

Male vocal behavior and phylogeny in deer

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Abstract

The phylogenetic relationships among 11 species of the Cervidae family were inferred from an analysis of male vocalizations. Eighteen characters, including call types (e.g. antipredator barks, mating loudcalls) and acoustic characteristics (call composition, fundamental frequency and formant frequencies), were used for phylogeny inference. The resulting topology and the phylogenetic consistency of behavioral characters were compared with those of current molecular phylogenies of Cervidae and with separate and simultaneous parsimony analyses of molecular and behavioral data. Our results indicate that male vocalizations constitute plausible phylogenetic characters in this taxon. Evolutionary scenarios for the vocal characters are discussed in relation with associated behaviors.

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Introduction

Since Lorenz (1941) first used behavioral characters to establish systematic relationships within the family Anatidae, the use of behavior as characters in reconstructing phylogenies has remained controversial (Atz, 1970; Baroni Urbani, 1989). Taxonomists have used two major arguments for supporting their lack of confidence in behavioral characters: doubts about the applicability of homology criteria to behavior, and suspicion of high levels of homoplasy in behavioral data (De Queiroz and Wimberger, 1993). However, these supposed drawbacks have been debated and vigorously rejected by numerous zoologists (Brooks and McLennan, 1991; Wenzel, 1992; Miller and Wenzel, 1995; Wimberger and De Queiroz, 1996; Grandcolas et al., 2001; Dessutter-Grandcolas and Robillard, 2003; Robillard et al., 2006), and, as originally argued by Tinbergen (1959), it is now increasingly recognized that behavioral characters are in fact just as reliable as morphological or molecular characters for inferring phylogenetic relationships. Despite this, the use of behavioral traits in systematics remains essentially limited to entomology (Carpenter,

1989; Proctor, 1992; Wenzel, 1992; Dessutter-Grandcolas, 1997; Crespi et al., 1998), where behavioral characters are typically added to the morphological data matrix. The phylogenetic relevance of behavior is more debated in vertebrates, where a handful of studies have been conducted on fish (McLennan et al., 1988; McLennan, 1994), amphibians (Cocroft and Ryan, 1995; Ryan and Rand, 1995; Robillard et al., 2006) and birds (Irwin, 1988; Prum, 1990; Irwin, 1996; Zyskowski and Prum, 1999; Tavares et al., 2006); and a couple of mammalian groups (Macedonia and Stranger, 1994; Kurt and Hartl, 1995). Even then, these studies typically consist of mapping behavioral traits on a morphological or molecular tree rather than actually using them as phylogenetic characters (McCracken and Sheldon, 1997; Cicero and Johnson, 1998; Lusseau, 2003). In order to further investigate the possible use of behavior—and particularly vocal behavior—in vertebrate phylogeny, we studied the family Cervidae, an appealing taxon because of its particularly conspicuous and increasingly well documented vocal habits.

Among ruminants, Cervidae are generally considered as constituting a monophyletic group of 51 species (Grubb, 2005) characterized by a strong and diverse vocal activity (Reby and McComb, 2003b). Acoustic and functional classifications of emitted sounds are

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Table 1
Taxa used in the study (higher taxa as in Gilbert et al., 2006)

Taxa	Common name	Bibliographic source
Bovidae		
<i>Ovis gmelini musimon</i>	European mouflon	
Moschidae		
<i>Moschus moschiferus</i>	Musk deer	
Cervidae, Cervinae		
Muntiacini		
<i>Muntiacus muntjak</i>	Indian muntjac	(Dubost, 1971; Wiles and Weeks, 1981; Barrette, 1997)
Cervini		
<i>Dama dama</i>	Fallow deer	(Alvarez et al., 1975; Reby and McComb, 2003b; McElligott et al., 2006; Vannoni and McElligott, 2007)
<i>Cervus elaphus elaphus</i>	European red deer	(Clutton-Brock and Albon, 1979; Reby and McComb, 2003a,b)
<i>Cervus elaphus corsicanus</i>	Corsican deer	
<i>Cervus nippon</i>	Sika deer	(Miura, 1984; Minami and Kawamichi, 1992)
<i>Cervus canadensis</i>	Wapiti	(Bowyer and Kitchen, 1987; Feighny et al., 2006)
Cervidae, Capreolinae		
Rangiferini		
<i>Rangifer tarandus</i>	Reindeer	(Lent, 1975; Frey et al., 2007)
Alceini		
<i>Alces alces</i>	Moose	(Reby et al., 1999)
Capreolini		
<i>Capreolus capreolus</i>	European roe deer	(Danilkin and Hewison, 1996)
<i>Hydropotes inermis</i>	Chinese water deer	(Cooke and Farrel, 1998; Reby et al., 1999)
Odocoileini		
<i>Odocoileus virginianus</i>	White-tailed deer	(Richardson et al., 1983; Atkeson et al., 1988)

fairly well established for a series of species: *H. inermis*, *C. capreolus*, *Odocoileus virginianus*, *Rangifer tarandus*, *Muntiacus muntjak*, *Dama dama*, *Cervus elaphus*, *Cervus canadensis* and *Cervus nippon* (references in Table 1), but they have never been submitted to phylogenetic analysis. The main call categories include “barking”, which is widespread in the group, “snorting”, a violent exhalation or inhalation through nostrils, which seems to serve the same functions, but is only manifested by New World deer (Odocoileini) and *R. tarandus*, “grunts”, present in *R. tarandus*, *O. virginianus* and *Alces alces* and the mating loudcalls specific to Cervini, the polygynous Eurasian deer and American *C. canadensis*.

Phylogenetic relationships within Cervidae have been debated for decades (Fig. 1). While molecular studies recently reached a state of consensus (Douzery and Randi, 1997; Randi et al., 1998; Hassanin and Douzery, 2003; Pitra et al., 2004; Gilbert et al., 2006), these results are in contradiction with phylogenies based on morphological characters (Groves and Grubb, 1987; Janis, 1993), particularly for the group gathering *A. alces*, *Capreolus capreolus* and *Hydropotes inermis* (the latter being the only antler-less species in the family). Similar problems are encountered in detailed intrageneric studies of *Cervus* species, whose taxonomy seems to be in need of revision (Ludt et al., 2004; Pitra et al., 2004; Gilbert et al., 2006), as the morphological characters supporting their diagnosis are increasingly considered as doubtful. In a previous study, we tentatively established phylogenetic relationships in a limited number of

Cervidae species, based on the female behavioral repertoire alone (Cap et al., 2002). While the results were reasonably coherent with morphological and molecular phylogenies, their weak cladistic support led us to try new analyses using an enlarged set of species and other kinds of behavioral data. Following recent encouraging results in phylogeny inference based on acoustic communication signals in insects (Desutter-Grandcolas, 1997; Robillard and Dessuter-Grandcolas, 2004), frogs (Robillard et al., 2006), birds (McCracken and Sheldon, 1997) and mammals (Macedonia and Stranger, 1994; Kurt and Hartl, 1995), we investigated the phylogenetic coherence of cervid male vocal behavior against that of molecular characters, which are generally considered more reliable. We devised a data matrix composed of only vocal characters, and we compared the issuing phylogenetic topology and character cladistic consistency relatively to molecular phylogeny reconstruction. A simultaneous parsimony analysis of both data sets was performed and evolutionary scenarios for vocal and associated behaviors are discussed.

Materials and methods

Studied taxa and sources

We examined the male vocal repertoire in 11 Cervidae taxa, plus one Moschidae and one Bovidae, introduced as outgroups in the analysis (Table 1). The vocal and

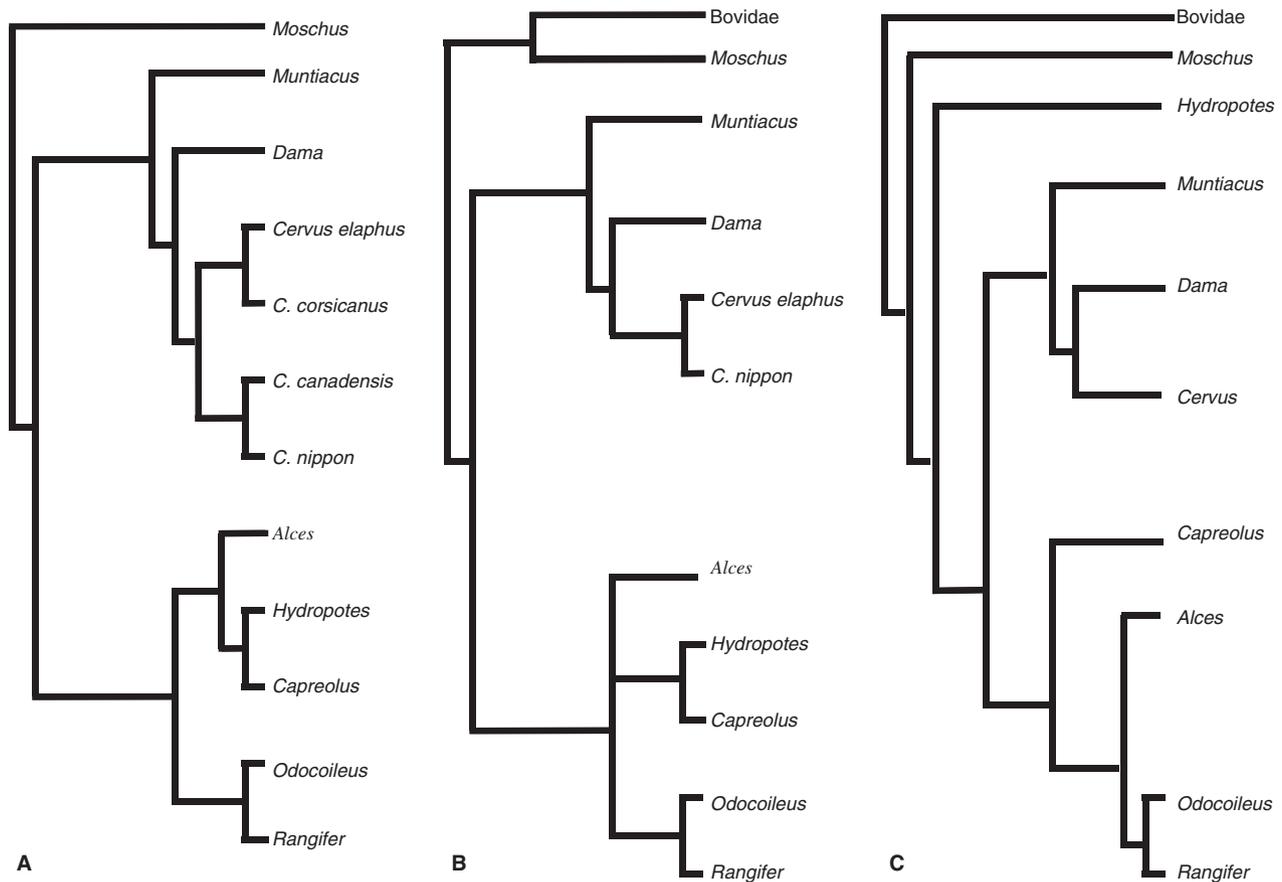


Fig. 1. Cladograms (maximum parsimony) of Cervids phylogenetic relationships based on: (A) *Cytochrome b* mtDNA gene data (Randi et al., 1998; Pitra et al., 2004); (B) *Cytochrome b* and *CO2* mtDNA and nuclear fragments: exon 2 of *alpha-lactalbumin* and intron 1 of the gene encoding *protein kinase C iota* data (Gilbert et al., 2006); (C) morphological data (Groves and Grubb, 1987).

contextual behavioral data used in this study came from an analysis of bibliographic sources (e.g. Richardson et al., 1983; Green, 1985; Atkeson et al., 1988; Minami and Kawamichi, 1992; Cooke and Farrel, 1998; Reby and McComb, 2003a,b), with the exception of some acoustic parameters (mean fundamental frequency F0 and call duration) derived from nine rutting grunts from a male moose (*A. alces*). These calls were recorded in Sweden in 1997 by D. Reby (Reby et al., 1999), using a Telinga pro-III-S/DAT Mike microphone and a DAT Sony TCD7 recorder (amplitude resolution: 16 bits, sampling rate: 48 kHz). Digital signals were directly transferred on to a G3 Macintosh computer using an Audiomeia II sound card and Sound Designer software. The duration and mean F0 of each call were extracted using the “To Pitch (cc)” command in the PRAAT 4.2.06 Digital Signal Processing freeware package (Boersma and Weenink, 2005; available from <http://www.fon.hum.uva.nl/praat/>). We also used PRAAT to produce the narrow band spectrograms presented in Fig. 3.

Phylogenetic analyses

The phylogenetic analysis of the male vocal behavior data was performed with PAUP* beta version 4 (Swofford, 2003), using maximum parsimony and equal costs of character change with the branch-and-bound algorithm. The topology of this vocalization-based phylogeny was compared with those of several molecular phylogenies: Pitra et al. (2004), based on *Cytochrome b* mtDNA, and containing all the taxa analysed in this study; Randi et al. (1998), also based on *Cytochrome b*; and Gilbert et al. (2006), based on *Cytochrome b*, *CO2* mtDNA and nuclear fragments (exon 2 of *alpha-lactalbumin* and intron 1 of the gene encoding *protein kinase C iota*), which does not include *C. canadensis*, *C. corsicanus* and *Ovis*. While Gilbert et al.’s (2006) analysis makes use of significantly more genes, the authors have only published the results of the ML and Bayesian analyses. In order to enable a more direct comparison of the phylogenetic coherence of vocal versus molecular data, we performed a parsimony

analysis of the molecular data. For the 10 species in common between the study of Gilbert et al. (2006) and ours, we used their complete molecular data set. For the two Cervidae species *C. canadensis* and *C. corsicanus* and for *O. gmelini*, we were limited to *Cytochrome b*. The aligned sequences of Gilbert et al. (2006) are available in the article's online supplementary material (doi.org/10.1016/j.ympev.2006.02.017), and the three extra *Cytochrome b* sequences have the following accession numbers: [canadensis cytb AB021096], [corsicanus cytb AY244489] and [musimons cytb SHPMTCBC]. Being without indels, their alignment is nonproblematic. Parsimony analysis of the complete molecular data matrix was performed with PAUP* beta version 4 (Swofford, 2003), with equal weights for all characters, a moderately differentiated TS/TV weight ratio of 1/3, and gaps coded as missing data. We used a TBR branch-swapping algorithm with 1000 bootstrap replicates and a bootstrap 50% majority-rule consensus tree. The simultaneous analysis of all characters, molecular and vocal, was performed using the same parameters, with all characters weighted equally, allowing the parsimonious inference of a phylogeny based on all the available relevant evidence.

The argument that behavioral characters are generally highly homoplastic can be tested by using measures of homoplasy for behavioral and e.g. molecular data sets on the same optimal phylogenetic topology. Two statistics are useful for this: the consistency index CI (Kluge and Farris, 1969) and the retention index RI (Farris, 1989a,b) which vary between 0 and 1, inversely to the homoplasy of the characters. Homoplasy is the difference between the change of a character optimized on a topology and the absolute minimum possible change for this character (the latter being: number of states—1). The CI expresses the partition of character change between minimal possible change and extra change (homoplasy) as the fraction of the former on the latter, while the RI expresses it as the fraction: greatest possible amount of change for the character on any tree minus the change on the tree/greatest possible amount of homoplasy (Farris, 1989a,b). Hence the CI and the RI enable a rough comparison of homoplasy in vocal versus molecular data in the studied taxa. The phylogenetic patterns of the characters were inferred from parsimonious optimizations using MESQUITE software (Maddison and Maddison, 2004).

Issues with the primary analysis of characters

The primary (or putative) homology of characters (De Pinna, 1991), and hence the constitution of the data matrix, heavily determines the subsequent phylogenetic analysis (Rieppel, 2004). For this reason, using behavior in systematics is generally considered as raising numerous methodological problems, and particularly that of

identifying actually inherited traits (Wenzel, 1992). In fact this problem is limited to higher vertebrates, as the inheritance of behavioral traits has long been asserted in other groups, notably in insects (Hoy and Paul, 1973; Hoy, 1990). In birds, the problem can be treated by excluding signals that include learnt components (like some aspects of passerine birds songs), and by using vocalizations where strong genetic determinism is established (like in herons—McCracken and Sheldon, 1997). While the importance of behavioral learning in mammals may lead us to question the inheritability of vocal characters (Macedonia and Stranger, 1994), the only groups known as capable of vocal imitation are, besides humans of course, marine mammals like pinnipeds and Cetaceans (Janik and Slater, 1997; Janik, 1999), elephants (Poole et al., 2005), and some cercopithecoid monkeys (Lemasson and Hausberger, 2004). In cervids no study has yet established any influence of learning in the vocal domain. Moreover, vocalizations are rather stereotyped, and the absence of known dialects, which could reflect “cultural” variation between isolated populations of the same species, suggests that learning is not involved in the development of vocal behavior in this taxon. This is further supported by the observation that hybrids of sika and red deer that are bred in complete isolation develop vocalizations that are acoustically intermediate between those of their parent species (Long et al., 1998).

Another major difficulty for using vocalizations in systematics, and particularly in mammals, comes from the difficulty of assessing homologies between the sequences of sound produced by different species (Macedonia and Stranger, 1994; Peters, 2002). While some aspects of a vocal character can be correlated with the morphology and physiology of the vocal apparatus of the caller (potentially giving supplementary clues for homology assessment), other aspects may reflect the habitat in which the species evolved, potentially misleading the determination of phylogenetic relationships in the case of strong selective convergence. For example, the fact that sound frequencies propagate differently through closed or open environments (Wiley and Richards, 1982) may lead to the evolution of common characteristics in the calls of species which evolved in the same type of habitat. Moreover, in contrast with the morphology, where anatomical features are compared essentially according to their relative localization and effective connection in space, the search for homology in vocal behavior is performed in additional dimensions: the timing of vocal utterances in behavioral sequences (e.g. during a chase or while approaching a female, etc.), and the ultimate function of the vocal utterance (as revealed by expressions like “pursuit deterrent signal”, “territorial call” or “mating call”). Hence, analyzing vocal features for phylogenetic investigations requires

cautiously defined parameters of character description, in order to try avoiding primary homology mistakes coming from either abusive or insufficient character similarity identification (i.e. problems of misleading evolutionary convergence or divergence, respectively).

Definition and coding of characters

In this study, eighteen characters were defined, according to different categories of criteria: presence/absence of typical vocal signals, temporal parameters (call repetition, duration, spacing), spectral parameters (fundamental frequency F0), phonation behaviors (lowering of the larynx, or configuration of the mouth) and functional aspects of vocal behavior as deduced from context of emission like mating and herding calls (Fig. 2). They are listed in Table 2. Numbers 0 to 6 are the conventional codes of character states in the data matrix, with no polarity implication, i.e. “unordered” coding.

When characters were absent in some taxa while being present under different character states in others, we applied the “inapplicable data” or “conventional coding” procedure (*sensu* Hawkins et al., 1997), which consists of: (1) considering the presence or absence of a character only once, through the use of a presence/absence column in the data matrix; and (2) coding for the different states of the character, when present, in a second column, with cells where the character is absent coded as “inapplicable”. This approach prevents counting character changes more than once while preserving the information from all character states. Hence, on a cladogram, if the emergence of a type of vocalization (under any state) is interpreted as an evolutionary change supporting a clade, the potential changes of the forms of this vocalization can be interpreted as informative synapomorphies only among the taxa already carrying this type of vocalization, with no impact on the taxa lacking it. The coding of such “inapplicable” cells is noted with “#” in Table 3, while the question marks “?” mean “non-documented”, hence potentially adopting any known character state. Such “inapplicable” missing entries can be managed a logical way in parsimony analysis, using standard programs. This is done through post-analysis discarding of possible extra equiparsimonious topologies obtained by the improper interpretation of inapplicable cells as having a positive character state content (Platnick et al., 1991). The polymorphic character 11 in *C. nippon* is noted “P”.

A particular difficulty for coding vocal characters arises from the fact that acoustically similar characters may serve different functions, when acoustically different ones may serve similar functions in different species, not to mention the possibility of structural polymorphism for specific functions. This leads to a

potential conflict of priority between structural and functional homology criteria. However, the very possibility of the different combinations mentioned above indicates that evolutionary changes can affect both function and structure, and that either kind of changes can occur independently. It therefore seems that a more neutral way to code the characters in such cases consists of separately coding the acoustic structure and the specific function of these vocalizations. For instance, the ability of emitting a “hiss” is structurally coded as such (character 5) in five species, despite the fact that both *C. capreolus* and *C. canadensis* use this type of vocalisation in agonistic contexts, while *H. inermis* uses it in a mating context. This coding implies that a change occurred in terms of vocal abilities in lineages acquiring or losing the ability to hiss (or the use of hissing), and therefore the presence/absence of the hiss is potentially a relevant character. However, the use of any type of vocalization, independently from its acoustic structure, during the approach of a single female is coded as a separate character (character 10). In our analysis *C. capreolus* and *H. inermis* share this functional character, despite the fact that while *C. capreolus* uses a rasp, *H. inermis* uses a hiss in this context. This implies that, in certain lineages, a change has occurred which consists in the acquisition or the loss of the ability (or usage) of vocalizing when approaching a female. In this example, the coding of these two characters is not redundant, since *C. Canadensis* has an aggressive hiss but no “non-herding” mating call. By coding both structural and functional changes, we are in agreement with Kluge’s (2007) point that phylogeny inference should be based on the notion of evolutionary changes, and that characters should be coded accordingly.

Results

Detailed description of characters

For all characters, non-applicable character states are noted “#” in Table 3.

(1) *Presence of vocal behavior.* Vocalizations of Cervidae, *M. moschiferus* and *O. gmelini* can be classified in three categories: (i) sounds generated in the larynx through vibration of vocal folds, such as loudcalls in Cervini and grunts in reindeer *R. tarandus*, *A. alces* and *O. virginianus* (Reby and McComb, 2003a,b), barks and contact calls in *M. moschiferus* (Flerov, 1952) and in most Cervidae (Reby et al., 1999), bleats in *O. gmelini* (Shillito Walser et al., 1981); (ii) sounds generated by strong inhalation or exhalation through the nostrils (snort) as found in *M. moschiferus* (Green, 1985),

Rutting call

<i>Ovis gmelini</i>	absent (Gonzalez, pers. comm.)
<i>Moschus moschiferus</i>	absent (Flerov 1952, Green 1985)
<i>Hydropotes inermis</i>	whistle (Cook and Farrel 1998)
<i>Capreolus capreolus</i>	rasping grunt (Danilkin and Hewison 1996)
<i>Alces alces</i>	grunt (Geist 1999)

Odocoileus virginianus complex grunt (Atkeson et al., 1988)

Rangifer tarandus grunts (Lent 1975)

Muntiacus muntjak cackling (Dubost, 1971)

Dama dama groan (Reby 1998)

Cervus elaphus elaphus roars (Reby and McComb 2003a)

Cervus elaphus corsicanus roars (Reby and McComb 2003b)

Cervus nippon moan (Minami and Kawamishi, 1992)

Cervus canadensis bugle (Feighny et al. 2006)

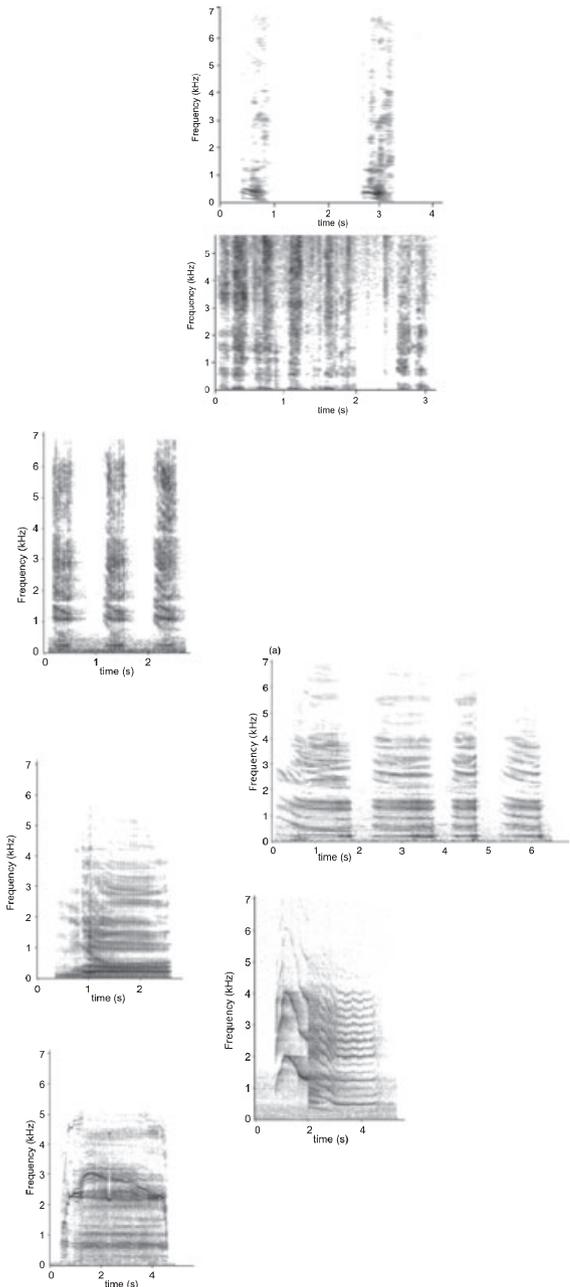


Fig. 2. Spectrograms of the rutting calls for 7 of the 13 taxa.

C. nippon (Minami and Kawamishi, 1992), *R. tarandus* (Lent, 1975) and South American Cervidae (Reby et al., 1999); (iii) hissing and whistling, present in *M. mos-*

chiferus (Flerov, 1952; Green, 1985), mouflon *O. gmelini* (Gonzalez, pers. comm.) and *C. capreolus* (Reby et al., 1999) in alarm context, as well as *H. inermis* while

Table 2
Character coding (equal weights, unordered)

(1) Vocal behavior: absent = 0, present = 1
(2) Bark: absent = 0, present = 1
(3) Barking pattern: repeated in a series = 0, unitary = 1
(4) Snorting: absent = 0, present = 1
(5) Hissing: absent = 0, present = 1
(6) Mating call: absent = 0, present = 1. (detailed in 7)
(7) Type of mating call: hiss = 0, grunt = 1 (associated with autapomorphic howl and moan in <i>C. nippon</i>), cackling = 2, groan = 3, roar = 4, bugle = 5
(8) F_0 of mating call: low < 250 Hz = 0, high > 600 Hz = 1
(9) Duration of mating call: short < 0.5 s = 0, long > 1.5 s = 1
(10) Non-herding mating calls: absent = 0, present = 1
(11) Herding mating calls: absent = 0, present = 1
(12) Emission pattern of mating call: single = 0, repeated = 1, polymorphic = P
(13) Stereotypy of repeated mating calls: stereotypical = 0, non-stereotypical = 1
(14) Vocal tract extension by larynx lowering in mating calls: absent = 0, present = 1
(15) Mouth configuration during herding mating calls: 0 = closed laterally, 1 = open with retracted lips and showing front teeth
(16) Male-female courtship dialogue: absent = 0, present = 1
(17) Chasing staccato call: present = 1, absent = 0
(18) Sparring call: present = 1, absent = 0

approaching a female (Cooke and Farrel, 1998) and in *C. canadensis* during agonistic interactions (Bowyer and Kitchen, 1987; Feighny et al., 2006).

(2) *Barking*. Barks are short and loud explosive vocalizations, typically aperiodic, but sometimes including a relatively high fundamental frequency and weak harmonics. Described in *M. moschiferus* as a rare behavior accompanying agonistic interactions (Flerov, 1952), barking is present in males and females of most Cervidae, with the exception of Odocoileini and *Rangifer* where it is replaced by the snort (Lent, 1975;

Richardson et al., 1983; Atkeson et al., 1988). Barking is typically categorized as an anti-predator, “pursuit deterrent signal”, informing the predator that it has been detected and that its approach is likely to be ineffective (Reby et al., 1999). It has also been shown that barks could function as territorial calls in *C. capreolus* (Reby et al., 1999). This type of call can thus be categorized as a “social and territorial status maintenance signal” together with long-term marking activities, like trampling or rubbing the antlers against small trees (Danilkin and Hewison, 1996). Barking has an anti-predator function as well as an agonistic function both during and outside the rutting period in territorial species like *M. muntjak* (Dubost, 1971), *H. inermis* (Flerov, 1952) and *C. capreolus* (Reby et al., 1999). In *A. alces* however, barking appears to be exclusively used in situations of imminent danger (Geist, 1999). While females of all Cervini species bark in alarm contexts, no barks or equivalent alarm calls have been identified in the adult male of *D. dama* (McElligott, pers. comm.) and *C. nippon* (Miura, 1984; Minami and Kawamichi, 1992). In *C. canadensis* a high pitched “cohesion” call is given by bulls in a similar postural context (Bowyer and Kitchen, 1987). *C. e. elaphus* and *C. e. corsicanus* males occasionally bark during the rutting period (Reby and McComb, 2003b), with the effect of increasing the cohesion of their harem.

(3) *Barking pattern*. In territorial species like *M. muntjak*, *H. inermis*, *C. capreolus* and *A. alces*, barking is given in stereotypical series of repeated calls. In contrast, in Cervini species, barking by males is infrequent outside the rutting period and is typically a unitary vocalization.

(4) *Snorting*. Snorts are short, aperiodic and noisy vocalizations, which do not involve the vibration of the vocal folds, and consist of a violent inhalation or

Table 3
Data matrix of vocal behavioral characters in eleven cervid species and two outgroups

	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8
<i>Ovis gmelini</i>	1	0	#	0	1	0	#	#	#	#	#	#	#	#	#	0	0	0
<i>Moschus moschiferus</i>	1	1	?	1	1	0	#	#	#	#	#	#	#	#	#	0	0	0
<i>Muntiacus muntjak</i>	1	1	0	0	0	1	2	?	?	1	0	1	?	?	?	0	1	0
<i>Dama dama</i>	1	0	#	0	0	1	3	0	0	0	1	1	0	1	0	0	1	0
<i>Cervus elaphus</i>	1	1	1	0	0	1	4	0	1	0	1	1	1	1	0	0	1	1
<i>Cervus corsicanus</i>	1	1	1	0	0	1	4	0	1	0	1	1	1	1	0	0	1	?
<i>Cervus canadensis</i>	1	1	?	0	1	1	5	1	1	0	1	0	#	1	1	0	1	1
<i>Cervus nippon</i>	1	0	#	1	0	1	1	1	1	1	1	P	0	0	1	0	1	1
<i>Hydropotes inermis</i>	1	1	0	0	1	1	0	?	?	1	0	1	?	0	?	1	0	0
<i>Odocoileus virginianus</i>	1	0	#	1	0	1	1	0	0	1	0	1	0	0	?	0	0	0
<i>Rangifer tarandus</i>	1	0	#	1	0	1	1	0	0	1	1	1	0	0	?	0	0	0
<i>Capreolus capreolus</i>	1	1	0	0	1	1	1	?	?	1	0	1	?	0	?	1	0	0
<i>Alces alces</i>	1	1	0	0	0	1	1	0	0	1	0	1	0	0	?	1	0	0

#, character absent, hence character state inapplicable in this column; ?, undocumented, all character states are possible; P, polymorph, both 0 and 1 states are present.

exhalation through the nostrils (Caro et al., 2004). Snorts are given all year round by *M. moschiferus* in alarm contexts, always preceding a hiss (Green, 1985). In *C. nippon*, the only snorting Cervinae species, snorts are emitted in agonistic contexts (Minami and Kawamichi, 1992). In all South-American Odocoileini (Richardson et al., 1983; Atkeson et al., 1988) and in *R. tarandus* (Lent, 1975), it is given either in a context of alarm, or after a dominant male has induced a subordinate male or a female into submission (Geist, 1999).

(5) *Hissing*. This call is frequently given by both *M. moschiferus* and *O. gmelini* in situations of alarm (Flerov, 1952; Green, 1985; Gonzalez, pers. comm.), in *C. canadensis* as an aggressive threatening behavior directed towards both males and females (Bowyer and Kitchen, 1987) as well as in *C. capreolus* along with charge blowing when a territorial male chases an intruder (Reby et al., 1999). Males of *H. inermis* also emit a hiss when approaching a female during the rutting period (Cooke and Farrel, 1998).

(6) *Mating calls: vocalizations specific to the rutting period*. Absent in both *M. moschiferus* (Flerov, 1952; Green, 1985) and *O. gmelini* (Gonzalez, pers. comm.), mating calls seem to be specific to cervids. These calls are defined as any vocalization addressed to the females during their oestrus period.

(7) *Types of mating calls*. This character codes the type of vocalization used as a mating call. Grunt (also called “rasping”) in *C. capreolus* (Danilkin and Hewison, 1996), grunt in *R. tarandus* and in *O. virginianus* (tending grunt) also addressed to dominated males during chasing runs (Lent, 1975; Richardson et al., 1983; Atkeson et al., 1988), grunt in *A. alces* males also given during threatening between males (Geist, 1999; Reby et al., 1999), cackling in *M. muntjak* (Dubost, 1971; Geist, 1999), hiss in *H. inermis* (Cooke and Farrel, 1998), and herding loudcalls of Cervini: groans and harsh groans in *D. dama* (Vannoni and McElligott, 2007); roars and harsh roars in *C. e. elaphus* (Reby and McComb, 2003a; b), roars in *C. e. corsicanus* (Kidjo et al., pers. comm.), bugles in *C. canadensis* (Bowyer and Kitchen, 1987; Feighny et al., 2006) and howls and moans in *C. nippon* (Minami and Kawamichi, 1992).

(8) *Fundamental frequency (F_0) of mating calls*. The fundamental frequency (F_0) of a vocalization corresponds to the rate of vibration of the vocal folds of the animal that vocalizes, and is responsible for the perceived “pitch” of the vocalization. F_0 of mating calls is highly variable in Cervidae, ranging from 34 Hz in *D. dama* to 2530 Hz in *C. canadensis*. F_0 below 250 Hz: *C. e. elaphus*, common roar: 112 Hz; *C. e. corsicanus*, roar: 36 Hz; *D. dama*, groan: 34 Hz; *R. tarandus*, grunt: 55 Hz; *A. alces*, grunt: 125 Hz; *O. virginianus*, tending grunt: 209 Hz. Undetermined: *M. muntjak*, *H. inermis*,

C. capreolus. F_0 above 600 Hz: *C. nippon*, howl: 1174 Hz, moan: 691 Hz; *C. canadensis*, bugle: 2530 Hz. (9) *Duration of mating calls*. This corresponds to the duration of the units that constitute mating calls. Most mating calls are relatively short, and only the genus *Cervus* is characterized by mating calls clearly exceeding 1 s in duration. Duration below 0.5 s: *D. dama*, groan: 0.38 s; *R. tarandus*, grunt: 0.18 s; *O. virginianus*, grunt: 0.2 s; *A. alces*, grunt: 0.24 s. Duration above 1.5 s: *C. nippon*, moan: 4.36 s, howl: 2.27 s; *C. canadensis*, bugle: 2.37 s; *C. e. elaphus*, roar: 1.9 s; *C. e. corsicanus*, roar: 1.6 s. Undetermined: *M. muntjak*, *H. inermis*, *C. capreolus*.

(10) *Non-herding mating calls*. This category gathers the soft, short range, vocalizations emitted by males as they approach a single oestrous female. Present: *C. capreolus* (rasps, Danilkin and Hewison, 1996), *H. inermis* (hiss, Cooke and Farrel, 1998), *O. virginianus* (tending grunt, Richardson et al., 1983; Atkeson et al., 1988), *A. alces* (grunts, Geist, 1999), *R. tarandus* (grunts, Lent, 1975), *M. muntjak* (cackling, Dubost, 1971), *C. nippon* (“successive grunt”, Minami and Kawamichi, 1992). Absent: *D. dama*, *C. e. elaphus*, *C. e. corsicanus*, *C. canadensis*.

(11) *Herding mating calls*. This category gathers the loud, long range vocalizations emitted by males towards females or rivals (usually associated with herding behavior, or leks). Present: *C. nippon* (howls and moans); *C. canadensis* (bugles), *C. e. elaphus* (common roars and harsh roars); *C. e. corsicanus* (common roars and harsh common roars); *D. dama* (common and harsh groans); *R. tarandus* (grunts, Lent, 1975); moose (grunts, Geist, 1999). Absent: *M. muntjak*, *H. inermis*, *C. capreolus*, *A. alces*, *O. virginianus*.

(12) *Emission pattern of mating call*. Calls can be given as isolated units (single), or typically in series. Single: *C. canadensis*, bugle. Repeated: *A. alces*, grunt; *D. dama*, groan, *R. tarandus*, grunt; *C. e. elaphus* and *C. e. corsicanus*, roars; *O. virginianus*, tending grunt; *M. muntjak*, cackling; *H. inermis*, hiss; *C. capreolus*, rasp. Polymorphic: *C. nippon* (single moan and repeated howl).

(13) *Level of stereotypy in repeated mating calls*. While some repeated mating calls consist of the repetition of a single stereotypical unit, others consist of a sequence of calls varying in their acoustic structure (duration, intensity and frequency modulation). Stereotypical: *D. dama*, groan; *A. alces*, grunt; *R. tarandus*, grunt; *C. nippon*, howl. Non-stereotypical: *C. e. elaphus*, roars and *C. e. corsicanus*, roars.

(14) *Vocal tract extension by larynx lowering and associated formant modulation in herding mating calls*. Formant frequencies correspond to broadband frequency components resulting from vocal tract filtering (in the pharynx, mouth and/or nasal cavity) of the “glottal wave” generated by vibration of vocal

folds (Fant, 1960). In most mammals, the larynx is tightly attached to the basis of the skull, limiting variations of the length and shape of the vocal tract. This typically results in the absence of significant modulation of the formant frequencies. The presence of formant frequency modulation is typical of the loudcalls of male Cervini (with the exception of *C. nippon* males) and reflects the ability of the males of these species to move their larynx downward, thereby extending their vocal tract as they vocalize (Fitch and Reby, 2001; Reby and McComb, 2003a,b; McElligott et al., 2006).

(15) *Configuration of the mouth during herding mating calls.* The mouth can be kept open with the lower lip retracted and the front teeth showing, as seen in *C. canadensis* and *C. nippon*. Alternatively, the mouth can also be opened vertically while narrowing laterally as observed in *D. dama* and in *C. elaphus* (Geist, 1999). Undetermined: all the other species.

(16) *Male-female courtship dialogue.* During approach a vocal interaction can take place between the male and the female, the latter giving a call similar to the young-contact call (Reby et al., 1999). Present in *C. capreolus* (Danilkin and Hewison, 1996), *A. alces* (Geist, 1999) and *H. inermis* (Cooke and Farrel, 1998).

(17) *Presence of a chase staccato call.* This rapid series of short calls is given during the rutting period by dominant males as they chase a female or a subordinate male. Present in *M. muntjak*, cackling (Dubost, 1971; Geist, 1999); *D. dama*, belch (Alvarez et al., 1975; Reby and McComb, 2003b); *C. elaphus*, chasing barks (Clutton-Brock and Albon, 1979; Reby and McComb, 2003b); *C. canadensis*, yelp (Bowyer and Kitchen, 1987); *C. sika*, “aggressive snort” (Minami and Kawamichi, 1992).

(18) *Presence of a sparring call.* This type of call is given by young or adult males before or during sparring (Minami and Kawamichi, 1992). Present in *C. nippon*, sparring mew; *C. canadensis*, sparring squeak; *C. e. elaphus*, sparring moan (Reby, pers. obs.). Undetermined: *C. e. corsicanus*.

Topologies and cladistic consistency

The parsimony analysis of the 18 vocal characters (16 parsimony informative) in the 13 taxa produced one most parsimonious tree with tree length = 29 steps, CI = 0.72 and RI = 0.78. With *Ovis* as outgroup the topology is (*Moschus* ((*Rangifer*, *Odocoileus*) (((*Hydropotes*, *Capreolus*) *Alces*) (*Muntiacus* (*Dama* ((*C. e. elaphus*, *C. e. corsicanus*) (*C. nippon*, *C. canadensis*)))))). This topology is very similar to the one presented in Fig. 3, except that the two clades in Capreolinae constitute a paraphyletic group, with (*Rangifer*, *Odocoileus*) in a more basal position than ((*Hydropotes*, *Capreolus*) *Alces*), while *Cervus* is split into two sister

clades with a consistent *C. elaphus* species. The CI and RI in our study can be compared to those in Randi et al. (1998), whose molecular cladogram had a CI = 0.42 and an RI = 0.59. A parsimony analysis with Hennig86 (Farris, 1989a,b) gave 10 equiparsimonious trees (including the one just described). The strict consensus shows *Moschus* as sister-group of the remaining taxa, a completely resolved clade for *Muntiacus*, *Dama* and *Cervus*, and no consensual resolution for the remaining taxa (Capreolinae), i.e.: (*Moschus* ((*Muntiacus* (*Dama* ((*C. e. elaphus*, *C. e. corsicanus*) (*C. nippon*, *C. canadensis*)))) (*Alces*) (*Hydropotes*) (*Capreolus*) (*Rangifer*) (*Odocoileus*)). While none of these trees should be discarded according to the “inapplicable data” coding logic, these extra solutions show zero-length branches, and should therefore be discarded on the basis that some character change must be inferred for supporting a phylogenetic topology. This indicates, nevertheless, that there is no strong resolution for Capreolinae in the vocal data analysis.

The molecular parsimony analysis (430 parsimony informative characters) gave the following topology: (*Ovis*, *Moschus* ((*Rangifer*, *Odocoileus*) ((*Capreolus*, *Hydropotes*) *Alces*) (*Muntiacus* (*Dama* (*C. e. corsicanus* (*C. nippon* (*C. Canadensis*, *C. e. elaphus*)))))). This topology is identical to the phylogeny based on the simultaneous analysis of all molecular and vocal data (Fig. 3). On this topology (measured with equal weights for all characters and all character states transformations), the molecular data (1460 steps) yields a CI = 0.56 and an RI = 0.38, the vocal data (35 steps) yields a CI = 0.60 and an RI = 0.64, while the combined data set (1495 steps) yields a CI = 0.56 and an RI = 0.39. Although this topology is not optimal for the vocal data, its homoplasy ratios remain comparable with those of molecular topologies also measured with equal weights for all characters and transformations.

The cladograms based on behavioral data only (see above) and molecular data only (Fig. 3) show some strong similarities: *Moschus* is unambiguously outside Cervidae, the general structure of Cervinae is resolved identically: (*Muntiacus* (*Dama* (*Cervus*))), and in Capreolinae, two clades are identified: (*Rangifer*, *Odocoileus*) and ((*Hydropotes*, *Capreolus*) *Alces*). The two differences are: a lack of resolution of the general structure of Capreolinae in the molecular analysis, the two previously mentioned clades standing as an unresolved polytomy with Cervinae; and a discordance in the internal structure of *Cervus*, appearing as (*C. e. corsicanus* (*C. nippon* (*C. Canadensis*, *C. e. elaphus*))) in the molecular and simultaneous analyses, and as ((*C. e. elaphus*, *C. e. corsicanus*) (*C. nippon*, *C. canadensis*)) in the vocal analysis. It should be noted that the vocal behavior based resolution in *Cervus* is the same as that issued from the Bayesian analysis of *Cytb* by Pitra et al. (2004) for the same four taxa. The combination of

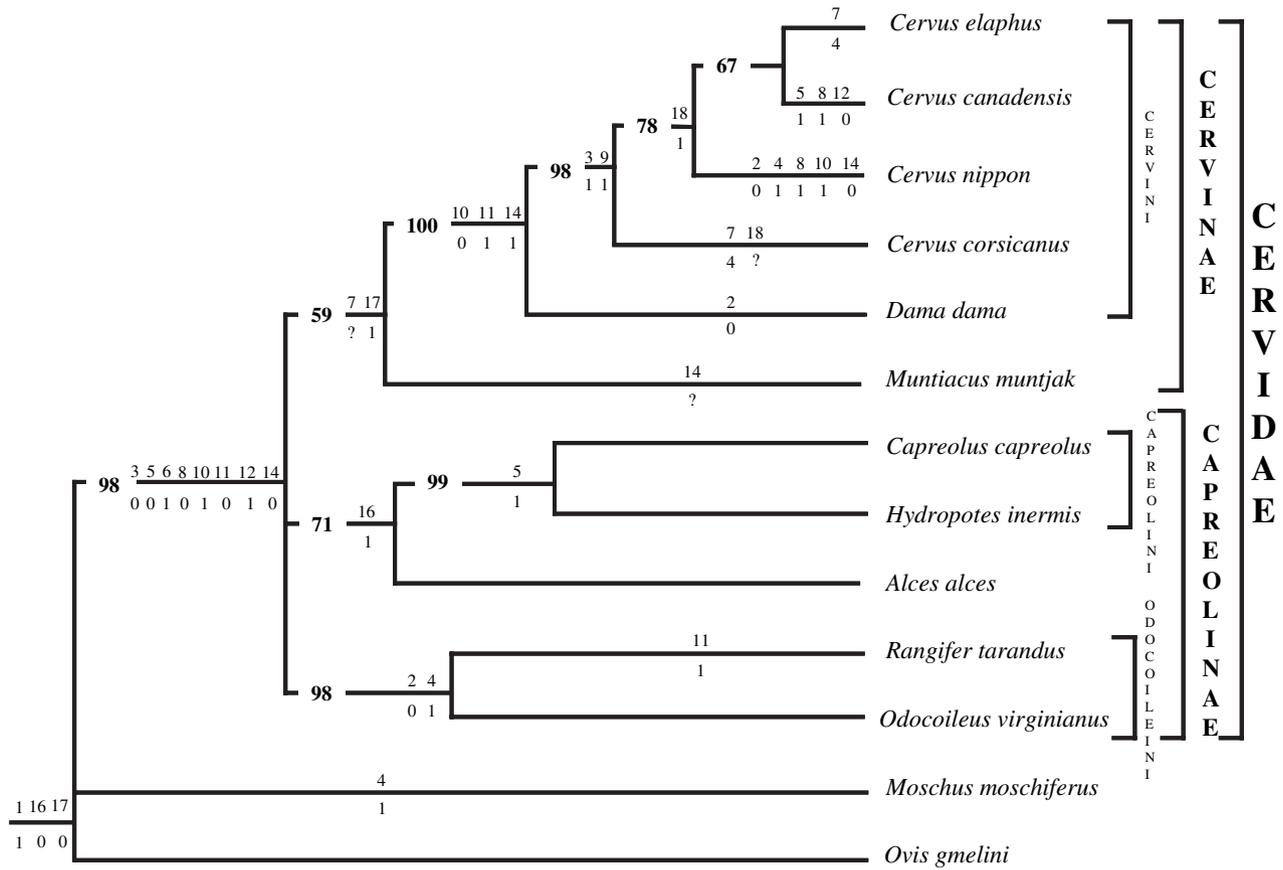


Fig. 3. Cladogram supported by vocal and molecular data (simultaneous parsimony analysis). Bold numbers on branches indicate bootstrap values. Vocal synapomorphies (except for ambiguous characters 13 and 15, see text) are indicated with character number (above branches) and character state number (below), referring to character descriptions and data matrix (Tables 2 and 3).

the vocal characters with the molecular ones does not influence the topology or the global CI, but there is an effect on the bootstrap values of some clades, notably slight raises for Cervinae (54 → 59) and *Cervus* (95 → 98), and a clear decrease for the somewhat surprising internal pectinate structure of *Cervus* splitting *C. elaphus* (88 → 78 and 85 → 67).

Behavioral phylogenetic patterns

The eighteen vocal characters were optimized on the topology of the simultaneous analysis tree (Fig. 3). The optimal phylogenetic patterns of the vocal characters are as follows:

Ovis: The only vocalizations recorded in mediterranean mouflon and sheep are the bleat, given all year round by rams, ewes and lambs (1), and the hiss (5) given by both rams and ewes in alarm contexts (Gonzalez, pers. comm.).

Moschus: in musk deer, the bark (2) is infrequent, and given as a contact call or during agonistic interactions, while barking is homologous and widespread in cervids,

where it also serves as an alarm and/or territorial display. In contrast with cervids, the musk deer alarm call is a hiss (5), which is often repeated twice. This call is also given (structural convergence) as a general threat signal in *C. canadensis* males, and along with the charge blowing given by territorial roe deer males when they chase an intruder. Musk deer also give a snort (4), sometimes preceding a blowing. In Cervinae, only *C. nippon* males are known to emit nasal snorts, also in agonistic contexts. Otherwise, all South American Odocoileini as well as *Rangifer* share this call, given either in alarm context or after a dominant male induces submission in a conspecific adult. Curiously, the hiss–snort association is found in dik-dik *Madoqua* sp. (Neotragini), and the snort is present in gazelles (Alcelaphini) and most bovid species (Walther, 1974). These observations tend to support the hypothesis that the musk deer is a sister-group of bovids (Hassanin and Douzery, 2003). Accordingly both kinds of blowing, either through the mouth (hiss) or through the nose (snort), still existing in a number of cervid species would constitute relic behaviors or reversals to a very ancient

behavioral trait antedating the origin of Cervioidea and corresponding to that of the common ancestor of bovids, moschids and cervids.

Cervidae: the exclusive common ancestor of Cervids would have shown stereotyped barks (3), as seen in *Muntiacus*, *Capreolus* or *Hydropotes*; as well as a mating call (6) with a low frequency (8), composed of short units (9) repeated in series in a stereotyped way (12, 13) somewhat similar to the male *Alces* mating call. The relatively low fundamental frequency observed in these calls may reflect the fact that the common ancestor of cervids lived in the relatively open landscape typical of the early Miocene (Janis, 1993), favorable to the propagation of low frequencies.

Cervinae: this group is characterized by the chasing staccato calls of different types, typically given during the rutting period and directed toward a female or a subordinate male (17). This is thus an exclusive synapomorphy for this group. In support for the homologous coding of this character in Indian muntjac (cackling) and other cervinae (i.e. Cervini), it must be noted that the examination of one male specimen of this species showed a descended larynx (14), a characteristic exclusively found in Cervini who present modulated formants (Reby, pers. obs.).

Cervini: their ancestral behavior would have comprised a unitary bark (3) and the replacement of non-herding mating call (10) by a typically louder herding call (11) with modulated formants (14). In this group, the bark does not seem to serve a territorial function any more, but rather a function of contact and dominance, the alarm function being mostly performed by females. Males in this group are typically polygynous and do not defend territories but form leks or defend harems. The original mating call evolved into a loud sexual vocalization, typically characterized by low and modulated vocal tract resonances (formants), as enabled by a descended and mobile larynx.

Cervus: this genus is originally characterized by a courting call with units reaching long durations (9), constituting a complex composition (single or non stereotyped series) with sparring calls (18). This behavioral profile is in contrast with that of the genus *Dama* in which the herding call is composed of identical and brief units repeated a stereotypic way.

C. elaphus: includes the common red deer *C. e. elaphus* and the Corsican red deer *C. e. corsicanus*, whose herding calls consist of bouts of non stereotyped units (13). While this species is surprisingly split in both the molecular only and molecular + vocal behavior analyses, the vocal behavior analysis supports the topology of Fig. 1A (Pitra et al., 2004) for *Cervus*, which suggests a monophyly of *Cervus elaphus* (and also a monophyly of *C. nippon*, *C. canadensis*).

C. nippon and **C. canadensis:** both species are characterized by a herding call with a high F_0 (8) composed of unitary but modulated units (12) and given with an open mouth and retracted lips (15). *C. canadensis:* the males of this species give a hiss (5) while threatening opponents. This hiss appears as a parallelism with the aggressive hiss in other taxa. *C. nippon:* its vocal repertoire comprises a strong nasal exhalation (snort) (4) in agonistic contexts. This character is convergent with similar behavior in territorial species (*Moschus*, *Odocoileus*). Contrary to the other *Cervus* species defending a moving harem, *C. nippon* males defend a territorial zone where they gather their harem (Minami and Kawamichi, 1992). Interestingly, this is concomitant with the re-emergence of the snort and a non-herding mating call in this species (10), whereas barking (2) and formant modulation in mating calls (14) are lost.

Capreolinae (including *Hydropotes*): these telemetacarpalian cervids show a courting approach call (10). This kind of behavior corresponds to all vocalizations performed by a male when approaching an immobile female at short range. It is found in *C. capreolus* under the form of rasp during slow approach before engaging in chasing the female, while *A. alces*, *R. tarandus* and *O. virginianus* males use a grunt when approaching females. These vocalizations could also be seen as an ancestral state with non-herding mating call as the original condition of mating call in cervids, and would be plesiomorphic in telemetacarpalian Cervinae. In Capreolinae, mating calls are grunts (7).

Alceini, Capreolini, Hydropotini: these three tribes show a vocal dialogue between male and female (16) which takes place after an approach by the male.

Alces: grunts are addressed to both males and females (10) and this species shows the characteristics of the ancestral mating call of the Cervidae (9, 10, 12 and 13).

Capreolini and Hydropotini: these groups present the hiss (5), given in two contexts: while charging another male in *Capreolus*, or while approaching a female in *Hydropotes*. This behavior could reflect a return to a habitat more closed than that of the early Cervidae, namely Euro-Asiatic forests for *Capreolus* and long grass vegetation of marsh borders for *Hydropotes*.

Odocoileini and Rangifer: this group is characterised by the loss of the bark (2), which is replaced by a loud snort (4) in either agonistic, alarm or rutting context.

Rangifer: herding calls (11) in *Rangifer* and Cervini are convergent behaviors. This fact can be explained by the similarity of their reproductive system showing an important polygamy. However, contrary to Cervini, *Rangifer* vocalizations are generally performed during slow, short-range approach of females. Herding is the only common behavior to these two taxa, although not all reindeer populations constitute harems.

Discussion

We found phylogenetic congruence between the studied behavioral characters and the molecular phylogenies of Pitra et al. (2004), Randi et al. (1998) and Gilbert et al. (2006), and the present parsimony analysis of molecular data. Topologies are identical for the general structure of Cervinae, and in Capreolinae they show a (*Rangifer*, *Odocoileus*) clade and a (*Capreolus*, *Hydropotes*, *Alces*) clade (considered as non-resolved for the position of *Alces* in Gilbert et al., 2006). On the simultaneous analysis topology, the CI and the RI of vocal-behavioral data are in no way weaker than those from molecular ones for equal character and character change weights. Interestingly, the lack of strong resolution with behavioral data (in Capreolinae) affects the same clade where molecular phylogenies show lowest bootstrap values (Gilbert et al., 2006), which could indicate some past events, like fast radiation, blurring a strictly dichotomous pattern for these taxa.

When considering the potential importance of behavioral characters for phylogeny inference, it must be underlined that this result was obtained without *a priori* sorting of the vocal behavioral characters, which were exclusively treated according to their acoustic and contextual parameters, and not selected according to any kind of expected phylogenetic consistency. While most students of behavioral phylogeny are typically wary about using behavior as characters (down to using only one character in some cases, e.g. Baroni Urbani, 1989), our study shows that the exclusive use of even a limited set of behavioral characters can yield consistent results. This confirms the value of vocalizations (and more generally behavior) for phylogenetic evolutionary studies (Mattern and McLennan, 2000; Dessutter-Grandcolas and Robillard, 2003; Lusseau, 2003; Robillard et al., 2006). Despite objections against the use of behavior in systematics (e.g. Wimberger and De Queiroz, 1996), this successful test of congruence supports the use of behavioral traits as characters directly in data matrices supporting phylogeny inference (Macedonia and Stranger, 1994; Irwin, 1996; Cap et al., 2002).

Based on scenarios inferred from the simultaneous analysis cladogram, a general optimal evolutionary scenario for cervids can be outlined as follows. According to fossil data, cervids would have diversified during the Miocene along with a general opening of the landscapes (Janis, 1993; Gentry, 1994; Webb, 2000). They would have been monogamous or slightly polygynous territorial species that used stereotypical barks as territorial, anti-predator and social maintenance calls, and a rutting call close to the *Alces* snort while approaching oestrous females.

In the Capreolinae group, our results tend to confirm the scenario derived from paleontological and molecular analyses (Randi et al., 1998; Pitra et al., 2004). The

Alceini tribe would comprise not only *Alces*, but also *H. inermis* and *C. capreolus*. The common behavioral repertoire for these three species points to a hypothetical ancestor fairly similar to the original cervid one: a territorial species, with female short distance approach involving a low frequency vocal dialogue between partners like in *Alces*. The likely return to a close habitat in ancestors of *Hydropotes* and *Capreolus* possibly favored the change of a low frequency courting call for hiss or rasp in these two species. For Odocoileini and *R. rangifer*, fossil data indicate a North-Asiatic origin (Webb, 2000) and, because of its passing through the Bering Strait before the early Pliocene, this group would have a more northern origin than Alceini. It is characterized by the loss of the bark, which is replaced by the exhalation snort, in agonistic, alarm and rutting contexts. During the rutting period these two taxa also perform a complex grunt during both inhalation and exhalation. In comparison, Alceini, Odocoileini and the reindeer seem to have colonized more open habitats, where territoriality would have been more problematic and gregariousness would have developed to the extreme form present in reindeer.

In Cervini, increased sexual selection associated with increased polygyny and sexual dimorphism would have led to the formation of harems rather than territories, and to the emergence of associated behaviors such as female herding and chasing of young male competitors. Such evolutionary pressures would have seen the loss of territorial stereotyped barks and the emergence of chase barks (*C. nippon*: Minami and Kawamichi, 1992; *C. e. elaphus*: Reby and McComb, 2003b; *C. e. corsicanus*: Kidjo, pers. comm.) as well as the deceptive use of single alarm barks to increase harem cohesion (Reby and McComb, 2003b). Considering acoustic structure, sexual selection for larger males would also have led to the use of formant frequencies for size assessment in intra-sexual (Reby et al., 2005) and inter-sexual (Charlton et al., 2007) interaction, favoring the evolution of size signal exaggeration by vocal tract elongation. This would have in turn led to the evolution of a permanently descended (*D. dama*, *C. e. elaphus*, *C. e. corsicanus*, *C. canadensis*) and/or mobile larynx (*D. dama*, *C. e. elaphus*, *C. e. corsicanus*, and also Pere David's deer *C. e. davidensis*). The general increase in size would have also led to a general frequency lowering of the spectral components (F_0 and formants) in all the call types.

The genus *Cervus* shows a complexification of the herding call with an increased modulation of the intensity contour and of the frequency components of the calls. In the western range of the genus *Cervus*, the taxa *C. e. elaphus* and *C. e. corsicanus* give bouts of non-stereotypical roars, in sharp contrast with the low-pitched stereotyped series found in *Dama*, while in the eastern range *C. nippon* and *C. canadensis* give unitary, highly modulated and high-pitched calls. Such an

evolution could be linked with an originally closed habitat, which would have favored a shortening of calling distances to the advantage of males with higher F_0 (Geist, 1999).

Another difference between the *Dama* and *Cervus* species is the presence of leks during the rutting period in *Dama* (Alvarez et al., 1975), which could represent the ancestral form of vocal contest organization in Cervini. Muntiacini, restricted to closed habitats, would have evolved very little relative to ancestral cervids. The courting call would have persisted only as a bark, which is much more frequent during the rutting period (Dubost, 1971), and charge barks performed while approaching females would have been replaced by cackling.

This evolutionary scenario is consistent with the one we previously established relatively to a phylogeny based on the female winter behavioral repertoire in nine species of Cervidae (Cap et al., 2002). This scenario suggested a more southern origin of Cervinae compared to Capreolinae (Alvarez et al., 1975) and close relationships between Moschids and Cervids (Gentry, 1994).

In conclusion, this study illustrates the effectiveness of behavioral characters as possible phylogenetic markers (McLennan et al., 1988; Wenzel, 1992; Kurt and Hartl, 1995; Miller and Wenzel, 1995; Lundrigan, 1996) as well as for testing evolutionary scenarios with reference to phylogenetic topologies (Carpenter, 1999; Cap et al., 2002). Our categorization of vocal behavior characters distinguished between the ability to perform specific types of vocalization (presence/absence of specific vocalization structure in the repertoire) from the ability of vocalizing in a particular context (differences in vocal communication functions), considering that an evolutionary change must have occurred in either cases. In the absence of more specific knowledge about evolutionary processes, this coding scheme avoided imposing an arbitrary hierarchy between structural and functional changes in such characters. Interestingly, Robillard et al. (2006) suggested an extension of the primary homology criteria for vocal characters, by considering the anatomical constraints and mechanisms at the basis of the production of the sounds on top of their acoustic structure. This enabled them to improve the categorization of vocal characters for phylogenetic analysis, compared with the more classical spectrographic similarity approach. Similarly, in our study, the impact of the position and mobility of the larynx on the range and modulation of formant frequencies in *Cervus* species enables us to homologize red deer low pitch herding calls with wapiti very high-pitched calls, despite the strong apparent dissimilarity of their spectrograms. While behavioral data can contribute to phylogeny inference, the requirements of phylogenetic analysis call for improved and diversified homology criteria, which might in turn prove beneficial for behavioral studies.

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