This is an electronic appendix to the paper by Hall *et al*. 2004 Telomere loss in relation to age and early environment in long-lived birds. *Proc. R. Soc. Lond.* B **271**, 1571–1576. (DOI 10.1098/rspb.2004.2768.)

Electronic appendices are refereed with the text. However, no attempt is made to impose a uniform editorial style on the electronic appendices.

Electronic appendix A

This appendix provides further details of the methods.

Preparation of DNA from blood

Blood samples were digested by mixing 10 - 15 µl blood with 300 µl SET, 30 µl 10% SDS, 12.5 - 15 µl proteinase K, and agitating overnight at 55°C. DNA was then extracted using phenol/ chloroform, and precipitated with ethanol and sodium acetate (Sambrook *et al*. 1989). DNA yields and integrity were verified by gel electrophoresis and spectrophotometry.

Production of TRFs by southern blot hybridisation

Roche Diagnostics Ltd., East Sussex, UK supplied the TeloTAGGG Telomere Length Assay Kit. In brief, the procedure was as follows. 2µg of DNA per sample was digested for 16 hours at 37ºC with the enzymes *Rsa*I and *Hinf*I (10U/µl, Invitrogen Ltd., Paisley, UK), 6U enzyme mix per µg of DNA. The digestion products were run on a 0.8% agarose gel at 120V for 4 hours, with a digoxigenin (DIG)-labelled molecular weight marker (23.1-0.12 kb, Roche Diagnostics Ltd., East Sussex, UK). The gel was denatured to separate the DNA into single strands (see TeloTAGGG Telomere Length Assay Instruction manual, Roche), and fragments were transferred to a Hybond N+ nylon membrane (Amersham Pharmacia Biotech. Ltd., Bucks, UK) by overnight southern blot transfer in 20 × SSC at room temperature. DNA was fixed to the membrane by UV cross-linking (optimal cross-link, Spectrolinker™ XL-1000, Spectronics Corporation). Hybridisation to a DIG-labelled probe (5' (TTAGGG) $_4$ 3') was carried out at 42ºC in a hybridisation oven (Hybaid maxi) for at least 3 hours. After stringency washes (2 ×SSC, 0.01% SDS at room temperature followed by 0.2 ×SSC, 0.1% SDS at 50ºC), the membrane was incubated for 30 minutes at room temperature with a DIG-specific antibody covalently coupled to alkaline phosphatase (anti-DIG-AP, DIG Luminescent Detection Kit, Roche Diagnostics Ltd., East Sussex, UK). A chemiluminescent substrate CSPD (DIG Luminescent Detection Kit, Roche Diagnostics Ltd., East Sussex, UK), which is metabolised by alkaline phosphatase, was later added to the membrane allowing visualisation of the TRF smears. Membranes were exposed to autoradiography film to form a permanent image of the telomere fragments in each lane. For each blood sample, duplicate digests were run on the same gel and the average of these two mean TRF lengths used as the estimate of telomere length. TRF lengths measured in this way correlate with those determined by other methods including quantitative fluorescence *in situ* hybridisation (Q-FISH) (Lansdorp *et al.* 1996; Hultdin *et al.* 1998) and quantitative polymerase chain reaction (PCR) (Cawthon 2002). The TotalLab software used to measure TRF length from scanned

autoradiograph images was Phoretix, version 1.10, ©1996- 2000 Nonlinear Dynamics Ltd., Newcastle-upon-Tyne, UK.

Natal environmental conditions

As part of the long-term monitoring of shags on the Isle of May, breeding attempts are followed from nest building to fledging at 16 plots around the island. Mean plot success is the number of chicks fledged per incubated nest, averaged across these sites. Each year a large proportion of shag chicks are ringed. From ringing records the total number ringed, average brood size at ringing, and median ringing date were obtained. Breeding success declines as median ringing date advances (Harris *et al*. unpublished data). To obtain a factor describing environmental conditions in each natal year, a PCA of mean plot success, total number of young ringed, average brood size at ringing and median ringing date was carried out.

References to the appendix

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