Multi-generational human provisioning predicts longer telomere length in Bali's temple macaques (*Macaca fascicularis*), consistent with the 'thrifty telomere' hypothesis

Short Title: Energetics and telomeres in urban primates

*Peter H. Rej¹, *Amy R. Klegarth^{1,2}, Crystal M. Riley Koenig³, Michael D. Gumert⁴,
Lisa Jones-Engel¹, Dan T. A. Eisenberg^{1,2}

*Co-first author

¹Department of Anthropology, University of Washington, Seattle, Washington

²Center for Studies in Demography and Ecology, University of Washington, Seattle, Washington

³Department of History, Sociology, and Anthropology, Southern Utah University, Cedar City, Utah

⁴Department of Psychology, Nanyang Technological University, Singapore

August 28, 2019 Draft

Corresponding Author: Peter H. Rej, PhD, Department of Anthropology, University of Washington, 314 Denny Hall, PO Box 353100, Seattle, WA 98185, USA Tel: (206) 221-4438, Email: peterrej@uw.edu

ABSTRACT

There is mounting evidence to suggest that short telomere length (TL) increases an organism's susceptibility to disease and accelerates aging. However, it is not well understood why these effects do not select for longer telomeres. The 'thrifty telomere' hypothesis suggests one possibility: while longer telomeres improve an organism's ability to repair tissue through less constrained cell proliferation, cell proliferation requires energy that might be more adaptively used for other physiological processes. Thus, natural selection may favor shorter telomeres in low resource environments. Here we present an experimental assessment of the thrifty telomere hypothesis carried out across urban long-tailed macaques (*Macaca fascicularis*) in Bali, Indonesia and Singapore. Groups of macaques in Bali have been variably provisioned by humans for over 1000 years and are somewhat genetically distinct. Thus, current provisioning levels likely index past natural selection. Conversely, in Singapore, groups are less genetically distinct, and regular human contact/provisioning is a much more recent phenomenon. Therefore, differences across Singaporean groups are more likely to be due to environmental exposures affecting TL within a generation. We measured TL in 168 wild macaques from across both islands. Two different measures of anthropogenic contact were collected from the Bali and Singapore animals. Consistent with the thrifty telomere hypothesis, we observed a positive association between TL and anthropogenic contact within Bali, but not Singapore. Inconsistent with predictions, there were no differences in TL across islands. These results provide tentative support for the thrifty telomere hypothesis, suggesting that increased energetic availability may allow selection for longer telomeres.

46

47 INTRODUCTION

48 Telomeres are the protective DNA caps of chromosomes that shorten with cell
49 division (Harley, Futcher, & Greider, 1990). Cells with critically short telomere length
50 (TL) enter into replicative senescence. Consistent with this occurrence, short TL
51 seems to cause impaired immune function and premature cellular aging (Blackburn,
52 Greider, & Szostak, 2006). Given these deleterious effects, it is likely that natural
53 selection shapes TL (e.g., Eisenberg, 2011; Haussmann & Mauck, 2008). However,
54 since short telomeres negatively impact health and longevity, it is not clear why
55 natural selection has not favored the evolution of longer telomeres. An increased risk
56 of cancer from long telomeres is one possibility (Stone et al., 2016), although TL
57 appears to play a diverse and complicated role in cancer etiologies (Cleal, Norris, &
58 Baird, 2018; Eisenberg & Kuzawa, 2018; Zhu et al., 2016) and cancer mortality is
59 typically restricted to late-life when the force of natural selection is considerably
60 attenuated (Eisenberg, 2011).

61 The ‘thrifty telomere hypothesis’ addresses the puzzle of the costs of long TL
62 by suggesting that increased cellular replication and replacement, and subsequently
63 improved long-term survival enabled by longer telomeres, may be energetically costly
64 (Eisenberg, 2011). For this reason, shorter, ‘thriftier’ telomeres may be favored in low
65 resource environments by natural selection over longer telomeres (Eisenberg, 2011).
66 Evidence in support of this hypothesis comes from mouse studies that assessed TL
67 across multiple species of domesticated laboratory mice and their wild counterparts.
68 For each species, the domesticated population had significantly longer telomeres than

69 their wild relatives (Bickle, Cantrell, Austad, & Wichman, 1998; Hemann, 2000;
70 Prowse & Greider, 1995). One key difference between wild and laboratory strains of
71 mice is that lab strains are usually fed *ad libitum* (Koteja, Carter, Swallow, & Garland,
72 2003). This unrestricted food availability, paired with reduced foraging and protection
73 from predators and pathogens, removes energetic constraints and may select for
74 longer telomeres among laboratory mice.

75 Diet-induced differences in TL between lab and wild individuals may also arise
76 due to within generation effects. In addition to cellular division, telomere shortening
77 may be hastened by environmental exposures experienced during the lifespan,
78 including poor nutrition and obesity in humans (Gielen et al., 2018; Muezzinler,
79 Zaineddin, & Brenner, 2014; Mundstock et al., 2015; but see Bateson & Nettle, 2018).
80 Diet and body composition influence oxidative stress levels, inflammation, and
81 infection susceptibility (Katona & Apte, 2008; Galland, 2010; Muñoz & Costa, 2013;
82 Nettleton, 2008)—all of which are pathways by which food may influence telomere
83 attrition (Ilmonen, Kotrschal, & Penn, 2008; Garcia-Calzon et al., 2015; Von Zglinicki,
84 2002).

85 In this paper, we examine TLs of long-tailed macaques (*Macaca fascicularis*)
86 on two islands in Southeast Asia - Bali, Indonesia and Singapore. In Singapore, urban
87 macaques gain access to anthropogenic food sources by raiding households,
88 businesses, and rubbish bins (Cheng, 2014; Riley et al., 2016). Whereas, the
89 situation on Bali is more complex, especially at the Hindu temples where some
90 populations of macaques have taken ‘refuge’ in this densely populated and cultivated
91 island. Many of these temples are visited daily by the local Balinese who offer

elaborate food, flower, and incense creations as part of their religious practice. This practice has persisted for over 1000 years (or roughly 72 macaque generations), and the Balinese tolerate the macaques that live on the temple grounds and consume the food offerings. In recent decades, however, a number of these temple sites have become inundated by tourists who routinely visit and feed the macaques (Lane-deGraaf, Fuentes, & Hollocher, 2014; Zimmermann & Radespiel, 2013). Recent population genetic studies showed that access to human food through provisioning at Bali's temples is linked to reduced emigration rates between groups (Klegarth et al., 2017; Lane-deGraaf et al., 2014), which may increase the speed and intensity with which natural selection acts to differentiate groups.

In contrast to Bali, Singapore's macaques are not as heavily provisioned. Singapore has only experienced significant increases in human population density and development within the last 70-200 years (~5-14 macaque generations) and is now the second most densely populated country in the world. Less than 2.5 km² of the island is designated as primary forest, the majority of which is protected by the Singapore National Parks Board (Brook, Sodhi, & Ng, 2003). Despite the rapid deforestation, around 1,900 macaques live in Singapore (Riley, Jayasri, & Gumert, 2015). The population is characterized by low genetic differentiation observed between sub-populations, which is consistent with a short, rapid period of urban development (Klegarth et al., 2017). Despite being less genetically distinct than macaques in Bali, extensive ecological records collected in Singapore provide strong evidence for the recent (70-200 years) separation of macaque sub-populations that serves to maintain differential access to anthropogenic resources between groups

(Corlett, 1992). These animals occupy a patchwork of interrupted primary and secondary forest fragments, recreational parks, and residential neighborhoods, although human-macaque contact is less common than in Bali (Fuentes et al., 2008; Schillaci et al., 2007). Unlike in Bali, the provisioning of Singapore's wild macaques is actively discouraged by both state and local agencies. Nevertheless, these animals are still fed by members of the public.

Here we tested whether the removal of energetic constraints due to food-supplementation via human provisioning over many generations selects for longer telomeres in wild *M. fascicularis*. Specifically, we compared TL dynamics between and within populations found in Bali, Indonesia and Singapore. Due to variations in food access, different timing of urbanization, and variable human attitudes towards macaques on the two islands, we hypothesized that temple macaques living in Bali would have evolved longer/less thrifty telomeres than their counterparts in Singapore. We also hypothesized that differential access to anthropogenic foods would influence within-island TL variation. Considering that the amount of provisioned, human-derived food varies across Bali's many temples, we tested whether the more heavily provisioned groups had longer telomeres. Since the Bali animals have had substantially more generations to accumulate changes in TL, these differences are more likely to be due to natural selection. Within Singapore, we assessed whether differences in access to anthropogenic foods between macaque groups (sampled at sites that were prime areas for human-macaque interactions) were associated with TL. However, since regular access to human-derived foods is a relatively recent phenomenon in Singapore, it is unlikely that there have been sufficient generations for

differences to evolve. Instead, differences across sites in Singapore are more likely to be due to developmental/environmental effects.

METHODS

Sampling

Whole blood samples from long-tailed macaques in Bali and Singapore were collected between 2000-2013, frozen while in the field, and stored at -80°C (Jones-Engel et al., 2007). Samples collected before 2005 underwent multiple freeze-thaws. The study protocol was approved by the University of Washington and the Notre Dame University Institutional Animal Care and Use Committees (UW - 4233-01; ND - 07-001 & 09-011), and adhered to the American Society of Primatologist Principles for the Ethical Treatment of Non-Human Primates. Briefly, macaques were trapped and anesthetized using cages or low-velocity dart guns. Biological and morphometric data were collected, and the monkeys were then placed in recovery cages to wake up prior to release back into their home range. To avoid resampling individual macaques, a variety of measures were employed, including microchip tagging and photo identifications. Body size/mass measurements were collected at the time of sampling and included crown-rump length (m) and weight (kg), which were used to calculate animal BMI (kg/m^2). Blood samples collected from Singapore were tested previously for SRV by western blotting (Grant et al., 2005). As infection predicts shorter TL in both humans and animal models (Asghar et al., 2015; Cohen et al., 2013; Ilmonen et al., 2008), we included SRV as a control variable in our Singapore analysis. A positive

score was assigned if at least one gag-encoded antigen and at least one env-encoded antigen were present (Lerche et al., 2001).

Environmental Data

The generational duration of macaque access to anthropogenic foods is variable between Bali and Singapore as is the intensity of access within both islands. In Bali, we compared 108 macaques across eight temple sites based on parameters of provisioning, population size, the number of tourism days, and human proximity (Table 1). Provisioning intensity was assessed for each site, which ranged from 1 to 4: 1 = infrequent ceremonial provisioning; 2 = low daily provisioning; 3 = moderate daily provisioning; 4 = heavy daily provisioning. Group sizes were estimated throughout 2002-2005 and ranged from 60 to 400 individuals (Fuentes, Southern, & Suaryana, 2005; Lane et al., 2010; Schillaci et al., 2010). Tourism days (defined as the number of days that non-Balinese tourists were present) were grouped as follows: 1 = low, 2 = moderate, 3 = high (Lane et al., 2010). Human proximity scores ranged from 1 to 3: 1 = rural temple, 2 = suburban temple, 3 = urban temple (Lane et al., 2010). Since these predictors measure similar aspects of anthropogenic contact, we incorporated these data into a comparable principal component analysis (PCA) to that run by Lane et al. (2011). PCA results are summarized in the supplementary file.

Table 1. Study site details for Bali temple macaques (N = 108)

Temple	n	Estimated Group Size	Provisioning [†]	Tourism Days [‡]	Human Proximity [§]
Alas Kedaton	18	400	4	3	3
Bedugul	4	200	2	2	2
Mekori	3	60	1	1	2
Padangtegal/Ubud	16	400	4	3	3

Pulaki	17	200	3	1	2
Sangeh	27	180	4	3	3
Teluk Termina	8	120	1	1	1
Uluwatu	15	300	4	3	3

Notes: †1 - infrequent ceremonial provisioning; 2 – low daily provisioning; 3 – moderate daily provisioning; 4 – heavy daily provisioning; ‡1 – low, 2 – moderate, 3 – high. §1 – rural, 2 – suburban, 3 – urban. Table adapted from Lane et al. 2010.

Macaque groups from 13 sites across Singapore were sampled (N = 60; Table 2). The average group size was 23 individuals (Riley, Jayasri, & Gumert, 2015). Data on habituation were collected for 46 of the 60 animals sampled (habituation data were not sampled at Bishan Park, Field Office, Lorong Sesuai, Pulau Ubin, and Venus Drive). Habituation is essentially synonymous with access to human-derived food (whether given willingly or acquired via raiding gardens, homes, and rubbish bins with varying concern about human retribution). Habituation scores were assigned to macaques at the group level (Table 2) by summing the results from tests assessing food response, flight response, and distance to the nearest anthropogenic food source (described by Riley et al., 2012). Briefly, food response timed animal responses to researcher-placed food in seconds: 1 = no response; 2 = 121 – 300; 3 = 61 – 120; 4 = 31 – 60; and 5 = 0 – 30. Flight response assessed how close (in meters) a researcher could get prior to a change in behavior (e.g., alert, flee, etc.): 1 = 8.1 – 9.5; 2 = 6.6 – 8.0; 3 = 5.1 – 6.5; 4 = 3.6 – 5.0; and 5 = 2.0 – 3.5. Distance to the nearest human-inhabited area measured in meters and scored as follows: 1 = 250+; 2 = 101 – 250; 3 = 51 – 100; 4 = 1 – 50; and 5 = 0. Habituation scores ranged from 5 to 15. Higher scores indicated increased habituation. Any group without at least one food and flight response test were excluded from the analysis.

Table 2. Study site details for Singapore macaques (N = 60)

Sampling Site	n	Group Habituation Score
Admiralty Park	5	11
Bishan Park	4	NA
Bukit Timah	4	15
Field Office	4	NA
Lorong Sesuai	1	NA
Lower Pierce Reservoir	13	6.66
Old Upper Thompson Road	1	12
Pulau Ubin	2	NA
Rifle Range Road	9	13.8
Sisters Island	5	6
Upper Pierce Reservoir	4	14.5
Upper Seletar	5	13
Venus Drive	3	NA

Note: habituation was not determined at Bishan Park, Field Office, Lorong Sesuai, Pulau Ubin, and Venus Drive.

DNA Extraction & Telomere Length Quantification

Whole blood samples (N = 168) were freshly extracted for this study using Qiagen Blood Mini Kits (Qiagen, Germantown, MD, USA). DNA was quantified using an Epoch Microplate Spectrophotometer (BioTek, Winooski, VT, USA) using UV clear plates (Greiner Bio-One, Item No.: 781801) as described in detail previously (Eisenberg, Kuzawa, & Hayes, 2015).

Relative TL was measured on a Bio-Rad CFX 384 real-time PCR detection system (Hercules, CA, USA) using an adapted version of Cawthon's monochrome multiplex quantitative PCR (MMQPCR; 2009) assay for humans (see Eisenberg, Hayes, & Kuzawa, 2012). T/S ratio for each sample was calculated based on their estimated starting quantity relative to a standard reference curve. For all reactions, high quality DNA extracted from human blood was used to create an eight-point, two-fold serially diluted standard curve (100 ng/reaction to 0.78 ng/reaction). Reaction size was 15 μ l. Macaque DNA isolated from the same individual was diluted to the

same working concentration as the sample DNA (8 ng/μl) and used as a positive control. Twelve positive controls were included on each plate. All standards and samples, as well as a negative control (water), were run in triplicate.

Validation of the MMQPCR assay for use on NHP samples included the examination of qPCR standard curve efficiencies and R^2 values, melt peak confirmation, age correlations, and measures of internal validation both between samples and between plates. Across all plates assayed, we observed an average efficiency of $E = 86.78\%$ (SD = 6.38%) and 84.23% (SD = 2.87%) for T and S respectively. The average R^2 value for T was 0.98 (SD = 0.01) and 0.99 (SD < 0.01) for S. Age correlation values are used as measures of external validity for human TL data (Eisenberg, 2016), and long-tailed macaque TL has been found to decrease with age across tissues (Gardner et al., 2007). We observe a correlation coefficient of -0.25 (95% CI = -0.39, -0.11), which is comparable to age-TL correlations observed in our human studies (Eisenberg, Hayes, & Kuzawa, 2012; Rej, Tennyson, Lee, & Eisenberg, 2019).

Because the coefficient of variation is a misleading statistic for qPCR derived TL data, we assessed our measurement error using the intraclass correlation coefficient (ICC; Eisenberg, 2016; Nettle, Seeker, Nussey, Froy, & Bateson, 2018; Verhulst et al., 2015). Seventy-seven samples were run twice: ICC1 = 0.86 (95% CI = 0.78, 0.91); ICC1k = 0.92 (95% CI = 0.88, 0.95). We excluded eleven sample replicates that were significant outliers identified by Dixon's-Q test.

Statistical analysis

Multiple OLS linear regression models were used to compare TL both between and within populations. Minimally and maximally controlled models were designed with telomere length replaced with a random number and deposited on Open Science Framework (<https://osf.io/w95bh/>) ahead of data analyses. Maximally controlled models included BMI, so we could assess if differences in TL were more driven by life course effects of BMI than evolutionary factors. We noted any deviations from our pre-registered models in the text as being "post hoc." First, we tested for differences in TL across the two islands (i.e., examined TL as a function of the number of generations with access to human-derived food; Table 4, Models 1 and 2). Next, we assessed whether long-term differences in anthropogenic contact/human provisioning predicted TL in Bali (Table 4, Models 3 and 4). Finally, we assessed whether differences in habituation predicted TL in the *M. fascicularis* living in Singapore only (Table 4, Models 5 and 6). We controlled for age, sex, and the year the sample was collected in all models. Age (months) was determined by dental eruption sequence and dental wear patterns using the protocol published by Schillaci et al. (2007--see Appendix S1). Three-quarters of the Bali temple macaques (81/108) and roughly two-thirds of Singapore macaques (36/60) were male. The male bias is likely due to males being bolder and easier to capture, as they tended to monopolize the resources in the trapping cages. Since telomere measures may be influenced by sample handling, and samples collected prior to 2005 underwent multiple freeze thaws, we controlled for year of sample collection. Specifically, we made a dummy variable, scoring samples collected between 2000-2004 as 0 and 2005-2010 as 1. We included BMI as a covariate in all of our maximally controlled models (Modes 2, 4, and 6), along with

exposure to (SRV) in the Singapore models (Model 5 and 6) only. We coded SRV infection as a binary variable (i.e., 0 = negative; 1 = positive). We performed all of our statistical analyses in R (R Development Core Team, 2019).

RESULTS

Our sample of 168 long-tailed macaques contained 108 individuals from Bali, Indonesia and 60 individuals from Singapore (Table 3). The Bali animals were more than a year older on average (Mean = 68.24 months, SD = 26.46) than the Singapore macaques (Mean = 49.84 months, SD = 34.19). On average, the Balinese temples regularly provisioned their macaques (Mean = 3.46, SD = 0.97) and saw moderate to high numbers of tourists (Mean = 2.60, SD = 0.67). As for the Singapore macaques, habituation scores were bimodally distributed, with many animals either being highly habituated (which in turn facilitates their access to food handouts or their ability to raid calorie dense rubbish and/or gardens) or having relatively little human contact (Mean = 10.77, SD = 3.67). However, it is important to note that Singapore is both highly urban and densely populated, so all of the macaques there have some degree of human overlap (Riley et al., 2016). Approximately 23% of the Singapore animals had antibodies to SRV.

Table 3. Sample Characteristics

	Whole Sample	Bali	Singapore
N	168	108	60
Age (months), mean (SD)	61.67 (30.66)	68.24 (26.46)	49.84 (34.19)
Sex, n (%)			
Male	117 (69.64)	81 (75.00)	36 (60.00)
Year Sampled, n (%)			
2000 – 2005	130 (77.38)	105 (97.72)	25 (41.67)
BMI ^a , mean (SD)	34.02 (11.70)	39.89 (10.50)	24.18 (5.16)

Group Size, mean (SD)	--	270.00 (93.35)	--
Provisioning ^b , mean (SD)	--	3.46 (0.97)	--
Tourism Days ^c , mean (SD)	--	2.60 (0.67)	--
Human Proximity ^d , mean (SD)	--	2.63 (0.62)	--
Habituation ^e , mean (SD)	--	--	10.77 (3.67)
Simian Retrovirus Type-D (SRV), n (%)			
Positive	--	--	14 (23.33)

Note: ^akg/m² (crown rump distance); ^b1-ceremonial, 2-low daily, 3-moderate daily, 4-high daily; ^c1-low, 2-moderate, 3-high; ^d1-rural temple, 2-suburban temple, 3-urban temple; ^e5-15 scored scale, higher values indicate increased habituation.

The results of testing our hypotheses are shown in Table 4. While Singaporean macaques had slightly shorter TL than those from Bali, this difference was nowhere near significant in either the minimally (Model 1: $\beta = -0.230$, $p = 0.561$) or maximally controlled models (Model 2: $\beta = -0.282$, $p = 0.631$).

In the Bali sample, we observe a significant, positive association between anthropogenic contact and TL, as predicted (Figure 1; Model 3: $\beta = 0.179$, $p = 0.026$). Larger values on the anthropogenic contact scale indicate more anthropogenic contact. Thus, animals living at temples with higher provisioning volumes and increased days of tourism tend to have longer telomeres than those living at smaller, less-visited sites. The 0.179 effect size of anthropogenic contact on TL in Model 3 corresponds to a 0.356 increase in TL with a one SD increase in anthropogenic contact. For comparison, one year of aging is associated with a 0.010 decrease in TL. In our maximally controlled Bali model, which additionally included BMI as a covariate, we removed fourteen individuals for which we did not have BMI measures (Model 4). In this model, the association between provisioning PC1 and TL attenuated slightly ($\beta = 0.160$, $p = 0.063$). Higher BMI showed no significant association with TL ($p = 0.478$).

In the Singapore sample, we did not observe any significant associations between habituation and TL in either our minimally (Model 5: $\beta = 0.066$, $p = 0.546$) or maximally controlled models (Model 6: $\beta = 0.085$, $p = 0.474$). BMI was also unrelated to TL ($p = 0.632$).

Since the age coefficient showed a greater association with TL in the Singapore than Bali sample, we ran a post hoc test identical to model 1, but with an interaction term between island and age. For this test, we centered age to avoid non-essential multicollinearity. Neither the main effect of island ($\beta = -0.307$, $p = 0.370$) nor the interaction term were significant ($\beta = -0.015$, $p = 0.125$), but both indicated a trend towards shorter TL in Singapore and greater declines in TL with age in Bali. The association with age also attenuated ($\beta = -0.009$, $p = 0.186$).

317 **Table 4.** Linear OLS regression models predicting TL.

	Dependent Variable: Relative Telomere Length					
	Across Islands		Within Bali		Within Singapore	
	Model 1 β (SE)	Model 2 β (SE)	Model 3 β (SE)	Model 4 β (SE)	Model 5 β (SE)	Model 6 β (SE)
Island (Singapore)	-0.230 (0.395)	-0.282 (0.585)	--	--	--	--
Anthropogenic Contact	--	--	0.179* (0.079)	<i>0.160†</i> (0.085)	--	--
Habituation	--	--	--	--	0.066 (0.109)	0.085 (0.117)
Age	-0.016** (0.005)	-0.015** (0.005)	<i>-0.010†</i> (0.006)	-0.011 (0.007)	-0.026* (0.011)	-0.026* (0.011)
Sex (Female)	0.178 (0.306)	0.194 (0.336)	0.288 (0.339)	0.292 (0.383)	0.046 (0.687)	0.095 (0.732)
Year (After 2005)	0.383 (0.434)	0.412 (0.493)	0.621 (0.953)	0.546 (0.997)	1.692* (0.808)	1.923* (0.896)
BMI	--	-0.015 (0.019)	--	-0.012 (0.017)	--	0.036 (0.075)
Island x BMI	--	0.015 (0.055)	--	--	--	--
SRV (Infected)	--	--	--	--	--	-0.633 (0.917)
N	168	150	108	94	46	44
Adjusted R ²	0.048	0.050	0.041	0.048	0.123	0.088

Note:

†p<0.10; **p*<0.05; ***p*<0.01

318

Figure 1. Anthropogenic contact predicting telomere length. Y-axis is telomere length residuals from Table 4, Model 3 with anthropogenic contact left out. Gray shaded area indicates the associated standard error of best-fit line

DISCUSSION

In this study, we compared TL in *M. fascicularis* populations from Bali, Indonesia and Singapore to assess the impact of variable levels of anthropogenic food access over many generations on TL. For over 1,000 years (> 72 generations), macaque populations in Bali have benefited from religious/cultural tolerances and sustained ritual provisioning, which allows them ample access to human-derived food resources (Lane-deGraaf et al., 2014; Wheatley, 1999; Zimmermann & Radespiel, 2013). This long-term access to human foods has been linked with reduced emigration rates between temples, as reflected by increased genetic diversity between groups (Klegarth et al., 2017). In contrast, Singapore's macaque population has only experienced significant increases in anthropogenic contact over the last 70-200 years (~5-14 macaque generations; Corlett, 1992), stemming from rapid human population growth and urban development. Thus, the fragmentation of Singapore's macaque population is a more recent phenomenon, as indicated by low genetic differentiation between subpopulations (Klegarth et al., 2017).

While we observed a slight tendency for shorter TL in the Singapore monkeys versus the Bali animals, this effect was not significant. Within Bali, however, increased anthropogenic contact predicted longer telomeres. Conversely, habituation score was not associated with macaque TL in Singapore. Assuming that the Bali animals had regular access to human-derived foods for at least five times as long as their Singapore counterparts, our results suggest that natural selection, not environmental effects experienced over the lifespan, has impacted long-tailed macaque TL. Further bolstering this interpretation is the lack of association between

BMI and TL within or between islands. Due to its central role in somatic maintenance, immune function, and cellular aging, TL is likely a target of natural selection. However, given the purported advantages of having longer telomeres, it remains unclear as to why selection would not favor their evolution. The thrifty telomere hypothesis provides a potential explanation: long telomeres promote energetically costly somatic maintenance effort, and thus, shorter telomeres may increase fitness in low resource environments (Eisenberg, 2011). Our results indicate that a millennium of constant supplementation of Bali's temple macaques with high-energy, human-derived foods may have minimized the energetic constraints and thereby selected for longer TL—consistent with the thrifty telomere hypothesis.

Over time, humans have seen considerable increases in life expectancies, in part due to improved nutrition (Vaupel, 2010). The thrifty telomere hypothesis, along with our findings in support of it here, suggests that longer telomeres could play a role in our increased longevity. According to this scenario, more stable and abundant nutrient access would tend to reduce the negative, energetically costly effects of long TLs, thereby allowing selection for long TL. Furthermore, TL may change more rapidly in response to this selection due to the paternal age effect on TL, wherein offspring of older fathers tend to have longer TL (Eisenberg & Kuzawa, 2018; Horvath et al., 2019). More stable and abundant nutritional environments likely allow fathers to be able to reproduce later in life and thereby pass on longer TL to their descendants. In turn, these longer telomeres may result in increased reproductive success for their offspring, who find themselves in similarly nutritionally replete environments wherein longer telomeres have fewer costs. Future work is required to elucidate this

relationship. For example, we would expect to see higher reproductive success in long versus short TL individuals in the contexts of high-energy availability.

While the long-term availability of anthropogenic foods may promote increased somatic maintenance, it is important to note that recent trends in global tourism continue to increase temple macaque access to these food sources (Fuentes, 2010), as the animals themselves are becoming an increasingly popular attraction. Today's Bali's temple macaques already appear markedly larger than their Singaporean counterparts. By our post hoc calculations, our Bali animals were not only significantly larger in overall body mass (ANOVA: $F = 109.36$, $p < 2.2 \times 10^{-16}$), but they also had over twice as much variation in BMI when compared to Singapore. The increasing number of very large monkeys in Bali's temples complicates the discussion of the benefits of intensified anthropogenic contact/provisioning on macaque health, as there are a number of well-established, adverse consequences associated with obesity, including high oxidative stress, infection susceptibility, and inflammation levels. Despite not observing a significant association between BMI and TL in our sample of Balinese temple macaques, we believe that monitoring the ongoing effects of global tourism on additional biomarkers of health warrants future investigation.

While we observed a significant, positive association between TL and anthropogenic contact in Bali's temple macaques, our study has some limitations. First, we cannot control for other possible sources of variation across groups that might co-occur with anthropogenic contact. Second, our Singapore analyses were limited by the nature of the habituation data. All of the macaques living on the island endure some degree of human contact. Third, increased provisioning in Bali may also

have beneficial effects on health over the lifespan of these animals, which could also explain our observation of longer telomeres in macaques with increased anthropogenic contact.

In conclusion, our study found evidence to suggest that increased anthropogenic contact predicted longer TL among long-tailed macaques living in Bali's Hindu temples. We did not observe such an association among Singapore's *M. fascicularis* population, which is subject to less frequent and less persistent human contact when compared to Bali (Fuentes et al., 2008; Schillaci et al., 2007). This result provides evidence in support of the thrifty telomere hypothesis, which suggests that longer telomeres promote increased somatic maintenance efforts, which are energetically costly (Eisenberg, 2011). Thus, natural selection may favor longer telomeres more in high resource environments, such as Bali's temples. Finally, these findings may be translatable to human evolution, as the telomeres of humans and NHP are generally similar, as telomere biology is conserved across anthropoid primates (Davis & Kipling, 2005; Steinert, White, Zou, Shay, & Wright, 2002). Namely, stable and abundant nutrient access allotted to many modern humans may offset energetic costs caused by long TL, thus allowing for the selection of longer TL in our species, a phenomenon that may be hastened by the paternal age effect on TL.

FUNDING INFORMATION

This work was funded by the University of Washington's Royalty Research Fund (#A111561), the Leakey Foundation, the National Science Foundation (NSF) IGERT GLOBES program (#0504495), the NSF East Asia Pacific Summer Institute

Singapore program 2012, the National Geographic Waitt Grant, the US Student Fulbright Program (Singapore 2013-2014), the National Geographic Young Explorer's Grant (9234-12).

ACKNOWLEDGEMENTS

We acknowledge Agustín Fuentes for his work in the original sampling of the Bali macaques and thank him for his input in the current study's design. We also thank Mona Xue her work in aging the Bali macaques.

REFERENCES

- Asghar, M., Hansson, B., Zehtindjiev, P., Hasselquist, D., Westerdahl, H., & Bensch, S. (2015). Hidden costs of infection: Chronic malaria accelerates telomere degradation and senescence in wild birds. *Science*, 347(6220), 436–438. <https://doi.org/10.1126/science.1261121>
- Bateson, M., Eisenberg, D. T. A., & Nettle, D. (2018). Controlling for baseline telomere length biases estimates of the effect of smoking on leukocyte telomere attrition in longitudinal studies. *Zenodo*, (July), 1–22. <https://doi.org/10.5281/zenodo.1009086>
- Bateson, M., & Nettle, D. (2018). Why are there associations between telomere length and behaviour? *Philosophical Transactions of the Royal Society B: Biological Sciences*, 373(1741). <https://doi.org/10.1098/rstb.2016.0438>
- Bickle, C. A., Cantrell, M. A., Austad, S. N., & Wichman, H. (1998). Effects of domestication on telomere length in *Mus musculus* and *Peromyscus maniculatus*. *FASEB Journal*, 12(4), 1845.
- Blackburn, E. H., Greider, C. W., & Szostak, J. W. (2006). Telomeres and telomerase: the path from maize, *Tetrahymena* and yeast to human cancer and aging. *Nature Medicine*, 12(10), 1133–1138. <https://doi.org/10.1038/nm1006-1133>
- Brook, B. W., Sodhi, N. S., & Ng, P. K. L. (2003). Catastrophic extinctions follow deforestation in Singapore. *Nature*, 424(6947), 420–423.
- Cawthon, R. M. (2009). Telomere length measurement by a novel monochrome multiplex quantitative PCR method. *Nucleic Acids Research*, 37(3), 1–7. <https://doi.org/10.1093/nar/gkn1027>
- Cheng, C. W. (2014). a review of the management measures of feral macaques in Hong Kong, 106. https://doi.org/10.5353/th_b5334219
- Cleal, K., Norris, K., & Baird, D. (2018). Telomere length dynamics and the evolution of cancer genome architecture. *International Journal of Molecular Sciences*,

- 19(2), 1–17. <https://doi.org/10.3390/ijms19020482>
- Cohen, S., Janicki-Deverts, D., Turner, R. B., Casselbrant, M. L., Li-Korotky, H. S., Epel, E. S., & Doyle, W. J. (2013). Association between telomere length and experimentally induced upper respiratory viral infection in healthy adults. *JAMA - Journal of the American Medical Association*, 309(7), 699–705. <https://doi.org/10.1001/jama.2013.613>
- Corlett, R. T. (1992). The Ecological Transformation of Singapore, 1819-1990. *Journal of Biogeography*, 19(4), 411. <https://doi.org/10.2307/2845569>
- Davis, T., & Kipling, D. (2005). Telomeres and telomerase biology in vertebrates: Progress towards a non-human model for replicative senescence and ageing. *Biogerontology*, 6(6), 371–385. <https://doi.org/10.1007/s10522-005-4901-4>
- Eisenberg, D. T. A., Hayes, M. G., & Kuzawa, C. W. (2012). Delayed paternal age of reproduction in humans is associated with longer telomeres across two generations of descendants. *Proceedings of the National Academy of Sciences*, 109(26), 10251–10256. <https://doi.org/10.1073/pnas.1202092109>
- Eisenberg, Dan T.A. (2011). An evolutionary review of human telomere biology: The thrifty telomere hypothesis and notes on potential adaptive paternal effects. *American Journal of Human Biology*, 23(2), 149–167. <https://doi.org/10.1002/ajhb.21127>
- Eisenberg, Dan T.A. (2016). Telomere length measurement validity: the coefficient of variation is invalid and cannot be used to compare quantitative polymerase chain reaction and Southern blot telomere length measurement techniques. *International Journal of Epidemiology*, (Cv), dyw191. <https://doi.org/10.1093/ije/dyw191>
- Eisenberg, Dan T.A., & Kuzawa, C. W. (2018). The paternal age at conception effect on offspring telomere length: Mechanistic, comparative and adaptive perspectives. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 373(1741). <https://doi.org/10.1098/rstb.2016.0442>
- Eisenberg, Dan T.A., Kuzawa, C. W., & Hayes, M. G. (2015). Improving qPCR telomere length assays: Controlling for well position effects increases statistical power. *American Journal of Human Biology*, 27(4), 570–575. <https://doi.org/10.1002/ajhb.22690>
- Fuentes, A. (2010). Naturalcultural encounters in bali: Monkeys, temples, tourists, and ethnoprimateology. *Cultural Anthropology*, 25(4), 600–624. <https://doi.org/10.1111/j.1548-1360.2010.01071.x>
- Fuentes, A., Kalchik, S., Gettler, L., Kwiat, A., Konecki, M., & Jones-Engel, L. (2008). Characterizing human-macaque interactions in Singapore. *American Journal of Primatology*, 70(8), 879–883. <https://doi.org/10.1002/ajp.20575>
- Fuentes, A., Southern, M. W., & Suarjana, K. (2005). Monkey forests and human landscapes: is extensive sympatry sustainable for *Homo sapiens* and *Macaca fascicularis* on Bali. *Commensalism and Conflict: The Human Primate Interface*, 168–195.
- García-Calzón, S., Zalba, G., Ruiz-Canela, M., Shivappa, N., Hébert, J. R., Martínez, J. A., ... Martí, A. (2015). Dietary inflammatory index and telomere length in subjects with a high cardiovascular disease risk from the PREDIMED-NAVARRA study: Cross-sectional and longitudinal analyses over 5 y. *American Journal of*

- Clinical Nutrition*, 102(4), 897–904. <https://doi.org/10.3945/ajcn.115.116863>
- 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 - Series A Biological Sciences and Medical Sciences*, 62(4), 367–374. <https://doi.org/10.1093/gerona/62.4.367>
- Gielen, M., Hageman, G. J., Antoniou, E. E., Nordfjall, K., Mangino, M., Balasubramanyam, M., ... Zeegers, M. P. (2018). Body mass index is negatively associated with telomere length: A collaborative cross-sectional meta-analysis of 87 observational studies. *American Journal of Clinical Nutrition*, 108(3), 453–475. <https://doi.org/10.1093/ajcn/nqy107>
- Grant, R., Watanabe, R., Kuller, L. H., Poland, B., Vinh, C., McLain, R., ... Anderson, D. (2005). Comparison of Wild-Type SRV in Indonesia and Singapore. *Journal of Medical Primatology*, 34, 332–333.
- Harley, C. B., Futcher, A. B., & Greider, C. W. (1990). Telomeres shorten during ageing of human fibroblasts. *Nature*, 345(6274), 458–460. <https://doi.org/10.1038/345458a0>
- Hausmann, M. F., & Mauck, R. A. (2008). Telomeres and longevity: Testing an evolutionary hypothesis. *Molecular Biology and Evolution*, 25(1), 220–228. <https://doi.org/10.1093/molbev/msm244>
- Hemann, M. T. (2000). Wild-derived inbred mouse strains have short telomeres. *Nucleic Acids Research*, 28(22), 4474–4478. <https://doi.org/10.1093/nar/28.22.4474>
- Horvath, K., Eisenberg, D., Stone, R., Anderson, J., Kark, J., & Aviv, A. (2019). Paternal Age and Transgenerational Telomere Length Maintenance: A Simulation Model. *Scientific Reports*, 9(1). <https://doi.org/10.1038/s41598-018-36923-x>
- Ilmonen, P., Kotrschal, A., & Penn, D. J. (2008). Telomere attrition due to infection. *PLoS ONE*, 3(5). <https://doi.org/10.1371/journal.pone.0002143>
- Jones-Engel, L., Steinkraus, K. A., Murray, S. M., Engel, G. A., Grant, R., Aggimarangsee, N., ... Linial, M. L. (2007). Sensitive Assays for Simian Foamy Viruses Reveal a High Prevalence of Infection in Commensal, Free-Ranging Asian Monkeys. *Journal of Virology*, 81(14), 7330–7337. <https://doi.org/10.1128/JVI.00343-07>
- Katona, P., & Apte, J. (2008). The Interaction between Nutrition and Infection. *Clinical Practice*, 46(5), 1582–1588. <https://doi.org/10.1086/587658>
- Klegarth, A.R., Sanders, S. A., Gloss, A. D., Lane-deGraaf, K. E., Jones-Engel, L., Fuentes, A., & Hollocher, H. (2017). Investigating biogeographic boundaries of the Sunda shelf: A phylogenetic analysis of two island populations of *Macaca fascicularis*. *American Journal of Physical Anthropology*, 163(4), 658–670. <https://doi.org/10.1002/ajpa.23235>
- Klegarth, Amy R., Hollocher, H., Jones-Engel, L., Shaw, E., Lee, B. P. Y. H., Feeney, T., ... Fuentes, A. (2017). Urban primate ranging patterns: GPS-collar deployments for *Macaca fascicularis* and *M. sylvanus*. *American Journal of Primatology*, 79(5). <https://doi.org/10.1002/ajp.22633>
- Koteja, P. S., Carter, P. A., Swallow, J. G., & Garland, T. (2003). Food wasting by house mice: Variation among individuals, families, and genetic lines. *Physiology*

- and Behavior, 80(2–3), 375–383. <https://doi.org/10.1016/j.physbeh.2003.09.001>
- Lane-deGraaf, K. E., Fuentes, A., & Hollocher, H. (2014). Landscape genetics reveal fine-scale boundaries in island populations of Indonesian long-tailed macaques. *Landscape Ecology*, 29(9), 1505–1519. <https://doi.org/10.1007/s10980-014-0069-0>
- Lane, K. E., Holley, C., Hollocher, H., & Fuentes, A. (2011). The anthropogenic environment lessens the intensity and prevalence of gastrointestinal parasites in Balinese long-tailed macaques (*Macaca fascicularis*). *Primates*, 52(2), 117–128. <https://doi.org/10.1007/s10329-010-0230-6>
- Lane, K. E., Lute, M., Rompis, A., Wandia, I. N., Putra, I. G. A. A., Hollocher, H., & Fuentes, A. (2010). Pests, pestilence, and people: the long-tailed macaque and its role in the cultural complexities of Bali. *Indonesian Primates, Developments in Primatology: Progress and Prospects*, 235–248. <https://doi.org/10.1007/978-1-4419-1560-3>
- Leo Galland, M. (2010). Diet and Inflammation. *Nutrition in Clinical Practice*, 25(6), 634–640.
- Lerche, N. W., Switzer, W. M., Yee, J. L., Shanmugam, V., Rosenthal, A. N., Chapman, L. E., ... Heneine, W. (2001). Evidence of Infection with Simian Type D Retrovirus in Persons Occupationally Exposed to Nonhuman Primates. *Journal of Virology*, 75(4), 1783–1789. <https://doi.org/10.1128/jvi.75.4.1783-1789.2001>
- Muezzinler, A., Zaineddin, A. K., & Brenner, H. (2014). Body mass index and leukocyte telomere length in adults: a systematic review and meta-analysis. *Obes.Rev.*, 15(1467-789X (Electronic)), 192–201.
- Mundstock, E., Sarria, E. E., Zatti, H., Mattos Louzada, F., Kich Grun, L., Herbert Jones, M., ... Mattiello, R. (2015). Effect of obesity on telomere length: Systematic review and meta-analysis. *Obesity*, 23(11), 2165–2174. <https://doi.org/10.1002/oby.21183>
- Muñoz, A., & Costa, M. (2013). Nutritionally mediated oxidative stress and inflammation. *Oxidative Medicine and Cellular Longevity*. <https://doi.org/10.1155/2013/610950>
- Nettle, D., Seeker, L., Nussey, D. H., Froy, H., & Bateson, M. (2018). Consequences of measurement error in qPCR telomere data: A simulation study. *BioRxiv*, 1–25. <https://doi.org/10.1101/491944>
- Nettleton, J. A. (2008). Dietary patterns, food groups, and telomere length in the Multi-Ethnic Study of Atherosclerosis (MESA)3. *Am J Clin Nutr.*, 28(30), 4439–4448. <https://doi.org/10.1037/a0013262>
- Prowse, K. R., & Greider, C. W. (1995). Developmental and tissue-specific regulation of mouse telomerase and telomere length. *Proceedings of the National Academy of Sciences*, 92(11), 4818–4822. <https://doi.org/10.1073/pnas.92.11.4818>
- R Development Core Team. (2019). R: A language and environment for statistical computing. Retrieved from <http://www.gbif.org/resource/81287>
- Rej, P. H., Tennyson, R. L., Lee, N. R., & Eisenberg, D. T. A. (2019). Years of caregiving for chronically ill and disabled family members is not associated with telomere length in the Philippines. *Psychoneuroendocrinology*, 103(October 2018), 188–194. <https://doi.org/10.1016/J.PSYNEUEN.2019.01.019>
- Riley, C. M., Jayasri, S. L., & Gumert, M. D. (2012). Singaporean long-tailed macaque

- (*Macaca fascicularis*) population census and status assessment: 2012. Report to the National Parks Board, Singapore.
- Riley, C. M., DuVall-Lash, A. S., Jayasri, S. L., Koenig, B. L., Klegarth, A. R., & Gumert, M. D. (2016). How Living Near Humans Affects Singapore's Urban Macaques. In M. T. Waller (Ed.), *Ethnoprimateology, Developments in Primatology: Progress and Prospects* (pp. 283–300). Springer International Publishing. https://doi.org/10.1007/978-3-319-30469-4_16
- Riley, C. M., Jayasri, S. L., & Gumert, M. D. (2015). Results of a nationwide census of the long-tailed macaque (*Macaca fascicularis*) population of singapore. *Raffles Bulletin of Zoology*, 63, 503–515.
- Schillaci, M. A., Engel, G. A., Fuentes, A., Rompis, A., Putra, A., Wandia, I. N., ... Jones-Engel, L. (2010). The Not-So-Sacred Monkeys of Bali: A Radiographic Study of Human-Primate Commensalism Indonesian Primates. *Indonesian Primates: Developments in Primatology. Progress and Prospects*, 249–256. <https://doi.org/10.1007/978-1-4419-1560-3>
- Schillaci, M. A., Jones-Engel, L., Lee, B. P. Y. H., Fuentes, A., Aggimarangsee, N., Engel, G. A., & Sutthipat, T. (2007). Morphology and somatometric growth of long-tailed macaques (*Macaca fascicularis fascicularis*) in Singapore. *Biological Journal of the Linnean Society*, 92(4), 675–694. <https://doi.org/10.1111/j.1095-8312.2007.00860.x>
- Steinert, S., White, D. M., Zou, Y., Shay, J. W., & Wright, W. E. (2002). Telomere biology and cellular aging in nonhuman primate cells. *Experimental Cell Research*, 272(2), 146–152. <https://doi.org/10.1006/excr.2001.5409>
- Stone, R. C., Horvath, K., Kark, J. D., Susser, E., Tishkoff, S. A., & Aviv, A. (2016). Telomere Length and the Cancer–Atherosclerosis Trade-Off. *PLOS Genetics*, 12(7), e1006144. <https://doi.org/10.1371/journal.pgen.1006144>
- Vaupel, J. W. (2010). Biodemography of human ageing. *Nature*, 464(7288), 536–542. <https://doi.org/10.1038/nature08984>
- 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(5), 1683–1686. <https://doi.org/10.1093/ije/dyv166>
- Von Zglinicki, T. (2002). Oxidative stress shortens telomeres. *Trends in Biochemical Sciences*, 27(7), 339–344. [https://doi.org/10.1016/S0968-0004\(02\)02110-2](https://doi.org/10.1016/S0968-0004(02)02110-2)
- Wheatley, B. P. (1999). The sacred monkeys of Bali, 189 p.
- Zhu, X., Han, W., Xue, W., Zou, Y., Xie, C., Du, J., & Jin, G. (2016). The association between telomere length and cancer risk in population studies. *Scientific Reports*, 6, 1–10. <https://doi.org/10.1038/srep22243>
- Zimmermann, E., & Radespiel, U. (2013). Primate Life Histories. In: Henke W., Tattersall I. (eds) .Springer, Berlin, Heidelberg. In W. Henke & I. Tattersall (Eds.), *Handbook of Paleoanthropology*. Springer Berlin Heidelberg. https://doi.org/https://doi.org/10.1007/978-3-642-27800-6_38-7

DATA ACCESSIBILITY

634 All data are freely available on the Open Science Framework public database [DOI to
635 be entered upon acceptance of manuscript]. The URL to access this data is [URL to
636 be entered upon acceptance of manuscript].

637

638 **AUTHOR CONTRIBUTIONS**

639 PHR – designed and conducted analysis, generated telomere length data, co-wrote
640 the paper; ARK – co-conceptualized the study, acquired funding, co-wrote the paper;
641 CMRK – collected data for Singapore animals and contributed to writing; MDG –
642 oversaw data collection for Singapore animals and contributed to writing; LJE – co-
643 conceptualized the study, acquired funding, collected data for Bali and Singapore
644 animals, and contributed to writing; DTAE – co-conceptualized the study, acquired
645 funding, supervised telomere length data generation, co-wrote the paper.