatively (P = 0.002, df = 29, parameter estimate = -0.087 ± 0.26, mean ± SE). There was no significant age by treatment interaction (P > 0.80).

Discussion

In the current study we found that telomeric attrition does occur in mosquitofish as they grow and develop. The chromosomal replication and cellular division that must occur can potentially explain the change in telomere length over time. We also found that mosquitofish exposed continually to 20°C had shorter telomeres than those first exposed to 30°C before being changed to the cooler temperature. The reasons for this are unclear, however it has been observed in zebrafish that exposure to different temperatures can cause great changes in antioxidant gene expression and we may be observing a similar effect here (Malek et al. 2004). In order to determine the particular mechanism that is leading to the telomeric attrition, be it chromosomal replication or ROS damage, future studies need to take into account the rates of growth of the fish, ROS levels and any antioxidant defences that may be mounted by the fish.

Telomeric attrition has long been believed to 'tick-away' in pre-determined fashion. However, recently it has been found that particularly stressful events, such as the loss of a tail in sand lizards, can increase attrition (Olsson et al. 2010; Monaghan and Haussmann 2006). In this study we have seen that temperature can affect the rate of attrition. Telomeres and their attrition are thus likely to be more complex than we initially anticipated and to be vital for our understanding of life-history evolution.

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Ethical Standards

All animal handling and experiments were conducted with the approval of the University of Sydney Animal Ethics Committee (L04/3-2008/3/4769).

Conflict of interest

The authors declare that they have no conflict of interest.

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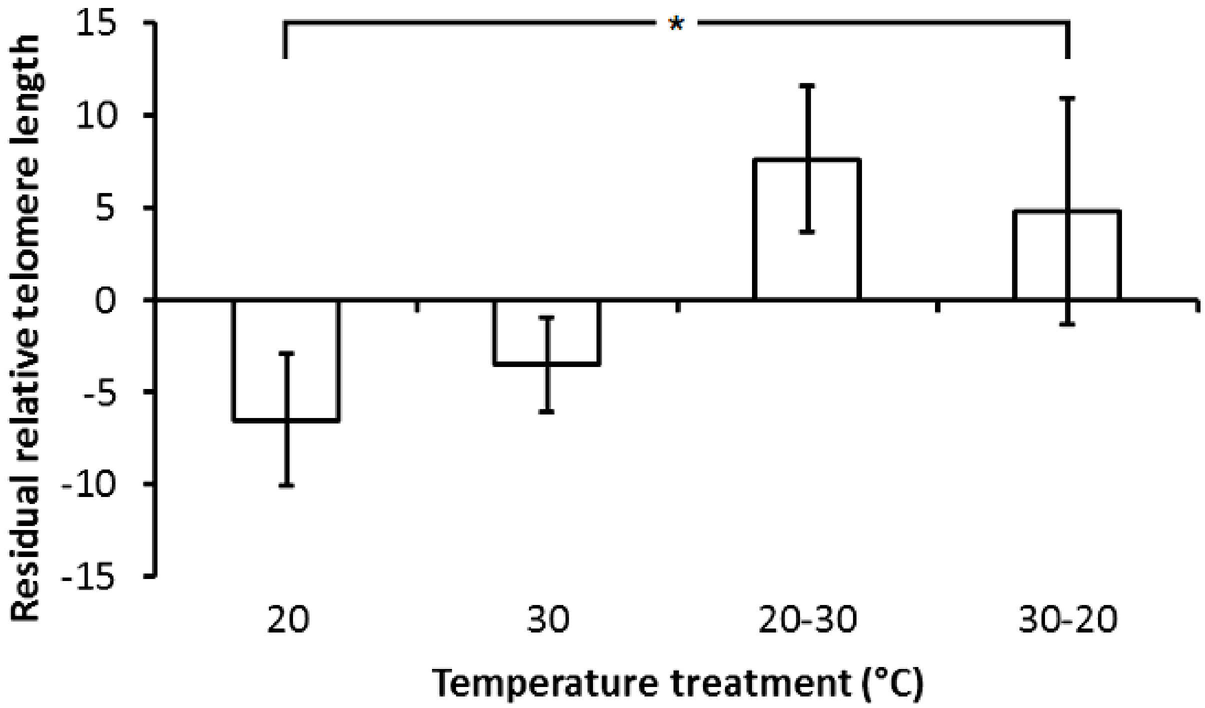
Legends to figures:

Fig. 1 The figure depicts the mean residual relative telomere length (± SE). The residual was found by regressing relative telomere length against age in days. The * denotes a significant difference between the 20˚C and 30-20˚C treatments (*P* = 0.0437)

Table 1 Mixed model analysis of the effect of age and temperature on relative telomere length in Eastern mosquitofish, *Gambusia holbrooki*. Standard length, mass and skeletal growth (length [mm] per day) were backwards-eliminated from the final model ($P > 0.40$). The 30-20˚C treatment was used as the standard to which the other temperature treatments were compared.

Parameter Estimates					
Variable	Estimate	Std Er	DF	t	Pr > |t|
Intercept	67.3304	13.3114	14	5.06	0.0002
Age	-0.8738	0.2619	29	-3.34	0.0023
20˚C	-12.8743	6.1031	29	-2.11	0.0437
30˚C	-9.6094	6.2463	29	-1.54	0.1348
20-30˚C	2.6259	6.3793	29	0.41	0.6836
30-20˚C	0	.	.	.	.

Figure 1.


15
10
5
0
-5
-10
-15
Residual relative telomere length
*
20
30
20-30
30-20
Temperature treatment (°C)