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# 1 Stochastic modelling of length dependent telomere shortening in *Corvus monedula*

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## 11 12 13 Abstract

14  
15 It was recently shown that, within individuals, longer telomeres shorten at a higher rate.  
16 This explorative study deals with a mathematical model of this process. It is a nonlinear  
17 differential equation describing length-dependent decrease that can be linked to a  
18 Poisson process. The model also takes in account telomere shortening due to the end  
19 replication problem. Parameters are fitted using data from samples of red blood cells of  
20 free-living juvenile corvids. The Poisson process can be related to oxidative stress  
21 causing DNA strand breaks. The shortest telomeres in a genome are the best predictors  
22 of survival, and one can therefore hypothesize on functional grounds that short  
23 telomeres should be better protected by some control mechanism in the cellular system.  
24 However, the present study shows that such a mechanism is not required to explain  
25 length-dependent telomere shortening: agents of telomere shortening such as oxidative  
26 stress with a certain strength modeled by a Poisson process with an appropriately  
27 chosen parameter suffices to generate the observed pattern.

28  
29 **Key words** telomere shortening, end replication, DNA strand break, oxidative stress,  
30 stochastic process

## 31 32 33 1. Introduction

34  
35 Telomeres are highly structured, non-coding DNA elements at the end of linear  
36 eukaryotic chromosomes. In vertebrates they consist of tandem repeated highly  
37 conserved DNA sequence (5'-TTAGGG-3')<sub>n</sub>. Telomeres play an important role in the  
38 protection of chromosome integrity (Blackburn, 1991) and in the longevity of  
39 organisms: associations between telomere length and survival were demonstrated in  
40 humans (Cawthon et al., 2003; Kimura et al., 2008; Bakaysa et al., 2007; Aviv, 2008),  
41 birds (Hausmann et al., 2005; Bize et al., 2009; Salomons et al., 2009) and nematodes  
42 (Joeng et al., 2004). Moreover, comparing species, it was found that the telomere  
43 shortening rate is correlated with maximum lifespan: short lived species lose their  
44 telomeres at a higher rate (Hausmann et al., 2003). It has been anticipated that telomere  
45 length, and its shortening rate in particular, may potentially be used as a proxy for the  
46 'life stress' experienced by individual organisms (Epel et al., 2006; Kotrschal et al.,  
47 2007) and hence serve as a marker of an individual's 'biological age'. Telomere  
48 shortening rate is accelerated by oxidative stress (Von Zglinicki, 2002; Tchirkov and  
49 Lansdorp, 2003). Since oxidative stress is often considered a major agent of senescence  
50 (Beckman and Ames, 1998), it provides a potential mechanistic link explaining  
51 associations between telomeres and life span, in that shorter telomeres may indicate that

1 the organism experienced higher levels of oxidative stress (Jennings et al., 2000;  
2 Monaghan and Haussmann, 2006). The length of the shortest telomere(s) has been  
3 suggested to determine when cellular senescence starts (e.g. Hemann et al., 2001;  
4 Capper et al., 2007; Di Fagagna, 2003; Shay and Wright 2005). On functional grounds  
5 one expects therefore that defensive mechanisms against DNA damage have evolved to  
6 act stronger on chromosomes with short telomeres (Karlseder et al., 2002), resulting in a  
7 lower shortening rate of shorter telomeres (Hemann et al., 2001; Martens et al., 2000).

8 In literature several models of telomere dynamics can be found (Proctor and  
9 Kirkwood, 2002a; Sidorov et al., 2004; Rodriguez-Brenes and Peskin, 2010). The  
10 present study differs from these studies in that we develop a stochastic model that  
11 specifically describes length-dependent telomere shortening. As an alternative for  
12 intensified DNA repair (negative feedback control), or some form of increased  
13 protection, that may explain why shorter telomeres decrease slower in length (Karlseder  
14 et al., 2002), we choose to test whether a simpler mechanism, not based on an active  
15 response of the cell, could be sufficient to generate the observed pattern. We assume  
16 that over a short telomere interval the risk of breaking of a telomere within a fixed time  
17 span is proportional with the length of that interval. Thus, we build on the assumption  
18 that telomeres are simply more vulnerable if they are larger targets. In line with Nitecki  
19 and Hoffman (1987) and Wilkinson (2006) we choose the Poisson process to describe  
20 this effect in a stochastic model. A mathematical model is formulated based on  
21 biological and physical arguments. In a next step the parameters of this model are fitted  
22 to data of empirical observations (Salomons et al., 2009). We consider data on telomere  
23 length of samples of blood cells of nesting jackdaws (*Corvus monedula*) from an  
24 observational study (Salomons et al., 2009). The data has random components requiring  
25 a robust model with a small number of parameters.

26 In an earlier study telomere length and its shortening in erythrocytes of jackdaws  
27 has been quantified (Salomons et al., 2009). Telomere length varies between  
28 erythrocytes and between chromosomes within a genome (Baird et al., 2003; Lansdorp  
29 et al., 1996; Martens et al., 1998). A degree of randomness within the population of  
30 hematopoietic stem cells that generate the erythrocytes is one of the sources of this  
31 variation. Salomons et al. (2009) used pulsed-field gel electrophoresis to measure  
32 telomeres. The method produces genome-wide distributions of telomere lengths for  
33 each sample. Hence it provides the opportunity to quantify telomere shortening  
34 separately for subsets of these size distributions (Haussmann et al., 2008). Indeed these  
35 data showed that, within adult and juvenile individuals, shortening of telomeres was size  
36 dependent, with longer telomeres losing base pairs at a higher rate.

37 In Section 2 we describe the way the data has been collected. In nestling  
38 jackdaws the shortening of telomeres was quantified by percentiles of the length  
39 distribution over the full interval (0, 100) at day 5 and day 30. From a graphical  
40 representation of the differences between equal percentiles at the two observation  
41 moments we already observe that in the subjects of our study length-dependent telomere  
42 shortening occurs in the sense that in the mean shorter telomeres exhibit a smaller  
43 shortening rate. In Section 3 we discuss mechanisms that may cause telomere  
44 shortening. First, there is the end replication problem being part of the cell division  
45 process (Olovnikov, 1996). Secondly, we consider the breaking of a telomere occurring  
46 spontaneously, e.g. due to oxidative stress, and model it by a Poisson process. In first  
47 instance we only consider a deterministic model based on the mean effect of these two  
48 mechanisms. We estimate the parameters in that model and give an estimate of the  
49 contribution of the different terms to the process. In Section 4 we use a Brownian  
50 motion process related to the Poisson process by choosing drift and diffusion that  
51 correspond with those of the Poisson process. Again we estimate parameters, now for

1 one individual, and compare the results of the different approaches. Finally, we  
 2 summarize our findings and draw conclusions in Section 5.

## 3 4 5 **2. Data collection**

6  
 7 We used data on free-living nestling jackdaws, a hole breeding semi-colonial bird  
 8 species, in the colony at the Biological Centre in Haren (The Netherlands), a semi-urban  
 9 environment, and in five smaller colonies located in a more rural area 5-10 kilometres  
 10 south of Haren, see Salomons et al. (2009) for more details. In brief, at the ages of 5 and  
 11 30 days a small blood sample was taken for DNA, and telomere length in erythrocytes  
 12 was measured using pulsed field electrophoresis. The size distribution of telomeres was  
 13 determined through densitometry using the open-source software ImageJ version 1.38x  
 14 (Haussmann et al., 2008). The distribution of terminal restriction fragments is usually  
 15 characterized using the average telomere length only. However, as explained above,  
 16 telomere shortening rate may not be the same for chromosomes with short as for the  
 17 chromosomes with long telomeres. The telomere distributions were characterized not  
 18 only by the mean, but also by the mode and by 21 equidistant percentiles.

19 From the two blood samples of 75 jackdaws, taken at 5 and 30 days of age, the  
 20 distribution of the mean change in length between these two days is given in Figure 1.  
 21 The global mean of change takes the value -223.7 bp being significantly negative. In  
 22 Figure 2a we give a graphical representation of the data of one jackdaw: the distribution  
 23 of the length is depicted by percentiles for both days. In Figure 2b an approximation of  
 24 the probability density function is derived from these data. Looking in more detail at the  
 25 change in length for a given percentile of all 75 jackdaws, we observe that percentiles at  
 26 the lower end (0, 5) behave irregularly compared with the larger percentiles (>10). A  
 27 similar, but less outspoken, behaviour is found at the upper end. The tails of the  
 28 distribution are more difficult to measure than the denser parts, and we therefore  
 29 attribute these fluctuations to measurement error. For this reason we only use the  
 30 percentiles  $p_i = 5i$ ,  $i = 2, \dots, 18$  in our penalty function when making parameter  
 31 estimates. In a graph for each of the 75 cases (Fig.3) we give the change of length  
 32 percentiles between day 5 and day 30 as a function of the mean

$$33 \quad \Delta p_i = \Delta p_i(\underline{p}_i), \quad \Delta p_i = p_i(30) - p_i(5) \quad \text{and} \quad \underline{p}_i = (p_i(5) + p_i(30))/2, \quad i = 2, \dots, 18.$$

34  
 35  
 36 The curves show large differences. As possible causes for this strong variation we  
 37 mention differences in genotype and (non)active cellular processes (telomerase).  
 38 Moreover, external circumstances like measurement errors are expected to play a role  
 39 From the figure we observe indeed that there is a mainstream of curves that have a  
 40 tendency of a smaller decrease at a smaller length, see Salomons et al. (2009) for a  
 41 different analysis with the same conclusion.

## 42 43 44 **3. Mechanisms for telomere shortening**

45  
 46 We next explore possible shortening mechanisms and formulated a model as follows. It  
 47 consists of a sequence of cell generations from which one cell is selected at each  
 48 generation. The cells have a mother-daughter relation and one of the daughters is  
 49 selected randomly. Events, such as replication and strand breaks, are only marked in  
 50 time by the rate they occur per unit of time in this process. In this way we choose a

1 single path in the branching process of cell proliferation. Repeating this selection of  
 2 descendents we obtain a set of histories that is representative for the cell population.  
 3 The fact that the population of cells grows does not play any role in this respect.  
 4 Compared with other studies in which the event of cell division and cell population  
 5 dynamics are also included (e.g. Olofsson and Kimmel, 1999) our choice makes the  
 6 model slightly less realistic. However, the advantage of this approach is that we  
 7 concentrate more on the mechanism of shortening itself. Instead of making the  
 8 assumption that length-dependent shortening is the result of some active control  
 9 mechanism that enhances DNA repair at shorter length (Karlseder et al., 2002), we  
 10 consider a passive process in which damage occurs at some rate due to external  
 11 conditions. Proctor and Kirkwood (2002b) gave a detailed description on the possible  
 12 effect of single strand breaks. In some cases full repair is likely, while in other partial  
 13 repair or no repair at all may occur leading to shortening of telomeres in daughter cells.  
 14 Since our modeling is based on realized shortening as accounted for in the data we only  
 15 consider the end result.

16 A fixed length at the end of the telomere is lost at the moment of replication  
 17 (Okazaki fragments), see e.g. Olovnikov (1996) for more about the *end replication*  
 18 *problem*. It is modeled by the fixed rate  $c$  of shortening. Base pairs form a string: if one  
 19 base pair is taken out, then the free end part will also be lost. Since we do not have  
 20 much information how the break of a strand from e.g. oxidative stress takes place, we  
 21 take the *Poisson process* as starting point of our model formulation, see Nitecki and  
 22 Hoffman (1987) and Wilkinson (2006) for general information on the prominent place  
 23 of Poisson processes in biology.

24 Let us consider a time interval  $(t, t + \Delta t)$  and compute the change of the telomere  
 25 length  $\Delta x$  over that time interval. The constant decrease of length due to the end  
 26 replication problem is then  $c\Delta t$ . Given that it was shown that the risk of a double strand  
 27 break is uniformly distributed (Petersen et al., 1998; Von Zglinicki, 2000; Sitte et al.,  
 28 2001), we can model the probability that a double strand break occurs by a *Poisson*  
 29 *distribution* with parameter  $\mu = ax(t)\Delta t$ . In fact we have a two dimensional Poisson  
 30 process: the risk of a break is assumed to be proportional with the length  $x(t)$  of the  
 31 telomere as well as with the length  $\Delta t$  of the time interval. For  $\Delta t$  sufficiently small the  
 32 event of two or more breaks can be neglected. The probability of a break follows from a  
 33 stochastic variable  $P(t)$  having a standard uniform distribution:  $P(t) \sim \text{Uniform}(0, 1)$ .

$$34 \quad \text{If } P(t) \leq \mu \quad \text{then} \quad x(t + \Delta t) = R(t) x(t)$$

35  
 36 with again  $R(t) \sim \text{Uniform}(0, 1)$ . In the Appendix we show that for  $\Delta t \rightarrow 0$  the  
 37 expected value of the length satisfies the differential equation  
 38  
 39

$$40 \quad x'(t) = -c - \frac{1}{2} ax(t)^2. \quad (1)$$

41  
 42 The above model only accounts for length decrease. It is well known from various  
 43 studies, see e.g. Jaskelioff et al. (2011), that there are processes in the cellular system  
 44 that cause an increase of the length of telomeres (telomerase). From the 75 cases of our  
 45 study 17 showed a slight mean increase of the telomere length over the 25 days. It is  
 46 also noted that in a small number of cases length decrease speeded up at shorter  
 47 telomere lengths, see Figure 3. Both phenomena are not taken up in our model. As  
 48 mentioned above, the data are rather noisy and it is not clear whether either biological  
 49 forces or measurement errors are responsible for this strong variation. This restriction

1 only allows us to introduce a model with a small number of parameters if we also want  
 2 to produce a reliable estimate for the parameters from the available data.

### 3 4 *Parameter estimation*

5 The differential equation (1) with initial value  $x(0) = x_5$  has an exact solution  
 6

$$7 \quad x(t; a, c) = \frac{q}{a} \tan\left(\arctan\left(\frac{ax_0}{q}\right) - \frac{1}{2}qt\right), \quad q = \sqrt{2ac} \quad \text{for} \quad ac > 0 \quad (2)$$

8  
 9 For  $t = 25$  the variable  $x$  denotes the telomere length at day 30. As a matter of fact in the  
 10 computations needed for doing the parameter estimation a numerical solution of (1) is  
 11 used because for  $q$  close to zero the accuracy of the solution produced by formula  
 12 manipulation software breaks down; a problem that does not show up in the numerical  
 13 integration of (1).

14 In a deterministic model of type (1) different chromosomes remain in time in the  
 15 same order with respect to their length. The model can be used to predict the length at  
 16 day 30 of a given percentile using the initial length of the same percentile at day 5. A  
 17 comparison of the outcome at day 30 with the observation will lead to an estimate of the  
 18 parameters  $a$  and  $c$  by minimizing the sum of the squared differences for percentiles  
 19 10(5)90 of all 75 cases (Table I). Moreover, we give an estimate  $f_{(a)}$  of the size of the  
 20 quadratic term of the right hand side of (1) by substituting  $x = (x_5 + x_{30})/2$  averaged over  
 21 all runs needed for computing the residual sum of squares for a given choice of the  
 22 parameters. A similar estimate is made for the other term giving  $f_{(c)}$ . The estimate for the  
 23 1-parameter model ( $a = 0$ ) is given as well. We see that for the 2-parameter model the  
 24 contribution to the length decrease is about equally distributed over the Poisson process  
 25 and the replication process (Table 1). It is noted that the difference in the residual sum  
 26 of squares is small. This is related to a rather high level of collinearity in the 2-  
 27 parameter model meaning that a fraction of the contribution  $f_{(a)}$  could be attributed to  $f_{(c)}$   
 28 and vice versa.

## 31 **4. Stochastic modelling of the process**

32  
 33 For given values of the parameters  $a$  and  $c$  a stochastic simulation of the process can be  
 34 carried out. At discrete times (stepsize  $\Delta t$ ) a fixed length  $c\Delta t$  is subtracted as well as a  
 35 randomly chosen part  $ax(t)^2R(t)\Delta t$  with  $R(t) \sim \text{Uniform}(0, 1)$ . The result depends on the  
 36 order in which these operations are carried out. For  $\Delta t$  sufficiently small the difference  
 37 between the two will become negligibly small. However, estimating parameters with  
 38 this stochastic simulation fails because of lack of convergence of the Monte Carlo  
 39 process in which simulation runs are repeated until the average of some penalty function  
 40 stabilizes. The stochastic process can also be described by a Fokker-Planck equation  
 41 being a linear diffusion equation for the probability density function  $p(t, x)$  at time  $t$ . In  
 42 the Appendix we describe a numerical simulation process based on a stochastic  
 43 differential equation which offers an alternative; it is called a *Brownian motion*. Using  
 44 (A7)-(A8) and the percentiles at day 5 of one specific nestling with parameter values  
 45 estimated for this case from (1),  
 46

$$47 \quad (a, c) = (0.73 \times 10^{-6}, 5.7), \quad (3)$$

48

1 we generate 1000 starting values and run the simulation over a time interval of 25 days.  
 2 The so obtained 1000 length values at day 30 are reordered and the percentiles 10(5)90  
 3 are read off (Fig. 4a). It is noted that this is only one realization of the process. If it is  
 4 repeated, slightly different values will be found.

#### 6 *Parameter estimation*

7 We may estimate the parameters using the above Brownian motion process. Again a  
 8 penalty function is specified for the difference between the observation and the outcome  
 9 for some choice of the parameters  $a$  and  $c$ . One has to take the average penalty over a  
 10 number  $n$  of runs. In this so-called Monte Carlo simulation the penalty converges to its  
 11 limit value at a rate  $n^{-1/2}$  for increasing  $n$ . In the present problem one has to take  $n \geq 25$   
 12 to obtain satisfactory results. Then the averaged penalty is at its minimum for

$$14 \quad (a, c) = (0.41 \times 10^{-6}, 7.5) \quad (4)$$

15  
 16 In Figure 4a we also give the outcome of one run for these parameter values. It is  
 17 anticipated that the result is better than that for parameter values (3) based on the  
 18 deterministic model. This is due to the fact that with (4) also the second order statistical  
 19 moment is taken in account. Figure 4b gives the outcome based on the Poisson process  
 20 itself. The resulting values are close to those of the Brownian motion. As already  
 21 remarked we did not succeed in estimating parameters based on a stochastic Poisson  
 22 type of simulation. The present result makes it likely that the best fitting parameter  
 23 values for the Poisson process are close to the ones of the Brownian motion model.

## 26 **5. Conclusions**

27  
 28 Telomere length and telomere shortening can be seen as biomarkers of ageing in that  
 29 they have repeatedly been shown to predict survival probabilities in humans and birds  
 30 (e.g. Cawthon et al., 2003; Hausmann et al., 2008; Bize et al., 2009; Salomons et al.,  
 31 2009). With the availability of detailed longitudinal data it has become possible to fit  
 32 new dynamical models to these data to develop insights in the mechanisms underlying  
 33 the shortening process. We explored possible physical processes for the terms of this  
 34 expansion that significantly may contribute to the decrease of telomere length in time.  
 35 We focused our attention upon two such mechanisms: a decrease at a fixed rate from the  
 36 replication process and random strand breaks. The replication of the DNA sequence  
 37 during cell division is incomplete due to the end-replication problem. As long as this  
 38 part consists of telomere base pairs only no essential information is lost. However,  
 39 eventually the telomere chain will become sufficiently short to induce cell death.  
 40 Processes acting in a cell, such as DNA repair and telomerase, may prevent or reverse  
 41 telomere shortening. These processes may still fall short in counterbalancing telomere  
 42 shortening or have harmful side-effects such as an increase in the risk of developing  
 43 cancer. In this study we did not consider these processes and attributed the net  
 44 shortening fully to the two physical processes of length decrease we incorporated.

45 Our main result is that the Poisson model for strand breaks shows that length-  
 46 dependent decrease of telomere length can be explained without requiring a feedback  
 47 mechanism that protects short telomeres. In our analysis we compared the result for the  
 48 2-parameter model with the minimal model allowing only a length decrease at a  
 49 constant rate ( $a = 0$ ). Although the approximation error went down only slightly the  
 50 contribution to the length decrease changed strongly in such a way that the two  
 51 mechanisms contribute about equally to the length shortening (Table I). In literature



1 several studies on the stochasticity of telomere shortening appeared. In some cases the  
 2 mathematical analysis dealt only with the behavior in the mean or telomere length  
 3 distributions at different times were presented without a formulation of the dynamical  
 4 process. In some other cases data of experiments were fitted to a dynamical model. Von  
 5 Zglinicki (2002) has demonstrated that oxidative stress increases the speed of telomere  
 6 shortening. Given a certain stress level we have shown that this process causes a length  
 7 dependent decrease. Op den Buijs et al. (2004) describe a type of feedback mechanism  
 8 for this length dependency based on the observation that the distribution of lengths is  
 9 close to a Weibull distribution.

10 It is noted that a stochastic model based on the Poisson process itself, instead of  
 11 the Brownian motion we introduced in Section 5, did not lead to successful parameter  
 12 estimation. The large influence of the higher statistical moments in such model requires  
 13 data with a lower external noise level. Finally, it is remarked that it might very well be  
 14 possible to analyze a differential equation model that describes length-dependent  
 15 telomere shortening as a process in which the cell actively decreases the shortening rate  
 16 by some negative feedback. More parameters are then needed and so in comparison it  
 17 should result in a much better fit in order to serve as a useful alternative. A same  
 18 argument applies to a model in which, next to telomerase, also a break in a specified end  
 19 part of the 3' strand is allowed (Rodriguez-Brenes and Peskin, 2010). As in our model  
 20 the risk of such a break is supposed to be uniformly distributed.

## 21 22 23 **Appendix**

24  
25 The stochastic model of telomere length decrease contains two stochastic variables: the  
 26 event of a possible break within a time interval  $(t, t + \Delta t)$  and the position of such a  
 27 break within the telomere chain. Furthermore there is the constant length decrease from  
 28 replication. The expected value of the change  $\Delta x$  of the length over the time interval is  
 29 computed as follows with  $K$  the stochastic variable denoting the event of a break ( $K =$   
 30 1) and the realisation  $X(t) = x$ :

$$31$$

$$32 \quad \Delta x = E_K E_X(\Delta X | K) = -c\Delta t + \sum_{k=0}^1 E_X(\Delta X | K = k)P(K = k)$$

$$33 \quad = -c\Delta t + E_X(\Delta X | K = 0)(1 - ax\Delta t) + E_X(\Delta X | K = 1)ax\Delta t. \quad (A1)$$

34  
35 Since the break position is uniformly distributed over the interval  $(0, x)$ , its expected  
 36 value equals  $x/2$  and its variance is  $x^2/12$ . This leads to the following expression for the  
 37 expected change

$$38 \quad \Delta x = -(c + \frac{1}{2}ax^2)\Delta t. \quad (A2)$$

39 Letting  $\Delta t \rightarrow 0$  we arrive at the deterministic differential equation for the mean change  
 40 in the telomere length

$$41$$

$$42 \quad x'(t) = -c - \frac{1}{2}ax(t)^2. \quad (A3)$$

43  
44 For the variance of  $\Delta X$  we obtain in a similar manner

45

$$\begin{aligned}
\text{Var}(\Delta X) &= \text{Var}_K \{E_X(\Delta X | K)\} + E_K \{\text{Var}_X(\Delta X | K)\} \\
&= E_K \{E_X(\Delta X | K)^2\} - (E_K \{E_X(\Delta X | K)\})^2 \\
&\quad + E_K \{E_X((\Delta X)^2 | K) - E_X(\Delta X | K)^2\} \\
&= -(E_K \{E_X(\Delta X | K)\})^2 + E_K \{E_X((\Delta X)^2 | K)\} \\
&= -E\{\Delta X\}^2 + E_K \{\text{Var}_X(\Delta X | K) + E_X(\Delta X | K)^2\} \\
&= -\{-c\Delta t - \frac{1}{2}ax^2\Delta t\}^2 + (0+0)(1-ax\Delta t) + (\frac{1}{12}x^2 + \frac{1}{4}x^2)ax\Delta t
\end{aligned}$$

2 or

$$3 \quad \text{Var}(\Delta X) = \frac{1}{3}ax(t)^3\Delta t + O\{(\Delta t)^2\}. \quad (\text{A4})$$

4

5 For a further description of the stochastic process of telomere shortening including a  
6 constant decrease from the end replication one may switch to a linear diffusion  
7 equation, the so-called Fokker-Planck equation (Gardiner, 1990). Let  $p(t, x)$  be the  
8 probability density function for the length  $X$  at time  $t$ . This function then satisfies the  
9 partial differential equation

10

$$11 \quad \frac{\partial p}{\partial t} = \frac{\partial}{\partial x} \left\{ \left( c + \frac{1}{2}ax^2 \right) p \right\} + \frac{1}{2} \frac{\partial^2}{\partial x^2} \left\{ \left( \frac{1}{3}ax^3 \right) p \right\}. \quad (\text{A5})$$

12

13 Together with a starting distribution at the initial time this equation yields the  
14 distribution at any time  $t$ . The stochastic process itself can be simulated by using the so-  
15 called Langevin equation to which the Ito calculus applies

16

$$17 \quad dX = -\left( c + \frac{1}{2}aX^2 \right) dt + \left( \frac{1}{3}aX^3 \right)^{1/2} dW(t), \quad (\text{A6})$$

18

19 see Gardiner (1990). In this stochastic differential equation  $dW(t)$  denotes a Wiener  
20 increment (Gardiner, 1990). Using a forward Euler scheme with step size  $\Delta t$  (Grasman  
21 and Van Herwaarden, 1999) a realization of the process can be derived from

22

$$23 \quad X(t + \Delta t) = X(t) - \left( c + \frac{1}{2}aX(t)^2 \right) \Delta t + \left( \frac{1}{3}aX(t)^3 \right)^{1/2} Z(t) \sqrt{\Delta t}, \quad X(0) = X_0 \quad (\text{A7})$$

24

25 with stochastic variable  $Z(t)$  having a standard normal distribution  $N(0, 1)$  and  $X_0$  having  
26 some prescribed distribution. If we have an approximation of the corresponding  
27 cumulative distribution function  $F(x_0)$  e.g. by means of a set of percentiles, we can  
28 easily generate starting values using the standard uniform distribution. Let  $R$  be  
29 Uniform(0,1), then

30

$$31 \quad X_0 = F^{-1}(R) \quad (\text{A8})$$

32

33 has the required distribution. In simulation one may as well take a large number of  
34 values for  $R$  being equally distributed over the interval  $(0, 1)$ .

35

36

37

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2  
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6  
7

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1 **Figure captions**

2  
3 Figure 1: Histogram of mean change of telomere length between day 5 and day 30 in  
4 blood cell samples of 75 jackdaws.

5  
6 Figure 2: Data of one individual: (a) length percentiles at day 5 and day 30  
7 approximating the cumulative distribution functions. (b) approximation of the  
8 probability density function using (a).

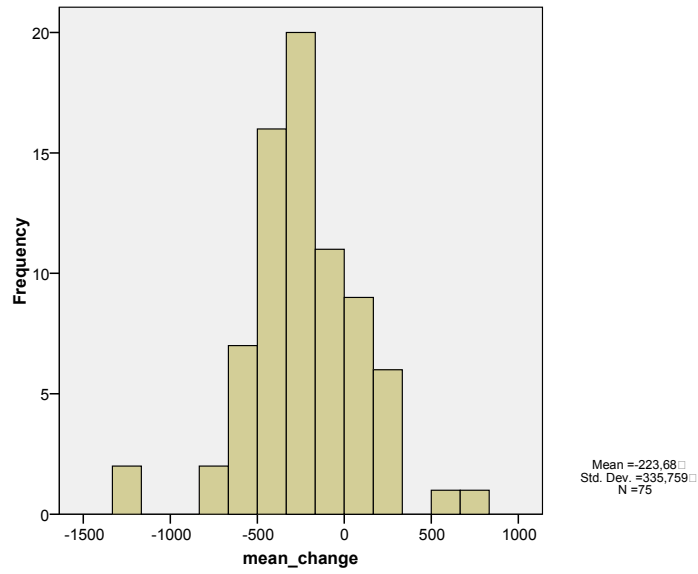
9  
10 Figure 3: From all 75 jackdaws the change of length of the population of telomeres from  
11 day 5 to day 30 is represented in a graph. From each of the length percentiles  $p =$   
12  $10(5)90$  at both days the change of length  $\Delta p = p(30) - p(5)$  is given as a function of  
13 the mean of the lengths at both days,  $\underline{p} = (p(5) + p(30))/2$ , and represented as a graph  
14 connecting the percentiles  $10(5)90$ . It is noted that at day 30 an interval between to  
15 consecutive percentiles may be composed out of cells different from the ones in the  
16 same interval at day 5.

17  
18 Figure 4. A comparison of model results with the observation of the telomere length  
19 percentiles at day 30 for one specific nestling. (a) The deterministic model is given by  
20 (1) with parameter values (3): the computed lengths at day 30 are connected by a curve.  
21 For the same parameter values the Brownian motion (A7)-(A8) gives the result  
22 indicated by (+). For the parameter values (4) obtained from a parameter estimation,  
23 based on the Brownian motion model, one run of that model gives percentiles indicated  
24 by ( $\times$ ). (b) The simulation result obtained by a Poisson process with values (4) is  
25 indicated by (O).

26  
27

1 Figure 1: Histogram of mean change of telomere length between day 5 and day 30 in  
2 blood cell samples of 75 jackdaws.

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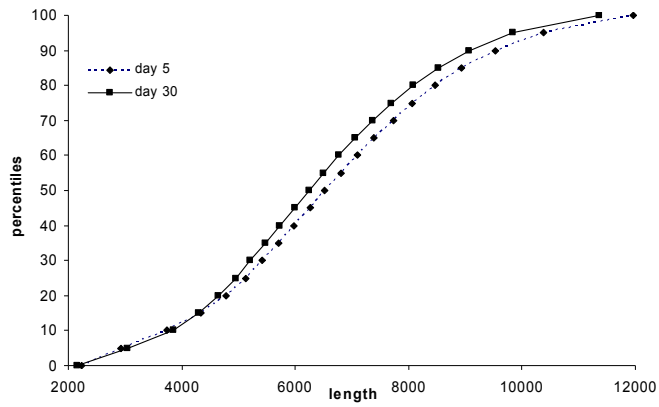
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3 probability density function using (a).

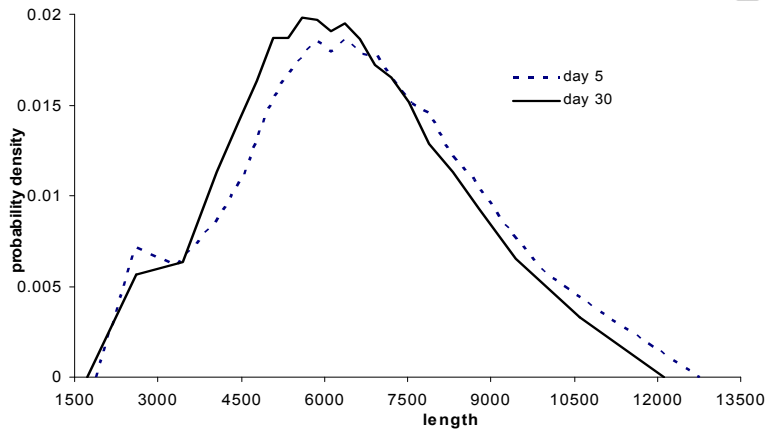
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5 (a)



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7 (b)

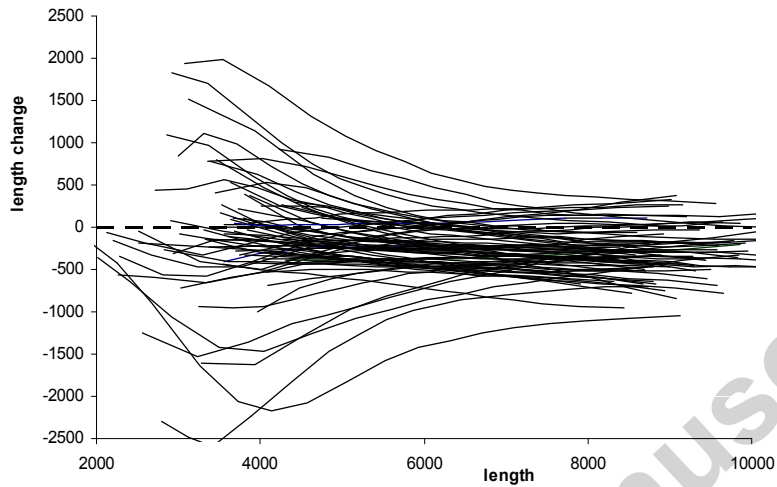


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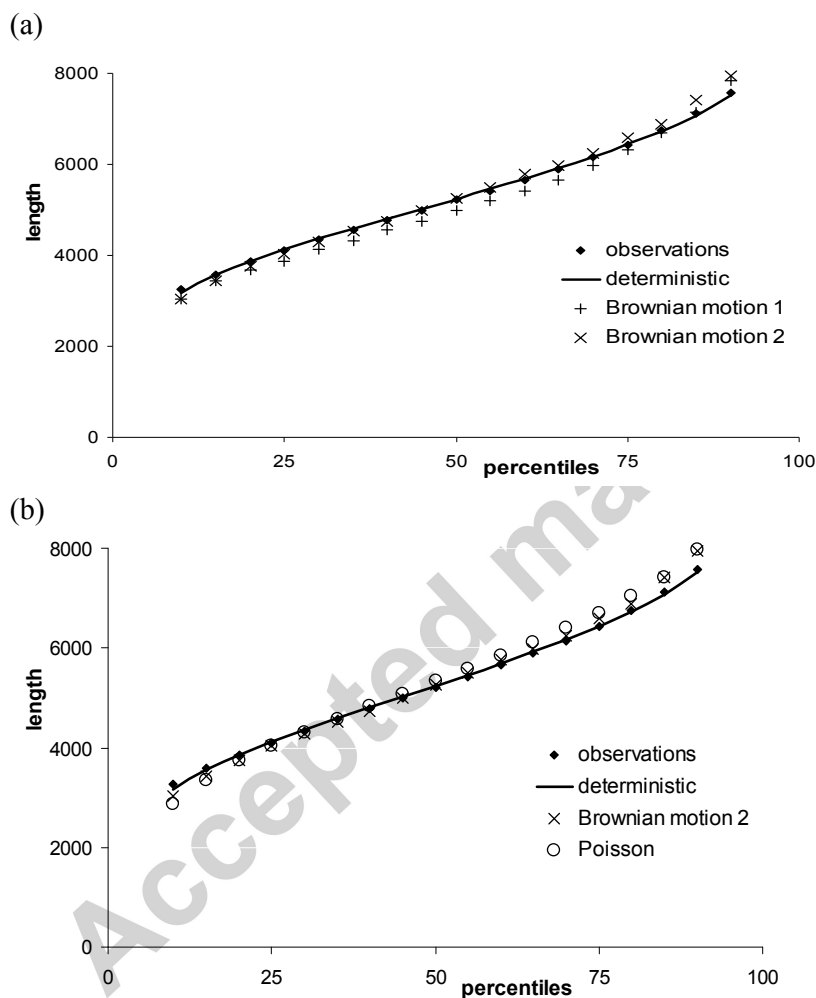
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 8 indicated by (O).



1 Table I. Estimates of the parameters  $a$  and  $c$  of the 2-parameter model and the  
 2 contribution of the corresponding two terms to the length decrease. In the text it is  
 3 explained how these contributions are computed. The estimate for the 1-parameter  
 4 model is given as well.

5

# parameters	$a$	$c$	*SSR ( $\cdot 10^8$ )	$f(a)$	$f(c)$
1	0	9.3	2.14	0	9.3
2	$0.22 \times 10^{-6}$	4.5	2.08	4.4	4.5

6 \* Sum of Squared Residuals

7

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- 1 >Data from juvenile Corvids shows length dependent telomere shortening.
- 2 >Telomere shortening from oxidative stress is modelled by a Poisson process.
- 3 >The 2-parameter telomere model covers shortening from replication and stress.
- 4

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