Received: 10 April 2017 | Revised: 30 June 2017 | Accepted: 7 August 2017

DOI: 10.1111/mec.14329

ORIGINAL ARTICLE

WILEY MOLECULAR ECOLOGY

Mind the cell: Seasonal variation in telomere length mirrors changes in leucocyte profile

Michaël Beaulieu[1] | Laure Benoit[2] | Steven Abaga[3] | Peter M. Kappeler[4] | Marie J. E. Charpentier[5]

[1]Zoological Institute and Museum, University of Greifswald, Greifswald, Germany

[2]CIRAD, Montpellier Cedex 5, France

[3]SODEPAL, Bakoumba, Gabon

[4]Behavioral Ecology and Sociobiology Unit, German Primate Center (DPZ), Göttingen, Germany

[5]ISEM UMR 5554, Montpellier Cedex 05, France

Correspondence
Michaël Beaulieu, Zoological Institute and Museum, University of Greifswald, Greifswald, Germany.
Email: miklvet@hotmail.fr

Funding information
Centre National de la Recherche Scientifique; Deutsche Forschungsgemeinschaft, Grant/Award Number: KA 1082-20-1

Abstract

Leucocytes are typically considered as a whole in studies examining telomere dynamics in mammals. Such an approach may be precarious, as leucocytes represent the only nucleated blood cells in mammals, their composition varies temporally, and telomere length differs between leucocyte types. To highlight this limitation, we examined here whether seasonal variation in leucocyte composition was related to variation in telomere length in free-ranging mandrills (*Mandrilllus sphinx*). We found that the leucocyte profile of mandrills varied seasonally, with lower lymphocyte proportion being observed during the long dry season presumably because of the combined effects of high nematode infection and stress at that time of the year. Interestingly, this low lymphocyte proportion during the long dry season was associated with shorter telomeres. Accordingly, based on longitudinal data, we found that seasonal changes in lymphocyte proportion were reflected by corresponding seasonal variation in telomere length. Overall, these results suggest that variation in lymphocyte proportion in blood can significantly affect telomere measurements in mammals. However, lymphocyte proportion did not entirely explain variation in telomere length. For instance, a lower lymphocyte proportion with age could not fully explain shorter telomeres in older individuals. Overall, our results show that telomere length and leucocyte profile are strongly although imperfectly intertwined, which may obscure the relationship between telomere dynamics and ageing processes in mammals.

KEYWORDS
infection, leucocytes, primates, seasonality, stress, telomeres

1 | INTRODUCTION

Telomeres are nucleoprotein complexes that consist of the repetition of the noncoding sequence 5′-TTAGGG-3′ at the end of eukaryotic chromosomes (Meyne, Ratliff, & Moyzis, 1989). During mitosis, they shorten because the 5′ end of DNA strands is not fully replicated (Blackburn, 1991; Lu, Zhang, Liu, Songyang, & Wan, 2013). Accordingly, old individuals having experienced more cell divisions than young ones typically exhibit shorter telomeres (Haussmann, Winkler, & O'Reilly, 2003). However, the negative relationship between telomere length and chronological age is far from being perfectly linear, and individuals of the same age can exhibit considerable variation in telomere length. One reason for this variability is that telomere shortening also depends on inbreeding, infection load,

stress or life history strategies (Epel, Blackburn, & Lin, 2004; Kotrschal, Ilmonen, & Penn, 2007; Blackburn & Epel, 2012; Monaghan, 2014; Asghar, Hasselquist, & Hansson, 2015; Bateson, 2016; Bebbington, Spurgin, & Fairfield, 2016). Telomeres have therefore received much attention in the fields of epidemiology and ecology, as they provide researchers with a mechanistic link between environmental conditions and health or fitness components (Monaghan & Haussmann, 2006; Haussmann & Marchetto, 2010; Sanders & Newman, 2013; Monaghan, 2014; Mathur, Epel, & Kind, 2016).

Once telomere length reaches a certain threshold, eukaryotic cells enter senescence or apoptosis, thereby contributing to the ageing of the organism. This may explain why short telomeres have been found to be associated with lower survival probability in several mammal and bird species (Haussmann, Winkler, & Vleck, 2005; Bakaysa, Mucci, & Slagboom, 2007; Heidinger, Blount, & Boner, 2012; Fairlie, Holland, & Pilkington, 2016). However, because telomere length and dynamics vary considerably among individuals, cross-sectional studies may fail to detect any negative relationship between telomere length and survival probability. Therefore, longitudinal studies measuring telomere shortening across time within individuals are more likely to detect such relationship, with rapidly shortening telomeres reflecting lower survival probability (Bize, Criscuolo, Metcalfe, Nasir, & Monaghan, 2009; Salomons, Mulder, & van de Zande, 2009; Barrett, Burke, Hammers, Komdeur, & Richardson, 2013; Boonekamp, Mulder, Salomons, Dijkstra, & Verhulst, 2014). In these longitudinal studies, telomere length is typically measured in tissues, such as blood, that can be sampled several times from the same individuals. In blood samples of birds, telomeres are primarily measured in erythrocytes, as these cells represent 99% of nucleated blood cells (Vinkler, Schnitzer, Munclinger, Votýpka, & Albrecht, 2010). In contrast, in blood samples of mammals (and of few other vertebrates (Mueller, Ryan Gregory, Gregory, Hsieh, & Boore, 2008)), telomeres can only be measured in leucocytes, as erythrocytes are enucleated (Ji, Murata-Hori, & Lodish, 2011).

Leucocytes are composed of five cell types with two different origins: neutrophils, eosinophils, basophils and monocytes belong to the myeloid lineage, while lymphocytes belong to the lymphoid lineage (Weng, 2001). One of the most striking differences between these two lineages lies in the replicative capacity of these cells. Indeed, while short-lived myeloid-derived cells do not undergo further cell divisions once in the blood stream, long-lived lymphocytes retain their proliferation potential. These functional differences between cell lineages are likely to be mediated by differences in telomerase activity, as this reverse transcriptase, which is able to elongate telomeric DNA strands, is only expressed in lymphocytes (Robertson, Gale, & Wynn, 2000; Weng, 2008; Lin, Damjanovic, & Metter, 2015). This difference in telomerase activity between cell lineages is likely to explain why telomeres are usually longer in lymphocytes than in myeloid-derived cells (Robertson et al., 2000; Lin et al., 2015) and indicates that variation in leucocyte composition in a given blood sample may affect the overall signal when measuring telomere length in mammals.

Leucocyte composition can first vary because of infection. Indeed, all leucocytes are primarily involved in the immune response, with myeloid-derived cells being involved in the innate response in the early stage of infection and lymphocytes acting later during the acquired response. Therefore, because of shorter telomeres in myeloid-derived cells than in lymphocytes (Robertson et al., 2000; Lin et al., 2015), blood samples collected from infected individuals are likely to show on average shorter telomeres in the early stages of infection and longer telomeres afterwards. However, lymphocyte proliferation may be inhibited by myeloid-derived cells, as it is the case during chronic nematode infection (Maizels & Yazdanbakhsh, 2003; Huang & Appleton, 2016). Moreover, telomere length may decrease in lymphocytes if telomerase is unable to compensate for faster telomere attrition due to lymphocyte proliferation (Ilmonen, Kotrschal, & Penn, 2008). Finally, the ability of infected organisms to invest into an appropriate immune response and to activate telomerase depends on the levels of resources they can invest into immunity (Forbes, Mappes, & Sironen, 2016). Indeed, immune defences are costly and therefore need to be traded off against other fitness-relevant functions (Lochmiller & Deerenberg, 2000; Rauw, 2012). Thus, during infection, overall changes in leucocyte profile and telomere length depend on (i) the preponderance of the acquired response over the innate response, (ii) the compensatory effects of telomerase relative to the rapid shortening of telomeres due to lymphocyte proliferation and (iii) the priority given to immunity and telomerase relative to other life functions.

Several hormones appear to regulate the trade-offs between immunity and other life functions (Martin, Weil, & Nelson, 2008). For instance, high levels of androgens during the mating season can suppress both innate and acquired immunity, which is likely to explain why females are generally better protected against infection than males (Klein, 2004; Marriott & Huet-Hudson, 2006; Trigunaite, Dimo, & Jørgensen, 2015). Similarly, high levels of glucocorticoids associated with high stress levels typically suppress immunity (Coutinho & Chapman, 2011). However, contrary to androgens, the action of glucocorticoids on the innate and the acquired immune response differs. Indeed, glucocorticoids favour myeloid-derived cells at the expense of lymphocytes (Havenstein, Langer, Stefanski, & Fietz, 2016), presumably because the developmental cost of lymphocyte proliferation and diversification is too high to sustain under chronic stress (McDade, Georgiev, & Kuzawa, 2016). Moreover, because of this high cost and because of increased maintenance costs with age (Monaghan, Charmantier, Nussey, & Ricklefs, 2008; McDade et al., 2016), the ratio between lymphocytes and myeloid-derived cells also decreases with age (Lin et al., 2015). Such stress and age effects on leucocytes may thus also contribute to variation in telomere measurements in mammals. A challenge in the interpretation of temporal variation in telomere length is therefore to distinguish actual changes in telomere length from apparent changes due to changes in leucocyte composition.

In this study, we examined whether changes in leucocyte profile were associated with changes in telomere length in free-ranging mandrills (*Mandrillus sphinx*) of known age. Mandrills are interesting to examine this relationship because they experience seasonal changes in infection and stress. Indeed, in their natural habitat (i.e., in the African rainforest), these Old World primates are more

strongly infected by nematodes during the dry season than during the rainy season (Poirotte, Basset, & Willaume, 2016). Moreover, they show higher levels of glucocorticoids at that time of the year, presumably because of mating activity, increased male–male competition and low food availability (Charpentier, Givalois, & Faurie, 2017; Setchell, Smith, Wickings, & Knapp, 2008, 2010). As both nematode infection and physiological stress favour myeloid-derived cells at the expense of lymphocytes (Coutinho & Chapman, 2011; Forbes et al., 2016; Huang & Appleton, 2016), we expected mandrills to show lower proportion of lymphocytes in their blood during the dry season than during the rainy season. As lymphocytes typically show longer telomeres than myeloid-derived cells (Robertson et al., 2000; Lin et al., 2015), this seasonal change in leucocyte profile was in turn expected to be reflected by shorter telomeres during the dry season.

2 | MATERIALS AND METHODS

2.1 | Study population and trapping

Since early 2012, we have been monitoring free-ranging mandrills inhabiting the Lékédi Park in Southern Gabon (Bakoumba) within the framework of the "Mandrillus Project" (http://www.projetmandrillus.com/). As part of this long-term study, we have regularly collected blood samples from mandrills at different times of the year. Towards this end, animals were captured by blowpipe intramuscular injections of ketamine (Imalgene® 1,000; 7 mg/kg for adults, 5 mg/kg for juveniles) and xylazine (Rompun®; 3 mg/kg for adults, 5 mg/kg for juveniles). Immediately after capture and sedation, blood was sampled from the iliac vein with EDTA-coated syringes. After blood collection, animals were antisedated with atipamezole (Antisedan ND, 0.5 mg/ml) to facilitate awakening. Across these field sessions conducted between 2012 and 2015, some individuals were repeatedly captured, which allowed us to collect data longitudinally. However, no samples were collected during the short rainy season (October–November), and too few samples were collected during the short dry season (December–January) to include them in our analyses, especially when analysing longitudinal seasonal changes. Consequently, this study is limited to the long rainy season (February–May) and the long dry season (June–September), for which we had enough data to analyse seasonal variation (long dry season → long rainy season, long rainy season → long dry season) longitudinally both in telomere length and lymphocyte proportion (see below). We used samples collected during five capture sessions, which took place in the long rainy season in 2012 and 2013 and in the long dry season in 2012, 2014 and 2015 (Figure 1). In total, we used 124 blood samples collected from 78 individuals (1–5 samples/individual). Among the sampled individuals, we knew the exact date of birth for 21 individuals and estimated it for 57 others using body condition, growth path and dental eruption pattern in juveniles (Galbany, Romero, & Mayo-Alesón, 2014). Among individuals for whom age was estimated, the possible error was estimated to be <1 year for 28 of them. At the time of capture, individuals' ages ranged from 0.3 to 20.3 years in males, and from 0.1 to 21.3 years in females.

2.2 | Lymphocyte proportion

Immediately after blood collection, we deposited a drop (~20 μl) of whole blood on a slide and performed a blood smear. The slide was stained using a kit RAL 555 (RAL Diagnostics, France) and associated protocol (derived from the May–Grünwald–Giemsa method). The total number of each of the five cell types (lymphocytes, neutrophils, eosinophils, basophils and monocytes) was counted and expressed thereafter as a frequency. For further analyses, we only considered lymphocyte proportion (i.e., the proportion of lymphoid-derived cells relative to all leucocytes). Two observers scored a subset of 40 blood smears in a double-blind fashion. The coefficient of correlation between both observers for lymphocyte proportion was 0.85.

2.3 | Telomere length

Following blood collection, blood samples were immediately centrifuged *in situ* (15 min, 3000 rpm) to obtain buffy coats, which were then stored at −20°C. Within a month, DNA was extracted from buffy coats using QIAamp DNA Blood Mini Kits (Hilden, Germany). We performed qPCR amplifications with oligonucleotide primers to hybridize to the TTAGGG and CCCTAA repeats (TelG and TelC; (Cawthon, 2009)). We used 36B4 (single-copy gene) as the endogenous reference gene (Cawthon, 2002). PCR quantifications were performed on a LightCycler 480 (Roche Diagnostics, Pleasanton, CA, USA) using the 384-multiwell plate format. For each sample and gene, three replicates were carried out in a total volume of 7 μl/reaction, which included 3.5 μl LightCycler 480 SYBR Green I Master Mix 2X (Roche Diagnostics), 900, 900, 300 and 500 nM of each primer, respectively, for TelG, TelC, 36B4u and 36B4d, 1 μl of DNA and RNase/DNase-free water to attain the final volume. The primer sequences were as follows: TelG: ACACTAAGGTTTGGGTTTGGGT

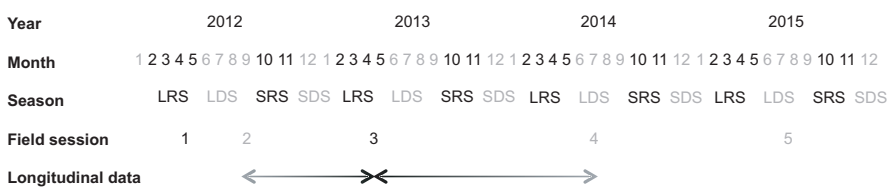

FIGURE 1 Chronogram illustrating the seasonal sequence in Gabon, and the field sessions when blood samples were collected in mandrills. Black refers to rainy seasons (LRS: long rainy season, SRS: short rainy season), and grey refers to dry seasons (LDS: long dry season, SDS: short dry season). Arrows show between which seasons blood samples were repeatedly collected from the same individuals (longitudinal data)

TTGGGTTTGGGTTAGTGT; TelC: TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA; 36B4u: CAGCAAGTGGGAAGGTGTAATCC and 36B4d: CCCATTCTATCATCAACGGGTACAA.

The qPCR amplification was performed as follows: a first denaturation step (95°C, 15 min) was followed by 55 PCR cycles including denaturation (95°C, 20 s), annealing (61°C, 15 s) and elongation (72°C, 12 s). To prove the specificity of the assay, melting curves for all reactions were determined. This procedure consisted of incubations (95°C, 5 s, then 70°C, 60 s) and a final slow heating (0.11°C/s up to 95°C) with continuous fluorescence measurement (five acquisitions per second). A negative control (water) was included on each plate to ensure the absence of contamination. A DNA standard was elaborated using an equimolar mix of all DNA samples. We used this standard as a positive control and to perform a calibrated range that served to calculate the efficiencies of the PCR. Average telomere length per cell was estimated for each sample using the method developed by Pfaffl (2001). As such, we calculated the following ratio = $E_{Tar}^{(CqSTar-CqTar)}/E_{Ref}^{(CqSRef-CqRef)}$ with E_{Tar} and E_{Ref} being, respectively, the PCR efficiencies for the targeted telomeres and reference genes, Cq_{Tar} and Cq_{Ref} the crossing points of the target and reference genes, and CqS_{Tar} and CqS_{Ref} the crossing points of the standard DNA of the target and reference genes. From the calibrated range obtained, we calculated PCR efficiencies using the second-derivative maximum method implemented in LightCycler 480 software (Roche Diagnostics, Pleasanton, CA, USA). All crossing point values were estimated using the LinRegPCR software (Ruijter, Ramakers, & Hoogaars, 2009). In the following analyses, we considered "Cq mean" corresponding to the average value of triplicate PCRs for target and reference genes (triplicates with $SD > 0.3$ were discarded; mean coefficient of variation for target gene: 0.50%, range: 0.06%–1.28%; mean coefficient of variation for reference gene: 0.33%, range: 0.02%–2.31%). Samples with a ratio >1.0 showed an average telomere length greater than that of the standard DNA, and samples with a ratio <1.0 presented an average telomere length shorter than that of the standard DNA.

2.4 | Statistical analyses

2.4.1 | Seasonal differences in lymphocyte proportion and telomere length

To examine seasonal differences in lymphocyte proportion and telomere length in the blood of mandrills, we considered all 124 samples obtained during the long rainy and the long dry season (from 2012 to 2015), and used general linear mixed models (GLMM) with the season of sampling, individual's age and sex as fixed factors. As some individuals were repeatedly measured, we also considered individual identity as a random factor and sampling session as a repeated factor (i.e., within-subject variable).

2.4.2 | Longitudinal seasonal variation in lymphocyte proportion and telomere length

To examine seasonal changes in lymphocyte proportion and telomere length within individuals, we considered the data from 22 individuals that were repeatedly sampled between the long dry season in 2012 and the long rainy season in 2013 ($LDS_{2012} \rightarrow LRS_{2013}$; time interval (mean ± *SD*): 219 ± 3 days; $N = 13$ individuals), and/or between the long rainy season in 2013 and the long dry season in 2014 ($LRS_{2013} \rightarrow LDS_{2014}$; time interval (mean ± *SD*): 464 ± 3 days; $N = 18$ individuals). As nine individuals among these 22 individuals were sampled in LDS_{2012}, LRS_{2013} and LDS_{2014}, we could analyse a total of 31 seasonal changes (LDS → LRS or LRS → LDS) in lymphocyte proportion (Δ lymphocytes) and telomere length (Δ telomeres). To compare seasonal variation (LDS → LRS vs. LRS → LDS) in lymphocyte proportion and telomere length, we subtracted the second measured value from the first measured value for both parameters (observed Δ) and divided these seasonal changes by the time elapsed between the two measurements (adjusted Δ = observed Δ/time interval) to correct for duration differences between the two time intervals. We used these values as dependent variables in GLMMs with seasonal variation (LDS → LRS or LRS → LDS), age and sex as fixed factors, and individual as a random factor. We also examined whether these values deviated from zero for each seasonal variation using one-sample *t* tests.

2.4.3 | Relationship between lymphocyte proportion and telomere length

To examine whether lymphocyte proportion and telomere length were related to each other, we used the same GLMM as described in 2.4.1 with telomere length as dependent variable and lymphocyte proportion and sex as fixed factors. To avoid collinearity problems, this model did not include season or age as fixed factors, as both factors were strongly related to lymphocyte proportion (see section 3). To examine whether the relationship between lymphocyte proportion and telomere length was consistent across seasons, we repeated our statistical analyses for the long dry season and for the long rainy season separately.

2.4.4 | Relationship between seasonal variation in lymphocyte proportion and seasonal variation in telomere length

To examine the relationship between seasonal variation in lymphocyte proportion (Δ lymphocytes) and seasonal variation in telomere length (Δ telomeres), we used the same GLMM as described in 2.4.2 to examine Δ telomeres, but replaced seasonal variation with Δ lymphocytes (sex and age were kept in this model, as they were unrelated to Δ lymphocytes; see results).

2.4.5 | Specifications on statistical analyses

Values of lymphocyte proportion were not skewed towards extreme values, and the residuals of all our models were normally distributed. In each model, we first included all two-way interactions. Then using a descending approach (i.e., starting with the interaction with the highest p value), we removed interactions with p values > .1. Final models therefore include all main effects and interaction terms with p values <.1 (Table 1). The complete models are provided in the supporting information (Tables S1 and S2). We also estimated effects sizes (η_p^2) and their 90% confidence intervals (as recommended by Steiger, 2004) for each fixed factor using general linear models including the same fixed factors as in the general linear mixed models described above. We did not examine the effects of social rank in our models, as social ranks correlate with age in male mandrills, and is maternally inherited in females. Therefore, female and male ranks are not directly comparable (Setchell, Wickings, & Knapp, 2006). All analyses were performed using SPSS 22.00 (SPSS Inc., Chicago, IL, USA), which uses the Satterthwaite's method to approximate degrees of freedom. Here, we report denominator degrees of freedom to the nearest integer.

3 | RESULTS

3.1 | Seasonal differences in lymphocyte proportion and telomere length

Lymphocyte proportion was 21% lower during the long dry season (mean ± *SE*: 46 ± 2%) than during the long rainy season (mean ± *SE*: 59 ± 3%). Similarly, telomeres were 25% shorter during the long dry season (mean ± *SE*: 1.20 ± 0.03 T/S) than during the long rainy season (mean ± *SE*: 1.61 ± 0.07 T/S). Effect size values suggested moderate-to-large effects of the season on lymphocyte proportion and telomere length, respectively (Table 1). Moreover, lymphocyte proportion and telomere length were negatively related to the age of the sampled animals, and effect size values suggested similarly large effects of age on both parameters (Table 1, Figures 2 and 4). The negative relationship between lymphocyte proportion and age was, however, stronger in males than in females, although lymphocyte proportion did not generally differ between sexes (Table 1, Figures 2 and 3). Males also exhibited longer telomeres than females (Table 1 and Figure 2) irrespective of any other factors (Table S1).

3.2 | Longitudinal seasonal variation in lymphocyte proportion and telomere length

Seasonal changes in lymphocyte proportion (Δ lymphocytes) and telomere length (Δ telomeres) followed the same pattern, and both parameters significantly differed between the long rainy season and the long dry season (LRS → LDS) and between the long dry season and the long rainy season (LDS → LRS, Table 1 and Figure 3). Effect size values suggested large effects of seasonal variation in both cases (Tables 1). The decrease in lymphocyte proportion (mean ± *SE*: −0.048 ± 0.008%) and in telomere length (mean ± *SE*: −0.00105 ± 0.00017 T/S) between the long rainy season and the long dry season was twice as important as the apparent increase in lymphocyte proportion (mean ± *SE*: 0.015 ± 0.021%) and in telomere length (mean ± *SE*: 0.00038 ± 0.00021 T/S) between the long dry season and the long rainy season. Accordingly, Δ lymphocytes and Δ telomeres were both significantly different from zero and negative between the long rainy season and the long dry season (Δ lymphocytes$_{(LRS\rightarrow LDS)}$: $t_{17} = -6.30$, $p < .001$; Δ telomeres$_{(LRS\rightarrow LDS)}$: $t_{17} = -7.04$, $p < .001$). In contrast, only Δ telomeres tended to be different from zero and positive between the long dry season and the long rainy season (Δ lymphocytes$_{(LDS\rightarrow LRS)}$: $t_{12} = 0.82$, $p = .429$; Δ telomeres$_{(LDS\rightarrow LRS)}$: $t_{12} = 2.08$, $p = .06$, respectively).

3.3 | Relationship between lymphocyte proportion and telomere length

Telomere length was positively related to lymphocyte proportion in both males and females (lymphocyte proportion: $F_{1,\ 120} = 21.65$, $p < .001$, $\eta_p^2 = 0.14$ (90% CI [0.06, 0.46]); sex: $F_{1,\ 119} = 0.59$,

TABLE 1 Results of general linear mixed models examining the effects of sex, age and season on lymphocyte proportion, telomere length, seasonal changes in lymphocyte proportion (Δ lymphocytes) and seasonal changes in telomere length (Δ telomeres) in blood samples of free-ranging mandrills

	% Lymphocytes	Telomere length	Δ Lymphocytes	Δ Telomere length
Sex	$F_{1,\ 75} = 2.60$, $p = .111$, $\eta_p^2 = 0.03$ [0.00, 0.09]	**$F_{1,\ 81} = 35.35$, $p < .001$, $\eta_p^2 = 0.25$ [0.14, 0.35]**	$F_{1,\ 27} = 0.01$, $p = .959$, $\eta_p^2 = 0.00$ [0.00, 0.00]	$F_{1,\ 14} = 0.69$, $p = .419$, $\eta_p^2 = 0.03$ [0.00, 0.18]
Season	**$F_{1,\ 92} = 15.40$, $p < .001$, $\eta_p^2 = 0.10$ [0.03, 0.19]**	**$F_{1,\ 92} = 50.89$, $p < .001$, $\eta_p^2 = 0.27$ [0.17, 0.37]**	**$F_{1,\ 27} = 10.24$, $p < .001$, $\eta_p^2 = 0.28$ [0.06, 0.46]**	**$F_{1,\ 13} = 33.30$, $p < .001$, $\eta_p^2 = 0.55$ [0.31, 0.68]**
Age	**$F_{1,\ 71} = 21.46$, $p < .001$, $\eta_p^2 = 0.16$ [0.07, 0.26]**	**$F_{1,\ 78} = 21.37$, $p < .001$, $\eta_p^2 = 0.18$ [0.08, 0.27]**	$F_{1,\ 27} = 0.01$, $p = .976$, $\eta_p^2 = 0.00$ [0.00, 0.00]	$F_{1,\ 12} = 0.55$, $p = .475$, $\eta_p^2 = 0.02$ [0.00, 0.16]
Sex*Age	**$F_{1,\ 72} = 12.20$, $p = .001$, $\eta_p^2 = 0.11$ [0.04, 0.20]**	$F_{1,\ 79} = 2.85$, $p = .095$, $\eta_p^2 = 0.03$ [0.00, 0.10]	–	–

Interaction terms associated with a p value > .10 were removed from the final models presented here (the full model are presented in Table S1). Effects sizes (η_p^2) and their 90% confidence intervals (in brackets) were estimated by general linear models with the same fixed factors as in the general linear mixed models. Significant results are highlighted in bold.

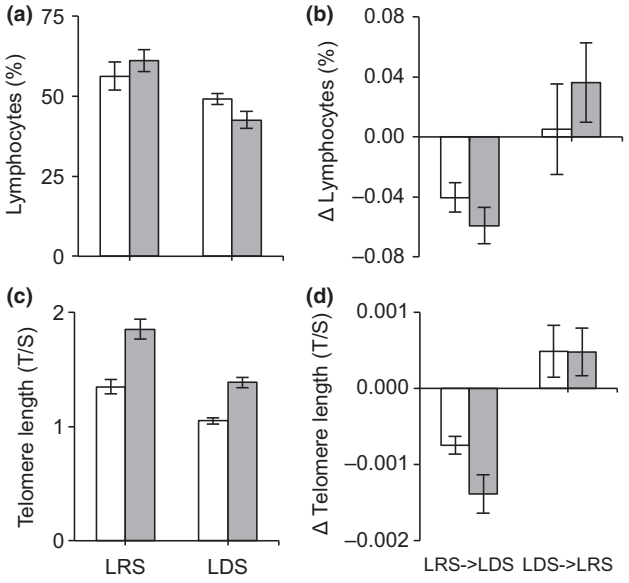

FIGURE 2 Proportion of lymphocytes (a), seasonal changes in lymphocyte proportion (Δ lymphocytes) (b), telomere length (c), and seasonal changes in telomere length (Δ telomeres) (d) in blood samples of free-ranging mandrills. Blood samples were collected during the long rainy season (LRS) and during the long dry season (LDS). Seasonal changes (Δ) were calculated between the long rainy season (first measure) and the long dry season (second measure; LRS → LDS), and between the long dry season (first measure) and the long rainy season (second measure; LDS → LRS) and were corrected for time differences. Females are illustrated in white and males in grey. Results are given as mean ± *SE*

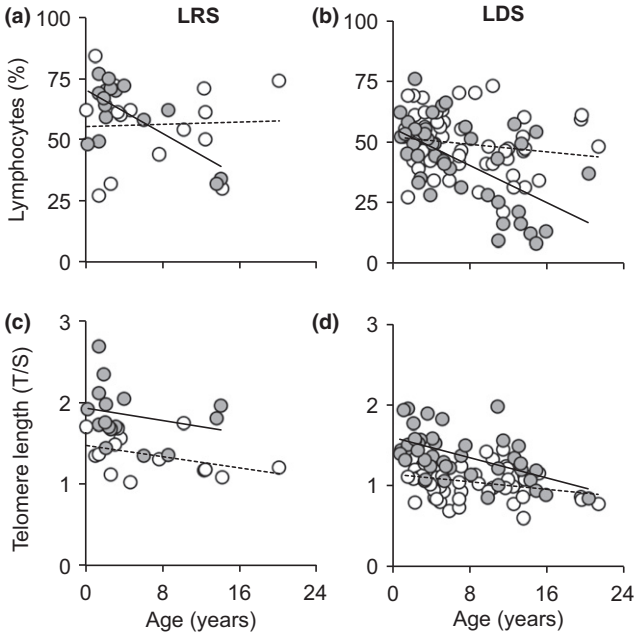

FIGURE 3 Relationships between lymphocyte proportion and age (a, b) and between telomere length and age (c, d) in mandrills during the long rainy season (LRS) and during the long dry season (LDS). Females are illustrated in white and males in grey. Dashed lines are regression lines for females, and solid lines are regression lines for males

p = .443, η_p^2 = 0.00 (90% CI [0.00, 0.05])), although this positive relationship tended to be stronger in males than in females (lymphocyte proportion*sex: $F_{1,\ 120}$ = 3.54, p = .062, η_p^2 = 0.02 (90% CI [0.00, 0.09]); Figure 4). A linear regression between lymphocyte proportion and telomere length showed that overall a 1% increase in lymphocyte proportion increases telomere length by 0.007 ± 0.002 T/S ($F_{1,\ 123}$ = 11.66, p = .001).

During the long dry season, we also found a positive relationship between telomere length and lymphocyte proportion (lymphocyte proportion: $F_{1,\ 87}$ = 5.81, p = .018, η_p^2 = 0.06 (90% CI [0.01, 0.16]); sex: $F_{1,\ 83}$ = 0.59, p = .230, η_p^2 = 0.00 (90% CI [0.00, 0.04])), which tended to be stronger in males than in females (lymphocyte proportion*sex: $F_{1,\ 87}$ = 3.17, p = .078, η_p^2 = 0.04 (90% CI [0.00, 0.12]). However, during the long rainy season, this relationship was not observed any more (lymphocyte proportion: $F_{1,\ 27}$ = 1.38, p = .250, η_p^2 = 0.05 (90% CI [0.00, 0.22]); sex: $F_{1,\ 27}$ = 0.41, p = .526, η_p^2 = 0.02 (90% CI [0.00, 0.15]; lymphocyte proportion*sex: $F_{1,\ 27}$ = 0.18, p = .672, η_p^2 = 0.00 (90% CI [0.00, 0.12]).

3.4 | Relationship between seasonal variation in lymphocyte proportion and seasonal variation in telomere length

When examining the concomitant effects of sex, age and variation in lymphocyte proportion (Δ lymphocytes) on variation in telomere length (Δ telomeres), we found that neither sex nor age affected Δ telomeres (sex: $F_{1,\ 27}$ = 0.57, p = .458, η_p^2 = 0.02 (90% CI [0.00, 0.16]); age: $F_{1,\ 27}$ = 0.49, p = 49, η_p^2 = 0.02 (90% CI [0.00, 0.16])). Interestingly, we found a significant and positive relationship between Δ lymphocytes and Δ telomeres ($F_{1,\ 27}$ = 7.63, p = .010, η_p^2 = 0.22 (90% CI [0.03, 0.41])) irrespective of any other factors (Table S2). In other words, increases in lymphocyte proportion were associated with increases in telomere length (Figure 4).

4 | DISCUSSION

4.1 | Seasonal variation in leucocyte profile and telomere length

As predicted, we found that seasonal changes in telomere length measured in the blood of free-ranging mandrills closely mirrored seasonal changes in leucocyte composition. Specifically, mandrills showed lower lymphocyte proportion and shorter telomeres during the long dry season than during the long rainy season. Accordingly, we found that lymphocyte proportion was positively related to telomere length, and seasonal variation in lymphocyte proportion was related to corresponding changes in telomere length.

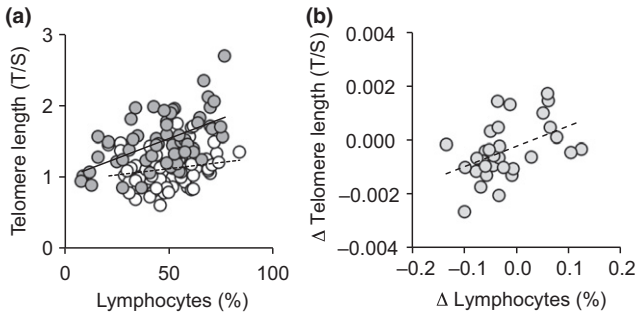

FIGURE 4 Relationship between lymphocyte proportion and telomere length (a), and between seasonal changes in lymphocyte proportion (Δ lymphocytes) and seasonal changes in telomere length (Δ telomeres) (b) in blood samples of free-ranging mandrills. In (a), females are illustrated in white and males in dark grey. The dashed and solid lines correspond to the regression lines for females and males, respectively. In (b), light grey symbols represent males and females together, and the dashed line corresponds to the regression line for males and females together

Seasonal changes in infection and stress levels likely underlie the occurrence of the seasonal fluctuations in leucocyte composition and telomere length that we observed in mandrills. Indeed, we previously found that mandrills are more heavily infected by nematodes during the dry season than during the rainy season, presumably because of lower rainfall at that time of the year (washout hypothesis; Poirotte et al., 2016). Moreover, we also found that the number of males present in the group increased during the mating season and slightly positively correlated with faecal glucocorticoid levels in both males and females (Charpentier et al., 2017). The fact that nematode infection and physiological stress favour myeloid-derived cells over lymphocytes (Huang & Appleton, 2016) likely explains why mandrills exhibit lower lymphocyte proportions during the long dry season than during the long rainy season. Moreover, the fact that myeloid-derived cells typically present shorter telomeres than lymphocytes (Robertson et al., 2000; Lin et al., 2015) likely explains why mandrills also exhibit shorter telomeres at the same time. Furthermore, the fact that we also found a positive relationship between lymphocyte proportion and telomere length during the long dry season (but not during the long rainy season) suggests that interindividual differences in responses to infection and stress levels at that time of the year are sufficient to make this relationship emerge. In mandrills, infection and stress are strongly intertwined, and their respective effects on leucocyte composition and telomere length therefore appear difficult to disentangle. A previous study conducted on humans in Costa Rica suggested that seasonal changes in infection pressure may be sufficient to lead to seasonal changes in telomere length, with shorter telomeres when infection pressure was maximal (although lymphocyte proportion and stress levels were not measured in this study) (Rehkopf, Dow, & Rosero-Bixby, 2014). Because infection and stress are likely to often co-occur in nature, further experimental studies should examine whether these factors really underlie the seasonal differences in leucocyte composition and telomere length that we observed, and, if so, whether their effects on leucocyte composition and telomere length are self-sufficient, intermingled, additive or synergistic.

4.2 | Seasonal variation in leucocyte profile as biological noise for telomere measurements

Irrespective of the reasons why leucocyte composition may vary, temporal changes in telomere length due to changes in leucocyte profile represent biological noise that likely decreases the resolution of studies examining telomere dynamics in mammals. For instance, in our study, even though we found that seasonal variation in telomere length (Δ telomeres) was negative between the long rainy season and the long dry season, we found that it tended to be positive between the long rainy season and the long dry season. The fact that we worked on a wild species prevented us from measuring Δ telomeres in a very large number of individuals, which might be the reason why we found that telomere length only tended to increase in mandrills between the long dry season and the long rainy season. However, several recent studies have reported significant telomere elongation in other mammal species, leading to an ongoing debate about the biological or methodological origin of such findings (Steenstrup, Hjelmborg, Kark, Christensen, & Aviv, 2013; Martin-Ruiz, Baird, & Roger, 2015; Verhulst, Susser, & Factor-Litvak, 2015; Bateson & Nettle, 2017). We propose that temporal changes in leucocyte profile may contribute to such unexpected observation.

To minimize the biological noise due to seasonal variation in leucocyte profile on telomere length, a first approach may consist in keeping infection and stress conditions constant when blood is collected. Even though such an approach may be feasible under laboratory conditions, ecologists appear unlikely to be able to apply it when working under natural conditions. In that case, telomeres could still be measured at the same time of the year to minimize seasonal effects on leucocyte composition. For instance, consistently measuring the telomeres of mandrills during the long rainy season when telomere length does not appear to depend on lymphocyte proportion appears to be an appropriate approach in our study population. It might also be possible to measure telomeres in tissues with less variable cellular composition than blood (e.g., muscle, mucosa cells), or even in leucocytes, but only after statistically controlling for their proportion in the blood.

Studies taking measures to avoid the effects of variable leucocyte composition on telomere length in free-ranging mammals are rare but emerging. For instance, a recent study reported that telomeres measured in buccal mucosa cells (assumed to be less variable than leucocytes) in dormice (*Glis glis*) shortened in young individuals but elongated in older ones (Hoelzl, Smith, & Cornils, 2016). Another recent study controlled for leucocyte composition when examining telomere length in male and female Soay sheep (*Ovis aries*) of different age (Watson, Bird, & Underwood, 2017). Similar to our study, the authors found that lymphocyte proportion decreased with age, especially in males. Moreover, they also found that lymphocyte proportion tended to be (p = .053) related to telomere length (Watson

et al., 2017). Hence, the relationship that we found between lymphocyte proportion and telomere length in mandrills appears to be generalizable to other mammalian species. However, even though we found that lymphocyte proportion was significantly related to telomere length, much variation remains unexplained. This variability may be due to the fact that we did not differentiate between lymphocyte types. Indeed, telomere dynamics differ not only among leucocyte types (as we considered here) but also among lymphocyte types (Weng, 2001; Lin, Cheon, & Brown, 2016). However, in Soay sheep, this methodological refinement led to weaker relationships between lymphocyte types and telomere length than the simple correlation between lymphocyte proportion and telomere length (Watson et al., 2017). Other factors, such as interindividual differences in infection load, ability to mount an immune response or stress levels, may therefore be responsible for this variability. Moreover, we found that old males had lower lymphocyte proportion than old females (which was not the case in young individuals), but these differences were not reflected at the telomere level (Figure 3). Age and sex differences between individuals therefore likely contribute to the variability surrounding the relationship between lymphocyte proportion and telomere length.

4.3 | Sex differences in telomere length

Although some studies found no differences in telomere length between males and females in mammals (Gardner, Kimura, & Chai, 2007; Hoelzl et al., 2016), most studies showed that females typically exhibit longer telomeres than males, presumably because of lower telomere loss due to stimulating effects of oestrogen on telomerase (Smith, Mattison, & Desmond, 2011; Müezzinler, Zaineddin, & Brenner, 2013; Watson et al., 2017). In sharp contrast, we found longer telomeres in male mandrills than in females. To our knowledge, this is the first description of longer telomeres in male mammals. The sex difference that we observed between males and females could not be explained by differences in telomere dynamics or in leucocyte profiles between males and females. Indeed, telomeres did not shorten more slowly in males, and males did not show higher lymphocyte proportion (Figures 2 and 3).

Four hypotheses have been suggested to explain sex differences in telomere length within species: (i) genetic heterogamety, (ii) sex differences in body size, (iii) sex differences in oxidative status and (iv) sex differences in telomere maintenance (Barrett & Richardson, 2011). The effects of genetic heterogamety on telomere length can be explained through the unguarded expression of deleterious recessive alleles on sex chromosomes in the heterogametic sex (e.g., male mammals and female birds), which may accelerate telomere shortening and increase mortality. In our study, the fact that heterogametic male mandrills show longer telomeres than females and similar telomere dynamics across time as females clearly refutes this hypothesis. This may be because genes influencing telomere length are not only located on sex chromosomes but also on autosomal chromosomes, as already described in humans (Barrett & Richardson, 2011). Longer telomere length in male mandrills is also unlikely to be related to the extreme sexual dimorphism in this species (males being two to three times as heavy as females), as larger individuals usually show shorter telomeres possibly because of more cellular replications and reduced telomerase expression and telomere maintenance (Barrett & Richardson, 2011; Ringsby, Jensen, & Pärn, 2015). In contrast, higher oxidative stress in females than in males especially during the mating season might underlie sex differences in telomere length in mandrills (Beaulieu, Mboumba, Willaume, Kappeler, & Charpentier, 2014). However, sex differences in oxidative status and telomere length do not appear to depend on sex differences in sex hormones and telomere maintenance mechanisms. Indeed, higher levels of oestrogens in females typically reduce oxidative damage and activate telomerase (thereby minimizing telomere attrition) whereas higher levels of testosterone in males typically increase oxidative damage (thereby accelerating telomere attrition if telomerase is not activated) (Barrett & Richardson, 2011). Moreover, in our study, telomere length differs between male and female mandrills before they reach sexual maturity and produce sex hormones (*c.* 3–4 years in females and 8–10 years in males) (Wickings & Dixson, 1992). Thus, a plausible explanation for longer telomeres in males may be that they have longer telomeres than females at birth. This early sex difference also appears to occur in the kakapo (*Strigops habroptilus*) and the lesser black-backed gull (*Larus fuscus*), where males also show longer telomeres than females from an early age (Foote, Gault, Nasir, & Monaghan, 2011; Horn, Robertson, & Will, 2011). In contrast, in humans, female newborns show longer telomeres than male newborns (Factor-Litvak, Susser, & Kezios, 2016). Altogether, these results suggest that sex differences in telomere length are initially determined during the embryonic stage. In mandrills, some gestational factors (e.g., differences in embryonic oxidative stress, telomerase activity, gestation duration) could therefore be responsible for the difference in telomere length that we observed after birth. Identifying these factors may be of prime importance, as they are likely to have health and fitness consequences later in life (Barrett & Richardson, 2011).

4.4 | Telomere length, leucocyte profile and survival

Longer telomeres are typically related to better survival (Haussmann et al., 2005; Bakaysa et al., 2007; Heidinger et al., 2012; Fairlie et al., 2016). Male mandrills with longer telomeres would therefore be expected to live longer than females. However, females appear to live longer in our natural population, and to the best of our knowledge, male mandrills older than 20 years have rarely been reported in their natural habitat, while females may live up to 35 years (M. J. E. Charpentier, personal observation). This result corroborates the observation in other species that sex differences in telomere length are not always reflected by sex differences in lifespan (Barrett & Richardson, 2011). One reason for the discrepancy between telomere length and longevity in mandrills may be that the effects of telomere length on survival are obscured by other factors in males. Indeed, male mandrills are more prone than females to die because

of external causes than because of physiological senescence, as they regularly engage in risky fights that can be fatal during the mating season (Setchell et al., 2006). Consequently, longer telomeres at birth in males appear adaptive, as they may allow males not to experience the effects of senescence (which would decrease their competiveness) before they die. Moreover, even if males escape death during fights, their survival may still be affected by physiological factors other than telomere length. For instance, lymphocyte proportion has also been described as predicting survival in several mammal species (Davis, Maney, & Maerz, 2008). Similarly, in humans, the relationship between telomere length and biomarkers of morbidity and mortality is 10%–20% weaker after controlling for leucocyte composition (Rehkopf, Needham, & Lin, 2016), thereby suggesting that leucocytes per se may also contribute to survival probability. The similar lymphocyte proportion that we observed in male and female mandrills may therefore also obscure the effects of telomere length on longevity. This further emphasizes the close connection between lymphocyte proportion and telomere length, and the need for future studies to disentangle the contribution of both factors on ageing processes.

ACKNOWLEDGEMENTS

We are grateful to past and present field assistants of the Mandrillus Project who collect daily behavioural data on study population. We also thank the SODEPAL-COMILOG society (ERAMET group) for their logistical contribution. We are grateful to Maxime Galan for his very helpful technical advice and to the molecular biology facilities at the CBGP laboratory (labex CeMEB, Montpellier). This study was funded by a grant of the Deutsche Forschungsgemeinschaft (DFG, KA 1082-20-1) to MJEC and PMK, a "Station d'Etudes en Ecologie Globale" (INEE-CNRS) to MJEC, a "Laboratoire International Associé" (CIRMF and INEE-CNRS) to MJEC and a PEPS ECOSAN (INEE-CNRS) to MJEC. This study was approved by an authorization from the CENAREST institute (permit number: AR0024/16/MESRS/CENAREST/CG/CST/CSAR). This is a Project Mandrillus publication number 10 [Correction added on 6 October 2017, after first Online publication. In the acknowledgment section, the last sentence "This is a Project Mandrillus publication number #" has been changed to "This is a Project Mandrillus publication number 10" at the request of the author.]

DATA ACCESSIBILITY

The data sets supporting this article have been uploaded as part of the Appendix S1.

CONFLICT OF INTEREST

The authors declare they have no conflict of interests.

AUTHOR'S CONTRIBUTION

M.J.E. and S.A. collected field data. L.B. conducted laboratory analyses. M.B. developed the concept of this article and conducted statistical analyses. All authors contributed to the writing of the article.

ORCID

Michaël Beaulieu http://orcid.org/0000-0002-9948-269X

REFERENCES

Asghar, M., Hasselquist, D., Hansson, B., Zehtindjiev, P., Westerdahl, H., & Bensch, S. (2015). Hidden costs of infection: Chronic malaria accelerates telomere degradation and senescence in wild birds. *Science*, *347*, 436–438.

Bakaysa, S. L., Mucci, L. A., Slagboom, P. E., Boomsa, D. I., McClearn, G. E., Johansson, B., & Pedersen, N. L. (2007). Telomere length predicts survival independent of genetic influences. *Aging Cell*, *6*, 769–774.

Barrett, E. L. B., Burke, T. A., Hammers, M., Komdeur, J., & Richardson, D. S. (2013). Telomere length and dynamics predict mortality in a wild longitudinal study. *Molecular Ecology*, *22*, 249–259.

Barrett, E. L. B., & Richardson, D. S. (2011). Sex differences in telomeres and lifespan. *Aging Cell*, *10*, 913–921.

Bateson, M. (2016). Cumulative stress in research animals: Telomere attrition as a biomarker in a welfare context? *BioEssays*, *38*, 201–212.

Bateson, M., & Nettle, D. (2017). The telomere lengthening conundrum—It could be biology. *Aging Cell*, *16*, 312–319.

Beaulieu, M., Mboumba, S., Willaume, E., Kappeler, P. M., & Charpentier, M. J. E. (2014). The oxidative cost of unstable social dominance. *Journal of Experimental Biology*, *217*, 2629–2632.

Bebbington, K., Spurgin, L. G., Fairfield, E. A., Dugdale, H. L., Komdeur, J., Burke, T., & Richardson, D. S. (2016). Telomere length reveals cumulative individual and transgenerational inbreeding effects in a passerine bird. *Molecular Ecology*, *25*, 2949–2960.

Bize, P., Criscuolo, F., Metcalfe, N. B., Nasir, L., & Monaghan, P. (2009). Telomere dynamics rather than age predict life expectancy in the wild. *Proceedings of the Royal Society B*, *276*, 1679–1683.

Blackburn, E. H. (1991). Structure and function of telomeres. *Nature*, *350*, 569–573.

Blackburn, E. H., & Epel, E. S. (2012). Telomeres and adversity: Too toxic to ignore. *Nature*, *490*, 169–171.

Boonekamp, J. J., Mulder, G. A., Salomons, H. M., Dijkstra, C., & Verhulst, S. (2014). Nestling telomere shortening, but not telomere length, reflects developmental stress and predicts survival in wild birds. *Proceedings of the Royal Society B*, *281*, 20133287.

Cawthon, R. M. (2002). Telomere measurement by quantitative PCR. *Nucleic Acids Research*, *30*, e47.

Cawthon, R. M. (2009). Telomere length measurement by a novel monochrome multiplex quantitative PCR method. *Nucleic Acids Research*, *37*, 1–7.

Charpentier, M. J. E., Givalois, L., Faurie, C., et al. (2017). Seasonal variation in glucocorticoid production in mandrills depends on a suite of small-magnitude effects.

Coutinho, A. E., & Chapman, K. E. (2011). The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. *Molecular and Cellular Endocrinology*, *335*, 2–13.

Davis, A. K., Maney, D. L., & Maerz, J. C. (2008). The use of leukocyte profiles to measure stress in vertebrates: A review for ecologists. *Functional Ecology*, *22*, 760–772.

Epel, E. S., Blackburn, E. H., Lin, J., Dhabhar, F. S., Adler, N. E., Morrow, J. D., & Cawthon, R. M. (2004). Accelerated telomere shortening in response to life stress. *Proceedings of the National Academy of Sciences*, *101*, 17312–17315.

Factor-Litvak, P., Susser, E., Kezios, K., McKeague, I., Kark, J. D., Hoffman, M., … Aviv, A. (2016). Leukocyte telomere length in newborns: Implications for the role of telomeres in human disease. *Pediatrics*, *137*, e20153927.

Fairlie, J., Holland, R., Pilkington, J. G., Pemberton, J. M., Harrington, L., & Nussey, D. H. (2016). Lifelong leukocyte telomere dynamics and survival in a free-living mammal. *Aging Cell*, *15*, 140–148.

Foote, C. G., Gault, E. A., Nasir, L., & Monaghan, P. (2011). Telomere dynamics in relation to early growth conditions in the wild in the lesser black-backed gull. *Journal of Zoology*, *283*, 203–209.

Forbes, K. M., Mappes, T., Sironen, T., Strandin, T., Stuart, P., Meri, S., … Huitu, O. (2016). Food limitation constrains host immune responses to nematode infections. *Biology Letters*, *12*, 20160471.

Galbany, J., Romero, A., Mayo-Aleson, M., Itsoma, F., Gamarra, B., Pérez-Pérez, A., … Charpentier, M. J. E. (2014). Age-related tooth wear differs between forest and savanna primates. *PLoS ONE*, *9*, e94938.

Gardner, J. P., Kimura, M., Chai, W., Durrani, J. F., Tchakmakjian, L., Cao, X., … Aviv, A. (2007). Telomere dynamics in macaques and humans. *Journals of Gerontology*, *62*, 367–374.

Haussmann, M. F., & Marchetto, N. M. (2010). Telomeres: Linking stress and survival, ecology and evolution. *Current Zoology*, *56*, 714–727.

Haussmann, M. F., Winkler, D. W., O'Reilly, K. M., Huntington, C. E., Nisbet, I. C. T., & Vleck, C. M. (2003). Telomeres shorten more slowly in long-lived birds and mammals than in short-lived ones. *Proceedings of the Royal Society B*, *270*, 1387–1392.

Haussmann, M. F., Winkler, D. W., & Vleck, C. M. (2005). Longer telomeres associated with higher survival in birds. *Biology Letters*, *1*, 212–214.

Havenstein, N., Langer, F., Stefanski, V., & Fietz, J. (2016). It takes two to tango: Phagocyte and lymphocyte numbers in a small mammalian hibernator. *Brain, Behavior, and Immunity*, *52*, 71–80.

Heidinger, B. J., Blount, J. D., Boner, W., Griffiths, K., Metcalfe, N. B., & Monaghan, P. (2012). Telomere length in early life predicts lifespan. *Proceedings of the National Academy of Sciences*, *109*, 1743–1748.

Hoelzl, F., Smith, S., Cornils, J. S., Aydinonat, D., Bieber, C., & Ruf, T. (2016). Telomeres are elongated in older individuals in a hibernating rodent, the edible dormouse (*Glis glis*). *Scientific Reports*, *6*, 36856.

Horn, T., Robertson, B. C., Will, M., Eason, D. K., Elliott, G. P., & Gemmell, N. J. (2011). Inheritance of telomere length in a bird. *PLoS ONE*, *6*, 1–5.

Huang, L., & Appleton, J. A. (2016). Eosinophils in helminth infection: Defenders and dupes. *Trends in Parasitology*, *32*, 798–807.

Ilmonen, P., Kotrschal, A., & Penn, D. J. (2008). Telomere attrition due to infection. *PLoS ONE*, *3*, 2143.

Ji, P., Murata-Hori, M., & Lodish, H. F. (2011). Formation of mammalian erythrocytes: Chromatin condensation and enucleation. *Trends in Cell Biology*, *21*, 409–415.

Klein, S. L. (2004). Hormonal and immunological mechanisms mediating sex differences in parasite infection. *Parasite Immunology*, *26*, 247–264.

Kotrschal, A., Ilmonen, P., & Penn, D. J. (2007). Stress impacts telomere dynamics. *Biology Letters*, *3*, 128–130.

Lin, J., Cheon, J., Brown, R., Coccia, M., Puterman, E., Aschbacher, K., … Blackburn, E. H. (2016). Systematic and cell type-specific telomere length changes in subsets of lymphocytes. *Journal of Immunology Research*, *2016*, 5371050.

Lin, Y., Damjanovic, A., Metter, E. J., Nguyen, H., Truong, T., Najarro, K., … Weng, N.-P. (2015). Age-associated telomere attrition of lymphocytes *in vivo* is co-ordinated with changes in telomerase activity, composition of lymphocyte subsets and health conditions. *Clinical Science*, *128*, 367–377.

Lochmiller, R. L., & Deerenberg, C. (2000). Trade-offs in evolutionary immunology: Just what is the cost of immunity? *Oikos*, *88*, 87–98.

Lu, W., Zhang, Y., Liu, D., Songyang, Z., & Wan, M. (2013). Telomeres-structure, function, and regulation. *Experimental Cell Research*, *319*, 133–141.

Maizels, R. M., & Yazdanbakhsh, M. (2003). Immune regulation by helminth parasites: Cellular and molecular mechanisms. *Nature Reviews Immunology*, *3*, 733–744.

Marriott, I., & Huet-Hudson, Y. M. (2006). Sexual dimorphism in innate immune responses to infectious organisms. *Immunologic Research*, *34*, 177–192.

Martin, L. B., Weil, Z. M., & Nelson, R. J. (2008). Seasonal changes in vertebrate immune activity: Mediation by physiological trade-offs. *Philosophical Transactions of the Royal Society B*, *363*, 321–339.

Martin-Ruiz, C. M., Baird, D., Roger, L., Boukamp, P., Krunic, D., Cawthon, R., … von Zglinicki, T. (2015). Reproducibility of telomere length assessment: An international collaborative study. *International Journal of Epidemiology*, *44*, 1673–1683.

Mathur, M. B., Epel, E., Kind, S., Desai, M., Parks, C. G., Sandler, D. P., & Khazeni, N. (2016). Perceived stress and telomere length: A systematic review, meta-analysis, and methodologic considerations for advancing the field. *Brain, Behavior, and Immunity*, *54*, 158–169.

McDade, T. W., Georgiev, A. V., & Kuzawa, C. W. (2016). Trade-offs between acquired and innate immune defenses in humans. *Evolution, Medicine, and Public Health*, *2016*, 1–16.

Meyne, J., Ratliff, R. L., & Moyzis, R. K. (1989). Conservation of the human telomere sequence (TTAGGG)n among vertebrates. *Proceedings of the National Academy of Sciences*, *86*, 7049–7053.

Monaghan, P. (2014). Organismal stress, telomeres and life histories. *Journal of Experimental Biology*, *217*, 57–66.

Monaghan, P., Charmantier, A., Nussey, D. H., & Ricklefs, R. E. (2008). The evolutionary ecology of senescence. *Functional Ecology*, *22*, 371–378.

Monaghan, P., & Haussmann, M. F. (2006). Do telomere dynamics link lifestyle and lifespan? *Trends in Ecology & Evolution*, *21*, 47–53.

Mueller, R. L., Ryan Gregory, T., Gregory, S. M., Hsieh, A., & Boore, J. L. (2008). Genome size, cell size, and the evolution of enucleated erythrocytes in attenuate salamanders. *Zoology*, *111*, 218–230.

Müezzinler, A., Zaineddin, A. K., & Brenner, H. (2013). A systematic review of leukocyte telomere length and age in adults. *Ageing Research Reviews*, *12*, 509–519.

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research*, *29*, 16–21.

Poirotte, C., Basset, D., Willaume, E., Makaba, F., Kappeler, P. M., & Charpentier, M. J. E. (2016). Environmental and individual determinants of parasite richness across seasons in a free-ranging population of Mandrills (*Mandrillus sphinx*). *American Journal of Physical Anthropology*, *159*, 442–456.

Rauw, W. M. (2012). Immune response from a resource allocation perspective. *Frontiers in Genetics*, *3*, 1–14.

Rehkopf, D. H., Dow, W. H., Rosero-Bixby, L., Lin, J., Epel, E. S., & Blackburn, E. H. (2014). Seasonal variation of peripheral blood leukocyte telomere length in Costa Rica: A population-based observational study. *American Journal of Human Biology*, *26*, 367–375.

Rehkopf, D. H., Needham, B. L., Lin, J., Blackburn, E. H., Zota, A. R., Wojcicki, J. M., & Epel, E. S. (2016). Leukocyte telomere length in relation to 17 biomarkers of cardiovascular disease risk: A cross-sectional study of US adults. *PLoS Medicine*, *13*, e1002188.

Ringsby, T. H., Jensen, H., Pärn, H., Kvalnes, T., Boner, W., Gillespie, R., … Monaghan, P. (2015). On being the right size: Increased body size is associated with reduced telomere length under natural conditions. *Proceedings of the Royal Society B*, *282*, 20152331.

Robertson, J. D., Gale, R. E., Wynn, R. F., Dougal, M., Linch, D. C., Testa, N. G., & Chopra, R. (2000). Dynamics of telomere shortening in

neutrophils and T lymphocytes during ageing and the relationship to skewed X chromosome inactivation patterns. *British Journal of Haematology*, *109*, 272–279.

Ruijter, J. M., Ramakers, C., Hoogaars, W. M. H., Karlen, Y., Bakker, O., van den Hoff, M. J. B., & Moorman, A. F. M. (2009). Amplification efficiency: Linking baseline and bias in the analysis of quantitative PCR data. *Nucleic Acids Research*, *37*, e45.

Salomons, H. M., Mulder, G. A., van de Zande, L., Haussmann, M., Linskens, M. H. K., & Verhulst, S. (2009). Telomere shortening and survival in free-living corvids. *Proceedings of the Royal Society B*, *276*, 3157–3165.

Sanders, J. L., & Newman, A. B. (2013). Telomere length in epidemiology: A biomarker of aging, age-related disease, both, or neither? *Epidemiologic Reviews*, *35*, 112–131.

Setchell, J. M., Smith, T., Wickings, E. J., & Knapp, L. A. (2008). Factors affecting fecal glucocorticoid levels in semi-free-ranging female mandrills (*Mandrillus sphinx*). *American Journal of Primatology*, *70*, 1023–1032.

Setchell, J. M., Smith, T., Wickings, E. J., & Knapp, L. A. (2010). Stress, social behaviour, and secondary sexual traits in a male primate. *Hormones and Behavior*, *58*, 720–728.

Setchell, J. M., Wickings, E. J., & Knapp, L. A. (2006). Life history in male mandrills (*Mandrillus sphinx*): Physical development, dominance rank, and group association. *American Journal of Physical Anthropology*, *510*, 498–510.

Smith, D. L., Mattison, J. A., Desmond, R. A., Gardner, J. P., Kimura, M., Roth, G. S., … Aviv, A. (2011). Telomere dynamics in rhesus monkeys: No apparent effect of caloric restriction. *Journals of Gerontology Series A Biological Sciences and Medical Sciences*, *66A*, 1163–1168.

Steenstrup, T., Hjelmborg, J. V. B., Kark, J. D., Christensen, K., & Aviv, A. (2013). The telomere lengthening conundrum—Artifact or biology? *Nucleic Acids Research*, *41*, 1–7.

Steiger, J. H. (2004). Beyond the F test: Effect size confidence intervals and tests of close fit in the analysis of variance and contrast analysis. *Psychological Methods*, *9*, 164–182.

Trigunaite, A., Dimo, J., & Jørgensen, T. N. (2015). Suppressive effects of androgens on the immune system. *Cellular Immunology*, *294*, 87–94.

Verhulst, S., Susser, E., Factor-Litvak, P. R., Simons, M. J. P., Benetos, A., Steenstrup, T., … Aviv, A. (2015). Commentary: The reliability of telomere length measurements. *International Journal of Epidemiology*, *44*, 1683–1686.

Vinkler, M., Schnitzer, J., Munclinger, P., Votýpka, J., & Albrecht, T. (2010). Haematological health assessment in a passerine with extremely high proportion of basophils in peripheral blood. *Journal of Ornithology*, *151*, 841–849.

Watson, R. L., Bird, E. J., Underwood, S., Wilbourn, R. V., Fairlie, J., Watt, K., … Nussey, D. H. (2017). Sex differences in leukocyte telomere length in a free-living mammal. *Molecular Ecology*, *26*, 3230–3240.

Weng, N. (2001). Interplay between telomere length and telomerase in human leukocyte differentiation and aging. *Journal of Leukocyte Biology*, *70*, 861–867.

Weng, N. P. (2008). Telomere and adaptive immunity. *Mechanisms of Ageing and Development*, *129*, 60–66.

Wickings, E. J., & Dixson, A. F. (1992). Development from birth to sexual maturity in a semi-free-ranging colony of mandrills (*Mandrillus sphinx*) in Gabon. *Journal of Reproduction and Fertility*, *95*, 129–138.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Beaulieu M, Benoit L, Abaga S, Kappeler PM, Charpentier MJE. Mind the cell: Seasonal variation in telomere length mirrors changes in leucocyte profile. *Mol Ecol*. 2017;26:5603–5613. https://doi.org/10.1111/mec.14329