



**Figure 1** A representative analysis of sheep TRF lengths. **a**, Genomic DNA from Finn Dorset sheep, the six-year-old Finn Dorset ovine mammary gland (OMG) tissue used to provide donor cells for nuclear transfer, OME cell primary cultures derived from the aforementioned tissue, Finn Dorset nuclear-transfer animal 6LL3 (Dolly), and from Poll Dorset animals was analysed by Southern blotting and hybridization with radiolabelled (TTAGGG)<sub>3</sub> oligonucleotide. Animal ages are indicated above their respective lanes; the duration in culture for primary cells (OME) is indicated as population doublings (PD). The presence of a consistent signal at approximately 1.5 kb was used as a comparative control for loading and sample integrity. **b**, Regression analysis of mean TRF lengths against age, showing the decline in telomere length with age for the controls (solid circles) together with the fitted line (solid line) and 95% prediction interval for an additional observation at any given age (dashed lines). Comparisons in the text between nuclear-transfer sheep and controls were made by using the prediction intervals from the regression and by Student *t*-tests on 17 degrees of freedom; the control sheep were used to estimate the mean response and its variance. Similar conclusions for 6LL3 were drawn by using a two-sided *t*-test against either the four controls aged 1 year or the four controls aged 6 years, with the use of the appropriate group of four control sheep to estimate the mean response and its variance.

The mean size of the terminal telomere fragment obtained by cutting with restriction enzyme (the mean terminal restriction fragment, or TRF) was found to decrease in control animals with increasing age, at a mean rate of 0.59 kilobases (kb) per year. A linear regression analysis of the sheep DNAs yielded a significant result ( $t=3.29$ ;  $P<0.01$ ) (Fig. 1b).

Mean TRF sizes were smaller in all three nuclear-transfer animals than in age-matched controls. DNA in 6LL3 showed the greatest diminution of mean TRF size for a one-year-old animal (19.14 versus  $23.9 \pm 0.18$  kb). The size difference is significant ( $P<0.005$ ) compared with the age-matched control animals. The smaller TRF in 6LL3 is consistent with the age of her progenitor mammary tissue (six years old) and with the time that OME cells derived from that tissue spent in culture before nuclear transfer. 6LL6 also showed a significant decrease in TRF size ( $20.37$  versus  $23.9 \pm 0.18$  kb;  $P<0.030$ ). As the number of animals analysed was small, it is possible that the difference was due to natural variation of the mean TRF size in these individuals, but the statistical significance of the data argues against this.

There was no significant difference between the DNA from the six-year-old progenitor mammary tissue and age-matched control DNA from fresh blood.

The influence of duration in culture, superimposed on the effect of the age of the

progenitor tissue, can be gauged from the diminution in mean TRF size of OME cells that have undergone up to 27 population doublings. A decrease in mean TRF size was observed at an average 0.157 kb per population doubling. This is an average derived from replicate experiments and is consistent with results from human somatic cells in culture<sup>9,10</sup>.

These observations indicate that the extent of shortening of the TRF might be mitigated, principally by minimizing the duration in culture and by a careful choice of the source of donor cells. This is particularly relevant for 6LL7, for which the use of fetal tissue and minimal culturing yielded an animal in which the mean TRF size was not significantly shorter at 95% confidence limits (21.19 versus  $23.9 \pm 0.18$  kb;  $P<0.088$ ) than age-matched controls. This is in contrast to results from 6LL3 and 6LL6, for which culturing was more prolonged.

The most likely explanation for the shorter mean TRFs in all three nuclear-transfer animals is that the mean TRF size observed in these animals reflects that of the transferred nucleus. Full restoration of telomere length did not occur because these animals were produced without germline involvement. It remains to be determined whether any telomerase activity, or an alternative telomere-lengthening mechanism, is present that could result in some telomere repair during the early development of sheep.

It is not known whether the actual physiological age of animals derived by nuclear transfer is accurately reflected by TRF measurement. Recent veterinary examination of the nuclear-transfer animals has confirmed that they are healthy and typical for sheep of their breeds, despite having a shorter mean TRF length. Furthermore, 6LL3 has undergone two normal pregnancies and has successfully delivered healthy lambs.

Telomere-based models of cellular senescence<sup>5-9</sup> predict that the nuclear-transfer-derived animal 6LL3 would reach a critical telomere length sooner than age-matched controls. However, considering the large size distribution of sheep TRFs, it remains to be seen whether a critical length will be reached during the animal's lifetime. The experimental inactivation of murine telomerase produced a phenotype only after five generations<sup>10</sup>, and similar observations have been made in telomerase-deficient yeast cells<sup>11</sup>. Mice have also been sequentially cloned by the transfer of adult cumulus-cell nuclei without any adverse effects<sup>12</sup>.

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## Did parrots exist in the Cretaceous period?

The timing of the origin of modern birds is much debated. The traditional view, based largely on the fossil record, suggests that most modern groups did not appear until the Tertiary, after the end-Cretaceous extinction event<sup>1</sup>, but recent work, based on molecular divergence data, has suggested that most, or all, of the major clades were present in the Cretaceous<sup>2,3</sup>. Verification of the latter proposal awaits the discovery of modern bird fossils in the Mesozoic which can be confirmed on the

basis of the derived features characterizing the major clades.

We do not consider that the recent description<sup>4</sup> of an avian dentary symphysis of a supposed psittaciform (parrot-like) bird from the Cretaceous Lance Formation of North America represents such a record. If it did, then this would be not only the oldest record of parrots by some 15 million years, but also the earliest recorded occurrence of a 'terrestrial' modern bird in the Cretaceous. Other reports from this period have been shown to be too fragmentary to be of any taxonomic value<sup>5,6</sup>, to occur with formations of uncertain Cretaceous age<sup>7,8</sup>, or to have been incorrectly assigned in the first place<sup>9</sup>.

We therefore recommend that this record be treated with caution until further fossil material of a similar age can be assigned with confidence to the Psittaciformes. This record should not be used to support hypotheses invoking an origin for the modern clades before the Cretaceous/Tertiary boundary<sup>2,3</sup> for the following reasons.

First, the characters listed by Stidham<sup>4</sup> to support the referral of this material to the Psittaciformes have a wider distribution among Cretaceous Maniraptoriformes (for example, a 'hook-like' dentary is seen in caenagnathid theropods<sup>10</sup>), and are variable within the group in question. Although a K-shaped neurovascular canal pattern is seen in some modern psittacids (such as *Cyanoramphus* sp.), this character is not seen in other taxa (*Polytelis* sp., for example). In a survey of large numbers of skeletal specimens of several modern species, we have observed that a K-shaped neurovascular canal pattern is variable in occurrence within individual psittaciform taxa (for instance, *Psittacula roseata*; G.J.D., personal observation), and that both the putative psittaciform characters cited by Stidham<sup>4</sup> are seen in other groups of modern birds (such as the Ciconiiformes; G.J.D., personal observation).

Second, the overall morphology of the Lance Formation specimen is markedly different from that of the oldest unequivocal parrots known in the fossil record, including well-preserved and complete skulls from the lower-middle Eocene of the London Clay, England, and Grube Messel, Germany<sup>11</sup>. These fossil birds, although exhibiting a typically psittaciform postcranial morphology (including the zygodactyl foot, which has the fourth toe directed backwards), lack the parrot-like beak of modern Psittaciformes. Moreover, the mandibular symphysis in Eocene forms is much smaller and narrower than in recent parrots and in the specimen from the Lance Formation<sup>4,11</sup>.

At present, the monophyly of the Psittaciformes, one of the most homogeneous of modern orders, is supported

exclusively by postcranial characters<sup>11</sup>, although the single recent family within the order, the Psittacidae, does have a single unique skull character: the presence of a 'parrot-like' beak (for example, maxilla broad dorsoventrally, with a sigmoidally curved ventral margin). We argue that, given the benefit of well-preserved and largely complete fossil material from the early Eocene, taxonomic assignments of material such as the Lance Formation specimen must remain tentative at present.

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*Stidham replies* — I have presented a hypothesis for the identification of a Late Cretaceous fossil as the oldest known parrot<sup>1</sup>. The specimen lacks the characters distributed more widely in non-avian maniraptoriforms, such as abundant teeth and unfused dentaries. It has many characters<sup>1</sup>, including the absence of an internal pillar of bone supporting a midline ridge, that are not present in oviraptoroids<sup>2</sup>. To assign the specimen to a non-avian clade requires a less parsimonious hypothesis of character evolution. The K-shaped neurovascular canal pattern character<sup>1</sup>, mapped onto various phylogenetic hypotheses of the relationships of crown group parrots<sup>3–5</sup>, is primitive for that clade. Like most characters, the K-shaped neurovascular canal pattern exhibits homoplasy and variation within natural populations. The complete absence of this character from some extant parrots seems to be the result of secondary loss due to the relative shortening of the jaw symphysis in some parrots (*Neophema*, for example). However, this character, as figured and described<sup>1</sup>, is not found outside crown-group parrots, although it superficially resembles the state in extant cathartid vultures. Although individual characters seen in the fossil can occur in other taxa, the combination of characters seen in the fossil

is not present outside crown-group parrots, and Dyke and Mayr have not demonstrated what clade, other than parrots, has this combination of characters.

It seems less than defensible to propose that we cannot have Cretaceous parrots because the oldest well-preserved fossils known so far are Eocene<sup>6</sup>. Previously proposed sister groups to parrots (reviewed in ref. 7), and the known fossil record of these sister taxa<sup>8,9</sup>, show that the parrot lineage should have been present at least 5 to 10 million years before the (middle Eocene) Messel and London Clay parrots<sup>6</sup>. This is the same amount of missing fossil record required by both my hypothesis of a latest Cretaceous parrot and Mayr and Daniels' suggestion<sup>6</sup> (made during their study of the Eocene parrots) of a Cretaceous origin of parrots.

The other known Cretaceous neornithines (listed earlier<sup>1</sup>: in contradiction with Dyke and Mayr, the New Jersey fossil birds are Cretaceous in age<sup>10</sup>) placed in various phylogenetic hypotheses of the ordinal level relationships of neognaths, including parrots<sup>7,11</sup>, show that parrots and most other neognath ordinal level clades are constrained to have diverged from other orders of modern birds in the Cretaceous or early Palaeocene.

If non-crown-group parrots are present in the Eocene<sup>6</sup>, then the sister group to those taxa (the stem leading to the crown group or the crown group itself, possibly with a modern-looking jaw) must have been present by the middle Eocene as well. The identification of the Cretaceous jaw as a parrot is subject to test and refutation, like any hypothesis. However, the accepted methods of the field, not statements about gaps in our current knowledge and preconceived notions of character evolution, must be used to falsify hypotheses and generate alternatives.

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