other Communication Disorders, National Institutes of Health, Bethesda, Maryland 20892, USA
e-mail: kacharb@nidcd.nih.gov

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Metabolism

Winter torpor in a large bird

Torpor is a natural state in which animals show a substantial and controlled reduction of body temperature to conserve energy[1,2]. A few small birds (weighing less than 80 g) are known to use it as a survival strategy in winter, but we have discovered that a large bird, the Australian tawny frogmouth, which weighs 500 g, can also enter this state. This surprising finding increases the size of birds known to use natural torpor by almost tenfold, suggesting that avian torpor is more widespread than is commonly believed, enabling birds to stay in their territory throughout the year.

Small endothermic birds and mammals have enormous food requirements because of the fast metabolic rate that is necessary to regulate a high body temperature (T_b). When this high metabolic rate is unsustainable, for example in periods of adverse weather and/or food shortage, many small mammals (body mass 2–10,000 g) survive by entering a state of torpor[1–3]. The study of torpor in birds has so far been restricted to species weighing less than 80 g (refs 2,4,5), and rather than using torpor to overcome adverse conditions, birds may migrate to avoid them. However, many birds are sedentary and often rely on ephemeral, weather-dependent food sources — so how do they overcome periodic energy bottlenecks?

Figure 1 Tawny frogmouth (*Podargus strigoides*).

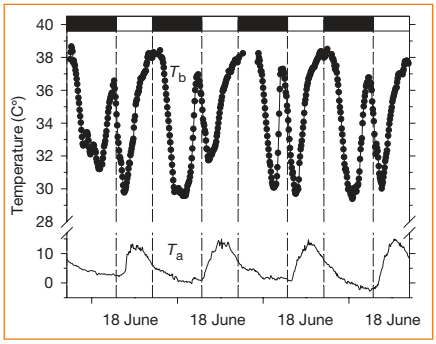

Figure 2 Fluctuations in body temperature of large Australian tawny frogmouth (T_b, filled symbols, measured by an internal transmitter) and in air temperature (T_a, solid line) over 4 days in June (winter). T_b was high at the beginning of the night (black bars), and then declined. The bird was aroused before dawn, after which it changed its roost and re-entered torpor. T_b increased from mid-morning to peak around sunset.

To answer this question, we investigated whether the Australian tawny frogmouth (*Podargus strigoides*; Fig. 1), a sedentary bird which feeds mainly on arthropods, uses torpor in the wild. The study was conducted from the Australian autumn to summer in an open woodland of *Eucalyptus* and *Acacia* at 1,000 m altitude in a cool temperate area near Armidale, New South Wales. We captured seven frogmouths and fitted them with temperature-sensitive transmitters (calibrated to the nearest 0.1 °C) weighing 3 g. All birds received an external backpack-style transmitter[4] (long range) to measure skin temperature (T_{skin}), and three birds had a second internal transmitter (short range), to measure core T_b and to determine $T_b - T_{skin}$ differentials, implanted under general anaesthesia. Transmitter signals were recorded at 10-min intervals for up to nine months[6].

All individuals entered torpor in winter: T_b fluctuated around 38–40 °C during activity and fell to about 36 °C during the rest phase, with a lower limit of 34 °C. On cold (less than 7 °C) winter nights in June–August, T_b fell below 34 °C on 202 of 462 observation days (44%). The minimum T_b recorded by an internal transmitter was 29.1 °C, the lowest T_b calculated from T_{skin} and the $T_b - T_{skin}$ differential was 27.2 °C, and the mean minimum T_b for the seven birds was 29.0 ± 1.0 °C. Birds usually entered torpor after a brief phase of activity in the evening (Fig. 2), became active again near sunrise and frequently entered a second bout of torpor after they had flown to their day roost. Dawn torpor was briefer (3.5 ± 1.2 hours) than night torpor (7.0 ± 1.2 hours) and was often terminated by passive rewarming in the sun.

Our study shows that the large frogmouth regularly enters torpor in winter. We argue that the use of torpor enables the bird to survive in a wide range of habitats and to remain resident in its territory throughout the year[7], despite feeding on arthropods. Because torpor occurs in this large bird and because birds are more diverse and smaller on average than mammals, we predict that avian torpor is much more common than is currently believed.

Gerhard Körtner, R. Mark Brigham*, Fritz Geiser
School of Biological Sciences, University of New England, Armidale 2351, Australia
e-mail: fgeiser@metz.une.edu.au
**Permanent address: Department of Biology, University of Regina, Regina, Saskatchewan S4S 0A2, Canada*

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Ageing

Cloning of mice to six generations

Mice have been cloned by nuclear transfer into enucleated oocytes[1–3], and here we describe the reiterative cloning of mice to four and six generations in two independent lines. Successive generations showed no signs of premature ageing, as judged by gross behavioural parameters, and there was no evidence of shortening of telomeres at the ends of chromosomes, normally an indicator of cellular senescence — in fact, these appeared to increase slightly in length. This increase is surprising, given that the number of mitotic divisions greatly exceeds that of sexually produced animals and that any deleterious effects of cloning might be expected to be amplified in sequentially cloned mice. Our results offer a new approach to the study of organismal ageing.

Founder clones (G1) of two lines, A and B, were generated using cumulus cells of adult female B6C3F1 mice (agouti coat colour) as nucleus donors, and oocytes from adult B6D2F1 (black) females as nucleus recipients. The cloning procedure

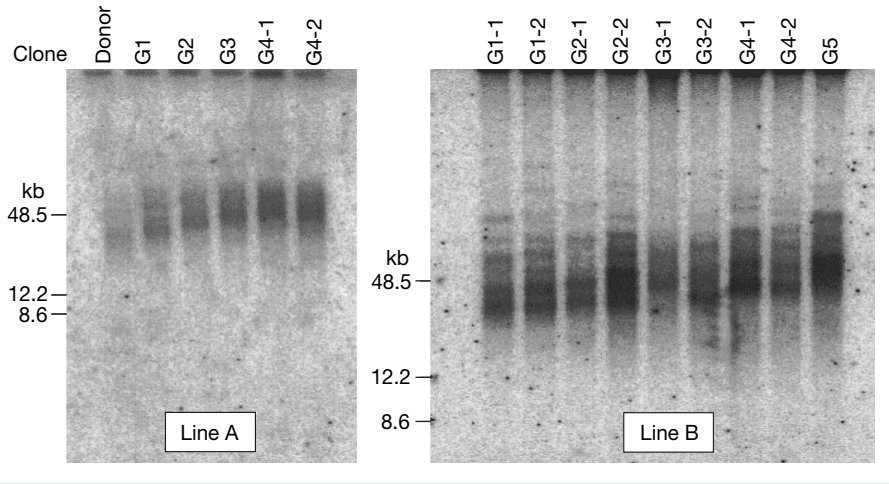

Figure 1 Telomere lengths in successive generations (G1–G5) of mice cloned from cumulus cells. Southern-blot analysis of terminal restriction-enzyme-cut fragments in five sequential generations shows that telomeres do not undergo incremental erosion in successive clonal generations. Genomic DNA isolated from peripheral-blood lymphocytes taken from representative animals from each generation was digested with the restriction enzyme *Hin*fI, resolved on a pulse field gel, transferred to a solid support and probed with a 5′-^{32}P-labelled $(T_2AG_3)_3$ oligonucleotide. Peripheral blood lymphocytes were sampled on the same day. Ages of mice (in months) were: in line A, donor, 18; G1, 16; G2, 14; G3, 12; G4, 9; G5, 9; in line B, G1, 15.5; G2, 13; G3, 11; G4, 9; G5, 7. Suffix numbers (G4-1 and G4-2, for example) identify different pups of each generation.

was repeated with cumulus cells from adult G1 mice as nucleus donors to produce the next clonal generation, G2, and so on. Table 1 summarizes the results obtained following the reconstruction of 3,920 enucleated oocytes.

Previously, about 2% of enucleated oocytes receiving a cumulus cell nucleus developed to live-born pups[1]. This value is comparable to the cloning efficiency for G1 in lines A (1.5%) and B (4.2%). However, the success rate dropped in successive cloned generations: line A did not produce a G5 clone from 670 reconstructed oocytes; in line B, the only live-born G6 clone was cannibalized by her foster mother, thereby terminating the line. Mouse lines A and B therefore represent totals of 9 and 26 clones from their respective donors. Placental size was consistently in the range previously reported for cloned mice[2] and did not increase in successive generations (data not shown).

Do sequentially cloned mice show signs of accelerated ageing? We assessed the behaviour of these mice and determined telomere lengths to assess organismal and cellular measures of ageing, respectively. We evaluated learning ability in the Morris water maze and Krushinsky tests, as well as strength and agility, and also used other assays designed to monitor signs of premature ageing, such as a decline in activity in the home cage and loss of coordination[4]. All cloned mice were, by these criteria, normal compared with age-matched controls (data not shown); the G5 mouse is alive and healthy at 1.5 years.

We measured telomere length in peripheral blood lymphocytes of clones G1–G6 by Southern-blot analysis of terminal restriction-enzyme-digested fragments (Fig. 1) and found no evidence of shortened telomeres in the cloned mice. In fact, our results show that the telomeres lengthen with each generation. As representative animals of each generation were sampled simultaneously, we cannot rule out an age-related contribution to this increase (with younger mice having longer telomeres). In addition, long telomeres in mice are optimally studied by means of different assays such as quantitative fluorescence *in situ* hybridization[5]. We have detected telomerase activity in cumulus cells (data not shown); it is therefore possible that telomeres in these cells are unusually long, resulting in offspring with concomitantly longer telomeres.

Shortened[6] and lengthened[7] telomeres have been reported in cloned livestock but, unlike ours, those experiments involved only a single round of cloning. Our results on sequentially cloned mice verify that telomere shortening is not a necessary outcome of the cloning process[8]. However, as only 1–2% of reconstructed oocytes yield live-born clones, the possibility of selection for donor nuclei with the longest telomeres cannot be excluded. Further investigation is required into the consequences of nuclear transfer on telomere length and lifespan.

Table 1 Effect of sequential cloning on full-term development

Line	*G1*	*G2*	*G3*	*G4*	*G5*	*G6*	*Total*
A	2/131 (1.5)	1/228 (0.4)	1/263 (0.4)	5/238 (2.1)	0/670 (0)	-	9/1,530 (0.6)
B	4/96 (4.2)	7/351 (2.0)	5/352 (1.4)	6/286 (2.1)	3/581 (0.5)	1/724 (0.1)	26/2,390 (1.1)

Successive generations are represented as G1, G2 and so on for two independent mouse lines, A and B. The number of pups born live after cumulus-cell nuclear transfer is compared to successfully reconstructed oocytes (pups/oocytes), with the corresponding percentages in parentheses. Significant χ^2 comparisons were derived for G4 and G5 from line A, G1 and G5, G6 from line B, and G2, G3, G4 versus G6 from line B ($P < 0.05$).

Teruhiko Wakayama*†, Yoichi Shinkai‡, Kellie L. K. Tamashiro*, Hiroyuki Niida§, D. Caroline Blanchard||, Robert J. Blanchard||, Atsuo Ogura¶, Kentaro Tanemura¶, Makoto Tachibana‡, Anthony C. F. Perry#, Diana F. Colgan#, Peter Mombaerts#, Ryuzo Yanagimachi*

**Department of Anatomy and Reproductive Biology, John A. Burns School of Medicine, University of Hawaii, Honolulu, Hawaii 96822, USA*
‡Department of Cell Biology, Institute for Virus Research, Kyoto University, Sakyo-Ku, Kyoto 606-8507, Japan
§Life Sciences Division, Lawrence Berkeley National Laboratory, University of California, Berkeley, Cailfornia 94720, USA
||Bekesy Laboratory of Neurobiology, University of Hawaii, Honolulu, Hawaii 96822, USA
¶Department of Veterinary Science, National Institute of Infectious Disease, Tokyo 162-8640, Japan
#The Rockefeller University, 1230 York Avenue, New York, New York 10021, USA
†Present address: The Rockefeller University, 1230 York Avenue, New York, New York 10021, USA
e-mail: wakayat@mail.rockefeller.edu

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Gene expression

Total silencing by intron-spliced hairpin RNAs

Post-transcriptional gene silencing (PTGS), a sequence-specific RNA degradation mechanism inherent in many life-forms, can be induced in plants by transforming them with either antisense[1] or co-suppression[2] constructs, but typically this results in only a small proportion of silenced individuals. Here we show that gene constructs encoding intron-spliced RNA with a hairpin structure can induce PTGS with almost 100% efficiency when directed against viruses or endogenous