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Assortative pairing by telomere length in king penguins and relationships with breeding success

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Abstract

Telomeres are non-coding genetic repeats protecting the ends of linear chromosomes. Long telomeres are often associated with high individual survival, and inter-individual variation in telomere length has recently been proposed as a proxy for individual quality. Therefore, one might expect individuals of either sex with long telomeres to be of higher intrinsic quality and to be preferred in the context of mate choice. Thus, in sexually monomorphic species where individuals discriminate mates on the basis of signals of intrinsic quality, mate choice should lead to assortative pairing by telomere length, and it should be associated with breeding performance. We tested these two predictions in the king penguin, a sexually monomorphic seabird. Over 3 years of study and 73 penguin pairs under contrasting environmental conditions, we found strong assortative pairing by telomere length. Interestingly, only female telomere length was positively associated to chick survival up to fledging, and this relationship was only apparent when foraging conditions at sea were average. The positive link between telomere length and breeding success confirmed that telomere length is somehow related to individual biological state at a given time. The proximate mechanisms by which birds assess individual state related to telomere length remains to be discovered.

Introduction

Darwin's (1871) theory of sexual selection was initially extremely controversial, but proved to be an highly studied topic in both empirical and theoretical evolutionary biology (Kirkpatrick and Ryan 1991; Andersson 1994; Badyaev and Landeen 2007; Kuijper et al. 2012; Lyon and Montgomerie 2012). Sexual selection can influence competition over mating opportunities and the choice of a mate at the commencement of breeding. With respect to mate choice, many species exhibit ornamental traits that occur in only one of the sexes, and appear to advertise individual quality to potential mates (Andersson 1994; Hill 2002). Of course, in some species ornamental traits that indicate mate quality occur in both sexes (Huxley 1916; Kraaijeveld et al. 2007; Jouventin and Dobson 2018). Thus, the theory of sexual selection has been developed to explain, in part, ornamental traits of animals that are condition-dependent signals, or indicators, of individual quality.

Telomeres are non-coding, double-stranded DNA sequences located at the ends of linear chromosomes that preserve the integrity of genomic information. During each cell division, the terminal end of telomeres is lost, so that telomeres progressively shorten as the organism ages (Blasco 2007): a process related to individual ageing and survival in the wild (Bize et al. 2009; Salomons et al. 2009). However, the idea of a simple causal relationship between telomere length and individual age has been criticized (Simons 2015). The decrease in telomere length over time does not occur at an identical rate in similar-aged individuals (Hall et al. 2004), and an increasing number of studies have advanced mechanistic explanations for the observed high variability in telomere length among similar-aged individuals in different species (reviewed in Monaghan and Haussmann 2006; Asghar et al. 2015; Nettle et al. 2015). Notably, telomeres are DNA structures highly susceptible to organism stress, including increases in oxidative stress (Costantini et al. 2011). Oxidative

stress induces DNA single–strand breaks during replication, leading to transient stalling of replication and telomere shortening (von Zglinicki 2002).

Thus, the rate of telomere loss depends strongly on individual life experiences and life-time accumulated stress (Epel et al. 2004; Kotrschal et al. 2007; Blackburn and Epel 2012; Aydinonat et al. 2014), including early growth conditions (Tarry-Adkins et al. 2009; Geiger et al. 2012; Haussmann et al. 2012; Reichert et al. 2014). An initial difference in growth in body size may persist among similar-aged adult individuals (Benetos, Kark et al. 2013). For instance, accelerated telomere loss has been shown to occur when environmental conditions during growth are poor (Tarry-Adkins et al. 2009; Geiger et al. 2012), when reproductive effort is high (Reichert et al. 2014), or when individuals are exposed to high levels of glucocorticoid stress hormones (Haussmann et al. 2012; reviewed in Angelier et al. 2017). Such heterogeneity in life experiences leads to high inter-individual variability in telomere length, both early in life (including through inheritance; Reichert et al. 2015) and adulthood (Hall et al. 2004). Moreover, counteracting mechanisms prevent or counterbalance telomere attrition. These mechanisms include DNA maintenance, telomerase activity (Haussmann et al. 2007), and anti-oxidant abilities (Badás et al. 2015).

Individuals differ in their ability to cope with environmental stressors, by DNA repair or counteraction of intracellular damages (Monaghan and Haussmann 2006; Asghar et al. 2015; Nettle et al. 2015). It has therefore been suggested that variability in telomere length may be a good proxy to assess variation in intrinsic quality amongst individuals, independently of age (Haussmann et al. 2005; Bauch et al. 2012; Nussey et al. 2014; Le Vaillant et al. 2015). For instance, individuals with higher anti-oxidant capacity, higher immunity or in generally better body condition may be less susceptible to external stressors and may suffer less from ensuing consequences on telomeres (von Zglinicki 2002; Ilmonen et al. 2008; Stier et al. 2014; Le Vaillant et al. 2015). One expects that extended lifespan and an

increase in the number of reproductive events, and ultimately individual fitness, should be associated with improved condition and longer telomere length (Pauliny et al. 2006). Previous studies support this prediction, highlighting that telomere length is often positively linked to physiological quality (e.g. higher immunity, higher body condition) or fitness traits (e.g. higher longevity; higher reproductive output) in different species (Haussmann et al. 2005; Pauliny et al. 2006; Bize et al. 2009; Salomons et al. 2009; Bauch et al. 2012; Le Vaillant et al. 2015), including humans (e.g. health status) (Verhulst, Dalgård et al. 2016).

Since telomere length appears to reflect the ability to cope with life stress and is perhaps a measure of overall quality, one might expect long telomeres to be associated with greater reproduction. Indeed, mate choice for high quality partners is one of the fundamental hypotheses in sexual selection theory (Zahavi 1975; Johnstone 1995; Kokko et al. 2003). The underlying idea is that by being selective in mate choice, individuals gain net fitness benefits from mating with high quality partners, either directly (*via* access to higher quality resources or greater parental care) or indirectly (*via* genetic benefits, i.e. good genes) (Burley 1977; Linville et al. 1998; Kempenaers 2007; García-Navas et al. 2009; Fromhage et al. 2009; Alonzo 2012). Thus, one might expect to find assortative mating patterns in terms of telomere length in regards to mate choice.

Positive assortative mating (i.e. when individuals pair according to similar phenotypic characteristics) have been previously found in birds (Cooke et al. 1976; Boag and Grant 1978; Coulter 1986; Delestrade 2001), the pattern was expected to result mainly from aged-derived breeding patterns. For example, first-time breeders and younger birds often start reproduction later than more experienced, older, individuals. A positive assortative mating pattern by foot colour was related to low oxidative stress in black guillemots (*Cepphus grylle*) (Fasanello et al. 2015). Oxidative stress is a labile marker that rapidly responds to intrinsic and extrinsic stress factors. This results suggest that, more than simply reflecting assortative age-pairing,

assortative mating could reflect active mate choice for high quality individuals in terms of oxidative physiology, with likely consequences on telomere length (von Zglinicki 2002).

In the present study, we tested the hypothesis that mutual mate choice for high quality partners results in assortative pairing by telomere length. We use breeding king penguins (*Aptenodytes patagonicus*) as our study species, as they are long-lived and survival (and thus more breeding opportunities) is expected to be under strong selection. This makes king penguins an ideal model for studying patterns of telomere length. Moreover, these penguins are monomorphic in appearance and choice of a high quality mate is crucial for both sexes (Nolan et al. 2010; Keddar et al. 2015a; Jouventin and Dobson 2018). Both parents must cooperate for 14 months to successfully raise their single chick to independence, and a single parent cannot succeed by itself (Stonehouse 1960; Weimerskirch et al. 1992). During this prolonged period, parents face strong energy constraints including long-term fasting on land (Groscolas and Robin 2001) and intense foraging periods at sea, several hundreds of kilometres from their breeding grounds (Charrassin and Bost 2001). Moreover, about 80% of successful pairs get new partners the next time they breed (Olsson 1998).

King penguins exhibit mutual mate choice, based on a ultra-violet colour ornaments on beak spots of the lower mandible (Nolan et al. 2010; Keddar et al. 2015a). These penguins also display monomorphic carotenoid based orange colour from the beak spots and yellow-orange auricular feather patches that contains an endogenous pterin-like spheniscin pigments (Thomas et al. 2013). Both pigments are known to have anti-oxidant properties (Edge et al. 1997; Oettl and Reibnegger 2002; Oettl et al. 2004). Compulsory bi-parental care is a more than favorable ground for high selection pressure on the mate choice process (Kokko and Monaghan 2001; Kokko and Johnstone 2002). Moreover, king penguins are monomorphic, meaning that both sexes present sexual ornaments that are important during mate choice process and that reflect individual qualities such as the immune system (Nolan et al. 2006;

Schull et al. 2016a), metabolism, the stress response (Viblanc et al. 2016), and behaviors (Keddar et al. 2015b).

However, whether mate-choice results in assortative pairing of high quality individuals is unknown. Our previous results for king penguins revealed that telomere length was associated with various indices of individual quality, including higher immune capacity and higher breeding performance, but telomere length did not appear to be associated with individual age (Le Vaillant et al. 2015). If telomere length is an integrative measure of the ability to cope with life stress, and by extension individual quality, we predict (i) that assortative pairing for high quality individuals with long telomere length should occur in king penguins, and (ii) that long telomere length should be associated high individual reproductive performance.

Material and Methods

Bird monitoring

We studied king penguins at Possession Island in a colony of ca. 20,000 breeding pairs in 2009-2011. As part of a long-term study on the ecophysiology of king penguins, we followed breeding pairs from egg-laying until fledging or breeding failure. In each year, in the same area of the breeding colony, we haphazardly selected adult king penguin pairs of unknown age once they had settled on their breeding territory (see below). None of the individuals were followed in more than one season. Pair selection started each year with marking of 20 pairs in January, and an additional 20 pairs in February (laying dates extend from November-March in this species; Weimerskirch et al. 1992) using a non-toxic human hair dye (Franck Provost, blue-black 2.1). No individual (identified by a radiofrequency PIT-Tag for long-term monitoring) was repeated between years. All birds were measured at a similar timing in the season. However, 2010 was a peculiar year in that none of the breeders sampled in February successfully resumed incubation or early chick brooding (see below). Due to natural breeding failure or timing constraints in the field, we were able to follow in total 87 couples with at least a chick at hatching (34 in 2009, 20 in 2010 and 33 in 2011). Telomere length was determined for both parents in 73 breeding pairs, distributed as follows: 33 breeding pairs in 2009 (laying dates from 14 January to 5 March), 20 breeding pairs in 2010 (laying dates from 16 January to 19 January) and 20 pairs in 2011 (laying from 19 January to 26 February).

For each breeding pair followed over their entire season, we proceeded as followed: ten days after hatching, the female and chick of each pair were caught and body size and mass (missing for some adults) were recorded to the nearest ± 4g using a platform balance (Kem IT60K2LIP). Flipper (±1mm) and bill length (±0.1mm) were measured using solid metal rulers. Blood (1mL) was collected from a flipper vein of adults using a heparinized syringe (2.5mL, G22- 1 ½ needle), centrifuged (4000 rpm for 5 min), and plasma and red blood cells

171 were immediately separated, frozen, and stored at -80°C (Stier et al. 2014; Reichert et al.
172 2015). Males were measured and sampled following the same protocol during their first
173 brooding shift (ca. 5-10 days later). Breeding pairs and their chicks were followed by daily
174 observations from the moment the couple settled in the colony until the end of the breeding
175 cycle, either when the chick fledged into the sea or the reproduction failed, allowing chick
176 death date to be recorded.
177
178 ***Environmental conditions and climatic anomalies***
179 Among the years of our study (2009-2011), marked differences in foraging conditions at sea
180 occurred: 2010 had favourable, 2011 unfavourable, and 2009 intermediate foraging conditions
181 (Bost et al. 2015). To evaluate yearly foraging conditions over the duration of the study, we
182 investigated large-scale climatic anomalies known to drastically affect marine resources and
183 the location of foraging zones, and produce strong demographic consequences on king
184 penguins. When tropical anomalies occur, areas where penguins foraged move further away
185 from their breeding colony, and their feeding depths increase, leading to a decrease of the size
186 of the population (Bost et al. 2015). We calculated South Atlantic and Indian Ocean dipole
187 (SAIOD) values over our study period (following Bost et al. 2015). The SIAOD is an index of
188 large-scale climatic anomalies in Sea Surface Temperatures (SST) in the Southern Atlantic-
189 Indian Ocean. Positive perturbations of SAIOD are associated both with SST anomalies and
190 with longer distances between the breeding colony and the Polar Front at sea (where king
191 penguins preferentially forage; Jouventin et al. 1994). Hence, positive perturbations of
192 SAIOD are associated with longer foraging trips due to wider spread of patchier foraging
193 resources, increased feeding depths and with a lower breeding success at our study colony
194 (Bost et al. 2015). Inversely, low values of SAIOD are associated with favourable
195 environmental conditions, shorter foraging trips and higher colony breeding success. We

196 computed the SAIOD estimate based on the time series from 1982-2014 for our study colony.
197 We then restricted our analyses to the periods beginning in February through the end of
198 March over 2009-2011. This corresponds to the peak of the summer period and it
199 encompasses the peak of energy demand for chick's growth and the highest constraint on
200 parent foraging effort. We extracted monthly mean SST (R, packages ncdf / ade4 / raster /
201 ggplot2) from the "NOAA Optimal Interpolation" database
202 (http://www.esrl.noaa.gov/psd/data/gridded/data.noaa.oisst.v2.html) for the known foraging
203 area of king penguins in our study colony (i.e. South Indian Ocean from 49°E to 55°E and
204 47°S to 53°S; Charrassin and Bost 2001). We calculated SAIOD as the first principal
205 component (PC1) of a principal component analysis of SST anomaly fields over the South
206 Atlantic-Indian Ocean area (10°N–50°S, 50°W–150°E) (R, packages ncdf / ade4 / raster /
207 ggplot2) (Bost et al. 2015). The distance to the Polar Front was estimated as the distance
208 between Baie du Marin, Possession Island, and the south 5°C isotherm (indicating the
209 position of the polar front; Bost et al. 2015) using the package GDAL (www.gdal.org).
210
211 ***Telomere length analyses***
212 Telomere length was obtained from DNA in red blood cells using the qPCR method (after
213 Cawthon 2002; Criscuolo et al. 2009). DNA was extracted from frozen red blood cells using
214 spin columns DNA purification kit (Nucleospin® Blood QuickPure, Macherey-Nagel, Düren
215 Germany). DNA purity was checked using absorbance ratios obtained when DNA
216 concentrations of each sample were measured with a Nanodrop ND-1000 spectrophotometer.
217 Primer sequences for telomere amplification were similar to those previously used (Geiger et
218 al. 2012; Stier et al. 2014; Reichert et al. 2015). For the single control gene (defined as non-
219 variable in copy numbers in our population; hereafter S; (Smith et al. 2011). We used the
220 *Aptenodytes patagonicus* zinc finger protein, primer sequences as defined by Primer 3

221 software: (Royal1: 5'-TACATGTGCCATGGTTTTGC-3'; Royal2: 5'-
222 AAGTGCTGCTCCCAAAGAAG-3'). Primer concentrations in the final reaction mix were
223 200 nM for telomere length and 300 nM for the control gene. Telomere and control gene
224 qPCR conditions were: 2min at 95°C followed by 40 cycles of 15s at 95°C, 30s at 56°C, 30s
225 at 72°C and 60s at 95°C. We used 2.5ng DNA per reaction and the BRYT Green® fluorescent
226 probe (GoTaq®qPCR Master Mix, Promega, France).

227 The control gene amplicons for this particular species were initially checked by gel
228 migration for having the expected size (based on primer sequencing for the Zinc Finger gene
229 in king penguins, see Geiger et al. 2012). A reference curve was included using 5 points of a
230 1/1 serial dilution which covered the whole range of sample Cq values for both control and
231 telomere amplification. The reference curve was present in each plate, including a negative
232 control without DNA – NTC. References curves were used to calculate the efficiency of each
233 specific amplification for each run, and were used for the final telomere length estimates; R^2
234 values of regression fits were > 0.95 in every case. All runs ended by a melting curve step to
235 check for non-specific primer-dimer artefact (see ESM 1A). Raw data are available in ESM 2.

236 The final value of telomere length was estimated following calculations recommended
237 by (Pfaffl 2001) as a ratio of amplification cycles between the telomere DNA sequence (T)
238 and a non-variable in copy number gene sequence (S, resulting in a T/S ratio). Telomere
239 lengths were measured on three different plates (i.e. runs) corresponding to each year of the
240 experiment (2009, 2010, 2011), to avoid a potential drift due to differences in storage duration
241 of red blood cells at -80°C. qPCR amplification efficiencies of the non-variable copy gene
242 (S) and of the telomere sequence were calculated specifically for each run, based on a serial
243 dilution of three different samples (one per year). These runs were checked for the presence
244 of consistent ranges in control and telomere Cq values (see ESM 1B and 1C). The reference
245 golden sample (which serves as a telomere length of 1) was not the same across the three

246 plates, but we verified that the amplification Cq values for both the control gene and the
247 telomere sequence were very close among them (ESM 1D) to avoid a drift due to calculation
248 over years. It is important to note that this did not have an effect on the main objective of the
249 paper, *i.e.* assortative paring in relation to telomere length within a given year. However, to
250 control for non-specific variation in telomere length measurement over years (i.e. runs), 15
251 additional samples (5 individuals taken randomly from each of the years) were run again on
252 an additional plate (Amplification efficiencies were 99.3% for both S and T). A repeatability
253 estimate (r) was calculated following (Lessells and Boag 1987) using r = (among individual
254 variance) / (within individual variance + among individual variance). Individual T/S ratio
255 repeatability was r = 0.822 (indicating low within individual variance over the two runs).
256 Intra-plate and inter-plate coefficients of variation are indicated in ESM 1E for control Cq
257 values, telomere Cq values and for the final relative telomere length value (T/S ratio).
258
259 ***Statistical analyses***
260 We used Pearson correlations to evaluate associations between telomere length (ln
261 transformed to achieve normality and homoscedasticity), body mass, and size proxies (flipper
262 and beak lengths) of mated pairs. To investigate the influence of year on the assortative
263 mating pattern in telomere length of paired males and females, we ran a linear model (LM)
264 with female telomere length as the response variable, and male telomere length and year as
265 independent variables, and the interaction of *male telomere length x year*. Differences in male
266 and female telomere length among years were assessed using a multivariate analyses of
267 variance (MANOVA) with Bonferroni post-hoc comparisons. We assessed whether breeding
268 partners' telomere length and breeding success were associated using (a) a Gaussian linear
269 model of adult males and females, and chick body mass at day 10, and (b) a generalized linear
270 model with a logistic binary distribution for chick survival at fledging (survival/death = 1/0).

Independent variables included female or male telomere length, sampling year, and the year x telomere length interaction. Chick body mass (for b) was included as a covariate. Post-hoc Tukey HSD tests were conducted using the 'multcomp' R package (Bretz et al. 2010). We used R3.1.3 (R Development Core Team 2008). Tests were two-tailed with $P< 0.05$ considered significant. Effect sizes (Z-transformed r) were calculated using from Equations 11, 15 and 20 following Nakagawa and Cuthill (2007), and are reported along with their 95% Confidence Intervals.

Results

Over 2009-2011, males and females were positively assorted by telomere length in all years of the study (LM; $Zr = 0.33$, $CI_{95} = [0.10 – 0.57]$, $P = 0.007$, non-significant year x telomere length interaction, Table 1, Fig. 1). A multivariate analysis revealed that female and male telomere length were different between years (Roy's greatest root, $P < 0.001$). The T/S ratio in 2009 was 1.19 ± 0.62 for females and 1.09 ± 0.48 for males; in 2010 it was 0.70 ± 0.29 for females and 0.65 ± 0.17 for males; and in 2011 it was 0.92 ± 0.49 for females and 0.99 ± 0.53 for males. Telomere length was significantly shorter in 2010 than in 2009, by 41% for males and 42% for females (Bonferroni post-hoc, $P = 0.001$ in both sexes, other tests were non-significant: $0.08 < P < 0.97$). However, there were no significant correlations of telomere length and body size (wing or beak length) or telomere length and body mass in either sex, nor were birds in pairs significantly assorted by body mass or size (see Table 2).

Chick body mass at ten days was not significantly associated with female ($Zr = 0.14$, $CI_{95} = [-0.09 – 0.37]$, $P = 0.26$), or male (LM; $Zr = -0.09$, $CI_{95} = [-0.33 – 0.14]$, $P = 0.45$) telomere length (see Table 3A & 3B). In 2010, chicks at 10 days were 18% lighter than in 2009 (521.0 ± 31.8 g *vs.* 633.3 ± 24.8 g) and 16% lighter than in 2011 (521.0 ± 31.8 g *vs.* 622.3 ± 31.8 g) (see Table 3). Chick survival rates were of 41.2% (14/34 chicks) in 2009,

296 80% (16/20) in 2010, and 42.4% (14/33) in 2011 (from 34 pairs in 2009 and 33 pairs in 2011,
297 we only acquired telomere length for both males and females in 33 and 20 pairs,
298 respectively). Both in female or male models (Table 3A or 3B), chick survival to fledging was
299 positively associated with chick body mass at ten days independently of the year (Zr = 0.29,
300 CI_{95} = [0.06 – 0.52], P = 0.017 and Zr = 0.32, CI_{95} = [0.09 – 0.55], P = 0.022; for female or
301 male models; see Table 3A and 3B). The interaction between female telomere length and year
302 was significantly related to chick survival. In 2009, females with longer telomere length were
303 more successful at raising their chick, but no such pattern was evident in 2010/2011 (see
304 significant interactions Table 3A; and Fig. 2). If laying date was included in the model,
305 female telomere length to fledging success relationship remained significant (Zr = 0.29, CI_{95} =
306 [0.06 – 0.52], P = 0.017). We did not keep laying date in the final model because variance in
307 laying dates was not comparable between years. Male telomere length was not related to chick
308 body mass or fledging success (Table 3B).

309 The SAOID value in the first year of the study (2009) indicated no large-scale climatic
310 perturbation (SAIOD = -2.1) and the polar front distance was intermediate (PF = 425 km; Fig.
311 3). In 2010, SAIOD reached its second lowest value for the period 1982-2014 (SAIOD = -
312 50.2), concomitant to a closer position of the polar front to the breeding site (PF = 400 km).
313 Finally, in 2011, SAIOD exhibited perturbations (SAIOD = 57.0), and the polar front was
314 further away (PF = 479 km).

315

316 **Discussion**

317 In species where both sexes express similar phenotypes and mate choice is mutual, assortative
318 pairing by individual quality is expected (Kraaijeveld et al. 2007). King penguins are known
319 to be mutually selective in mate choice (Nolan et al. 2010; Keddar et al. 2015a), most of them
320 change partner every breeding season (about 80%; Olsson 1998; Bried et al. 1999) and

individuals frequently changes partners during the pairing period (Olsson et al. 2001). We found that over 3 contrasting breeding seasons, assortative pairing by telomere length occurred in this species, supporting the hypothesis of mutual mate choice for high quality partners. Pairing with a high-quality partner is likely essential to reproductive success, given the strong constraints faced by this species during reproduction (i.e. a 14-month period of chick-rearing, long-term fasting, and alternating visits to feeding grounds several hundreds of kilometres distant from the breeding colony; Weimerskirch et al. 1992; Charrassin and Bost 2001; Groscolas and Robin 2001).

Individual telomere length has been suggested to be an integrative measure of individual quality (Nussey et al. 2014), since it is susceptible to a range of stressors the organism encounters during life (Epel et al. 2004; Kotrschal et al. 2007; Ilmonen et al. 2008; Entringer et al. 2011; Geiger et al. 2012; Haussmann et al. 2012; Boonekamp et al. 2014; Reichert et al. 2014; Aydinonat et al. 2014; Stier et al. 2014), and is positively associated with components of fitness (reproduction, survival rates) in the wild (Bize et al. 2009; Salomons et al. 2009; Bauch et al. 2012; Le Vaillant et al. 2015). In king penguins, measures of telomere length using qPCR have previously been positively related to crucial individual fitness features such as time of arrival on the breeding site and breeding performance. Consistent with the individual quality hypothesis, telomere length has also been positively related to individual immune capacity (i.e. natural antibody levels, Le Vaillant et al. 2015).

An alternative explanation for the observed pattern is that birds preferentially paired with similar aged partners, because of active mate choice or because of different dates of arrivals of young and older adults at breeding grounds as documented in other bird species (Cooke et al. 1976; Boag and Grant 1978; Coulter 1986; Delestrade 2001). The *age hypothesis* requires that variation in telomere length is mostly explained by age within a cohort of individuals. This means that inter-individual telomere loss is comparable over

346 lifetime, and that differences in telomere length that are measured at one age only reflect
347 difference in early life telomere length (Heidinger et al. 2012). This idea is opposed to the
348 *exposome hypothesis*, which suggests that the inter-individual variability in telomere length is
349 due to the inter-individual ability to respond to life stress (Monaghan and Haussmann 2006;
350 Haussmann and Heidinger 2015).

351 The age and exposome hypotheses are not mutually exclusive, since individuals that
352 survive to the oldest ages may be those in the best condition or of the highest quality.
353 However, if the exposome hypothesis were more influential, we would expect age-matched
354 individuals to exhibit highly variable telomere lengths due to variation in their past-exposure
355 to stress. In this case, the assortative mating pattern by telomere length would be little
356 influenced by age. On the other hand, if the age hypothesis were more influential, then age
357 should explain a large part of the variance in telomere length in samples of assortative mating
358 pairs. Bauch et al. (2013) studied individually marked common terns over several years, and
359 successful reproduction was associated with shorting of telomere lengths, regardless of age.
360 However, the least telomere shortening was found in the most successful pairs and in those
361 that failed early. Further, longer telomeres were strongly associated with fitness components
362 such as early breeding and greater reproductive success (Bauch et al. 2014). Thus, the
363 mechanisms that underlie assortative pairing by telomere length may be complicated, and
364 disentangling causal relationships among telomere length, individual quality, and mate choice
365 may inevitably prove to be difficult.

366 In king penguins, however, age assortative pairing may be unlikely. First, telomere
367 length in king penguins appears to be unrelated to age in 5-8 year-old adults (Le Vaillant et al.
368 2015; see also Monaghan 2010 for a review on telomere length and individual state). Second,
369 young king penguins are typically inexperienced birds of lower foraging efficiency (Le
370 Vaillant et al. 2012; Le Vaillant et al. 2013) and breeding success (Weimerskirch et al. 1992).

371 This appears to contradict the observation that long telomeres (i.e. hypothetically young birds)
372 are associated with higher breeding success (Le Vaillant et al. 2015). Finally, the breeding
373 phenology of king penguins (especially the 14-month period until chick independence)
374 produces a low success rate for initial pairs (only 30% Jouventin and Mauget 1996; Olsson
375 1996), and mate fidelity among subsequent breeding seasons is relatively low (about 21 %;
376 Olsson 1998; Jouventin 1999). Further, only about half of initially paired couples stay
377 together to breed (Olsson 1998a; Keddar et al. 2015a). King penguins thus frequently change
378 mates within and among breeding seasons. However, more information on age and pairing
379 patterns are required to test for an influence of age on assortative mating and breeding with
380 respect to telomere length.

381 Interestingly, the association between telomere length and reproductive success in the
382 present study was only significant for females and depended on year. Perhaps individual
383 quality has less influence when reproductive constraints are overwhelming, even for high
384 quality individuals, or universally absent in respectively particularly harsh or favourable
385 conditions. Years of poor environmental resources negatively influenced appearance of colour
386 ornaments used in mate choice and later social dynamics (Keddar et al. 2015b,c). Individual
387 quality may have the strongest consequences when competition over relatively limited but not
388 limiting resources occurs (Kirkwood and Rose 1991; Kirkwood and Austad 2000; Ricklefs
389 and Scheuerlein 2001). Our results on breeding success, based on chick survival rates,
390 suggested that reproduction was particularly successful in 2010 (16 chicks survived out of 20,
391 an 80% survival rate), while being lower the two other years (at about 40%). In 2010, all late
392 breeders failed at our study colony, mostly during incubation, which is consistent with
393 frequently observed low breeding success for late season breeders (Weimerskirch et al. 1992;
394 Olsson 1996; Dobson et al. 2008).

Chick survival rates were significantly associated with SAIOD values and were previously found to be reliable proxies of environmental conditions and yearly population breeding success at our study colony (Bost et al. 2015). Females with long telomeres exhibited higher breeding success in 2009 (higher chick survival), when environmental conditions (based on SAIOD estimates) were intermediate compared to 2010 (+) and 2011 (-). Our results also raise the question of whether telomere length is actually indicative of fitness in this species (fitness associations were only found for females and only in one out of three years). However, other results in different years and on different king penguins (both chicks and adults) repeatedly suggested strong associations between telomere length and fitness (Geiger et al. 2012; Stier et al. 2014; Le Vaillant et al. 2015). Alternatively, yearly differences in environmental conditions may preclude the detection of consistent telomere length-fitness relationships (e.g. 2011 was disastrous in terms of breeding success for the entire colony).

King penguins alternate early and late breeding dates mainly because a successful attempt is not compatible with early breeding the subsequent year (Weimerskirch et al. 1992; Jouventin and Mauget 1996; Olsson 1996; Dobson et al. 2008). However, more data are needed to better examine the actual impact of environmental conditions on links between telomere length and fitness. Male telomere length was unrelated to breeding success in our study, begging the question of why females mate with males of similar telomere length. Male king penguins take charge of the longest fasting period on-land during reproduction of *ca* 30 days (including courtship and the first incubation shift), while the female forages at sea (Weimerskirch et al. 1992). Our previous results highlighted an oxidative debt to prolonged fasting (Schull et al. 2016b), so that females may choose males with high antioxidant capacities, which would in turn affect telomere length (von Zglinicki 2002).

In a species where mutual mate choice is known to occur (Nolan et al. 2010; Keddar et al. 2015a), and where partner cooperation is essential for reproductive success, the present

results of assortative mating by telomere length provide support for the idea that telomere length might be a good proxy of individual condition. Those observations raise intriguing questions on the underlying mechanisms and fitness consequences of the significant and strong association of telomere lengths within breeding pairs.

Acknowledgements

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634 **Figures**
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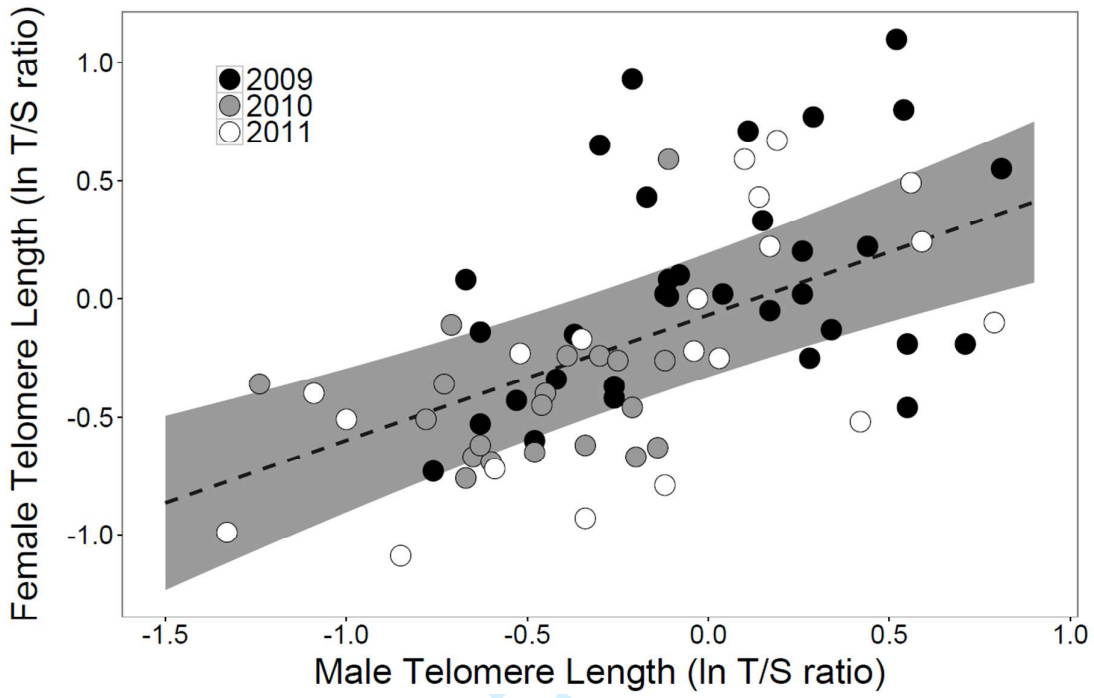

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639 **Figure 1.** Assortative pairing by telomere length in 73 king penguin pairs followed in 2009,
640 2010 or 2011 in the Crozet archipelago. Relationship between male and female telomere
641 lengths over the three years (LM; y = 0.45x - 0.063; $r^2 = 0.39$, $F_{1,73} = 36.10$, $P < 0.001$, for
642 detail see Table 1A).

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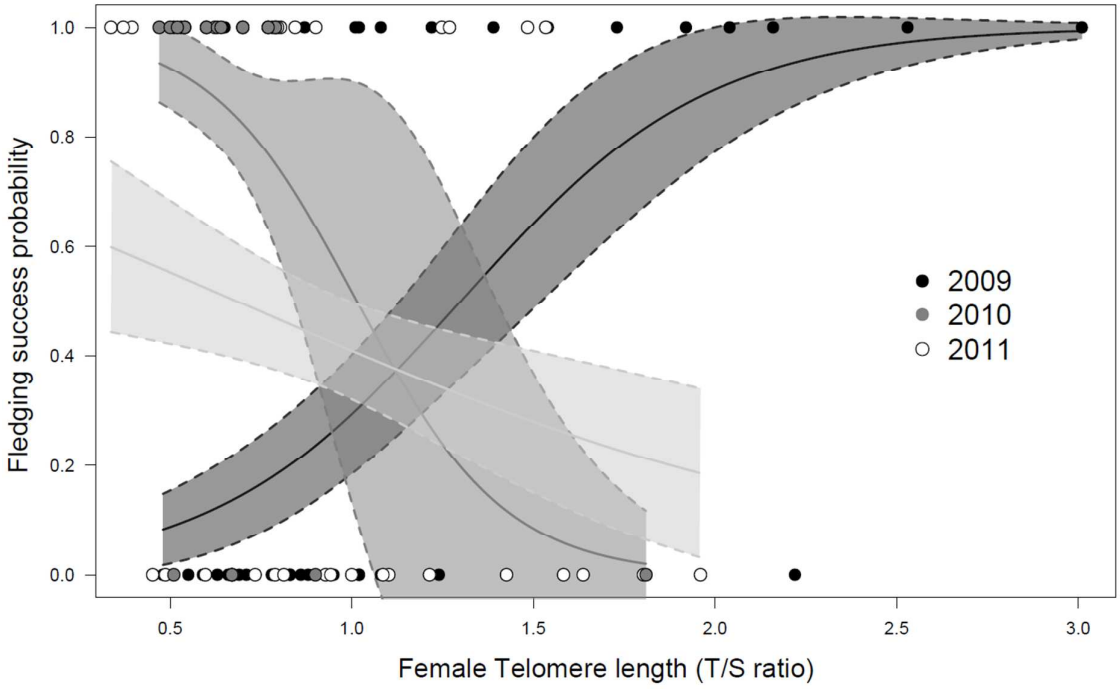

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646 **Figure 2.** Estimated ±SE Fledging success (in percentage) with in relation to Female
647 Telomere length (T/S ratio). Individual observed data are plotted as symbols. In 2009, females
648 with longer RTL were more successful at raising their chick (GLM, z = 2.75, P = 0.006) but
649 no such pattern was evident in 2010 and 2011 (GLMs, z = -0.783, *P* = 0.43380 and *z* = -1.369,
650 *P* = 0.1709 respectively)

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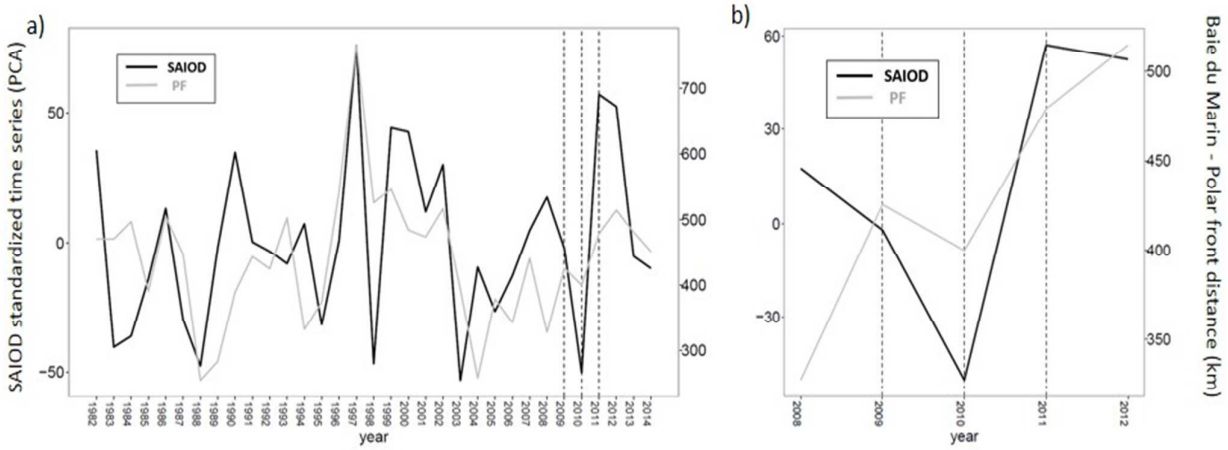

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654 **Figure 3:** (a) South Atlantic and Indian Oceans dipole (SAIOD) and polar front distance from
655 the Baie du Marin colony, time series over 1982 to 2014; (b) zoom on years 2008-2012. The
656 dashed lines represent the three years considered in our study. Based on SAIOD estimations,
657 2011 was an unfavourable breeding year in terms of distances of foraging, while 20010 was
658 more favourable, 2009 being characterized by intermediate distances of the polar front.

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665 **Table 1.** Linear model estimates for the relationship between male and female telomere
666 length (TL) in king penguins breeding pairs in years 2009, 2010 and 2011. The factor *Years* in
667 the model is compared against 2009 as a reference level.
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Female TL (N=73)	**Estimates**	**± SE**	***t*-value**	***P*-value**
Intercept	0.06	0.07	0.91	0.36
Male TL	0.45	0.16	2.78	0.007
Years$_{[2009]}$				
2010	-0.36	0.19	-1.89	0.06
2011	-0.18	0.11	-1.56	0.12
Male TL x Years$_{[2009]}$	-0.19	0.36	-0.53	0.60
2010	0.14	0.22	0.63	0.53
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704 **Table 2.** Correlation coefficients (Pearson's *r*), *P*-values and samples sizes for relationships
705 between (A) male and female morphological traits (i.e. flipper and beak lengths, body mass);
706 and (B) between morphological traits and telomere lengths in females and males (ln-
707 transformed TL).

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(A) Correlations between male and female morphological traits			
Males / Females	*Flipper length*	*Beak length*	*Body mass*
Flipper length	r = 0.057 P = 0.636 *n* = 73	-0.092 0.437 73	0.039 0.886 16
Beak length	-0.019 0.876 73	-0.017 0.88 73	0.268 0.315 16
Body mass	0.316 0.408 9	0.409 0.274 9	-0.219 0.636 7
(B) Correlation between male or female telomere length and morphological traits			
	Flipper length	*Beak length*	*Body mass*
Female telomere length	r = -0.175 P = 0.140 *n* = 72	-0.019 0.874 72	-0.504 0.055 15
Male telomere length	-0.070 0.555 73	0.197 0.094 73	0.199 0.609 9

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728 **Table 3.** Linear model estimates for the relationship between (A) female or (B) male telomere
729 length (TL) and chick body mass at 10-days or chick survival until fledging. The factor *Years*
730 in both model is compared against 2009 as a reference level. For chick survival, a Generalized
731 Linear Model was used, fitted with a binomial error distribution.
732

(A) Females (N=73)	**Estimates**	**± SE**	***t (or z)*-value**	***P*-value**
Chick body mass (g) at day 10				
Intercept	629.45	25.26	24.92	<0.001
Female TL	61.78	54.30	1.14	0.259
Years$_{[2009]}$				
2010	-132.98	61.25	-2.17	0.034
2011	-10.76	42.95	-0.25	0.803
Female TL x Years$_{[2009]}$				
2010	-120.39	121.76	-0.99	0.326
2011	-78.39	81.98	-0.96	0.342
Chick survival to fledging				
Intercept	-4.39	1.67	-2.63	0.008
Female TL	3.57	1.30	2.75	0.006
Years$_{[2009]}$				
2010	1.39	1.31	1.06	0.288
2011	0.94	0.71	1.32	0.187
Chick body mass at day 10	0.006	0.002	2.39	0.017
Female TL x Years$_{[2009]}$				
2010	-7.17	2.94	-2.44	0.015
2011	-4.31	1.61	-2.68	0.007
(B) Males (N=73)				
Chick body mass (g) at day 10				
Intercept	633.15	25.08	25.25	<0.001
Male TL	-44.64	58.88	-0.76	0.451
Years$_{[2009]}$				
2010	-158.43	68.55	-2.31	0.024
2011	-7.70	41.85	-0.18	0.855
Male TL x Years$_{[2009]}$				
2010	-53.19	130.45	-0.41	0.685
2011	64.22	81.38	0.79	0.433
Chick survival to fledging				
Intercept	-3.99	1.44	-2.77	0.006
Male TL	0.20	0.97	0.21	0.834
Years$_{[2009]}$				
2010	2.78	1.26	2.22	0.027
2011	0.75	0.63	1.19	0.233
Chick body mass at day 10	<0.01	<0.01	2.65	0.008
Male TL x Years$_{[2009]}$				
2010	0.53	2.21	0.239	0.811
2011	-0.80	1.29	-0.62	0.533

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Electronic Supplementary Material. *"Assortative pairing by telomere length in king penguins and relationships with breeding success"*

ESM1 Telomere length qPCR measurements in king penguins

A. Examples of amplification (left panels) and melting curves (right panels) of king penguin DNA amplification by qPCR of control gene (Figure 1A) and telomere sequences (Figure 1B).

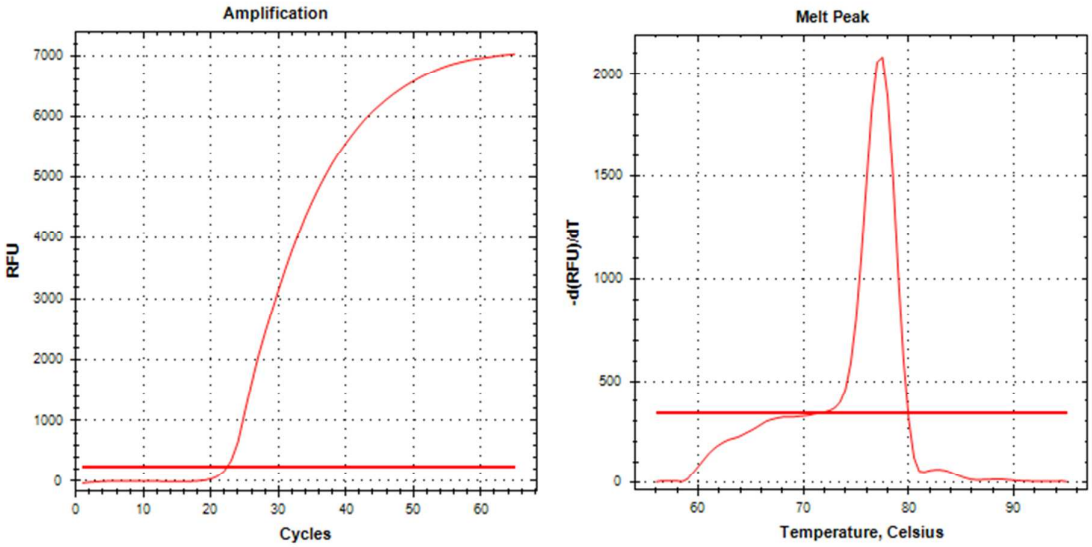

Figure 1A: Amplification and melting curve of king penguin DNA obtained using *Aptenodytes patagonicus* zinc finger protein primers.

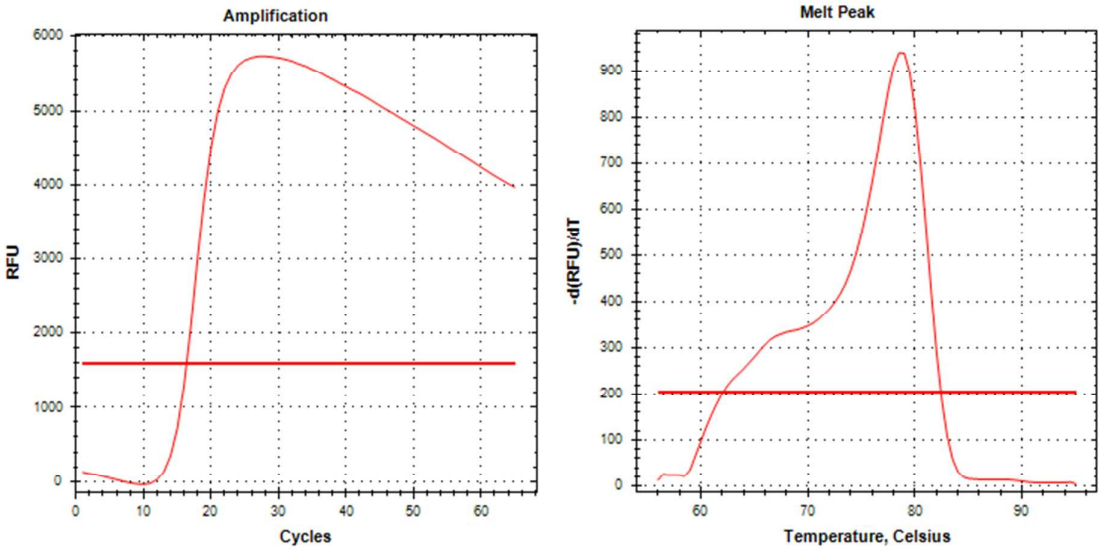

Figure 1B: Amplification and melting curve of king penguin DNA obtained using telomere primers.

B. Amplification values of the standard curves obtained in the run 2010 (A: telomere sequence amplification, B: control gene amplification). Those standard curves were obtained based on serial dilutions of one random king penguin sample of a given year. Each sample was done in duplicate (Cq1 and Cq2). Values of the 2009 and 2011 years were of similar ranges.

A. Standard curve (TEL)	Mean Cq value 2009	Mean Cq value 2010	Mean Cq value 2011
NTC-01	34.41	34.56	34.55
Std-01	16.63	16,56	16.57
Std-02	17.61	17,34	17.67
Std-03	18.53	18,34	18.34
Std-04	19.46	19,49	19.78
Std-05	20.50	20,62	20.62
B. Standard curve (Ctrl)			
NTC-01	>40	39.31	>40
Std-01	23.98	23,53	24.32
Std-02	24.89	24,72	24.98
Std-03	25.79	25,72	25.78
Std-04	26.47	26,63	26.99
Std-05	28.27	27,56	27.89

C. Amplification efficiencies of standard curves (done based on serial dilutions of one random king penguin sample of a given year) measured on the runs 2009, 2010, 2011.

Amplification efficiencies %	Ctrl	TEL
2009	102,9	103,1
2010	100,6	100,9
2011	102.5	102.3

D. Identities and amplification values of the golden standards used to calculate the relative telomere lengths of adult king penguins in the qPCR runs of 2009, 2010, 2011. The T/S value of the golden standards is 1 in each year.

Golden samples values	Identity	Crtl Cq	Tel Cq
Plate 2009	PE21	25,89	18,65
Plate 2010	ADE04	26,62	18,19
Plate 2011	54	25,97	18,83

E. Intra and inter-plates coefficients of variation of telomere (Tel Cq) and single control gene (Ctrl Cq) amplification cycle numbers, and of the final relative telomere length value (T/S ratio calculated following Pfaffl (2001)).

Intra-plate CVs (%)	Tel Cq	Ctrl Cq	T/S
2009	1,31	0,75	0,13
SE	0,09	0,06	0,01
2010	1,52	0,74	13,70
SE	0,13	0,07	1,28
2011	1,15	0,87	10,19
SE	0,22	0,21	1,56
Inter-plate CVs (%)	**Tel Cq**	**Ctrl Cq**	**T/S**
2009-2010-2011	1,86	0,82	10,30
SE	0,24	0,14	1,80

References

1. Pfaffl, M.W. 2001 A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research* **29**, 2003-2007.

N°	ID	father/mother	group	year	T/S
1	1.1	father	**EARLY**	**2009**	**0.92**
2	1'	mother	**EARLY**	**2009**	**1.10**
3	5.1	father	**EARLY**	**2009**	**0.53**
4	5'	mother	**EARLY**	**2009**	**0.87**
5	10	father	**EARLY**	**2009**	**0.90**
6	1.10'	mother	**EARLY**	**2009**	**1.01**
7	11.1	father	**EARLY**	**2009**	**0.74**
8	11'	mother	**EARLY**	**2009**	**1.92**
9	12.1	father	**EARLY**	**2009**	**1.74**
10	12'.1	mother	**EARLY**	**2009**	**0.63**
11	14.1	father	**EARLY**	**2009**	**1.18**
12	14'	mother	**EARLY**	**2009**	**0.95**
13	16.1	father	**EARLY**	**2009**	**0.90**
14	16'	mother	**EARLY**	**2009**	**1.08**
15	18.1	mother	**EARLY**	**2009**	**1.02**
16	18'	father	**EARLY**	**2009**	**1.04**
17	20	mother	**EARLY**	**2009**	**1.24**
18	20'	father	**EARLY**	**2009**	**1.55**
19	L2	mother	**LATE**	**2009**	**0.48**
20	L2'	father	**LATE**	**2009**	**0.47**
21	L4	father	**LATE**	**2009**	**0.53**
22	L4'	mother	**LATE**	**2009**	**0.59**
23	L7	father	**LATE**	**2009**	**0.77**
24	L7'	mother	**LATE**	**2009**	**0.69**
25	L9	father	**LATE**	**2009**	**0.62**
26	L9'	mother	**LATE**	**2009**	**0.55**
27	L12	mother	**LATE**	**2009**	**0.83**
28	L12'	father	**LATE**	**2009**	**2.03**
29	L17	father	**LATE**	**2009**	**1.74**
30	L17'	mother	**LATE**	**2009**	**0.83**
31	L22	father	**LATE**	**2009**	**0.59**
32	L22'	mother	**LATE**	**2009**	**0.65**
91	4	father	**EARLY**	**2009**	**0.84**
92	4'	mother	**EARLY**	**2009**	**1.54**
93	6	father	**EARLY**	**2009**	**0.81**
94	6'	mother	**EARLY**	**2009**	**2.53**
95	7.1	father	**EARLY**	**2009**	**0.51**
96	7'	mother	**EARLY**	**2009**	**1.08**
97	9.1	father	**EARLY**	**2009**	**1.68**
98	9'	mother	**EARLY**	**2009**	**3.01**
99	13.1	father	**EARLY**	**2009**	**1.30**
100	13'	mother	**EARLY**	**2009**	**1.02**
101	15.1	father	**EARLY**	**2009**	**1.12**
102	15'	mother	**EARLY**	**2009**	**2.04**
103	17.1	father	**EARLY**	**2009**	**1.71**
104	17'	mother	**EARLY**	**2009**	**2.22**

105	19.1	mother	**EARLY**	**2009**	**1.02**
106	19'	father	**EARLY**	**2009**	**0.89**
107	21	mother	**EARLY**	**2009**	**0.78**
108	22	mother	**EARLY**	**2009**	**1.22**
109	22'	father	**EARLY**	**2009**	**1.30**
110	L3	father	**LATE**	**2009**	**0.68**
111	L5	father	**LATE**	**2009**	**0.77**
112	L5'	mother	**LATE**	**2009**	**0.66**
113	L8	mother	**LATE**	**2009**	**0.86**
114	L8'	father	**LATE**	**2009**	**0.69**
115	L11	mother	**LATE**	**2009**	**1.73**
116	L11'	father	**LATE**	**2009**	**2.24**
117	L13	father	**LATE**	**2009**	**1.40**
118	L13'	mother	**LATE**	**2009**	**0.88**
119	L14	mother	**LATE**	**2009**	**1.39**
120	L14'	father	**LATE**	**2009**	**1.16**
121	L16	mother	**LATE**	**2009**	**2.16**
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123	L18	father	**LATE**	**2009**	**1.32**
124	L18'	mother	**LATE**	**2009**	**0.78**
125	L20	mother	**LATE**	**2009**	**0.71**
126	L20'	father	**LATE**	**2009**	**0.66**
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129	A03	mother	**EARLY**	**2010**	**1.81**
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131	A04	mother	**EARLY**	**2010**	**0.53**
132	A04*	father	**EARLY**	**2010**	**0.87**
133	A05	mother	**EARLY**	**2010**	**0.47**
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137	A12	mother	**EARLY**	**2010**	**0.70**
138	A12*	father	**EARLY**	**2010**	**0.48**
139	A13	father	**EARLY**	**2010**	**0.82**
140	A13*	mother	**EARLY**	**2010**	**0.51**
141	A19	mother	**EARLY**	**2010**	**0.77**
142	A19*	father	**EARLY**	**2010**	**0.78**
143	A20	mother	**EARLY**	**2010**	**0.90**
144	A20*	father	**EARLY**	**2010**	**0.49**
145	A21	mother	**EARLY**	**2010**	**0.79**
146	A21*	father	**EARLY**	**2010**	**0.68**
147	A22	mother	**EARLY**	**2010**	**0.77**
148	A22*	father	**EARLY**	**2010**	**0.89**
181	A06	mother	**EARLY**	**2010**	**0.60**
182	A06*	father	**EARLY**	**2010**	**0.46**
183	A07	mother	**EARLY**	**2010**	**0.79**

184	A07*	father	**EARLY**	**2010**	**0.74**
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186	A08*	father	**EARLY**	**2010**	**0.71**
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191	A14	mother	**EARLY**	**2010**	**0.50**
192	A14*	father	**EARLY**	**2010**	**0.55**
193	A15	father	**EARLY**	**2010**	**0.63**
194	A15*	mother	**EARLY**	**2010**	**0.64**
195	A16	father	**EARLY**	**2010**	**0.53**
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220	ADE*03* 18.02.11	father	**EARLY**	**2011**	**1.026**
221	ADE*04* 30.01.11	father	**EARLY**	**2011**	**0.986**
222	ADE*05* 26.01.11	father	**EARLY**	**2011**	**0.962**
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226	ADE*10* 15.02.11	father	**EARLY**	**2011**	**0.452**
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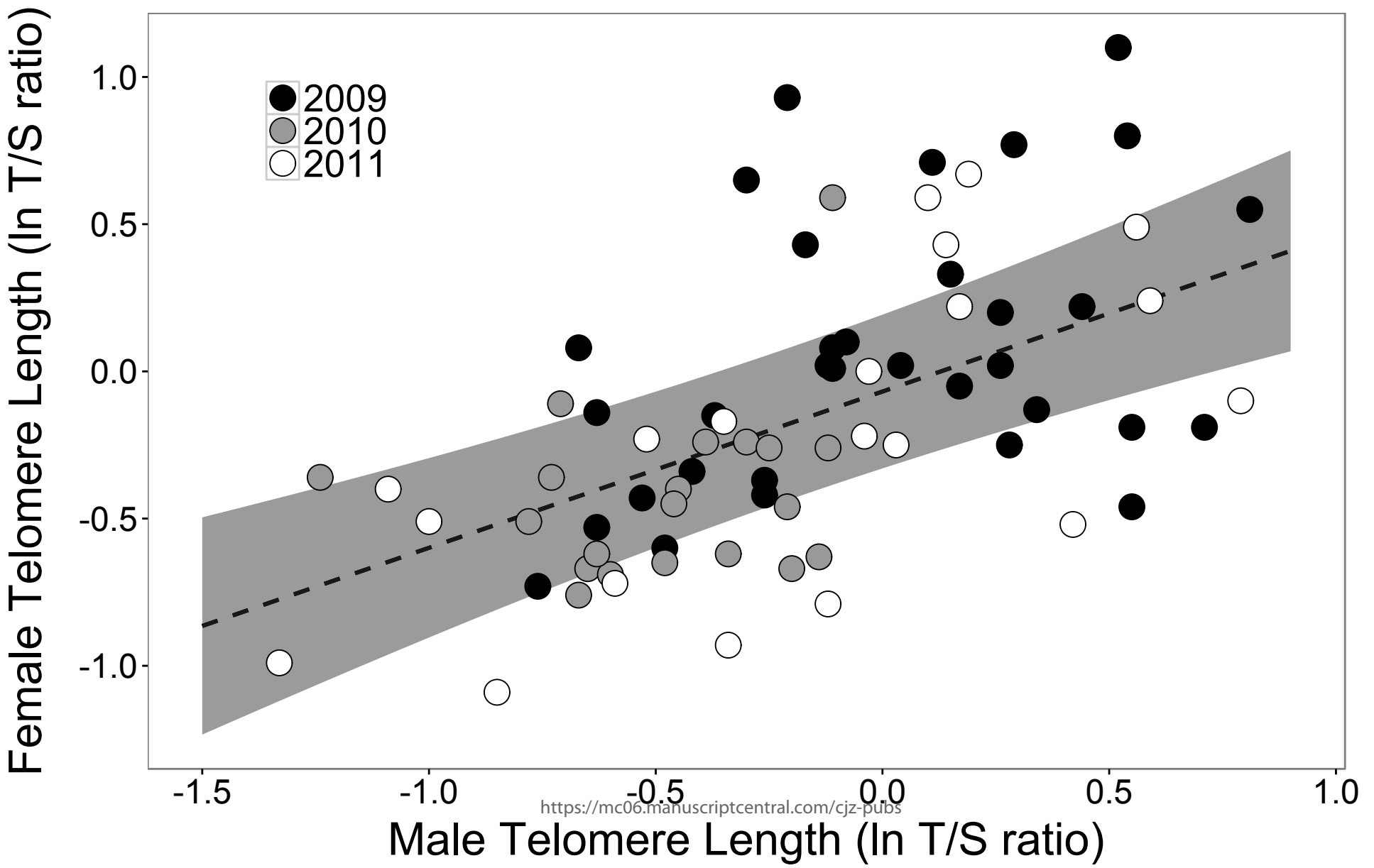
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2010
2011
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Male Telomere Length (ln T/S ratio)
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0.0
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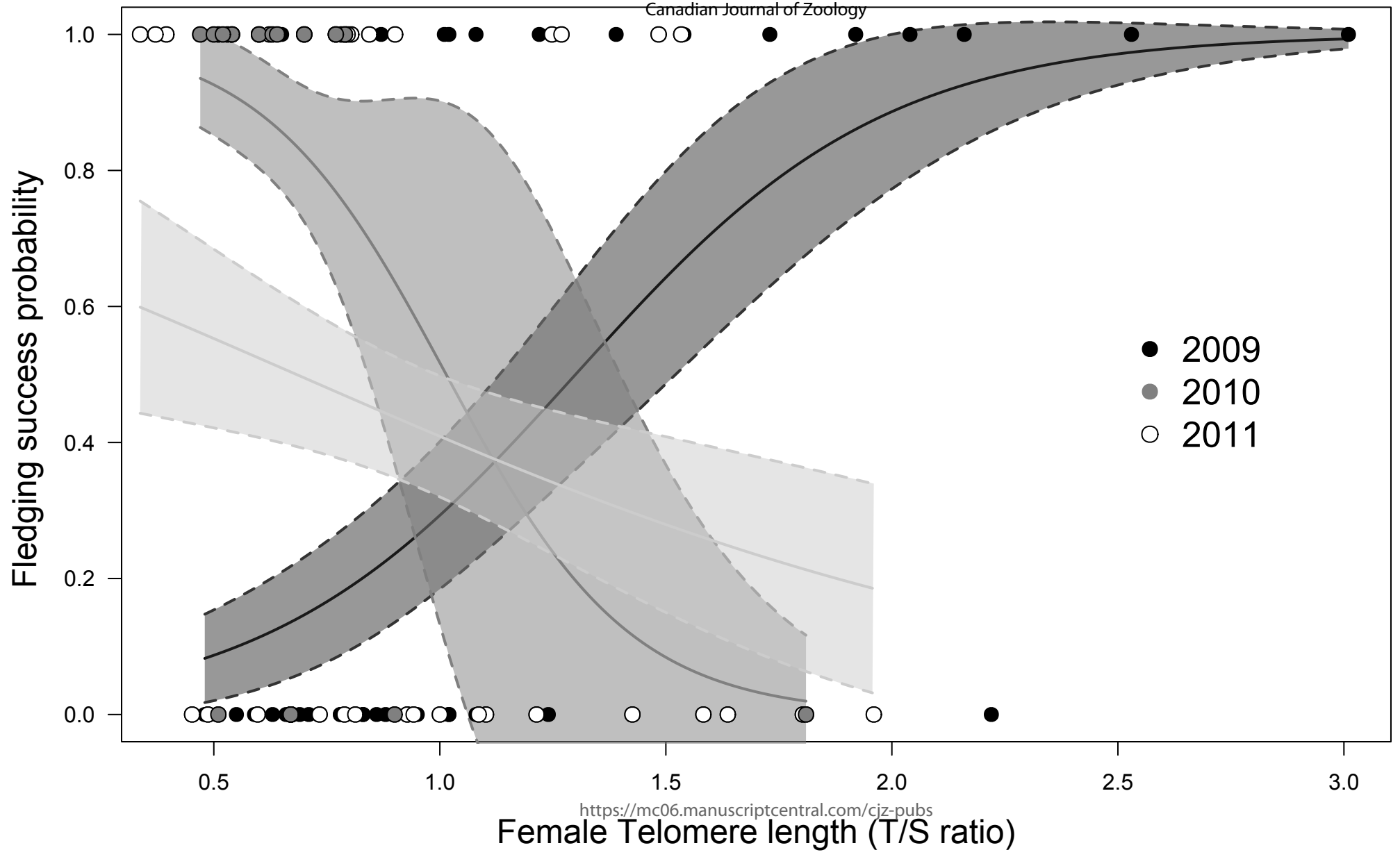
1.0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0.4
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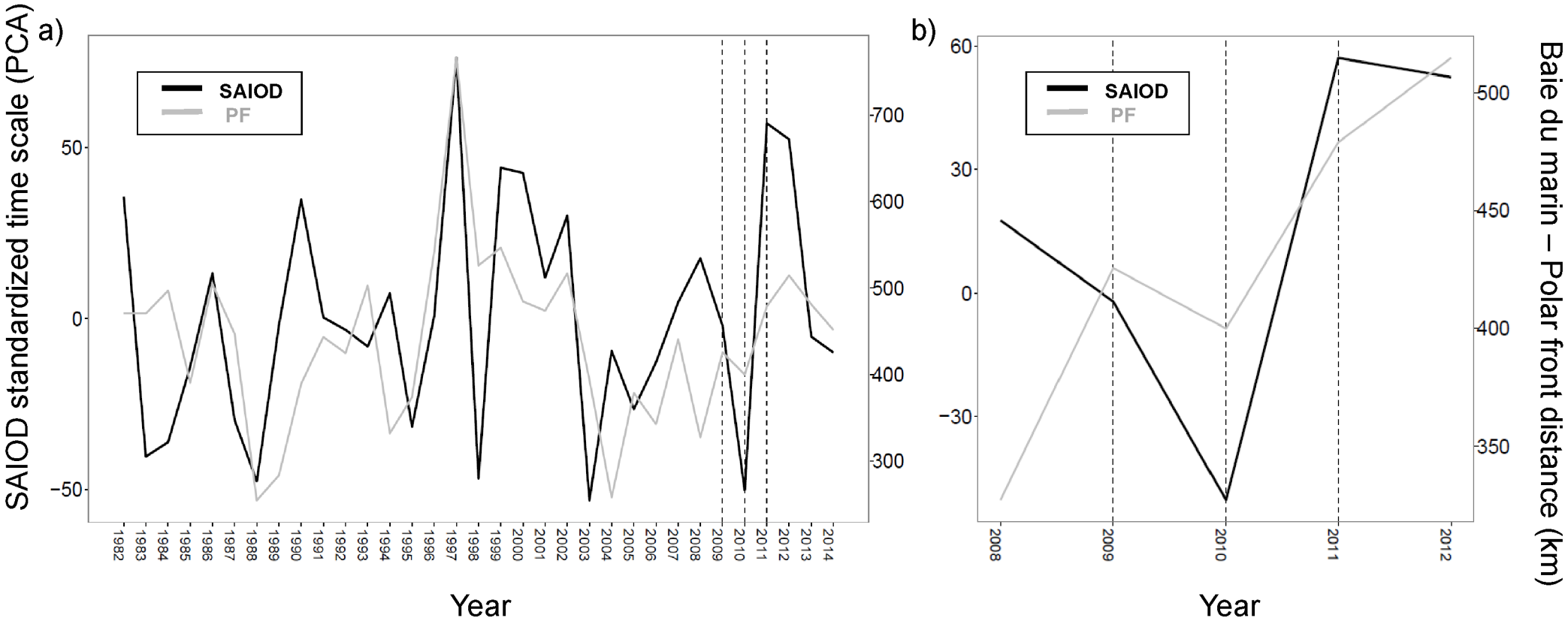
2.0
2.5
3.0
2009
2010
2011
Female Telomere length (T/S ratio)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SAIOD
PF
SAIOD standardized time scale (PCA)
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600
500
400
300
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1983
1984
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1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
Year
b)
SAIOD
PF
60
30
0
−30
500
450
400
350
2008
2009
2010
2011
2012
Year
Baie du marin – Polar front distance (km)