Effects of early-life competition and maternal nutrition on telomere lengths in wild meerkats

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Early-life adversity can affect health, survival and fitness later in life, and recent evidence suggests that telomere attrition may link early conditions with their delayed consequences. Here, we investigate the link between early-life competition and telomere length in wild meerkats. Our results show that, when multiple females breed concurrently, increases in the number of pups in the group are associated with shorter telomeres in pups. Given that pups from different litters compete for access to milk, we tested whether this effect is due to nutritional constraints on maternal milk production, by experimentally supplementing females’ diets during gestation and lactation. While control pups facing high competition had shorter telomeres, the negative effects of pup number on telomere lengths were absent when maternal nutrition was experimentally improved. Shortened pup telomeres were associated with reduced survival to adulthood, suggesting that early-life competition for nutrition has detrimental fitness consequences that are reflected in telomere lengths. Dominant females commonly kill pups born to subordinates, thereby reducing competition and increasing growth rates of their own pups. Our work suggests that an additional benefit of infanticide may be that it also reduces telomere shortening caused by competition for resources, with associated benefits for offspring ageing profiles and longevity.

1. Introduction

The early period of an animal’s life can have a disproportionately influential role in determining health, survival and reproductive success later in life, even though it accounts for a relatively minor proportion of total lifespan [1]. Despite the importance of the early-life environment, our understanding of the physiological mechanisms underpinning its lasting and delayed consequences remains poor [2].

Telomere loss has recently been proposed as a potential molecular mechanism linking early-life adversity with later-life performance and ageing [3]. Telomeres are non-coding sequences at the ends of eukaryotic chromosomes that play a critical role in protecting genome integrity [4]. Telomeres shorten with each cell division, and this shortening is accelerated during early development and by stressors including oxidative damage and stress hormone exposure ([5–7], but see [8]). When telomeres shorten beyond a critical point, the cell enters replicative senescence, and accumulation of senescent cells can impair tissue function in later life when cell renewal capacity is reduced [9]. A number of studies have shown that telomere length or rate of loss predicts survival and longevity in vertebrates [10–12] including humans (reviewed in [13]), and short telomeres are associated with the systemic loss of function frequently observed in ageing individuals [14]. Accelerated telomere loss early in life may...
therefore advance the onset of senescence, thereby linking early-life conditions with later-life health and survival.

Early-life adversity promotes telomere shortening in a range of species, including salmon, humans and several birds [15–19]. In birds, offspring competing with more rivals, or rivals higher in the competitive hierarchy, exhibit accelerated telomere loss [20–25]. Studies of the consequences of offspring competition on telomere dynamics have thus far focused almost exclusively on biparental species; the importance of early-life competition in species with other social systems therefore remains unclear.

In animal societies where multiple females breed the effects of early-life competition on telomere lengths are likely to be particularly pronounced, because the number of competing offspring is expected to be higher and competition therefore more intense. Where females breed asynchronously, greater age asymmetries between offspring will likely further exacerbate telomere loss for offspring that are younger or lower in the competitive hierarchy [23,25]. Alternatively, sharing offspring care between multiple females may buffer offspring against unpredictable environments [26] and improve growth and health [27], thus relaxing competition and slowing telomere attrition. Whether the effects of early-life competition on the rate of telomere attrition in animal societies are exacerbated by increased offspring number, or mitigated by cooperative care of young, remains unknown.

Where early-life adversity promotes the accumulation of age-related damage and poor telomere integrity, we would predict that selection would favour parental strategies that protect offspring, either by improving the environment or by enhancing offspring resilience to adversity. Despite extensive evidence that early-life adversity is reflected in enduring deleterious effects on telomere lengths [15–20], and that short telomeres are linked with poor health and curtailed survival [12,14,28,29], little is known about parental strategies associated with slowed offspring telomere attrition, and how effective they are [30].

Here, we investigate whether early-life adversity, in the form of intense pup competition, is associated with shortened telomeres in wild Kalahari meerkat pups at emergence from the natal burrow. Meerkats (Suricata suricatta) live in stable cooperatively breeding groups of up to 50 individuals. Reproduction is largely monopolized by a single dominant female, but older subordinate females also attempt to breed at a lower frequency [31]. Mean litter size is 4.1 pups (range 1–8) [32]. Mixed litters are suckled indiscriminately by all lactating females [32], and pups therefore compete both with their littermates and with pups from other litters. Previous research suggests that pups compete for access to milk before emerging from the natal burrow, as experimental contraception of subordinate females leads to increased growth of the dominant’s pups at emergence from the birth burrow [33]. Pups are also frequently observed aggressively competing for access to provisioning helpers after emergence [34]. After investigating whether variation in the number of competing pups affects their telomere lengths, we test whether supplementing the mother’s food intake during gestation and lactation mitigates the effects of competition on pup telomeres. We then investigate whether early-life telomere lengths predict survival into adulthood. Finally, we explore the extent to which mothers reduce pup competition by killing litters born to other females and discuss how this strategy might impact telomere dynamics in their own pups. Such infanticide is common in meerkat groups and is almost always perpetrated by heavily pregnant females [35].

2. Material and methods

(a) Study population

Data collection was conducted in the context of a long-term study, monitoring a naturally regulated population of wild meerkats at the Kuruman River Reserve, South Africa (26°58′ S, 21°49′ E), between 1994 and 2015. All meerkats were habituated to close observation (less than 1 m) and individually recognizable using small dye-marks (approx. 2 cm², for adults and older pups) or trimming small patches of fur (approx. 0.5 cm², for newly emerged pups) [36]. Virtually all (greater than 95%) meerkaks could be voluntarily weighed on electronic scales (+0.1 g, Durascale, UK) before they commenced foraging in the morning, at midday and after sunset. Groups were visited two to three times per week to collect behavioural, life-history and bodyweight data. Observations of pregnancy, birth, infanticide, dominance, group size and rainfall were made using protocols detailed elsewhere [36,37]. Mother and father identity were assigned genetically [38,39].

(b) Pup tail tip sampling

Meerkats are born in an underground burrow, emerging for the first time at age 3–4 weeks. Shortly after the litter’s first emergence, a small biopsy of skin from the tail tip was collected from each pup (age 28.3 ± 3.4 days) for the determination of telomere length and parentage [39]. Skin samples were immediately transferred to 96% ethanol and stored at −20°C until DNA extraction.

(c) Supplementary feeding experiment

To investigate the effects of early nutritional environment on telomere lengths, we fed pregnant females during gestation and lactation. To minimize inter-individual differences in body condition, our experimental procedure was limited to dominant females. The supplementary feeding protocol consisted of one hard-boiled egg per day (divided equally between the morning and afternoon observation sessions) commencing 6 weeks after the end of a dominant female’s pregnancy, and continuing until the next parturition [40]. Thereafter, fed dominant females received four eggs per week until the pups were weaned. This feeding protocol occurred between August and November in 2011 and 2012. Control females were pregnant during the same period and did not receive supplemental food.

(d) Observations of infanticide

We investigated how infanticide by dominant females affects the number of competing pups and the likely consequences for telomere lengths in her own litter. While previous analyses of the distribution of infanticide have focused on consequences for the victim mother (i.e. whether her litter survives or is killed [35,37]), we quantified the benefits of infanticide for the perpetrator (i.e. how many competitor pups she removes). We identified periods when the dominant female is most likely to kill pups born to other females (the 30 days prior to her own parturition, hereafter termed ‘high infanticide period’) and least likely (the 30 days immediately after giving birth, hereafter termed ‘low infanticide period’) [27]. We then assessed subordinate litter survival probabilities and the total number of subordinate pups surviving to emergence during these two periods. Parturition for all females could be identified by sudden weight loss and change in body shape [36], and pup production for each period was measured as the number of pups born that survived to emergence from the birth burrow.
(e) Quantitative PCR determination of telomere lengths
We used quantitative PCR (qPCR) analysis to measure telomere length in skin samples, based on published protocols with some modifications [41,42]. This measure represents the average telomere length across cells in a sample and is reported as the level of telomeric sequence abundance relative to a reference non-variable copy number gene (T/S ratio). Further details of DNA extraction and qPCR analysis can be found in the electronic supplementary methods.

(f) Statistical analysis
Statistical analyses were carried out in R v. 3.2.3, using a stepwise model simplification approach [43,44]. Initially all fixed terms of interest were fitted, followed by the stepwise removal of terms whose removal from the model resulted in a non-significant change in deviance (using maximum log-likelihood estimation), until the minimal adequate model (MAM) was obtained, in which only significant terms remained. Dropped terms were then added back in to the MAM to confirm their non-significance. The homoscedasticity and normality of residuals were confirmed by visual inspection, and all continuous predictors were scaled to a mean of 0 and standard deviation of 1. The significance of all terms was tested either by removing the terms from the MAM (if the term was in the MAM) or by adding the terms to the MAM (if the term was not included in the MAM). Analysis using Akaike’s information criterion correcting for small sample size (AICc) and inspection of the top model set (for which AICcc differed by less than 2) yielded qualitatively identical results [45]. We ran four sets of statistical models: first to investigate the determinants of pup telomere lengths in the large correlative dataset, second to investigate how experimental supplementary feeding of mothers impacted pup telomere lengths, third to investigate whether early-life telomere lengths predict survival into adulthood and fourth to investigate the consequences of infanticide for pup competition.

(i) What are the determinants of pup telomere lengths?
Our primary interest was the effect of the number of competing pups on telomere lengths at emergence from the natal burrow. For each sampled pup, we assessed the number of rival pups (aged under 90 days) present in the group, every day between the focal pup’s birth and day of sampling for telomere length. The average of these daily rival counts represents our measure of overall competition experienced by the focal pup prior to sampling, hereafter termed ‘pup number’. This estimate of pup competition includes littersmates and pups from older and younger litters born to the dominant female and subordinate females.

We controlled for maternal factors that may influence offspring quality, including weight at conception, age (mean 4.9 years, range 1.2–8.0) and dominance status (dominant or subordinate) [46]. Social group size (average number of adult group members calculated as above for pup number) and rainfall (mm) in the month before birth can also both influence offspring quality [47]. We also controlled for the number of daily counts of emerging subordinate pups born during the two periods (30 days before and after dominant parturition). Infanticide typically takes place shortly after birth, so we classed each subordinate parturition as a ‘success’ or ‘infanticide’ according to whether the litter survived its first 2 days (litter loss after this point is more likely to be due to starvation or predation [35,37]). Although newborn litters remained in the burrow for up to 4 weeks, their survival could be recorded daily by observing whether the group continued to leave babysitters during foraging trips [35]. The number of successes and infanticides were then used as the response term in a binomial mixed-effects model, with the high/low infanticide period fitted as a two-level predictor. The random terms were dominant female pregnancy ID, dominant female ID and group ID. Second, for each dominant female parturition, we calculated the total number of emerging subordinate pups born during the two infanticide periods, and fitted this as the response term in a GLMM with a Poisson distribution. The main predictor of interest was the two-level high/low dominant female infanticide period, and we controlled for the number of subordinate females giving birth.

3. Results
(a) What are the determinants of pup telomere lengths?
Male and female pups had similar telomere lengths at 4 weeks ($\chi^2 = 1.14, p = 0.47$; electronic supplementary material, table S1). Pup telomere lengths were not associated
In both figures, the points represent raw data, which are translucent for clarity. Shaded areas represent the 95% confidence intervals of each model prediction. and its telomere length at emergence from the natal burrow. The line represents the model predictions from a GLMM, with an average maternal age of 4.86 years. The negative association between the number of competitors a pup encounters in the first weeks of life and its telomere length at emergence from the natal burrow. The line represents the model predictions from a GLMM, with a mean pup number of 5.43. (b) Does an experimental feeding of mothers mitigate the effects of pup number? The effect of maternal supplementary feeding on pup telomere lengths was evident as a significant interaction between experimental treatment and pup number (figure 2). While control pups had shorter telomeres under greater pup competition, no similar pattern was observed in pups from fed mothers. In contrast to our larger, correlative dataset, in our restricted experimental dataset, pup telomere lengths were not significantly affected by maternal age, either as a single term or in the interaction with treatment (both \( \chi^2 < 2.23, p > 0.14 \)). Retention of the non-significant maternal age in the model did not qualitatively change the results.

(c) Do pup telomere lengths predict survival to adulthood? A pup’s probability of survival to adulthood was positively predicted by its weight (\( \chi^2 = 16.24, p < 0.001 \); electronic supplementary material, table S3) and its mother’s age (\( \chi^2 = 4.88, p = 0.027 \)). In this dataset, pups born to dominant females were less likely to survive to adulthood compared with those born to subordinates (\( \chi^2 = 14.03, p < 0.001 \)); however, this may be driven by poor data availability for subordinates: only 17 pups (9% of this dataset) were born to subordinates. Pups were less likely to survive in larger groups (\( \chi^2 = 4.15, p = 0.042 \)). Controlling for these significant effects, pups with longer telomeres were significantly more likely to survive to adulthood (\( \chi^2 = 17.93, p < 0.001 \); figure 3). Survival to adulthood was not significantly predicted by pup sex or rainfall (both \( \chi^2 < 0.82, p > 0.36 \)).
Pups with short telomeres show a lower probability of survival to adulthood. Dominant females can reduce pup telomere lengths, but further work would be needed to clarify the role of paternal age at conception in meerkat telomere lengths, as paternal age has a weak negative effect on pup telomere lengths. This result is surprising, given that paternal age at conception is unrelated to offspring telomere length in other mammals [63]; whether subsequent litters of pups have longer telomeres as the mother grows older is therefore unclear. We also find that paternal age has a weak negative effect on pup telomere lengths. This result is surprising, given that paternal age at conception is typically positively associated with offspring telomeres (e.g. in humans [63] and chimpanzees (Pan troglodytes) [64]). It is possible that older male meerkats lose condition faster than humans or chimpanzees, with concomitant decreases in sperm and pup telomere lengths, but further work would be needed to clarify the role of paternal age at conception in meerkat telomere dynamics.

Shortened pup telomeres following early-life competition may be associated with significant fitness costs, given our
finding that reduced telomere lengths predicted low survival to adulthood. Short telomeres and rapid telomere attrition are associated with reduced survival and curtailed longevity in a number of species, both in captivity and in the wild [10–12,28]. Given that we found short telomeres were linked to reduced survival during meerkats’ first year of life, this likely does not reflect accelerated senescence, as senescence is typically only manifest after meerkats reach 3 years old [65]. Similarly, early-life telomere dynamics are linked with survival during the first years of life in other wild mammals [29], suggesting that telomeres are not only linked to ageing-related mortality, but provide an integrative biomarker of somatic damage which can be associated with mortality at any age [66]. Whether telomere dynamics in adult meerkats are predictive of age-related mortality requires further investigation.

Our results suggest that infanticide by dominant females leads to marked reductions in the number of competitors faced by their own litters. Previous evidence suggests that experimental reductions of pup number, either by temporary pup removal or by contraception of subordinate females, leads to increased weight gain in the remaining pups [33,67]. Heavier pups are subsequently more likely to survive to adulthood and acquire dominance [67,68], suggesting that this accelerated growth does not exceed the optimal growth rate and therefore confers little costs. By eliminating rival offspring, dominant females are therefore likely to improve the condition, survival and probability of dominance acquisition of their own litters.

Our findings highlight a further potential benefit of infanticide: the removal of competitor pups may be associated with a significant increase in pup telomere lengths. Longer telomeres are associated with improved early-life survival in meerkats and later-life benefits including delayed senescence and improved longevity in a number of other species [10–12,14]. Such later-life benefits may be particularly important in meerkats: in dominants (who monopolize reproduction), the primary determinant of lifetime reproductive success is dominance tenure length [69]. Dominants of both sexes exhibit senescence and their tenure typically ends when they are unable to repel same-sex challengers [36,39,65,70]. In addition to the above benefits for offspring condition and dominance acquisition, infanticide may therefore allow dominant females to improve pup telomere lengths, thus delaying their onset of senescence, extending their dominance tenures and increasing their lifetime reproductive success. While the level and type of parental care has been shown to influence offspring telomere lengths in humans and captive rhesus monkeys (Macaca mulatta) [30,71,72], to our knowledge this is the first evidence that a specific maternal strategy (killing competitor pups) has associated benefits for offspring telomere lengths.

5. Conclusion
Our results suggest that in a social species, where offspring competition may be particularly pronounced, an unfavourable early-life competitive environment accelerates telomere loss under natural conditions, with potentially lifelong consequences [12]. Despite the observed enduring detrimental effects of early-life adversity on telomere dynamics [20–25,28,58], and the clear selection pressure this places on parents, few studies have investigated whether parents are able to protect offspring telomeres by improving the early environment. In meerkats, dominant females kill rival litters to reduce competition for their own pups, resulting in improved pup condition and likely benefits for telomere lengths and longevity. Overall, our results highlight that both the early environment and protective parental strategies may affect offspring telomere lengths, and without detailed consideration of both, we are likely to underestimate the role of telomere dynamics in shaping life-histories, ageing profiles and fitness.

Ethics. Our work was approved by the Animal Ethics Committee of the University of Pretoria, South Africa (no. EC010-13) and by the Northern Cape Department of Environment and Nature Conservation, South Africa (FAUNA 1020/2016).

Data accessibility. All data used in analyses and figures are included in the electronic supplementary material.

Authors’ contributions. This study was designed by D.L.C. and T.C.-B.; P.M. and R.G. planned and implemented the laboratory analyses and advised on interpretation of telomere data. D.L.C. planned and implemented the statistical analyses; D.L.C. and T.C.-B. wrote the paper, with extensive advice from P.M. and R.G. All authors contributed to the manuscript, approved the final version and are accountable for the work.

Competing interests. We declare we have no competing interests.
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