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**Short-term telomere dynamics is associated  
with glucocorticoid levels in wild populations  
of roe deer**

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## ABSTRACT

While evidence that telomere length is associated with health and mortality in humans and birds is accumulating, a large body of research is currently seeking to identify factors that modulate telomere dynamics. We tested the hypothesis that high levels of glucocorticoids in individuals under environmental stress should accelerate telomere shortening in two wild populations of roe deer (*Capreolus capreolus*) living in different ecological contexts. From two consecutive annual sampling sessions, we found that individuals with faster rates of telomere shortening had higher concentrations of fecal glucocorticoid metabolites, suggesting a functional link between glucocorticoid levels and telomere attrition rate. This relationship was consistent for both sexes and populations. This finding paves the way for further studies of the fitness consequences of exposure to environmental stressors in wild vertebrates.

## *Keywords*

Aging - *Capreolus capreolus* - Fecal glucocorticoid metabolites - Life-history - Stress

## 1. Introduction

Telomeres are non-coding and repetitive DNA sequences located at the extremity of eukaryotic linear chromosomes. The shortening of telomere sequences observed in somatic cells over the lifetime of most species is expected to be associated with the aetiology of age-related diseases and an increased risk of mortality (see Blackburn et al. 2015; Wilbourn et al. 2018 for evidence in humans and birds). Therefore, identifying ecological factors modulating telomere dynamics has become an important challenge (Blackburn et al., 2015; Monaghan et al., 2018). In vertebrates, including humans (Epel et al., 2004), it has been repeatedly suggested that environmental stressors (e.g. exposure to predators, food shortage, psychosocial stress) should accelerate telomere attrition rate (Epel et al., 2004; Haussmann and Marchetto, 2010; Monaghan, 2014). In agreement with this prediction, a recent meta-analysis based on 109 studies investigating the association between environmental stressors and telomere length across vertebrates revealed that individuals facing stressful conditions have, on average, shorter telomeres (Chatelain et al. 2020). This relationship was particularly pronounced in birds and mammals, and was consistent irrespective of the type of stressor considered (Chatelain et al. 2020). From a mechanistic point of view, environmental stressors have been suggested to modulate telomere dynamics through stimulation of the hypothalamic-pituitary-adrenal axis, triggering the release of glucocorticoids into the bloodstream by the adrenal gland cortex (reviewed in Haussmann and Marchetto 2010). Chronically elevated glucocorticoids are thought to accelerate telomere shortening through diverse physiological pathways (Haussmann and Marchetto, 2010), especially through increased oxidative stress (Angelier et al., 2018; Gil et al., 2019; Reichert and Stier, 2017).

In the wild, most of our knowledge about the influence of glucocorticoids on telomere length or dynamics comes from avian studies (Angelier et al., 2018). These studies have revealed that, in most cases, high levels of plasma corticosterone are associated with short

telomeres. This is well illustrated in black-legged kittiwakes (*Rissa tridactyla*) where individuals carrying a corticosterone implant during the reproductive season experienced more marked telomere shortening than control individuals (Schultner et al., 2014) and in black-browed albatross (*Thalassarche melanophrys*) where a negative association was found between baseline corticosterone levels and telomere length in both sexes (Angelier et al., 2019). Interestingly, some studies have reported no association between glucocorticoid level and telomere length, or even a positive relationship in some cases (reviewed in Angelier et al. 2018), and empirical studies have suggested that the direction of the association might be influenced by both environmental conditions and sex (Bauch et al., 2016; Jiang et al., 2019; Young et al., 2016). Our knowledge is much more limited in mammals, although a few experiments on laboratory rodents suggest that stressful environments can induce shorter telomeres (Ilmonen et al., 2008). Overall, our current understanding of the link between physiological markers of stress and telomere dynamics remains limited, especially in wild mammals. In this study, we tested whether individuals with high glucocorticoid levels had shorter telomeres and a faster year-to-year telomere attrition rate in both sexes of two free-ranging populations of roe deer (*Capreolus capreolus*) living in environments that differ markedly in terms of habitat quality. We predicted that the relationship between glucocorticoid level and telomere loss should be steeper in the roe deer population facing marked resource limitation (Chizé) than in the population living in a more productive environment (Trois-Fontaines). We also predicted that this relationship should be steeper in males, as they might experience a higher baseline stress level than females (Carbillet et al., 2019). A stronger impact of glucocorticoids on telomere shortening in males compared to females could explain, at least to some extent, why male roe deer have a shorter lifespan compared to females (e.g. Garratt et al., 2015).

## 2. Material and Methods

### 2.1. Study population and sample collection

We studied two populations of roe deer living in enclosed forests. Trois-Fontaines forest (TF - 1,360 ha), located in north-eastern France (48°43'N, 4°55'E), has rich soils and provides high quality habitat for roe deer. In contrast, Chizé forest (CH - 2,614 ha), located in western France (46°05'N, 0°25'W), has a low productivity due to poor soils and frequent summer droughts, providing a less suitable habitat for roe deer (Pettorelli et al., 2006). For the last 40 years, 10-12 days of capture have been organized each winter as part of a long-term Capture-Mark-Recapture program (Gaillard et al., 1993). During winter, the diet composition is very similar at both study sites (mostly brambles, *Rubus* sp. and ivy, *Hedera helix*, see Tixier and Duncan 1996) and should not generate differences in faecal composition between populations which otherwise may cause a bias in FGM measurements (see below). In two consecutive years (2016 and 2017), blood samples were collected between January and February, minimizing potential confounding variation in stress levels due to individual reproductive state (Cheynel et al., 2017). Indeed, at this time of the year, nearly all females are in the very early phase of gestation, while males have not yet established their mating territories (Andersen et al., 1998). Upon capture, individuals are sexed, weighed ( $\pm$  50g) and a basic clinical examination is performed. We collected blood samples (up to 1mL/kg) from the jugular vein. We sampled only individuals of known age, which have been captured during the first year of life. Within 30 min of sampling, whole blood was spun at 2000 g for 10 min and the plasma layer drawn off and replaced with the same quantity of 0.9% w/v NaCl solution and spun again. The intermediate buffy coat layer, comprising mainly leukocytes, was collected in a 1.5-mL Eppendorf tube and immediately frozen at  $-80^{\circ}\text{C}$  in a portable freezer (Telstar SF 8025) until further use.

## 2.2. *Telomere assays*

Relative telomere length (hereafter RTL) was measured by quantitative PCR as previously described for these populations (Wilbourn et al., 2017). Genomic DNA was extracted from white blood cells using the Macherey-Nagel NucleoSpin® Blood QuickPure kit. DNA yield and purity was quantified using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington DE, USA) and DNA integrity was assessed by running 200 ng total DNA on a 0.5% agarose gel and DNA bands scored on a scale of 1-5 by visual examination. Samples passed QC with a DNA yield of  $\geq 20$  ng/ $\mu$ l, an acceptable purity absorption range of 1.7 - 2.0 for the 260/280 nm ratio and  $> 1.8$  for the 260/230 nm ratio, and a DNA integrity score of either 1 or 2 (Seeker et al., 2016). We measured relative leukocyte telomere length (RTL) using a real-time quantitative PCR method (qPCR; Cawthon 2002) which has previously been optimized and validated in sheep and cattle (Seeker et al., 2016) and previously used in roe deer (Wilbourn et al., 2017). This method measures the total amount of telomeric sequence present in a DNA sample relative to the amount of a non-variable copy number reference gene (beta-2-microglobulin (B2M)). B2M was previously identified as an appropriate reference gene for use in qPCR studies of telomere length in sheep (Fairlie et al., 2016) and cattle (Seeker et al., 2018, 2016). The selection was based on comparison of panels of candidate genes supplied as part of the GeNorm kit by Primerdesign (12 candidate reference genes for sheep, 6 for cattle). B2M showed a consistent amplification profile, clean melting curve and stable qPCR results in preliminary analyses with roe deer samples, and was therefore deemed to be a suitable reference gene for our study (Wilbourn et al., 2017). For telomere reactions, we used the following HPLC purified primers, Tel 1b (5'-CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT-3') and Tel 2b (5'-GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT-3') (from Epel et al. 2004). For B2M reactions, primers were supplied by Primer Design (Catalogue number: HK-SY-Sh-900, Southampton, UK).

Using an automated liquid handling robot (Freedom Evo-2 150; Tecan), we were able to load both the DNA samples and qPCR master mix in 384 well plates, allowing us to run both telomeric and B2M reactions in separate wells, but on a single plate. A separate master mix for each primer set was prepared containing 5µl LightCycler 480SYBR Green I Master Mix (Cat # 04887352001, Roche, West Sussex, UK), 0.5µl B2M (300nm) primer or 0.6µl each telomeric primer (900nm), and 1 ng of sample DNA per individual PCR reaction. DNA was amplified in 10µl reactions. Each plate included a non-treated control (water; NTC) for each amplicon, a calibrator sample (1ng) on each row to account for plate to plate variation and robot pipetting error, as well as a five step 1:4 serial dilution starting at 20ng/µl to inspect visually the qPCR curves. The calibrator sample was DNA that had been extracted from a large quantity of blood obtained from a single wild roe deer. In this case, the calibrator was extracted using the Qiagen DNeasy Blood and Tissue kit (Cat# 69581. Manchester. UK), pooled and quality controlled in the same way as our DNA samples of interest. All samples, calibrators and NTC's were run in triplicate and all qPCR were performed using a Roche LC480 instrument using the following reaction protocol: 10min at 95°C (enzyme activation), followed by 50 cycles of 15s at 95°C (denaturation) and 30s at 58°C (primer annealing), then 30s at 72°C (signal acquisition). Melting curve protocol was 1 min at 95°C, followed by 30s at 58°C, then 0.11°C/s to 95°C followed by 10s at 40°C.

We used the LinRegPCR software package (version 2016.0) (Ruijter et al., 2009) to correct for baseline fluorescence, to set a window of linearity for each amplicon group and to calculate well-specific reaction efficiencies and Cq values (Fairlie et al., 2016). A constant fluorescence threshold was set within the window of linearity for each amplicon group, calculated using the average Cq across all three plates. The threshold values used were 0.304 and 0.394, and the average efficiency across all plates was 1.89 for both the B2M and telomere amplicon groups. Samples were excluded from further analysis if the coefficient of variation



(CV) across triplicate Cq values for either amplicon was > 5%, or if at least one of their triplicate reactions had an efficiency that was 5% higher or lower than the mean efficiency across all wells on that plate for the respective amplicon. Overall, thirteen samples failed quality control at either the DNA extraction or qPCR stage and were excluded from the study. We calculated relative telomere length (RTL) for each sample following (Pfaffl, 2001) as follows:

$$\text{RTL} = (\text{ETEL}(\text{CqTEL}[\text{Calibrator}] - \text{CqTEL}[\text{Sample}])) / (\text{EB2M}(\text{CqB2M}[\text{Calibrator}] - \text{CqB2M}[\text{Sample}]))$$

where ETEL and EB2M are the mean reaction efficiencies for the respective amplicon group across all samples on a given plate; CqTEL[Calibrator] and CqB2M[Calibrator] are the average Cqs for the relevant amplicon across all calibrator samples on the plate; and CqTEL[Sample] and CqB2M[Sample] are the average of the triplicate Cqs for the sample for each amplicon.

Data on RTL change between years were available for 43 individuals (Chizé: 12 females, 6 males; Trois Fontaines: 13 females, 12 males, see Table 1 for sample sizes).

### 2.3. Glucocorticoid assays

Glucocorticoid levels were assessed by measuring fecal glucocorticoid metabolites (hereafter FGMs). In roe deer, FGM levels represent an integrated measure of adrenocortical activity about half a day prior to capture (Dehnhard et al., 2001; Palme, 2019). Feces were collected rectally and frozen immediately at -80°C until assayed (except in 2016 at TF when samples were stored at -20°C). FGMs were extracted following a methanol-based procedure and assayed using a group-specific 11-oxoetiocholanolone enzyme immunoassay (EIA) as previously described and validated for roe deer (Möstl et al., 2002; Zbyryt et al., 2017). To determine the amount of glucocorticoid metabolites in feces of roe deer from the 2016 and 2017 field seasons, we sampled  $500 \pm 5$  mg of each homogenized fecal sample that we vortexed for 30 min with 5 ml of 80% methanol before being centrifuged (15 min at 2500 g) (Palme et al., 2013). We

determined the amount of FGMs (with a 3 $\alpha$ , 11-oxo structure) in an aliquot of the supernatant (after a further 1+9 dilution with assay buffer). Measurements were carried out in duplicate (intra- and inter-assay coefficients of variation were <10% and <15%, respectively) and results expressed as nanograms per gram of wet feces. To ensure that there were no differences in the moisture content of feces between populations that could bias our FGM measurements, we measured the moisture content of 60 randomly selected fecal samples (30 per population). In each sample, the moisture content was calculated by subtracting dry mass (after total dehydration in an oven at 60°C for 120 hours) from wet mass. The weighing was carried out to the nearest 0.1 mg on an electronic scale and the result was expressed as % of wet mass. The results did not reveal any difference in feces moisture content between sites (mean moisture content  $\pm$  SD: 43  $\pm$  7% vs. 44  $\pm$  11% at Chizé and Trois Fontaines, respectively).

Overall, FGM concentrations were similar in Chizé and Trois Fontaines (Chizé: 973.98ng/g, 95% CI [716.55;1231.42]; Trois Fontaines: 800.59ng/g, 95% CI [649.60;951.47]). One male from the TF population had an extremely low non-physiological (and unexplained) FGM level in 2017 (8 ng/g, see Fig. S1) and was removed from the analyses. Individual concentrations of FGMs in 2016 and 2017 were correlated ( $r = 0.42$ ,  $p = 0.02$ ,  $n = 30$ , Fig. 1). However, this relationship was strongly influenced by a single male in Chizé, which had the highest level of FGMs in the dataset in both 2016 and 2017. Once this individual was removed, individual concentrations of FGMs in 2016 and 2017 were no longer correlated ( $r = 0.18$ ,  $p = 0.34$ ,  $n = 29$ ), but the repeatability of FGM concentrations (i.e. ratio of among-individual variance to total variance, Nakagawa and Schielzeth, 2010) between 2016 and 2017 was 0.35, which is within the range generally observed for glucocorticoids in vertebrates (Taff et al., 2018) and more specifically in roe deer (Carbillet et al., 2020).

#### 2.4. Statistical analyses

We performed a set of analyses seeking to test whether either raw RTL values (in 2016 and 2017) or the change in RTL between years (RTLc, computed as the difference in RTL values between 2016 and 2017 for a given individual) were related to glucocorticoid levels. We first analyzed the relationship between FGMs on RTL within each year. As both habitat and sex have been suggested to mediate the relationship between FGMs and telomere length in wild animals (Angelier et al., 2018), we included possible additive or interactive effects of sex and population. Finally, we included age to control for possible age-dependence of telomere length (Wilbourn et al., 2017), and body mass (log-transformed) to control for possible growth-mediated covariation between body mass and telomere length (Monaghan and Ozanne, 2018) as covariates (see Tables S1, S2 Appendix A for the full set of models).

To test for a link between FGMs and RTLc, we analyzed the relationship between RTLc and FGM levels (log-transformed) measured in 2016 and 2017 (e.g. Table S4). In addition to the effects of FGMs measured in 2016 or 2017, we tested the influence of the mean FGM level (log-transformed) as a proxy of the mean baseline stress level experienced during two consecutive winters on RTLc. We followed the same model selection procedure as the one described above using RTL as the response variable. We thus included log-transformed body mass (using body mass measured in 2016, 2017 or mean body mass for models with FGMs in 2016, FGM in 2017 and mean FGMs, respectively) and age as potential covariates. We also included possible additive or interactive effects of sex and population on RTLc (see Table S3 for a full list of models).

We repeated the analyses excluding individuals sampled in their first year of life (i.e. at about eight months of age, Tables S2 and S4), because telomere attrition may be faster during early life compared to adulthood (Fairlie et al., 2016), and including the birth cohort (2003 to 2015) as a random effect (Table S5) to control for non-independence among animals born in the same year using linear mixed effects models (using the R-package lme4). In all cases, results

were qualitatively unchanged (Tables S2, S4 and S5). Model selection was based on the Akaike Information Criterion (AIC) and we retained the model with the lowest AIC, except when the difference in AIC ( $\Delta AIC$ ) between two competing models was less than 2, in which case we retained the simplest model (Burnham and Anderson 2002).

### 3. Results

Within years, we detected no association between FGMs and RTL (Table S1,  $\beta \pm SE = -0.04 \pm 0.07$ ,  $n = 38$  in 2016;  $\beta = -0.11 \pm 0.08$  in 2017,  $n = 32$ ; Fig. 2). The repeatability of RTL values was 0.24, while we observed a decrease in telomere length between 2016 and 2017 in 51.2% (22 out of 43) of individuals (Fig. 3). Our model selection procedure revealed that high average FGM levels in 2016 and 2017 were associated with a stronger decrease in RTL between these two consecutive years (Table S3,  $\beta = -0.23 \pm 0.11$ ,  $n = 30$ , Fig. 4a). Likewise, FGM levels of roe deer captured in 2017 were negatively associated with RTLc ( $\beta = -0.20 \pm 0.09$ ,  $n = 32$ , Fig. 4b). Population, sex and body mass had no detectable influence on RTLc. The level of FGMs measured in 2016 was not related to RTLc (the constant model was selected, Table S3, Fig 4c). Finally, our results were not impacted by individual differences in the number of days elapsed between the 2016 and 2017 capture sessions (Table 2).

### 4. Discussion

Previous reports in birds have revealed that high levels of glucocorticoids are associated with shorter telomeres in nestlings (e.g. Pegan et al., 2019; Powolny et al., 2020) and among adults (see Angelier et al., 2018 for a review; but see Cerchiara et al., 2017 for a counter-example in Magellanic penguins, *Spheniscus magellanicus*). In our study, we did not observe any within-year association between relative telomere length and FGM level in roe deer. While substantial inter-individual differences in telomere dynamics occur in wild vertebrates (Fairlie et al., 2016;

Spurgin et al., 2018), the large variation in telomere length observed among juveniles (Wilbourn et al., 2017) might explain these results if, to some extent, telomere length in adulthood can be predicted by telomere length during early-life. However, we found that high average FGM levels in two consecutive winters predicted the rate of telomere attrition in both roe deer populations. Here, it is noteworthy that, between 2016 and 2017, telomere length increased with age in 48.8% of individuals, in line with a few recent reports of within-individual elongation of telomere length in wild populations of vertebrates (e.g. Soay sheep, *Ovis aries*, Fairlie et al., 2016; edible dormouse, *Glis glis*, Hoelzl et al., 2016; Seychelles warbler, *Acrocephalus sechellensis* Spurgin et al., 2018). While measurement error could contribute to such an apparent elongation of telomere length (Steenstrup et al., 2013), we cannot totally rule out that this pattern might have a biological foundation (Bateson and Nettle, 2017). For instance, this pattern could arise from year-to-year changes in the leukocyte formula of individuals (Spurgin et al., 2018), which has already been observed in roe deer (Cheynel et al., 2017). In addition, the elongation of telomeres with increasing age could result from a possible expression of telomerase (Blackburn et al., 2015). Currently, our knowledge on telomerase activity in cervids is both limited and unclear (no expression in the fibroblasts of two Muntjac species, *Muntiacus muntjak* and *Muntiacus reevesi*, see Gomes et al., 2011; expression in the antlers of sika deer, *Cervus nippon*, see Sun et al., 2010) so that complementary analyses are thus required to disentangle these different scenarios that challenge the common view of an inevitable telomere loss over the life course. In roe deer, repeatability in telomere length was relatively high (i.e. 0.24) compared to previous published values (e.g. 0.068 in Seychelles warbler, *Acrocephalus sechellensis*, but see Bichet et al., 2020), which might be due to the fact that our measurements were performed in two consecutive years, so that a lower rate of change is expected compared to measurements taken across a longer time span.

Our findings indicate that the relationship between glucocorticoid levels and telomere dynamics is not sex-specific in roe deer. This contrasts with the relationship between baseline corticosterone levels and telomere length (measured in two consecutive years) reported in males only in a study in common terns (*Sterna hirundo*) (Bauch et al., 2016). In terns, corticosterone levels reflect male, but not female, reproductive expenditure (Bauch et al., 2016) and males allocate three times more effort to chick feeding than females (Wiggins and Morris, 1987), which may explain these sex-specific responses of telomere attrition. In roe deer, males grow antlers each year and actively defend mating territories, while female reproductive allocation is high, because they generally give birth to twins that have fast post-natal growth rates (Andersen et al., 1998). Given that the overall reproductive effort is high in both males and females and that this species is weakly dimorphic in size, this is consistent with the lack of sex differences in telomere dynamics that we report. From this, it appears particularly important to accurately assess how reproductive expenditure differs between males and females and to consider how the stress response following increased reproductive effort might differ between the sexes when investigating the possible sex-specific effects of stress on telomere dynamics.

In recent years, a few studies in birds have investigated whether the relationship between telomere length and corticosterone level can be modulated by environmental conditions. A comparison of nestlings from two populations of Thorn-tailed Rayadito (*Aphrastura spinicauda*) located at different latitudes and thus exposed to different climatic conditions revealed that individuals living at high latitude have higher levels of baseline corticosterone and somewhat shorter telomeres than individuals living at low latitude (Quirici et al., 2016). However, the negative association found between telomere length and baseline corticosterone level was broadly similar across the two populations (Quirici et al., 2016). On the contrary, a comparison of three colonies of thick-billed murre (*Uria lomvia*) revealed a much more contrasted picture (Young et al., 2016). While no relationship between telomere length and

baseline corticosterone levels was found in individuals living in habitat of medium quality, the relationship was negative in the high quality habitat but, surprisingly, positive in the low quality habitat, emphasizing the complex role played by the environment in mediating these relationships (Young et al., 2016). One possible approach to decipher these relationships is to focus on telomere loss rather than telomere length to control for inter-individual differences in telomere length (Quirici et al., 2016). In the present study, we found that the slope of the relationship between telomere length and glucocorticoid level did not differ between our two populations of roe deer living in contrasting environments. Since glucocorticoid levels also did not differ between the two populations, we can hypothesize that an adaptive down-regulation of the stress response in Chizé, where the roe deer experience the harshest environmental conditions (see Le Saout et al. 2016 for similar observations in black-tailed deer, *Odocoileus hemionus sitkensis*) might lead to a similar pattern of telomere loss as a function of physiological stress in both habitats. Note, however, that we cannot rule out that the absence of sex- or population-specific variation in our study might be due to low statistical power.

In vertebrates, the release of glucocorticoids is the last stage in a cascade of physiological responses initiated by environmental stressors (Hausmann and Marchetto, 2010). While these pathways are well described (Boonstra, 2013), the physiological mechanisms linking high levels of glucocorticoids to a faster pace of telomere attrition - as we report here - are yet to be identified in wild vertebrate populations. Studies performed to date have focused on the alteration of the redox homeostasis (Angelier et al., 2018; Hausmann et al., 2012) and on the modulation of the mitochondrial metabolism (Casagrande et al., 2020; Casagrande and Hau, 2019) by glucocorticoids. In addition, in vitro experiments have shown that high levels of glucocorticoids reduce the activity of telomerase, the main enzyme responsible for preserving chromosome length through telomeric DNA synthesis, in human leukocytes (Choi et al., 2008). However, modulation of telomerase activity by stress is a

complex phenomenon that depends not only on glucocorticoid level, but also on the magnitude of stress reactivity and recovery (Beery et al., 2012; Epel et al., 2010). Moreover, the relative influence of this third pathway remains unknown in large herbivores, as our knowledge on telomerase activity is particularly limited within this taxa (Gomes et al., 2011). Future work should seek to integrate multiple measures of oxidative damage and antioxidant defenses with measures of mitochondrial and telomerase activity in leukocytes to decipher the physiological connections linking environmental stressors and telomere dynamics.

Overall, our study highlights that the level of stress (as measured by FGM levels) influences telomere dynamics, even over a short time scale, which could potentially generate substantial individual differences in telomere length in late adulthood. As short telomeres are associated with poor health (Beirne et al., 2014), and thereby greater mortality risk in populations of vertebrates in the wild (Bichet et al., 2020; Wilbourn et al., 2018), we suggest that telomere dynamics might mediate the long-term survival cost of repeated exposure to environmental stressors.

#### **Declaration of Competing Interest**

No conflict of interest.

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#### **Authors' contributions**

JFL & EGF conceived and designed the study. JFL, BR, LC, JMG, AJMH, HV, FD, JD, SP, MP, EGF performed fieldwork. BR, CR and LC extracted DNA. RP, JC, BR ran FGM assays. HF, RW, SLU and DN ran telomere assays. JFL performed the statistical analysis, wrote the



first draft of the paper and then received input from all other co-authors. All authors approved the final version of the manuscript and agree to be held accountable for the content therein.

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## Ethics

The protocol of capture and blood sampling under the authority of the Office Français de la Biodiversité (OFB) was approved by the Director of Food, Agriculture and Forest (Prefectoral order 2009–14 from Paris). The land manager of both sites, the Office National des Forêts (ONF), permitted the study of the populations (Partnership Convention ONCFS-ONF dated 2005-12-23). All experiments were performed in accordance with guidelines and regulations of the Ethical Committee of Lyon 1 University (project DR2014-09, June 5, 2014).

## Data accessibility

Data available have been uploaded on dryad (<https://datadryad.org/stash/share/YuyEniPCdMFLAZzmIja29re6JmRktx09ITzi15UMoL8>) and will be made publicly available upon acceptance.

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564 **Table 1:** Sex- and population-specific sample sizes used in the analyses. (a) Number of individuals with data on relative telomere length (RTL)  
565 in 2016 and 2017, (b) Number of individuals with data on fecal glucocorticoid metabolites (FGMs) in 2016 and RTL in both 2016 and 2017, (c)  
566 Number of individuals with data on FGMs in 2017 and RTL in both 2016 and 2017, (d) Number of individuals with data on FGMs in both 2016  
567 and 2017 and RTL in both 2016 and 2017. The age range of individuals from a given subset is given in brackets.  
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<b>(a) RTL (2016 and 2017)</b>				<b>(b) FGMs (2016)</b>			
	<b>Males</b>	<b>Females</b>	<b>Both sexes</b>		<b>Males</b>	<b>Females</b>	<b>Both sexes</b>
<b>Trois-Fontaines</b>	12 (age: 1-9)	13 (age: 1-13)	25 (age: 1-13)	<b>Trois-Fontaines</b>	11 (age: 1-9)	9 (age: 1-11)	20 (age: 1-11)
<b>Chizé</b>	6 (age: 1-7)	12 (age: 2-13)	18 (age: 1-13)	<b>Chizé</b>	6 (age: 1-7)	12 (age: 2-13)	18 (age: 1-13)
<b>Both populations</b>	18 (age: 1-9)	25 (age: 1-13)	43 (age: 1-13)	<b>Both populations</b>	17 (age: 1-9)	21 (age: 1-13)	38 (age: 1-13)

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<b>(c) FGMs (2017)</b>				<b>(d) FGMs (2016 and 2017)</b>			
	<b>Males</b>	<b>Females</b>	<b>Both sexes</b>		<b>Males</b>	<b>Females</b>	<b>Both sexes</b>
<b>Trois-Fontaines</b>	8 (age: 2-8)	6 (age: 2-14)	14 (age: 2-14)	<b>Trois-Fontaines</b>	8 (age: 2-8)	4 (age: 2-9)	12 (age: 2-9)
<b>Chizé</b>	6 (age: 2-8)	12 (age: 3-14)	18 (age: 2-14)	<b>Chizé</b>	6 (age: 2-8)	12 (age: 2-14)	18 (age: 2-14)
<b>Both populations</b>	14 (age: 2-8)	18 (age: 2-14)	32 (age: 2-14)	<b>Both populations</b>	14 (age: 2-8)	16 (age: 2-14)	30 (age: 2-14)

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**Table 2:** Parameter estimates of the selected model describing the change in RTL as a function of concentration in fecal glucocorticoid metabolites (FGMs) measured in 2017, n=32 (A) or the mean concentration (across 2016 and 2017) in fecal glucocorticoid metabolites (FGMs), n=30 (B) with the number of days elapsed between the two capture events entered as a covariate.

<b>(A)</b>	Estimate	SE	t	<i>p</i>
Intercept	-0.64	1.21	-0.53	0.60
FGMs (2017)	-0.24	0.09	-2.61	0.01
Number of days between captures	0.01	0.00	1.92	0.07

<b>(B)</b>	Estimate	SE	t	<i>p</i>
Intercept	-0.12	1.44	-0.08	0.94
Mean FGMs	-0.24	0.11	-2.15	0.04
Number of days between captures	0.00	0.00	1.35	0.19

**CAPTION FOR FIGURES:**

**Fig. 1:** Relationship between the concentration of fecal glucocorticoid metabolites (FGMs) measured in 2017 (ng/g, log-transformed) and the FGM concentration measured in 2016 (ng/g, log-transformed). Individual FGM concentrations in 2016 and 2017 were correlated ( $r = 0.42$ ,  $p = 0.02$ ,  $n = 30$ ), with a repeatability of 0.22. This relationship was strongly influenced by a single male in Chizé with the highest FGMs in both 2016 and 2017. After removing this individual the relationship was no longer statistically significant:  $r = 0.18$ ,  $p = 0.34$ ,  $n = 29$ , repeatability of 0.32). Males in Chizé are in dark blue, females in Chizé are in light blue, males in Trois-Fontaines are in dark red and females in Trois-Fontaines are in light red.

**Fig. 2:** Relationship between RTL and fecal glucocorticoid metabolite concentration (FGMs, (ng/g, log-transformed) using samples collected in 2016 (a) and 2017 (b).

**Fig. 3:** Within-individual change in RTL across the two consecutive years of our study (2016 and 2016) ( $n = 43$ ).

**Fig. 4:** Relationship between the within-individual difference in RTL between 2017 and 2016 and the individual mean FGM over both years (a), 2017 (b) and 2016 (c). Males in Chizé are in dark blue, females in Chizé are in light blue, males in Trois-Fontaines are in dark red and females in Trois-Fontaines are in light red.

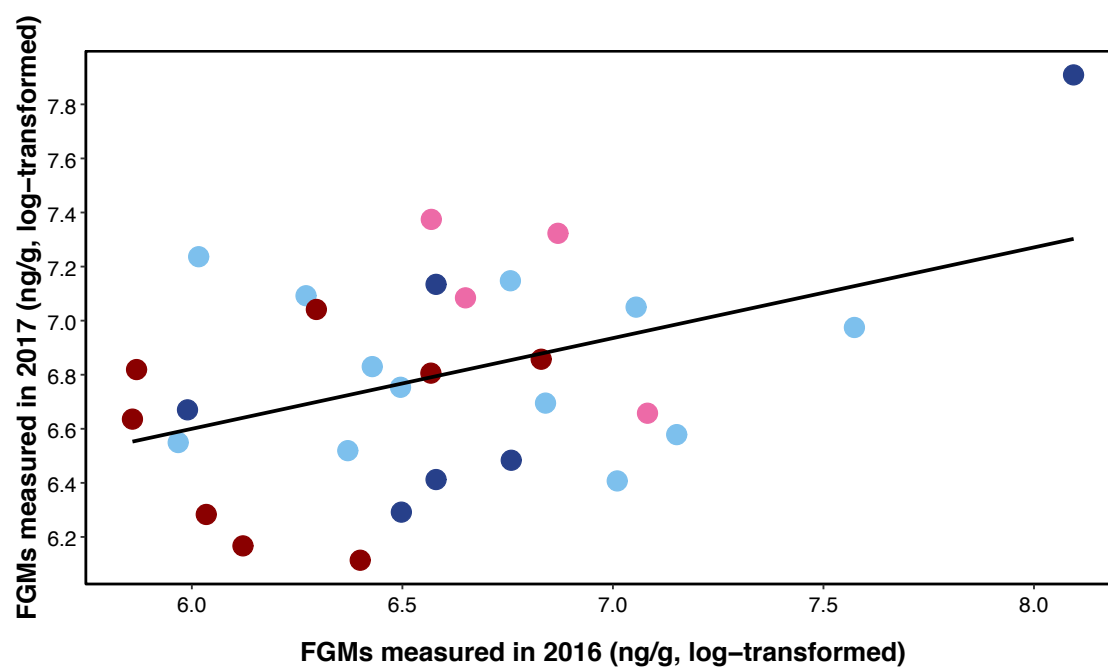
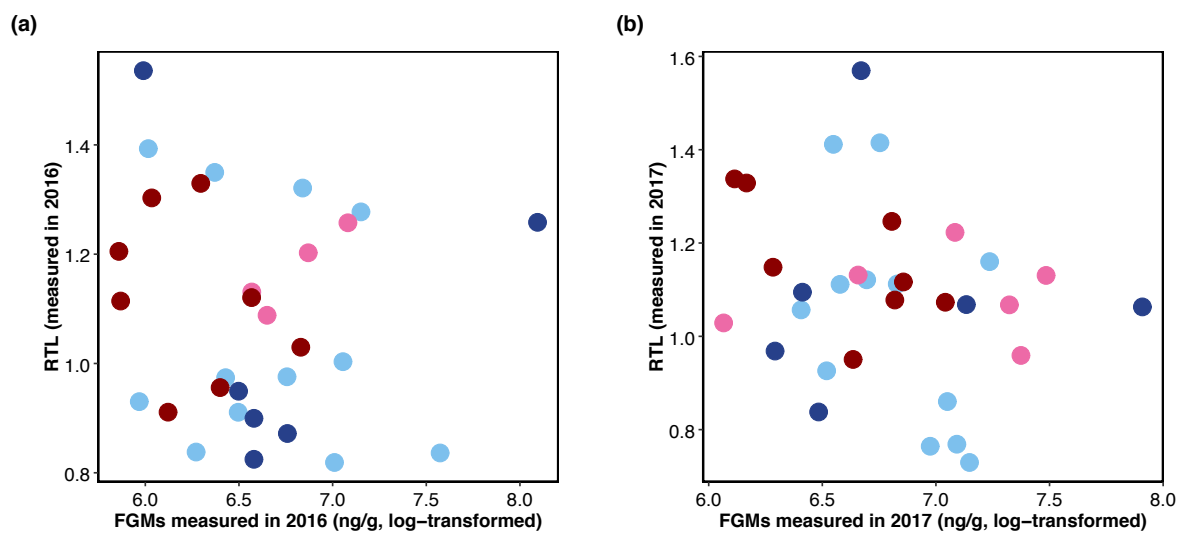


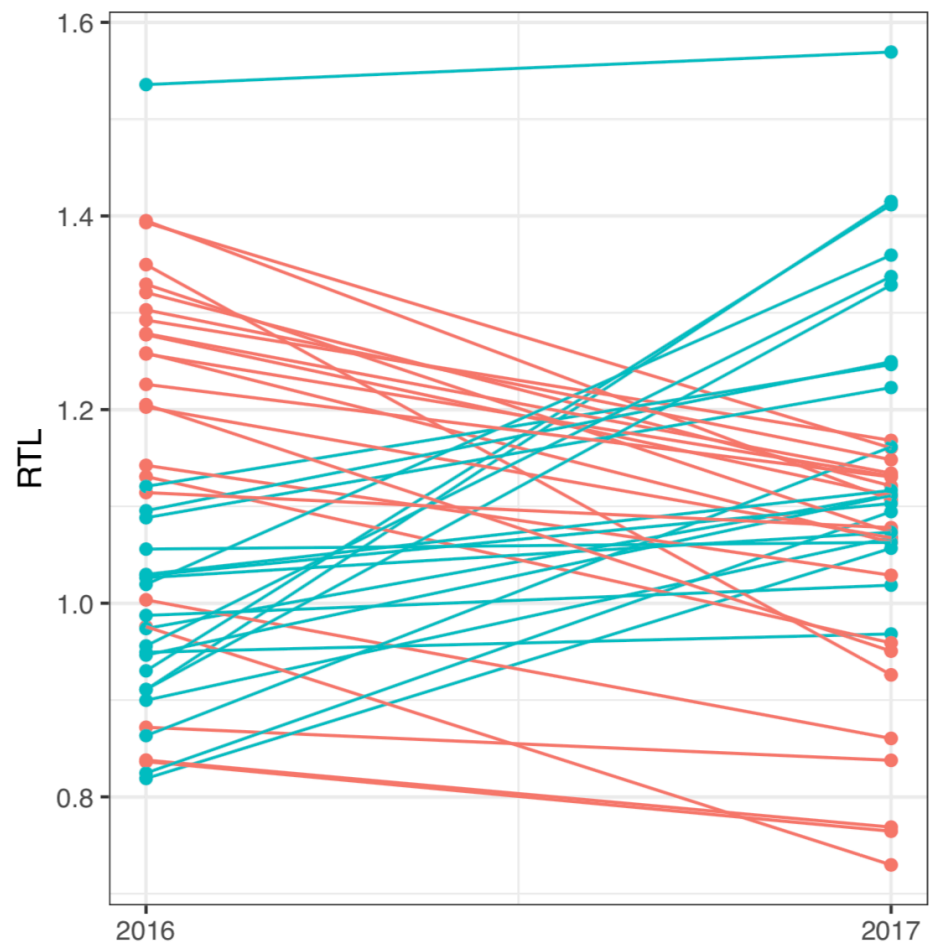
Fig. 1





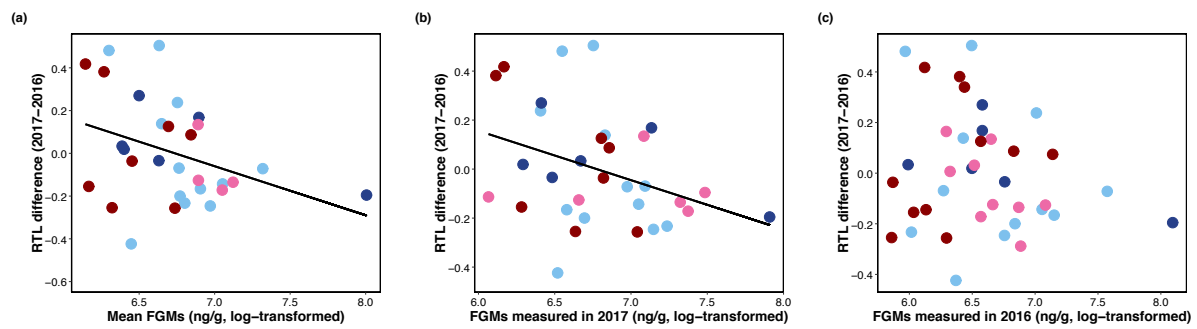
**Fig. 2**

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**Fig. 3**



**Fig. 4**