

Evolution und Phylogeny of the Old World deer (Cervinae)

The Old World deer fulfill important ecological functions within the Eurasian faunal community. Their inter-species relationships, however, have been controversially discussed for many years. Therefore, we aimed to construct a molecular phylogeny as the foundation for an evolution-based systematics of this taxonomic group.

Evolution and phylogeny of Old World deer (Cervinae)

The phylogenetic pattern and timing of the radiation of Old World deer was determined based on the complete mitochondrial cytochrome *b* gene from 33 Cervinae taxa. Using rooted and unrooted phylogenies derived from distinct theoretical approaches, strong support was achieved for monophyly of the Old World deer with muntjacs as sister group as well as for the divergence of at least three distinct genera: *Rucervus*, *Dama*, and *Cervus*. The latter clade comprises what have previously been regarded as the genera or subgenera *Panolia*, *Rusa*, *Cervus*, *Sika*, and probably *Przewalskium*. Our data also consistently confirmed paraphyly of nominate *C. elaphus*. We used these molecular phylogenies to assess the homoplastic evolution of morphological, geographical, ecological, and selected behavioural character state differences within the Cervinae. Reliable fossil calibrations, large molecular data sets, and improved dating methods are shaping a molecular time scale for the evolutionary radiation of Old World deer that occurred at the Miocene/Pliocene transition and is largely compatible with existing palaeontological evidence. Using node ages estimated from sequence data, we estimated an average per-lineage diversification rate of 0.51 ± 0.1 species per million years (my) over roughly the last 6 my.

Unequal rates of change and dating cervid divergences

Our phylogram for OW deer clearly portrays violation of a molecular clock, with interspersed long and short branches. A general clock-like behavior was also rejected because the constrained and unconstrained analyses were significantly different in a likelihood ratio test of the OW deer (without clock, $-\ln = 6098.72$; with clock, $-\ln = 6275.15$; $P < 0.005$) and of the NW deer lineage (without clock, $-\ln = 5870.02$; with clock, $-\ln = 5905.57$; $P < 0.005$). Because the tests of rate heterogeneity among lineages were significant, we dated the nodes by using a tree-based methodology (Sanderson, 1997) that relies on fossil calibration of nucleotide substitution rates. Assignment of fossils to appropriate nodes followed guidelines discussed in

Magallon and Sanderson, 2001. To circumvent problems associated with employing a single calibration point to calculate rates of nucleotide substitution and subsequent estimation of divergence time, minimal age estimates were assigned to two nodes on the cervid phylogeny. The oldest known New World deer fossil is *Eocoileus gentryorum* from sediments of the upper Bone Valley Formation in Florida, dated in the late Hemphillian age of the Pliocene, about 5.0 mya (Webb, 2000). The second fossil-calibrated node is the Muntiacinae-Cervinae split. The oldest fossil remains assignable to this node appear in the Miocene deposit of Lufeng in China. This fossil bed is dated at 7.0 mya (Han 1985), and was used as the minimal age estimate for the node that includes the most recent common ancestors (MRCA) of the two lineages. Despite uncertainty in assigning minimal age estimates to nodes in the NPRS phylogeny using the fossil record, the two calibration points independently resulted in very similar age estimates. Estimation of divergence times using both calibrations points yielded an age estimate of 5.92 ± 0.49 mya (CI_{95%} : 5.78-6.06) and 6.87 ± 0.42 mya (CI_{95%} : 6.73-7.00) for the MRCA of the extant species of OW deer and the NW deer, respectively (Fig. 1). The rate of nucleotide substitution was estimated by linear regression as 0.0257 substitutions/site/my/lineage ($r = 0.95$), giving a pair wise rate of 5.14% per million years.

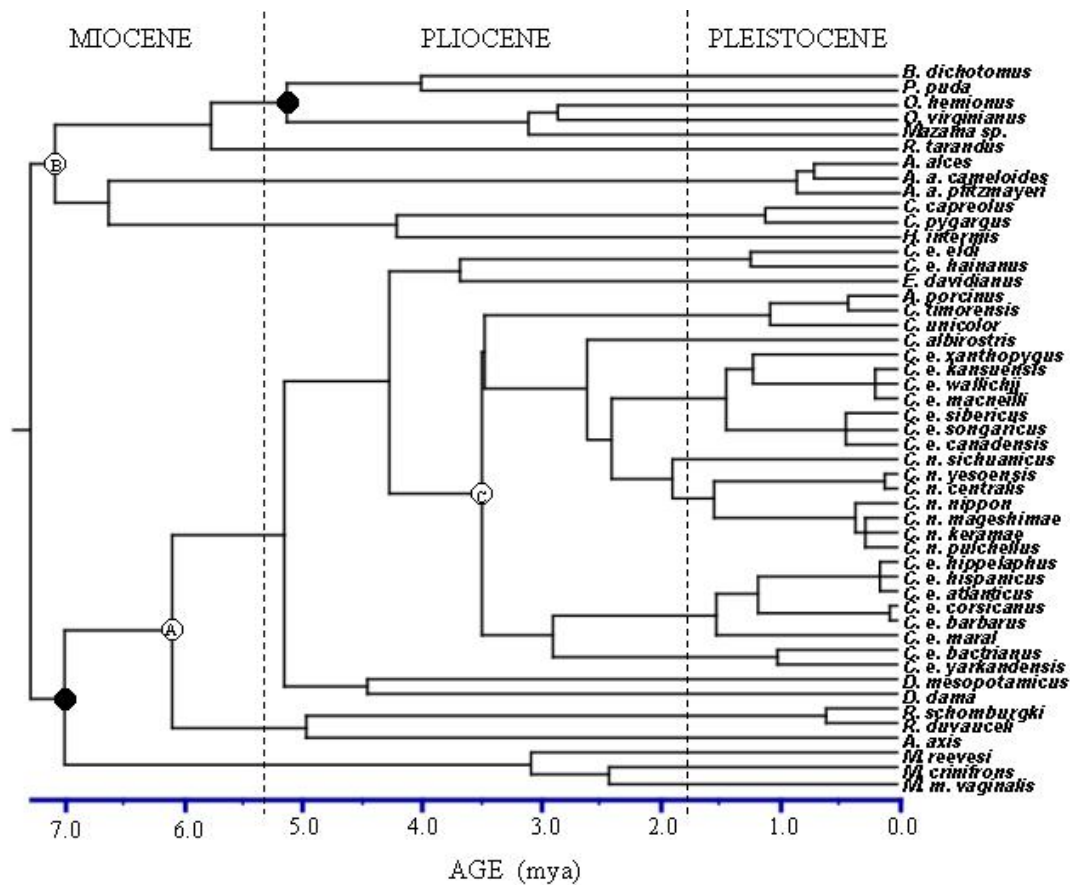


Figure. 1 Fossil-constrained phylogram (chronogram) of Cervidae. The node ages were estimated according to Sanderson's (1997) nonparametric rate smoothing (NPRS) method using TreeEdit and PAUP*. The fossil-calibrated nodes are the Muntiacinae-Cervinae split (A) and the origin of the New World Odocoileinae (B) constrained to have a minimum age of 7.0 and 5.0 mya, respectively. Numbered circles mark fossil dates explained in the discussion section. Geological time scale is given in millions of years.

Integration of molecular and fossil data

In this study, we minimized errors of topology, calibration and rate heterogeneity among lineages, to explore the correspondence between the fossil record and our molecular-based age estimates (Fig. 3). The following checks of the accuracy of the divergence time estimates include minimum ages estimated conservatively by the first appearance of fossils referable to one of the constituent OW deer lineages. We realize, of course, that the fossil record inevitably incorporates many biases and contains real gaps.

1. Di Stefano and Petronio (2002) named the Late Miocene *Cervocerus novorossiae* as the most primitive member of the Cervini. While conflicting with the conclusions of Azanza (1993), this is much more consistent with the DNA, except that it would probably push back somewhat the date of the basal cervine node. Azanza's (1993) schema probably relates rather to the earliest radiations of the antlered Cervidae as a whole.
2. If indeed the Asian genus *Procapreolus* is ancestral to *Capreolus*, its estimated age of Late Miocene-Early Pliocene (Zdansky, 1925) would suggest that our nodes are in the right order of magnitude. It is unclear, however, whether the species, *Procapreolus latifrons*, aged at ca. 6-5.5 mya, is or is not also ancestral to *Hydropotes*. We think probably not: *Procapreolus* has short, 3 pointed antlers, scarcely developed and usually lacking tracks, crest, and pearls; the angle between the brow tine and the beam is always strongly acute, while the b span and the pedicles are very long; the pliomerine fold is present, even if sometimes scarcely developed; molars are columnar and with weak interlobal styles (Di Stefano and Petronio, 2002).
3. Di Stefano and Petronio (2002) named *Axis shansius*, which dates back to ca. 5 mya, as the earliest fossil of the lineage leading to *Axis axis*.
4. Di Stefano and Petronio (2002) identify Early/Middle Pliocene *Cervus magnus* as the very first 4-point deer, i.e. part of the *elaphus*-like group. The implications are that the node that includes the *Elaphus/ Canadensis/ Nippon/ Rusa/ Hyelaphus* group needs to be pushed back.
5. Di Stefano and Petronio (2002) reported that *Rusa elegans* (Middle Pliocene of Shaanxi is "very close" to *unicolor*, as are the Pliocene European fossils referred to *Pseudodama*. This is considerably earlier (about twice as early) than in the present DNA study. However, the *unicolor* antler is doubtless the primitive one for the entire *unicolor/porcinus/timorensis* clade, so we can suppose that *elegans* could be anywhere along it, not necessarily on the actual *unicolor* clade.
6. *A. lyra* of the European Mid/Late Pliocene follows *A. shansius* (Di Stefano and Petronio, 2002).

7. Di Stefano and Petronio's (2002) assignment of late Pliocene *R. hilzheimeri* to the *albirostris* clade is in excellent agreement with our DNA dating.

8. The earliest fossils of *Elaphurus* date back to the Late Pliocene (c. 3-2 mya) (Taru and Hasegawa, 2002), agreeing well with the present study.

9. *C. nippon* fossils were first found in the Late Pliocene deposits of Europe (Thenius and Hofer, 1960 in Geist, 1998). This agrees well with the first divergence of the *nippon* clade (node N) in the Late Pliocene.

10. *A. lydekkeri*, a fossil species from Java dating back to ca. 1.5 mya (van den Bergh 1999) could be related to *Axis axis* but is probably part of the *Hyelaphus* clade (Meijaard and Groves, 2004). This does not appear to be congruent with our phylogeny as it would push back the porcinus/timorensis node by ca. 1.1 my. On the other hand, we could make the case that the *Hyelaphus* morphology is plesiomorphic for the entire *Rusa*, so that the *A. lydekkeri* date might represent any point along the stem of the hog deer/sambar/rusa clade.

11. Di Stefano and Petronio (2002) identified *Cervus grayi* as the latest common ancestor to *Cervus nippon* and a lineage leading to the temperate *C. elaphus* and the wapiti group. According to them these two lineages split during the late Villafranchian (ca. 1.3 mya), which is ca. 1 my later than suggested by the present mtDNA phylogeny. The DNA data of this and all other studies are quite clear about the non-monophyly of the *elaphus* group; we therefore take *C. grayi* as denoting the last common ancestry of the sika- and wapiti group.

12. According to Di Stefano and Petronio (2002), *bactrianus* is a relict of the acornate red deer that lived in Europe in the Early Pleistocene (Galerian, about 1 my or a bit less).

13. The first coronate (i.e. real *C. elaphus*) deer occur in the Late Galerian (about 0.5 mya). These data seem consistent with the present phylogeny. At the same time, there are wapiti in "central-eastern Asia".

In conclusion, reliable fossil calibrations, large molecular data sets, and improved dating methods are shaping a molecular time scale for the evolution of deer that is largely compatible

with existing palaeontological evidence. Few discrepancies may be caused by insufficient correction for rate heterogeneity among OW deer lineages, a too-recent dating of the Muntiacinae-Cervinae split, or a combination of these factors.