Socioecological variables predict telomere length in wild spotted hyenas

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Telomeres are regarded as important biomarkers of ageing and serve as useful tools in revealing how stress acts at the cellular level. However, the effects of social and ecological factors on telomere length remain poorly understood, particularly in free-ranging mammals. Here, we investigated the influences of within-group dominance rank and group membership on telomere length in wild adult spotted hyenas (Crocuta crocuta). We found large effects of both factors; high-ranking hyenas exhibited significantly greater mean telomere length than did subordinate animals, and group membership significantly predicted mean telomere length within high-ranking females. We further inquired whether prey availability mediates the observed effect of group membership on telomere length, but this hypothesis was not supported. Interestingly, adult telomere length was not predicted by age. Our work shows for the first time, to the best of our knowledge, the effects of social rank on telomere length in a wild mammal and enhances our understanding of how social and ecological variables may contribute to organismal senescence.

1. Introduction

Social and ecological factors can have important effects on animal health, yet the proximate mechanisms mediating such effects are still being determined. Within the past decade, telomere length has been identified as a candidate mechanism through which individual fitness and life expectancy may vary [1]. Telomeres are highly conserved, non-coding, repetitive DNA sequences that cap the ends of eukaryotic chromosomes, confer chromosomal stability and buffer against degradation of coding sequences following semiconservative DNA replication [2]. When telomeres shorten to a critical length, a regulatory cascade is triggered, potentially culminating in DNA damage and cell death [2]. Hence, telomeres may figure importantly in organismal ageing and the mediation of life-history variation [1].

Although telomeres tend to shorten with age [1–3], the rate at which this happens may be influenced by the quality of an organism’s environment [1,3–5]. In animal societies structured by linear dominance hierarchies, social rank can act as a filter through which ecological factors affect the individual. An individual’s dominance status often determines its priority of access to key resources and has important downstream effects on health [6]. Previous work in despotic animal societies has shown large effects of social rank on stress physiology [7], but we know nothing about its influence on telomere length. Here, we tested predictions of a hypothesis suggesting that telomere length varies with socioecological conditions experienced by adult free-living spotted hyenas (Crocuta crocuta). Because the lives of high-ranking hyenas are much less challenging in many respects than are those of low-ranking individuals [8], we expected that leucocyte telomeres would be longer in the former than the latter. Ecological conditions varied among...
our five study groups, so we also expected that telomere length might vary among groups, perhaps in association with varying prey abundance. We also examined effects of age within a subset of individuals that were sampled repeatedly as adults. Lastly, we inquired whether maternal telomere length predicted telomere length in adult offspring.

2. Material and methods
Full methodological details are given in the electronic supplementary material.

(a) Data collection
We measured telomere length in 64 whole blood samples drawn from 54 adult hyenas. For nine individuals, we had repeated samples taken at different ages during adulthood (electronic supplementary material, table S1). Ages at sampling ranged from 2.5 to 13 years, based on known birthdates (±7 days) or tooth wear data (± six months) [9]. Adult hyenas were members of five social groups, called clans, sampled between 1999 and 2013 in the Masai Mara National Reserve, Kenya (electronic supplementary material, table S2). All clans contained multiple matrilineages comprising females and their descendants as well as multiple immigrant males. Among both natal females from different matrilineages and male immigrants, within-clan relatedness is effectively zero [10].

(b) Telomere length analysis
Mean leucocyte telomere length was determined using the telomere restriction fragment length assay, as described previously [11]. Intra- and interassay coefficients of variation were 2.79% and 3.16%, respectively.

(c) Statistics
We used a Shapiro–Wilk normality test to confirm that the data were normally distributed ($W = 0.99, p = 0.79$). We then ran a linear-mixed-effects model (LMM) of restricted maximum likelihood to assess the effects of dominance rank and age on adult mean telomere length using the lme4 package [12] in R v. 3.1.1 [13]. We included random effects of clan membership, sampling year, and ID because we sampled among five different clans, across multiple years and had repeated measures on nine hyenas. Clan membership and ID, but not sampling year, significantly improved the fit of the model based on likelihood ratio tests ($p < 0.05$) and were included in the final LMM. We further tested for effects of clan membership, prey density and age on telomere length using only samples from high-ranking females. This allowed us to control for dominance rank while retaining a large enough sample size for analysis. Neither random effect of ID nor sampling year improved the model, so we employed a multiple linear regression for our analysis. Pairwise comparisons among clans were calculated by least-square means and Satterwaithe’s approximation for degrees of freedom [14]. To identify whether there was an age effect on telomere length, we ran an LMM with ID as a random effect for individuals that were sampled repeatedly as adults. We employed a simple regression model to investigate whether maternal telomere length predicted telomere length in adult offspring. Here, mothers and offspring with multiple samples were assigned a single mean adult telomere length.

3. Results
Among all adults, relative dominance rank significantly predicted telomere length ($F_{1,34.9} = 8.43, p = 0.006$; figure 1), but...
The random effects for hyena ID and clan explained 26.8% and 24.6% of the residual variation, respectively, and significantly improved the fit of the model (ID: $\chi^2 = 4.83$, d.f. = 1, $p = 0.028$; clan: $\chi^2 = 4.24$, d.f. = 1, $p = 0.039$). Among high-ranking females ($n = 30$ telomere samples from 26 hyenas), only clan membership significantly predicted telomere length (table 1 and figure 2). For nine individuals with repeated sampling, the random effect of ID explained 58.7% of the residual variation and improved the fit of the model ($\chi^2 = 4.71$, d.f. = 1, $p = 0.030$), but there was no significant effect of age on telomere length ($F_{1,13.7} = 1.57$, $p = 0.20$). We also found no relationship between telomere lengths in mothers and their adult offspring ($R^2 = 0.11$, $p = 0.39$; electronic supplementary material, table S3).

4. Discussion

In hyena societies, high social rank ensures superior food access at kills; this forces many low-ranking animals to forage near territory edges or commute great distances in search of uncontested food [15,16]. Low-ranking female and male hyenas may thus incur high maintenance costs, similar to birds subjected to environmental challenges [3–5]. Metabolic stress may cause high concentrations of reactive oxidative species and stress hormones, which are known correlates of telomere shortening [17]. In spotted hyenas in Tanzania, dominance rank predicted circulating glucocorticoid concentrations among non-lactating females, with lower concentrations found in high- than low-ranking females [18]. Our finding that high-ranking hyenas display longer telomeres than low-ranking hyenas is consistent with earlier work, and further suggests that social subordination has important consequences at the cellular level.

Age was not a significant predictor of telomere length in any of our analyses and appears not to affect telomere length strongly among adult hyenas. Although counterintuitive, this is consistent with findings in long-lived birds, where the rate of telomere shortening is notably slower during adulthood than early development [1,3]. Hyenas from high-ranking matrilines might be born with longer telomeres than those from low-ranking matrilines, but we could not test this, nor

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**Figure 2.** Mean telomere length among high-ranking females from five clans. Box plots show group mean (line within box) ± s.d. (box) and range (whiskers).

**Table 1.** Results from a multiple linear regression model predicting telomere length among high-ranking females only ($n = 30$ telomere samples from 26 hyenas). The Talek clan is set as the reference category. Italicized $p$-values indicate statistically significant differences at $\alpha = 0.05$.

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did we find any evidence that maternal telomere length predicts offspring telomere length (electronic supplementary material, table S3). Increasing evidence shows that pre- and post-natal stress can have long-lasting effects on adult telomere length [3,19,20]. Spotted hyenas experience the highest mortality rates during the period immediately after weaning [21], and this may coincide with a high rate of telomere shortening. We speculate that the rank-related variation observed in adult telomere length may have early developmental origins. As a random effect, clan membership explained a large proportion of the residual variance in telomere length among adult hyenas. We investigated the clan effect further by modelling it as a fixed effect where it significantly predicted telomere length among high-ranking females. Within-clan relatedness among spotted hyenas averages around zero [10], allowing us to rule out clan-specific genetic effects. Therefore, we tested the hypothesis that telomere length might be influenced by local prey availability, which varies among clan territories (electronic supplementary material, table S2). However, this was not a significant predictor within high-ranking females (table 1), implying that other factors that vary among clans are likely in play. Our analysis also revealed pronounced individual variation in telomere length even though sampled females all occupied top positions in their respective dominance hierarchies. Thus, there appears to be considerable inter- and intraclan variation in the relationship between dominance rank and telomere length. This is the first study to investigate the influence of social dominance on telomere length in a non-human species, and we encourage future experimental testing of this relationship in other species.

References