Estimation of environmental and genetic contributions to telomere length variation in a wild mammal

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Abstract

Understanding individual variation in fitness-related traits requires separating the environmental and genetic determinants. Telomeres are protective caps at the ends of chromosomes that are thought to be a biomarker of senescence as their length predicts mortality risk and reflect the physiological consequences of environmental conditions. The relative contribution of genetic and environmental factors to individual variation in telomere length is however unclear, yet important for understanding its evolutionary dynamics. In particular, the evidence for transgenerational effects, in terms of parental age at conception, on telomere length is mixed. Here, we investigate the heritability of telomere length, using the 'animal model', and parental age at conception effects on offspring telomere length in a wild population of European badgers (*Meles meles*). While we found no heritability of telomere length, our power to detect heritability was low and a repeatability of 2%

across individual lifetimes provides a low upper limit to ordinary heritability. However, year (25%) and cohort (3%) explained greater proportions of the phenotypic variance in telomere length. There was no support for parental age at conception effects, or for longitudinal within-parental age effects on offspring telomere length. Our results indicate a lack of transgenerational effects through parental age at conception and a low potential for evolutionary change in telomere length in this population. Instead, we provide evidence that individual variation in telomere length is largely driven by environmental variation in this wild mammal.

Keywords: Telomere length, heritability, parental age at conception, senescence, wild mammal

1. Introduction

The extrinsic environment can have individual-specific effects on physiology, which are key to variation in fitness (Lindstrom, 1999), life-history strategies (Metcalfe & Monaghan, 2001) and senescence patterns (Nussey, Kruuk, Morris, & Clutton-Brock, 2007). However, in wild populations it is challenging to quantify how the extrinsic environment affects physiology. Consequently, biomarkers reflecting how such physiological costs are related to fitness are required. The forces of natural selection acting on the heritability of such a biomarker (the proportion of phenotypic variance explained by additive genetic variance), can describe its evolutionary potential (Lynch & Walsh, 1998; Charmantier, Brommer, & Nussey, 2014). It is therefore important to separate environmental and genetic components that contribute to individual variation in fitness-related traits in order to understand the evolution of such traits (Charmantier et al., 2014; Wilson, Charmantier, & Hadfield, 2008; Nussey, Froy, Lemaitre, Gaillard, & Austad, 2013).

Telomeres are a biomarker of senescence in some species (Monaghan & Haussmann, 2006), and understanding the heritability of telomere length may provide insight into the evolution of senescence (Dugdale & Richardson, 2018). In addition, telomeres can quantify the physiological costs incurred by environmental conditions (Monaghan, 2014). Telomeres are repetitive non-coding

sequences (5'-TTAGGG-3') at the ends of eukaryotic chromosomes that, along with shelterin proteins, maintain genomic integrity and prevent end-to-end fusion of linear chromosomes (Blackburn, 1991). Due to the end-replication problem, telomeres shorten with each cell division (Olovnikov, 1973). Telomere shortening can, however, be accelerated by adverse environmental conditions (e.g. Boonekamp, Mulder, Salomons, Dijkstra, & Verhulst, 2014; Nettle et al., 2015) and metabolically demanding activities (Heidinger et al., 2012; Epel et al., 2004). *In vitro* evidence shows that oxidative damage contributes to telomere shortening (von Zglinicki, 2002), but there is no evidence for such effects *in vivo* (Reichert & Stier, 2017; Boonekamp, 2017). Telomeres can also be restored by telomerase, although this enzyme is transcriptionally repressed after initial development (Blackburn et al., 1989). However, alternative telomere lengthening pathways exist (Cesare & Reddel, 2010; Mendez-Bermudez et al., 2012). Critically short telomeres can result in replicative senescence, where accumulation of senescent cells can impair tissue functioning (Armanios & Blackburn, 2012; Campisi, 2005) and may lead to organismal senescence (Young, 2018).

Individual variation in telomere length occurs in wild populations (Fairlie et al., 2016; Spurgin et al., 2017; van Lieshout et al., 2019) which is linked to individual life-histories (Wilbourn et al., 2018). Understanding the degree to which individual variation in telomere length is due to genetic and environmental effects, in addition to the strength of natural selection acting on telomere length, allows estimation of the potential for evolutionary change (Lynch & Walsh, 1998; Charmantier et al., 2014). Heritability of telomere length has been estimated in over seven wild species and in >26 studies in humans (see Table 1 in Dugdale & Richardson, 2018). These studies primarily used parent–offspring regressions to determine the heritability of telomere length, with estimates ranging from 0 to 1. The majority, however, of these heritability estimates were relatively high, which is unexpected given that heritabilities of traits closely related to fitness are often low (Price & Schluter, 1991; Postma, 2014; Mousseau & Roff, 1987). However, parents and offspring often live in similar environments, and parent–offspring regressions are frequently confounded by these 'shared environment' effects, which can inflate heritability estimates (Kruuk, 2004).

The 'animal model' provides a statistical approach that can overcome the drawbacks of parent–offspring regressions because it allows partitioning of variance components into additive genetic and shared environment sources (Kruuk & Hadfield, 2007; Wilson et al., 2010). Because heritability is the proportion of phenotypic variation due to additive genetic variance, any changes to the amount of environmental variation will impact heritability estimates, even if the additive genetic variance does not itself change (Kruuk & Hadfield, 2007; Dugdale & Richardson, 2018). Environmental effects (e.g. Boonekamp et al., 2014; Nettle et al., 2015) therefore need to be accounted for to derive accurate heritability estimates (Dugdale & Richardson, 2018). The 'animal model' is a mixed-effects model that uses either the expected proportion of the genome that individuals share by descent (from a pedigree) or by state (from genomic data) to partition phenotypic variance into environmental and genetic components (Wilson et al., 2010). The two studies applying an animal model approach in wild populations of non-human vertebrates found no heritability of telomere length in white-throated dippers (*Cinclus cinclus*; 0.007 ± 0.013 SE; Becker et al., 2015), but high heritability in great reed warblers (*Acrocephalus arundinaceus*; 0.480 ± 0.120 SE; Asghar, Bensch, Tarka, Hansson, & Hasselquist, 2015). However, although these were pioneering studies, the sample sizes were relatively low for quantitative genetic analyses and the power to detect heritability was not stated. Additionally, neither study had repeated measures to estimate permanent environment effects, which may inflate additive genetic effects (Kruuk & Hadfield, 2007). More studies in wild populations, and from a wider range of taxa, with larger sample sizes and repeated measures, are required to distentangle the genetic and environmental contributions to variation in telomere length.

The influence of environmental conditions on variation in telomere length is not only important to account for statistically, but informs about which environmental factors shape individual telomere length. Previous studies have shown that cohort (Hall et al., 2004; Watson, Bolton, & Monaghan, 2015; Fairlie et al., 2016), year (Mizutani, Tomita, Niizuma, & Yoda, 2013; Wilbourn et al., 2017), social group (Cram, Monaghan, Gillespie, & Clutton-Brock, 2017; Boonekamp et al., 2014; Nettle et al., 2015) and parental effects (Asghar et al., 2015; Cram et al., 2017) affect individual

105 telomere length. Understanding the relative contribution of these different sources of environmental
106 variation on telomere length sheds light on its evolution.

107 In addition to these environmental and additive genetic effects, offspring telomere length may
108 also be influenced by paternal age at conception (PAC) according to two mutually non-exclusive
109 hypotheses. First, to compensate for telomere loss due to sperm production and progressive cell
110 replication, telomerase activity in germ stem cells is high. Telomerase expression might, beyond
111 restoring telomere length, overcompensate and result in elongation of telomeres in germ stem cells
112 (Kimura et al., 2008; Aviv & Susser, 2013). Second, stem cells with longer telomeres are better able to
113 withstand repeated cell replication and therefore may become predominant in the stem cell pool with
114 age due to the selective loss of germ stem cells with shorter telomeres (Kimura et al., 2008; Hjelmborg
115 et al., 2015). In humans, there is cross-sectional evidence that older men produce sperm with longer
116 telomeres (r = 0.127 – 0.160; Aston et al., 2012; de Meyer et al., 2007; Kimura et al., 2008; Nordfjall,
117 Svenson, Norrback, Adolfsson, & Roos, 2010).

118 The evidence for a positive cross-sectional PAC effect is even stronger in captive chimpanzees
119 (*Pan troglodytes*; r = 0.378) compared to humans (Eisenberg, Tackney, Cawthon, Cloutier, & Hawkes,
120 2017). An explanation for this stronger effect is that chimpanzees have relatively larger testes and
121 higher rates of sperm production than humans, due to their more promiscuous mating system
122 (Birkhead & Møller, 1998). Stronger sperm competition could therefore result in the PAC effect,
123 because stronger postcopulatory competition should select for high quality sperm to be produced at
124 a fast rate (Eisenberg et al., 2017). We would therefore expect that species with high levels of sperm
125 competition and high rates of sperm production, such as in polygynandrous species, should show the
126 strongest PAC effect.

127 PAC effects are often confounded with maternal age at conception (MAC), as these are
128 typically highly correlated in human populations (Table 1 in Froy et al., 2017). The presence of MAC
129 effects in humans is generally considered to be due to the correlation with PAC instead of a true
130 independent biological effect (de Meyer et al., 2007; Kimura et al., 2008), because oocytes are

produced prenatally, while sperm is produced throughout life (Eisenberg & Kuzawa, 2018). Additionally, parental age effects on fitness may be sex-specific (Bouwhuis, Vedder, & Becker, 2015). For example, male sparrows with older fathers, or females with older mothers, had lower lifetime reproductive success than sparrows with younger same-sex parents, and a hypothesised potential mechanism is sex-specific telomere shortening (Schroeder, Nakagawa, Rees, Mannarelli, & Burke, 2015), although this was not the case in common terns (Bouwhuis, Verhulst, Bauch, & Vedder, 2018). Sex-specific parental age at conception effects may therefore be present, but are rarely tested in wild populations.

Studies in wild populations have provided mixed evidence for PAC and MAC effects. Studies from different taxa, with a variety of mating systems, have shown a negative PAC effect (Bouwhuis et al., 2018; Criscuolo, Zahn, & Bize, 2017; Olsson et al., 2011), including a longitudinal (Bauch, Boonekamp, Korsten, Mulder, & Verhulst, 2019) and an experimental manipulation (Noguera, Metcalfe, & Monaghan, 2018) study. However, other studies have reported no PAC or MAC effect on offspring telomere length (Heidinger et al., 2016; McLennan et al., 2018; Froy et al., 2017; Belmaker, Hallinger, Glynn, Winkler, & Haussmann, 2019) or a positive MAC effect (Asghar et al., 2015). The variation in PAC and MAC effects on offspring telomere length among species requires more studies to disentangle potential causes and mechanisms underlying such variation in transgenerational effects.

Here, we investigate PAC and MAC effects and the heritability of telomere length in polygynandrous European badgers (*Meles meles*; henceforth 'badgers'). Individual variation in badger telomere length in early-life (<1 year old), but not adult life, is associated with survival probability (van Lieshout et al., 2019). However, a low heritability is expected, as within-individual repeatability in telomere length is very low (0.022, 95% CI = 0.001 – 0.103; van Lieshout et al., 2019). While this sets the upper limit for ordinary heritability (Bijma, 2011), understanding the relative importance of environmental (i.e. cohort, year, social group, maternal and paternal effects) and additive genetic variance components is important to understand the evolution of telomere length. Badgers respond

to year-specific weather variation which affects their behaviour, physiology and fitness (Macdonald, Newman, Buesching, & Nouvellet, 2010; Nouvellet, Newman, Buesching, & Macdonald, 2013; Noonan et al., 2014; Bilham et al., 2018) and because they are group-living, they may be impacted by social group attributes (Woodroffe & Macdonald, 2000; Beirne, Delahay, & Young, 2015). Cubs are born in February, which is followed by a post-partum mating peak after which matings can occur throughout the year (Macdonald, Newman, & Buesching, 2015). Badgers are highly promiscuous, which may promote sperm competition (Dugdale, Griffiths, & Macdonald, 2011a). However, female badgers are induced ovulators thus requiring long matings (Yamaguchi, Dugdale, & Macdonald, 2006), and associated testes ascendence in males in autumn/winter (Woodroffe & Macdonald, 1995), lead to reduced sperm production rates (Sugianto, Newman, Macdonald, & Buesching, 2019) that may reduce the potential for transgenerational effects (i.e. PAC/MAC effects) on offspring telomere length.

We therefore test for: (i) sex-specific and longitudinal PAC and MAC effects on offspring relative leukocyte telomere length (RLTL), after assessing whether PAC and MAC are correlated; and (ii) the proportion of variance in juvenile RLTL (≤29 months old) and RLTL across individual lifetimes, that is explained by additive genetic and environmental effects.

2. Methods

2.1 *Study system*

We conducted this study in Wytham Woods, Oxfordshire, UK (51°46'24"N, 1°20'04"W), a 424 ha mixed semi-natural woodland site surrounded by mixed arable and permanent pasture (Macdonald & Newman, 2002; Macdonald, Newman, Dean, Buesching, & Johnson, 2004). The resident badger population forms an almost closed population (immigration/emigration <3%; Macdonald & Newman, 2002). Badgers live in social groups with a mean of 11.3 individuals (range = 2 – 29; da Silva, Macdonald, & Evans, 1994) and a mean number of 19 social groups (95% CI = 17 – 21; range = 14 – 26; Dugdale, Macdonald, Pope, Johnson, & Burke, 2008) in the population between 1987–2010. Cohort-dependent cub survival probability varies from 0.61 to 0.94 (mean ± SE = 0.67 ± 0.03;

183 Macdonald, Newman, Nouvellet, & Buesching, 2009), whereas mean annual adult survival probability
184 in the population is 0.83 (± 0.01 SE; Macdonald et al., 2009) with a mean lifespan of 3.31 years (± 3.51
185 SD; Bright Ross, J., Pers. Comm.).
186 Trapping sessions were conducted three or four times per year over two weeks in May–June
187 (Spring), August–September (Summer) and November (Autumn), with trapping in January (Winter) in
188 focal years, for two to three consecutive days per social group. Trapped badgers were anaesthetised
189 using an intra-muscular injection of 0.2 ml ketamine hydrochloride per kg body weight (McLaren et
190 al., 2005). Badgers were identified by a unique tattoo number on the left inguinal region. Sex, age
191 class, sett (group den system), social group and capture date were recorded for each badger. Badgers
192 were aged by the number of days elapsed since the 14th of February in the respective birth year
193 (Yamaguchi et al., 2006). Individuals first caught as adults were aged through tooth wear, where tooth
194 wear 2 indicates a 1-year old adult (van Lieshout et al., 2019). Blood was collected by jugular
195 venipuncture into vacutainers with an EDTA anticoagulant and stored at -20°C immediately. Badgers
196 were released at their setts, after full recovery from anaesthesia.
197
198 2.2 *Molecular analyses*
199 We extracted genomic DNA from whole blood samples (*n* = 1248 samples; 612 badgers) using the
200 DNeasy Blood & Tissue kit (Qiagen, Manchester, UK) according to the manufacturer's protocol, with
201 modifications by conducting a double elution step (2x 75 µl AE buffer) and using 125 µl of
202 anticoagulated blood. We checked DNA integrity by running a random selection of DNA extracts (ca.
203 20%) on agarose gels to ensure high molecular weight. DNA concentration of all samples was
204 quantified using the Fluostar Optima fluorometer (BMG Labtech, Ortenberg, Germany) and
205 standardized to 20 ng/µl, after which samples were stored at -20 °C. We used monochrome multiplex
206 quantitative PCR (MMqPCR) analysis to measure RLTL (Cawthon, 2009). This measure is the
207 abundance of telomeric sequence relative to a reference gene, which are both analysed in the same

well, and represents the mean telomere length across cells in a sample. A detailed description of the MMqPCR analysis can be found in van Lieshout et al. (2019).

2.3 *Pedigree*

The pedigree was constructed using DNA extracted from blood or guard hair samples, genotyped for 35 microsatellite loci (Dugdale, Macdonald, Pope, & Burke, 2007; Annavi et al., 2014a), and *MasterBayes* 2.47 (Hadfield, 2010). The pruned pedigree (which excludes non-informative individuals) contained 753 unique individuals, from 7 generations, trapped between 1987 and 2010 (Table S1).

2.4 *Statistical analyses*

2.4.1 *PAC and MAC effects*

Statistical analyses were conducted in R 3.3.1 (R Development Core Team, 2019). PAC and MAC effects were analysed in general linear mixed models (GLMMs), with RLTL measurements square-root transformed to meet assumptions of Gaussian error distributions and subsequently turned into Z-scores (Verhulst, 2020). We checked fixed effects for collinearity through variance inflation factors (VIF < 3).

We first determined the correlation between PAC and MAC to investigate whether analyses for PAC and MAC effects needed to be conducted separately. There were 471 RLTL measurements from 240 offspring (121 females and 119 males; with 108 unique fathers and 120 unique mothers) where MAC and PAC were known. PAC and MAC both spanned ages 1–12 years and there was a weak positive correlation between PAC and MAC (Pearson's $r = 0.160$, $P < 0.001$; Figure S1), allowing for PAC and MAC effects to be tested in the same model.

The effects of PAC and MAC on offspring RLTL were subsequently tested using linear mixed effect models in *lme4* 1.1–14 (Bates, Machler, Bolker, & Walker, 2015). The model included fixed covariates for the best-fitting age relationship with RLTL, which was a threshold model (van Lieshout et al., 2019), and a fixed factor for season. Individual ID, cohort, year, qPCR plate, row on qPCR plate,

maternal ID, paternal ID and social group were included as random effects. MAC and PAC were added to this model as fixed effects, and their interaction with sex, where significance was tested using likelihood ratio tests (n = 471 measurements; 240 badgers). Based on our dataset and model structure, we have 80% statistical power to detect a PAC effect of 0.00067 or greater (Figure S2) using a simulation-based power analysis in *simr* 1.0.5 (Green & MacLeod, 2016). This is equivalent to a correlation coefficient of 0.131 or greater (with the PAC effect size multiplied by its standard deviation and divided by the standard deviation of RLTL), providing statistical power to detect correlation coefficients found previously in humans (r = 0.127–0.160; de Meyer et al., 2007; Eisenberg et al., 2017; Nordfjall et al., 2010) and chimpanzees (r = 0.378; Eisenberg et al., 2017). Additional models were run, where only offspring RLTL measurements from cubs (<1 year old) were included, to ensure the inclusion of adults did not mask effects of PAC or MAC. There were 194 measurements from 194 cubs (94 females, 100 males) that had 97 unique fathers and 109 unique mothers. The cub model was similar to the full model, but did not include random effects for individual ID (i.e. no repeat measures) and year (i.e. equivalent to cohort). We then separated, including all offspring RLTL measurements, within- from between-parental effects (n = 441 measurements; 210 badgers) for each parent to test for longitudinal PAC and MAC effects, by taking the mean age at reproduction for each parent (between-parent effect) and subtracting this mean from each of the ages at reproduction of the parent (within-parent effect; van de Pol & Wright, 2009).

2.4.2 *Partitioning variance in RLTL*

We determined the relative contribution of environmental and genetic components to variation in RLTL with a quantitative genetic 'animal model', using pedigree relatedness based on parent-offspring assignments (n = 1248 measurements; 612 badgers). We had 80% power to detect a heritability of RLTL of ≥0.27 (Figure S3), estimated using *pedantics* 1.7 (Morrissey & Wilson, 2010). We used a stepwise addition approach to facilitate the detection of confounding random effects (Charmantier et al., 2014), while estimating the changes in heritability in response to addition of random effects.

Additionally, we present results without fixed effects, as random effects are conditioned on the fixed effects (Wilson, 2008). We used *MCMCglmm* 2.25 (Hadfield 2010), with the number of iterations set to 600,000, a thinning of 300 and burn-in period of 15,000 iterations. The response variable was untransformed RLTL to gain variance estimates on the scale the trait was measured on (de Villemereuil, Schielzeth, Nakagawa, & Morrissey, 2016); only a square-root tranformation of RLTL met Gaussian assumptions, however, a square-root link is not available in *MCMCglmm*. Three thresholds of age at measurement (van Lieshout et al., 2019) were included as fixed covariates and season as a fixed factor. The random effects included: additive genetic, permanent environment (to account for environmental and non-additive genetic between-individual variation), parental effects (mother and father ID), year effects (cohort and capture years), resident social group, and measurement effects (qPCR plate and row, to account for variance generated during the laboratory analysis). We present results with qPCR plate and row included and excluded from the total phenotypic variance when calculating heritability, since qPCR plate and row represent technical, not biological, variance (de Villemereuil, Morrissey, Nakagawa, & Schielzeth, 2018).

Since badgers exhibit increases as well as decreases in RLTL in later life, and juvenile RLTL (≤29 months old) does not vary with age cross-sectionally (van Lieshout et al., 2019), we also estimated variance components and heritability just using a dataset of juvenille RLTL (≤29 months old; n = 837 measurements; 556 badgers). We had 80% power to detect a heritability of ≥0.28 (Figure S4). The random effects were the same as in the full dataset. For the fixed effects the difference was that age was included as a linear covariate rather than a threshold model (as the first threshold is at 29 months; van Lieshout et al., 2019).

For random effects we used parameter expanded priors (F distribution: V = 1, nu = 1, alpha.mu = 0, alpha.V = 1,000) since variance components were close to zero. Model convergence was checked through low autocorrelation between successive thinned samples (<0.1), Heidelberg and Welch's diagnostics (tests if samples are drawn from stationary distribution), Geweke diagnostic (equality of means of first 10% and last 50% of Markov chain), and whether the effective size was >1000 for both

fixed and variance components. Fixed effects were considered significant if the 95% credibility intervals of the posterior mode did not overlap zero.

We also conducted a frequentist analysis in *ASReml-R* 3 using the same model structure to determine the robustness of our variance component estimates. In *ASReml-R*, the significance of fixed effects was determined through Wald Z tests, whereas significance of random effects was determined through twice the difference in log-likelihood (Visscher, 2006).

3. Results

Neither MAC nor PAC showed an overall, or sex-specific, association with variation in offspring RLTL at any age (Figure 1a & 1b, respectively), or as cubs (Figure 1c & 1d, respectively; Table S2). Additionally, within- and between-parental age at conception effects for each parent were not linked to variation in offspring RLTL (Table S2).

The additive genetic variance explained near zero of the total phenotypic variance in RLTL (Table S3, Models 1–9). Heritability (h^2) was < 0.001 (95% CrI = <0.001–0.026) with qPCR plate and row variance included in the phenotypic variance (Table S3, Model 7) and 0.001 (95% CrI = <0.001–0.028) when qPCR plate and row variance were excluded (Table S3, Model 8). In contrast, year (0.251, 95% CrI = 0.143–0.459) and cohort (0.030, 95% CrI = 0.007–0.074) explained a greater proportion of the phenotypic variance in RLTL (Figure 2; Table S3, Model 7). Social group (<0.001, 95% CrI = <0.001–0.014), paternal (<0.001, 95% CrI = <0.001–0.025) and maternal (<0.001, 95% CrI = <0.001–0.030) effects explained near zero variance in RLTL (Figure 2; Table S3, Model 7).

There was also no detectable heritability of juvenile RLTL (≤29 months old; h^2 <0.001, 95% CrI = <0.001–0.043), moderate year (0.216, 95% CrI = 0.107–0.431) and small cohort (0.037, 95% CrI = 0.003–0.123) effects, and no detectable social group (<0.001, 95% CrI = <0.001–0.020), paternal (<0.001, 95% CrI = <0.001–0.026) or maternal (<0.001, 95% CrI = <0.001–0.032) effects (Table S3, Model 9).

A frequentist approach in *ASReml–R* showed similar results with additive genetic variance explaining near zero of the phenotypic variance, but with cohort and year effects explaining variation in RLTL (Table S4 & S5).

4. Discussion

Our study found no evidence for PAC or MAC associations with offspring RLTL in the European badger. For primates there is extensive evidence for a positive PAC effect (e.g. Njajou et al., 2007; Eisenberg et al., 2017; Kimura et al., 2008). However, previous studies in non-primate vertebrates have reported a variety of relationships between offspring telomere length and PAC or MAC (e.g. Olsson et al., 2011; Asghar et al., 2015; Heidinger et al., 2016; McLennan et al., 2018; Bouwhuis et al., 2018). Only one study has investigated PAC and MAC effects in a non-primate mammal. Soay sheep, which also have a promiscuous mating system with likely stronger sperm competition than in badgers (Preston, Stevenson, Pemberton, Coltman, & Wilson, 2003), also found no relationship between offspring RLTL (either measured across all ages or only as lambs) and PAC or MAC (Froy et al., 2017). Our results add to the growing literature of mixed PAC/MAC results in wild populations (Olsson et al., 2011; Heidinger et al., 2016; Eisenberg et al., 2017; Njajou et al., 2007; McLennan et al., 2018), despite positive PAC effects in humans and chimpanzees (Njajou et al., 2007; Eisenberg et al., 2017; Kimura et al., 2008). Variation in PAC and MAC effects among species may be due to differences in mating systems and associated sperm production rates (Bouwhuis et al., 2018). Additionally, variation may be present but masked by sex-specific effects on offspring, however, we tested for but did not detect these.

Counter to our expectation for a highly promiscuous species that exhibits multiple and repetitive mounting behaviour (Dugdale et al., 2007; Dugdale et al., 2011a), we found no PAC effect, for which there are several potential reasons. First, telomerase activity may be more tightly regulated, or even lower, in the germline in badgers. However, while we know telomerase activity varies among tissue types and species (Davis & Kipling, 2005; Gomes et al., 2011), we require a better understanding of telomerase activity in species with different mating systems to validate this hypothesis. Secondly,

337 higher sperm competiton may reduce the variability in RLTL among germ stem cells, negating selection
338 for germ stem cells with longer telomeres at older ages and therefore longer offspring RLTL (Kimura
339 et al., 2008). Thirdly, female badgers exhibit various postcopulatory mechanisms (i.e. embryonic
340 diapause, superfetation, superfecundation) which may obscure the relationship between PAC or MAC
341 and offspring RLTL. While replication is suppressed during embryonic diapause, maternal stress could
342 impact offspring RLTL through glucocorticoids (Haussmann, Longenecker, Marchetto, Juliano, &
343 Bowden, 2012; Angelier, Costantini, Blevin, & Chastel, 2018; Yamaguchi et al., 2006) or superfetation
344 could result in less exposure of the fertilised egg to maternal glucocorticoids. However, the effects of
345 these postcopulatory mechanisms on PAC and MAC effects are difficult to quantify. Finally, badgers
346 have a much lower life expectancy than humans and chimpanzees (Macdonald & Newman, 2002), as
347 do Soay sheep (Froy et al., 2017). While reproductive senescence is observed in both sexes (Dugdale,
348 Pope, Newman, Macdonald, & Burke, 2011b), the effects of telomere elongation in sperm may not
349 become apparent due to the shorter life expectancy, compared to humans and chimpanzees. Even
350 though in male badgers the testes ascend in autumn with no spermatogenesis (Sugianto et al., 2019),
351 sperm production is likely highest in the peak mating season immediately after parturition (Macdonald
352 et al., 2015). Despite the high potential for sperm competition in this species, seasonal mating peaks
353 in badgers may explain the lack of a PAC effect through the lack of continuity and rate of sperm
354 production in badgers, as recently hypothesised in Bouwhuis et al. (2018). PAC and MAC effects are
355 less consistent in wild populations than in humans, and the underlying mechanisms may entail more
356 than just the degree of promiscuity in a system.

357 While our study reveals no heritability of RLTL, we did not have the statistical power to detect
358 heritability of RLTL <0.27. The low power may be attributable to the pedigree structure, in terms of a
359 realtively low number of full-sibs (Table S1), due to high extra-group paternity in badgers (Dugdale et
360 al., 2007; Annavi et al., 2014b), and a low mean pairwise relatedness (Table S1). Given that variance
361 in RLTL explained by individual was very low at 2%, which forms the upper limit to ordinary heritability,
362 the contribution of additive genetic variance to total phenotypic variance in RLTL in this wild mammal

363 population is low. The low heritability of RLTL is consistent with low heritability of fitness-related traits
364 in other species (Kruuk et al., 2000; Teplitsky, Mills, Yarrall, & Merila, 2009). We have previously
365 identified associations between early-life RLTL (<1 year old) and survival probability in this species (van
366 Lieshout et al., 2019), so selection may have eroded genetic variation underlying RLTL in this
367 population (Price & Schluter, 1991; Postma, 2014; Mousseau & Roff, 1987).

368 Partitioning of variation in RLTL in badgers into genetic and environmental factors showed
369 that variation in RLTL was largely driven by environmental variation. Of the environmental factors
370 investigated, we found no evidence for social group, maternal or paternal effects explaining variation
371 in RLTL. Even though nest or social group (Nettle et al., 2015; Boonekamp et al., 2014; Cram et al.,
372 2017; Becker et al., 2015) and maternal effects (Asghar et al., 2015) have been important for telomere
373 length in other species, this is not the case for our badger population. Badgers provide neonatal care
374 up to independence at around 14–16 weeks (Fell, Buesching, & Macdonald, 2006; Dugdale, Ellwood,
375 & Macdonald, 2010), and we therefore cannot capture badgers until at least 3 months of age
376 (Protection of Badgers Act, 1992). As the strength of maternal effects on offspring decline with the
377 age of the offspring (Moore, Whiteman, & Martin, 2019), maternal effects explaining variation in
378 offspring RLTL will be more difficult to detect. In contrast, we found that variation in RLTL was
379 explained by a small cohort and moderate year effects.

380 The small effect of cohort on RLTL is in accordance with previous studies in mammals and
381 birds which had shorter telomeres, or accelerated telomere shortening, when subject to sub-optimal
382 natal conditions (Hall et al., 2004; Nettle et al., 2015; Watson et al., 2015; Fairlie et al., 2016). However,
383 the variance explained by the year in which the individual was captured was about eight times greater
384 than the cohort effect, even though we could not separate cohort and year effects for 163 badgers
385 since they died as cubs. Although we cannot identify the specific drivers of the association between
386 year and variation in RLTL, badgers are sensitive to annual weather variation (Nouvellet et al., 2013;
387 Macdonald et al., 2010), which affects their food availability, and can lead to elevated levels of
388 oxidative stress (Bilham et al., 2018). Additionally, exposure to diseases may vary among years and

could contribute to variation in RLTL (Newman, Macdonald, & Anwar, 2001; Sin et al., 2014). Furthermore, the size of the extant population varied by a factor of almost three-fold over the study interval (with no change in range), causing substantial inter-annual variation in population density (Macdonald & Newman, 2002; Macdonald et al., 2009) that could lead to RLTL variation in badgers.

Since an evolutionary response depends on the magnitude of both natural selection and the heritability of the trait (Kruuk 2004; Lynch & Walsh 1998), the evolutionary potential of telomere length, in this badger population, appears to be low. Instead, variation in badger RLTL is largely driven by non-additive genetic sources such as variation between cohorts and years. Further research is required to understand which and how specific environmental and social factors impact an individual's physiology and contribute to variation in RLTL.

Ethics

All work was approved by the University of Oxford's Animal Welfare and Ethical Review Board, ratified by the University of Leeds, and carried out under Natural England Licenses, currently 2017-27589-SCI-SCI and Home Office Licence (Animals, Scientific Procedures, Act, 1986) PPL: 30/3379.

Acknowledgements

We thank all members of the Wytham badger team, past and present, for their help in data collection. We also thank Geetha Annavi for her help with the pedigree and Natalie dos Remedios and Mirre Simons for their help and advice on telomere analyses. S.H.J.v.L. was funded by a Leeds Anniversary Research Scholarship from the University of Leeds with support of a Heredity Fieldwork Grant from the Genetics Society and a Priestley Centre Climate Bursary from the University of Leeds. Telomere length analyses were funded by a Natural Environment Research Council (NERC) Biomolecular Analysis Facility – Sheffield, grant to H.L.D. and A.B. (NBAF984) and a Royal Society Research Grant to H.L.D. (RG170425).

Authors' contributions

The study was conceived by S.H.J.v.L., A.B. and H.L.D., and developed by A.M.S.; Samples were collected by S.H.J.v.L., C.N., C.D.B., D.W.M. and H.L.D.; S.H.J.v.L. conducted laboratory work with advise from T.B. and statistical analyses with input from A.M.S. and H.L.D.; The paper was written by S.H.J.v.L. and H.L.D. and all authors contributed critically and gave final approval for publication.

Data accessibility

Data will be deposited in the Dryad Digital Repository upon acceptance.

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Figures and tables

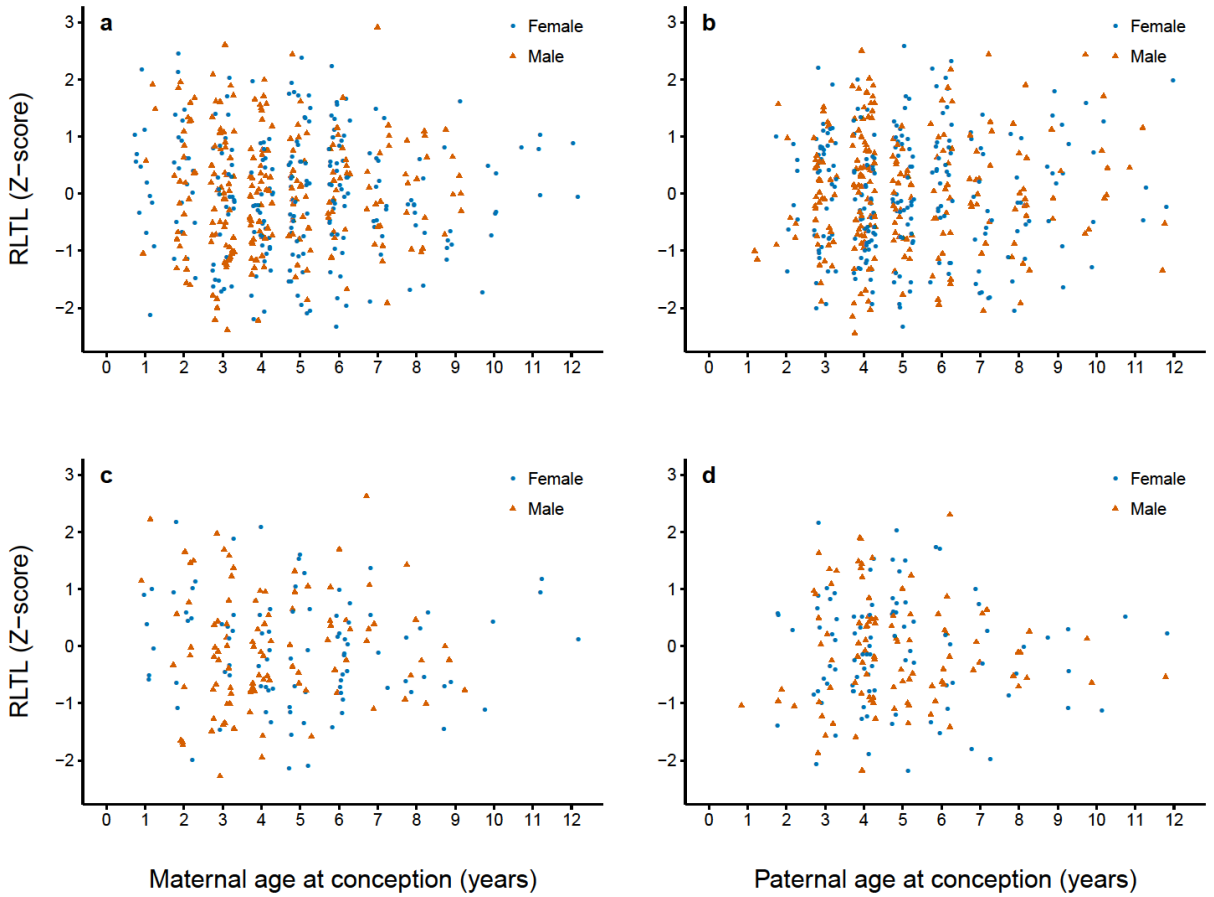

Figure 1 Associations between offspring relative leukocyte telomere length (RLTL) and either maternal (a & c) or paternal (b & d) age at conception (years) in European badgers. Scatterplots show raw data (blue for females and brown for males) for all ages (a & b; *n* = 417 measurements; 240 badgers) or only offspring measured as cubs (<1 year; c & d; 194 measurements; 194 badgers), and jittered for clarity.

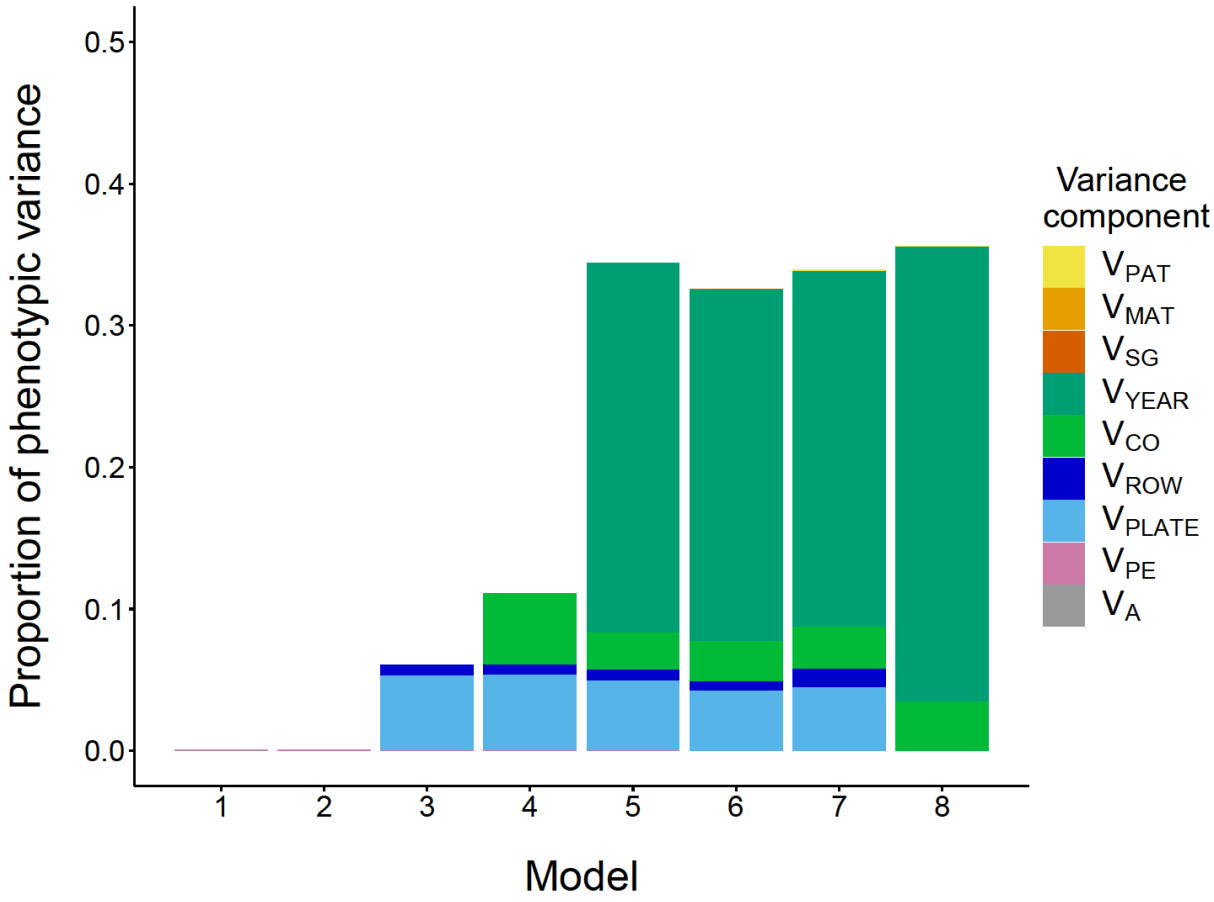

Figure 2 Proportion of variance explained in relative leukocyte telomere length (RLTL; models 1–8) in European badgers of all ages. Variance components: V_A = additive genetic, V_{PE} = permanent environment, V_{PLATE} = plate, V_{ROW} = row, V_{CO} = cohort, V_{YEAR} = year, V_{SG} = social group, V_{MAT} = maternal, and V_{PAT} = paternal. Model numbers on the x-axis correspond with Table S3.

Supporting information

Estimation of environmental and genetic contributions to telomere length variation in a wild mammal

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Table S1 Information from pruned pedigree of the Wytham badger population (1987 – 2010).

Relationship	***n***	**Relationship**	***n***
Records	753	Paternal grandmothers	261
Max. pedigree depth	7	Paternal grandfathers	214
Maternities	486	Founders	206
Paternities	458	Mean maternal sibship size	2.48
Full sibs	194	Mean paternal sibship size	2.59
Maternal sibs	691	Non-zero F	29
Paternal sibs	880	F > 0.125	11
Maternal half sibs	497	Mean pairwise relatedness	0.007
Paternal half sibs	686	Pairwise relatedness ≥ 0.125	0.023
Maternal grandmothers	196	Pairwise relatedness ≥ 0.25	0.013
Maternal grandfathers	174	Pairwise relatedness ≥ 0.5	0.004

Table S2 Parameter estimates from mixed model testing paternal and maternal age at conception (PAC & MAC, respectively) effects on offspring relative leukocyte telomere length (Z-score) in European badgers. β = direction and magnitude of effect, S.E. = standard error, 95 % CI = 95 % confidence interval, β_W = within-individual effect, β_B = between-individual effect, χ^2 = chi-squared value and associated p-value, reference terms in brackets = reference level for factors; * = interaction. Significant parameters (p-value < 0.05) are in bold.

Parameter	β	S.E.	95% CI	χ^2	*p-value*
PAC/MAC model (cubs + adults – *n* = 471 measurements; 240 badgers)†					
Intercept	-0.014	0.127	-0.258 to 0.228		
Age (≤ 29 months)	-0.016	0.068	-0.162 to 0.120	0.115	0.735
(>29 and ≤ 65 months)	**0.180**	**0.079**	**0.030 to 0.338**	**5.317**	**0.021**
(>65 and ≤ 112 months)	**-0.176**	**0.071**	**-0.311 to -0.038**	**6.138**	**0.013**
(> 112 months)	**0.125**	**0.055**	**0.018 to 0.231**	**5.119**	**0.024**
Sex (female)	-0.021	0.093	-0.201 to 0.169	0.023	0.879
Season (Spring)				0.738	0.864
Summer	0.091	0.105	-0.120 to 0.294		
Autumn	-0.017	0.191	-0.387 to 0.353		
Winter	0.015	0.025	-0.459 to 0.509		
PAC	0.046	0.066	-0.081 to 0.176	0.491	0.491
MAC	-0.031	0.060	-0.148 to 0.088	0.238	0.627
Sex (female) * PAC	-0.018	0.092	-0.197 to 0.162	0.037	0.848
Sex (female) * MAC	-0.007	0.096	-0.194 to 0.181	0.006	0.939
Within/Between-individual PAC/MAC (cubs + adults – *n* = 441 measurements; 210 badgers)††					
Intercept	0.026	0.115	-0.199 to 0.248		
Age (≤ 29 months)	-0.043	0.069	-0.188 to 0.092	0.506	0.477
(>29 and ≤ 65 months)	**0.197**	**0.080**	**0.043 to 0.359**	**6.055**	**0.014**
(>65 and ≤ 112 months)	**-0.181**	**0.072**	**-0.320 to -0.040**	**6.265**	**0.012**
(> 112 months)	**0.125**	**0.056**	**0.014 to 0.235**	**4.797**	**0.029**
Season (Spring)				0.224	0.974
Summer	0.059	0.108	-0.165 to 0.269		
Autumn	0.024	0.196	-0.355 to 0.405		
Winter	-0.012	0.249	-0.485 to 0.482		
PAC (β_W)	0.042	0.049	-0.051 to 0.140	0.838	0.360
MAC (β_W)	0.001	0.049	-0.092 to 0.098	0.005	0.945
PAC (β_B)	0.023	0.048	-0.070 to 0.116	0.236	0.627
MAC (β_B)	-0.063	0.050	-0.160 to 0.034	1.602	0.206
PAC/MAC model (cubs – 194 measurements; 194 badgers)†††					
Intercept	0.037	0.204	-0.360 to 0.432		
Age	0.056	0.213	-0.354 to 0.468	0.078	0.780
Sex (female)	-0.107	0.133	-0.365 to 0.145	0.694	0.405
Season (Spring)				5.021	0.170
Summer	0.064	0.278	-0.489 to 0.610		
Autumn	-0.845	0.649	-2.116 to 0.402		
Winter	-0.416	0.906	-2.167 to 1.306		
PAC	-0.040	0.091	-0.213 to 0.134	0.210	0.664
MAC	-0.075	0.084	-0.236 to 0.086	0.753	0.385
Sex (female) * PAC	0.183	0.130	-0.064 to 0.435	2.035	0.154
Sex (female) * MAC	0.015	0.136	-0.246 to 0.276	0.012	0.912

Random effect estimates (variance): †Cohort ($1.920*10^{-2}$), Social group ($2.116*10^{-2}$), Year ($5.795*10^{-2}$), Plate ($6.640*10^{-2}$), Row ($1.613*10^{-2}$), individual ID ($3.927*10^{-8}$), mother ID ($3.248*10^{-10}$), father ID ($1.340*10^{-8}$), Residual ($8.503*10^{-1}$); ††Cohort ($8.221*10^{-3}$), Social group ($1.330*10^{-2}$), Year ($6.593*10^{-2}$), Plate ($6.992*10^{-2}$), Row ($1.538*10^{-2}$), individual ID ($1.368*10^{-6}$), mother ID ($<1.000*10^{-12}$), father ID ($<1.000*10^{-12}$), Residual ($8.537*10^{-1}$); †††Cohort ($8.221*10^{-2}$), Social group ($<1.000*10^{-12}$), Plate ($1.669*10^{-1}$), Row ($3.728*10^{-2}$) mother ID ($<1.000*10^{-12}$), father ID ($3.631*10^{-3}$), Residual ($6.391*10^{-1}$)

Table S3 Additive genetic and environmental effects on relative leukocyte telomere length in European badgers, estimated using the 'animal model' with *MCMCglmm* 2.25 (Hadfield, 2010). Nine models are presented with random effects for additive genetic and permanent environment variance components. Subsequently, fixed and random effects are sequentially added to determine their effect on heritability. Values represent the posterior modes and 95% credible intervals of the variance estimates (V_A = additive genetic, V_{PE} = permanent environment, V_{PLATE} = plate, V_{ROW} = Row, V_{CO} = cohort, V_{YEAR} = year, V_{SG} = social group, V_{MAT} = maternal, V_{PAT} = paternal, V_R = residual, V_P = phenotypic). Age = threshold age, h^2 = heritability.

Model	Parameters	V_A	V_{PE}	V_{PLATE}	V_{ROW}	V_{CO}	V_{YEAR}	V_{SG}	V_{MAT}	V_{PAT}	V_R	h^2
1	TL = V_A + V_{PE} (n = 1248)	$1.514*10^{-5}$ ($3.251*10^{-9}$ – $2.903*10^{-3}$)	$1.195*10^{-5}$ ($1.727*10^{-10}$ – $2.252*10^{-3}$)								$3.278*10^{-2}$ ($2.940*10^{-2}$ – $3.514*10^{-2}$)	$3.847*10^{-4}$ ($9.000*10^{-8}$ – $8.442*10^{-2}$)
2	TL = V_A + V_{PE} + Age + Season (n = 1248)	$1.994*10^{-5}$ ($9.028*10^{-11}$ – $2.574*10^{-3}$)	$1.551*10^{-5}$ ($1.386*10^{-11}$ – $2.141*10^{-3}$)								$3.173*10^{-2}$ ($2.888*10^{-2}$ – $3.475*10^{-2}$)	$3.408*10^{-4}$ ($2.974*10^{-9}$ – $7.599*10^{-2}$)
3	TL = V_A + V_{PE} + Age + Season + V_{PLATE} + V_{ROW} (n = 1248)	$1.371*10^{-5}$ ($8.968*10^{-11}$ – $2.679*10^{-3}$)	$8.569*10^{-6}$ ($4.241*10^{-10}$ – $2.157*10^{-3}$)	$1.842*10^{-3}$ ($7.412*10^{-4}$ – $3.728*10^{-3}$)	$2.281*10^{-4}$ ($2.168*10^{-7}$ – $2.087*10^{-3}$)						$2.960*10^{-2}$ ($2.682*10^{-2}$ – $3.248*10^{-2}$)	$3.589*10^{-4}$ ($2.636*10^{-9}$ – $7.943*10^{-2}$)
4	TL = V_A + V_{PE} + Age + Season + V_{PLATE} + V_{ROW} + V_{CO} (n = 1248)	$1.544*10^{-5}$ ($2.218*10^{-9}$ – $1.816*10^{-3}$)	$6.133*10^{-6}$ ($1.152*10^{-9}$ – $1.739*10^{-3}$)	$2.014*10^{-3}$ ($7.811*10^{-4}$ – $3.681*10^{-3}$)	$2.541*10^{-4}$ ($3.329*10^{-14}$ – $2.050*10^{-3}$)	$1.755*10^{-3}$ ($7.539*10^{-4}$ – $5.310*10^{-3}$)					$2.898*10^{-2}$ ($2.617*10^{-2}$ – $3.124*10^{-2}$)	$3.445*10^{-4}$ ($5.715*10^{-8}$ – $5.102*10^{-2}$)
5	TL = V_A + V_{PE} + Age + Season + V_{PLATE} + V_{ROW} + V_{CO} + V_{YEAR} (n = 1248)	$8.043*10^{-6}$ ($4.165*10^{-12}$ – $1.571*10^{-3}$)	$5.448*10^{-6}$ ($1.352*10^{-9}$ – $1.528*10^{-3}$)	$1.898*10^{-3}$ ($1.011*10^{-3}$ – $4.073*10^{-3}$)	$3.323*10^{-4}$ ($6.150*10^{-9}$ – $2.372*10^{-3}$)	$1.147*10^{-3}$ ($3.697*10^{-4}$ – $3.632*10^{-3}$)	$1.196*10^{-2}$ ($4.353*10^{-3}$ – $2.470*10^{-2}$)				$2.574*10^{-2}$ ($2.339*10^{-2}$ – $2.807*10^{-2}$)	$2.284*10^{-4}$ ($8.823*10^{-11}$ – $3.443*10^{-2}$)
6	TL = V_A + V_{PE} + Age + Season + V_{PLATE} + V_{ROW} + V_{CO} + V_{YEAR} + V_{SG} (n = 1248)	$4.427*10^{-6}$ ($4.073*10^{-10}$ – $1.455*10^{-3}$)	$8.682*10^{-6}$ ($3.660*10^{-10}$ – $1.522*10^{-3}$)	$1.874*10^{-3}$ ($9.780*10^{-4}$ – $4.132*10^{-3}$)	$3.104*10^{-4}$ ($8.686*10^{-9}$ – $2.325*10^{-3}$)	$1.215*10^{-3}$ ($4.251*10^{-4}$ – $3.651*10^{-3}$)	$1.027*10^{-2}$ ($4.887*10^{-3}$ – $2.563*10^{-2}$)	$3.283*10^{-6}$ ($1.556*10^{-10}$ – $7.129*10^{-4}$)			$2.554*10^{-2}$ ($2.355*10^{-2}$ – $2.817*10^{-2}$)	$1.786*10^{-4}$ ($1.122*10^{-8}$ – $3.214*10^{-2}$)
7	TL = V_A + V_{PE} + Age + Season + V_{PLATE} + V_{ROW} + V_{CO} + V_{YEAR} + V_{SG} + V_{MAT} + V_{PAT} (n = 1248)	$8.544*10^{-6}$ ($1.012*10^{-9}$ – $1.185*10^{-3}$)	$8.635*10^{-6}$ ($3.867*10^{-10}$ – $1.227*10^{-3}$)	$2.035*10^{-3}$ ($9.682*10^{-4}$ – $4.117*10^{-3}$)	$5.146*10^{-4}$ ($6.728*10^{-7}$ – $2.456*10^{-3}$)	$1.210*10^{-3}$ ($2.855*10^{-4}$ – $3.360*10^{-3}$)	$1.161*10^{-2}$ ($4.662*10^{-3}$ – $2.586*10^{-2}$)	$2.560*10^{-6}$ ($3.780*10^{-10}$ – $6.397*10^{-4}$)	$1.119*10^{-5}$ ($7.704*10^{-12}$ – $1.352*10^{-3}$)	$5.819*10^{-6}$ ($6.900*10^{-11}$ – $1.107*10^{-3}$)	$2.525*10^{-2}$ ($2.322*10^{-2}$ – $2.783*10^{-2}$)	$7.660*10^{-5}$ ($2.033*10^{-8}$ – $2.580*10^{-2}$)
8	TL = V_A + V_{PE} + Age + Season + V_{PLATE} + V_{ROW} + V_{CO} + V_{YEAR} + V_{SG} + V_{MAT} + V_{PAT} (n = 1248; without V_{PLATE} and V_{ROW} in V_P)	$8.544*10^{-6}$ ($1.012*10^{-9}$ – $1.185*10^{-3}$)	$8.635*10^{-6}$ ($3.867*10^{-10}$ – $1.227*10^{-3}$)	$2.035*10^{-3}$ ($9.682*10^{-4}$ – $4.117*10^{-3}$)	$5.146*10^{-4}$ ($6.728*10^{-7}$ – $2.456*10^{-3}$)	$1.210*10^{-3}$ ($2.855*10^{-4}$ – $3.360*10^{-3}$)	$1.161*10^{-2}$ ($4.662*10^{-3}$ – $2.586*10^{-2}$)	$2.560*10^{-6}$ ($3.780*10^{-10}$ – $6.397*10^{-4}$)	$1.119*10^{-5}$ ($7.704*10^{-12}$ – $1.352*10^{-3}$)	$5.819*10^{-6}$ ($6.900*10^{-11}$ – $1.107*10^{-3}$)	$2.525*10^{-2}$ ($2.322*10^{-2}$ – $2.783*10^{-2}$)	$8.604*10^{-5}$ ($2.168*10^{-8}$ – $2.758*10^{-2}$)
9	Juvenile TL = V_A + V_{PE} + Age + Season + V_{PLATE} + V_{ROW} + V_{CO} + V_{YEAR} + V_{SG} + V_{MAT} + V_{PAT} (n = 837)	$1.270*10^{-5}$ ($1.787*10^{-9}$ – $1.800*10^{-3}$)	$1.120*10^{-5}$ ($1.308*10^{-9}$ – $2.119*10^{-3}$)	$1.566*10^{-3}$ ($6.601*10^{-4}$ – $3.729*10^{-3}$)	$4.296*10^{-5}$ ($9.762*10^{-9}$ – $1.435*10^{-3}$)	$1.767*10^{-3}$ ($1.347*10^{-4}$ – $5.652*10^{-3}$)	$8.607*10^{-3}$ ($3.172*10^{-3}$ – $2.303*10^{-2}$)	$3.139*10^{-6}$ ($1.736*10^{-9}$ – $9.264*10^{-4}$)	$5.353*10^{-6}$ ($3.911*10^{-12}$ – $1.401*10^{-3}$)	$5.807*10^{-6}$ ($1.214*10^{-9}$ – $1.159*10^{-3}$)	$2.492*10^{-2}$ ($2.278*10^{-2}$ – $2.886*10^{-2}$)	$2.490*10^{-4}$ ($4.057*10^{-8}$ – $4.009*10^{-2}$)

Table S4 Additive genetic and environmental effects on relative leukocyte telomere length in European badgers of all ages, estimated using *ASReml-R*. Est. = fixed effect estimate, Prop. = proportion of variance explained, S.E. = Standard error, h^2 = heritability. Age parameters refer to threshold model where Age 1 = ≤29 months old, Age 2 = >29 and ≤65 months old, Age 3 = >65 and ≤112 months old and Age 4 = >112 months old. Variance components are: V_A = additive genetic, V_{PE} = permanent environment, V_{CO} = cohort, V_{YEAR} = year, V_{SG} = social group, V_{MAT} = maternal, V_{PAT} = paternal, V_R = residual, V_{PLATE} = plate, V_{ROW} = row. Total phenotypic variance (V_p) = $3.970*10^{-2}$. Reference terms in brackets = reference level for factors. Significance of fixed effects was determined through Wald Z tests, and for random effects through twice the difference in log-likelihood. Significant parameters ($p < 0.05$) are in bold.

Trait: Relative leukocyte telomere length (*n* = 1248 measurements; 612 badgers)
ASReml-R

	Est.	**S.E.**	**F-value**	**p-value**
Fixed effects				
Intercept	0.4796	$2.933*10^{-2}$		
Age 1	$-2.888*10^{-4}$	$6.920*10^{-4}$	0.174	0.677
Age 2	**$2.156*10^{-3}$**	**$6.755*10^{-4}$**	**10.18**	**0.001**
Age 3	**$-2.570*10^{-3}$**	**$8.358*10^{-4}$**	**9.454**	**0.002**
Age 4	**$4.766*10^{-3}$**	**$2.036*10^{-3}$**	**5.481**	**0.019**
Season (Spring)			**3.627**	**0.013**
Summer	**$2.448*10^{-2}$**	**$1.123*10^{-2}$**		
Autumn	**$1.841*10^{-2}$**	**$1.799*10^{-2}$**		
Winter	**$-5.365*10^{-2}$**	**$2.521*10^{-2}$**		
Random effects	**Est. (S.E.)**	**h^2/Prop. (S.E.)**	**χ^2**	**p-value**
V_A[a]	$5.110*10^{-9}$ ($2.260*10^{-10}$)	$1.286*10^{-7}$ ($1.420*10^{-8}$)		
V_{PE}[a]	$7.610*10^{-9}$ ($3.360*10^{-10}$)	$1.914*10^{-7}$ ($2.115*10^{-8}$)		
V_{CO}	**$1.411*10^{-3}$ ($6.970*10^{-4}$)**	**$3.551*10^{-2}$ ($1.750*10^{-2}$)**	**16.92**	**<0.001**
V_{YEAR}	**$1.161*10^{-2}$ ($4.230*10^{-3}$)**	**0.292 ($7.632*10^{-2}$)**	**79.94**	**<0.001**
V_{SG}	$1.494*10^{-4}$ ($2.163*10^{-4}$)	$3.761*10^{-3}$ ($5.458*10^{-3}$)	0.700	0.403
V_{MAT}	$5.086*10^{-4}$ ($5.612*10^{-4}$)	$1.280*10^{-2}$ ($1.414*10^{-2}$)	0.544	0.461
V_{PAT}	$1.974*10^{-4}$ ($3.658*10^{-4}$)	$4.968*10^{-3}$ ($9.214*10^{-3}$)	0.412	0.521
V_R	$2.585*10^{-2}$ ($1.142*10^{-3}$)	0.651 ($7.189*10^{-2}$)		
V_{PLATE}[b]	**$2.085*10^{-3}$ ($7.034*10^{-4}$)**		**46.11**	**<0.001**
V_{ROW}[b]	**$4.354*10^{-4}$ ($3.256*10^{-4}$)**		**8.824**	**0.003**

[a] Significance not tested because variance components were at boundary

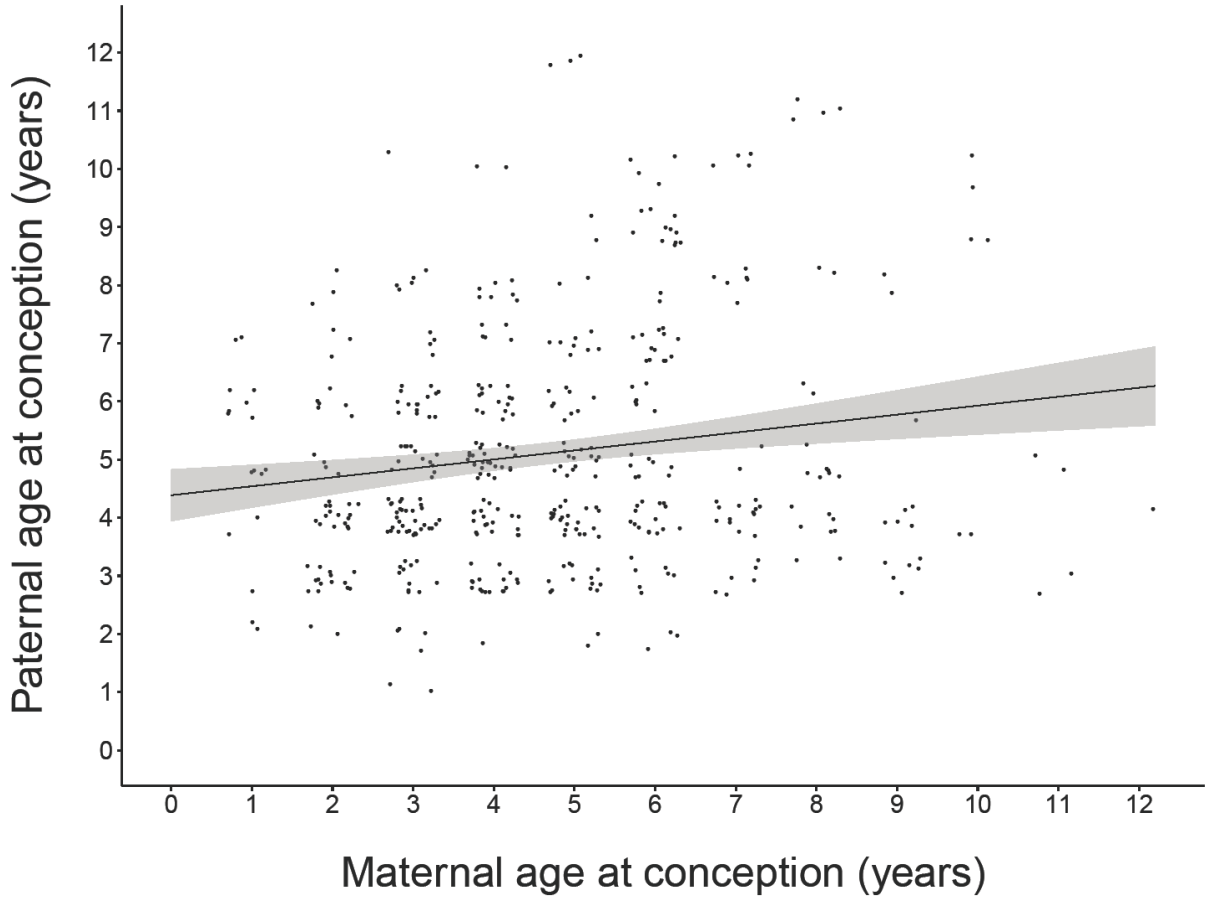
[b] Measurement error and not biological variance so no proportion and associated standard error provided

Table S5 Additive genetic and environmental effects on relative leukocyte telomere length (Age ≤ 29 months) in European badgers, estimated using *ASReml-R*. Est. = fixed effect estimate, Prop. = proportion of variance explained, S.E. = Standard error, h^2 = heritability. Variance components are: V_A = additive genetic, V_{PE} = permanent environment, V_{CO} = cohort, V_{YEAR} = year, V_{SG} = social group, V_{MAT} = maternal, V_{PAT} = paternal, V_R = residual, V_{PLATE} = plate, V_{ROW} = row. Total phenotypic variance (V_p) = $3.910*10^{-2}$. Reference terms in brackets = reference level for factors. Significance of fixed effects was determined through Wald Z tests, and for random effects through twice the difference in log-likelihood. Significant parameters ($p < 0.05$) are in bold.

Trait: Early-life relative leukocyte telomere length (*n* = 837 measurements; 556 badgers) ASReml-R				
	Est.	**S.E.**	**F-value**	**p-value**
Fixed effects				
Intercept	0.4771	$2.874*10^{-2}$		
Age	$1.120*10^{-4}$	$8.403*10^{-4}$	0.018	0.894
Season (Spring)			**2.933**	**0.033**
Summer	**$1.576*10^{-2}$**	**$1.398*10^{-2}$**		
Autumn	**$1.516*10^{-2}$**	**$2.408*10^{-2}$**		
Winter	**$-7.998*10^{-2}$**	**$3.150*10^{-2}$**		
Random effects	**Est. (S.E.)**	**h^2/Prop. (S.E.)**	**χ^2**	**p-value**
V_A	$1.280*10^{-4}$ ($1.075*10^{-3}$)	$3.271*10^{-3}$ ($2.746*10^{-2}$)	0.014	0.906
V_{PE}[a]	$8.770*10^{-9}$ ($5.560*10^{-10}$)	$2.240*10^{-7}$ ($2.569*10^{-8}$)		
V_{CO}	**$1.945*10^{-3}$ ($1.138*10^{-3}$)**	**$4.970*10^{-2}$ ($2.862*10^{-2}$)**	**11.13**	**<0.001**
V_{YEAR}	**$1.004*10^{-2}$ ($4.062*10^{-3}$)**	**0.257 ($7.890*10^{-2}$)**	**40.45**	**<0.001**
V_{SG}	$2.412*10^{-4}$ ($3.169*10^{-4}$)	$6.162*10^{-3}$ ($8.107*10^{-3}$)	0.879	0.348
V_{MAT}[a]	$2.710*10^{-9}$ ($1.720*10^{-10}$)	$6.924*10^{-8}$ ($7.940*10^{-9}$)		
V_{PAT}[a]	$2.360*10^{-9}$ ($1.500*10^{-10}$)	$6.024*10^{-8}$ ($6.907*10^{-9}$)		
V_R	$2.679*10^{-2}$ ($1.699*10^{-3}$)	0.684 ($7.846*10^{-2}$)		
V_{PLATE}[b]	**$1.771*10^{-3}$ ($7.239*10^{-4}$)**		**19.49**	**<0.001**
V_{ROW}[b]	$1.942*10^{-4}$ ($2.461*10^{-4}$)		1.319	0.251

[a] Significance not tested because variance components were at boundary

[b] Measurement error and not biological variance so no proportion and associated standard error provided



826

827 **Figure S1** Scatterplot showing the correlation between paternal and maternal ages at conception for
828 badgers with relative leukocyte telomere length measures at any age (*n* = 471 samples; 240 badgers).
829 Parental ages are integers, jittered for clarity on the amount of data.

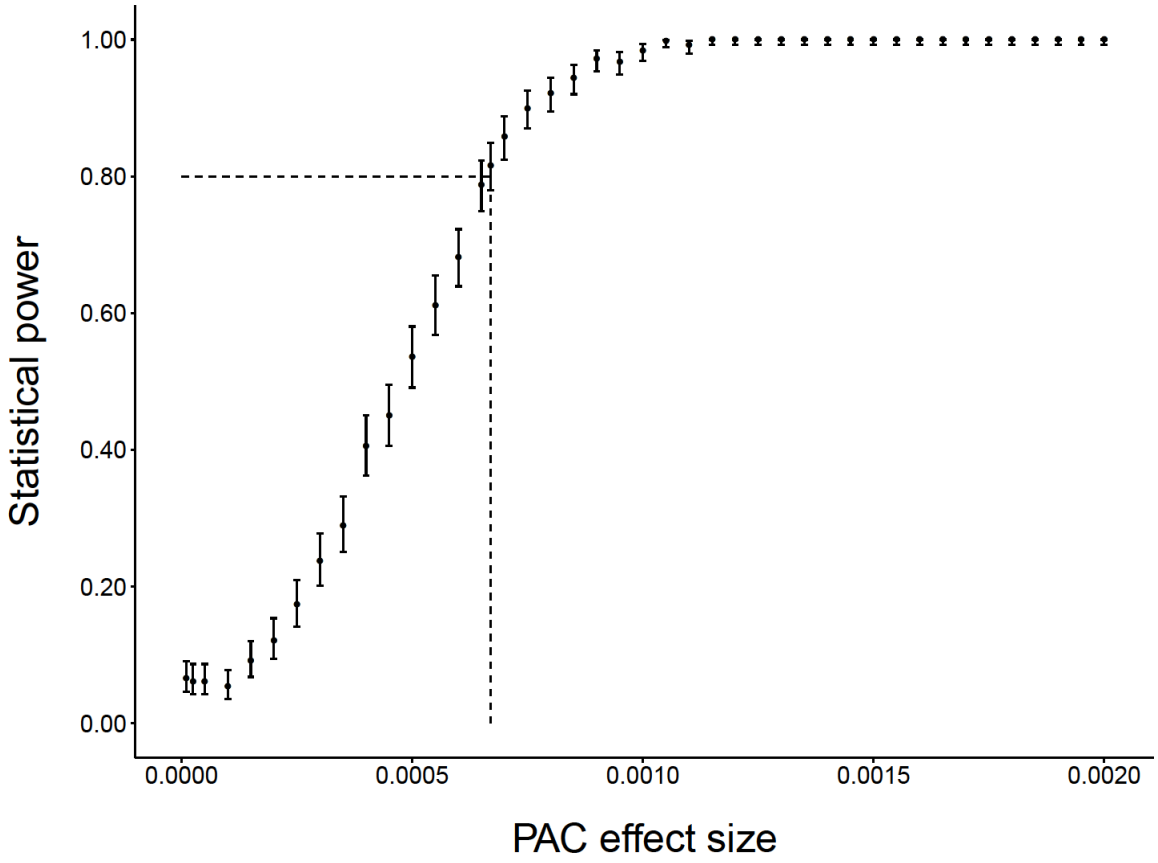


Figure S2 Statistical power to detect paternal age at conception (PAC) effect sizes in our European badger dataset using *simr* v1.0.5 (Green & MacLeod, 2016). Point estimates and error bars show mean power with associated 95% confidence intervals estimated from 500 simulations. Dashed line represents 80% power to detect a PAC effect size of 0.00067 or greater, with the specifications of our model and structure of our data. This is similar to a correlation coefficient of 0.131 (where correlation coefficient = $(\beta_{PAC}*SD_{PAC})/SD_{RLTL}$ = (0.00067*24.57207)/0.1254953).

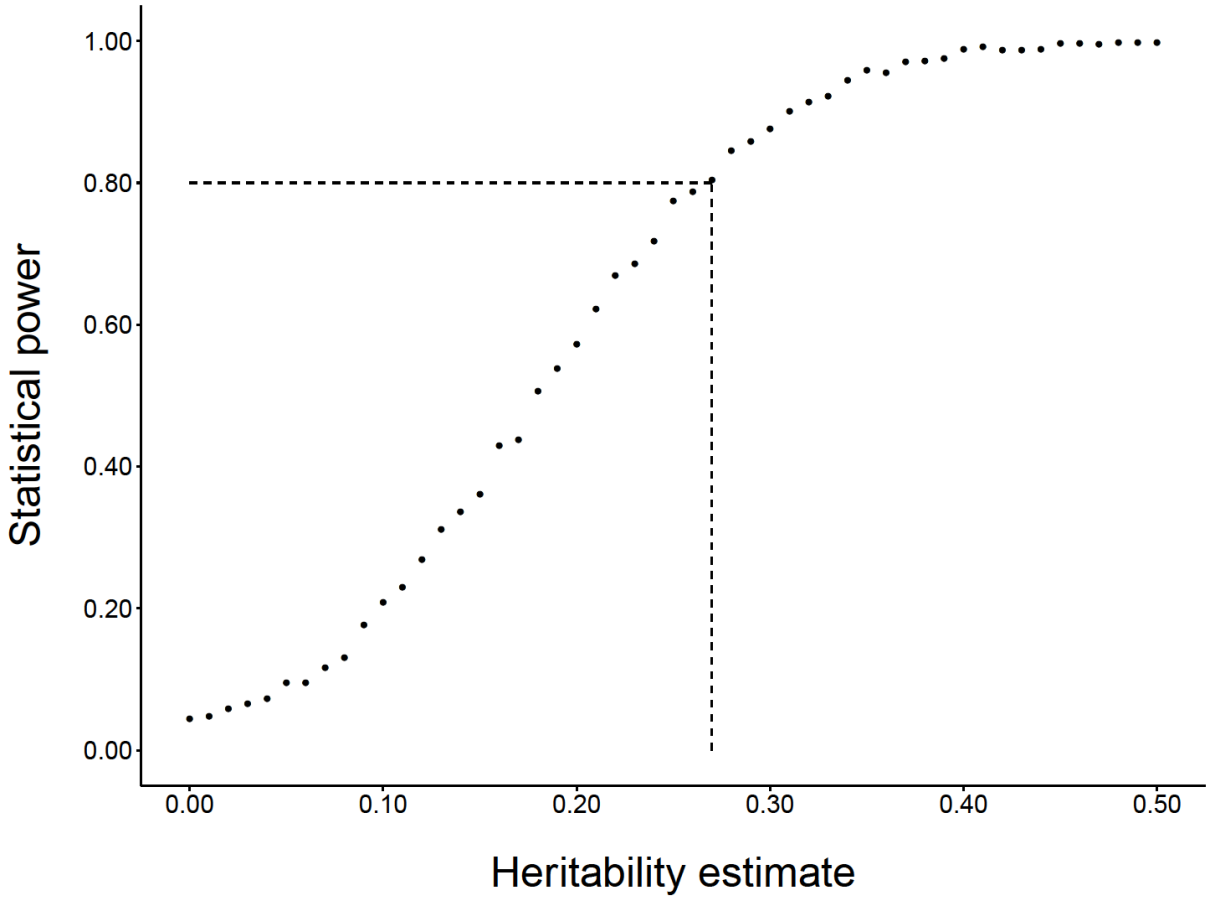


Figure S3 Statistical power to detect varying heritability estimates of telomere length in the European badger with our dataset (*n* = 1248 measurements; 612 badgers) and pruned pedigree structure using *pedantics* 1.7 (Morrissey & Wilson, 2010). Point estimates show mean power estimated from 1000 simulations. Dashed line represents 80% power to detect a heritability estimate of 0.27 or greater, with the specifications of our model and structure of our data.

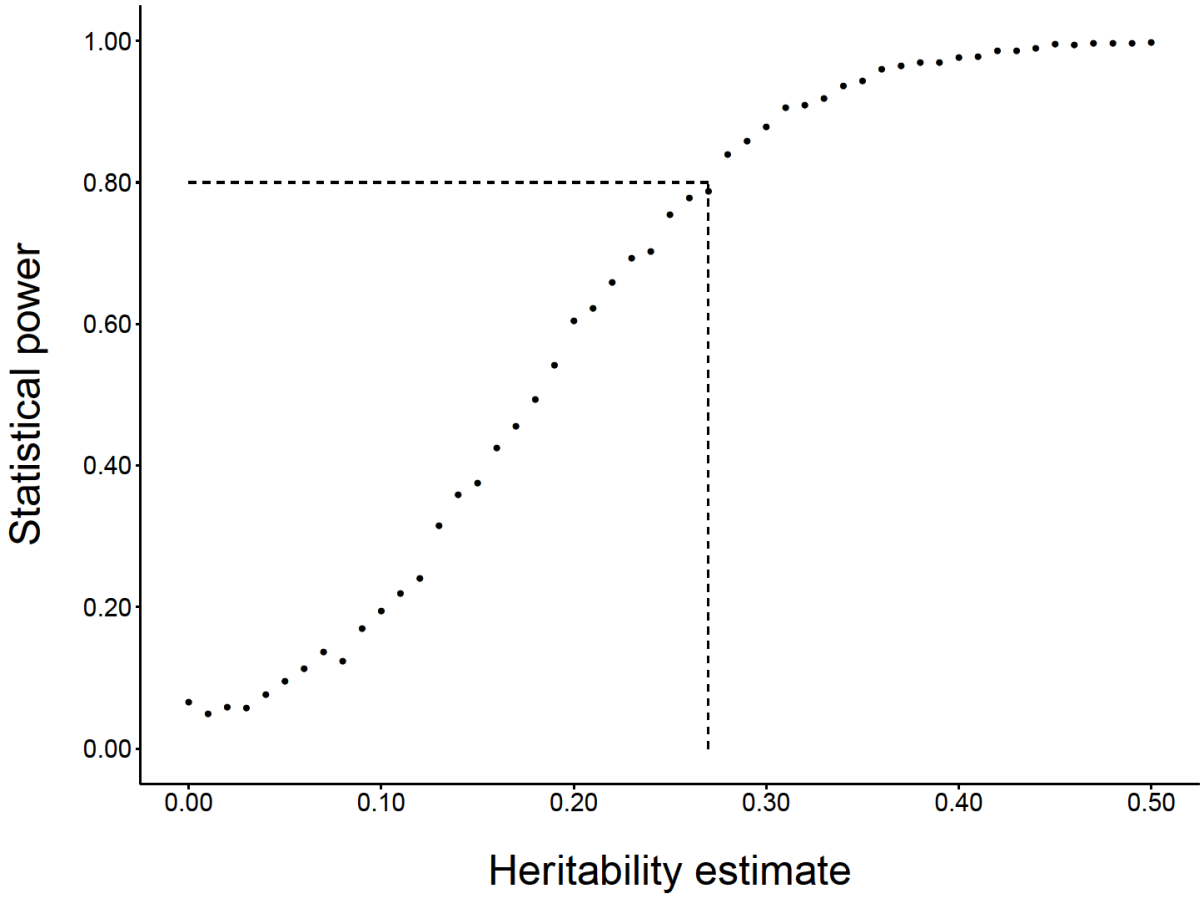


Figure S4 Statistical power to detect varying heritability estimates of juvenile telomere length (≤29 months old) in the European badger with our dataset (*n* = 837 measurements; 556 badgers) and pruned pedigree structure using *pedantics* 1.7 (Morrissey & Wilson, 2010). Point estimates show mean power estimated from 1000 simulations. Dashed line represents 80% power to detect a heritability estimate of 0.28 or greater, with the specifications of our model and structure of our data.

References

Green P., MacLeod C. J. (2016). simr: an R package for power analysis of generalized linear mixed models by simulation. *Methods in Ecology and Evolution*, *7*, 493-498. https://doi.org/10.1111/2041-210x.12504

Hadfield J. D. (2010). MCMC methods for multi-response generalised linear mixed models: the MCMCglmm R package. *Journal of Statistical Software*, *33*, 1-22. https://doi.org/10.18637/jss.v033.i02

Morrissey M. B., Wilson A. J. (2010). pedantics: an r package for pedigree-based genetic simulation and pedigree manipulation, characterization and viewing. *Molecular Ecology Resources*, *10*, 711-719. https://doi.org/10.1111/j.1755-0998.2009.02817.x