LETTERS

The phylogenetic position of the 'giant deer' *Megaloceros giganteus*

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The giant deer, or 'Irish elk', has featured extensively in debates on adaptation, sexual selection, and extinction. Its huge antlers—the largest of any deer species, living or extinct—formed a focus of much past work[1–4]. Yet the phylogenetic position of the giant deer has remained an enigma. On the basis of its flattened antlers, the species was previously regarded as closely related to the living fallow deer[5–7]. Recent morphological studies[8], however, have challenged that view and placed the giant deer closer to the living red deer or wapiti. Here we present a new phylogenetic analysis encompassing morphological and DNA sequence evidence, and find that both sets of data independently support a sister-group relationship of giant and fallow deer. Our results include the successful extraction and sequencing of DNA from this extinct species, and highlight the value of a joint molecular and morphological approach.

With a fossil record extending from 400 kyr ago to its extinction about 8 kyr ago[1–4], the giant deer (*Megaloceros giganteus*) ranged from Ireland to central Siberia. Reaching a shoulder height of about 2 m and with antlers spanning up to 3.5 m, it was the largest known member of the 'Old World deer' (subfamily Cervinae)[7].

The core taxa included in the study were the giant deer and its two putative alternative living sister-groups within the Cervinae, the fallow deer (European fallow, *Dama dama*, and Mesopotamian fallow, *D. mesopotamica*) and red deer (*Cervus elaphus*). To these were added the southeast Asian axis deer (*Axis axis*) and hog deer (*Axis porcinus*), in view of suggestions that *Axis* is a close living relative of fallow deer[3]. To broaden taxonomic sampling, wapiti (*C. canadensis*), sika (*C. nippon*) and Eld's deer (*C. eldi*) were added. The small muntjac deer of eastern Asia (*Muntiacus* spp.), which have been shown on morphological and molecular grounds to be basal living members of the Cervinae[9–11], were used as an outgroup. In this way, every major clade of the Cervinae, identified in recent molecular studies[11], was represented.

A total of 988 base pairs (bp) of *M. giganteus* mitochondrial DNA (mtDNA) was obtained (see Methods) from two specimens of *M. giganteus* of widely divergent geographical origin. The first, from Ballynamintra Cave, Waterford, Ireland, has an uncalibrated AMS radiocarbon date of 11,567 ± 42 yr BP (KIA25446); the other, from Kamyshlov Mire in western Siberia, has an uncalibrated date of 7,065 ± 38 yr BP (ref. 4). The sequences of the two specimens are 99.9% similar (one substitution) and so only the Siberian sequence was used in subsequent phylogenetic analyses. Homologous sequences were obtained for all the species in the study (Table 1).

Analysis of the mtDNA data under parsimony, likelihood, distance and bayesian criteria gave the same tree topology (Fig. 1a), and indicates a sister-group relationship between *M. giganteus* and *Dama*. To determine whether the molecular data could discriminate between the two previous morphologically derived hypotheses regarding the position of *Megaloceros*, a Kishino–Hasegawa (KH) test[12] was used to determine the significance of differences in likelihood values between on the one hand a topology in which *Megaloceros* forms a monophyletic group with species of *Dama*, and on the other a topology in which *Megaloceros* forms a monophyletic group with all or any species of the genus *Cervus*. Using the parameters derived above, a two-tailed KH test identifies the second of these as significantly less likely than the first, at $P < 0.01$.

A thorough examination of antlers, skulls, teeth and postcranial bones among the selected species led to a preliminary list of 250 variable characters. After discarding those with a high degree of intraspecific variability or difficulty of scoring (see Methods and Supplementary Information), the remaining set comprised 74 characters (Supplementary Table S1), of which 69 were phylogeneti-

Table 1 | Deer species and GenBank accession numbers of mtDNA sequences in this study

Taxon	Common name	ATP8	Control region	Cytochrome *b*
Dama dama	Fallow deer	AM072730	AF291895	AJ000022
Dama mesopotamica	Mesopotamian fallow deer	AM072731	AM072738	AM072742
Axis axis	Axis deer	AM072732	AM072739	AM072743
Axis porcinus	Hog deer	AM072733	AF291897	AY035874
Cervus canadensis	Wapiti	AM072737	AF058369	AF423199
Cervus elaphus	Red deer	AF104683	AF291886	AF423195
Cervus nippon yesoensis	Hokkaido Sika deer	AB108507	D50129	AB021095
Cervus eldi	Eld's deer	AM072734	AY137117	AY157735
Muntiacus muntjak	Indian muntjak	AY225986	AY225986	AY225986
Muntiacus crinifrons	Black muntjak	AY239042	AY239042	AY239042
Megaloceros giganteus	Giant deer (Russia)	AM072736	AM072740	AM072744
Megaloceros giganteus	Giant deer (Eire)	AM072736	AM072740	AM072745

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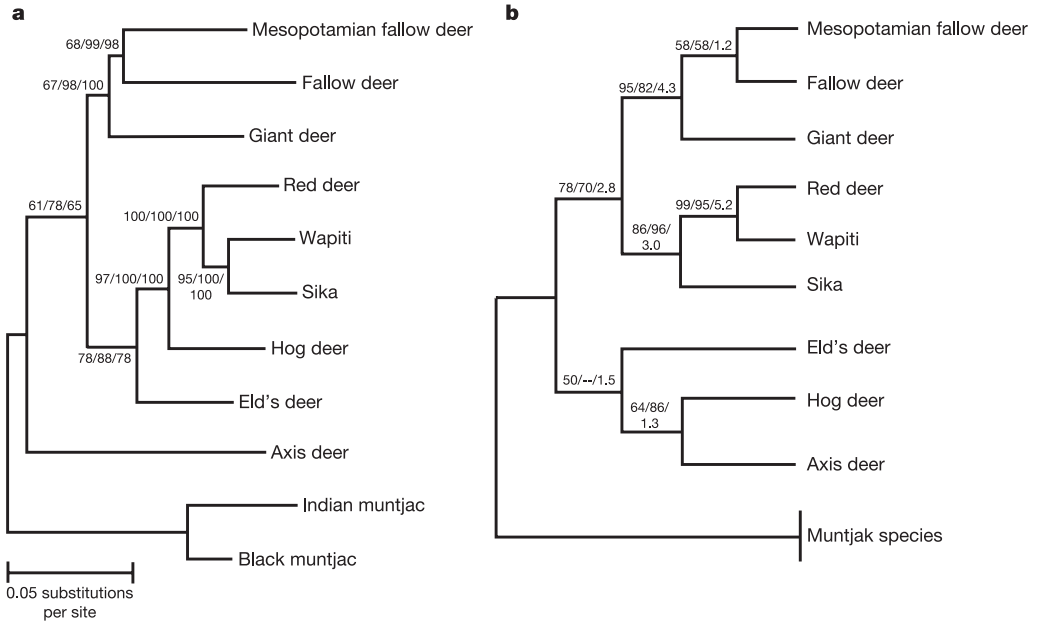

Figure 1 | Phylogenetic relationships among deer species based on molecular and morphological analyses. a, Maximum-likelihood phylogram constructed from 986–989 bp of mtDNA. Numbers above branches indicate, respectively, the percentage of trees that upheld that branch in an analysis of 1,000 bootstrap replicates, in a bayesian analysis of the molecular data set, and in a bayesian analysis of the combined morphological and molecular data set. **b**, The single most parsimonious morphological cladogram. Tree length 123 steps, consistency index = 0.61, retention index = 0.58. Numbers above branches indicate, respectively, the percentage of trees (over 50%) in which a clade occurred in a maximum-parsimony analysis of 20,000 bootstrap replicates of the data, posterior probabilities from a bayesian analysis, and Bremer support indices.

cally informative. A single most-parsimonious tree was recovered, with strong statistical support for a monophyletic *M. giganteus–Dama* clade (Fig. 1b). Bayesian analysis produced a compatible but slightly less resolved tree, again with strong support for the *Megaloceros–Dama* grouping. The monophyletic clade of red deer, wapiti and sika was also recovered in both analyses, supporting the traditional view that red deer and wapiti are sister-species, in contrast to the wapiti-sika clade found in mtDNA (Fig. 1a; ref. 11). Axis, hog deer and Eld's deer were generally more basal on the tree but their topology was unstable. Neither our molecular analyses nor our morphological analyses provide evidence of a close relationship between *Axis* and the *Megaloceros–Dama* clade.

Combined analyses of the molecular and morphological data were undertaken with both bayesian and maximum-parsimony methods. The analyses consistently supported the *Megaloceros–Dama* and *Cervus elaphus–canadensis–nippon* clades. The bayesian topology was identical to the DNA-only tree (Fig. 1a), whereas using parsimony, detailed features such as the internal relationships of *Cervus*, depended on the relative weighting of morphological and molecular characters (Supplementary Information).

Eight derived morphological characters are unique to the *M. giganteus–Dama* clade in our data set (Supplementary Table S2); six of them occur in *M. giganteus* and both *Dama* species, and two in *M. giganteus* and *D. dama* only. They comprise two antler, one skull, two dental, one vertebral and two limb-bone characters (Fig. 2). Three further derived dental and one derived postcranial character are shared, apparently convergently, by the *Megaloceros–Dama* clade and one other species in the study. A final possible shared character, not included in the analysis, is the characteristic 'swollen larynx' ('Adam's apple') of *Dama*, seen in Palaeolithic representations of *M. giganteus*[13] (Fig. 2a).

The sister-group relationship of *M. giganteus* and living *Dama*, shown by both our morphological and molecular data, corroborates the hypothesis of their relationship but must be viewed in the context of other Plio-Pleistocene fossil deer. *M. giganteus* is part of an array of well-documented species of 'giant deer' restricted to Eurasia, which have been grouped as Tribe Megacerini Viret 1961 (refs 14–17). These share characters such as thickened mandible bones and, in most species, palmated antlers, although many authors[15,18] have argued that they fall into two groups that might even have a biphyletic origin, one (*Praemegaceros*) derived from the Eurasian Plio-Pleistocene genus *Eucladoceros*, the other (including *M. giganteus*) of uncertain origin. However, a cladistic analysis[19] found strong support for megacerine monophyly.

Our preliminary study of the other megacerine taxa indicates that the characters here identified as synapomorphies of *M. giganteus* and living *Dama* are common among them, but much less so among *Eucladoceros*, suggesting that *Dama* and all of the megacerine deer form a natural group. In this respect, we might invert the nineteenth-century appellation of *M. giganteus* as a 'giant fallow buck'[5] and consider the living fallow deer rather as the last representatives of a formerly speciose giant deer tribe. Although earlier forms have been claimed[18], the first appearance of unquestionable megacerines is in the middle part of the Early Pleistocene epoch (about 1.4 Myr ago (ref. 16)), that of the *Dama dama/D. mesopotamica* lineage in the early Middle Pleistocene (about 700 kyr ago) and that of *M. giganteus*, plus related oriental forms, in the late Middle Pleistocene (about 400 kyr ago). This indicates the possibly rapid radiation of the clade in this relatively recent interval.

However, a series of European Pliocene to Early Pleistocene medium-sized cervids (spanning the approximate interval 3.0–1.0 Myr ago) have been regarded as forming a stem-group to modern *Dama* and have been placed in that genus by some authors[8,20]. A recent cladistic analysis[8,19] found derived characters linking these species and later *Dama*, although our preliminary observations on these taxa indicate that they lack most of the synapomorphies of modern *Dama* and *M. giganteus* identified here. This indicates homoplasy in the data. Either the entire clade of megacerines and modern *Dama* is of recent origin and postdates the split with the earlier '*Dama*-like' forms, or else the latter are the sister-group of modern *Dama* and the split with megacerines is older. The latter would conform more closely to the deep divergence in mtDNA

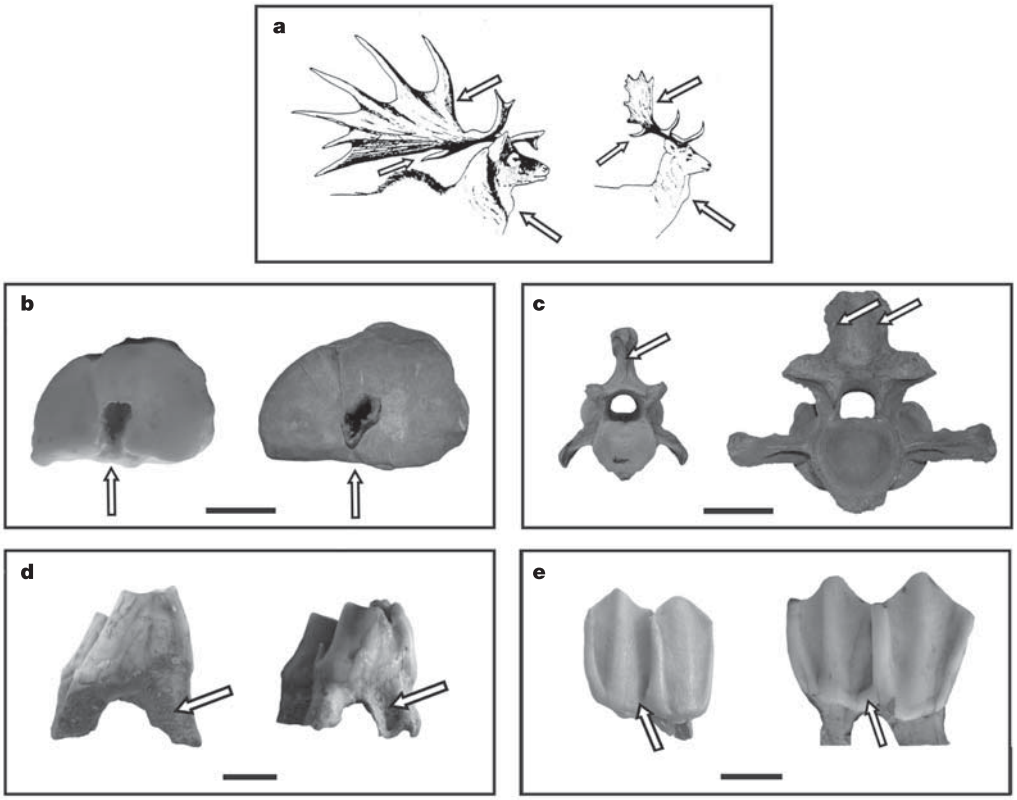

Figure 2 | Examples of morphological characters. **a**, *M. giganteus* (left) and *D. dama* (right), showing antler palmation, back tine, and expanded larynx. (Modified from Valerius Geist, *Deer of the World*, Stackpole Books, ref. 3.) **b**, Metacarpal of *C. canadensis* (left) and *M. giganteus* (right), showing separation of facets in *Cervus*. **c**, Posterior view of axis vertebra in *C. elaphus* (left) and *M. giganteus* (right), showing single medial ridge in *C. elaphus*, two ridges bounding groove in *M. giganteus*. **d**, Posterior view of M^3 in *C. elaphus* (left) and *D. dama* (right), showing convex root in *C. elaphus*, concavity and labial ridge in *D. dama*. **e**, Labial view of upper molar of *C. elaphus* (left) and *M. giganteus* (right), showing horizontal basal ridges in *M. giganteus*. Scale bars, 2 cm (**b**), 5 cm (**c**) and 1 cm (**d**, **e**). See Supplementary Information for details of specimens.

sequence, with the *Dama–Megaloceros* split placed at 4–5 Myr ago (assuming a molecular clock and a divergence of the muntiacine and cervine deer at about 7 Myr ago[11]). Resolving the relationships between these taxa, *Eucladoceros* spp., and other fossils sometimes implicated in megacerine ancestry[17,18], would enable us to explore palaeobiological questions such as the direction of size change, antler evolution, and associated adaptive issues. Some features of modern *Dama*, such as the robust axis vertebra (Fig. 2c) and relict parietal foramen[21], suggest former adaptation to very large antler size and could correspond to ancestry from a large megacerine, but a comprehensive cladistic analysis of all these taxa is needed to address these issues further.

Last, the deep (7.85%) mtDNA divergence between European fallow deer and the endangered[22] Mesopotamian fallow deer *Dama mesopotamica* highlights the distinctive nature of the latter taxon (corroborated by data in a recent study[11]), in spite of its current demotion to a subspecies on the basis of similarities in gross morphology and in behaviour, and ease of cross-breeding in captivity[9,23]. The morphological closeness of red deer and wapiti, to the exclusion of sika, is also notable, recalling earlier hypotheses of their relationship[24], but is in conflict with mtDNA data, inviting further investigation.

METHODS

Morphological analysis. Because of extensive intra-specific variability, all 250 original characters were scored on a series of individuals for each species. Variation was quantified as described previously[25], and characters were regarded as fixed only if they reached a high level of consistent expression (Supplementary Information). Intraspecific polymorphism was recoded as an ordered series with scaled weighting, and the data were analysed under parsimony by exhaustive search with PAUP 4.0b10 (ref. 26), as well as by bayesian analysis (Supplementary Information).

Extraction and identification of ancient DNA. Sequence data were generated from about 0.1 g of cortical bone or tooth samples (Supplementary Information) with the use of established protocols for ancient DNA (University College London and the Ancient Biomolecules Centre, as in ref. 27; Trinity College Dublin, as in ref. 28).

In ancient mtDNA analysis, sequences can be recovered that are not authentic but derive from some external contaminant or the nuclear genome. We regard our *Megaloceros* sequences as genuine for the following reasons. First, entirely independently and without any exchange of materials, the two lead laboratories (University College London and Trinity College Dublin) generated near-identical (99.9%) sequences from different specimens obtained, directly in each case, from distant collections (in Eire and western Siberia respectively), by different workers (I.B. and C.J.E.) using different extraction methods and primer pairs. Second, by using separate samples of bone, a subset of sequences from the Kamyshlov specimen (from primers amplifying ATP8, Cerv_cytb160F/288R, Cerv_cytb269F/403R and Cerv_cytb467F/604R) were independently replicated by M.B. at the Ancient Biomolecules Centre; and from the Ballynamintra specimen (using primers amplifying ATP8 and Cerv_cytb63F/176R, Cerv_cytb643F/760R and Cerv_cytb675F/786R) by I.B. at University College London. Third, the mtDNA sequences of the two *M. giganteus* specimens are clearly of cervid origin but are unique, sharing eight base substitutions not found in the other cervid taxa studied. Fourth, each sequence in the cyt*b* contigs had a perfect match to overlapping fragments. Last, sequences of PCR product clones for several cyt*b* fragments revealed a damage pattern characteristic for ancient DNA but not indicative of the presence of another contaminating sequence (Supplementary Information).

Molecular phylogenetic analyses. Between 986 and 989 characters (118 bp of ATPase 8, 113–116 bp of the control region, 755 bp of cytochrome *b*) were obtained for all taxa in the study (Table 1), of which 267 (27%) were variable and 158 (16%) were phylogenetically informative. We used PAUP 4.0b10 (ref. 26) for the initial analyses. Likelihood ratio tests supported the use of a GTR model (six

substitution types), incorporating site-specific rates for each of the four site categories (first, second, third and non-coding). For maximum-likelihood analyses, heuristic searches were conducted with starting trees generated by ten randomly derived stepwise addition sequences, with branch swapping by tree bisection-reconnection (TBR) and re-estimation of parameters. The maximum-likelihood topology (Fig. 1a) was also recovered by using both maximum parsimony and neighbour-joining with the HKY85 substitution model. Bootstrap support values were obtained by an analysis of 1,000 replicate data sets, with the use of maximum-likelihood analysis under a GTR + SS model with re-estimation of parameters at each step. Replicate data sets that maintained proportionality of each site type were generated with PAML v3.14 (ref. 29). Bayesian analyses of the morphological, molecular and combined data sets were conducted with MrBayes v3.1. Topology searches were initiated from random starting trees, with molecular data assigned a GTR + SS model, and morphological data analysed with distributed rates and likelihoods corrected for scoring bias caused by the presence of only variable characters in the data set. Both combined and molecular analyses were run for 5,000,000 generations, and the morphology-only analysis was run for 2,500,000 generations. Trees were sampled every 100 generations, with the first 25% discarded as burn-in[30].

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Information Sequences are deposited in GenBank under accession numbers AM072730–AM072749. Reprints and permissions information is available at npg.nature.com/reprintsandpermissions. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to I.B. (I.Barnes@ucl.ac.uk).