UNIVERSIDAD COMPLUTENSE DE MADRID

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Departamento de Paleontología

ANÁLISIS EVOLUTIVOS Y ECOLÓGICOS DE RUMIANTES BASADOS EN DATOS FILOGENÉTICOS

MEMORIA PARA OPTAR AL GRADO DE DOCTOR
PRESENTADA POR

Juan López Cantalapiedra

Bajo la dirección de los doctores

Jorge Morales Romero
Manuel Hernández Fernández

Madrid, 2012

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Basados en Datos Filogenéticos

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Universidad Complutense de Madrid
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Consejo Superior de Investigaciones Científicas
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BAJO LA DIRECCIÓN DE LOS DIRECTORES
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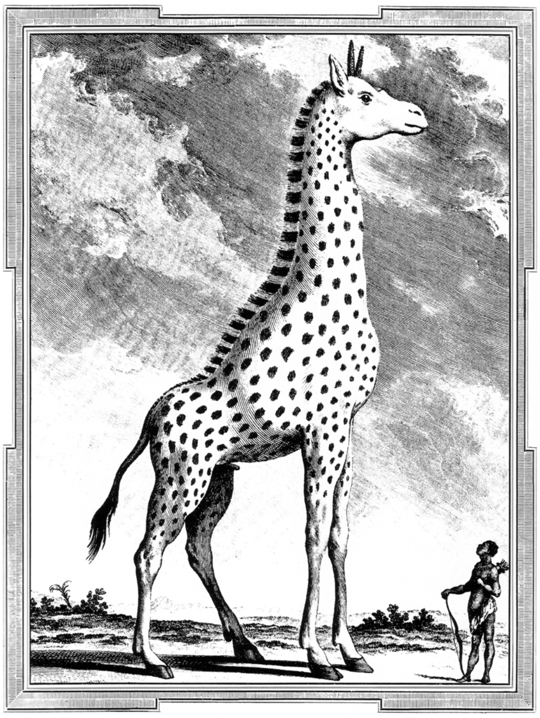
A mis padres y a mis amigos.



It is the beginning of an exciting new era in evolutionary reconstruction.

- Fredrik Ronquist -

Bayesian inference of character evolution (2004)

To emphasize that the three turnover responses of species are closely related by a common causal principle, they may be compared to the three faces of the Hindu Triad of deities: the passive response, distribution change without macroevolution, corresponds to Vishnu the Preserver, extinction to Siva the Destroyer, and speciation to Brahma the Creator.

- Elisabeth Vrba -

Turnover-pulses, the Red Queen, and related topics (1993)

I thought of the long ages of the past, during which the successive generations of this little creature had run their course–year by year being born, and living and dying amid these dark and gloomy woods, with nointelligent eye to gaze upon their loveliness; to all appearance such a wanton waste of beauty. [...]. It seems sad, that on the one hand such exquisite creatures should live out their lives and exhibit their charms only in these wild inhospitable regions, [...]. This consideration must surely tell us that all living things were not *made for man. Many of them have no relation to him. The cycle of their existence has gone on independently of his, and is disturbed or broken by every advance in man's intellectual development; and their happiness and enjoyment, their loves and hates, their struggles for existence, their vigorous life and early death, would seem to be immediately related to their own well-being and perpetuation alone[...].*

- Alfred Russell Wallace -
The Malay Archipelago (1869)

Índice

AGRADECIMIENTOS

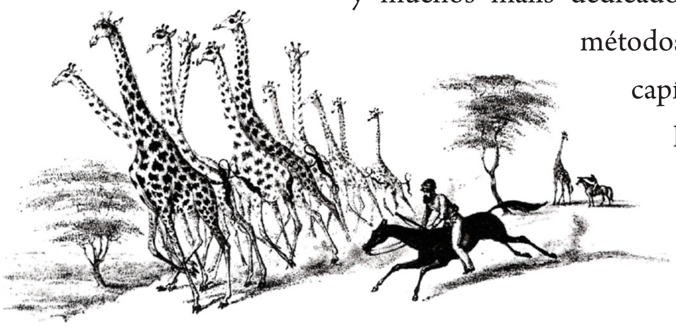
Que yo terminara estudiando paleontología y trabajando en esta tesis fue fruto de la conjunción de dos oportunidades. La primera fue que mis padres me apoyaran desde el principio en todas mis decisiones, las que me llevaron a estudiar Biología en Madrid y más tarde a emprender un doctorado en Paleontología. Es inmenso mi agradecimiento a esa libertad de elección y a su apoyo a lo largo de todos estos años. La segunda oportunidad me la ofrecieron los directores de esta tesis, Jorge Morales y Manuel Hernández Fernández. Su confianza en un recién llegado al mundillo paleontológico fue un gesto que espero se vea en parte recompensado con la consecución del presente trabajo. En estos años ambos me han regalado su apoyo, un inmenso conocimiento que se extiende más allá de la *paleo*, y la experiencia de trabajar en sus proyectos, incluyendo el privilegio de excavar en los yacimientos de Somosaguas y Cerro de los Batallones. Su aportación humana y científica durante estos años es impagable.

Una parte muy importante de este trabajo nace gracias a la colaboración con Beatriz Azanza, Daniel de Miguel y Gema Alcalde, quienes además de prestar los datos de dietas y locomoción de rumiantes han contribuido en parte significativa a la discusión de varios de los capítulos. Israel Sánchez contribuyó de manera sustancial a la revisión de la introducción y me ayudó con la sección sobre Moschidae que se incluye en la misma.

He tenido la suerte de que los proyectos y las excavaciones en los que he colaborado han estado siempre impregnados de una gran dimensión humana. Así, mi aportación ha sido siempre motivada por las relaciones de amistad que me unen a la gente con la que trabajo. Es el momento de agradecer a todos los becarios (y *adjuntos*) con los que he compartido tantas horas de trabajo, de carcajadas y de momentos inolvidables ya fuera en un despacho, en una excavación o en una furgoneta camino de un congreso. Sin que el orden de nombramiento haga referencia más que a mi entropía cerebral, son Óscar, Sole y Laura, Adriana, Enrique, Miriam, Blanca, Ana, Paloma, Vero, María, Omid, Isra y Vicky. Incluyo en el agradecimiento a todos los miembros del Equipo de Introducción a la Investigación GeoPaleoBiológica en Somosaguas (que responde al pegadizo acrónimo EIIGPBS) que han colaborado tanto en las excavaciones como en los trabajos de investigación asociados al yacimiento. Son ellos los que dan energía a un concepto tan maravilloso como el que se gesta alrededor del yacimiento de Somosaguas y que, a mi juicio, marca la pauta a seguir si creemos en una paleontología divulgativa, cercana y para todos. Nieves López, fallecida a finales de 2010, fue sin duda quien visionó esa paleontología para la gente, quien potenció todas las caras del poliedro Somosaguas: investigación, divulgación y educación. A ella dedico un agradecimiento muy especial. La paleontología en España dará un gran paso si decide seguir las ideas que Nieves proyectó en Somosaguas.

También quiero agradecer a Marian Álvarez, quien me animó a excavar en el Cerro de los Batallones por primera vez; por sus sabios consejos durante todos estos años. A Robert Asher, por sus interesantes conversaciones en Batallones y por ser mi guía en el 68º congreso de la *Society of Vertebrate Paleontology* celebrado en Cleveland en 2008, todo un viaje iniciático para mí.

Estos años de doctorado he tenido la gran suerte de poder disfrutar de tres estancias en el extranjero. Dos de ellas en la Simon Fraser University, en Vancouver, y una en Berkeley. Vancouver, donde estuve un total de seis meses, marcó la trayectoria de mi tesis. Bajo la tutela de Arne Mooers aprendí tantas cosas útiles que mi deuda con él y su equipo está a la altura de la que tengo con mis directores de tesis. Se podría decir que Arne me adoptó y en numerosas ocasiones compartí muy buenos momentos con él, su mujer y su pequeña hija. Trabajar en su equipo me abrió la puerta a la biología evolutiva moderna. Volví de Vancouver con ideas sobre cómo casar lo que allí aprendí con la paleontología, algunas de las cuales se han materializado en capítulos de esta tesis. En el agradecimiento a Arne tengo que incluir a Jeff Joy y Rich FitzJohn, a quienes debo su paciencia, muchas horas y muchos mails dedicados a mostrarme los entresijos de los métodos filogenéticos más punteros. Varios capítulos de esta tesis se benefician de la inestimable ayuda y el método desarrollado por Tyler Kuhn para



generar distribuciones de árboles filogenéticos resueltos, siendo inmensa mi gratitud hacia él. Mis estancias en Vancouver se enriquecieron en gran medida gracias a los numerosos comentarios en los seminarios que impartí para la gente del FAB* Lab y Les Ecologistes. Siempre hay montones de cosas que aprender de gente como ellos. El otoño de 2009 pasé dos meses en la Universidad de California en Berkeley. Allí tuve el privilegio de trabajar con Anthony Barnosky y su equipo. Pude ver cómo trabaja un equipo que es referencia en la paleontología mundial. Al propio Barnosky le agradeceré siempre su inmensa amabilidad, su trato afectivo y su compromiso. También le agradezco la invitación a la cena navideña que celebró en su casa de Standford, agradecimiento que extiendo a su mujer, Liz Hadly. Quiero agradecer especialmente la ayuda y la simpatía de la gente del *lab*: Susumu Tomiya, Kaitlin Maguire y Jenny McGuire. También a Nicholas Matzke por los ratos de trasteo con Lagrange y la maravillosa fiesta de Halloween que organizó en su casa. Las estancias breves tienen un importante componente fuera de la universidad que es fundamental para la supervivencia de la mente cuando estás fuera de casa. No puedo dejar fuera de este apartado a Daniel y Jenny, Terry, Nick, Scotty, Will, Aaron, Anni, Nimal, Laura, Adam, Ricardo, Darko, Diego, Ana, Lina y Maggie. Sin ellos no habría tenido vida social más allá del *lab* y mis estancias hubieran sido bastante menos llevaderas.

No puedo concluir estos agradecimientos sin mencionarles a ellos. A mis amigos les debo mi supervivencia. En Casa Camacho, deambulando por Palencia, en Varsovia o en un ger en el valle del río Orkhon, ellos están siempre a bordo. Muchas gracias por entenderme, hacerme reir, consolarme, acompañarme en todos los caminos que recorro. Mi vida sin vosotros sería otra más gris; mucho menos divertida.

PUBLICACIONES

Capítulo 2: Cantalapiedra JL, Hernández Fernández M, Alcalde G, Azanza B, DeMiguel D, Morales J (in press) Ecological correlates of ghost lineages in ruminants. *Paleobiology*.

Capítulo 3: Cantalapiedra JL, Hernández Fernández M, Azanza B, Morales J (in prep.) Global phylogenetic data show major shifts in diversification of ruminant lineages associated to Miocene climatic episodes.

Capítulo 4: Cantalapiedra JL, FitzJohn RG, Hernández Fernández M, DeMiguel D, Azanza B, Morales J, Mooers A (in revision) Mixed-feeding and the diversication of ruminants through the Tertiary. *BMC Evol Biol*.

Capítulo 5: Cantalapiedra JL, Hernández Fernández M, Morales J (in revision) Biomic specialization and speciation rates in ruminants (Cetartiodactyla, Mammalia): a test of the resource-use hypothesis at the global scale. *PLoS One*.

Capítulo 6: Cantalapiedra JL, Hernández Fernández M, Morales J (in revision) Historical factors of continental and biogeographic regions are responsible for phylogenetic structure in ruminant metacommunities. *J Biogeogr*.

Breve Prefacio

Éste es un trabajo sobre la evolución de los rumiantes, con un énfasis especial en las conexiones entre su ecología y los cambios físicos globales. También explora algunas de las razones que han llevado a este grupo a protagonizar una de las radiaciones adaptativas más exitosas de la historia evolutiva de los mamíferos cuyo resultado es la gran diversidad de ecomorfotipos y la amplia distribución geográfica que presentan hoy en día. Si bien se tiene un conocimiento razonablemente extenso sobre sus características e historia natural, todavía existen muchas incógnitas acerca de cómo aparecieron sus rasgos ecológicos más notables, cómo éstos han cambiado a lo largo del tiempo y hasta qué punto se correlacionan con la evolución de la Tierra durante los últimos 50 millones de años, época en la que se ha producido su radiación evolutiva. Siendo un grupo que ha jugado un papel ecológico tan importante en los ecosistemas terrestres, conformando parte fundamental en las comunidades de herbívoros del Neógeno, profundizar en la comprensión de todas estas cuestiones puede ayudarnos a comprender mejor, no sólo a los rumiantes, sino también la historia de los complejos sistemas ecológicos de los que forman y formaron parte. Es la finalidad de esta tesis acercarnos desde diferentes puntos de vista, desde diferentes alternativas metodológicas, a la naturaleza de las conexiones entre ecología, cambio del entorno y la evolución de estos herbívoros.

1

Introducción

Los rumiantes

Con alrededor de 200 especies, los rumiantes (Ruminantia Scopoli, 1777) forman actualmente el suborden más diverso dentro de los cetartiodáctilos. Su evolución abarca los últimos 50 millones de años y su íntima relación con nuestra propia historia así como su importancia económica los ha convertido en el objetivo de muchos estudios desde muy diversas disciplinas (Vrba y Schaller, 2000). En concreto, los trabajos de ecología y macroevolución encuentran en las especies de este suborden una serie de características que lo convierten en el grupo de estudio ideal. En primer lugar conforman un grupo muy diverso lo que permite realizar diferentes aproximaciones para las cuales el número de observaciones sea transcendental para la validez estadística de los análisis. Además poseen gran variedad de adaptaciones ecomorfológicas que no tiene igual entre el resto de ungulados y grandes herbívoros (Wilson y Reeder, 2005; Marcot, 2007). Su diversidad abarca desde formas de talla pequeña y costumbres solitarias como los ciervos ratón (*Tragulus javanicus*), ramoneadores que habitan los densos bosques tropicales de Asia, hasta especies gregarias de gran talla que pastan en manada en las grandes llanuras del este de África como los búfalos cafres (*Sincerus caffer*) o los ñúes (*Connochaetes taurinus*). Algunos rumiantes incluso superan la tonelada de peso, como es el caso del búfalo de agua asiático (*Bubalus bubalis*). Su amplio espectro ecológico ha permitido a este grupo distribuirse por todos los continentes a excepción de Oceanía y Antártida, y estar presente en todos los biomas, desde los desiertos a las tundras; desde los bosques ecuatoriales a las frías estepas. Esta diversidad ha permitido a los investigadores incluirlos en trabajos de ecología evolutiva y abordar diversas cuestiones sobre la coevolución de aspectos tales como talla, dieta, comportamiento social y antipredador (Roberts, 1996; Nieto, 1998; Berger y Gompper, 1999; Brashares et al., 2000; Blob y LaBarbera, 2001; Christiansen, 2002; Bro-Jorgensen, 2008). Los rumiantes son herbívoros y, como tales, están más sujetos que las especies omnívoras o carnívoras a las variaciones de los recursos alimenticios que les ofrecen los ecosistemas. Tienen además requerimientos muy específicos en cuanto a tipo de vegetación, temperatura y precipitación. Todo esto convierte a los rumiantes en un interesante grupo de estudio para contrastar hipótesis que relacionen los cambios físicos (producidos por los ciclos astronómicos y los periodos actividad tectónica) con su especiación, dispersión y extinción; en definitiva, con su evolución

El interés de los paleontólogos por este grupo tiene una razón más allá de su vertiginosa diversificación durante el Neógeno y su extraordinaria riqueza ecológica. Muchos de los restos fósiles de rumiantes del Plio-Pleistoceno aparecen asociados a los primeros homínidos conocidos, y su estudio ha permitido entender cuáles fueron los cambios en los paisajes y las faunas que acompañaron los primeros pasos de la evolución de nuestro propio linaje. Desde aquellos tiempos, nuestra propia historia ha estado estrechamente ligada a los rumiantes, que durante millones de años nos han proporcionado alimento, herramientas y abrigo (Vrba y Schaller, 2000). Las primeras manifestaciones de arte los representan en paredes de cuevas y huesos tallados, y deidades de diversas religiones, muchas ya extintas, los escogieron para su representación terrenal. Tal era nuestra dependencia de los recursos que nos ofrecían que no fue hasta su domesticación cuando las poblaciones humanas pudieron abandonar el nomadismo y asentarse en poblaciones donde florecería el comercio, el arte y el conocimiento. Todavía hoy gran parte del ganado que nos aporta alimento está constituido por rumiantes y su importancia en la economía está fuera de duda. No es de extrañar, por tanto, que se haya dedicado un gran esfuerzo científico al estudio de este grupo. Sin embargo queda mucho por hacer. El rápido deterioro de sus hábitats y la caza incontrolada en muchos países en desarrollo, último refugio de algunas especies, a veces se suma al desconocimiento de las organizaciones conservacionistas sobre el estado de las poblaciones y sus distribuciones reales. En un futuro cercano el conocimiento exhaustivo de la genética de las poblaciones y de su ecología, y una caracterización del estado de conservación de las especies (algunas de las cuales se encuentra en zonas donde los conflictos bélicos hacen difícil su estudio) es vital para que se tomen las medidas acertadas a tiempo. En este sentido, el conocimiento del pasado de los rumiantes nos ayudará a entender y anticiparnos a las variaciones en la diversidad que presumiblemente se deribarán de la situación de cambio global en la que nos encontramos actualmente.

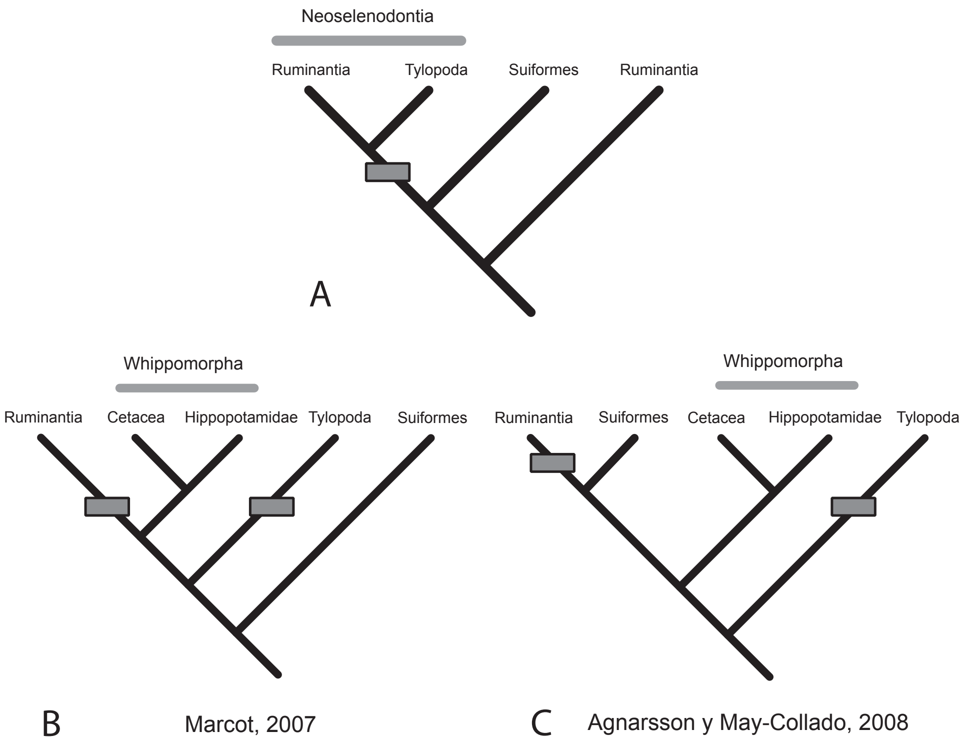


Figura 1.1. Representación de las relaciones filogenéticas de los grupos dentro de Cetartiodactyla. A) el esquema tradicional contempla Neoselenodontia como un grupo monofilético; B) en la propuesta de Marcot (2007) se incluye el clado Whippomorfa (Hippopotamidae+Cetacea) como grupo hermano de Ruminantia; C) en la propuesta de Agnarsson y May-Collado (2008) Ruminantia y Suiformes forman un clado que a su vez es grupo hermano de Whippomorpha, mientras que Tylopoda queda en una posición más basal. Las propuestas de Marcot y Agnarsson implican que el desarrollo de la rumia y la morfología neoselenodonta de los molares (rectángulo gris) se produjo de forma independiente en Ruminantia y Tylopoda.

Ruminantia en Cetartiodactyla

El suborden Ruminantia es un grupo monofilético que se incluye dentro de Cetartiodactyla, el orden que actualmente, y de acuerdo a las últimas evidencias filogenéticas, contiene los grupos tradicionalmente conocidos como artiodáctilos y cetáceos, estos últimos situados ahora como grupo hermano de los hipopótamos (Marcot, 2007; Agnarsson y May-Collado, 2008; Geisler y Theodor, 2009). Además de Ruminantia, Cetartiodactyla incluye Tylopoda (camellos y llamas), Suiformes (cerdos y pecaríes), Hippopotamidae (hipopótamos) y Cetacea (cetáceos) (Fig.1.1). Durante muchos años los sistemáticos han considerado a los rumiantes como grupo hermano de Tylopoda (Webb y Taylor, 1980), constituido actualmente por los géneros *Camelus* (camellos y dromedarios), *Lama* (guanacos) y *Vicugna* (vicuñas). Dicha relación es el resultado de que Ruminantia y Tylopoda comparten, no sólo la capacidad de efectuar el proceso de rumia, sino también una morfología tretraselenodonta en sus molares superiores que se interpreta como una sinapomorfía del clado que conformarían ambos grupos: Neoselenodontia (Fig.1.1A). No obstante, la aparición de filogenias moleculares de Cetartiodactyla en los últimos años ha cuestionado esta estrecha relación (Marcot, 2007; Agnarsson y May-Collado, 2008), considerando Neoselenodontia como un grupo parafilético, lo que implicaría que la evolución de la rumia y de los molares con morfología neoselenodonta se dio de manera independiente en Tylopoda y Ruminantia (Fig.1.1B y 1.1C). Este hecho no parece algo excepcional si tenemos en cuenta la presencia en el registro fósil de otros grupos de artiodáctilos selenodontos, como es el caso de Bunoselenodontia, cuyas relaciones filogenéticas son controvertidas (Stucky, 2005).

Los principales caracteres que diferencian a los rumiantes de los otros cetartiodáctilos están estrechamente relacionados con la dieta y la locomoción y aunque no todos están presentes en todos los grupos dentro de Ruminantia permiten realizar una descripción general del grupo. Los rumiantes actuales presentan un estómago con cuatro cámaras, que les permite realizar el proceso de la rumia, y molares selenodontos (molares con cuatro cúspides incurvadas en forma de media luna). Los incisivos superiores son vestigiales o se han perdido y los caninos inferiores son incisiviformes (con forma espatulada). El navicular y el cuboides se han fusionado formando el cubonavicular y el astrágalo presenta dos poleas alineadas (salvo en los tragúlidos que no llegan a estarlo completamente). El tercer y cuarto metápodos se han fusionado y alargado y los primeros, segundos y quintos metápodos y falanges se han reducido, al igual que la ulna y la fíbula.

Origen y evolución de los rumiantes

Pese a ser uno de los grupos de mamíferos con mejor registro fósil conocido en el Terciario (Vrba y Schaller, 2000), el origen evolutivo y biogeográfico de los rumiantes en el Eoceno sigue siendo materia de debate. Existe, por ejemplo, gran controversia a cerca del grupo extinto más relacionado con los rumiantes. Mientras algunos autores apuntan a Protoceratidae, otros señalan a Amphimerycidae, Dichobunidae o Bunoselenodontia, algunos de los cuales son admitidos por diversos autores como rumiantes basales (Norris, 2000; Geisler y Uhen, 2005; Prothero, 2005; Stucky, 2005; O'Leary y Gatesy, 2008). Nuestro conocimiento sobre la aparición y la radiación basal del grupo se debe principalmente a un registro bastante completo de formas selenodontas del Eoceno Medio norteamericano (Métais y Vislobokova, 2007), mientras que el registro de linajes basales en Asia ha sido prácticamente desconocido durante años. Sin embargo, el hallazgo de *Archaeomeryx* en Mongolia (Shana Murun, Eoceno Medio) ha centrado la atención en el continente asiático como posible cuna del grupo, y desde entonces más formas han ido engrosando el registro fósil conocido de rumiantes basales en este continente (Webb y Taylor, 1980; Métais et al., 2000, 2001, 2007). Para muchos es precisamente *Archaeomeryx* el rumiante más primitivo, aunque su posición filogenética dentro de "Tragulina" (ver siguiente apartado) no está demasiado clara y otros lo han propuesto como más próximo a Pecora (Vislobokova, 1990).

Primera radiación.

Controversias aparte, existe amplio consenso sobre una primera radiación de rumiantes basales a partir de artiodáctilos selenodontos en Asia y Norteamérica durante el Eoceno. Esta radiación de carácter explosivo dio como resultado una amplia diversidad de grupos sucesivos basales a Pecora que se conocen con el nombre de "Traguloidea" o "Tragulina" (Webb y Taylor, 1980), grupo parafilético que incluye varias familias (Métais y Vislobokova, 2007). Si bien el origen de estas familias dentro de Tragulina no está del todo claro, lo cierto es que todas ellas estaban distribuidas en Asia y Norteamérica ya en el Eoceno medio. Los linajes fruto de esta primera radiación parecen llegar a Europa hacia el Eoceno superior y a partir de la *Grand Coupure*, en el Oligoceno Inferior, estos grupos empezaron a diversificarse en este continente.

De entre todos los traguloideos, sólo la familia Tragulidae persistió durante el Neógeno y sobrevive hoy en día (Sánchez et al., 2010b). Son menos avanzados que el resto de rumiantes tanto en sus características morfológicas como en la fisiología y anatomía de su aparato digestivo, lo que les ha convertido en el arquetipo del rumiante primitivo. Pese a que su registro fósil anterior al Mioceno es muy pobre, sabemos que los tragúlidos alcanzaron una notable y repentina diversidad a principios de este periodo y que su amplia distribución geográfica ya incluía África, el sur de Asia y Europa. Otro resultado de esta gran radiación de los rumientes al inicio del Neógeno fue la aparición de un linaje de rumiantes más derivados denominado Pecora, que sería protagonista de la segunda gran radiación del grupo.

La radiación Pecora y evolución en el Neógeno.

Hoy en día menos de una docena de especies de rumiantes pertenecen a Tragulidae, familia superviviente de aquella primera radiación. El resto de las doscientas especies de rumiantes pertenecen a un clado compuesto por taxones más derivados: los Pecora. Los Pecora son también conocidos como "rumiantes superiores" y, en rasgos generales, se caracterizan por el desarrollo de un estómago compartimentado más complejo, un esqueleto postcraneal más derivado (astrágalo con lados paralelos, metápodos con fusión completa...) y diferentes tipos de apéndices craneales, aunque todavía sobreviven algunos grupos de Pecora inermes. Si bien los Pecora aparecieron durante el Oligoceno inferior en Asia Central (Hernández Fernández y Vrba, 2005a; Métais y Vislobokova, 2007), la gran diversificación de las subfamilias y tribus dentro del grupo comenzó en el Mioceno. En el Mioceno inferior, y en relación con el cambio hacia climas más estacionales, los Pecora protagonizaron una radiación asociada al incremento de tamaño así como a la aparición de apéndices craneales de forma independiente en varios linajes de rumiantes (Morales et al., 1993), incluidos grupos extintos como climacocerátidos, lagomerícidos o paleomerícidos. En general, la evolución de los Pecora ha estado marcada por sus adaptaciones locomotoras y alimenticias, existiendo en ellas un alto grado de direccionalidad y homoplasia entre los diferentes grupos. Actualmente los Pecora incluyen cinco familias: Antilocapridae (berrendo o antílope americano), Giraffidae (jirafa y okapi), Bovidae (bisontes, búfalos, antílopes, gacelas y cabras), Moschidae (ciervos almizcleros) y Cervidae (ciervos).

Antilocapridae. A finales del Mioceno Inferior se produjo la entrada de Dromomerycidae y Merycodontinae en Norteamérica. Los antilocaprinos evolucionaron a partir de mericodontinos en el Mioceno medio y probablemente estuvieran bien adaptados a climas áridos y más fríos, lo que les permitió convertirse en el grupo más exitoso en diversificaciones posteriores durante el Mioceno superior y el Plioceno. Actualmente, el antílope americano (*Antilocapra americana*) sobrevive como único representante de la familia Antilocapridae que, además de alcanzar una gran diversidad de formas, jugó un papel fundamental en las comunidades de herbívoros del Neógeno Norteamericano (Janis et al., 1998; Janis et al., 2000; Janis et al., 2004).

Giraffidae. Los jirafoideos *sensu lato* probablemente tienen un origen asiático, a juzgar por el registro fósil; de hecho taxones braquiodontos atribuidos a jirafoideos se han encontrado en los sedimentos de Bugti Hills en el Mioceno Inferior de Pakistán (MN3; Ginsburg et al., 2001). En la península Ibérica, también se han determinado especies atribuidas a jirafoideos muy basales, también datados en la biozona MN3, como es el caso de los géneros *Teruelia* y *Lorancameryx* (Moyà-Solà, 1987; Morales et al., 1993). Coetáneos con estas formas se registran en Africa los climacocerátidos, familia estrechamente relacionada con los Giraffidae, representada por especies que presentaban ya cierta tendencia a la hipsodoncia (Morales et al., 1999; Morales et al., 2003a; Morales et al., 2008). Durante la MN4, hace unos 17.5 Ma, aparecen en Africa del Norte, en Gebel Zelten (Libia) los Giraffidae más antiguos conocidos, como *Canthumeryx sirtensis* (Hamilton, 1978).

Aunque existe cierta controversia sobre qué incluir dentro de la familia Giraffidae, parece que *Palaeotragus* y *Giraffokeryx* del Mioceno medio son generalmente considerados como formas muy primitivas, próximas a *Canthumeryx* (Janis y Scott, 1987). Durante el Mioceno los jiráfidos se extendieron ampliamente por Europa y Asia, caracterizándose por presentar morfotipos de gran talla y complexión masiva, como *Samotherium*, lejos del diseño esbelto y el cuello alargado típicos de las jirafas actuales. En esta época los sivaterinos, caracterizados por tener cuatro osiconos, también evolucionaron hacia formas de gran tamaño y extremidades cortas. Los representantes de principios del Plioceno están ya ligados a *Sivatherium* y *Giraffa*. Pese a que se conocen unas treinta especies de jiráfidos y jirafoideos extintos (Solounias et al., 2000), en la actualidad esta familia cuenta tan sólo con dos especies: la jirafa (*Giraffa camelopardalis*) y el okapi (*Okapia johnstoni*).

Bovidae. Aunque algunos han propuesto a algunas formas del Oligoceno Medio de Mongolia como bovoideos, los análisis moleculares apuntan a una posible primera cladogénesis de bóvidos próxima a la transición Oligoceno-Mioceno (hace unos 23 Ma) (Hassanin y Douzery, 2003; Hernández Fernández y Vrba, 2005a), y la primera forma fósil considerada como un verdadero bóvido es *Eotragus* (Solounias et al., 1995). Estos bóvidos primitivos, que ya presentaban núcleos óseos cónicos en los cuernos, eran de talla pequeña y aparecen en Europa y Pakistán hace unos 18 millones de años (Solounias et al., 1995; Ginsburg et al., 2001). La aparición de otras formas más basales en África como *Namacerus* de principios del Mioceno Medio de Namibia (unos 17 Ma, Morales et al., 2003b), así como la amplia diversidad de morfotipos craneales de bóvidos en este continente parece apoyar un origen africano del grupo en esa época y una rápida dispersión. Incluso se ha propuesto que la aparición de cuernos se pudo dar más de una vez en el grupo.

En el Mioceno superior, y en relación con el incremento en la aridez y un enfriamiento global, los paisajes de tipo sabana se hicieron más comunes y se dio una expansión de praderas y de taxones de plantas C4 (Cerling et al., 1993; Beerling y Osborne, 2006). Este cambio propició la aparición y diversificación en África de varias tribus de bóvidos adaptadas a los nuevos ecosistemas como Aepycerotini, Alcelaphini, Hippotragini, Reduncini y Tragelaphini (Hassanin y Douzery, 1999; Bibi et al., 2009). Algunos “boselafinos” evolucionaron hacia formas de gran talla y originaron la tribu Bovini, mientras que Antilopini estaba ya ampliamente distribuida por África y Eurasia en el Mioceno superior (Gentry, 2000). Durante los pulsos climáticos que caracterizaron el Plio-Pleistoceno se dio un importante cambio faunístico en los bóvidos africanos, apareciendo varias de las tribus que todavía persisten en la actualidad (Vrba, 1995b). Con alrededor de 130 especies, Bovidae es actualmente la familia más diversa dentro de los rumiantes.

Moschidae. Los mósquidos o ciervos almizcleros son un grupo de rumiantes pécora inermes y de pequeña talla que se definen como el clado que agrupa a *Micromeryx*, *Hispanomeryx*, *Moschus* y su último antecesor común, junto con todos los descendientes de éste (Sánchez et al., 2010a). Esta definición basada en un análisis cladístico excluye del grupo a otros pécoras inermes más primitivos como los blastomerícidos norteamericanos, amén de otros grupos. Por ello, Moschidae debe considerarse un grupo exclusivamente eurasiático y Mioceno que quedó restringido a una distribución exclusivamente asiática a finales del Mioceno (Sánchez et al., 2010a). Los mósquidos aparecieron en el Mioceno medio, siendo muy comunes en las faunas del Mioceno medio y superior. Los mósquidos alcanzaron aparentemente su máximo de diversidad a finales del Mioceno medio (Sánchez y Morales, 2006; Sánchez et al., 2010a); actualmente sólo sobrevive el género *Moschus*, el ciervo almizclero asiático, con cinco especies (Nowak, 1999) que presentan una amplia distribución geográfica en Asia. Como muchos grupos de rumiantes, la mayoría extintos, los machos de las especies de Moschidae se caracterizan por presentar grandes caninos en daga. Aunque de forma clásica se les ha asociado con los ciervos, algunos estudios moleculares y morfológicos recientes han propuesto una relación de grupos hermanos entre los bóvidos y los mósquidos, con una serie de grupos "stem" que vivieron en el sur de África a finales del Mioceno inferior (Sánchez et al., 2010a).

Cervidae. Los cérvidos se definen principalmente por la presencia de astas que se pierden y regeneran estacionalmente. Una de las preguntas abiertas radica en la identificación de las astas de los ciervos más basales como auténticas astas y su consiguiente inclusión dentro del grupo. La evidencia paleontológica presenta a *Procervulus*, que aparece hace unos 19 Ma (MN3) en Europa, como el ciervo más primitivo. Sin embargo sus apéndices no son como los de los ciervos actuales: carecen de roseta, su microestructura es diferente y su caducidad seguramente también lo era. Otras formas coetáneas son *Ligeromeryx*, *Lagomeryx*, y *Stephanocemas*. Habrá que esperar hasta el Mioceno Medio, hace unos 13 Ma, para que aparezca el primer cérvido con verdaderas astas con roseta: *Euprox*. Para algunos especialistas no deberían considerarse como cérvidos todo lo anterior a *Euprox*, y aunque algunas filogenias moleculares arrojan fechas acordes (Pitra et al., 2004; Gilbert et al., 2006), otras estimas de aparición de los linajes dentro de Cervidae son mucho anteriores (Hassanin y Douzery, 2003; Bininda-Emonds et al., 2007; Gatesy, 2009). Durante el Mioceno medio aparecen también *Heteroprox* y

Dicrocerus, aunque sus astas no son tan avanzadas como las de *Euprox*. La diversidad de cérvidos del Mioceno medio está probablemente relacionada con un cambio a nivel global hacia inviernos más fríos, menor precipitación estival y la consiguiente aridificación de los ecosistemas. Más tarde, durante el Plio-Pleistoceno, los cérvidos experimentan una nueva cladogénesis de grupos de gran talla en Eurasia, así como una nueva dispersión como resultado de su entrada a Norteamérica (Geist, 1998). A partir de los cérvidos establecidos en Norteamérica se da una nueva cladogénesis en zonas tropicales de linajes que entrarán en Sudamérica tras el establecimiento del istmo de Panamá hace unos 3 Ma. No obstante, en este punto no hay demasiada concordancia entre los datos fósiles y los datos moleculares. Mientras no se conoce ninguna evidencia en el registro de una radiación pre-istmo, varios estudios filogenéticos apuntan a una diversificación previa, durante el Plioceno (Pitra et al., 2004; Gilbert et al., 2006; Duarte et al., 2008).

Existe un consenso casi absoluto sobre la posición de Tragulidae como grupo hermano del resto de rumiantes actuales, que formarían un grupo monofilético (Pecora). Sin embargo, las relaciones filogenéticas entre las familias que conforman Pecora son todavía discutidas. Casi todas las opciones posibles han sido propuestas en la literatura, y la aparición de filogenias moleculares no ha hecho más que ampliar el abanico de posibilidades (Su et al., 1999; Liu et al., 2001; Hassanin y Douzery, 2003; Geisler y Uhen, 2005; Hernández Fernández y Vrba, 2005a; Bininda-Emonds et al., 2007; Marcot, 2007; Agnarsson y May-Collado, 2008; O'Leary y Gatesy, 2008; Gatesy, 2009). Sin embargo, más que un conflicto directo entre filogenias morfológicas y moleculares estamos probablemente ante una falta de resolución de ambos métodos. La razón la encontramos en la propia evolución de los Pecora, que, como hemos mencionado anteriormente, se caracteriza por la adquisición de una serie de caracteres que han surgido como respuesta a exigencias locomotoras y alimenticias (Gentry, 2000). La selección direccional de este tipo de adaptaciones ha podido ser tan fuerte en los diferentes grupos que podemos estar ante un caso de evolución paralela de caracteres, lo que supone que en análisis filogenéticos de caracteres morfológicos taxones que debieran estar separados por largas historias evolutivas independientes aparezcan agrupados compartiendo una sinapomorfía que en realidad es una homoplasia. En el caso de Antilocapridae y Giraffidae, con sólo una y dos especies actuales respectivamente, este artefacto analítico se acentúa aún más debido a la conocida "atracción entre ramas largas" (Marcot, 2007). Los análisis moleculares, a su vez, encuentran obstáculos a la hora de definir las relaciones entre las familias Pecora. La historia evolutiva del grupo está caracterizada por una serie de radiaciones adaptativas muy rápidas que a finales del Oligoceno y durante el Mioceno Inferior y Medio dieron lugar a

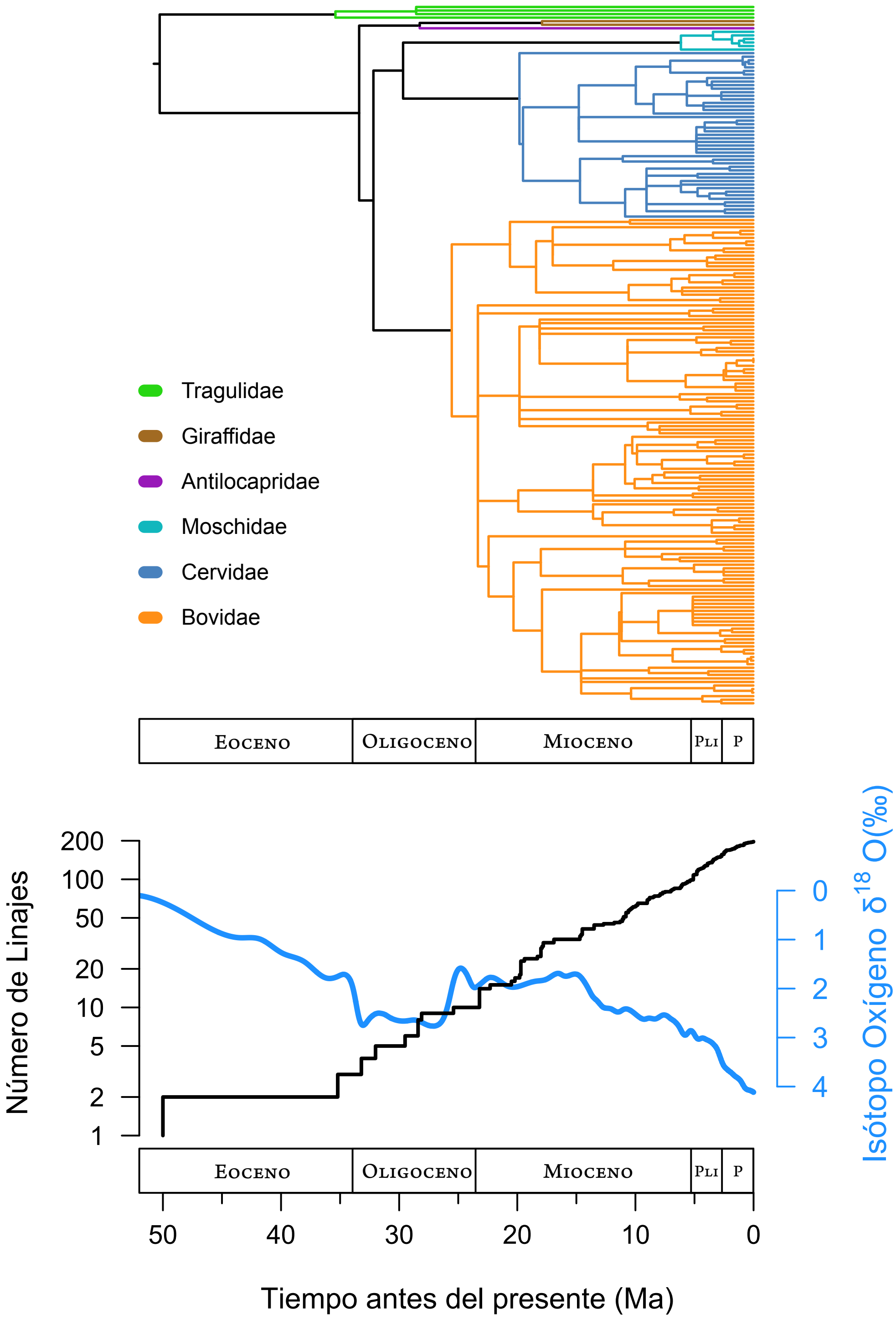


Figura 1.2. Hipótesis filogenética para las 197 especies actuales de rumiantes propuesta por Hernández Fernández y Vrba (2005a). En diferentes colores se representan las seis familias incluidas en el suborden Ruminantia. Debajo se muestra el número de linajes de dicha filogenia a lo largo del tiempo con escala logarítmica. La curva azul representa los valores del isótopo $^{\delta 18}$O como representación de la temperatura oceánica media en el hemisferio norte (Zachos et al., 2001).

las principales familias que conocemos hoy en día además de otras muchas. Esta cladogénesis explosiva se traduce en una escasa acumulación de cambios evolutivos (moleculares y morfológicos) entre eventos de ramificación filogenética, lo que revierte negativamente en el poder resolutivo de los análisis (Gatesy et al., 1992; Hassanin y Douzery, 2003; Hernández Fernández y Vrba, 2005a; Marcot, 2007).

La filogenia de estudio

La aplicación del método comparativo en cuestiones como la ecología y la evolución requiere de una filogenia a nivel de especie y la inclusión de la totalidad de los representantes de un grupo en dicha filogenia es crucial si no queremos que nuestros análisis carezcan de poder estadístico y presenten problemas debido a un sesgo de muestreo (Harvey y Pagel, 1991; Agnarsson y May-Collado, 2008). En la presente tesis hemos utilizado el "super-árbol" de rumiantes publicado por Hernández Fernández y Vrba (Fig. 1.2; Hernández Fernández y Vrba, 2005a) que representa el consenso de la evidencia total de información molecular, fósil, morfológica y etológica. Además de cumplir las condiciones mencionadas al incluir las 197 especies de rumiantes (siguiendo la nomenclatura de Wilson y Reeder, 1993), presenta el 80% de sus nodos datados con información fósil y molecular, una proporciónn mucho mayor que en la inmensa mayoría de los "super-árboles" existentes. Si bien este árbol presenta politomías en algunos nodos debido a la particular historia evolutiva del grupo (como hemos mencionado anteriormente y se discute en Gatesy et al., 1992; Hassanin y Douzery, 2003; Hernández Fernández y Vrba, 2005a; Marcot, 2007), dichas politomías han sido tratadas de manera coherente y apropiada a la metodología de cada capítulo en los casos en los que suponían un problema analítico.

Análisis metodológicos

La revolución filogenética y el método comparativo.

Esta tesis aborda varias cuestiones sobre la macroevolución de los rumiantes empleando un marco analítico filogenético. Durante el desarrollo de la investigación muchas de las metodologías comparativas han ido perfeccionándose y haciéndose cada vez más accesibles a los biólogos evolutivos y paleontólogos. Es por ello que la presente tesis representa, además de una ventana a cuestiones evolutivas sobre los rumiantes, una exploración a través algunas de las múltiples metodologías que podemos encontrar actualmente para la realización de análisis comparativos dentro de un contexto filogenético. A modo de revisión, voy a comentar la filosofía del método así como la evolución de algunas de sus principales herramientas.

Los últimos veinte años han sido testigos de una revolución que ha cambiado el modo en el que los paleontólogos y los biólogos evolutivos se asoman al pasado (Felsenstein, 1985; Harvey y Pagel, 1991; Smith, 1994). Este cambio se ha producido a medida que los trabajos científicos han ido incluyendo y complementando sus análisis con información filogenética de las unidades taxonómicas bajo estudio. Es decir, a medida que se han tenido en cuenta las relaciones evolutivas entre ellas, en vez de tratar dichas unidades como elementos o fenómenos independientes.

Las filogenias son importantes en dos aspectos principales del método comparativo: para realizar análisis estadísticos apropiadamente y para inferir carateres ancestrales, estados transicionales y eventos evolutivos del pasado (Losos, 2011). Si queremos estudiar la relación existente entre, por ejemplo, el peso corporal y el tipo de dieta en un conjunto de especies, tradicionalmente se usaba una ANOVA en la que las especies eran tratadas como elementos aislados. Sin embargo, si las especies próximas tienden a presentar un fenotipo más parecido, entonces dos caracteres podrían covariar entre especies incluso si ambos caracteres no evolucionan de una manera correlacionada (Felsenstein, 1985). Los métodos filogenéticos incorporan esa no-independencia en la estructura estadística. En cuanto a la reconstrucción de la historia de uno o más caracteres (Fig. 1.3; ver siguientes apartados), la importancia de trabajar en este marco analítico filogenético radica en que permite identificar los eventos evolutivos que producen cambios en esos caracteres y trabajar sobre la distribución de los caracteres y sus cambios a lo largo de la filogenia en vez de fijarnos simplemente en la distribución de sus valores observados en las unidades biológicas objeto del trabajo (Felsenstein, 1985; Martins y Hansen, 1996).

De la parsimonia a la probabilidad. Los primeros trabajos que emplearon este método utilizaban cladogramas no datados y caracteres discretos. Por tanto las metodologías aplicadas no podían tener en cuenta la longitud de las ramas que unían las especies y la reconstrucción de los caracteres a lo largo del cladograma se hacía empleando el método de máxima parsimonia (Sillén-Tullberg, 1988; Maddison, 1990). Este método busca la reconstrucción de un carácter que implica el menor número de cambios posibles de dicho carácter (Fig. 1.3A). En este caso la reconstrucción del carácter viene determinada directamente por la topología del cladograma, es muy sencilla y puede realizarse sin ningún cálculo matemático. El estudio de la correlación de caracteres binarios en un marco parsimonioso podía realizarse utilizando el denominado test de cambios concentrados incluido en el célebre software MacClade (Maddison, 1990; Ortolani, 1999; Ord et al., 2002),

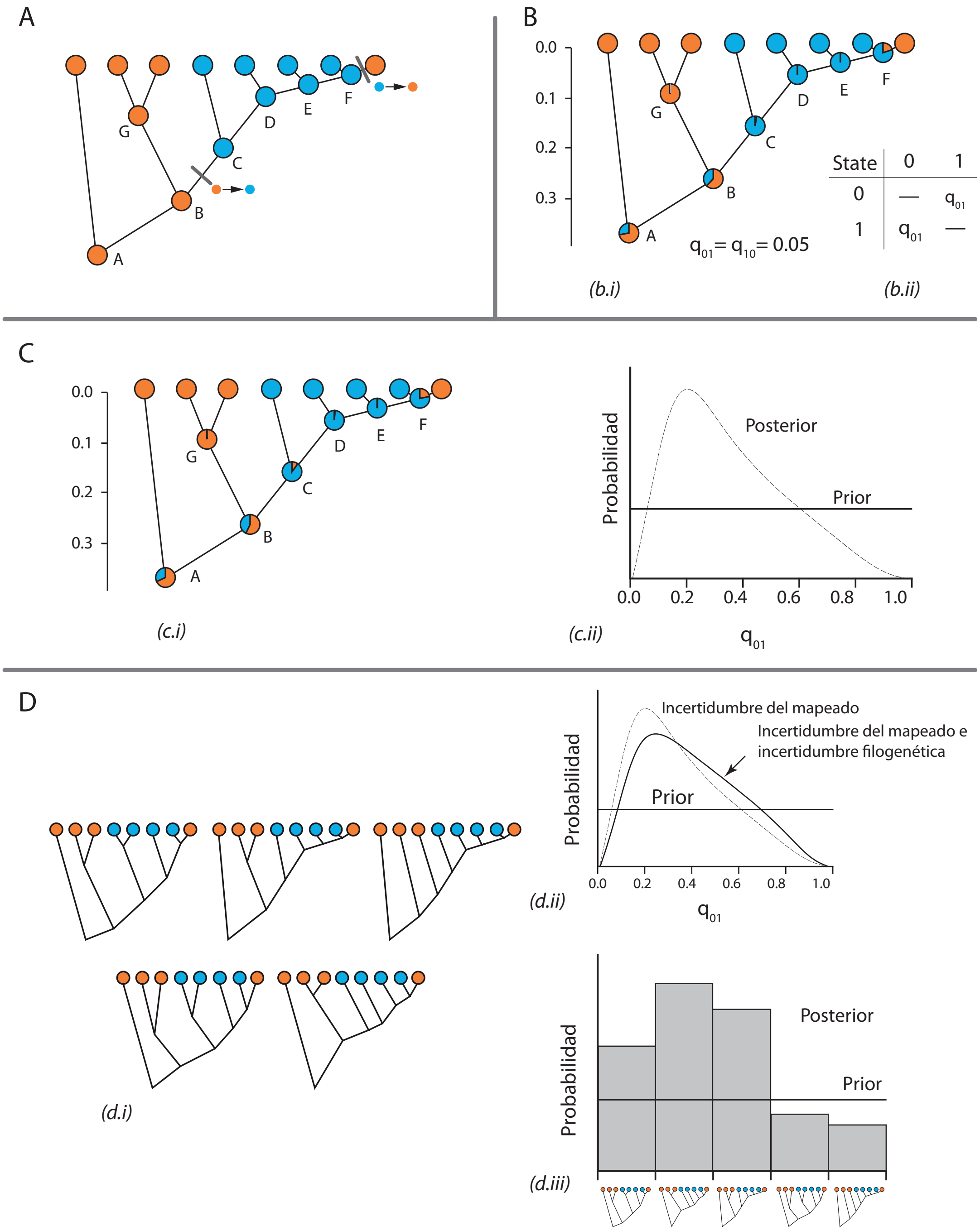


Figura 1.3. Método Comparativo. Dada una filogenia con ocho taxones terminales y una distribución de un carácter binario (naranja y azul), se muestran varias metodologías de reconstrucción de estados ancestrales de dicho carácter. A, máxima parsimonia. B, máxima probabilidad con un modelo de Markov con dos tasas de cambio para un carácter binario (tabla b.ii) donde las probabilidades de cambio entre estados del carácter (q_{01} y q_{10}) son conocidas e iguales a 0.5 (b.i). C, en la inferencia Bayesiana q_{01} y q_{10} siguen siendo iguales pero no se conocen y son estimadas; a priori todos los valores posibles de q_{01} y q_{10} son igualmente probables (prior; c.ii) y la propia estima nos devuelve una distribución (posterior; cii) en la que algunos valores son más probables que otros (c.ii). D, en esta inferencia bayesiana se añade la incertidumbre filogenética a la incertidumbre sobre q_{01} y q_{10}; en lugar de un solo árbol, el punto de partida es un conjunto de árboles posibles producto de un análisis de parsimonia (árboles igualmente parsimoniosos) o de una estima filogenética bayesiana (d.i). Tras la estima podemos mirar la distribución de las probabilidades de los los parámetros de interés (distribuciones marginales) tanto para q_{01} (d.ii) como para las diferentes filogenias (d.iii). Modificado de Ronquist (2004).

que analiza la distribución de ganancias y pérdidas del carácter dependiente en referencia a la distribución del carácter independiente.

A mediados de los años noventa del pasado siglo se desarrolló software más complejo para el análisis comparativo de datos continuos y discretos que permitía introducir topologías filogenéticas datadas (como CAIC, siglas de Comparative Analysis by Independent Contrasts, Purvis y Rambaut, 1995). Paulatinamente, la aparición de árboles filogenéticos (cladogramas datados), gracias en gran medida al desarrollo de los análisis filogenéticos moleculares calibrados con fósiles, ha permitido aumentar la complejidad de las reconstrucciones de caracteres así como el estudio de su coevolución. La inclusión de la escala temporal en este contexto ha permitido trabajar con tasas de cambio instantáneas que permitían mayor realismo en las reconstrucciones, planteando la inevitable incertidumbre en la reconstrucción de los caracteres, y ha dado lugar a la aparición de métodos para analizar la correlación de dos caracteres utilizando el criterio de máxima probabilidad (Pagel, 1994, 1997, 1999). En este tipo de análisis se construye o estima un modelo para las transiciones entre estados de los caracteres y se calcula su probabilidad. Dicho modelo es un modelo de Markov en el que las probabilidades de cambio entre estados de un carácter en un determinado lugar del árbol dependen del estado del carácter en ese momento y no de la distribución de los estados del carácter en el resto del árbol (Fig. 1.3B). La comparación entre un modelo en el que dos caracteres evolucionen independientemente y otro en el que coevolucionan, se realiza por comparación de los valores de probabilidad de cada uno. Este tipo de modelos es aplicable a caracteres discretos con, normalmente, pocos estados.

En los últimos años el análisis de la correlación entre variables en un contexto filogenético ha seguido desarrollándose. Nuevos métodos permiten incorporar las relaciones filogenéticas entre especies como varianzas y covarianzas que acompañan a los datos observados como parte de un modelo lineal de mínimos cuadrados (Generalized Least Squares o GLS, Martins y Hansen, 1997) o como una matriz de correlación en un modelo lineal a partir de Ecuaciones Estimadas Generalizadas (Generalized Estimated Equations o GEE, Paradis y Claude, 2002; Paradis, 2006). Tratar caracteres y relaciones filogenéticas como variables de modelos lineales permite explorar la correlación de más de dos variables a la vez de una manera sencilla sin tener que estimar reconstrucciones de las mismas, y la forma en la que la información filogenética se añade al modelo permite trabajar con filogenias que presentan politomías (Paradis, 2006).

La inferencia bayesiana. Todos los métodos anteriores trabajan a partir de unos datos observados para las especies y una filogenia "conocida" que añade información sobre sus relaciones evolutivas. Algunos de ellos, como los métodos de máxima probabilidad desarrollados por Pagel mencionados anteriormente (Pagel, 1994, 1997, 1999), incluyen la incertidumbre en la reconstrucción y correlación entre los caracteres como parte de sus cálculos. No obstante, los modelos de Markov que emplean estos últimos parten de tasas de cambio entre estados de carácter conocidas a priori (q_{01} y q_{10} en la Fig. 1.3B). Además, trabajar sólo con una filogenia supone que damos por hecho que es la verdadera y que conocemos perfectamente las relaciones evolutivas entre las unidades biológicas que estudiamos, obviando la incertidumbre implícita en el proceso de obtención de esa filogenia, desde la elección de caracteres, la toma y codificación de los mismos, hasta el método empleado en la estima de la filogenia en sí (máxima parsimonia, máxima probabilidad, árboles consenso,...). La elección de una sola filogenia puede entrañar, si ésta no es la correcta, un problema ya que el mismo análisis comparativo realizado con diferentes filogenias podría dar diferentes respuestas (Pagel et al., 2004; Ronquist, 2004). La inferencia Bayesiana surgió como respuesta a esta problemática ya que permite incluir tanto la incertidumbre filogenética como la incertidumbre sobre el valor de los parámetros en un análisis comparativo (ver figura 1.3C y 1.3D y Huelsenbeck et al., 2000; Lewis, 2001; Pagel et al., 2004; Ronquist, 2004; Pagel y Meade, 2006). En este nuevo marco analítico no se emplea una sola filogenia, sino que varias hipótesis filogenéticas son añadidas al análisis (Fig. 1.3d.i). Cuando se construyen filogenias, ya sea mediante máxima parsimonia (ver Swofford, 2000; Goloboff et al., 2008) o mediante métodos de búsqueda bayesiana (Huelsenbeck y Ronquist, 2001), es inusual encontrar una sola filogenia resultado, y normalmente se realizan árboles consenso que sirven como resumen de los árboles resultantes. La reconstrucción de caracteres mediante inferencia bayesiana permite incluir en el análisis todos esos árboles resultado en lugar de colapsar los resultados en un único árbol consenso, con la pérdida de información que esto conlleva. Imaginemos una tabla con tantas dimensiones como parámetros queramos estimar en nuestro modelo (estados de carácter, transiciones entre estados,...), donde cada celda es una combinación de valores de dichos parámetros. Para añadir la incertidumbre filogenética el modelo añade una nueva dimensión formada por todas las filogenias posibles (Ronquist, 2004). El análisis genera cadenas exploratorias que surcan dicho espacio multidimensional calculando la probabilidad de cada celda (de cada combinación de valores de parámetros, incluidas las posibles filogenias) buscando aquellas con mayores valores de probabilidad. Si se deja correr el suficiente número de pasos, las cadenas llegan a un valor de probabilidad en el que se estabilizan (en inglés "stationarity") y a partir del cual se puede empezar a muestrear los modelos encontrados. A este tipo de metodología se la conoce como MCMC (del inglés Markov Chain Monte Carlo, Gamerman, 1997). El resultado son frecuencias de muestreo y distribuciones de probabilidad que pueden ser observadas para el modelo global (para cada celda) o para un parámetro de particular interés para el investigador. Las frecuencias de uno sólo de los parámetros del modelo recibe el nombre de distribución marginal (porque nos fijamos en uno de los lados de esa tabla multidimensional) y nos permite estudiar por separado las frecuencias de un parámetro numérico (la tasa de cambio entre 0 y 1, q_{01}, en la figura 1.3.c.ii y 1.3.d.ii) o de las frecuencias de los árboles incluidos en el análisis (Fig. 1.3.d.iii).

Filogenias y patrones evolutivos: tasas de diversificación

Uno de los avances más significativos que se ha dado en los últimos años en el campo de la biología evolutiva es nuestra capacidad para recuperar información de procesos evolutivos a partir de la forma de los árboles filogenéticos (Harvey et al., 1994; Mooers y Heard, 1997, 2002). Aunque inicialmente los árboles filogenéticos se empleaban como meras representaciones de las relaciones evolutivas entre taxones, los patrones de ramificación guardan una información muy valiosa sobre los procesos evolutivos que han operado durante la historia de los grupos. Procesos como extinciones masivas, radiaciones adaptativas o aumento de las tasas de extinción dejan su particular huella en las filogenias (Figura 1.4); y en los últimos años la potencia y la fiabilidad analítica para exprimir esa información ha crecido exponencialmente (Rabosky, 2006a, b, 2010; Alfaro et al., 2009; Stadler, 2011). En palabras de Emmanuel Paradis (Paradis, 2011):

> *"Las filogenias moleculares contribuyen al estudio de los patrones y procesos macroevolutivos a pesar de que los eventos pasados (fósiles) no están guardados en este tipo de datos".*

Para contrastar la precisión de estos métodos los científicos se basan en simulaciones controladas. Ellos mismos generan la historia evolutiva de un grupo, teniendo absoluto control sobre los cambios en las tasas de extinción, momentos de radiación, etc. Una vez concluida la evolución de ese grupo ficticio se traza la correspondiente filogenia que contiene los taxones que han llegado hasta el presente y a partir de esa filogenia intentan recuperar los procesos evolutivos que ellos mismos han fijado a priori y que conocen a la perfección. Este proceso se realiza muchas veces hasta tener una significación de la credibilidad del método. El incremento en la fiabilidad de este tipo de metodología junto con la posibilidad de incorporar al análisis la incertidumbre filogenética (trabajar sobre árboles igualmente parsimoniosos o probables) hace de este campo de estudio uno de los

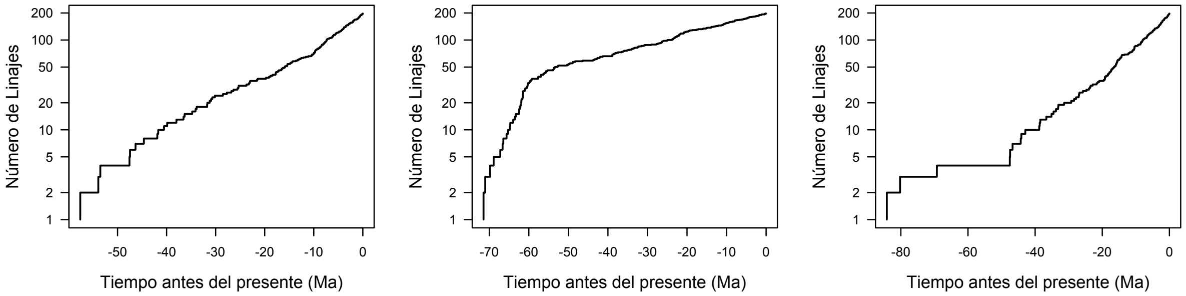


Figure 1.4. Representación del número de linajes a lo largo del tiempo para tres filogenias simuladas con TreeSim (Stadler, 2010), todas con 197 especies en el presente. A) filogenia de un grupo que ha mantenido sus tasas de especialización y extinción constantes a lo largo de su historia evolutiva. B) filogenia de un grupo que se originó como resultado de una radiación adaptativa, donde la tasa de especiación inicial es muy alta y va descendiendo paulatinamente según se van llenando los nichos ecológicos disponibles. C) filogenia de un grupo que sufrió un evento de extinción hace 50 Ma en el que se perdieron el 80% de los linajes existentes en ese momento; la filogenia no puede recuperar la existencia de esos linajes desaparecidos, pero sí la magnitud del evento que los hizo desaparecer.

más prometedores para el entendimiento de los procesos evolutivos.

Tasas de diversificación asociadas a caracteres. Durante décadas paleontólogos y biólogos evolutivos han propuesto que la adquisición de determinados caracteres puede permitir a un grupo acceder a nuevos nichos ecológicos y protagonizar una radiación adaptativa. Las nuevas técnicas comparativas nos permiten estudiar la relación entre estas adaptaciones clave y la diversificación de los linajes que las presentan. Por ejemplo, Maddison (2007) desarrolló un método para estimar si la presencia o ausencia de un carácter dado se identificaba con tasas de especiación y extinción significativamente diferentes. Inicialmente el método sólo permitía el estudio de caracteres binarios (su nombre, BiSSE, procede de binary-state speciation and extinction), aunque en los años sucesivos nuevos métodos más completos han visto la luz y ahora permiten trabajar con caracteres multiestado (MuSSE; FitzJohn, 2011) como en el trabajo sobre dietas presentado en el capítulo 4 de esta tesis, o con caracteres continuos (QuaSSE; FitzJohn, 2010) como se ha hecho en el capítulo 5 sobre la especialización biómica.

Filogenias, ecología y biogeografía

Además de dirigir la mirada hacia el pasado, la creciente disponibilidad de filogenias datadas en seguida ha sido aprovechada en otros campos. Un ejemplo claro es su utilidad en el estudio de asociaciones de flora o de fauna y en biogeografía. La incorporación de información filogenética en estas áreas de estudio se ha disparado en los últimos diez años con la aparición de herramientas como Phylocom (ver capítulo 6; Webb et al., 2008). Esta nueva corriente metodológica la iniciaron los ecólogos de plantas, pero poco a poco se ha ido extendiendo, facilitando una mayor comprensión de procesos ecológicos como la exclusión por competencia, el filtro ambiental de hábitat y el papel de la conservación filogenética de caracteres ecológicos o la convergencia en la formación de comunidades. Aún hoy muchos estudios se basan en análisis de presencia/ausencia de especies, géneros y familias en una determinada celda de muestreo con el sesgo que supone trabajar con estas tres jerarquías taxonómicas. En un reciente trabajo, Kreft et al. (2010) reconocen la necesidad de incluir la información filogenética en cuestiones biogeográficas y ecológicas:

> *"La taxonomía presenta una importante limitación cuando se comparan agrupaciones entre celdas de una cuadrícula basándose en la presencia y ausencia de los taxones. Por ejemplo, la capacidad inclusiva y la edad de los géneros y familias varía entre taxones y regiones, y los análisis a nivel de especies basados en los típicos índices de semejanza de presencia-ausencia tampoco tienen en cuenta las diferencias de edad y el grado de separación evolutiva de las especies."*

En el capítulo 6 exploramos si los procesos que operan en la configuración de la estructura filogenética de las comunidades de rumiantes a gran escala responden a interacciones interespecíficas (e.g. exclusión por competencia) o si son de naturaleza física, ambiental o histórica.

El método Monte Carlo

Además de métodos basados en datos filogenéticos, hemos empleado simulaciones Monte Carlo para abordar cuestiones macroevolutivas. Este método consiste en generar distribuciones al azar contra las que comparar los datos observados. Por ejemplo, podemos establecer la significación de que nuestro valor observado sea superior o inferior a lo esperado por azar simplemente viendo la proporción de valores simulados que caen por debajo o por encima, respectivamente, de ese valor observado. Una de las bondades de esta comparación es que permite diferenciar si las observaciones han sido producidas por procesos al azar o si deben ser explicados por la actuación de determinados procesos macroevolutivos (Gotelli, 2000). Los parámetros de las simulaciones se pueden fijar para cumplir determinadas "reglas", en nuestro caso ecológicas, que serán respetadas durante las simulaciones. Las simulaciones de presencia/ausencia en los diferentes biomas que se presenta en el capítulo 5 se realizó manteniendo el número de especies existentes en cada bioma, ya que diferentes regímenes climáticos tienen diferentes condiciones de productividad y de capacidad de carga. Éste tipo de simulaciones ya se ha aplicado con éxito en la contrastación de la hipótesis del uso de los recursos en los grandes mamíferos africanos (Hernández Fernández y Vrba, 2005b) y para el conjunto de los mamíferos sudamericanos (Moreno Bofarull et al., 2008). Nosotros recogemos este testigo y utilizamos simulaciones Monte Carlo, junto con el método QuaSSE (FitzJohn, 2010) mencionado anteriormente, para contrastar las predicciones de esta hipótesis sobre el suborden Ruminantia a escala global.

Objetivos

Hay una finalidad transversal en esta tesis: abordar cuestiones macroevolutivas de los rumiantes y contribuir con nuestra aproximación al conocimiento que ya se tiene de este grupo a partir de trabajos paleontológicos o ecológicos. Tenemos especial interés en comprobar cómo la ecología del grupo conecta con sus procesos evolutivos y cómo los cambios globales que se han registrado durante el Cenozoico han modulado estos procesos. La comprensión de cómo estos procesos interactúan entre sí es fundamental para entender el pasado y anticiparnos al futuro incierto de las faunas de rumiantes y otros mamíferos.

Dado que la base de buena parte de este trabajo es una filogenia, un primer paso es explorar cómo se compagina la información filogenética con la información que tenemos del registro fósil para los rumiantes. Hasta el desarrollo de las metodologías filogenéticas, la única evidencia directa sobre la evolución procedía del registro fósil (Norell, 1993). La información contenida en los árboles filogenéticos de especies actuales, que nos muestran una hipótesis sobre las relaciones entre linajes y sus momentos de aparición, puede ser cotejada con los patrones de aparición y desaparición de los taxones en el registro fósil asociados a dichos linajes. De esta manera es posible estimar qué proporción de los linajes que llegan hasta las especies actuales conocemos a través de dicho registro. La proporción de linajes para los que no se conoce registro fósil recibe el nombre de linaje fantasma o extensión de rango (Norell, 1992; Smith, 1994). En el capítulo 2 presentamos un trabajo en el que buscamos correlaciones entre mayores proporciones de linajes fantasma y diversas variables ecológicas.

En capítulos posteriores estudiamos la repercusión de los cambios físicos globales en la evolución del grupo. Las implicaciones de estos grandes procesos en la evolución han sido estudiadas desde hace años y varias hipótesis en relación con ellos se han agrupado en lo que se conoce como Teoría del Hábitat (Vrba, 1992, 1995a). En el capítulo 3 comprobamos si existen cambios en la tasa de diversificación del grupo a lo largo de su historia evolutiva y si existe alguna sincronía entre dichos cambios y eventos climáticos conocidos e identificados gracias a otras aproximaciones (isótopos estables, registro fósil...).

La perspectiva de una historia evolutiva afectada por este tipo de eventos a gran escala hace que nos planteemos otro tipo de preguntas. En un momento de cambio del entorno, de aparición de nuevos nichos ecológicos o de fragmentación y desaparición de ecosistemas, ¿existe alguna adaptación clave de las especies capaz de afectar a sus tasas de especiación? En el caso de los rumiantes, numerosos autores han planteado que la adaptación a diferentes dietas es uno de los factores determinantes en su evolución (Janis, 1982; Gentry, 2000; Janis et al., 2000; Pérez-Barbería et al., 2001; Janis et al., 2004; DeMiguel et al., 2008). Sin embargo, el efecto de diferentes dietas en las tasas de especiación de los rumiantes no ha sido contrastado directamente hasta la fecha. En el capítulo 4 planteamos esta cuestión haciendo uso de modelos de diversificación dependiente de caracteres.

En este mismo marco, algunos autores han propuesto modelos evolutivos en los que diferentes rasgos ecológicos tienen un papel clave en la diversificación de los grupos. Dentro de las teorías macroevolutivas, una de las que más repercusión ha tenido fue la hipótesis del uso de los recursos propuesta por Vrba (1980, 1987), que conecta el grado de especialización ecológica de las especies con la tolerancia a los cambios físicos del hábitat y la forma en la que éstos condicionarán la evolución de un grupo. En el capítulo 5 contrastamos diferentes predicciones del modelo de Vrba mediante el empleo conjunto de simulaciones Monte Carlo y metodologías filogenéticas.

Finalmente, y siguiendo con la idea de la influencia del escenario global en los procesos evolutivos, abordamos el estudio de la estructura filogenética de las comunidades de rumiantes. Los procesos que configuran las asociaciones faunísticas que observamos actualmente están condicionados por diversos factores que operan a muy diferente escala (Heard y Cox, 2007; Vamosi et al., 2009; Kamilar y Guidi, 2010). La configuración de las comunidades puede darnos pistas, no sólo de procesos ecológicos a escala local, sino también de procesos pretéritos que operaron (y operan) a gran escala. Estos procesos tienen particular interés cuando se estudian procesos de macroevolución y tienen su origen en la cambiante configuración de los dominios bioclimáticos, a su vez influidos por la deriva continental y otros procesos como los ciclos astronómicos (los ciclos de Milankovitch) y las fluctuaciones en el nivel de los mares. Se cree que dichos cambios en la distribución de las condiciones climáticas, junto con la disposición de las conexiones entre masas terrestres, son el motor que determina los intercambios faunísticos y los eventos de especiación y extinción asociados. Dada la importante presencia de rumiantes en las comunidades de mamíferos y su amplia distribución en todos los biomas terrestres, dedicamos el capítulo 6 a estudiar cuestiones de macroevolución y biogeografía desde un punto de vista nuevo, siguiendo las pistas que estos procesos han ido dejando en la estructura filogenética de las comunidades de rumiantes actuales.

Referencias

Agnarsson I, May-Collado LJ (2008) The phylogeny of Cetartiodactyla: the importance of dense taxon sampling, missing data, and the remarkable promise of cytochrome b to provide reliable species-level phylogenies. *Mol Phylogenet Evol* 48(3):964-85.

Alfaro ME, Santini F, Brock C, Alamillo H, Dornburg A, Rabosky DL, Carnevale G, Harmon LJ (2009) Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. *Proc Natl Acad Sci U S A* 106(32):13410-4.

Beerling D, Osborne C (2006) The origin of the savanna biome. *Glob Change Biol* 12(11):2023-2031.

Berger J, Gompper ME (1999) Sex ratios in extant ungulates: products of contemporary predation or past life histories? *J Mammal* 80(4):1084-113.

Bibi F, Bukhsianidze M, Gentry A, Geraads D, Kostopoulos D, Vrba E (2009) The Fossil Record and Evolution of Bovidae: State of the Field. *Palaeontologia Electronica* 12(3).

Bininda-Emonds ORP, Cardillo M, Jones KE, MacPhee RDE, Beck RMD, Grenyer R, Price SA, Vos RA, Gittleman JL, Purvis A (2007) The delayed rise of present-day mammals. *Nature* 446(7135):507-512.

Blob RW, LaBarbera M (2001) Correlates of variation in deer antler stiffness: age, mineral content, intra-antler location, habitat, and phylogeny. *Biol J Linn Soc* 74(1):113-120.

Brashares J, Garland T, Arcese P (2000) Phylogenetic analyses of coadaptation in behavior, diet, and body size in the African antelope. *Behav Ecol* 11(4):452-463.

Bro-Jorgensen J (2008) Dense habitats selecting for small body size: a comparative study on bovids. *Oikos* 117(5):729-737.

Cerling TE, Wang Y, Quade J (1993) Expansion of C4 ecosystems as indicator of global ecological change in the late Miocene. *Nature* 361(6410):344-345.

Christiansen P (2002) Locomotion in terrestrial mammals: the influence of body mass, limb lenght and bone proportions on speed. *Zool J Linn Soc* 136(4):685-714.

DeMiguel D, Fortelius M, Azanza B, Morales J (2008) Ancestral feeding state of ruminants reconsidered: earliest grazing adaptation claims a mixed condition for Cervidae. *BMC Evol Biol* 8(13).

Duarte JMB, González S, Maldonado JE (2008) The surprising evolutionary history of South American deer. *Mol Phylogenet Evol* 49(1):17-22.

Felsenstein J (1985) Phylogenies and the comparative method. *Am Nat* 125(1):1-15.

FitzJohn RG (2010) Quantitative traits and diversification. *Syst Biol* 59(6):619-33.

FitzJohn RG (2011) diversitree: comparative phylogenetic tests of diversification. An R package. http://www.zoology.ubc.ca/prog/diversitree.

Gamerman D (1997) Markov Chain Monte Carlo: Stochastic Simulation for Bayesian Inference. Chapman & Hall, Boca Ratón, Florida.

Gatesy J (2009) Whales and even-toed ungulates (Cetartiodactyla). In *The Timetree of Life*, eds Hedges SB, Kumar S. Oxford University Press, Oxford. Pp. 511-515

Gatesy J, Yelon D, DeSalle R, Vrba E (1992) Phylogeny of the Bovidae (Artiodactyla, Mammalia), based on mitochondrial ribosomal DNA sequences. *Mol Biol Evol* 9(3):433.

Geisler JH, Theodor JM (2009) Hippopotamus and whale phylogeny. *Nature* 458(7236):E1-4.

Geisler JH, Uhen M (2005) Phylogenetic Relationships of Extinct Cetartiodactyls: Results of Simultaneous Analyses of Molecular, Morphological, and Stratigraphic Data. *J Mamm Evol* 12(1-2):145-160.

Geist V (1998) Strategies of deer evolution. In *Deer of the World: Their Evolution Behaivour and Ecology*, ed Geist V. Stackpole Books, Mechanisburg. Pp. 1-18

Gentry AW (2000) The Ruminant radiation. In *Antelopes, Deer, and Relatives: Fossil Record, Behavioral Ecology, Systematics and Conservation*, eds Vrba ES, Schaller GB. Yale University Press, New Haven. Pp. 11-25

Gilbert C, Ropiquet A, Hassanin A (2006) Mitochondrial and nuclear phylogenies of Cervidae (Mammalia, Ruminantia): systematics, morphology, and biogeography. *Mol Phylogenet Evol* 40(1):101-17.

Ginsburg L, Morales J, Soria D (2001) Les ruminantia (Artiodactyla, Mammalia) du Miocene des Bugti (Balouchistan, Pakistan). *Estud Geol* 57(3-4):155-177.

Goloboff PA, Farris KC, Nixon KC (2008) TNT, a free program for phylogenetic analysis. *Cladistics* 24(4):774-786.

Gotelli NJ (2000) Null model analysis of species co-occurrence patterns. *Ecology* 81(9):2606-2621.

Hamilton WR (1978) Fossil giraffes from the Miocene of Africa and a revision of the phylogeny of the Giraffoidea. *Philos Trans R Soc Lond B Biol Sci* 283(996):165-229.

Harvey PH, Holmes EC, Mooers AØ, Nee S (1994) Inferring evolutionary processes from molecular phylogenies. In *Models in Phylogeny Recosntruction*, eds Scotland RW, Siebert DJ, Williams DM. Clarendon Press, Oxford. Pp. 313-333

Harvey PH, Pagel MD (1991) The comparative method in evolutionary biology. Oxford University Press, Oxford.

Hassanin A, Douzery EJ (2003) Molecular and morphological phylogenies of ruminantia and the alternative position of the Moschidae. *Syst Biol* 52(2):206-28.

Hassanin A, Douzery EJP (1999) The tribal radiation of the family Bovidae (Artiodactyla) and the evolution of the mitochondrial cytochrome b gene. *Mol Phylogenet Evol* 13(2):227-243.

Heard SB, Cox GH (2007) The shapes of phylogenetic trees of clades, faunas, and local assemblages: Exploring spatial pattern in differential diversification. *Am Nat* 169(5):E107-E118.

Hernández Fernández M, Vrba ES (2005a) A complete estimate of the phylogenetic relationships in Ruminantia: a dated species-level supertree of the extant ruminants. *Biol Rev* 80(2):269-302.

Hernández Fernández M, Vrba ES (2005b) Macroevolutionary processes and biomic specialization: testing the resource-use hypothesis. *Evol Ecol* 19(3):199–219.

Huelsenbeck JP, Rannala B, Masly JP (2000) Accommodating phylogenetic uncertainty in evolutionary studies. *Science* 288(5475):2349-50.

Huelsenbeck JP, Ronquist F (2001) MrBayes: bayesian inference of phylogenetic trees. *Bioinformatics* 17(8):754-755.

Janis C (1982) Evolution in horns in ungulates: ecology and paleoecology. *Biol Rev Camb Philos Soc* 57(2):261-317.

Janis CM, Damuth J, Theodor JM (2000) Miocene ungulates and terrestrial primary productivity: where have all the browsers gone? *Proc Natl Acad Sci U S A* 97(14):7899-7904.

Janis CM, Damuth J, Theodor JM (2004) The species richness of Miocene browsers, and implications for habitat type and primary productivity in the North American grassland biome. *Palaeogeogr Palaeoclimatol Palaeoecol* 207(3-4):371-398.

Janis CM, Scott KM (1987) The interrelationships of higher ruminant families: with special emphasis on the members of the Cervoidea. *Am Mus Novit* 2893:80-85.

Janis CM, Scott KM, Jacobs LL (1998) Evolution of Tertiary Mammals of North America. Volume 1: Terrestrial Carnivores, Ungulates, and Ungulatelike Mammals. Cambridge University Press, Cambridge.

Kamilar JM, Guidi LM (2010) The phylogenetic structure of primate communities: variation within and across continents. *J Biogeogr* 37(5):801-813.

Kreft H, Jetz W (2010) A framework for delineating biogeographical regions based on species distributions. *J Biogeogr* 37(11):2029-2053.

Lewis PO (2001) Phylogenetic systematics turns over a new leaf. *Trends Ecol Evol* 16(1):30-37.

Liu FR, Miyamoto MM, Freire NP, Ong PQ, Tennant MR, Young TS, Gugel KF (2001) Molecular and Morphological Supertrees for Eutherian (Placental) Mammals. *Science* 291(5509):1786-1789.

Losos JB (2011) Seeing the forest for the trees: the limitations of phylogenies in comparative biology. *Am Nat* 177(6):709-727.

Maddison WP (1990) A method for testing the correlated character evolution of two binary characters: are gains and losses concentrated on certain branches of a phylogenetic tree? *Evolution* 44(3):539-557.

Maddison WP, Midford PE, Otto SP (2007) Estimating a binary character's effect on speciation and extinction. *Syst Biol* 56(5):701-710.

Marcot JD (2007) Molecular phylogeny of terrestrial Artiodactyls: conflicts and resolution. In *The Evolution of Artiodactyls,* eds Prothero DR, Foss SE. The John Hopkins University Press, Baltimore. Pp. 4-18

Martins EP, Hansen TF (1996) The statistical analysis of interspecific data: a review and evaluation of phylogenetic comparative methods. In *Phylogenetics and the*

Comparative Method, ed Martins EP. Oxford University Press, Oxford. Pp. 22-75

Martins EP, Hansen TF (1997) Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *Am Nat* 149(4):646-667.

Métais G, Benammi M, Chaimanee Y, Jaeger J, Tun T, Thein T, Ducrocq S (2000) Discovery of new ruminant dental remains from the Middle Eocene Pondaung Formation (Myanmar): reassessment of the phylogenetic position of Indomeryx. *C R Acad Sci Paris Ser II* 330(11):805-811.

Métais G, Chaimanee Y, Jaeger J, Ducrocq S (2007) Eocene bunoselenodont Artiodactyla from southern Thailand and the early evolution of Ruminantia in South Asia. *Naturwissenschaften* 94(6):493-498.

Métais G, Chaimanee Y, Jaeger JJ, Ducrocq S (2001) New remains of primitive ruminants from Thailand: evidence of the early evolution of the Ruminantia in Asia. *Zool Scr* 30(4):231-248.

Métais G, Vislobokova I (2007) Basal Ruminants. In *The Evolution of Artiodactyls*, eds Prothero DR, Foss SE. The John Hopkins University Press, Baltimore. Pp. 189-212

Mooers AØ, Heard S (2002) Using tree shape. *Syst Biol* 51(6):833.

Mooers AØ, Heard SB (1997) Inferring evolutionary process from phylogenetic tree shape. *Quart Rev Biol* 72(1):31-54.

Morales J, Pickford M, Soria D (1993) Pachyostosis in a Lower Miocene giraffoid from Spain, *Lorancameryx pachyostoticus* nov. gen. nov. sp. and its bearing on the evolution of bony appendages in artiodactyls. *Geobios* 26(2):207-230.

Morales J, Soria D, Nieto M, Peláez-Campomanes P, Pickford M (2003a) New data regarding *Orangemeryx hendeyi* Morales et al., 2000, from the type locality, Arrisdrift, Namibia. *Memoir Geol Surv Namibia* 19:305-344.

Morales J, Soria D, Pickford M (1999) New stem giraffoid ruminants from the early and middle Miocene of Namibia. *Geodiversitas* 21(2):229-253.

Morales J, Soria D, Pickford M (2008) Pecoran ruminants from the Early Miocene of the Sperrgebiet, Namibia. *Memoir Geol Surv Namibia* 20:397-464.

Morales J, Soria D, Pickford M, Nieto M (2003b) A new genus and species of Bovidae (Artiodactyla, Mammalia) from the early Middle Miocene of Arrisdrift, Namibia, and the origins of the family Bovidae. *Memoir Geol Surv Namibia* 19:371-384.

Moreno Bofarull A, Arias Royo A, Hernández Fernández M, Ortiz-Jaurequizar E, Morales J (2008) Influence of continental history on the ecological specialization and macroevolutionary processes in the mammalian assemblage of South America: differences between small and large mammals. *BMC Evol Biol* 8(97):1-18.

Moyà-Solà S (1987) Los Rumiantes (Cervoidea y Bovidea, Artiodactyla, Mammalia) del Ageniense (Mioceno inferior) de Navarrete del Río (Teruel, España). *Paleontol i Evol* 21:247--270.

Nieto M (1998) Relaciones entre comportamiento, ecología y morfología en formas actuales de bóvidos: aplicaciones a la paleoecología (Tesis Doctoral). Universidad Autónoma de Madrid, Madrid.

Norell MA (1992) Taxic origin and temporal diversity: the effect of phylogeny. In *Extintion and Phylogeny*, eds Novacek MJ, Wheeler QD. Columbia University Press, New York. Pp. 89-118

Norell MA (1993) Tree-based approaches to understanding history: comments on ranks, rules, and the quality of fossil record. *Am J Sci* 293(A):407-417.

Norris C (2000) The cranium of Leptotragulus, a hornless protoceratid (Artiodactyla: Protoceratidae) from the Middle Eocene of North America. *J Vertebr Paleontol* 20(2):341-348.

Nowak RM (1999) Walker's mammals of the world. The Johns Hopkins Univiversity Press, Baltimore.

O'Leary M, Gatesy J (2008) Impact of increased character sampling on the phylogeny of Cetartiodactyla (Mammalia): combined analysis including fossils. *Cladistics* 24(4):397-442.

Ord TJ, Blumstein DT, Evans CS (2002) Ecology and signal evolution in lizards. *Biol J Linn Soc* 77(1):127-148.

Ortolani A (1999) Spots, stripes, tail tips and dark eyes: predicting the function of carnivore colour patterns using the comparative method. *Biol J Linn Soc* 67(4):433-476.

Pagel M (1994) Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proc R Soc B* 255(1342):37-45.

Pagel M (1997) Inferring evolutuonary processes from phylogenies. *Zool Scr* 26(4):331-348.

Pagel M (1999) The Maximun Likelihood Approach to Reconstructing Ancestral Character States of Discrete Characters on Phylogenies. *Syst Biol* 48(3):612-622.

Pagel MD, Meade A (2006) Bayesian Analysis of Correlated Evolution of Discrete Characters by Reversible-Jump Markov Chain Monte Carlo. *Am Nat* 167(6).

Pagel MD, Meade A, Barker D (2004) Bayesian estimation of ancestral character states on phylogenies. *Syst Biol* 53(5):673-84.

Paradis E (2006) Analysis of Phylogenetics and Evolution with R. Springer, New York.

Paradis E (2011) Time-dependent speciation and extinction from phylogenies: a least squares approach. *Evolution* 65(3):661-72.

Paradis E, Claude J (2002) Analysis of comparative data using generalized estimating equations. *J Theor Biol* 218(2):175-85.

Pérez-Barbería FJ, Gordon IJ, Nores C (2001) Evolutionary transitions among feeding styles and habitats in ungulates. *Evol Ecol Res* 3(2):221-230.

Pitra C, Fickel J, Meijaard E, Groves PC (2004) Evolution and phylogeny of old world deer. *Mol Phylogenet Evol* 33(3):880-895.

Prothero DR (2005) Protoceratidae. In *Evolution of Tertiary Mammals of North America*, eds Janis CM, Scott KM, Jacobs LL. Cambridge University Press, Cambridge. Pp. 431-438

Purvis A, Rambaut A (1995) Comparative analysis by independent contrasts (CAIC):

an Apple Macintosh application for analysing comparative data. *Comp Appl Biosci* 11(3):247-251.

Rabosky DL (2006a) LASER: a maximum likelihood toolkit for detecting temporal shifts in diversification rates from molecular phylogenies. *Evol Bioinform* 2(6):273-260.

Rabosky DL (2006b) Likelihood methods for detecting temporal shifts in diversification rates. *Evolution* 60(6):1152-1164.

Rabosky DL (2010) Extinction rates should not be estimated from molecular phylogenies. *Evolution* 64(6):1816-1824.

Roberts SC (1996) The evolution of hornedness in female ruminants. *Behaviour* 133(5-6):399-442.

Ronquist F (2004) Bayesian inference of character evolution. *Trends Ecol Evol* 19(9):475-81.

Sánchez I, Domingo M, Morales J (2010a) The genus *Hispanomeryx* (Mammalia, Ruminantia, Moschidae) and its bearing on musk deer phylogeny and systematics. *Palaeontology* 53(5):1023-1047.

Sánchez I, Quiralte V, Morales J, Pickford M (2010b) A new genus of tragulid ruminant from the early Miocene of Kenya. *Acta Palaeontol Pol* 55(2):177-187.

Sánchez IM, Morales J (2006) Distribución biocronológica de los Moschidae (Mammalia, Ruminantia) en España. *Estud Geol* 62(1):533-546.

Scopoli JA (1777) Introdvctio ad historiam natvralem sistens genera lapidvm, plantarvm, et animalivm hactenvs detecta, caracteribvs essentialibvs donata, in tribvs divisa, svbinde ad leges natvrae. Gerle, Pragae.

Sillén-Tullberg B (1988) Evolution of gregariousness in aposematic butterfly larvae: a phylogenetic analysis. *Evolution* 42(2):293-305.

Smith AB (1994) Systematics and the fossil record. Backwell Scientific Publications, Oxford.

Solounias N, Barry JC, Bernor RL, Lindsay E, Raza SM (1995) The oldest bovid from the Siwaliks, Pakistan. *J Vertebr Paleontol* 15(4):806-814.

Solounias N, McGraw WS, Hayek L-A, Werdelin L (2000) The Paleodiet of the Giraffidae. In *Antelopes, Deer, and Relatives: Fossil Record, Behavioral Ecology, Systematics and Conservation*, eds Vrba ES, Schaller GB. Yale University Press, New Haven. Pp. 84-95

Stadler T 2010. TreeSim in R - Simulating trees under the birth-death model. Available at http://cran.r-project.org/web/packages/TreeSim/index.html.

Stadler T (2011) Mammalian phylogeny reveals recent diversification rate shifts. *Proc Natl Acad Sci U S A* 108(15):6187-6192.

Stucky RK (2005) Eocene bunodont and bunoselenodont Artiodactyla ("dichobunids"). In *Evolution of Tertiary Mammals of North America*, eds Janis CM, Scott KM, Jacobs LL. Cambridge University Press, Cambridge. Pp. 358-374

Su B, Wang YX, Lan H, Wang W, Zhang Y (1999) Phylogenetic study of complete cytochrome b genes in musk deer (genus Moschus) using museum samples. *Mol Phylogenet Evol* 12(3):241-9.

Swofford DL 2000. *PAUP*: Phylogenetic Analysis Using Maximum Parsimony* (*and other methods), Version 4.10b. Released by the author.

Vamosi SM, Heard SB, Vamosi JC, Webb CO (2009) Emerging patterns in the comparative analysis of phylogenetic community structure. *Mol Ecol* 18(4):572-592.

Vislobokova IA (1990) The basic features of historical development and classification of the Ruminantia. *Paleontol J* 4:3-14.

Vrba ES (1980) Evolution, species and fossils: how does life evolve? S Afr J Sci 76:61-84.

Vrba ES (1987) Ecology in relation to speciation rates: some case histories of Miocene-Recent mammal clades. *Evol Ecol* 1(4):283–300.

Vrba ES (1992) Mammals as a Key to Evolutionary Theory. *J Mammal* 73(1):1-28.

Vrba ES (1995a) The fossil record of African Antelopes (Mammalia, Bovidae) in relation to human evolution and paleoclimate. In *Paleoclimate and Evolution with Emphasis on Human Origins*, eds Vrba ES, Denton GH, Patridge TC, Burcke LH. Yale University Press, New Haven. Pp. 385-324

Vrba ES (1995b) On the connections between paleoclimate and evolution. In *Paleoclimate and Evolution with Emphasis on Human Origins*, eds Vrba ES, Denton GH, Patridge TC, Burcke LH. Yale University Press, New Haven. Pp. 24-45

Vrba ES, Schaller GB (2000) Introducction. In *Antelopes, Deer, and Relatives: Fossil Record, Behavioral Ecology, Systematics and Conservation*, eds Vrba ES, Schaller GB. Yale University Press, New Haven. Pp. 1-8

Webb CO, Ackerly DD, Kembel SW (2008) Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics* 24(18):2098-2100.

Webb SD, Taylor BE (1980) The phylogeny of hornless ruminants and a description of the cranium of *Archaeomeryx*. *Bull Am Mus Nat Hist* 167:121-157.

Wilson DE, Reeder DM (1993) Mammal Species of the World: a taxonomic and geographic reference. Smithsonian Institution Press, Washington.

Wilson DE, Reeder DM (2005) Mammal Species of the World: a taxonomic and geographic reference. The Johns Hopkins University Press, Baltimore.

Zachos J, Pagani M, Sloan L, Thomas E, Billups K (2001) Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* 293(5517):686-693.

2

Ecological correlates of ghost lineages in ruminants

> *How do we understand the history of life? Traditionally patterns of preservation have been used as direct indicators of taxic and faunal origin and demise.*
>
> *- Mark A. Norell -*

Abstract

Integration between phylogenetic systematics and paleontological data has proved to be an effective method for identifying periods that lack fossil evidence in the evolutionary history of clades. In this study we aim to analyze whether there is any correlation between various ecomorphological variables and the duration of these underrepresented portions of lineages, which we call ghost lineages for simplicity, in ruminants. Analyses within phylogenetic (Generalized Estimating Equations) and non-phylogenetic (ANOVAs and Pearson correlations) frameworks were performed on the whole phylogeny of this suborder of Cetartiodactyla (Mammalia). This is the first time ghost lineages are focused in this way. To test the robustness of our data, we compared the magnitude of ghost lineages among different continents and among phylogenies pruned at different ages (4, 8, 12, 16, and 20 Ma). Differences in mean ghost lineage were not significantly related to either geographic or temporal factors. Our results indicate that the proportion of the known fossil record in ruminants appears to be influenced by the preservation potential of the bone remains in different environments. Furthermore, large geographical ranges of species increase the likelihood of preservation.

Introduction

An important part of our knowledge about evolution is based on information from fossils. Patterns of preservation in the fossil record have provided a key tool for estimating dates of appearance of new living forms on Earth and understanding events of diversification and extinction (Norell, 1993). Nevertheless, our comprehension of the many factors that may influence the preservation of species in the fossil record is still incomplete. For example, although stratigraphic sampling appears to be relevant (Alroy et al., 2001; Crampton et al., 2003), we do not know whether ecological characteristics of species are also important in this context. The use of ecological data on modern species along with phylogenetic systematics synthesizing information from both extant and extinct species may provide a novel approach to this issue.

Traditionally, cladistic hypotheses have been adjusted to stratigraphic ranges of species by adding inferred lineages for which no fossil has been recovered. Following this view, ghost lineages were defined as complete branches in an evolutionary tree that lacks a known fossil record, but whose presence is inferred from the tree topology obtained by phylogenetic analysis (Norell, 1992; Smith, 1994). These ghost lineages can be recovered only by a phylogenetic approach calibrated with paleontological data (Norell, 1996). Moreover, Smith (1994) also identified range extensions, which are temporal gaps that must be added to the stratigraphic ranges of taxa in order to build an evolutionary tree that fits temporal relationships with a phylogenetic analysis. In this context some authors have surveyed the extent to which assumptions of different cladistic hypotheses may influence the estimations of our paleontological knowledge (Norell and Novacek, 1992a, b; Weishampel, 1996; O'Keefe and Sander, 1999; Benton et al., 2000; Wagner, 2000a, b; Wills, 2002; Pol and Norell, 2006; Worthy et al., 2006). For example, given two fossil taxa with different first appearances and known fossil ranges that do not overlap, different lengths of inferred range must be added depending on different cladistic assumptions. If we assume that as organisms evolve they give rise to new taxa in dichotomous splits, the evolutionary histories of two sister groups sharing a common ancestor should have equal duration (Paul, 1982). Thus, a range from the first appearance of the younger taxon to the date of first appearance of the older taxon must be added (Norell, 1996). Conversely, an ancestor-descendant relationship could be assumed and we would only have to infer a range between the last appearance of the older taxon (inferred ancestor) and the first appearance of the younger taxon (Wagner, 1995, 2000a, b). In phylogenies containing fossil taxa, cladistic hypotheses and temporal calibration come from the same source: fossils. In such cases sampling bias directly affects phylogenetic accuracy, which in turn may bias the assessment of the fossil record. Only well-resolved topologies yield a correct interpretation of gaps in the fossil record (Wagner, 2000a).

Molecular phylogenies provide a new tool in this scenario. Molecular-based phylogenetic analyses usually generate origin dates earlier than the first appearance of known fossil taxa (Hartenberger, 1998; Adkins et al., 2001; Huchon et al., 2002; Teeling et al., 2005). Comparing both fossil and molecular estimates of lineages origin may shed some light on the accuracy with which the fossil record represents the evolutionary history of lineages leading to living species (Teeling et al., 2005; Johnson et al., 2006). This approach, according to the criteria of Teeling et al. (2005), places the oldest known fossil for each branch of the molecular tree and calculates the percentage of unrepresented basal branch length. Nevertheless, although taxonomic sampling bias in molecular trees of extant species is theoretically smaller than in phylogenies of extinct taxa, assessing the accuracy of the fossil record in this manner is applicable only to lineages leading to extant species and it is not exempt from limitations (see further discussion in "Limitations of the Methods," below).

For the sake of simplicity, hereafter we use the term "ghost lineages" to refer to ghost lineages, range extensions, and unrepresented basal branch lengths. All of these have been included in studies assessing the congruence among divergence dates from molecular phylogenies and fossil ranges (Teeling et al., 2005; Johnson et al., 2006), paleodiversity estimates (Lane et al., 2005), inferences about patterns of character acquisition (Sidor and Hopson, 1998), and the magnitude of critical events (Cavin and Forey, 2007; Ruta and Benton, 2008). In this work, we tested whether several ecomorphological attributes of the species (body mass, presence in biomes, range size, diet, and locomotor modes) may influence the duration of ghost lineages and, therefore, the likelihood of generating a complete fossil record. Our test focused on the suborder Ruminantia, which is the most speciose extant clade of large land herbivores and presents a fossil record that covers a time span of 50 Myr. Ruminants have developed a spectacular diversity of ecomorphological specializations, with wide geographical and ecotypic ranges and existing species inhabiting every terrestrial biome (Walter, 1970). Such ecological diversity and taxonomic richness, with 197 extant species in 79 genera and about 300 extinct genera (Grubb, 1993; Hernández Fernández and Vrba, 2005a), prove ruminants to be a valuable target for evolutionary research (Vrba and Schaller, 2000).

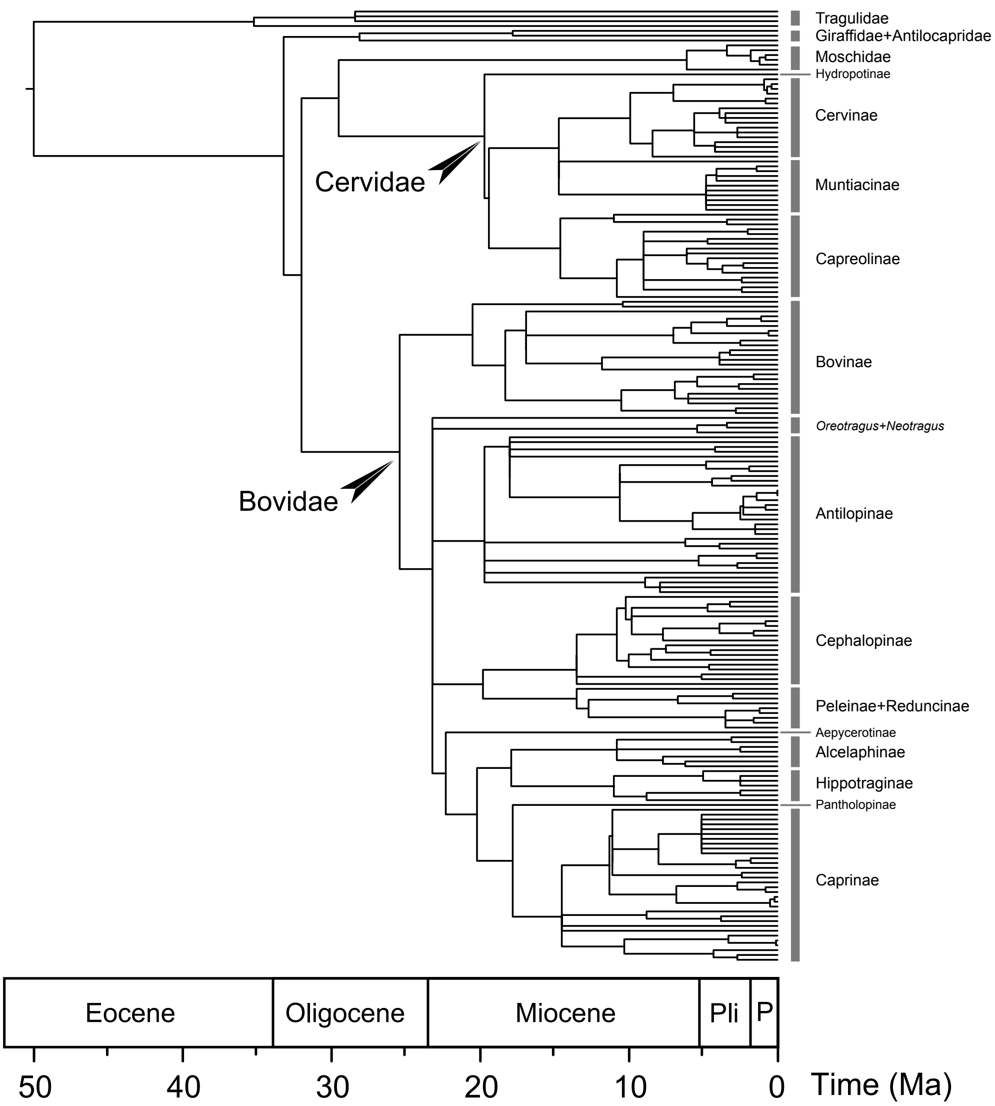


Figure 2.1. Supertree of all 197 extant and recently extinct species of ruminants (Hernández Fernández and Vrba 2005a) showing the names of families and subfamilies.

Materials and Methods

Data. The phylogeny of suborder Ruminantia was taken from the supertree published by Hernández Fernández and Vrba (2005a), which includes all the 197 extant and recently extinct ruminant species. This supertree is a consensus tree combining morphological, ethological and molecular information from every phylogeny published up to date, and includes a time calibration using paleontological data (Fig. 2.1).

To identify the correlations among ghost lineages' durations and the different ecomorphological characters, we compiled data for 19 binary variables and 2 continuous variables (Table 2.1). Following Telling et al. (2005) we collated

Dependent	*Independent*					
Continuous	**Continuous**		**Binary**			
% Ghost Lineage	*Body Mass (Kg)*	*Range Extension (latitude extent)*	*Locomotor mode*	*Diet*	*Biomes*	*Stenobiomy*
			Gallop	Browser	Evergreen Tropical Rain Forest (I)	Species inhabiting only one biome
			Zigzag	Mixed Diet	Deciduous Tropical Forest (II)	
			Bounding Gallop	Grazer	Savanna (II/III)	
			Stotting		Subtropical Desert (III)	
			Climbing		Mediterranean Forest (IV)	
					Temperate Evergreen Forest (V)	
					Temperate Broad-Leaf Deciduous Forest (VI)	
					Steppe and Cold Desert (VII)	
					Boreal Coniferous Forest (Taiga) (VIII)	
					Tundra (IX)	

Table 2.1. Variables used in the non-phylogenetic and the phylogenetic analyses of ghost lineage percentages.

the oldest known fossil for each branch of the supertree and compared its age with the ages representing the beginning and end of that branch (see table in Appendix 2.1 and figures in Appendix 2.2). We calculated ghost lineage durations as the percentage of the total branch length that contains no fossil record. Unlike Johnson et al.'s (2006) study, in which, for example, "an old Lynx species fossil was interpreted as representing the entire fossil history of this group (i.e. 0% missing)," we used each fossil for calculating the underrepresented length of only one branch.

Information on body mass was compiled for the 197 extant species of the group. We also differentiated five locomotor modes (Alcalde et al., 2006) and three diets (DeMiguel et al., 2008). Biogeographic data for the 197 species were taken from distribution information obtained from several sources (Answell, 1971; Corbet, 1978; Hall, 1981; Eisenberg, 1989; Corbet and Hill, 1992; Redford and Eisenberg, 1992; Grubb, 1993; Kingdon, 1997; Mitchell-Jones et al., 1999; Eisenberg and Redford, 2000; IUCN, 2008). We used the method described by Hernández Fernández (2001) to estimate the presence/absence in the terrestrial biomes described by Walter (1970), who defined them as particular combinations of climate and vegetation. Because altitudinal gradients represent a habitat series analogous to that of biomes, vegetation belts in mountains were also borne in mind when estimating the occurrence of species in a given biome (Hernández Fernández and Vrba, 2005c). Furthermore, following Hernández Fernández and Vrba (Hernández Fernández and Vrba, 2005b) we considered as stenobiomic species those occupying only one biome.

To test the correlation between ghost lineage durations and the different ecomorphological variables, we performed both non-phylogenetic and phylogenetic tests.

Non-Phylogenetic Test. We conducted conventional analyses treating all branches in the supertree as cases (356 in total). Ghost lineage percentages for each branch were assessed as explained above, and values of the independent variables were reconstructed by using parsimony reconstruction methods as implemented in Mesquite (Maddison and Maddison, 2007). When character reconstruction was ambiguous for binary variables (both "0" and "1" values were equally parsimonious in some branch of the tree) we used the "most parsimonious reconstruction mode" and chose those reconstructions with the most gains (changes form "0" to "1") and the fewest losses (changes from "1" to "0"), and those with the fewest gains and the most losses. We carried out our analyses for both of them (Ortolani and Caro, 1996; Ortolani, 1999). To gauge relationships between ghost lineage percentage and the continuous and binary ecomorphological variables, we used Pearson correlations and one-factor ANOVAs, respectively.

Phylogenetic Test. Closely related species are more likely to share similar ecological features because of common ancestry, so data for different species cannot be considered as independent points in comparative studies (Felsenstein, 1985; Harvey and Pagel, 1991). Therefore, by using the comparative method in a phylogenetic framework, we avoid phylogenetic biases that might be present in our ecological variables. Phylogenetic analyses were performed using Generalized Estimating Equations (GEE) (Paradis and Claude, 2002; Paradis, 2006), which incorporates species relatedness as a correlation matrix and uses a generalized linear model approach. Because data for these analyses must be introduced for the tips of the tree, we calculated for each tip of the tree the average of the values of ghost lineage percentage of every branch leading to that tip from the root of the tree.

Tests for Data Robustness. It may be argued that some clades exhibit a great deal of ghost range because they are all located in one part of the world or are all of a particular age, which could be related to differential paleontological sampling. In order to address this issue we conducted two different ANOVA tests. The first one compared the ghost lineage percentages of the branches implied in the evolution of ruminant species from different continents (North America, South America, Eurasia, and Africa). The second analysis compared ghost lineage percentages among the branches of the ruminant phylogeny when pruned at different ages (4, 8, 12, 16 and 20 Ma) to establish whether ghost lineages were more important in some geologic ages than others. We did not use phylogenies pruned at ages older than 20 Ma because the number of branches implied in the analyses would be too low to develop statistically powerful analyses.

Furthermore, as an additional test for the robustness of the data on ghost lineage length, we performed linear regression analyses between the ghost lineage percentages of the branches and the age of their previous node. A statistically strong relationship would indicate that the age of the branch may influence the importance of the ghost range in it. We analyzed the four continental data sets as well as the complete data set.

Limitations of the Methods. Because the phylogeny used for the analyses is a supertree, it could change as new phylogenetic studies are published [see, for example, the case of the supertree for mammalian families (Liu et al., 2001; Bininda-Emonds et al., 2007)]. Our results are therefore contingent on the degree to which future studies affect the interpretation of phylogenetic relationships within Ruminantia. Nevertheless, future variations in the topology or higher resolution of the tree will have little influence on our conclusions as long as such changes do not affect many branches. Taking into account the high number of studies Hernández Fernández and Vrba (2005a) used to develop the ruminant supertree, as well as the supertree's high consistency and retention indices, profound changes in the topology of the tree are unlikely in the near future.

Another possible drawback is related to the selection of fossils for the definition of ghost lineages in every branch. Some uncertainty in the phylogenetic relationships of extinct taxa is warranted and the position of single taxa along the ruminant phylogeny may affect the inference of ghost lineage durations. This issue, however, is also dependent on the development of new studies on phylogenetic relationships of extinct ruminants, including the occurrence of new discoveries. Such uncertainty cannot be accounted for in this work, but future reviews of our conclusions may be needed in order to confirm their robustness.

Finally, our estimate of the duration of ghost lineages for ruminants might differ substantially from one that considers the entire fossil record of the clade, because including more taxa and branches could lead to differences in the calculation of ghost lineages. Such a problem could be solved by using a complete supertree, one that also includes all the extinct taxa of Ruminantia. Although the development of such a new supertree is in progress, however, it is beyond the scope of the current study.

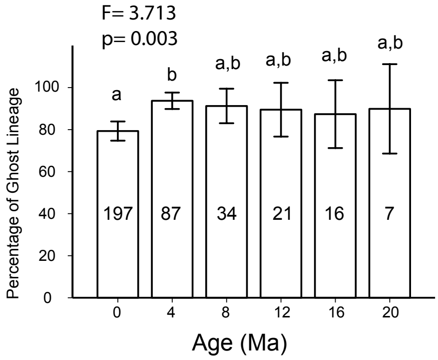


Figure 2.2. Variation of the average ghost lineage percentages at different ages. Number of tips for each age is shown within the bars. Error bars: 95% confidence interval.

Results

Data Robustness. The results of the post hoc ANOVAs for different ages point to a slight difference between the current percentages of ghost lineage in extant lineages and those at 4 Ma. Nevertheless, the results from 0 and 4 Ma didn't differ significantly from any of the other time periods (Fig. 2.2).

The relationship between ghost lineage percentage in each branch and the age of the prior node does not fit a linear model, neither for the whole tree, or when the lineages are examined separately on each continent (Fig. 2.3 and Fig. 2.4), with the exception of Eurasia. Nevertheless, in the latter case this relationship is very weak, explaining less than 4% of the variability in the data set.

Finally, the post hoc ANOVAs did not show any significant differences when ghost lineage percentages of each continent were compared ($p = 0.593$) (Fig. 2.5).

All these results indicate that our data on ghost lineage percentage are not influenced by either geographical or temporal factors.

Ghost Lineages and their Ecological Correlates. The total percentage of ghost lineage in Ruminantia, measured as the proportion of ghost lineage durations and total range, is 80% (Table 2.2). Average durations of ghost lineages for each family and subfamily range from 11%, in the lineage that gave rise to the only species included in the modern Antilocapridae (*Antilocapra americana*), to 97.5% in Hydropotinae (Table 2.2).

Pearson correlations and one-factor ANOVAs showed significant relationships between ghost lineage percentage and ten ecomorphological variables. Negative correlations were found between ghost lineages percentages and body mass, geographic range, gallop, stotting, grazer diet, and presence in

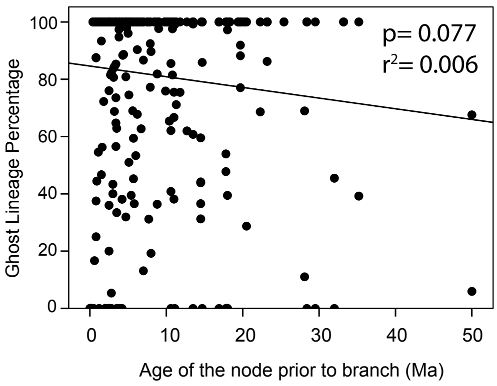


Figure 2.3. Relationship between the age of each branch of the supertree of ruminants and the associated ghost lineage percentage. The determination coefficient and significance of the linear regression are shown.

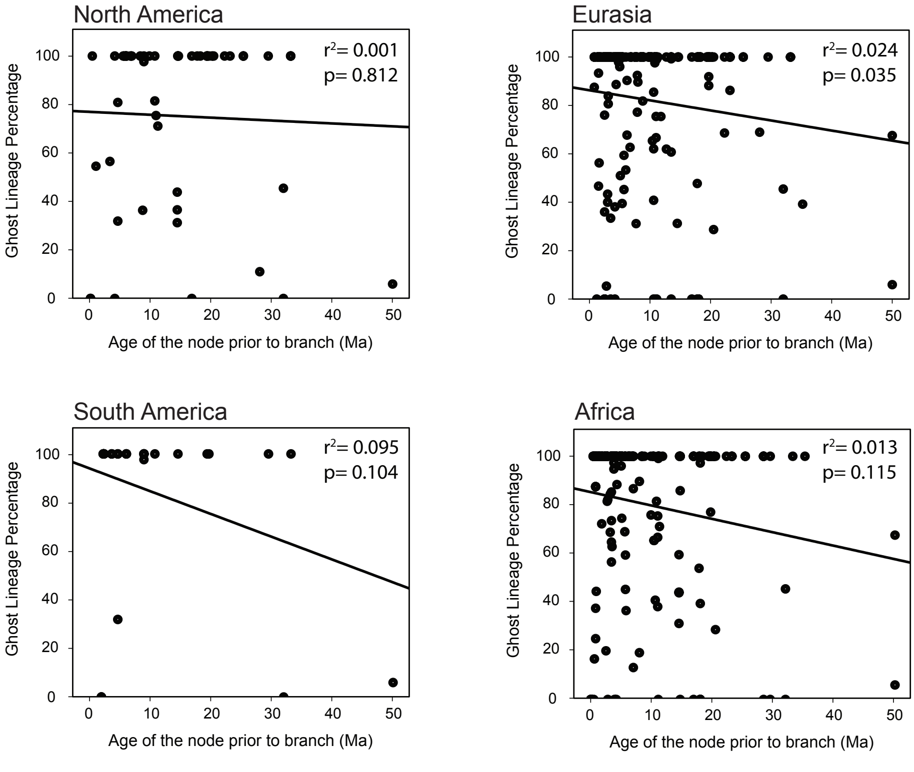


Figure 2.4. Relationship between the age of each branch and the associated ghost lineage percentage analyzed separately for the extant species from each continent. For each linear regression, the determination coefficient and the significance of the relationship are shown.

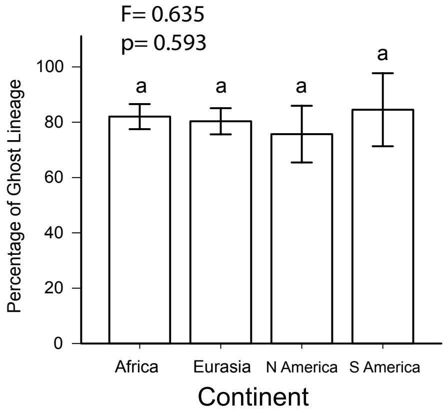


Figure 2.5. Ghost lineage percentages inferred in the evolution of ruminant species from different continents. Error bars: 95% confidence interval.

Family	Sp	B	Total GL%	Mean GL%	Subfamily	Sp	B	Total GL%	Mean %GL
Tragulidae	4	6	81.55	72.40					
Antilocapridae	1	1	11.03	11.03					
Giraffidae	2	3	33.99	38.89					
Moschidae	6	11	46.77	90.90					
Cervidae	47	80	90.18	82.44	Hydropotinae	1	1	97.5	97.5
					Cervinae	18	33	90.01	76.85
					Muntiacinae	10	13	97.36	93.39
					Capreolinae	18	31	85.11	82.18
Bovidae	137	249	83.57	80.74	Bovinae	24	46	82.32	78.92
					Antilopinae	33	58	84.99	81.18
					Cephalophinae	19	36	98.26	95.83
					Peleinae	1	1	60.74	60.74
					Reduncinae	8	15	70.00	66.50
					Aepycerotinae	1	1	68.61	68.61
					Alcelaphinae	7	12	73.94	73.94
					Hippotraginae	7	12	81.47	76.70
					Pantholopinae	1	1	53.93	53.93
					Caprinae	32	53	80.04	75.40
					incertae sedis	4	6	94.20	97.70
TOTAL	197	356	80.30	81.50					

Table 2.2. Total percentage and mean percentage of ghost lineage (%GL) for different taxonomic groups in Ruminantia. Sp, number of extant species; B, number of branches.

	Non-Phylogenetic *356 branches*		Phylogenetic *197 species*	
Body Mass	***0.014***	-	0.106	+
Log (Body Mass)	***0.000***	-	0.924	+
Range Extension	***0.000***	-	***0.002***	-
Log (Range)	***0.001***	-	***0.004***	-
Gallop	***0.000***	-	*0.074*	-
Gallop.2	***0.001***	-	(NA)	
Zigzag	***0.005***	+	***0.019***	+
Zigzag.2	***0.007***	+	(NA)	
Bounding Gallop	***0.018***	+	0.476	+
Bounding Gallop.2	***0.004***	+	(NA)	
Stotting	***0.022***	-	0.608	-
Stotting.2	***0.027***	-	(NA)	
Climbing	0.619	-	0.156	+
Browser	***0.008***	+	0.810	-
Browser.2	***0.005***	+	(NA)	
Mixed Diet	0.878	-	0.580	-
Mixed Diet.2	0.414	-	(NA)	
Grazer	***0.002***	-	0.403	+
Grazer.2	***0.010***	-	(NA)	
I	0.139	+	*0.061*	+
I.2	***0.043***	+	(NA)	
II	0.692	-	0.786	+
II.2	0.673	+	(NA)	
II/III	***0.037***	-	***0.013***	-
II/III.2	***0.008***	-	(NA)	
III	0.801	-	0.960	-
III.2	*0.092*	-	(NA)	
IV	0.561	-	0.738	+
IV.2	0.665	-	(NA)	
V	0.350	+	0.313	+
V.2	0.202	+	(NA)	
VI	0.696	-	0.288	+
VII	0.380	+	0.111	+
VII.2	0.698	+	(NA)	
VIII	0.631	+	0.740	-
VIII.2	0.977	+	(NA)	
IX	0.286	-	0.689	-
IX.2	0.510	-	(NA)	
Stenobiomic	0.159	+	0.403	+
Stenobiomic.2	0.232	+	(NA)	

Table 2.3. Significance level (p) of the relationships between ghost lineage percentage and the ecomorphological variables under study (Table 2.1) yielded from both the conventional and the phylogenetic tests. Some variables are duplicated (denoted by ".2"), representing the most-parsimonious reconstructions for the inner branches of the tree with more losses and more gains, respectively (see methods). Bold-italic, significant correlation ($p < 0.05$); italic, marginal significance ($p < 0.1$); +, positive relationship; –, negative relationship; NA, not available.

savannahs. Therefore, it seems that all these variables are associated with a better representation in the known fossil record. On the other hand, positive correlations were found for zigzag, bounding gallop, browser diet, and presence in tropical rain forest (biome I) (Table 2.3).

The phylogenetic analyses confirm several of these trends (Table 2.3). They corroborate negative correlations between ghost lineage presence and both geographic range extension and presence in savannahs. Moreover, they also verify positive correlations with zigzag locomotor mode and presence in evergreen tropical rain forests.

To confine our conclusions to the most consistent results, below we discuss only the correlations showing significance in both the non-phylogenetic and the phylogenetic tests.

Discussion

Total Ghost Lineage Percentage for Ruminantia. Our calculations yielded 80% of ghost lineage for the supertree of the 197 extant and recently extinct species of ruminants. This does not necessarily imply a poor fossil record. In fact, Ruminantia have one of the most abundant fossil records associated with any mammalian group. Therefore, our results suggest that the known fossil record of ruminants is not intimately related to the evolution of extant species. That is, many extinct lineages of ruminants are not closely related to extant species and, consequently, were not included in the calculations of ghost lineage percentages.

In any case, this value is similar to those estimated by Teeling et al. (2005) for 30 genera inside Chiroptera (73%) and by Johnson et al. (2006) for the 37 living species of Felidae (76%). The slightly higher value of ghost lineage percentage for Ruminantia may be related to two different issues. First, it follows the positive correlation between the number of tips in a tree and the global percentage of ghost lineage ($r = 0.198$, $p < 0.001$, according to the analyses of 1000 trees included in the supplementary data of Benton et al., 2000). In fact, if we downsample our data to the genus level (74 tips), the mean percentage of ghost lineage in ruminants decreases to 70.7%, which seems to indicate a substantially better fossil record than the ones for Chiroptera and Felidae. Second, it also might be caused by the exclusion from the calculations of fossil taxa that pre-dated the molecular age of the associated branch and whose relatedness to the earlier lineage was unclear, unlike in the analyses of Teeling et al. (2005) and Johnson et al. (2006). As described in our methods, we used each fossil for the calculations of the unrepresented proportion of a single branch, whereas in some cases Johnson et al. (2006) based an assessment of 0% unrepresented lineage length in several adjacent branches on a single fossil.

Ecological Variables Enhancing the Probability of Preservation in the Fossil Record (Negative Correlations). The study showed a significant relationship between gallop locomotor mode and low percentages of ghost lineage. Gallop is associated with open substrates with scant or grassy vegetation. In these types of substrates, edaphic activity and acidity from dead leaves are usually absent, and thus conditions are more favorable for preservation of fossil bones (Table 2.3).

In the same way, those species exhibiting wide latitudinal ranges are understood to be widespread; thus, because they are more likely to appear in fossils sites, we might have a more complete knowledge of their evolutionary history. It has been argued that widespread species are usually larger and more generalist than species with restricted ranges (Mayr, 1963; MacArthur, 1972; Jackson, 1974; Glazier, 1980; Brown, 1984; Brown and Maurer, 1987, 1989; Brown, 1995; Gaston and Blackburn, 1996a, b; Thompson et al., 1998). Nevertheless, the relationships among these ecomorphological factors are highly variable (Hernández Fernández and Vrba, 2005b), which could explain why our results indicate consistent statistical significance between ghost lineage percentage and range extent, but not with other apparently related variables.

According to our results, the presence in the biome savannah seems to underlie the congruence between stratigraphic and phylogenetic information. Savannahs are generally located in sedimentary basins where rainfall is highly seasonal and thus wildlife is attracted to marginal lacustrine environments. Although savannahs are typically associated with the formation of fossils sites (Behrensmeyer, 1976; Lyman, 1994; Polonio and López Martínez, 2000; Alberdi et al., 2001), this biome has developed substantially during the late Neogene (Potts and Behrensmeyer, 1992; Hernández Fernández and Vrba, 2006). The origin of savannah environments like those found today has been associated with the spread of C_4 grasses in the late Miocene (Cerling et al., 1993). We must clarify that in this work we do not refer to biomes as geographical areas but as ecosystems, which are prone to latitudinal shift due to global climatic changes. In this sense herbivore species are not constrained to particular geographic areas but rather are adapted to vegetation types; ruminants especially are usually assumed to have occurred in the same habitats across climatic changes, although they were forced to shift their geographic ranges pursuing the shifts of biomes (Vrba, 1987; Hernández Fernández and Vrba, 2005b; Moreno Bofarull et al., 2008).

Ecological Variables Decreasing the Probability of Preservation in the Fossil Record (Positive Correlations). Only one of all the ecomorphological variables studied in this work is consistently identified by both phylogenetic and non-phylogenetic analyses as having a significant influence on the decrease of preservation probability within the fossil record of ruminants. There is a significant correlation (marginally significant in the phylogenetic analysis) between high percentages of ghost lineages and the presence in the evergreen tropical rain forest (biome I). This biome type is usually found in locations with dense forest canopy, which hinders finding fossil sites. Finally, these locations are in areas not much studied from a paleontological point of view (Kerbis et al., 1993).

Zigzag locomotion mode showed a significant or marginally significant positive correlation with ghost lineage percentage. Adaptations to different locomotor modes reflect the type of environment inhabited by each species (Smith and Savage, 1956; Köhler, 1993; DeGusta and Vrba, 2003). In this way, zigzagging is related to forests characterized by developed undergrowth and intense soil activity due to the presence of roots, fungi, microorganisms, and soil fauna (Walter, 1970) (Walter 1970). All these factors make preservation of organic remains difficult.

Conclusions

The study of ghost lineages and the causes of their existence and duration are still barely explored. To tackle this issue we applied the Generalized Estimating Equations, ANOVAs and Pearson correlations to phylogenetic data on ruminants. Our intent was to clarify whether biometric, biogeographic, or ecological variables our knowledge of ruminant paleontology and phylogeny, as well as how these factors influence the fit between paleontological and phylogenetic data within this suborder.

The results indicate that the proportion of the known fossil record of ruminants is determined largely by the potential for bone preservation in each environment. The likelihood of such preservation is also correlated with geographical distribution.

Additional analyses studying ghost lineages and their duration patterns along phylogenies of different groups could shed light on the biases that affect our knowledge of the fossil record. Finally, comprehensive studies of the species' ecomorphological characteristics (Hernández Fernández et al., 2009) in the global fossil record of those groups could help us to test hypotheses generated by studies of modern ruminant species.

Literature Cited

Adkins RM, Gelke EL, Rowe D, Honeycutt RL (2001) Molecular phylogeny and divergence time estimates for major rodent groups: evidence from multiple genes. *Mol Biol Evol* 18(5):777-791.

Alberdi MT, Alonso MA, Azanza B, Hoyos M, Morales J (2001) Vertebrate taphonomy in circum-lake environments: three cases in the Guadalix-Baza Basin (Granada, Spain). *Palaeogeogr Palaeoclimatol Palaeoecol* 161(1-2):1-26.

Alcalde G, Alberdi MT, Azanza B, Hernández Fernández M (2006) Inferencias ambientales a partir del estudio de la locomoción en comunidades de ruminates. In *XXII Jornadas de las Sociedad Española de Paleontología*, ed Fernández Martínez E. León University, León (Spain). Pp. 13-15

Alroy J, Marshall CR, Bambach RK, Bezusko K, Foote M, Fürsich FT, Hansen TA, Holland SM, Ivany LC, Jablonski D, Jacobs DK, Jones DC, Kosnik MA, Lidgard S, Low S, Miller AI, Novack-Gottshall PM, Olszewski TD, Patzkowsky ME, Raup DM, Roy K, Sepkoski JJ, Sommes MG, Wagner PJ, Webber A (2001) Effects of sampling standarization on estimates of Phanerozoic marine diversification. *Proc Natl Acad Sci U S A* 98(11):6261-6266.

Answell WFH (1971) Order Artiodactyla. In *The Mammals of Africa: an Identification Manual*, eds Meester J, Setzer HW. Smithsonian Institution Press, Washington. Pp. 1-84

Behrensmeyer AK (1976) Fossil assemblages in relation to sedimentary environments in the East Rudolf sucession. In *Earliest man and environment in the Lake Rudolf Basin. Stratigraphy, paleoecology and evolution*, eds Coppens Y, Howell FC, Isaac GL, Leakey EF. University Chicago Press, Chicago and London. Pp. 383-401

Benton MJ, Wills MA, Hitchin R (2000) Quality of the fossil record through time. *Nature* 403(6769):534-537.

Bininda-Emonds ORP, Cardillo M, Jones KE, MacPhee RDE, Beck RMD, Grenyer R, Price SA, Vos RA, Gittleman JL, Purvis A (2007) The delayed rise of present-day mammals. *Nature* 446(7135):507-512.

Brown JH (1984) On the relationship between abundance and distribution of species. *Am Nat* 124(2):255-279.

Brown JH (1995) Macroecology. University of Chicago, Chicago, IL.

Brown JH, Maurer BA (1987) Evolution of species assemblages: effects of energetic constraints and species dynamics on the diversification of the North American avifauna. *Am Nat* 130(1):1-17.

Brown JH, Maurer BA (1989) Macroecology: the division of food and space among species on continents. *Science* 243(4895):1145-1150.

Cavin L, Forey PL (2007) Using ghost lineages to identify diversification events in the fossil record. *Biol Lett* 3(2):201-204.

Cerling TE, Wang Y, Quade J (1993) Expansion of C4 ecosystems as indicator of global ecological change in the late Miocene. *Nature* 361(6410):344-345.

Corbet GB (1978) The mammals of the Palaearctic region: a taxonomic review. Cornell

University Press, London.

Corbet GB, Hill JE (1992) The mammals of the Indomalayan Region: a systematic review. Oxford University Press, Oxford.

Crampton JS, Beu AG, Cooper RA, Jones CM, Marshall B, Maxwell PA (2003) Estimating the rock volume bias in paleobiodiversity studies. *Science* 301(5631):358-360.

DeGusta D, Vrba ES (2003) A method for inferring paleohabitats from the functional morphology of bovid astragali. *J Archaeol Sci* 30(8):1009-1022.

DeMiguel D, Fortelius M, Azanza B, Morales J (2008) Ancestral feeding state of ruminants reconsidered: earliest grazing adaptation claims a mixed condition for Cervidae. *BMC Evol Biol* 8(13).

Eisenberg HJF (1989) Mammals of the Neotropics Vol. 1: The Northern Neotropics. University of Chicago Press, Chicago.

Eisenberg HJF, Redford K (2000) Mammals of the Neotropics. Vol. 3: The Central Neotropics. University of Chicago Press, Chicago.

Felsenstein J (1985) Phylogenies and the comparative method. *Am Nat* 125(1):1-15.

Gaston KJ, Blackburn TM (1996a) Conservation implications of geographic range size–body size relationships. *Conserv Biol* 10(2):638-646.

Gaston KJ, Blackburn TM (1996b) Range size–body size relationships: evidence of scale dependence. *Oikos* 75(3):479-485.

Glazier DS (1980) Ecological shifts and the evolution of geographically restricted species of North American Peromyscus (mice). *J Biogeogr* 7(1):63-83.

Grubb P (1993) Order Artiodactyla. In *Mammal Species of the World: a taxonomic and geographic reference*, eds Wilson DE, Reeder DM. Smithsonian Institution Press, Washington. Pp. 377-414

Hall ER (1981) The mammals of North America. John Wiley & Sons, New York.

Hartenberger J (1998) Description de la radiation des Rodentia (Mammalia) du Paléocène supérieur au Miocène; incidences phylogénétiques. *C R Acad Sci Paris Ser II* 326(6):439-444.

Harvey PH, Pagel MD (1991) The comparative method in evolutionary biology. Oxford University Press, Oxford.

Hernández Fernández M (2001) Bioclimatic discriminant capacity of terrestrial mammal faunas. *Glob Ecol Biogeogr* 10(2):189-204.

Hernández Fernández M, Alcalde G, DeMiguel D, García-Yelo B, Azanza B (2009) Functional groups in ruminants as environmental proxies. *J Vertebr Paleontol* 29(3):113.

Hernández Fernández M, Vrba ES (2005a) A complete estimate of the phylogenetic relationships in Ruminantia: a dated species-level supertree of the extant ruminants. *Biol Rev* 80(2):269-302.

Hernández Fernández M, Vrba ES (2005b) Macroevolutionary processes and biomic specialization: testing the resource-use hypothesis. *Evol Ecol* 19(3):199–219.

Hernández Fernández M, Vrba ES (2005c) Rapoport effect and biomic specialization in

African mammals: revisiting the climatic variability hypothesis. *J Biogeogr* 32(5):903-918.

Hernández Fernández M, Vrba ES (2006) Plio-Pleistocene climatic change in the Turkana Basin (East Africa): Evidence from large mammal faunas. *J Hum Evol* 50(6):595-626.

Huchon D, Madsen O, Sibbald MJ, Ament K, Stanhope MJ, Catzeflis F, de Jong WW, Douzery EJ (2002) Rodent phylogeny and a timescale for the evolution of Glires: evidence from an extensive taxon sampling using three nuclear genes. *Mol Biol Evol* 19(7):1053-65.

IUCN. 2008. *Digital distributions maps of the mammals of te world. Version 1.0.* Available at: http://www.iucnredlist.org/initiatives/mammals/description/download_gis_data.

Jackson JBC (1974) Biogeographic consequences of eurytopy and stenotopy among marine bivalves and their evolutionary significance. *Am Nat* 108(962):541-560.

Johnson WE, Eizirik E, Pecon-Slattery J, Murphy WJ, Antunes A, Teeling E, O'Brien SJ (2006) The late radiation of modern Felidae: a genetic assesement. *Science* 311(5757):73-77.

Kerbis JC, Wrangham RW, Carter ML, Hauser HD (1993) A contribution to tropical rain-forest taphonomy: retrieval and documentation of chimpanzee remains from Kibale Forest, Uganda. *J Hum Evol* 25(6):485-514.

Kingdon J (1997) The Kingdon field guide to African mammals. Academic Press, London.

Köhler M (1993) Skeleton and Habitat of recent and fossil Ruminants. Münchner Geowissenschaftliche Abhandlungen, München.

Lane A, Janis CM, Sepkoski JJ (2005) Estimating paleodiversities: a test of the taxic and phylogenetic methods. *Paleobiology* 31(1):21-34.

Liu FR, Miyamoto MM, Freire NP, Ong PQ, Tennant MR, Young TS, Gugel KF (2001) Molecular and Morphological Supertrees for Eutherian (Placental) Mammals. *Science* 291(5509):1786-1789.

Lyman RL (1994) Vertebrate Taphonomy. Cambridge University Press, Cambridge.

MacArthur RH (1972) Geographical ecology: patterns in the distribution of species. Harper and Row, New York.

Maddison W, Maddison D (2007) Mesquite: A modular system for evolutionary analysis, version 2.0 http://mesquiteproject.org.

Mayr E (1963) Animal species and evolution. Belknap Press, Cambridge.

Mitchell-Jones AJ, Amori G, Bogdanowic W, Krystufek B, Reijnders P (1999) The Atlas of European Mammals. T. & A.D. Poyser, London.

Moreno Bofarull A, Arias Royo A, Hernández Fernández M, Ortiz-Jaurequizar E, Morales J (2008) Influence of continental history on the ecological specialization and macroevolutionary processes in the mammalian assemblage of South America: differences between small and large mammals. *BMC Evol Biol* 8(97):1-18.

Norell MA (1992) Taxic origin and temporal diversity: the effect of phylogeny. In *Extintion and Phylogeny*, eds Novacek MJ, Wheeler QD. Columbia University Press, New York. Pp. 89-118

Norell MA (1993) Tree-based approaches to understanding history: comments on ranks, rules, and the quality of fossil record. *Am J Sci* 293(A):407-417.

Norell MA (1996) Ghost taxa, ancestors, and assumptions: a comment on Wagner. *Paleobiology* 22(3):453-455.

Norell MA, Novacek MJ (1992a) Congruence between superpositional and phylogenetic patterns: comparing cladistic patterns with fossil record. *Cladistics* 8(4):319-337.

Norell MA, Novacek MJ (1992b) The fossil record and evolution: comparing cladistic and paleontological evidence for vertebrate history. *Science* 255(5052):1690-1693.

O'Keefe FR, Sander PM (1999) Paleontological paradigms and inferences of phylogenetic pattern: a case study. *Paleobiology* 25(4):518-533.

Ortolani A (1999) Spots, stripes, tail tips and dark eyes: predicting the function of carnivore colour patterns using the comparative method. *Biol J Linn Soc* 67(4):433-476.

Ortolani A, Caro TM (1996) The adaptive significance of color patterns in carnivores: phylogenetic tests of classic hypotheses. In *Carnivore behaviour, ecology and evolution,* ed Gittleman JL. Cornell University Press, Ithaca, NY. Pp. 132-188

Paradis E (2006) Analysis of Phylogenetics and Evolution with R. Springer, New York.

Paradis E, Claude J (2002) Analysis of comparative data using generalized estimating equations. *J Theor Biol* 218(2):175-85.

Paul CRC (1982) The adequacy of the fossil record. In *The adequacy of the fossil record,* eds Donovan S, Paul CRC. John Wiley and Sons, Chichester. Pp. 75-117

Pol D, Norell MA (2006) Uncertainty in the age of fossils and the stratigraphic fit to phylogenies. *Syst Biol* 55(3):512-21.

Polonio I, López Martínez N (2000) Taphonomic analyses of the Somosaguas fossil site (Middle Miocene, Madrid). *Coloquios de Paleontología* (51):235-266.

Potts R, Behrensmeyer AK (1992) Late Cenozoic terrestrial ecosystems. In *Terrestrial Ecosystems Trough Time,* eds Behrensmeyer AK, Damuth JD, DiMichele WA, Sues HD, Potts R, Wing SL. The University of Chicago Press, Chicago. Pp. 419-541

Redford K, Eisenberg HJF (1992) Mammals of the Neotropics Vol. 2: The Southern Cone. University of Chicago Press, Chicago.

Ruta M, Benton MJ (2008) Calibrated diversity, tree topology and the mother of mass extinctions: the lesson of temnospondyls. *Palaeontology* 51(6):1261-1288.

Sidor CA, Hopson JA (1998) Ghost lineages and "mammalness": assessing the temporal pattern of caracter acquisition in the Synapsida. *Paleobiology* 24(2):254-273.

Smith AB (1994) Systematics and the fossil record. Backwell Scientific Publications, Oxford.

Smith JM, Savage RJG (1956) Some locomotory adaptations in mammals. *Zool J Linn Soc* 42(288):603-622.

Teeling EC, Springer MS, Madsen O, Bates P, O'Brien SJ, Murphy WJ (2005) A molecular phylogeny for bats iluminates biogeography and the fossil record. *Science* 307(5709):580-584.

Thompson K, Hodgson JG, Gaston KJ (1998) Abundance-range size relationships in the herbaceous flora of central England. *J Ecol* 86(3):439-448.

Vrba ES (1987) Ecology in relation to speciation rates: some case histories of Miocene-Recent mammal clades. *Evol Ecol* 1(4):283–300.

Vrba ES, Schaller GB (2000) Introducction. In *Antelopes, Deer, and Relatives: Fossil Record, Behavioral Ecology, Systematics and Conservation,* eds Vrba ES, Schaller GB. Yale University Press, New Haven. Pp. 1-8

Wagner PJ (1995) Stratigraphic tests of cladistic hypotheses. *Paleobiology* 21(2):153-178.

Wagner PJ (2000a) Phylogenetic analyses and the fossi record: tests and inferences, hypotheses and models. *Paleobiology* 26(sp4):341-371.

Wagner PJ (2000b) The quality of the fossil record and the accuracy of phylogenetic inferences about sampling and diversity. *Syst Biol* 49(1):65-86.

Walter H (1970) Vegetation and Clima. Eugen Ulmer, Stuttgart.

Weishampel DB (1996) Fossils, phylogeny, and discovery: a cladistic study of history of tree topologies and ghost lineages durations. *J Vertebr Paleontol* 16(2):191-197.

Wills MA (2002) The tree of life and the rock of ages: are we getting better at estimating phylogeny? *BioEssays* 24(3):203-207.

Worthy TH, Tennyson AJ, Archer M, Musser AM, Hand SJ, Jones C, Douglas BJ, McNamara JA, Beck RM (2006) Miocene mammal reveals a Mesozoic ghost lineage on insular New Zealand, southwest Pacific. *Proc Natl Acad Sci U S A* 103(51):19419-23.

Appendix 2.1

Calculations of the Ghost Lineages percentages for every branch of the supertree of Ruminantia. Age of nodes and their numeration were taken from Hernández Fernández & Vrba (2005a). Number of branches according to Appendix 2.2

Branch #	***Node Prior to Branch***	***Age of Previous Node***	***Node After Branch***	***Age of Node After Branch***	***Oldest Fossil***	***Age of Oldest Fossil***	***Time Interval Branch***	***Age of Lineage***	***Ghost Lineage Duration***	***Ghost Lineage %***
1	4	28.4					28.4 - 0.0	28.4	28.4	100.0
2	4	28.4					28.4 - 0.0	28.4	28.4	100.0
3	3	28.4	4	28.4			28.4 - 28.4	0	28.4	0.0
4	3	28.4					28.4 - 0.0	28.4	28.4	100.0
5	2	35.2	3	28.4			35.2 - 28.4	6.8	35.2	100.0
6	2	35.2			*Dorcatherium*	21.4	35.2 - 0.0	35.2	13.8	39.2
7	1	50	2	35.2	*Archaeotragulus*	40	50.0 - 35.2	14.8	10.0	67.6
8	7	17.8			*Praepalaeotragus*	17.8	17.8 - 0.0	17.8	0.0	0.0
9	7	17.8			*Bohlinia*	9.3	17.8 - 0.0	17.8	8.5	47.8
10	6	28.1	7	17.8	*Propalaeoryx/Sperrgebiotomeryx*	21	28.1 - 17.8	10.3	7.1	68.9
11	6	28.1			*Bedenomeryx milaloquensis*	25	28.1 - 0.0	28.1	3.1	11.0
12	5	33.2	6	28.1			33.2 - 28.1	5.1	33.2	100.0
13	14	0.8					0.8 - 0.0	0.8	0.8	100.0
14	14	0.8					0.8 - 0.0	0.8	0.8	100.0
15	13	1.3	14	0.8			1.3 - 0.8	0.5	1.3	100.0
16	13	1.3					1.3 - 0.0	1.3	1.3	100.0
17	12	1.9	13	1.3			1.9 - 1.3	0.6	1.9	100.0
18	12	1.9					1.9 - 0.0	1.9	1.9	100.0
19	11	3.5	12	1.9			3.5 - 1.9	1.6	3.5	100.0
20	11	3.5					3.5 - 0.0	3.5	3.5	100.0
21	10	6.4	11	3.5			6.4 - 3.5	2.9	6.4	100.0
22	10	6.4					6.4 - 0.0	6.4	6.4	100.0
23	9	29.5	10	6.4	*Dremotherium*	29.5	29.5 - 6.4	23.1	0.0	0.0
24	57	2.4					2.4 - 0.0	2.4	2.4	100.0
25	57	2.4					2.4 - 0.0	2.4	2.4	100.0
26	49	9	57	2.4			9.0 - 2.4	6.6	9.0	100.0
27	56	2.4					2.4 - 0.0	2.4	2.4	100.0
28	56	2.4					2.4 - 0.0	2.4	2.4	100.0
29	49	9	56	2.4			9.0 - 2.4	6.6	9.0	100.0
30	55	2.3					2.3 - 0.0	2.3	2.3	100.0
31	55	2.3					2.3 - 0.0	2.3	2.3	100.0
32	54	3.7	55	2.3			3.7 - 2.3	1.4	3.7	100.0
33	54	3.7					3.7 - 0.0	3.7	3.7	100.0
34	53	4.7	54	3.7			4.7 - 3.7	1	4.7	100.0
35	53	4.7					4.7 - 0.0	4.7	4.7	100.0

Branch #	Node Prior to Branch	Age of Previous Node	Node After Branch	Age of Node After Branch	Oldest Fossil	Age of Oldest Fossil	Time Interval Branch	Age of Lineage	Ghost Lineage Duration	Ghost Lineage %
36	52	6.1	53	4.7			6.1 - 4.7	1.4	6.1	100.0
37	52	6.1					6.1 - 0.0	6.1	6.1	100.0
38	52	6.1					6.1 - 0.0	6.1	6.1	100.0
39	49	9	52	6.1			9.0 - 6.1	2.9	8.9	100.0
40	51	4.7			*Odocoileus virginianus*	3.2	4.7 - 0.0	4.7	1.5	31.9
41	51	4.7			*Odocoileus hemionus*	0.9	4.7 - 0.0	4.7	3.8	80.9
42	49	9	51	4.7	*Odocoileus sp.*	4.8	9.0 - 4.7	4.3	4.2	97.7
43	50	2			*Ozotoceros*	2	2.0 - 0.0	2	0.0	0.0
44	50	2			*Antifer ensenadense*	2	2.0 - 0.0	2	0.0	0.0
45	49	9	50	2	*Eocoileus gentryorum y Bretzia pseudalces*	5	9.0 - 2.0	7	4.0	57.1
46	48	10.8	49	9			10.8 - 9.0	1.8	10.8	100.0
47	48	10.8			*Rangifer sp*	3	10.8 - 0.0	10.8	7.8	72.2
48	45	14.6	48	10.8			14.6 - 10.8	3.8	14.6	100.0
49	47	3.4			*Capreolus sussenbornensis*	3	3.4 - 0.0	3.4	0.4	11.8
50	47	3.4			*Caproelus caproelus*	0.5	3.4 - 0.0	3.4	2.9	85.3
51	46	11	47	3.4	*Procaproelus loczyi*	8.1	11.0 - 3.4	7.6	2.9	38.2
52	46	11			*Cervalces (Libralces) gallicus / Alcinae indet*	3	11.0 - 0.0	11	8.0	72.7
53	45	14.6	46	11			14.6 - 11.0	3.6	14.6	100.0
54	26	19.4	45	14.6			19.4 - 14.6	4.8	19.4	100.0
55	44	1.4					1.4 - 0.0	1.4	1.4	100.0
56	44	1.4					1.4 - 0.0	1.4	1.4	100.0
57	43	4.1	44	1.4			4.1 - 1.4	2.7	4.1	100.0
58	43	4.1			*M. muntjak*	0.5	4.1 - 0.0	4.1	3.6	87.8
59	43	4.1			*M. feae*	0.5	4.1 - 0.0	4.1	3.6	87.8
60	42	4.8	43	4.1			4.8 - 4.1	0.7	4.8	100.0
61	42	4.8					4.8 - 0.0	4.8	4.8	100.0
62	42	4.8					4.8 - 0.0	4.8	4.8	100.0
63	42	4.8					4.8 - 0.0	4.8	4.8	100.0
64	42	4.8			*M. rooselvetorum*	0.1	4.8 - 0.0	4.8	4.7	97.9
65	42	4.8			*M. reevesi*	1.3	4.8 - 0.0	4.8	3.5	72.9
66	42	4.8					4.8 - 0.0	4.8	4.8	100.0
67	41	14.7	42	4.8	*Muntiacus leilaoensis*	8	14.7 - 4.8	9.9	6.7	67.7
68	41	14.7			*Elaphodus cephalophus*	0.5	14.7 - 0.0	14.7	14.2	96.6
69	27	14.7	41	14.7			14.7 - 14.7	0	14.7	0.0
70	40	4.2					4.2 - 0.0	4.2	4.2	100.0

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71	40	4.2			*C. elaphus acoronatus*	0.8	4.2 - 0.0	4.2	3.4	81.0
72	39	4.2	40	4.2			4.2 - 4.2	0	0.8	0.0
73	39	4.2					4.2 - 0.0	4.2	4.2	100.0
74	35	5.6	39	4.2			5.6 - 4.2	1.4	5.6	100.0
75	38	2.7					2.7 - 0.0	2.7	2.7	100.0
76	38	2.7					2.7 - 0.0	2.7	2.7	100.0
77	38	2.7					2.7 - 0.0	2.7	2.7	100.0
78	35	5.6	38	2.7	*Rucervus sivalensis*	3.6	5.6 - 2.7	2.9	2.0	69.0
79	37	3.5			*Rusa unicolor*	1.3	3.5 - 0.0	3.5	2.2	62.9
80	37	3.5			*R. timorensis*	0.1	3.5 - 0.0	3.5	3.4	97.1
81	37	3.5					3.5 - 0.0	3.5	3.5	100.0
82	36	3.9	37	3.5			3.9 - 3.5	0.4	3.9	100.0
83	36	3.9					3.9 - 0.0	3.9	3.9	100.0
84	35	5.6	36	3.9			5.6 - 3.9	1.7	5.6	100.0
85	34	8.4	35	5.6			8.4 - 5.6	2.8	8.4	100.0
86	34	8.4			*Elaphurus bifurcatus / Arvernoceros ardei*	3	8.4 - 0.0	8.4	5.4	64.3
87	28	9.9	34	8.4			9.9 - 8.4	1.5	9.9	100.0
88	33	0.8			*D.c. mugharensis / Dama clactoniana*	0.78	0.8 - 0.0	0.8	0.0	2.5
89	33	0.8			*Dama dama tiberina*	0.78	0.8 - 0.0	0.8	0.0	2.5
90	29	7	33	0.8	*Megacerini-Eucladoceros o Pseudodama*	3	7.0 - 0.8	6.2	4.0	64.5
91	32	0.4					0.4 - 0.0	0.4	0.4	100.0
92	32	0.4					0.4 - 0.0	0.4	0.4	100.0
93	31	0.7	32	0.4			0.7 - 0.4	0.3	0.7	100.0
94	31	0.7					0.7 - 0.0	0.7	0.7	100.0
95	30	3	31	0.7			3.0 - 0.7	2.3	3.0	100.0
96	30	3			*Axis axis fossilis / Axis shansius*	3	3.0 - 0.0	3	0.0	0.0
97	29	7	30	3	*Axis sp./ Axis speciosus*	6.2	7.0 - 3.0	4	0.8	20.0
98	28	9.9	29	7	*Cervocerus*	7.7	9.9 - 7.0	2.9	2.2	75.9
99	27	14.7	28	9.9			14.7 - 9.9	4.8	14.7	100.0
100	26	19.4	27	14.7			19.4 - 14.7	4.7	19.4	100.0
101	25	19.7	26	19.4			19.7 - 19.4	0.3	19.7	100.0
102	25	19.7			*Hydropotes inermis / Hydropotes sp*	0.5	19.7 - 0.0	19.7	19.2	97.5
103	9	29.5	25	19.7			29.5 - 19.7	9.8	29.5	100.0
104	8	32	9	29.5	*Eumeryx*	32	32.0 - 29.5	2.5	0.0	0.0
105	79	2.8			*Tragelaphus gaudryi*	2.65	2.8 - 0.0	2.8	0.2	5.4

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106	79	2.8					2.8 - 0.0	2.8	2.8	100.0
107	73	10.5	79	2.8			10.5 - 2.8	7.7	10.5	100.0
108	78	6					6.0 - 0.0	6	6.0	100.0
109	78	6			*Tragelaphus gridei*	2.8	6.0 - 0.0	6	3.2	53.3
110	110	4.7					4.7 - 0.0	4.7	4.7	100.0
111	74	6.9	78	6			6.9 - 6.0	0.9	6.9	100.0
112	77	2.6					2.6 - 0.0	2.6	2.6	100.0
113	77	2.6					2.6 - 0.0	2.6	2.6	100.0
114	75	5.4	77	2.6			5.4 - 2.6	2.8	5.4	100.0
115	76	1.6					1.6 - 0.0	1.6	1.6	100.0
116	76	1.6					1.6 - 0.0	1.6	1.6	100.0
117	75	5.4	76	1.6	*Taurotragus nakuse*	3.9	5.4 - 1.6	3.8	1.5	39.5
118	74	6.9	75	5.4			6.9 - 5.4	1.5	6.9	100.0
119	73	10.5	74	6.9			10.5 - 6.9	3.6	10.5	100.0
120	73	18.3	73	10.5			18.3 - 10.5	7.8	18.3	100.0
121	72	3.9					3.9 - 0.0	3.9	3.9	100.0
122	72	3.9					3.9 - 0.0	3.9	3.9	100.0
123	70	3.9	72	3.9			3.9 - 3.9	0	3.9	0.0
124	71	3.2			*Bubalus teilandi*	0.5	3.2 - 0.0	3.2	2.7	84.4
125	71	3.2			*Bubalus palaeokerabau*	1	3.2 - 0.0	3.2	2.2	68.8
126	70	3.9	71	3.2			3.9 - 3.2	0.7	3.9	100.0
127	69	11.8	70	3.9			11.8 - 3.9	7.9	11.8	100.0
128	69	11.8			*Syncerus sp*	2.9	11.8 - 0.0	11.8	8.9	75.4
129	62	16.9	69	11.8			16.9 - 11.8	5.1	16.9	100.0
130	68	2.5			*Bos javanicus*	2	2.5 - 0.0	2.5	0.5	20.0
131	68	2.5					2.5 - 0.0	2.5	2.5	100.0
132	63	7	68	2.5	*Leptobos falconeri*	3.1	7.0 - 2.5	4.5	3.9	86.7
133	67	0.6			*Bos primigenius*	0.5	0.6 - 0.0	0.6	0.1	16.7
134	67	0.6					0.6 - 0.0	0.6	0.6	100.0
135	64	5.8	67	0.6	*Bos acutifrons*	3.9	5.8 - 0.6	5.2	1.9	36.5
136	66	1.1					1.1 - 0.0	1.1	1.1	100.0
137	66	1.1			*Bison latifrons*	0.5	1.1 - 0.0	1.1	0.6	54.5
138	65	3.4	66	1.1	*Bison tamanensis*	2.1	3.4 - 1.1	2.3	1.3	56.5
139	65	3.4			*Bos grunniers*	0.9	3.4 - 0.0	3.4	2.5	73.5
140	64	5.8	65	3.4			5.8 - 3.4	2.4	5.8	100.0

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141	63	7	64	5.8			7.0 - 5.8	1.2	7.0	100.0
142	62	16.9	63	7			16.9 - 7.0	9.9	16.9	100.0
143	61	16.9	62	16.9			16.9 - 16.9	0	16.9	0.0
144	61	16.9					16.9 - 0.0	16.9	16.9	100.0
145	60	18.3	61	16.9			18.3 - 16.9	1.4	18.3	100.0
146	58	20.5	60	18.3			20.5 - 18.3	2.2	20.5	100.0
147	59	10.4			*Tetracerus daviesi*	3.6	10.4 - 0.0	10.4	6.8	65.4
148	59	10.4			*Boselaphus sp*	3.6	10.4 - 0.0	10.4	6.8	65.4
149	58	20.5	59	10.4	*Boselaphini indet*	17.6	20.5 - 10.4	10.1	2.9	28.7
150	15	25.4	58	20.5	*Eotragus sansanensis*		25.4 - 20.5	4.9	25.4	100.0
151	159	2.7					2.7 - 0.0	2.7	2.7	100.0
152	159	2.7					2.7 - 0.0	2.7	2.7	100.0
153	158	4.3	159	2.7			4.3 - 2.7	1.6	4.3	100.0
154	158	4.3			*Capricornis sumatrensis*	0.5	4.3 - 0.0	4.3	3.8	88.4
155	155	10.3	158	4.3			10.3 - 4.3	6	10.3	100.0
156	157	0.1			*Naemorhedus goral*	0.1	0.1 - 0.0	0.1	0.0	0.0
157	157	0.1			*Naemorhedus caudatus*	0.1	0.1 - 0.0	0.1	0.0	0.0
158	156	3.3	157	0.1			3.3 - 0.1	3.2	3.3	100.0
159	156	3.3					3.3 - 0.0	3.3	3.3	100.0
160	155	10.3	156	3.3			10.3 - 3.3	7	10.3	100.0
161	139	14.5	155	10.3	*Rupicaprini indet*	12	14.5 - 10.3	4.2	2.5	59.5
162	154	3.8			*Rupicapra rupicapra*	0.1	3.8 - 0.0	3.8	3.7	97.4
163	154	3.8			*Rupicapra pyrenaica*	0.2	3.8 - 0.0	3.8	3.6	94.7
164	153	8.8	154	3.8			8.8 - 3.8	5	8.8	100.0
165	153	8.8			*Neotragoceros*	5.6	8.8 - 0.0	8.8	3.2	36.4
166	139	14.5	153	8.8	*Rupicaprini indet*	12	14.5 - 8.8	5.7	2.5	43.9
167	152	0.2			*Ovis dalli*	0.2	0.2 - 0.0	0.2	0.0	0.0
168	152	0.2			*Ovis canadiensis*	0.2	0.2 - 0.0	0.2	0.0	0.0
169	151	0.5	152	0.2			0.5 - 0.2	0.3	0.5	100.0
170	151	0.5			*Ovis nivicola*	0.5	0.5 - 0.0	0.5	0.0	0.0
171	148	6.8	151	0.5			6.8 - 0.5	6.3	6.8	100.0
172	150	0.8			*Ovis vignei*	0.1	0.8 - 0.0	0.8	0.7	87.5
173	150	0.8			*Ovis aries*	0.1	0.8 - 0.0	0.8	0.7	87.5
174	149	2.7	150	0.8			2.7 - 0.8	1.9	2.7	100.0
175	149	2.7			*Ovis amon*	0.5	2.7 - 0.0	2.7	2.2	81.5

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176	148	6.8	149	2.7			6.8 - 2.7	4.1	6.8	100.0
177	140	11.3	148	6.8	*Pseudotragus parvidens*	8.1	11.3 - 6.8	4.5	3.2	71.1
178	147	2.4					2.4 - 0.0	2.4	2.4	100.0
179	147	2.4					2.4 - 0.0	2.4	2.4	100.0
180	142	11.1	147	2.4			11.1 - 2.4	8.7	11.1	100.0
181	146	1.8			*Hemitragus jayakari*	0.5	1.8 - 0.0	1.8	1.3	72.2
182	146	1.8					1.8 - 0.0	1.8	1.8	100.0
183	145	2.8	146	1.8	*Hemitragus orientalis*	2.8	2.8 - 1.8	1	0.0	0.0
184	145	2.8			*Hemitragus bonali*	0.5	2.8 - 0.0	2.8	2.3	82.1
185	143	8	145	2.8	*Tossunoria*	7	8.0 - 2.8	5.2	1.0	19.2
186	144	5.1					5.1 - 0.0	5.1	5.1	100.0
187	144	5.1					5.1 - 0.0	5.1	5.1	100.0
188	144	5.1					5.1 - 0.0	5.1	5.1	100.0
189	144	5.1			*Capra ibex*	1.3	5.1 - 0.0	5.1	3.8	74.5
190	144	5.1					5.1 - 0.0	5.1	5.1	100.0
191	144	5.1					5.1 - 0.0	5.1	5.1	100.0
192	144	5.1					5.1 - 0.0	5.1	5.1	100.0
193	144	5.1					5.1 - 0.0	5.1	5.1	100.0
194	144	5.1					5.1 - 0.0	5.1	5.1	100.0
195	143	8	144	5.1	*Norbertia hellenica*	5.4	8.0 - 5.1	2.9	2.6	89.7
196	142	11.1	143	8			11.1 - 8.0	3.1	11.1	100.0
197	141	11.1	142	11.1			11.1 - 11.1	0	11.1	0.0
198	141	11.1			*Ammotragus lervia*	0.1	11.1 - 0.0	11.1	11.0	99.1
199	140	11.3	141	11.1			11.3 - 11.1	0.2	11.3	100.0
200	139	14.5	140	11.3	*Pachytragus solignaci*	13.5	14.5 - 11.3	3.2	1.0	31.3
201	139	14.5			*Mesenbriacerus*	9.2	14.5 - 0.0	14.5	5.3	36.6
202	139	14.5			Palaeoryx	8.1	14.5 - 0.0	14.5	6.4	44.1
203	24	17.8	139	14.5			17.8 - 14.5	3.3	17.8	100.0
204	24	17.8			*Qurlignoris*	8.2	17.8 - 0.0	17.8	9.6	53.9
205	22	20.2	24	17.8			20.2 - 17.8	2.4	20.2	100.0
206	138	2.5			*Hippotragus leucophaeus*	0.6	2.5 - 0.0	2.5	1.9	76.0
207	138	2.5			*Hippotragus equinus*	2.5	2.5 - 0.0	2.5	0.0	0.0
208	137	8.8	138	2.5			8.8 - 2.5	6.3	8.8	100.0
209	137	8.8			*Hippotragus niger*	1.6	8.8 - 0.0	8.8	7.2	81.8
210	134	11	137	8.8			11.0 - 8.8	2.2	11.0	100.0

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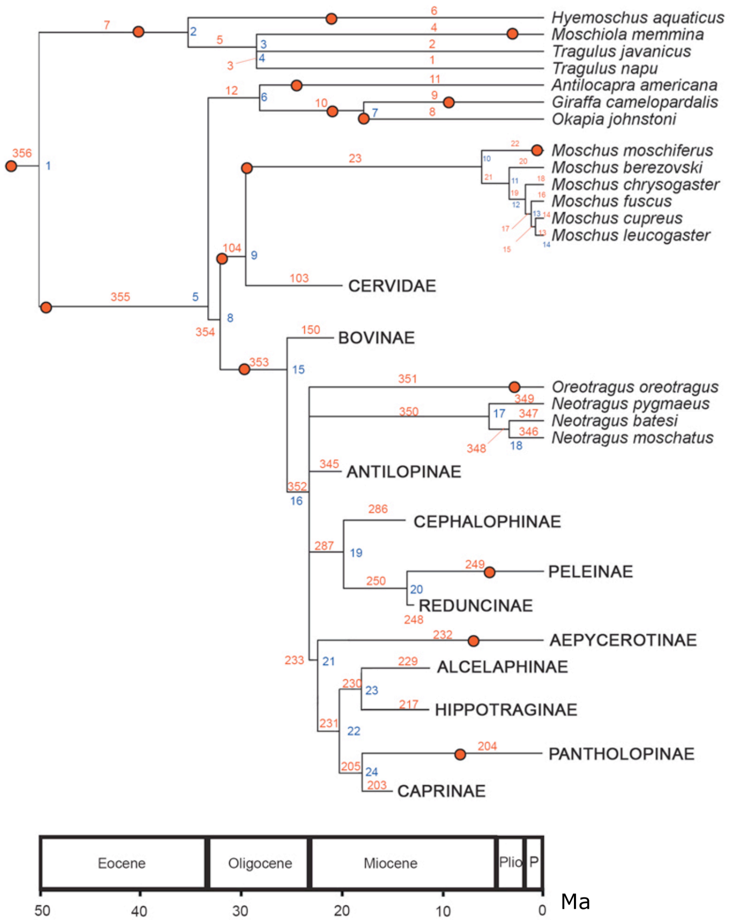
211	136	2.5					2.5 - 0.0	2.5	2.5	100.0
212	136	2.5			*Oryx gazella*	2.5	2.5 - 0.0	2.5	0.0	0.0
213	136	2.5					2.5 - 0.0	2.5	2.5	100.0
214	135	5	136	2.5	*Oryx sp*	2.6	5.0 - 2.5	2.5	2.4	96.0
215	135	5					5.0 - 0.0	5	5.0	100.0
216	134	11	135	5	*"Predamalis" sp Damalacra*	7	11.0 - 5.0	6	4.0	66.7
217	23	17.9	134	11			17.9 - 11.0	6.9	17.9	100.0
218	133	6.2			*Damaliscus pygargus*	2	6.2 - 0.0	6.2	4.2	67.7
219	133	6.2			*Damaliscus lunatus*	0.6	6.2 - 0.0	6.2	5.6	90.3
220	132	7.7	133	6.2			7.7 - 6.2	1.5	7.7	100.0
221	132	7.7			*Damalacra neanica*	5.3	7.7 - 0.0	7.7	2.4	31.2
222	129	10.8	132	7.7			10.8 - 7.7	3.1	10.8	100.0
223	131	2.5			*Connochaetes taurinus*	2.5	2.5 - 0.0	2.5	0.0	0.0
224	131	2.5			*Connochaetes africanus*	1.6	2.5 - 0.0	2.5	0.9	36.0
225	129	10.8	131	2.5	*Connochaetes gentry*	2.7	10.8 - 2.5	8.3	8.1	97.6
226	130	3.1			*Sigmoceros lichensteinii*	0.5	3.1 - 0.0	3.1	2.6	83.9
227	130	3.1			*Alcelaphus buselaphus*	0.6	3.1 - 0.0	3.1	2.5	80.6
228	129	10.8	130	3.1			10.8 - 3.1	7.7	10.8	100.0
229	23	17.9	129	10.8			17.9 - 10.8	7.1	17.9	100.0
230	22	20.2	23	17.9			20.2 - 17.9	2.3	20.2	100.0
231	21	22.3	22	20.2			22.3 - 20.2	2.1	22.3	100.0
232	21	22.3			*Aepyceros proemelampus*	7	22.3 - 0.0	22.3	15.3	68.6
233	16	23.2	21	22.3			23.2 - 22.3	0.9	23.2	100.0
234	128	1.6					1.6 - 0.0	1.6	1.6	100.0
235	128	1.6			*Kobus leche*	0.7	1.6 - 0.0	1.6	0.9	56.3
236	127	3.5	128	1.6			3.5 - 1.6	1.9	3.5	100.0
237	127	3.5			*Kobus ellipiprymnus*	2.33	3.5 - 0.0	3.5	1.2	33.4
238	125	3.5	127	3.5			3.5 - 3.5	0	3.5	0.0
239	126	1.2					1.2 - 0.0	1.2	1.2	100.0
240	126	1.2			*Kobus kob*	1.2	1.2 - 0.0	1.2	0.0	0.0
241	125	3.5	126	1.2			3.5 - 1.2	2.3	3.5	100.0
242	122	12.7	125	3.5	*Kobus presigmoidalis*	7	12.7 - 3.5	9.2	5.7	62.0
243	124	3			*Redunca redunca*	1.7	3.0 - 0.0	3	1.3	43.3
244	124	3			*Redunca arundinum*	1.8	3.0 - 0.0	3	1.2	40.0
245	123	6.7	124	3	*Redunca darti*	2.8	6.7 - 3.0	3.7	3.9	100.0

Branch #	Node Prior to Branch	Age of Previous Node	Node After Branch	Age of Node After Branch	Oldest Fossil	Age of Oldest Fossil	Time Interval Branch	Age of Lineage	Ghost Lineage Duration	Ghost Lineage %
246	123	6.7			*Redunca fulvorufula*	2.5	6.7 - 0.0	6.7	4.2	62.7
247	122	12.7	123	6.7			12.7 - 6.7	6	12.7	100.0
248	20	13.5	122	12.7			13.5 - 12.7	0.8	13.5	100.0
249	20	13.5			*Pelea sp*	5.3	13.5 - 0.0	13.5	8.2	60.7
250	19	19.8	20	13.5			19.8 - 13.5	6.3	19.8	100.0
251	121	5.1			*Philantomba monticola*	2.5	5.1 - 0.0	5.1	2.6	51.0
252	121	5.1					5.1 - 0.0	5.1	5.1	100.0
253	106	13.5	121	5.1			13.5 - 5.1	8.4	13.5	100.0
254	120	4.6					4.6 - 0.0	4.6	4.6	100.0
255	120	4.6					4.6 - 0.0	4.6	4.6	100.0
256	116	10	120	4.6			10.0 - 4.6	5.4	10.0	100.0
257	119	4.5					4.5 - 0.0	4.5	4.5	100.0
258	119	4.5					4.5 - 0.0	4.5	4.5	100.0
259	118	7.5	119	4.5			7.5 - 4.5	3	7.5	100.0
260	118	7.5					7.5 - 0.0	7.5	7.5	100.0
261	117	8.5	118	7.5			8.5 - 7.5	1	8.5	100.0
262	117	8.5					8.5 - 0.0	8.5	8.5	100.0
263	116	10	117	8.5			10.0 - 8.5	1.5	10.0	100.0
264	107	10.8	116	10			10.8 - 10.0	0.8	10.8	100.0
265	115	1.6					1.6 - 0.0	1.6	1.6	100.0
266	115	1.6					1.6 - 0.0	1.6	1.6	100.0
267	113	3.9	115	1.6			3.9 - 1.6	2.3	3.9	100.0
268	114	0.8					0.8 - 0.0	0.8	0.8	100.0
269	114	0.8					0.8 - 0.0	0.8	0.8	100.0
270	113	3.9	114	0.8			3.9 - 0.8	3.1	3.9	100.0
271	112	7.7	113	3.9			7.7 - 3.9	3.8	7.7	100.0
272	112	7.7					7.7 - 0.0	7.7	7.7	100.0
273	109	9.8	112	7.7			9.8 - 7.7	2.1	9.8	100.0
274	111	3.2					3.2 - 0.0	3.2	3.2	100.0
275	111	3.2					3.2 - 0.0	3.2	3.2	100.0
276	110	4.7	111	3.2			4.7 - 3.2	1.5	4.7	100.0
277	110	4.7					4.7 - 0.0	4.7	4.7	100.0
278	109	9.8	110	4.7			9.8 - 4.7	5.1	9.8	100.0
279	109	9.8					9.8 - 0.0	9.8	9.8	100.0
280	108	10.2	109	9.8			10.2 - 9.8	0.4	10.2	100.0

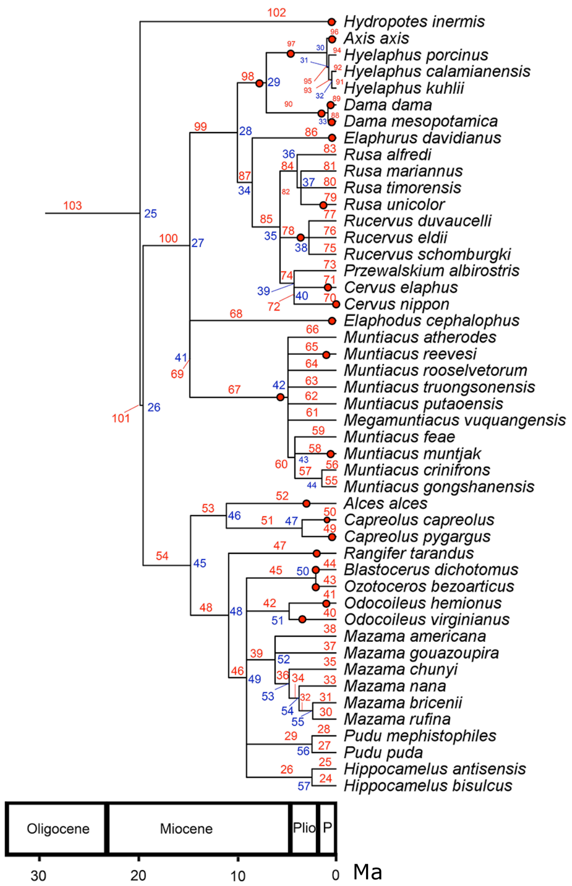
Branch #	Node Prior to Branch	Age of Previous Node	Node After Branch	Age of Node After Branch	Oldest Fossil	Age of Oldest Fossil	Time Interval Branch	Age of Lineage	Ghost Lineage Duration	Ghost Lineage %
281	108	10.2					10.2 - 0.0	10.2	10.2	100.0
282	107	10.8	108	10.2			10.8 - 10.2	0.6	10.8	100.0
283	106	13.5	107	10.8			13.5 - 10.8	2.7	13.5	100.0
284	105	13.5	106	13.5			13.5 - 13.5	0	13.5	0.0
285	105	13.5			*Sylvicapra gimnia*	0.1	13.5 - 0.0	13.5	13.4	99.3
286	19	19.8	105	13.5			19.8 - 13.5	6.3	19.8	100.0
287	16	23.2	19	19.8			23.2 - 19.8	3.4	23.2	100.0
288	104	7.9					7.9 - 0.0	7.9	7.9	100.0
289	104	7.9			*Raphicerus melanotis*	0.6	7.9 - 0.0	7.9	7.3	92.4
290	104	7.9			*Raphicerus campestris*	1.8	7.9 - 0.0	7.9	6.1	77.2
291	103	8.9	104	7.9			8.9 - 7.9	1	8.9	100.0
292	103	8.9					8.9 - 0.0	8.9	8.9	100.0
293	80	19.7	103	8.9			19.7 - 8.9	10.8	19.7	100.0
294	102	2.7					2.7 - 0.0	2.7	2.7	100.0
295	102	2.7					2.7 - 0.0	2.7	2.7	100.0
296	100	5.3	102	2.7			5.3 - 2.7	2.6	5.3	100.0
297	101	1.4					1.4 - 0.0	1.4	1.4	100.0
298	101	1.4					1.4 - 0.0	1.4	1.4	100.0
299	100	5.3	101	1.4			5.3 - 1.4	3.9	5.3	100.0
300	80	19.7	100	5.3	*Madoqua sp*	7	19.7 - 5.3	14.4	12.7	88.2
301	99	3.9					3.9 - 0.0	3.9	3.9	100.0
302	99	3.9					3.9 - 0.0	3.9	3.9	100.0
303	98	6.2	99	3.9			6.2 - 3.9	2.3	6.2	100.0
304	98	6.2					6.2 - 0.0	6.2	6.2	100.0
305	80	19.7	98	6.2	*Gazella schlosseri*	9.3	19.7 - 6.2	13.5	10.4	77.0
306	97	1.5			*Gazella tingitana*	0.1	1.5 - 0.0	1.5	1.4	93.3
307	97	1.5					1.5 - 0.0	1.5	1.5	100.0
308	97	1.5			*Gazella pomeli*	0.8	1.5 - 0.0	1.5	0.7	46.7
309	91	5.7	97	1.5	*Gazella borbonica*	3.8	5.7 - 1.5	4.2	1.9	45.2
310	96	0.8			*Gazella dorcas*	0.1	0.8 - 0.0	0.8	0.7	87.5
311	96	0.8					0.8 - 0.0	0.8	0.8	100.0
312	93	2.3	96	0.8			2.3 - 0.8	1.5	2.3	100.0
313	95	0			*Gazella gazella*	0.01	0.0 - 0.0	0	0.0	0.0
314	95	0			*Gazella arabica*	0.01	0.0 - 0.0	0	0.0	0.0
315	94	1.4	95	0			1.4 - 0.0	1.4	1.4	100.0

Branch #	***Node Prior to Branch***	***Age of Previous Node***	***Node After Branch***	***Age of Node After Branch***	***Oldest Fossil***	***Age of Oldest Fossil***	***Time Interval Branch***	***Age of Lineage***	***Ghost Lineage Duration***	***Ghost Lineage %***
316	94	1.4					1.4 - 0.0	1.4	1.4	100.0
317	93	2.3	94	1.4			2.3 - 1.4	0.9	2.3	100.0
318	93	2.3					2.3 - 0.0	2.3	2.3	100.0
319	92	2.5	93	2.3			2.5 - 2.3	0.2	2.5	100.0
320	92	2.5					2.5 - 0.0	2.5	2.5	100.0
321	91	5.7	92	2.5	*Gazella borbonica*	3.8	5.7 - 2.5	3.2	1.9	59.4
322	85	10.6	91	5.7	*Gazella deperdita*	8.6	10.6 - 5.7	4.9	2.0	40.8
323	90	3.1					3.1 - 0.0	3.1	3.1	100.0
324	90	3.1					3.1 - 0.0	3.1	3.1	100.0
325	89	4.4	90	3.1			4.4 - 3.1	1.3	4.4	100.0
326	89	4.4			*Nanger granti*	0.5	4.4 - 0.0	4.4	3.9	88.6
327	86	10.6	89	4.4	*Gazella sp*	5.3	10.6 - 4.4	6.2	5.3	85.5
328	88	1.9					1.9 - 0.0	1.9	1.9	100.0
329	88	1.9					1.9 - 0.0	1.9	1.9	100.0
330	87	4.8	88	1.9			4.8 - 1.9	2.9	4.8	100.0
331	87	4.8			*Eudorcas rufina*	0.1	4.8 - 0.0	4.8	4.7	97.9
332	86	10.6	87	4.8	*Gazella sp*	7	10.6 - 4.8	5.8	3.6	62.1
333	85	10.6	86	10.6			10.6 - 10.6	0	10.6	0.0
334	84	18	85	10.6	*Gazella nigerensis*	18	18.0 - 10.6	7.4	0.0	0.0
335	84	18			*Prostrepsiceros*	10.9	18.0 - 0.0	18	7.1	39.4
336	82	18	84	18			18.0 - 18.0	0	18.0	0.0
337	83	4.2			*Antidorcas recki*	2.6	4.2 - 0.0	4.2	1.6	38.1
338	83	4.2					4.2 - 0.0	4.2	4.2	100.0
339	82	18	83	4.2			18.0 - 4.2	13.8	18.0	100.0
340	82	18					18.0 - 0.0	18	18.0	100.0
341	81	18	82	18			18.0 - 18.0	0	18.0	0.0
342	81	18			*Saiga tatarica*	0.5	18.0 - 0.0	18	17.5	97.2
343	80	19.7	81	18			19.7 - 18.0	1.7	19.7	100.0
344	80	19.7			*Ourebia ourebi*	1.6	19.7 - 0.0	19.7	18.1	91.9
345	16	23.2	80	19.7			23.2 - 19.7	3.5	23.2	100.0
346	18	3.4					3.4 - 0.0	3.4	3.4	100.0
347	18	3.4					3.4 - 0.0	3.4	3.4	100.0
348	17	5.4	18	3.4			5.4 - 3.4	2	5.4	100.0
349	17	5.4					5.4 - 0.0	5.4	5.4	100.0
350	16	23.2	17	5.4			23.2 - 5.4	17.8	23.2	100.0
351	16	23.2			*Oreotragus major*	3.2	23.2 - 0.0	23.2	20.0	86.2
352	15	25.4	16	23.2			25.4 - 23.2	2.2	25.4	100.0
353	8	32	15	25.4	*Hanhaicerus qii*	29	32.0 - 25.4	6.6	3.0	45.5
354	5	33.2	8	32			33.2 - 32.0	1.2	33.2	100.0
355	1	50	5	33.2	*Archaeomeryx*	49	50.0 - 33.2	16.8	1.0	6.0
356			1	50	*Pseudamphimeryx*	53.3	0.0 - 50.0	-50	-53.3	100.0

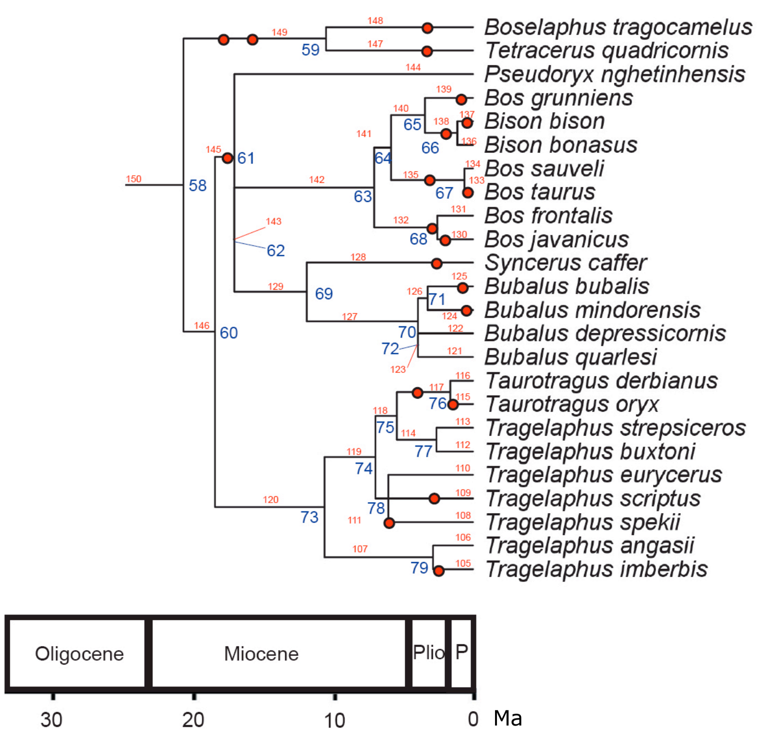
Appendix 2.2



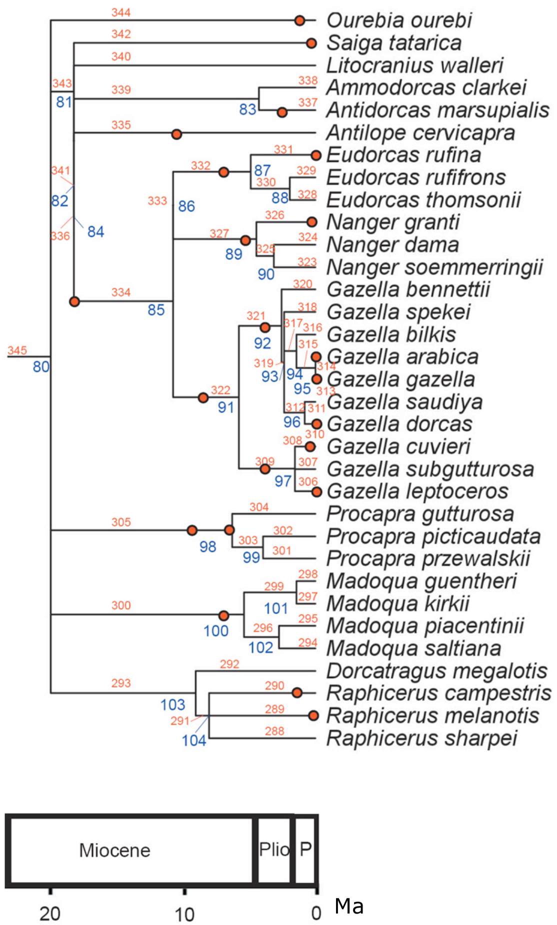
Appendix 2.2.A. Phylogeny of Ruminantia (Hernández Fernández & Vrba 2005a) showing the main subfamilies and Tragulidae, Giraffidae and Moschidae. Red spots, oldest fossil record for each branch. Numbers of nodes and branches match those in Appendix I. Red Numbers, number of branches. Blue Numbers, numbers of nodes.



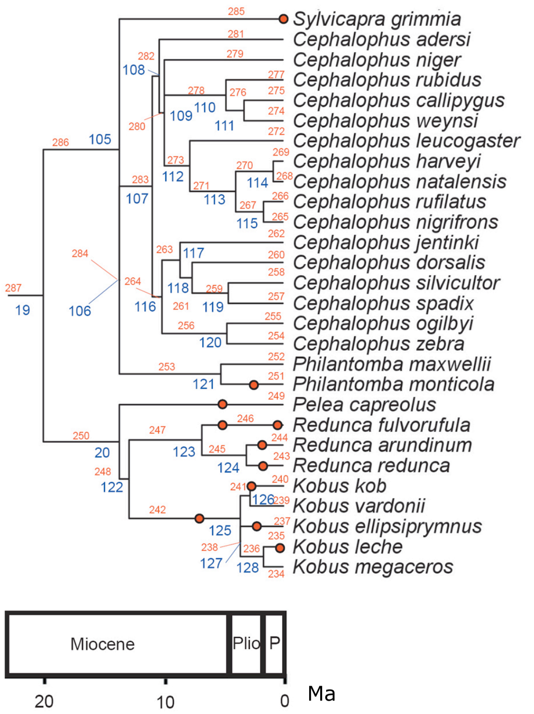
Appendix 2.2.B. Phylogeny of Cervidae (Hernández Fernández & Vrba 2005a). Legend as in Appendix 2.A.



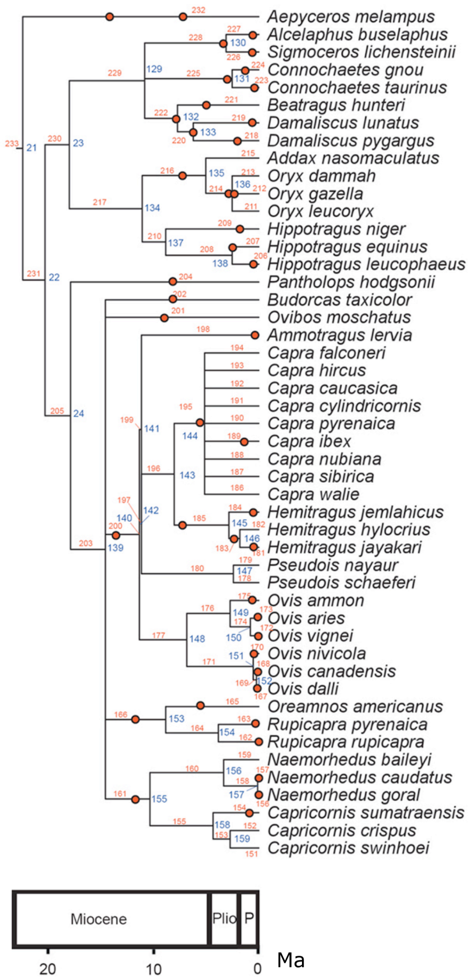
Appendix 2.2.C. Phylogeny of Bovinae (Hernández Fernández & Vrba 2005a). Legend as in Appendix 2.A.



Appendix 2.2.D. Phylogeny of Antilopinae (Hernández Fernández & Vrba 2005a). Legend as in Appendix Appendix 2.A.



Appendix 2.2.E. Phylogeny of Cephalophinae, Peleinae and Reduncinae (Hernández Fernández & Vrba 2005a). Legend as in Appendix 2.A.



Appendix 2.2.F. Phylogeny of Aepycerotinae, Alcelophinae, Hippotraginae, Pantholopinae and Caprinae (Hernández Fernández & Vrba 2005a). Legend as in Appendix 2.A.

3

Global phylogenetic data show major shifts in diversification of ruminant lineages associated to Miocene climatic episodes

> “*The prediction […] is that most lineage turnover has occurred in pulses, varying from minute to massive in scale, across disparate groups of organisms and in predictable temporal association with changes in the physical environment.*”
>
> *- Elisabeth Vrba -*

Abstract

Diversification processes that led to the present-day diversity can be recovered from phylogenetic trees of living species. We here draw on the information of branching times included in the phylogeny of the living ruminants and use a maximum-likelihood method (Likelihood Analysis of Speciation/Extinction Rates, LASER) to assess substantial changes in the rate of diversification of the group. Our results suggest that a first peak in the net diversification rate took place at the beginning of the Miocene, which involved the cladogenesis of both deer and bovids and is related to a basal branching event in Giraffidae as well. During the Middle Miocene, ca. 15 Mya, there was a second mayor diversification rate shift in bovid and deer lineages, which was concomitant with the onset of a global cooling. Later on, we found evidence supporting an increase in diversification of bovid lineages ca. 11 Ma and a peak in the rate of diversification in cervids around 5 Mya involving radiations of lineages in Eurasia and the New World. All these diversification events appear to be in synchrony with major environmental and faunal episodes as evidenced by fossil and paleontological data.

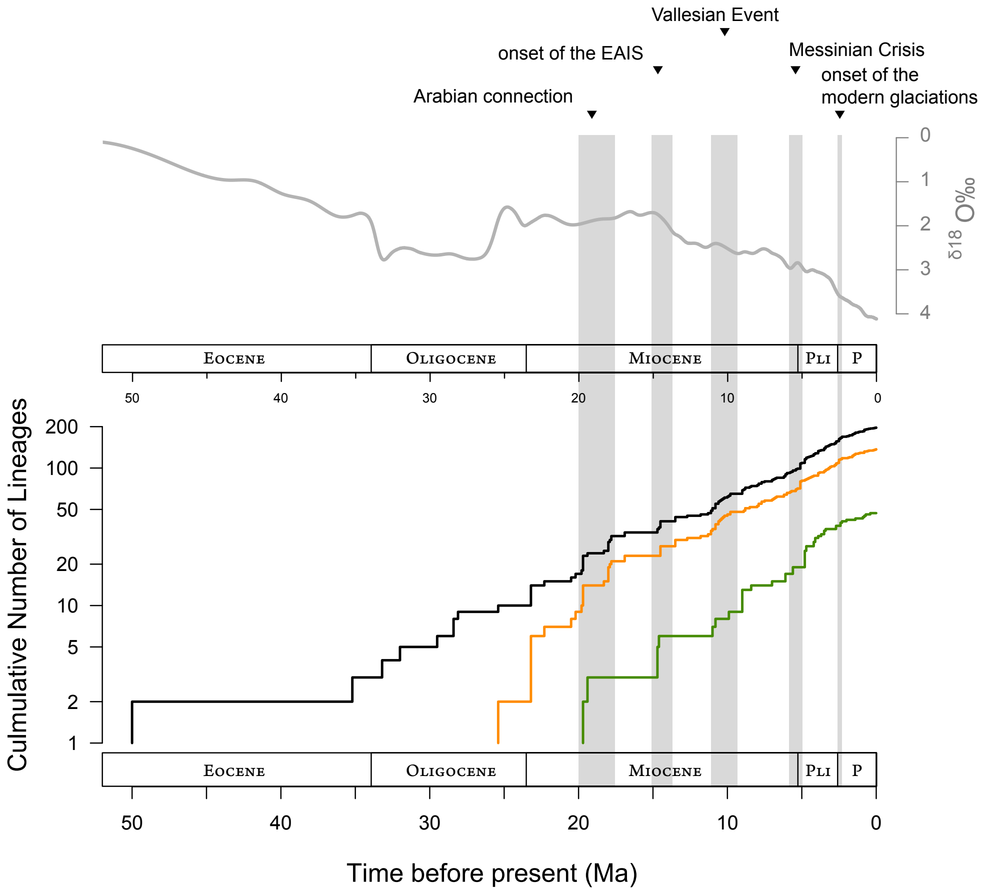


Figure 3.1. Global temperature curve during the last 50 Ma after Zachos et al. (2001) and cumulative number of lineages for ruminants (black), bovids (orange) and cervids (green) plotted against time following the phylogenetic hypothesis of Hernández Fernández and Vrba (2005). Major climatic and faunal events of the last 30 My are shown. EAIS, East Antarctic Ice Sheet.

Introduction

The evolution of ruminants and the signal of turnover pulses in their fossil record has intrigued paleontologists for decades (Vrba, 1984, 1995b; Hassanin and Douzery, 1999; Gentry, 2000). In these pulses, first appearances of new taxa with distinct morphologies are clustered in time and concomitant with last appearances of already existing taxa. The result is a major renovation of the faunas in a relatively short period of time, a phenomenon named *faunal turnover*. These events may be triggered by local cladogenesis and also by allopatric speciation and changes in geographic distribution of taxa elsewhere. Such changes in the biotas has been proposed to be a consequence of physical change spurred in turn by climatic shifting. Following this idea, events of speciation and extinction require initiation by climatic forcing, which causes these events to be bunched in time (Vrba, 1992, 1995a, b, 1999). However, pulses in the fossil record must be interpreted with caution. Physical changes in habitats may in turn influence taphonomic biases by changing their potential of preservation and the abundance, rarity and distribution of species (Vrba, 1995b; Cantalapiedra et al., in press). In many cases the fossil record is sparsely sampled, showing no peaks of species origination, although high morphological diversity among the recovered specimens may point to cladogenesis pulses. In this situation other evidences of cladogenesis should be found and tested including similar fossil patterns of simultaneous cladogenesis in other groups (following the turnover pulse hypothesis; Vrba, 1993), the identification of tectonic shifting and local and global climatic change that could have driven such pulse and, finally, the patterns observed in phylogenetic trees (Vrba, 1995b). In addition to depict evolutionary relationships among species, dated phylogenies contain valuable information on the processes that have shaped the evolutionary history of a group and have given rise to those species (Figure 3.1). These processes are responsible for phylogenetic trees being far from balanced and presenting odd distributions of splitting times (see Figure 3.1; Harvey et al., 1994; Mooers and Heard, 1997). Interestingly, these properties are quantifiable (Mooers and Heard, 2002) and recent methods allow for statistical analyses of such patterns (Rabosky, 2006; Rabosky and Lovette, 2008). Additionally, these methods allow to fit and compare different models of diversification, which makes them suitable for assessing macroevolutionary patterns from highly resolved phylogenies such as the one of the ruminants. Since this suborder (Ruminantia) is found in five continents and its history spans through 50 million years (Hernández Fernández and Vrba, 2005), it is an ideal group for examining these issues (Mercer and Roth, 2003). We investigate here whether significant changes in net diversification rates took place along the evolutionary history of ruminants and the potential connections of such changes with past physical changes and faunal events.

Material and Methods

The supertree of the ruminants published by Hernández Fernández and Vrba (Hernández Fernández and Vrba, 2005) is the most complete phylogeny of living ruminants publised to this date. All its nodes are dated, 80% of them being calibrated with fossil information. This particular feature makes it a reliable phylogenetic tree for analyses based on the distribution of splitting times as the one used here. This tree, however, contains some non-dichotomous nodes in the form of soft polytomies that may bias this kind of approach. We dealt with this by generating a distribution of 1000 fully resolved trees following Kuhn et al. (2011), which allows to incorporate phylogenetic uncertainty into consideration. The ages of the nodes resulting from the resolved polytomies and those originally estimated using a pure birth model (20%, see Hernández Fernández and Vrba, 2005) were estimated using a Birth-Death tree prior and estimated birth and death rates. In this way all the estimated branching times follow the same model. The identification of temporal shifts in diversification rates was carried out using LASER (Likelihood Analysis of Speciation/Extinction rates ; Rabosky, 2006). LASER compares different models of diversification, both with a constant rate of diversification (λ) over time (rate-constant models) and with a λ that may change up to three times in temporal breakpoints (rate-variable model). The rate-constant models include a Pure Birth model (PB) and a Birth-Death model (BD). The rate-variable models analyzed here include a model where the rate of diversification changes once and takes two different values (Yule 2 model), a model where the rate of diversification changes two times and takes three different values (Yule 3 model) and a model where the rate of diversification changes three times and takes four different values (Yule 4 model). A model with different λ will find time points for major shifts in λ, but this does not mean that there are no other minor changes in λ. Since we work with 1000 different fully resolved trees, slight differences between trees may cause that the major changes in diversification are in different time points. In each case the model identifies these different points for the shifts and estimates different values for λ. All the models were compared using the ratio of their Akaike Information Criteria (AIC), which measures the goodness of fit of a statistical model while penalizes the number of parameters (the complexity) of the model. The best model gets the lower AIC score, and the fit of a model is significantly better than other when the different in their AIC scores is greater than two units (Burnham and Anderson, 2002). We run LASER over the 1000 fully resolved ruminant trees and the corresponding subtrees of the two most specious families, Cervidae and Bovidae, in order to pick up particular signals within these subclades.

Results

Accordingly to the AIC scores, the Yule 4 model, in which the rate of diversification changes three times through the tree, is the best model for explaining the diversification of ruminants in 896 out of 1000 trees (89.6%) (Table 3.1). In the other 104 cases the best-fit model was the Yule 3 model (ΔAIC=1.2). For bovids, a Yule 4 model gets better support in 615 cases (61.5%; ΔAIC=0.9 for the Yule3; Table 3.2), and in the case of cervids this model provides the best fit in all the trees (100%; ΔAIC=5.4 for the Yule3; ; Table 3.3). In no case a rate-constant model got best support than a rate-variable model.

Model Type	***Mean lnL and range***	***n***	***Mean AIC and range***	***ΔAIC***
Rate-Constant Models				
Pure Birth	235.4 (226.6 – 244.7)	1	-469.9 (-486.9 – -453.1)	4.3
Birth-Death	237.0(228.6 – 245.4)	2	-468.9 (-487.3 – -451.1)	5.3
Rate-Variable Models				
Yule 2	237.8 (229.7 – 247.3)	3	-469.6 (-488.6 – -453.4)	4.6
Yule 3	*241.5 (231.9 – 250.5)*	*5*	*-473.0 (-491.0 – -453.7)*	*1.2*
Yule 4	***244.1 (233.8 – 254.7)***	***7***	***-474.2 (-495.4 – -453.6)***	***0.0***

Table 3.1. Summary of model fits in LASER for ruminants. *lnL* is the log Likelihood of the fit, *n* is the number of parameters, *AIC* is the Akaike Information Criterion as a measure of the support of each model and *ΔAIC* is the difference of *AIC* relative to the best model (Yule 4, in bold). Models showing no significant differences with the best one are shown in italics. Mean and range of *lnL* and *AIC* values from the 1000 trees analysed are shown.

Model Type	***Mean lnL and range***	***n***	***Mean AIC and range***	***ΔAIC***
Rate-Constant Models				
Pure Birth	126.9 (118.2 – 134.7)	1	-251.8 (-267.5 – -234.4)	8.8
Birth-Death	126.9 (118.2 – 134.7)	2	-249.8 (-265.5 – -232.4)	10.8
Rate-Variable Models				
Yule 2	132.2 (124.2 – 139.3)	3	-258.4 (-272.7 – -242.4)	2.1
Yule 3	*134.8 (126.1 – 143.6)*	*5*	*-259.6 (-277.2 – -242.3)*	*0.9*
Yule 4	***137.2 (127.3 – 145.0)***	***7***	***-260.5 (-276.0 – -240.6)***	***0.0***

Table 3.2. Summary of model fits in LASER for bovids. *lnL* is the log Likelihood of the fit, *n* is the number of parameters, *AIC* is the Akaike Information Criterion as a measure of the support of each model and *ΔAIC* is the difference of *AIC* relative to the best model (Yule 4, in bold). Models showing no significant differences with the best one are shown in italics. Mean and range of *lnL* and *AIC* values from the 1000 trees analysed are shown.

Model Type	***Mean lnL and range***	***n***	***Mean AIC and range***	***ΔAIC***
Rate-Constant Models				
Pure Birth	6.8 (2.4 – 11.1)	1	-11.5 (-20.2 – -2.8)	5.2
Birth-Death	6.8 (2.4 – 12.3)	2	-9.6 (-20.5 – -0.8)	7.1
Rate-Variable Models				
Yule 2	8.6 (5.7 – 13.6)	3	-11.2 (-21.3 – -5.5)	5.5
Yule 3	10.6 (7.3 – 14.6)	5	-11.3 (-19.2 – -4.5)	5.4
Yule 4	***15.3 (10.8 – 22.3)***	***7***	***-16.7 (-30.5 – -7.5)***	***0.0***

Table 3.3. Summary of model fits in LASER for cervids. *lnL* is the log Likelihood of the fit, *n* is the number of parameters, *AIC* is the Akaike Information Criterion as a measure of the support of each model and *ΔAIC* is the difference of *AIC* relative to the best model (Yule 4, in bold). Models showing no significant differences with the best one are shown in italics. Mean and range of *lnL* and *AIC* values from the 1000 trees analysed are shown.

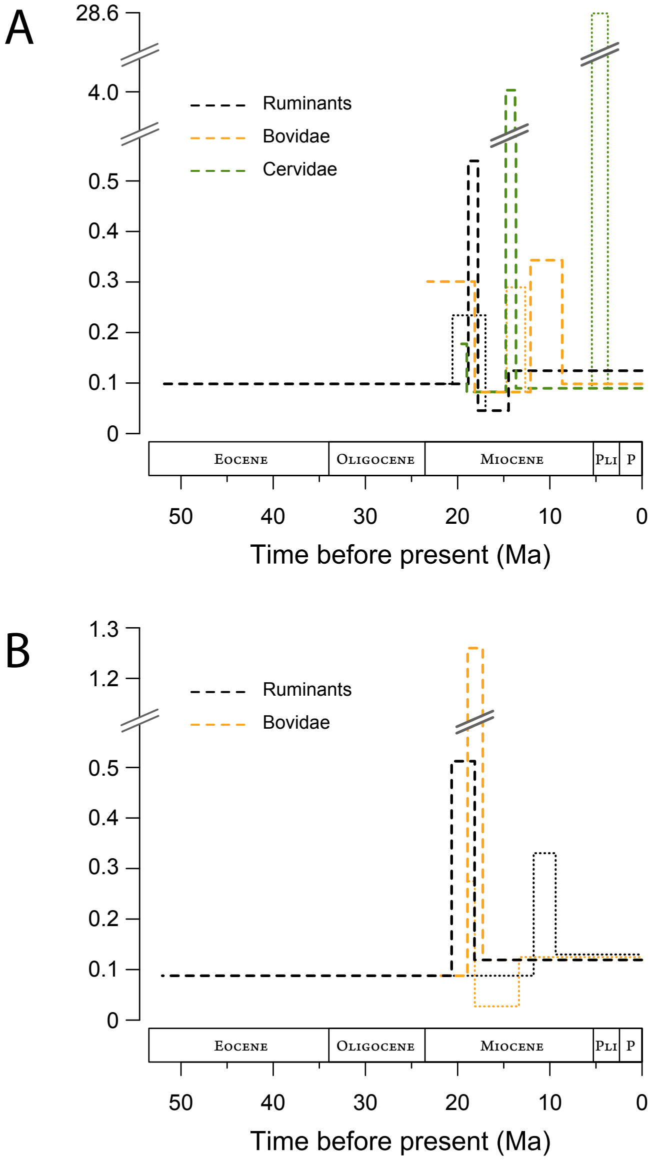


Figure 3.2. Net diversification rate estimates (per million years) for the phylogenies of the ruminants (black) and the families Bovidae (orange) and Cervidae (green) according to LASER Yule 4 model (A) and Yule 3 model (B). Dashed lines represent the evolutionary trend shown by the mayority of the 1000 trees. Pointed lines depict the evolutionary trend shown by an other important group of the 1000 trees.

The diversification rates and the temporal shift points show certain variation as a result of analyzing a distribution of trees. Nevertheless, in spite of the different configurations shed by the Yule 3 and Yule 4 rate-variable models along the 1000 trees, a few temporal breakpoints are found with high frequency. This points to a congruent trend of λ (increase or decrease) in all the cases (see Fig. 3.2). Since the Yule 4 model usually includes the same two temporal breakpoints of the Yule 3 model (see Fig. 3.2A and 3.2B) adding a third one, in order to simplify both results and discussion, hereafter we only summarize the output of the Yule 4 model.

Beginning with a low rate of diversification (0.09), the ruminant lineages (black lines in Fig. 3.2) underwent an extraordinary increase of this rate from c.a. 18.3 to 17.7 Mya (λ = 0.50). Some trees (28.3%) depict a diversification peak from 20.9 to 17.3 Mya (λ = 0.23). Diversification decreased just after 17 Mya (λ = 0.04) and experiences another increase from around 14.7 onwards (λ = 0.13).

LASER estimates very high initial diversification rates for bovids (λ = 0.31; see Fig. 3.2), until a temporal shift at a mean age of 17.9 Mya, when the diversification rate drops to 0.08 (Fig. 3.2). After this moment, two different but analogous trends are shown. For an important bunch of trees (55.8%), LASER picks up a high diversification rate (λ = 0.34) between 12.3 and 8.6 Mya. Some trees (8.8%) depict an increase of the rate of diversification (λ = 0.29) from 14.6 to 13.3 Mya. In any case, 74.2% of all the trees show a final decrease in diversification (λ = 0.11) at around 10.2 Mya along bovid lineages.

The origin of cervids is related with a high diversification rate around 19.4 Mya in 54.1% of the trees (λ values around 1.68). After this first period, diversification drops to lower levels (0.08). For some trees (41.3%), LASER shows an explosive increase in λ (3.9) from 14.7 to 14.6 Ma. In other cases (20.6%), LASER picks a peak of diversification (up to 28.6) around 5.8 to 3.7 Mya.

Discussion

Along the 1000 trees, exclusively rate-variable models were chosen as the best fit, which points to a changing rate of net diversification along ruminant evolution. Far from an explosive initial radiation, ruminants show relatively low rates of diversification during their first 30 million years of evolution, which encompass most part of the Eocene and the Oligocene (Fig. 3.2). As a result of a radiation of selenodont artiodactyls in Asia and North America during the Eocene, primitive ruminants coexisted with a wide diversity of ecomorphogically similar groups, many of which did not survive the Paleogene (Webb and Taylor, 1980). The ecological role and phyletic position of these ancestral artiodactyls, however, are far form understood, and some of them (eg. Protoceratidae, Amphimericidae, Dichobunidae and Bunoselenodontia) have been proposed as the fossil sister group of the ruminants themselves. This low net diversification rate could reflect that ecological niches were fully occupied by all this variety of forms preventing an early radiation of ruminants. Nevertheless, it could also be the case that high turnover with associated high extinction rates may have operated during this period, making difficult the detection of early diversification in a phylogeny of extant species. Our results show that the initial low net diversification rates abruptly increases 2-3 fold at 20.9 Mya, reaching a maximum of diversification rates at 18.3 Mya. This high diversification rate will last during the rest of the Early Miocene, until ca. 17 Mya. Coincident with the beginning of the Miocene Climatic Optimum, this burst in the diversification is common to both bovids and cervids (Fig. 3.2A). This outcome is also in concert with extensive paleontological evidence. The Early Miocene (23-16 Ma) was a period of change. Globally, the beginning of the Early Miocene was a uniform and relatively humid time interval, characterized by shallow temperature gradients and weak seasonality (Miller et al., 1991; Flower and Kennett, 1994; van Dam et al., 2006). Through the late Early Miocene, a climate change that have been recorded globally caused the onset of higher seasonality, marked regional differentiation in precipitation regimes (Bruch et al., 2007; Eronen et al., 2010) and a stepper latitudinal thermal gradient (Janis, 1993). This shift in the physical conditions of ecosystems spurred synchronous radiations in different groups of animals, including ungulates. This moment marks the dawn of large-sized forms in ruminants and other artiodactyls and the appearance of cranial appendages in several ruminant lineages from the late Early Miocene onwards, including cervids, bovids, giraffids and some extinct groups such as climacoceratids, lagomericids and paleomericids (Janis, 1989; Morales et al., 1993; Geist, 1998). The spectacular abrupt increase in ecomorphotypes in all these lineages suggest an adaptive radiation that filled new ecological niches as new ecosystems spread, particularly the open landscapes favored by the lower global humidity (Schluter, 2000; Delsuc et al., 2004; Steeman et al., 2009; Fordyce, 2010). Adapting to these new open scenarios involved changes in body size and physiology as a response to new diets, seasonal fluctuation of resources (Morales et al., 1993) and new social structures, antipredator strategies or mating systems (Janis, 1982, 1989).

Accordingly to the tree branching pattens, this period coincides with diversification events inside Bovidae, giving rise to Antilopinae and Bovinae, and differentiating Cephalophinae from Reduncinae and Alcelaphinae, Hippotraginae and Caprinae (Hernández Fernández and Vrba, 2005). The branching event in Giraffidae giving rise to giraffe's and okapi's lineages took also place at this point. Moreover, extensive evidence strongly suggest that giraffoids were already diversified in the Early Miocene of Asia (Ginsburg et al., 2001), Africa (Hamilton, 1978) and Europe (Moyà-Solà, 1987; Morales et al., 1993). In cervids, the end of the Early Miocene marks the basal configuration of lineages leading to Hydropotinae, Cervinae and Capreolinae (Hernández Fernández and Vrba, 2005).

Within this period of change at the end of the Early Miocene, we found strong support for a peak of extraordinary high rates of diversification around 18 to 17 Ma (a 2 fold increase respect the previous rate; Fig. 3.2A). In addition to the changing trend of global ecosystems through the Early Miocene, at this point Africa and Eurasia collided creating the *Gomphoterium* landbridge, which allowed faunas interchanges between both continents (Rögl, 1999; van der Made, 1999; van der Made et al., 2006). This faunal event, named the "proboscidean event", entailed changes on community assemblages widespread in Eurasia and Africa (Pickford, 1990; Agustí, 1999). Fossil evidence supports a bovid radiation at the end of the Early Miocene. Although the work of Hernández Fernández and Vrba (2005) indicates that bovid lineages had their origin some time earlier, the first known fossil definitive bovid, *Eotragus*, comes from deposits in Europe and Pakistan with around 18 Mya (Solounias et al., 1995; Ginsburg et al., 2001), and the presence of basal forms in Africa (like *Namacerus*, Morales et al., 2003) with a wide morphodiversity of craneal appendages c.a. 17 Mya are in concert with LASER results for bovids. The initial explosive radiation of cervids presented by our analyses is also congruent with first appearances of fossil taxa considered related to true antlered deer, as *Procervulus* and *Acteocemas* (Azanza, 1993; Agustí, 1999).

After the diversification peak at 18 -17 Mya, a period of low net diversification begun, which lasted until 15 - 14 Mya. The fact that the diversification of the lineages leading to extant ruminants dropped to basal levels between 17 and 15 Mya could be the result of the organization and stabilization of the new ecosystems recently developed. However, this could be also due to an increased diversification among other ruminant groups today extinct, which in turn maintained the diversification of lineages of extant ruminants back to low levels. Nevertheles, as it has been pointed before, the use of phylogenies of living species only allows us to track the evolutionary patterns of extant clades.

From the Middle Miocene (15-14 Mya) onwards diversification in ruminant lineages increased again. For bovids, LASER also yielded a significant increase of the diversification from around 14.6 to 13.6 Mya, with especially high rates between 12.3 and 8.6 Ma (Fig. 3.2A). There was also a peak of diversification in deer lineages between 15 and 14 Mya for an important bunch of the 1000 trees. All these phylogenetic signals seem to point to a common driver: the Middle Miocene Global Cooling Event. Around 15 to 14 Mya, variations in sea level, deep ocean circulation and a major growth of the Eastern Antarctic Ice Sheet (EAIS) had large effects on global temperature, global carbon cycling and, subsequently, on the terrestrial biosphere (Fig. 3.1; Miller et al., 1991; Flower and Kennett, 1994; Pickford and Morales, 1994; Barry, 1995; Zachos et al., 2001). There was a climatic trend to cooler winters, decreased summer rainfall and a subsequently expansion of semiarid ecosystems and xerophyllous vegetation in low and mid-latitudes. These effects brought about the creation of a wide-spread corridor of open woodland landscapes known as the Greek-Iranian Province, which connected northwestern Africa to the eastern Mediterranean area, south and western Asia and acted as an axis for intercontinental migration of all the new arid-adapted faunas (De Bonis et al., 1992). New adaptations involved an increase of body size diversity, hypsodonty and cursoriality (Janis, 1993). Changes in ruminant faunas at this moment are particularly well documented in the Siwaliks (Pakistan), where the presence of large bovids and giraffids increased in local assemblages as new immigrants arrived from Europe (Barry et al., 1991; Barry, 1995). This moment is coincident with the origin of extant tribes in Bovinae, Antolopinae, Cephalophinae, Alcelaphinae, Hippotraginae and Caprinae, as a result of independent bovid radiations in southern Asia and Africa (Hassanin and Douzery, 1999; Hernández Fernández and Vrba, 2005). The Middle Miocene radiation of cervids is associated to a basal radiation in Cervinae and Capreolinae. The fossil record reflects the appearance of the first antlers with rosettes in euproxines at this moment (Azanza, 1993). The Yule4 model in LASER did not show any significant diversification shift for ruminants after 14 Ma. This does not mean that there are no other shifts in diversification after that moment, but they are not among the three most important shifts, previously mentioned here. However, there are two additional temporal points that have shown diversification peaks when bovid and cervids were analyzed separately.

For many of the 1000 resolved trees of bovids analyzed, LASER detected a diversification peak between 12.3 and 8.6 Mya (Fig. 3.2A). This peak is in synchrony with a moment of global relative high humidity, environmental heterogeneity and faunal turnover in Eurasia known as the Vallesian Climax (Fortelius et al., 2006). Vallesian faunas were among the richest mammal assemblages of the Cenozoic (Fortelius et al., 1996; Morales et al., 1999) and, as suggested by our results, that environmental heterogeneity may have enhanced the diversification in ruminant lineages, as part of the Vallesian faunal turnover. After the climax, a new restructuring of deep oceanic circulation and the growth of ice sheets in western Antarctica led to a cooling climatic pulse (Miller et al., 1991; Zachos et al., 2001). These changes brought about higher continental uniformity and an impoverishment of mammal assemblages in concert with an abrupt replacement of sub-tropical evergreen forests by more deciduous woodlands (Agustí and Moyà-Solà, 1990; Morales et al., 1999; Merceron et al., 2010). The extinctions recorded during this Vallesian Crisis especially affected those taxa with tropical-forest affinities (Agustí and Antón, 2002; Eronen et al., 2010). There is a noticeable synchronism between the Vallesian Crisis and the drop of the rate of diversification in bovids to lower values that will last until the present (Fig. 3.2A).

In the case of cervids, while the Yule4 model does not pick up a major shift in diversification rates at this point, it finds a significant shift in diversification rates for deer lineages around 5 Mya, right after the Miocene-Pliocene boundary (Fig. 3.2). Accordingly to the calibrated topology of the tree, several deer lineages splits bunch at this temporal point. In the Old World, a major basal radiation of muntjacs is recorded, substantially increasing the diversity of this tropical clade. This moment also marks the splits among *Przewalskium* and *Cervus* and the lineages inside *Cervus* (Hernández Fernández and Vrba, 2005). In the New World, molecular analyses report several basal splits associated with the lineages to *Pudu*, *Mazama* and *Odocoileus* during this period (Pitra et al., 2004; Gilbert et al., 2006; Duarte et al., 2008).

Final remarks

Since net diversification rates of ruminant lineages have been subject to changes through their existence, we have been able to identify several moments in their history that have modeled the evolution of the group. Our results strongly suggest that shifts in the diversification patterns of the ruminants are connected to climatic events at the global scale.

In any case, the outcome of studies based on branching patterns within phylogenies of living species are to be combined with other evidences of past biotic events. A preliminary analysis of the fossil data appears to be highly concordant with our results (Fig. 3.3). Nevertheless, we foresee a future combination of both types of data because this will provide an important contribution to studies on macroevolution complementing and improving our knowledge on evolutionary patterns.

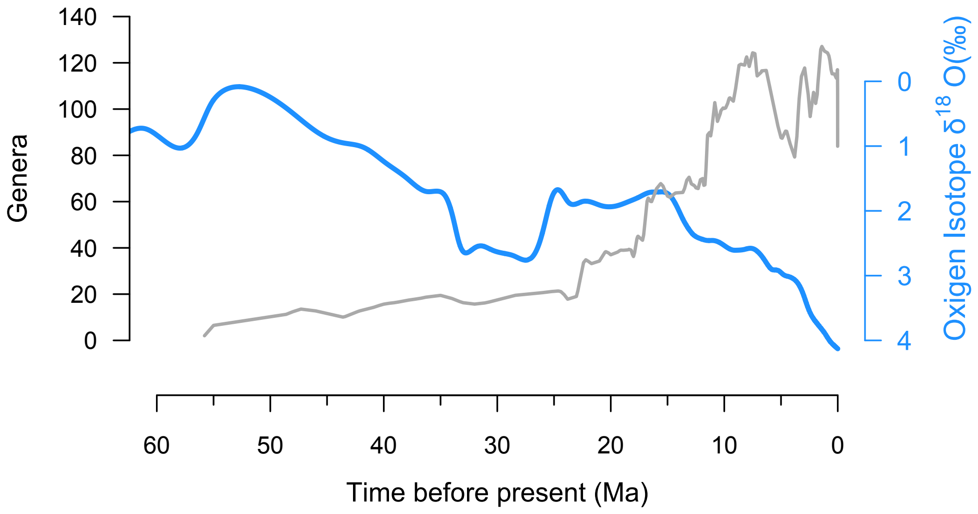


Figure 3.3. Fossil-based diversity curve at the genus level (grey line). Raw data are from the Paleobiology Database (Alroy, 2011) and the Neogene of the Old World Database (Fortelius, 2011). The blue smoothed curve represents the mean global temperature for the last 50 My (Zachos et al., 2001)

Literature Cited

Agustí J (1999) A critical re-evaluation of the Miocene mammal units in the Western Europe: dispersal events and problems of correlation. In *The Evolution of Neogene Terrestrial Ecosystems in Europe*, eds Agustí J, Rook L, Andrews P. Cambridge University Press, Cambridge. Pp. 84-112

Agustí J, Antón M (2002) Mammoths, Sabertooths, and Hominids. Columbia University Press, New York.

Agustí J, Moyà-Solà S (1990) Mammal extinctions in the Vallesian (Upper Miocene). *Lecture Notes Earth Sci* 30:425-432.

Alroy J (2011) Paleobiology Database. Macquarie University, Sydney. http://paleodb.org.

Azanza B (1993) Sur la nature des appendices frontaux des cervidés (Artiodactyla, Mammalia) du Miocène inférieur et moyen. Remarques sur leur systématique et leur phylogénie. *C R Acad Sci Paris Ser II* 316(8):1163-1169.

Barry J, Morgan M, Winkler A, Flynn L, Lindsay E, Jacobs L, Pilbeam D (1991) Faunal interchange and Miocene terrestrial vertebrates of southern Asia. *Paleobiology* 17(3):231-245.

Barry JC (1995) Faunal Turnover and Diversity in the Terrestrial Neogene of Pakistan. In *Paleoclimate and Evolution with Emphasis on Human Origins*, eds Vrba ES, Denton GH, Patridge TC, Burcke LH. Yale University Press, New Haven. Pp. 115-134

Bruch A, Uhl D, Mosbrugger V (2007) Miocene climate in Europe – patterns and evolution: a first synthesis of NECLIME. *Palaeogeogr Palaeoclimatol Palaeoecol*

253(1-2):1-7.

Burnham KP, Anderson GC (2002) Model selection and multimodel inference: a practical information-theoretic approach. Springer-Verlag, New York.

Cantalapiedra JL, Hernández Fernández M, Alcalde G, Azanza B, DeMiguel D, Morales J (in press) Ecological correlates of ghost lineages in ruminants. *Paleobiology*.

De Bonis L, Bouvrain G, Geraads D, Koufos G (1992) Diversity and paleoecology of Greek late Miocene mammalian faunas. *Palaeogeogr Palaeoclimatol Palaeoecol* 91(1-2):99-121.

Delsuc F, Vizcaino SF, Douzery EJP (2004) Influence of Tertiary paleoenvironmental changes on the diversification of South American mammals: a relaxed molecular clock study within xenarthrans. *BMC Evol Biol* 4(11):1-13.

Duarte JMB, González S, Maldonado JE (2008) The surprising evolutionary history of South American deer. *Mol Phylogenet Evol* 49(1):17-22.

Eronen J, Puolamäki K, Liu L, Lintulaakso K, Damuth J, Janis C, Fortelius M (2010) Precipitation and large herbivorous mammals II: application to fossil data. *Evol Ecol Res* 12(2):235-248.

Flower B, Kennett J (1994) The middle Miocene climatic transition: East Antarctic ice sheet development, deep ocean circulation and global carbon cycling. *Palaeogeogr Palaeoclimatol Palaeoecol* 108(3-4):537-555.

Fordyce JA (2010) Host shifts and evolutionary radiations of butterflies. *Proc R Soc B* 277(1701):3735-43.

Fortelius M (coordinator) (2011). Neogene of the Old World Database of Fossil Mammals (NOW). University of Helsinki. http://www.helsinki.fi/science/now/.

Fortelius M, Eronen J, Liu LP, Pushkina D, Tesakov A, Vislobokova I, Zhang ZQ (2006) Late Miocene and Pliocene large land mammals and climatic changes in Eurasia. *Palaeogeogr Palaeoclimatol Palaeoecol* 238(1-4):219-227.

Fortelius M, Werdelin L, Andrews P, Bernor RL, Gentry A, Humphrey L, Mittmann HW, Viratana S (1996) Provinciality, Diversity, Turnover, and Paleoecology in Land Mammal Faunas of the Later Miocene of Western Eurasia. In *The Evolution of Western Eurasian Neogene Mammal Faunas*, eds Bernor RL, Fahlbusch V, Mittmann H-W. Columbia University Press. Pp. 414-448

Geist V (1998) Strategies of deer evolution. In *Deer of the World: Their Evolution Behaivour and Ecology*, ed Geist V. Stackpole Books, Mechanisburg. Pp. 1-18

Gentry AW (2000) The Ruminant radiation. In *Antelopes, Deer, and Relatives: Fossil Record, Behavioral Ecology, Systematics and Conservation*, eds Vrba ES, Schaller GB. Yale University Press, New Haven. Pp. 11-25

Gilbert C, Ropiquet A, Hassanin A (2006) Mitochondrial and nuclear phylogenies of Cervidae (Mammalia, Ruminantia): systematics, morphology, and biogeography. *Mol Phylogenet Evol* 40(1):101-17.

Ginsburg L, Morales J, Soria D (2001) Les ruminantia (Artiodactyla, Mammalia) du Miocene des Bugti (Balouchistan, Pakistan). *Estud Geol* 57(3-4):155-177.

Hamilton WR (1978) Fossil giraffes from the Miocene of Africa and a revision of the phylogeny of the Giraffoidea. *Philos Trans R Soc Lond B Biol Sci* 283(996):165-229.

Harvey PH, Holmes EC, Mooers AØ, Nee S (1994) Inferring evolutionary processes from molecular phylogenies. In *Models in Phylogeny Recosntruction*, eds Scotland RW, Siebert DJ, Williams DM. Clarendon Press, Oxford. Pp. 313-333

Hassanin A, Douzery EJP (1999) The tribal radiation of the family Bovidae (Artiodactyla) and the evolution of the mitochondrial cytochrome b gene. *Mol Phylogenet Evol* 13(2):227-243.

Hernández Fernández M, Vrba ES (2005) A complete estimate of the phylogenetic relationships in Ruminantia: a dated species-level supertree of the extant ruminants. *Biol Rev* 80(2):269-302.

Janis C (1982) Evolution in horns in ungulates: ecology and paleoecology. *Biol Rev Camb Philos Soc* 57(2):261-317.

Janis C (1989) A climatic explanation for patterns of evolutionary diversity in ungulate mammals. *Palaeontology* 32(3):463-481.

Janis CM (1993) Tertiary mammal evolution in the context of changing climates, vegetation, and tectonic events. *Annu Rev Ecol Syst* 24(1):467-500.

Kuhn TS, Mooers AØ, Thomas GH (2011) A simple polytomy resolver for dated phylogenies. *Meth Ecol Evol* doi: 10.1111/j.2041-210X.2011.00103.x.

Mercer JM, Roth VL (2003) The effects of Cenozoic global change on squirrel phylogeny. *Science* 299(5612):1568-72.

Merceron G, Kaiser TM, Kostopoulos DS, Schulz E (2010) Ruminant diets and the Miocene extinction of European great apes. *Proc R Soc B* 277(1697):3105-12.

Miller K, Wright J, Fairbanks R (1991) Unlocking the ice house: Oligocene-Miocene oxygen isotopes, eustasy, and margin erosion. *J Geophys Res* 96(B4):6829-6848.

Mooers AØ, Heard S (2002) Using tree shape. *Syst Biol* 51(6):833.

Mooers AØ, Heard SB (1997) Inferring evolutionary process from phylogenetic tree shape. *Quart Rev Biol* 72(1):31-54.

Morales J, Nieto M, Kholer M, Moyà-Solà S (1999) Large mammlas from the Vallesian of Spain. In *The evolution of Neogene Terrestrial Ecosystems in Europe*, eds Agustí J, Rook L, Andrews P. Cambridge University Press, Cambridge. Pp. 113-126

Morales J, Pickford M, Soria D (1993) Pachyostosis in a Lower Miocene giraffoid from Spain, *Lorancameryx pachyostoticus* nov. gen. nov. sp. and its bearing on the evolution of bony appendages in artiodactyls. *Geobios* 26(2):207-230.

Morales J, Soria D, Pickford M, Nieto M (2003) A new genus and species of Bovidae (Artiodactyla, Mammalia) from the early Middle Miocene of Arrisdrift, Namibia, and the origins of the family Bovidae. *Memoir Geol Surv Namibia* 19:371-384.

Moyà-Solà S (1987) Los Rumiantes (Cervoidea y Bovidea, Artiodactyla, Mammalia) del Ageniense (Mioceno inferior) de Navarrete del Río (Teruel, España). *Paleontol i Evol* 21:247--270.

Pickford M (1990) Dynamics of Old World biogeographic realms during the Neogene: implications for Biostratigraphy. In *European Neogene Chronology*, eds Lindsay EH, Fahlbusch V, Mein P. Plenum Press, New York. Pp. 413-442

Pickford M, Morales J (1994) Biostratigraphy and palaeobiogeography of East Africa and the Iberian Peninsula. *Palaeogeogr Palaeoclimatol Palaeoecol* 112(3-4):297-322.

Pitra C, Fickel J, Meijaard E, Groves PC (2004) Evolution and phylogeny of old world deer. *Mol Phylogenet Evol* 33(3):880-895.

Rabosky DL (2006) LASER: a maximum likelihood toolkit for detecting temporal shifts in diversification rates from molecular phylogenies. *Evol Bioinform* 2(6):273-260.

Rabosky DL, Lovette IJ (2008) Explosive evolutionary radiations: decreasing speciation or increasing extinction through time? *Evolution* 62(8):1866-75.

Rögl F (1999) Mediterranean and Paratethys palaeogeography during the Oligocene and Miocene. In *The evolution of Neogene Terrestrial Ecosystems in Europe*, eds Agustí J, Rook L, Andrews P. Cambridge University Press, Cambridge. Pp. 8-22

Schluter D (2000) The Ecology of Adaptive Radiation. Oxford University Press, New York.

Solounias N, Barry JC, Bernor RL, Lindsay E, Raza SM (1995) The oldest bovid from the Siwaliks, Pakistan. *J Vertebr Paleontol* 15(4):806-814.

Steeman ME, Hebsgaard MB, Fordyce RE, Ho SY, Rabosky DL, Nielsen R, Rahbek C, Glenner H, Sorensen MV, Willerslev E (2009) Radiation of extant cetaceans driven by restructuring of the oceans. *Syst Biol* 58(6):573-85.

van Dam J, Aziz HA, Álvarez Sierra MA, Hilgen F, van den Hoek Ostende L, Lourens L, Mein P, van der Meulen A, Peláez-Campomanes P (2006) Long-period astronomical forcing of mammal turnover. *Nature* 443(7112):687-91.

van der Made J (1999) Intercontinental relationship Europe–Africa and the Indian subcontintent. In *The Miocene Land Mammals of Europe*, eds Rössner G, Heissig K. Verlag Dr. Friedrich Pfeil, München. Pp. 457–472

van der Made J, Morales J, Montoya P (2006) Late Miocene turnover in the Spanish mammal record in relation to paleoclimate and the Messinian Salinity Crisis. *Palaeogeogr Palaeoclimatol Palaeoecol* 238(1-4):228-246.

Vrba ES (1984) Evolutionary pattern and process in the siter-group Alcelaphini-Aepycerotini (Mammalia: Bovidae). In *Living Fossils*, eds Eldredge N, Stanley SM. Springer-Verlag, New York. Pp. 62-79

Vrba ES (1992) Mammals as a Key to Evolutionary Theory. *J Mammal* 73(1):1-28.

Vrba ES (1993) Turnover-pulses, the Red Queen, and related topics. *Am J Sci* 293A(A):418-452.

Vrba ES (1995a) The fossil record of African Antelopes (Mammalia, Bovidae) in relation to human evolution and paleoclimate. In *Paleoclimate and Evolution with Emphasis on Human Origins*, eds Vrba ES, Denton GH, Patridge TC, Burcke LH. Yale University Press, New Haven. Pp. 385-324

Vrba ES (1995b) On the connections between paleoclimate and evolution. In *Paleoclimate and Evolution with Emphasis on Human Origins*, eds Vrba ES, Denton GH, Patridge TC, Burcke LH. Yale University Press, New Haven. Pp. 24-45

Vrba ES (1999) Habita theory in relation to the evolution in African Neogene biota and hominids. In *African Biogeography, Climate Change and Human Evolution*, eds Bromage TG, Schrenk F. Oxford University Press, New York. Pp. 19-39

Webb SD, Taylor BE (1980) The phylogeny of hornless ruminants and a description of the cranium of *Archaeomeryx*. *Bull Am Mus Nat Hist* 167:121-157.

Zachos J, Pagani M, Sloan L, Thomas E, Billups K (2001) Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* 293(5517):686-693.

4

Mixed-feeding and the diversification of ruminants through the Tertiary

> “*As such, tree shape is the signature of the forces that produce biodiversity, and its study informs one of the major areas in evolutionary biology.*”
>
> *- Arne Ø. Mooers -*

Abstract

Global abiotic change and ecological flexibility are two major factors influencing rates of speciation and extinction across clades. The evolution of feeding styles is though to be key in the explosive radiation of ruminants. Classic scenarios depict a browsing as the ancestral state and gradual evolution towards mixed-feeding and grazer lineages concomitant with increasing aridity and subsequent expansion of open habitats during the Neogene. However new insights have challenged this view, suggesting mixed-feeding ancestors for several of the ruminant families. Here, we explored which the most likely scenario explaining the evolutionary transitions between diets and asked whether ruminant lineages underwent differential rates of speciation under different feeding styles (browsing, grazing and mixed feeding) and global temperature regimes. In order to assess these issues, we use new multi-state speciation and extinction (MuSSE) models on the supertree of the group, an accurate synthesis of dietary categories of all 197 extant species of ruminants, and a precise record of global Tertiary climate. MuSSE inferred higher diversification rates in mixed-feeding and grazing lineages than in browsers. The inclusion of global temperature data did not significantly improve the model fits. Our ancestral diet reconstructions depicted a browser ancestor for ruminants and for Giraffidae, Moschidae and Tragulidae, while a mixed feeding ancestor is inferred for Bovidae and Cervidae. A browser deer ancestor and a mixed feeding at the basal node of the tree might be also consistent with our data.

Introduction

The connection between broad-scale habitat change and broad-scale evolutionary process is a major question in evolution (Vrba, 1980a; Janis, 1993; Vrba et al., 1995; Agustí et al., 1999; Vrba, 1999; Benton, 2009). For instance, diversification rates in both marine and terrestrial mammals may have shifted in concert with global climatic shifts and continental drifting (Vrba, 1992, 1993; Pickford and Morales, 1994; Janis et al., 2002; Delsuc et al., 2004; Johnson et al., 2006; van der Made et al., 2006; Steeman et al., 2009). Food quality and its availability in the environment are important factors influencing the biogeography of many taxa, and the physiological, morphological and behavioural adaptations to particular resources may be associated with modifications on other key ecological aspects like body size, body shape, social behavior or antipredator strategies (Brashares et al., 2000; Gagnon and Chew, 2000; Clauss et al., 2003; Gordon, 2003; Brown and Sibly, 2006; Bro-Jorgensen, 2008). In a context of physical change and environmental reorganization, key innovations may be important for radiating into newly-created niches (Luo, 2007; Lynch, 2009; Slater et al., 2010). Herbivores are more constrained to a particular vegetation physiognomy than other groups (Vrba, 1980a, b, 1987, 1992), suggesting that key innovations in feeding may mediate a causal connection between habitat change and their macroevolutionary patterns. In this paper we evaluate diet as a possible key character influencing diversification patterns in ruminants.

Herbivore diets of ungulates are classically classified (Hofmann and Stewart, 1972) as browsing (leaf-eating), grazing (grass eating) and mixed feeding (both leaf-eating and grass eating). Fossil ruminants have been frequently utilized as paleoecological indicators due to their wide range of ecomorphological adaptations and their ubiquity in the fossil record (Vrba and Schaller, 2000; Hernández Fernández and Vrba, 2006; DeMiguel et al., 2010). Currently, paleontological estimates of diets are based on techniques for the analysis of micro- and mesowear (Walter et al., 1978; Solounias et al., 1988; Fortelius and Solounias, 2000; Solounias and Semprebon, 2002) and the analysis of stable isotopes on tooth enamel (Quade et al., 1992; Cerling et al., 1993; Leethorp et al., 1994) together with more traditional approaches (e.g. measure of the degree of dental crown height or hypsodonty). All these methods have provided valuable information on connections between paleoclimate, paleoecology and faunal turnovers (Janis, 1982; Gentry, 2000; Janis et al., 2000; Jernvall and Fortelius, 2002; Kaiser, 2003; Janis et al., 2004; Fortelius et al., 2006; Kaiser and Rossner, 2007; Codron et al., 2008; DeMiguel et al., 2008; Domingo et al., 2009; DeMiguel et al., 2010; Eronen et al., 2010; DeMiguel et al., 2011). This work has also drawn on the development of reasonable phylogenies for ruminants, allowing tests of hypotheses of covariation of different feeding styles in ungulates with body size (Brashares et al., 2000; Bro-Jorgensen, 2008), and habitat use (Pérez-Barbería et al., 2001).

One open question on the role of diet in Ruminant diversification concerns mixed feeding. Some interpretations place mixed feeding as a predominantly middle step leading from a hypothesised ancestral browsing to derived grazing, concomitant with the cooling and drying trend during the Neogene that lead to the end-Miocene expansion of open grass-dominated habitats (Janis, 1982; Pérez-Barbería et al., 2001; Janis et al., 2002). However, the only evidence supporting a browser ancestry for ruminants is the fact that all basal fossils share low-crowned cheek teeth; the connection between "low-crowned" and "leaf-eater" is now in question (DeMiguel et al., 2008). Mixed feeders defined by microwear are by far more abundant in the fossil record than browsers or grazers, which is consistent with the hypothesis that generalist mixed feeders were most successful in responding to the dramatic changes in vegetal resources through the Tertiary (DeMiguel et al., 2010).

Against this background, we test several hypotheses concerning the relationship between feeding style and diversification in Ruminants in a comparative framework (MuSSE; FitzJohn, 2011). Utilizing a time-calibrated tree of all extant ruminants and a classification of current feeding modes, we first test three models with different constraints for transition rates between feeding styles. We then test specific time-dependent diversification models, asking whether lineages exhibiting different feeding styles diversified differentially as global temperature varied across the Tertiary. All the models were compared using the ratio of their Akaike Information Criteria (AIC), which measures the goodness of fit of a statistical model while penalizes the number of parameters (the complexity) of the model. The best model gets the lower AIC score, and the fit of a model is significantly better than other when the different in their AIC scores is greater than two units (Burnham and Anderson, 2002).

Finally, in order to characterize the diversification of ruminants in relation to feeding style further and following the results obtained by Solounias and Moelleken (1994) and DeMiguel et al. (2008), we use the best fit model of trait-change to asses whether browsing versus mixed-feeding was the ancestral state at the root of the Ruminant tree and at the base of the major clades Bovidae, Cervidae, Moschidae, Tragulidae and Giraffidae. We found higher diversification rates in mixed-feeding and grazing lineages than in browsers and a high backward transition from "grazer" to “mixed-feeding”. Our ancestral diet reconstruction depicts a browser ancestor for the group and for the basal node of Tragulidae, Moschidae and Giraffidae and a mixed feeding style for the most specious families, Cervidae and Bovidae.

Methods

Feeding modes. Extant herbivorous ungulates, primarily ruminants, have traditionally been placed in three broad dietary categories proposed by Hofmann and Stewart (1972) according to which predominant type of forage they prefer. We performed an extensive review of the ecological literature to classify all 197 extant and recently extinct taxa (see Appendix 4.1). Browsers (or concentrate feeders) focus feeding on herbaceous and woody material such as forbs, leaves and fruits (e.g. mouse-deer *Tragulus napu*, moose *Alces alces*, giraffe *Giraffa camelopardalis*). Grazers (or bulk and roughage feeders) concentrate feeding on grasses, rushes and sedges (e.g. hartebeest *Alcelaphus buselaphus*, sable antelope *Hippotragus niger*). Mixed (intermediate) feeders have a composite diet of grasses, rushes and sedges, and browse (e.g. impala *Aepyceros melampus*, sambar *Cervus unicolor*). While different classification schemes have been proposed (Hofmann, 1973; Jarman, 1974; Langer, 1988; Bodmer, 1990; Mysterud, 1998), Hofmann and Stewart’s categorization(Hofmann and Stewart, 1972), based on observations of the natural feeding behaviour, is almost universally accepted.

Phylogenetic tree. The basis of our analysis is a recently-published consensus supertree that includes all 197 extant and recently extinct species of the suborder Ruminantia (Hernández Fernández and Vrba, 2005). This supertree is a consensus from 124 trees published from 1970 to 2003, including morphological, ethological and molecular information. The supertree was constructed using matrix representation with parsimony (MRP). Importantly, 80% of the nodes on the tree are dated using a large compendium of molecular and fossil data, with the remaining 20% of the nodes being interpolated using a pure birth model. Most of the remaining polytomies in the tree are soft, and would bias almost any statistic that draws on tree shape (as we do here). The polytomies were therefore resolved using the pure birth model as proposed by Kuhn et al. (2011) [see also and example of its use by FitzJohn (2010)]. While we make no claims that the Ruminants evolved in a fashion consistent with this model, this should be unbiased with respect to our analyses of feeding mode, and offer only noise with respect to time-based analyses (see below). 100 randomly drawn trees from the resulting distribution were retained for analysis (Fig. 4.1).

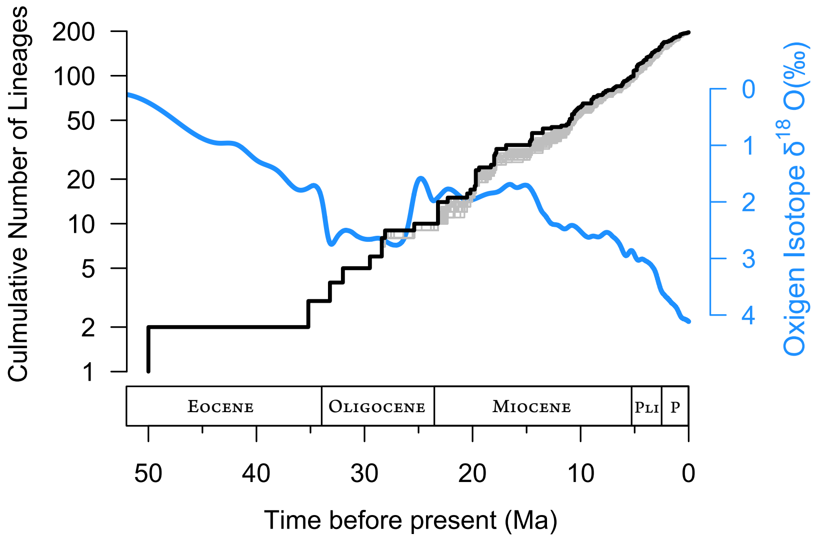


Figure 4.1. Lineages-through-time plot of the 100 trees used in our analyses. Cumulative number of lineages against time for the supertree of ruminants published by Hernández Fernández & Vrba (2005) (black line) and the 100 resolved phylogenies of ruminants used in this work (grey lines). The blue smoothed curve represents the mean global temperature for the past 50 My (Zachos et al., 2001).

Analyses. This is a likelihood-based approach that computes the probability of a tree, including branch lengths and character states, under a model where speciation and extinction rates may vary with a character state. The character evolves under a simple Markov model of evolution (Pagel, 1994). Ancestral state estimates and shifts in rates of diversification of lineages in different states were analyzed using a multi-state extension (MuSSE: Multi-State Speciation and Extinction, see FitzJohn, 2011) of the BiSSE trait-based diversification modeling approach (Maddison et al., 2007; for an example, see Lynch, 2009) that allows analysis of our three-state diet character

We competed three candidate models for transitions among the three dietary states while also estimating the diversification rates of lineages exhibiting each of the three states in a maximum likelihood framework. The overall rate of diversification for a diet state under these three models are constant through time and we named these "time-constant models". These time-constant MuSSE models for a three-state trait may have up to 12 free parameters: three pairs of forward and backward transition rates among states, and three rates of diversification and three rates of extinction under each state. In order to asses the contribution of the parameters to the models, several constrained and unconstrained extra models were

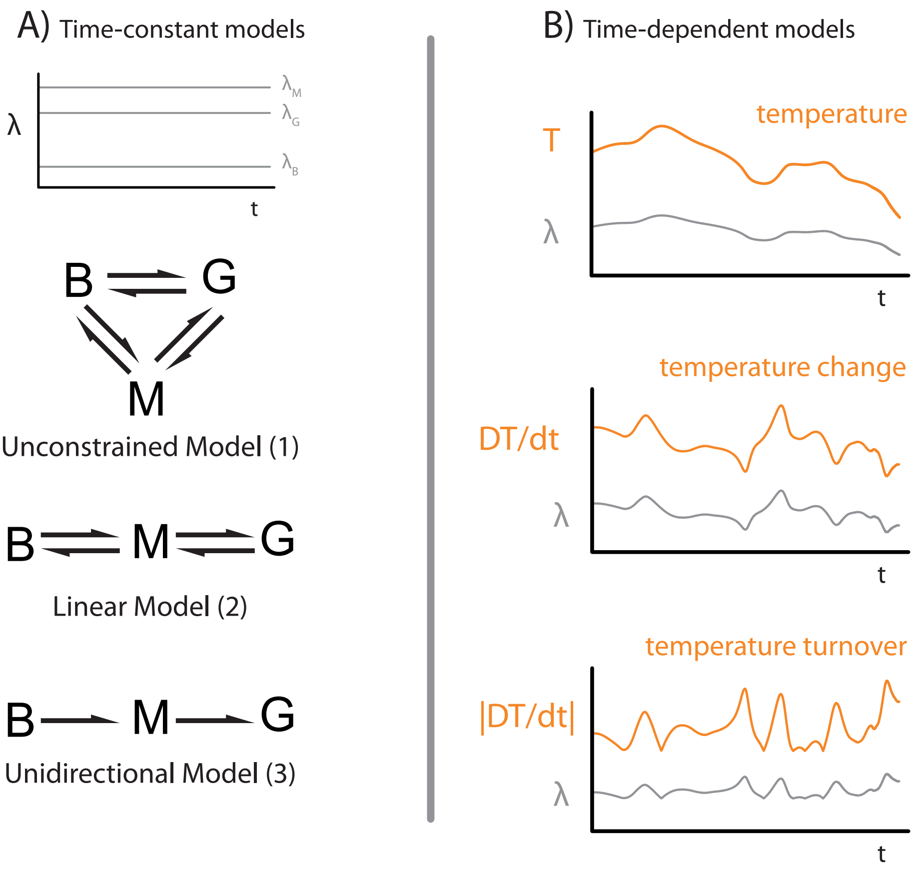


Figure 4.2. Schematic representation of the multi-state speciation and extinction (MuSSE) models tested in our analyses. A) time-constant models where rates of diversification (λ) are constant through time; each model has different constrains of the transitions; model numbers as referenced in the text; B) three examples of how rates of diversification (λ) may vary in concert with temperature, temperature change (first derivative of temperature) and temperature turnover (as the absolute derivative of the temperature) in our time-dependent models.

also compared using their likelihood ratios and AIC values (see Appendix 4.2). The three time-constant models tested were (Fig. 4.2A): (1) a free model where all transitions between browser, grazer and mixed-feeder states were allowed; (2) a linear model where transitions between browser and grazer states were forbidden, forcing those transitions to be made through the mixed-feeder state, following Pérez-Barbería (2001); (3) a unidirectional linear model where only transitions from browser to mixed-feeder and from mixed-feeder to grazer were allowed (Janis, 1982; Fortelius, 1985). For each, diversification rates were allowed to vary among the three diet categories. We then chose the best-fit model among the three time-constant models and use it to developed six time-dependent models where rates of diversification not only varied among diets but, were also allowed to vary as a function of the global temperature profile. For these time-dependent models, we drew on the precise and complete isotope-based record of Tertiary global climate change (Zachos et al., 2001).

We tested six a priori time-dependent models depicted in Fig. 4.2B. These models are not exhaustive, but focus on mixed feeding and on testing whether ruminant clades with different feeding styles diversified more during warm or cold periods (called temperature-dependent models), or more during episodes of warming or cooling (change-dependent models) or more during episodes of absolute change of global temperature (turnover-dependent models). The six models were (1) a model where relative rates of diversification under the three states vary in concert with temperature (temperature-dependent); (2) a model where rates of diversification under browser and grazer states vary in concert with temperature as above, but the rate of diversification for mixed-feeder lineages vary in concert with the derivative of the temperature profile (change-dependent mixed-feeders). This model predicts that mixed feeders diversify most during times of global temperature warming or cooling; (3) a model where rates of diversification under browser and grazer states vary in concert with temperature and the rate of diversification for mixed-feeder lineages vary in concert with the absolute value of the derivative of the temperature profile. This turnover-dependent mixed-feeder model has mixed-feeders diversifying during both warming and cooling periods; (4) a model where relative rates of diversification under the three states vary in concert with the derivative of the temperature (change-dependent); (5) a model where rates of diversification under browser and grazer states vary in concert with the derivative of the temperature and the rate of diversification for mixed-feeder lineages vary in concert with the absolute value of the derivative of the temperature profile (change-dependent browsers and grazers; turnover-dependent mixed-feeders); and (6) a model where rates of diversification for all three states vary in concert with the absolute value of the derivative of the temperature profile (turnover-dependent). MuSSE models were run over each of the 100 resolved tree topologies. The estimate of parameters was assessed from the 100 trees distribution and models were compared using the set of 100 ΔAIC scores (Burnham and Anderson, 2002).

We also used MuSSE to estimate ancestral dietary states for the basal node of the families Bovidae, Cervidae, Moschidae, Tragulidae and Giraffidae and for the root of the entire tree under the best-fit model of the nine presented above. Trait-base diversification models present less biased estimates of ancestral states in cases where diversification is trait-dependent as we expect here. For each tree, distributions of parameters were computed over 2000 steps of a Markov chain Monte Carlo (MCMC) starting from the maximum likelihood point. The marginal probabilities of ancestral state reconstructions were computed for each sample of the chain and the first 500 steps were discarded as burn in.

Time-constant	*Mean lnLH and range*		*P*	*Mean AIC and range*		*Mean ΔAIC*
Linear	***-701.73***	***(-709.15 – -694.04)***	***10***	***1423.46***	***(1408.08–1438.31)***	–
Unconstrained	-701.52	(-708.89 – -694.04)	12	1427.04	(1412.08–1441.79)	3.58
Unidirectional	-722.65	(-731.88 – -714.09)	8	1461.31	(1444.18–1479.76)	37.85

Time-dependent	*Mean lnLH and range*		*P*	*Mean AIC and range*		*Mean ΔAIC*
all λ absolute derivative	-699.81	(-707.45 – -692.23)	13	1425.63	(1410.46–1440.89)	2.17
all λ derivative	-700.40	(-707.72 – -693.01)	13	1426.80	(1412.03–1441.43)	3.34
λB λG derivative; λM abs. derivative	-700.49	(-707.84 – -692.93)	13	1426.99	(1411.85–1441.68)	3.53
all λ temperature	-700.78	(-708.85 – -693.55)	13	1427.56	(1413.10–1443.70)	4.10
λB λG temperature; λM derivative	-701.14	(-708.73 – -693.55)	13	1428.27	(1413.10–1443.47)	4.81
λB λG temperature; λM abs. derivative	-701.21	(-708.85 – -693.45)	13	1428.41	(1412.91–1443.69)	4.95

Table 4.1. Likelihood and AIC (Akaike Information Criterion) values obtained for the models competing in this study. Models are ordered by mean ΔAIC rank. Given are the mean, minimum and maximum likelihood (lnLH) and AIC values obtained from the posterior distributions. P, number of parameters of the model. The model with the lowest ΔAIC is shown in bold-italic.

Results and Discussion

Models of Diversification. Under MuSSE, the free time-constant linear model (2) with different rates of diversification under each diet provided the best fit to the data across the 100 trees (Table 4.1; parameters of the nine competed models are shown in Table 4.2). The ynthesis of the dietary cathegories for the 197 ruminant species and the estimated parameters of the linear model are shown in Figure 4.3. The scenario of mixed-feeding being a transitional step between browsing and grazing states (c.f. Pérez-Barbería et al., 2001) is highly preferable to the unconstrained character evolution model (1) (ΔAIC= 3.584; Table 4.1). Constraining the linear model to have unidirectional transitions among diets, however (model 3) caused a significant decrease in the fit (ΔAIC= 37.847; Table 4.1). Furthermore, we found that backward transition from grazer to mixed-feeder (q_{GM}) is needed, as removing this path caused a large decrease in fit (ΔAIC=14.294; see Appendix 4.2). Though the transition from grazer to mixed-feeder is higher than the other transitions among states under the linear model (q_{BM}= 0.003; q_{MB}= 0.023; q_{MG}= 0.012; q_{GM}= 0.044; see Fig. 4.3), constraining all transition rates to be equal has little effect on fit (ΔAIC=0.568; see Appendix 4.2). Thus, the comparative evidence points to an appreciable probability for bidirectional transitions between grazing and mixed feeding.

TIME-CONSTANT	Mean ΔAIC	Diversification rates			Extinction rates			Transitions					
		λ_B	λ_M	λ_G	μ_B	μ_M	μ_G	q_{BM}	q_{MB}	q_{MG}	q_{GM}	q_{BG}	q_{BG}
Linear	–	***0.077***	***0.172***	***0.131***	***0.006***	***0.029***	***0.000***	***0.003***	***0.023***	***0.012***	***0.044***	–	–
Unconstrained	3.58	0.075	0.173	0.131	0.004	0.035	0.000	0.002	0.023	0.012	0.045	0.001	0.000
Unidirectional	37.85	0.099	0.175	0.117	0.000	0.014	0.055	0.033	–	0.031	–	–	–

TIME-DEPENDENT	Mean ΔAIC	Diversification rates ($\lambda = c+bT$)						Extinction rates			Transitions					
		λ_B		λ_M		λ_G										
		c_B	b_B	c_M	b_M	c_G	b_G	μ_B	μ_M	μ_G	q_{BM}	q_{MB}	q_{MG}	q_{GM}	q_{BG}	q_{BG}
all λ absolute derivative	2.17	0.062	-0.517	0.173	0.016	0.195	0.634	0.011	0.029	0.000	0.002	0.023	0.013	0.045	–	–
all λ derivative	3.34	0.070	-0.453	0.183	0.190	0.108	-1.000	0.002	0.040	0.000	0.003	0.022	0.012	0.046	–	–
λB λG derivative; λM abs. derivative	3.53	0.070	-0.445	0.173	0.023	0.108	-1.000	0.002	0.028	0.000	0.003	0.023	0.013	0.045	–	–
all λ temperature	4.10	0.063	0.511	0.256	-0.217	0.158	-0.277	0.000	0.112	0.000	0.005	0.019	0.010	0.053	–	–
λB λG temperature; λM derivative	4.81	0.063	0.501	0.183	0.183	0.151	-0.240	0.000	0.039	0.000	0.003	0.021	0.012	0.045	–	–
λB λG temperature; λM abs. derivative	4.95	0.063	0.483	0.174	0.033	0.150	-0.237	0.000	0.027	0.000	0.003	0.022	0.012	0.044	–	–

Table 4.2. Parameters of the nine competed models. Models are ordered by mean ΔAIC rank. Parameters include diversification rates (λ), extinction rates (μ) and transition rates between states (q_{ij}, transition rates from state i to state j). B, browser; M, mixed-feeder ; G, grazer. In Time-Dependent models, λ is a function of the temperature (or its first derivative or its absolute derivative; see Methods), where c is the mid point of λ around which the climate effect wobbles and b is the amplitude of the climate effect, scaled by c.

This flexibility at the mixed-grazer part of the dietary spectrum may account in part for the ecological success of ruminants and their explosive radiation during the Neogene. Others (Codron et al., 2008; DeMiguel et al., 2010) have suggested lineages may change their dietary strategy towards leaf-grass mixed intake as a response to adjustments in vegetal resource abundance during episodes of climatic and habitat change. Facultative flexible diets ("facultative mixed state" proposed by DeMiguel et al., 2008) may increase the "threat tolerance" of isolated populations during habitat fragmentation in relation to climatic pulses (Waldron, 2010) and represent a favorable strategy during episodes of faunal turnovers, when changes in habitat and faunas may entail new ecological opportunities but also the appearance of unexpected competitors (DeMiguel et al., 2010). This tolerance of isolated populations to habitat fragmentation and resources limitation may be associated with increased speciation (Waldron, 2010). Indeed, under MuSSE, mixed-feeder lineages had higher rates of diversification than grazers and browsers for our best model (lineal model; see parameters distributions in Fig. 4.2; AIC score in Table 4.1). Constraining the linear model to have equal rates of diversification under the three feeding styles produced a noticeable drop in fit (ΔAIC= 4.713, see Appendix 4.2). Comparing models with different a priori constrains of the diversification rates (λ) highlights that the difference between λ_{Mx} and λ_B is significantly important

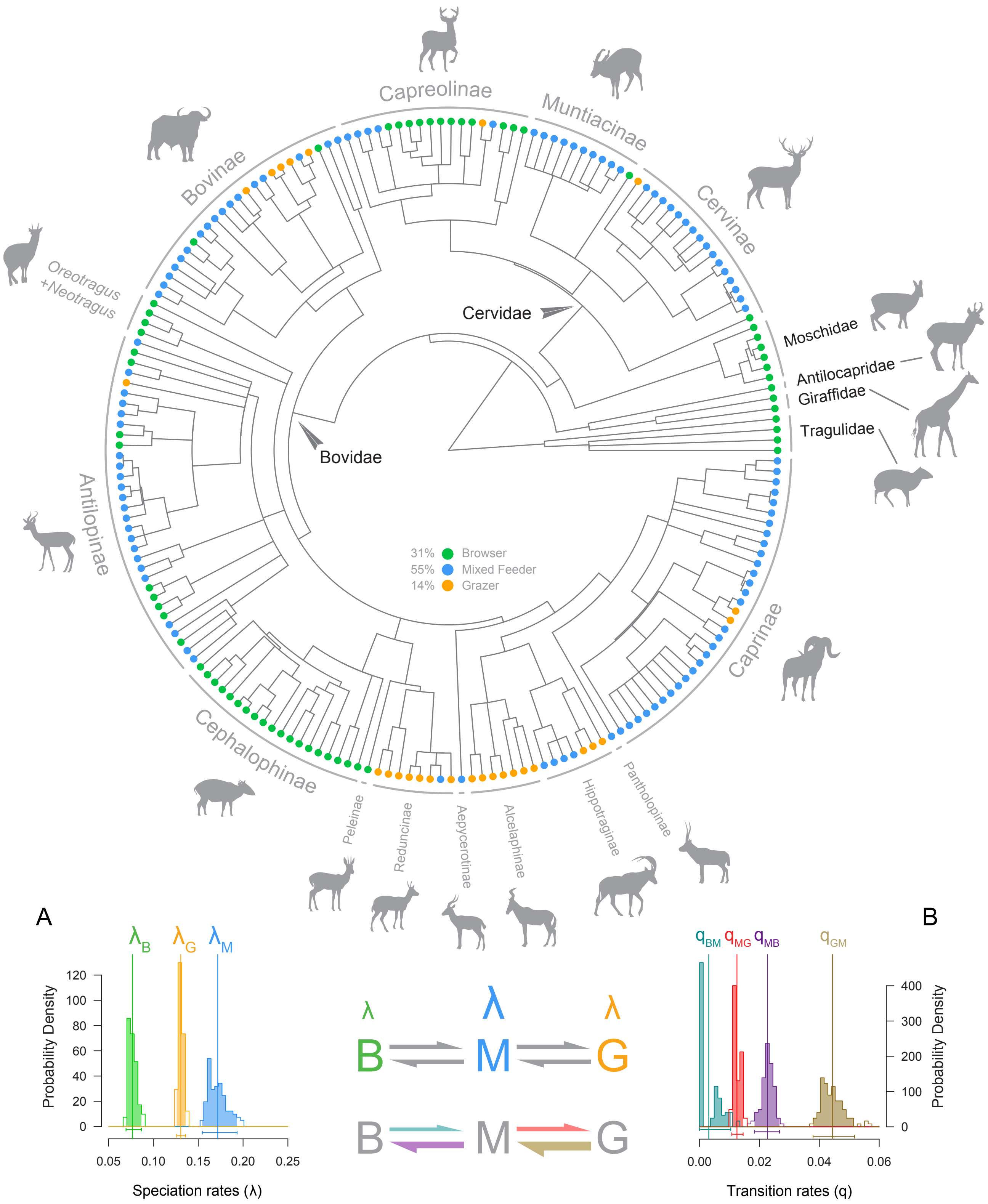


Figure 4.3. Distribution of feeding styles across ruminants and estimated parameters of the MuSSE linear model. Phylogeny of the ruminants and dietary categories for the 197 species shown at the tips. Shown are the names of the main families (black) and subfamilies (grey). Distribution of maximum likelihood parameters over the tree uncertainty; A) reconstructed rates of speciation (λ) and B) transitions rates between feeding styles (q) under MuSSE. B, browser (green); M, mixed-feeder (blue); G, grazer (orange). Rates of diversification and transition between states are colour coded. Mean values are indicated by the solid vertical lines. Bars at the bottom of the distributions and the shaded areas correspond to the 95% confidence intervals.

for the validity of the linear model (ΔAIC=6.484; see Appendix 4.2), though differences of λ_G from λ_{Mx} and λ_B are not (ΔAIC=0.737; ΔAIC=1.105; see Appendix 4.2).

These results agree with new evidence supporting that the dynamics of some open ecosystems may involve a positive feedback for herbivores that both reduce tree survivorship and maintain the biomass burning processes (Beerling and Osborne, 2006). A higher rate of diversification of mixed feeders is also consistent with their abundance in the fossil record of ruminants, evidenced by the study on dental micro- and mesowear of basal giraffids (Solounias et al., 1988; Solounias et al., 2000), dromomerycids (Semprebon et al., 2004), antilocaprids (Semprebon and Rivals, 2007), basal cervids (Solounias and Moelleken, 1994; DeMiguel et al., 2008; DeMiguel et al., 2010) and bovids (Merceron et al., 2004; Merceron et al., 2005).

None of the six time-dependent models produced better fits to the data than the time-constant models (see Table 4.1 and Appendix 4.1). Our time-dependent analyses did not pick up any signal of global temperature on ruminant diversification and do not capture a relationship between temperature and the diversification rates of different types of feeders. Although previous work relied on global paleoclimate records in order to fit diversification models with time windows (Lynch, 2009; Steeman et al., 2009), the paleotemperature information itself is rarely included directly in the model. The paleoclimate record used here (see temperature curve in Fig. 4.1; Zachos et al., 2001), which includes high-latitude sea surface estimates of temperature, depicts the global trend of climate during the last 65 My. This may be the wrong scale: it may be that the tempo and nature of the repercussions of global climate on diet adaptations operate at regional scales where environmental reorganization may be influenced by other factors, such as topography, sea level fluctuations and sea currents (Fortelius et al., 2006; Eronen et al., 2010). If regional models of climate change can be produced, our approach can be repeated to test this.

Ancestral diets reconstruction. Although previous work has tackled the reconstruction of ruminant feeding styles using phylogenetic approaches (DeMiguel et al., 2008), this is the first time that differential diversification under different diet strategies have been taken into account. Distributions of the marginal probabilities of ancestral state reconstructions from the MCMC analyses are shown in Fig. 4.4. The reconstruction depicts a mixed feeding style at the basal node of Bovidae and browser diets at the basal nodes of Giraffidae, Moschidae and Tragulidae. For the ancestral node of Cervidae the estimations point to a mixed

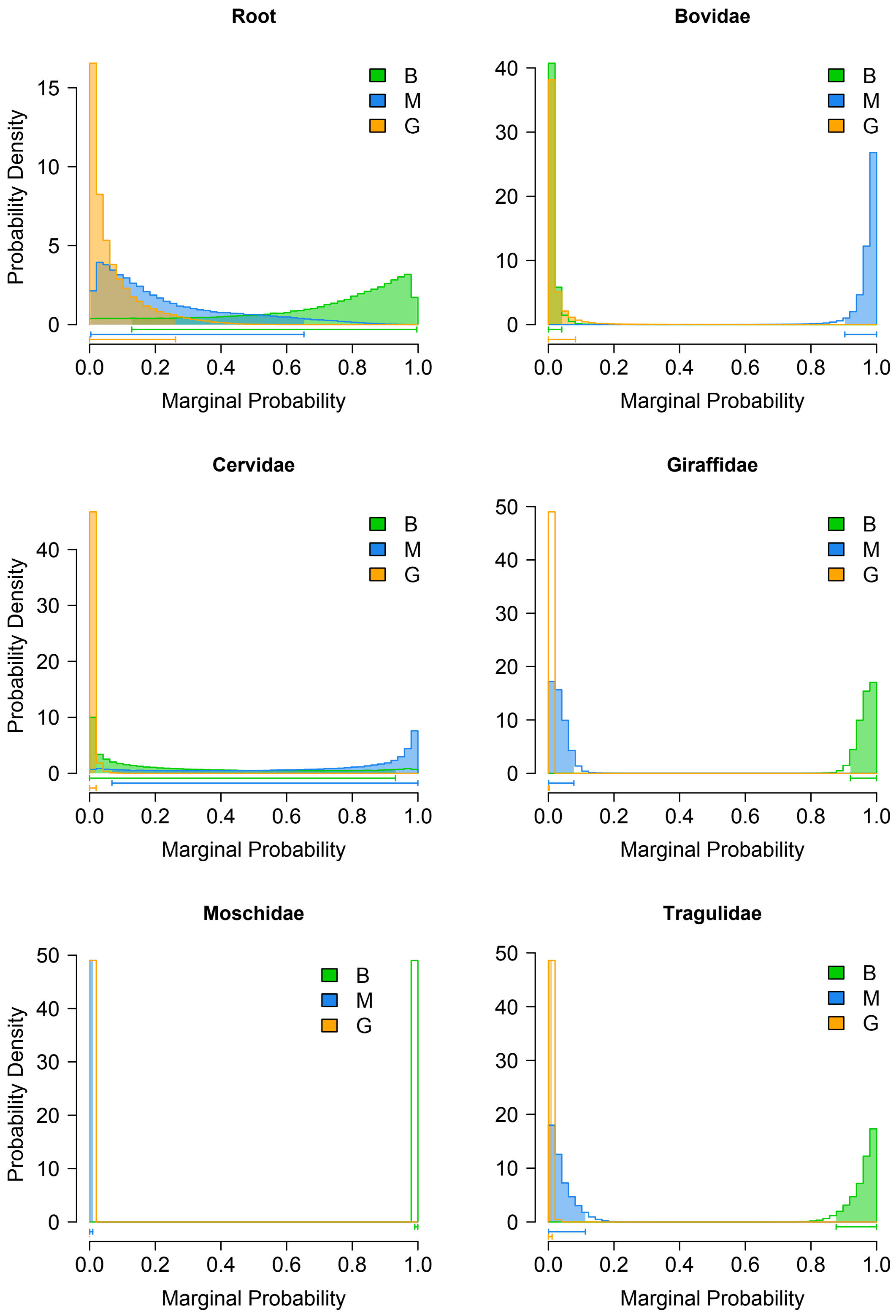


Figure 4.4. Posterior distributions of ancestral diet reconstructions. Posterior distributions of marginal probabilities of the ancestral diet reconstructions after 1500 steps of our MCMC analyses for the root of the tree and five of the ruminant families. B, browser (green); M, mixed-feeder (blue); G, grazer (orange). Bars at the bottom of the distributions and the shaded areas correspond to the 95% confidence intervals.

feeder state in most cases, although in a few cases a browser ancestor might be consistent with the data. Although most of the estimations of marginal probabilities point to a browser diet as the state in the root of the tree, in some cases a mixed feeding ancestor at the root is reasonably consistent with the data as well. These findings provide support for the recent hypothesis proposed by DeMiguel and co-workers (DeMiguel et al., 2008) that posited mixed feeding as the ancestral feeding style of Cervidae. The dietary preference of the oldest Cervidae lineage, *Procervulus*, has been crucial to help elucidate this hypothesis (DeMiguel et al., 2008; DeMiguel et al., 2010). Comparing the diets at the beginning and end of this lineage (e.g., roughly 18 to 14 Mya) shows a marked difference in the incorporation of grass and abrasives, but species were always shifted between the browse-dominated and the grass-dominated end of the mixed feeder continuum. As such, cervids were certainly physiologically able to both browse and graze from their first occurrences, and the morphological expression of this "facultative mixed" condition probably depended on environmental circumstances. The reconstruction supports a mixed feeding style at the basal ingroup node of Bovidae as well. If, as noted above, cervids were originally mixed feeders and taking into account that earliest bovids, such as *Namacerus, Eotragus* or *Tethytragus,* could already have incorporated grass in their diet having a precocious grade of hypsodonty (Azanza and Morales, 1994; Morales et al., 2003), it is also to be expected a variable spectrum of feeding strategies among basal bovid forms.

The basal nodes of Giraffidae, Moschidae and Tragulidae are all reconstructed as browsers (Fig. 4.4). The diet of Giraffidae was until recently thought to be similar to that of the living members, the giraffe (*Giraffa camelopardalis*) and the okapi (*Okapia johnstoni*); all species of Giraffidae have been commonly described as browsers (Franz-Odendaal and Solounias, 2004). Solounias and co-workers (1988; 2000) found, using tooth microwear analyses and premaxillary shape, a higher heterogeneity (browsing, mixed feeding and grazing) in early species than previously thought. As a consequence, we consider that an ancestral mixed condition different to browsing cannot be totally rejected as a feasible ancestral feeding type for this clade.

With respect to the ancestral reconstruction for Moschidae, since nutrient requirements are allometrically related to body size (Bell, 1971; Jarman, 1974; Demment and Van Soest, 1985; Illius and Gordon, 1987), we would expect a tendency for small species such as moschids to be browsers. An age of 6.4 Ma (Late Miocene) has been estimated for the origin of the crown group of living moschids (Hernández Fernández and Vrba, 2005), which is in agreement with fossil evidences (Sánchez et al., 2010). As far as we know, the only information regarding feeding types of moschids close to this epoch concerns *Micromeryx flourensianus* from the Late Miocene locality of Rudabánya (Hungary), and is consistent with fruit-browsing (Merceron et al., 2007). Studies for earlier species attributed to this family (e.g. Late Oligocene *Dremotherium* and *Bedenomeryx,* and Early Miocene *Pomelomeryx*) reveal both browsing and grazing strategies (Kaiser and Rossner, 2007; Novello and Brunet, 2010), although these "moschids" seem to be more related with other pecoran groups (Vislobokova, 1990; Vislobokova, 2000; Sánchez et al., 2010).

Regarding living tragulids, these forms seem to have had a diet consisting of a variety of foods (Solounias and Semprebon, 2002), even including animal matter. The African water chevrotain *Hyaemoschus* is well known to eat occasionally arthropods and small animals (Kingdon, 1982; Barrette, 1987; Geist, 1998). This strategy could be considered as an opportunism feeding, and as the beginning of the facultative mixed feeding mentioned above for higher ruminants. However, no direct evidence on the paleodiet of basal tragulids is yet available from fossils. In general, the ancestral diet reconstructions both agree (for Cervidae and Bovidae) and disagree (for Giraffidae) with evidence from fossil micro- and mesowear. What is needed is a framework where fossil and contemporary data and phylogenetics can be incorporated into the same inference framework.

Conclusions

We found evidence to support that lineages showing facultative mixed feeding have higher speciation rates than those than browse or graze. We interpret that facultative flexible diets may increase the "threat tolerance" of isolated populations during climatic pulses and the subsequent habitat fragmentation. Bidirectional transition rates between "grazer" and "mixed feeding" appear necessary to the fit of the model. In particular, we rule out the classic unidirectional scenario from "browser" to "grazer" through "mixed feeding". Adding global climate information did not improve the fit of the models, probably owing to regional heterogeneity in the effects of global temperature on habitat change. The reconstruction of the paleodiets suggests mixed feeding as the ancestral diet for basal bovids and deer, whereas the ancestral diet of tragulids, giraffids and moschids was estimated to be browsing. We conclude that flexible diets have been of paramount importance for the evolution of ruminants, their diversification during the Neogene and their ecological success.

Literature Cited

Agustí J, Rook L, Andrews P (1999) The Evolution of Neogene Terrestrial Ecosystems in Europe. Cambridge University Press, Cambridge.

Azanza B, Morales J (1994) *Tethytragus* nov. gen. et *Gentrytragus* nov. gen. Deux nouveaux Bovidés (Artiodactyla, Mammalia) du Miocène moyen. Relations phylogénétiques des Bovidés ante-vallésiens. *Proc K Ned Akad Wet Ser C Biol Med Sci* 97:249-282.

Barrette C (1987) The comparative behaviour and ecology of chevrotains, musk deer, and morphologically conservative deer. In *Biology and Management of the Cervidae*, ed Wemmer CM. Smithsonian Institution, Washington, DC. Pp. 200-213

Beerling D, Osborne C (2006) The origin of the savanna biome. *Glob Change Biol* 12(11):2023-2031.

Bell RHV (1971) A grazing system in the Serengeti. *Sci Am* 224:86-93.

Benton MJ (2009) The Red Queen and the Court Jester: Species Diversity and the Role of Biotic and Abiotic Factors Through Time. *Science* 323(5915):728-732.

Bodmer RE (1990) Ungulate frugivores and the browser-grazer continuum. *Oikos* 57(3):319-325.

Brashares J, Garland T, Arcese P (2000) Phylogenetic analyses of coadaptation in behavior, diet, and body size in the African antelope. *Behav Ecol* 11(4):452-463.

Bro-Jorgensen J (2008) Dense habitats selecting for small body size: a comparative study on bovids. *Oikos* 117(5):729-737.

Brown JH, Sibly RM (2006) Life-history evolution under a production constraint. *Proc Natl Acad Sci U S A* 103(47):17597-17599.

Burnham KP, Anderson GC (2002) Model selection and multimodel inference: a practical information-theoretic approach. Springer-Verlag, New York.

Cerling TE, Wang Y, Quade J (1993) Expansion of C4 ecosystems as indicator of global ecological change in the late Miocene. *Nature* 361(6410):344-345.

Clauss M, Frey R, Kiefer B, Lechner-Doll M, Loehlein W, Polster C, Rössner GE, Streich WJ (2003) The maximum attainable body size of herbivorous mammals: morphophysiological constraints on foregut, and adaptations of hindgut fermenters. *Oecologia* 136(1):14-27.

Codron D, Brink JS, Rossouw L, Clauss C (2008) The evolution of ecological specialization in southern African ungulates: competition- or physical environmental turnover? *Oikos* 117(3):344-353.

Delsuc F, Vizcaino SF, Douzery EJP (2004) Influence of Tertiary paleoenvironmental changes on the diversification of South American mammals: a relaxed molecular clock study within xenarthrans. *BMC Evol Biol* 4(11):1-13.

DeMiguel D, Azanza B, Morales J (2010) Trophic flexibility within the oldest Cervidae lineage to persist through the Miocene Climatic Optimum. *Palaeogeogr Palaeoclimatol Palaeoecol* 289(1-4):81-92.

DeMiguel D, Azanza B, Morales J (2011) Paleoenvironments and paleoclimate of the Middle Miocene of central Spain: A reconstruction from dental wear of ruminants. *Palaeogeogr Palaeoclimatol Palaeoecol* 302(3-4):452-463.

DeMiguel D, Fortelius M, Azanza B, Morales J (2008) Ancestral feeding state of ruminants reconsidered: earliest grazing adaptation claims a mixed condition for Cervidae. *BMC Evol Biol* 8(13).

Demment MW, Van Soest PJ (1985) An nutritional explanation for body-size patterns of ruminants and nonruminants herbivores. *Am Nat* 125(5):631-672.

Domingo L, Cuevas-González J, Grimes ST, Hernández Fernández M, Lopez-Martinez N (2009) Multiproxy reconstruction of the palaeoclimate and palaeoenvironment of the Middle Miocene Somosaguas site (Madrid, Spain) using herbivore dental enamel. *Palaeogeogr Palaeoclimatol Palaeoecol* 272(1-2):53-68.

Eronen J, Puolamäki K, Liu L, Lintulaakso K, Damuth J, Janis C, Fortelius M (2010) Precipitation and large herbivorous mammals II: application to fossil data. *Evol Ecol Res* 12(2):235-248.

FitzJohn RG (2010) Quantitative traits and diversification. *Syst Biol* 59(6):619-33.

FitzJohn RG (2011) diversitree: comparative phylogenetic tests of diversification. An R package. http://www.zoology.ubc.ca/prog/diversitree.

Fortelius M (1985) Ungulate cheek teeth: developmental, functional, and evolutionary interrelations. *Acta Zool Fenn* 180:1-76.

Fortelius M, Eronen J, Liu LP, Pushkina D, Tesakov A, Vislobokova I, Zhang ZQ (2006) Late Miocene and Pliocene large land mammals and climatic changes in Eurasia. *Palaeogeogr Palaeoclimatol Palaeoecol* 238(1-4):219-227.

Fortelius M, Solounias N (2000) Functional characterization of ungulate molars using the abrasion-attrition wear gradient: a new method for reconstructing paleodiets. *Am Mus Novit* 3301:1-35.

Franz-Odendaal TA, Solounias N (2004) Comparative dietary evaluations of an extinct giraffid (*Sivatherium hendeyi*) (Mammalia, Giraffidae, Sivatheriinae) from Langebaanweg, South Africa (early Pliocene). *Geodiversitas* 26(4):675-685.

Gagnon M, Chew AE (2000) Dietary preferences in extant African Bovidae. *J Mammal* 81(2):490-511.

Geist V (1998) Early deer and their primitive descendants. In *Deer of the World: Their Evolution Behaivour and Ecology*, ed Geist V. Stackpole Books, Mechanisburg. Pp. 19-37

Gentry AW (2000) The Ruminant radiation. In *Antelopes, Deer, and Relatives: Fossil Record, Behavioral Ecology, Systematics and Conservation*, eds Vrba ES, Schaller GB. Yale University Press, New Haven. Pp. 11-25

Gordon IJ (2003) Browsing and grazing ruminants: are they different beasts? *For Ecol Manag* 181(1-2):13-21.

Hernández Fernández M, Vrba ES (2005) A complete estimate of the phylogenetic relationships in Ruminantia: a dated species-level supertree of the extant ruminants. *Biol Rev* 80(2):269-302.

Hernández Fernández M, Vrba ES (2006) Plio-Pleistocene climatic change in the Turkana Basin (East Africa): Evidence from large mammal faunas. *J Hum Evol* 50(6):595-626.

Hofmann RR (1973) Ruminant Stomach: stomach structure and feeding habits of eastern African game ruminants. East African Literature Bureau, Nairobi.

Hofmann RR, Stewart DRM (1972) Grazer or Browser: a classification based on the stomach structure and feeding habits of East African ruminants. *Mammalia* 36(2):226-240.

Illius AW, Gordon IJ (1987) The allometry of food intake in grazing ruminants. *J Anim Ecol* 56(3):989-999.

Janis C (1982) Evolution in horns in ungulates: ecology and paleoecology. *Biol Rev Camb Philos Soc* 57(2):261-317.

Janis CM (1993) Tertiary mammal evolution in the context of changing climates, vegetation, and tectonic events. *Annu Rev Ecol Syst* 24(1):467-500.

Janis CM, Damuth J, Theodor JM (2000) Miocene ungulates and terrestrial primary productivity: where have all the browsers gone? *Proc Natl Acad Sci U S A* 97(14):7899-7904.

Janis CM, Damuth J, Theodor JM (2004) The species richness of Miocene browsers, and implications for habitat type and primary productivity in the North American grassland biome. *Palaeogeogr Palaeoclimatol Palaeoecol* 207(3-4):371-398.

Janis CM, Damuth J, Theodora JM (2002) The origins and evolution of the North American grassland biome: the story from the hoofed mammals. *Palaeogeogr Palaeoclimatol Palaeoecol* 177(1-2):183-198.

Jarman PJ (1974) The social organization of antelope in relation to their ecology. *Behaviour* 48(3-4):215-267.

Jernvall J, Fortelius M (2002) Common mammals drive the evolutionary increase of hypsodonty in the Neogene. *Nature* 417(6888):538-540.

Johnson WE, Eizirik E, Pecon-Slattery J, Murphy WJ, Antunes A, Teeling E, O'Brien SJ (2006) The late radiation of modern Felidae: a genetic assesement. *Science* 311(5757):73-77.

Kaiser TM (2003) The dietary regimes of two contemporaneous populations of *Hippotherium primigenium* (Perissodactyla, Equidae) from the Vallesian (Upper Miocene) of Southern Germany. *Palaeogeogr Palaeoclimatol Palaeoecol* 198(3-4):381-402.

Kaiser TM, Rossner GE (2007) Dietary resource partitioning in ruminant communities of Miocene wetland and karst palaeoenvironments in Southern Germany. *Palaeogeogr Palaeoclimatol Palaeoecol* 252(3-4):424-439.

Kingdon J (1982) East African Mammals: An Atlas of Evolution in Africa, Volume 3, Part D:Bovids. University of Chicago Press, Chicago.

Kuhn TS, Mooers AØ, Thomas GH (2011) A simple polytomy resolver for dated phylogenies. *Meth Ecol Evol* doi: 10.1111/j.2041-210X.2011.00103.x.

Langer P (1988) The Mammalian Herbivore Stomach. Comparative Anatomy, Function and Evolution. G. Fisher, New York.

Leethorp JA, Vandermerwe NJ, Brain CK (1994) Diet of *Australopithecus robustus* at Swartkrans from stable carbon isotopic analysis. *J Hum Evol* 27(4):361-372.

Luo ZX (2007) Transformation and diversification in early mammal evolution. *Nature* 450(7172):1011-9.

Lynch VJ (2009) Live-birth in vipers (Viperidae) is a key innovation and adaptation to global cooling during the Cenozoic. *Evolution* 63(9):2457-65.

Maddison WP, Midford PE, Otto SP (2007) Estimating a binary character's effect on speciation and extinction. *Syst Biol* 56(5):701-710.

Merceron G, Blondel C, Brunet M, Sen S, Solounias N, Viriot L, Heintz E (2004) The late Miocene paleoenvironments of Afghanistan as inferred from dental microwear in artiodactyls. *Palaeogeogr Palaeoclimatol Palaeoecol* 207(1-2):143-163.

Merceron G, Bonis L, Viriot L, Blondel C (2005) Dental microwear of fossil bovids from northern Greece: paleoenvironmental conditions in the eastern Mediterranean during the Messinian. *Palaeogeogr Palaeoclimatol Palaeoecol* 217(3-4):173-185.

Merceron G, Schullz E, Kordos L, Kaiser TM (2007) Paleoenvironment of *Dryopithecus brancoi* at Rudabánya, Hungary: evidence from dental meso- and micro-wear analyses of large vegetarian mammals. *J Hum Evol* 53(4):331-349.

Morales J, Soria D, Pickford M, Nieto M (2003) A new genus and species of Bovidae (Artiodactyla, Mammalia) from the early Middle Miocene of Arrisdrift, Namibia, and the origins of the family Bovidae. *Memoir Geol Surv Namibia* 19:371-384.

Mysterud A (1998) The relative roles of body size and feeding type on activity time of temperate ruminants. *Oecologia* 113(3):442-446.

Novello A, Brunet M (2010) Feeding behavior and ecology of the Late Oligocene Moschidae (Mammalia, Ruminantia) from La Milloque (France): evidence from dental microwear analysis. *C R Palevol* 9(8):471-478.

Pagel M (1994) Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proc R Soc B* 255(1342):37-45.

Pérez-Barbería FJ, Gordon IJ, Nores C (2001) Evolutionary transitions among feeding styles and habitats in ungulates. *Evol Ecol Res* 3(2):221-230.

Pickford M, Morales J (1994) Biostratigraphy and palaeobiogeography of East Africa and the Iberian Peninsula. *Palaeogeogr Palaeoclimatol Palaeoecol* 112(3-4):297-322.

Quade J, Cerling TE, Barry JC, Morgan ME, Pilbeam DR, Chivas AR, Lee-Thorp JA, van der Merwe NJ (1992) A 16-Ma record of paleodiet using carbon and oxygen isotopes in fossil teeth from Pakistan. *Chem Geol Isot Geosci Sect* 94(3):183-192.

Sánchez I, Domingo M, Morales J (2010) The genus *Hispanomeryx* (Mammalia, Ruminantia, Moschidae) and its bearing on musk deer phylogeny and systematics. *Palaeontology* 53(5):1023-1047.

Semprebon G, Janis C, Solounias N (2004) The diets of the Dromomerycidae (Mammalia: Artiodactyla) and their response to Miocene vegetational change. *J Vertebr Paleontol* 24(2):427-444.

Semprebon G, Rivals F (2007) Was grass more prevalent in the pronghorn past? an assessment of the dietary adaptations of Miocene to recent Antilocapridae (Mammalia: Artiodactyla). *Palaeogeogr Palaeoclimatol Palaeoecol* 253(3-4):332-347.

Slater GJ, Price SA, Santini F, Alfaro ME (2010) Diversity versus disparity and the radiation of modern cetaceans. *Proc R Soc B* 277(1697):3097-104.

Solounias N, McGraw WS, Hayek L-A, Werdelin L (2000) The Paleodiet of the Giraffidae.

In *Antelopes, Deer, and Relatives: Fossil Record, Behavioral Ecology, Systematics and Conservation*, eds Vrba ES, Schaller GB. Yale University Press, New Haven. Pp. 84-95

Solounias N, Moelleken S (1994) Dietary differences between two archaic ruminant species from Sansan, France. *Hist Biol* 7(3):203-220.

Solounias N, Semprebon G (2002) Advances in the reconstruction of ungulate ecomorphology with application to early fossil equids. *Am Mus Novit* 3366:1-49.

Solounias N, Teaford M, Walker A (1988) Interpreting the diet of extinct ruminants: the case of a non-browsing giraffid. *Paleobiology* 14(3):287-300.

Steeman ME, Hebsgaard MB, Fordyce RE, Ho SY, Rabosky DL, Nielsen R, Rahbek C, Glenner H, Sorensen MV, Willerslev E (2009) Radiation of extant cetaceans driven by restructuring of the oceans. *Syst Biol* 58(6):573-85.

van der Made J, Morales J, Montoya P (2006) Late Miocene turnover in the Spanish mammal record in relation to paleoclimate and the Messinian Salinity Crisis. *Palaeogeogr Palaeoclimatol Palaeoecol* 238(1-4):228-246.

Vislobokova IA (1990) The basic features of historical development and classification of the Ruminantia. *Paleontol J* 4:3-14.

Vislobokova IA (2000) Evolution and Classification of Tragulina (Ruminantia, Artiodactyla). *Paleontol J* 35(sp2):S69-S145.

Vrba ES (1980a) Evolution, species and fossils: how does life evolve? *S Afr J Sci* 76:61-84.

Vrba ES (1980b) The significance of bovid remains as indicators of environment and predation patterns. In *Fossils in the Making. Vertebrate Taphonomy and Paleoecology*, eds Behrensmeyer AK, Hill AP. University of Chicago Press, Chicago. Pp. 247-271

Vrba ES (1987) Ecology in relation to speciation rates: some case histories of Miocene-Recent mammal clades. *Evol Ecol* 1(4):283–300.

Vrba ES (1992) Mammals as a Key to Evolutionary Theory. *J Mammal* 73(1):1-28.

Vrba ES (1993) Turnover-pulses, the Red Queen, and related topics. *Am J Sci* 293A(A):418-452.

Vrba ES (1999) Habita theory in relation to the evolution in African Neogene biota and hominids. In *African Biogeography, Climate Change and Human Evolution*, eds Bromage TG, Schrenk F. Oxford University Press, New York. Pp. 19-39

Vrba ES, Denton GH, Partridge TC, Burckle LH (1995) Paleoclimate and Evolution, with Emphasis on Human Origins. Yale University Press, New Haven.

Vrba ES, Schaller GB (2000) Introducction. In *Antelopes, Deer, and Relatives: Fossil Record, Behavioral Ecology, Systematics and Conservation*, eds Vrba ES, Schaller GB. Yale University Press, New Haven. Pp. 1-8

Waldron A (2010) Lineages that cheat death: surviving the squeeze on range size. *Evolution* 64(8):2278-2292.

Walter A, Hoek HN, Pérez L (1978) Microwear of mammalian teeth as an indicator of diet. *Science* 201(4359):908.

Zachos J, Pagani M, Sloan L, Thomas E, Billups K (2001) Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* 293(5517):686-693.

Appendix 4.1

Literature used for assessing the dietary strategy of every ruminant species

Barrette C (1985) Antler eating and antler growth in wild Axis deer. *Mammalia* 49(4):491-499.

Barrette C (1987) The comparative behaviour and ecology of chevrotains, musk deer, and morphologically conservative deer. In *Biology and Management of the Cervidae,* ed Wemmer CM. Smithsonian Institution, Washington, DC. Pp. 200-213

Bell RHV (1971) A grazing system in the Serengeti. *Sci Am* 224:86-93.

Bergerund AT (1972) Food habits of the Newfoundland caribou. *J Wildl Manag* 36(3):913-923.

Bodmer RE (1989) Ungulate biomas in relation to feeding strategy within Amazonian forest. *Oecologia* 81(4):547-550.

Bodmer RE (1990) Ungulate frugivores and the browser-grazer continuum. *Oikos* 57(3):319-325.

Branan WV, Wekhoven MC, Marchinton RL (1985) Food habits of brocket and white-tailed deer in Suriname. *J Wildl Manag* 49(4):972-976.

Bugalho MN, Milne JA, Racey PA (2001) The foraging ecology of red deer (*Cervus elaphus*) in a Mediterranean environment: is a larger body size advantageous? *J Zool* 255(3):285-289.

Bullock DJ (1985) Annual diets of hill sheep and feral goats in southern Scotland. *J Appl Ecol* 22(2):423-433.

Campos-Arceiz A, Takatsuki S (2005) Food habits of sika deer in the Shiranuka Hills, eastern Hokkaido: a northern example from the north–south variations in food habits in sika deer. *Ecol Res* 20(2):129-133.

Chapman JA, Feldhammer GA (1982) Wild mammals of North America: Biology, Management, and Conservation. The Johns Hopkins Univiversity Press, Baltimore.

Du Toit JT (1990) Feeding-height stratification among African browsing ruminants. *Afr J Ecol* 28(1):55-61.

Estes RD (1991) The Behavior Guide to African Mammals: Including Hoofed Mammals, Carnivores, Primates. University of California Press, Berkeley and Los Angeles, California.

Geist V (1977) Mountain Sheep: a Study in Behavior and Evolution. University of Chicago Press, Chicago.

Geist V (1998) Deer of the World: Their Evolution, Behaviour, and Ecology. Stackpole Books, Mechanisburg, PA.

Gordon IJ, Illius AW (1996) The nutritional ecology of African ruminants: A reinterpretation. *J Anim Ecol* 65(1):18-28.

Hansen RM, Clark RC (1977) Foods of elk and other ungulates at low elevations in northwestern Colorado. *J Wildl Manag* 41(1):76-80.

Hoefs M (1974) Food selection by Dall's sheep (*Ovis dalli dalli* Nelson). In *The Behaviour of Ungulates and its Relation to Management,* eds Geist V, Walther F. IUCN Publications new series, Morges, Switzerland. Pp. 759–786

Hofmann RR (1973) Ruminant Stomach: stomach structure and feeding habits of eastern African game ruminants. East African Literature Bureau, Nairobi.

Hofmann RR (1985) Digestive physiology of the deer-their morphophysiological specialization and adaptation. *R Soc New Zealand Bull* 22:393-407.

Hofmann RR (1989) Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. *Oecologia* 78(4):443- 457.

Homolka M (1996) Foraging strategy of large herbivores in forest habitats. *Folia Zool* 45(2):127-136.

Illius AW, Gordon IJ (1999) The physiological ecology of mammalian herbivores. In *Nutritional Ecology of Herbivores*, eds Jung HJG, Fahey GC. American Society of Animal Science, Savoy, Ill. Pp. 71-96

Janis CM. 1988. An estimation of tooth volume and hypsodonty indices in ungulate mammals, and the correlation of these factors with dietary preference. Pp. 367-387. In Russell D, Santoro J, Sigogneau-Russell D, eds. Teeth Revisited: Proceedings of the VII International Symposium on Dental Morphology. París.

Kingdon J (1982) East African Mammals: An Atlas of Evolution in Africa, Volume 3, Part D:Bovids. University of Chicago Press, Chicago.

Labaô-Tello J, Van Gelder RJ (1975) The natural history of the nyala (*Tragelaphus angasi*) (Mammalia Bovidae). *Bull Am Mus Nat Hist* 155:319-386.

Laca EA, Demment MW (1996) Foraging strategies of grazing animals. In *The Ecology and Management of Grazing Systems*, eds Hodgson J, Illius AW. CAB International, Wallingford. Pp. 137-158

Liu B, Jiang Z (2002) Foraging strategy of Przewalski's gazelle. *Acta Zool Sin* 48(3):309-316.

Lu CD (1988) Grazing behavior and diet selection of goats. *Small Ruminant Res* 1(3):205-216.

Lumpkin S, R. KK (1984) Cephalophus sylvicultor. *Mamm Sp* 225(225):1-7.

McDonald JN (1981) North American Bison: their classification and evoltion. University of California Press, Berkeley.

McNaughton SJ, Georgiadis NJ (1986) Ecology of African grazing and browsing mammals. *Annu Rev Ecol Syst* 17:39-65.

Milne JA (1991) Diet selection by grazing animals. *Proc Nutr Soc* 50(1):77-85.

Nowak RM (1991) Walker's mammals of the world. The Johns Hopkins Univiversity Press, Baltimore, MD.

Nudds TD (1980) Forage "preference": theoretical consideration of diet selection by deer. *J Wildl Manag* 44(3):735-740.

O'Gara BW, Yoakum JD (2004) Pronghorn ecology and management. University Press of Colorado, Boulder.

Papachristou TG, Dziba LE, Provenza FD (2005) Foraging ecology of goats and sheep on wooded rangelands. *Small Ruminant Res* 59(2):141-156.

Pérez-Barbería FJ, Gordon IJ (1998) The influence of sexual dimorphism in body size and mouth morphology on diet selection and sexual segregation in cervids. *Acta Vet Hung* 46(3):357-367.

Pérez-Barbería FJ, Gordon IJ, Nores C (2001) Evolutionary transitions among feeding styles and habitats in ungulates. *Evol Ecol Res* 3(2):221-230.

Schaller G, Qitao T, Wenshi P, Zisheng HJ, Heming S (1986) Feeding behavior of Sichuan takin (*Budorcas taxicolor*). *Mammalia* 50(3):311-322.

Schaller GB (1967) The Deer and the Tiger. University Chicago Press, Chicago.

Schaller GB (1977) Distribution. In *Mountain Monarchs: Wild Sheep and Goats of the Himalaya.*, ed Schaller GB. University of Chicago Press, Chicago. Pp. 45-82

Seligman NG (1996) Management of Mediterranean Grasslands. In *The Ecology and Management of Grazing Systems*, eds Hodgson J, Illius AW. CAB International. Pp. 359-392

Sheng HL (1992) The Deer in China. East China Normal University Press, Shanghai.

Sinclair ARE (1977) The African buffalo: a study of resource limitation of populations. University Chicago Press, Chicago

Stronge DC, Fordham RA, Minot EO (1997) The foragins ecology of feral goats *Capra hircus* in the Mahoenui Giant Weta Reserve, southern King Country, New Zeland. *N Z J Ecol* 21:81-88.

Tener JS (1965) Muskoxen in Canada: a biological and taxonomic review. Canadian Wildlife Service, Ottawa (Canada).

Van Soest PJ (1994) Nutritional Eecology of the Ruminant. Comstock Publishing, Ithaca, New York.

Appendix 4.2

Contrained parameters	*Model*	*Mean lnLH*	*param*	*Mean AIC*	*Min Lh*	*Max Lh*	*Min AIC*	*Max AIC*	*ΔAIC*
Transition rates (q)	B<M<>G	-701.66	9	1421.32	-709.15	-694.04	1406.08	1436.31	-2.14
	linear	**-701.73**	**10**	**1423.46**	**-709.15**	**-694.04**	**1408.08**	**1438.31**	**0.00**
	all q equal	-705.01	7	1424.03	-713.07	-698.61	1411.23	1440.14	0.57
	q23=q32	-703.20	9	1424.40	-710.66	-696.03	1410.06	1439.32	0.94
	q12=q21	-703.44	9	1424.88	-711.53	-696.72	1411.44	1441.06	1.42
	unconstrained	**-701.52**	**12**	**1427.04**	**-708.89**	**-694.04**	**1412.08**	**1441.79**	**3.58**
	B<M>G	-709.79	8	1435.57	-717.95	-702.33	1420.67	1451.89	12.11
	B<>M>G	-709.88	9	1437.75	-717.95	-702.33	1422.67	1453.89	14.29
	B<M<G	-712.77	8	1441.54	-720.65	-702.79	1421.58	1457.31	18.08
	unidirectional	**-722.65**	**8**	**1461.31**	**-731.88**	**-714.09**	**1444.18**	**1479.76**	**37.85**
Diversification rate (λ)	λM=λG	-702.18	9	1422.35	-709.32	-694.84	1407.68	1436.64	-1.11
	all λ free (linear)	**-701.73**	**10**	**1423.46**	**-709.15**	**-694.04**	**1408.08**	**1438.31**	**0.00**
	λB=λG	-703.10	9	1424.20	-710.28	-695.29	1408.58	1438.57	0.74
	all λ equal	-706.09	8	1428.17	-712.24	-698.67	1413.35	1440.48	4.71
	λB=λM	-705.97	9	1429.94	-712.26	-698.57	1415.14	1442.51	6.48

Likelihood and AIC (Akaike Information Criterion) values obtained for the constrained models compared in this study. Models are ordered by mean ΔAIC rank. Given are the mean, minimum and maximum likelihood and AIC values obtained from the posterior distributions. Models used for testing evolutionary models (see methods) are shown in bold letters. Constrained models used to assess the contribution of parameters to improve the fit of the evolutionary models are shown in regular letters.

5

Biomic specialization and speciation rates in ruminants (Cetartiodactyla, Mammalia): a test of the resource-use hypothesis at the global scale

> *[…] we are far from a complete understanding of the causes and consequences of rarity and extinction, and therefore of the processes of evolution.*
>
> *- Alexander Harcourt -*

Abstract

The resource-use hypothesis proposed by Elisabeth S. Vrba predicts that specialist species have higher speciation and extinction rates than generalists because they are more susceptible to environmental changes and vicariance. In this work, we test some of the predictions derived from this hypothesis on the 197 exant and recently extinct species of Ruminantia (Cetartiodactyla, Mammalia) using the biomic specialization index (BSI) of each species, which is based on its distribution within different biomes. We ran 10000 Monte Carlo simulations of our data in order to get a null distribution of BSI values against which to contrast the observed data. Additionally, we drew on a supertree of the ruminants and a phylogenetic likelihood-based method (QuaSSE) for testing whether the degree of biomic specialization affects speciation rates in ruminant lineages. Our results are consistent with the predictions of the resource-use hypothesis, which foretells a higher speciation rate of lineages restricted to a single biome (BSI = 1) and higher frequency of specialist species in biomes that underwent high degree of contraction and fragmentation during climatic cycles. Bovids and deers present differential specialization across biomes; cervids show higher specialization in biomes with a marked hydric seasonality (tropical deciduous woodlands and schlerophyllous woodlands), while bovids presents higher specialization in a greater variety of biomes. This might be the result of divergent physiological constraints as well as a different biogeographic and evolutionary history.

Introduction

Species biogeography is influenced by present environmental conditions, but it is also true that large-scale processes in the past have a major impact on the distribution of the living forms that we see today (Vrba, 1992; Janis, 1993; Pickford and Morales, 1994; Vrba et al., 1995; Gentry, 2000; Van Dam et al., 2006; Eronen et al., 2009). The changing connections among land masses, the vicariance due to the creation and alternate expansion-contraction of climatic dominions as well as the establishment of geographic barriers have influenced the way lineages evolved during millions of years. Some researchers have identified such large-scale processes as major forces triggering faunal turnovers and some of the hypotheses based on these ideas were gathered together in what is called the habitat theory (Vrba, 1992, 1995, 1999). The resource-use hypothesis, which is included as part of this theory, suggests that the degree of specialization of species has an important role on the differential evolution of clades (Vrba, 1980a, 1987, 1993). Specialist species are more prone to suffer limitation of their resources and, thus, they are more susceptible to environmental changes, vicariance and strong directional selection. Accordingly, this hypothesis predicts higher speciation and extinction rates in specialist species. On the other hand, generalists are expected to present higher flexibility, which allows them to survive through climatic cycles and to maintain slow speciation rates through time.

Different indices of ecological specialization have been proposed (Eeley and Foley, 1999; Harcourt, 2000; Harcourt et al., 2002; Hernández Fernández and Vrba, 2005b). However, some of these measures are difficult to apply in global comparisons and may be biased for rare and less-sampled taxa (Doherty and Harcourt, 2004). Following previous works (Vrba, 1987; Hernández Fernández and Vrba, 2005b; Moreno Bofarull et al., 2008), we consider the biomic specialization as a reliable measure of the ecological specialization of a species, which has been proved to be applicable in global scale studies like the study herein. We consider a species to be stenobiomic or eurybiomic according to the number of biomes it is able to inhabit, which are characterized by gross vegetation physiognomy. Stenobiomic lineages are predicted to inhabit a particular biome and, thus, a relatively narrow range of vegetation physiognomy, which makes them more prone to suffer vicariance due to climatic forcing and the subsequent fragmentation of that biome. Conversely, generalist species can use resources from a wider range of biomes, which allows them to overcome climatic changes and habitat fragmentation. Here, the term “resource” encompasses a wide range of physical and biotic factors, including moisture, temperature, substrate, vegetation

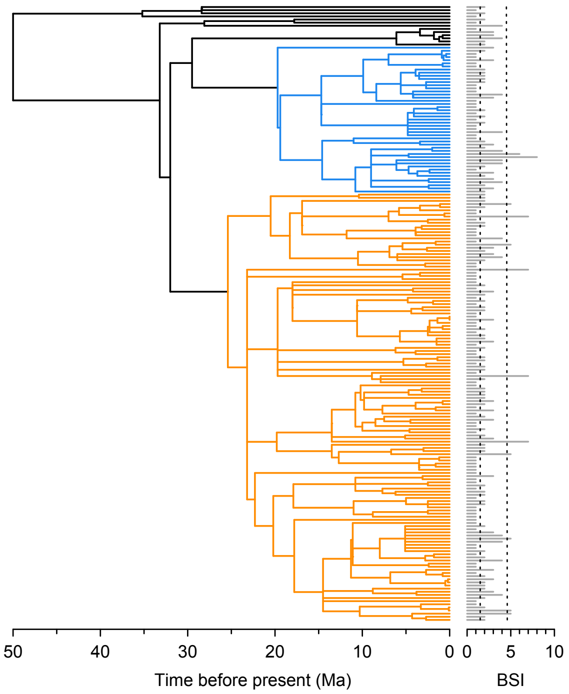


Figure 5.1. Phylogenetic tree of the ruminants and BSI values distribution. Shown with colours are the most speciouse families: Cervidae (blue) and Bovidae (orange). BSI is shown by the horizontal bar for each species. The vertical dashed lines indicate the limits between stenobiomic species (BSI= 1), semi-eurybiomic species (1< BSI< 5) and extreme eurybiomic species (BSI ≥ 5).

cover, food items and any other environmental components usable by an organism (Vrba, 1987, 1993; Hernández Fernández and Vrba, 2005b).

The resource-use hypothesis was originally tested with the fossil record of the African large mammals (Vrba, 1980a, 1987). Following the work by Hernández Fernández and Vrba (2005b), we have compared the observed biomic specialization index (BSI), which is the number of biomes occupied by a species, with a null distribution of BSI from Monte Carlo simulations . This method allows testing hypothesis connecting ecological specialisation and macroevolutionary processes while avoiding the incompleteness of the fossil record and has been previously applied to the mammalian assemblages of Africa and South America (Hernández Fernández and Vrba, 2005b; Moreno Bofarull et al., 2008). These works offered support for several predictions of the resource-use hypothesis: 1) given a clade, we should find more specialist species than expected by chance, due to their higher rates of diversification; 2) we should expect higher specialization of species inhabiting biomes that underwent high degree of fragmentation and contraction during climatic cycles, since populations in those biomes are subject to a high incidence of vicariance; 3) different clades are expected to show different degrees of specialization, because they may be adapted to very different climates and environments. Here, we tested these predictions on the 197 extant and recently extinct species of ruminants because this group is of major interest in studies on macroevolution and ecology. Ruminants are distributed world-wide, naturally occurring in five continents, present a high diversity of ecological adaptations and inhabit in all the world biomes. Therefore, we were able to compare, for the first time, the biomic specialization among all the biomes at the global scale. We also tested the first prediction, which links higher speciation rates to more specialized lineages, by applying a phylogenetic likelihood-based method (QuaSSE) that fits quantitative-trait-dependent models of speciation-extinction on the BSI dataset of the ruminants and the phylogeny of the group (Fig. 5.1).

We found that a high frequency of ruminant species is restricted to a single biome as a consequence of high speciation rates in stenobiomic lineages and higher specialization of species inhabiting biomes that underwent a high degree of fragmentation and contraction during climatic cycles. Finally, our results show a disparate specialization across biomes in bovid and cervids owing to their different biogeographic histories, resource requirements and adaptations.

Material and Methods

Data

For each species, we computed the biomic specialization index (BSI) developed by Hernández Fernández and Vrba (2005b), which is the number of biomes that they inhabit. We follow the biome classification of Walter (1970), summarized in Table 5.1. The starting point of our data set consists of the complete geographical distributions of all 197 species of the suborder Ruminantia, encompassing living species and those that became extinct in the last two centuries (Answell, 1971; Corbet, 1978; Eisenberg, 1989; Corbet and Hill, 1992; Redford and Eisenberg, 1992; Grubb, 1993; Kingdom, 1997; Mitchell-Jones et al., 1999; Eisenberg and Redford, 2000; IUCN, 2008). Distribution areas due to introduction by humans were omitted. For taxonomic consistency, we have followed the species-level taxonomy proposed by Wilson and Reeder (1993). The number of climatic zones inhabited by a species was assessed by the relative size of its geographic range in relation to the distribution of the different biomes and climatic dominions (Hernández Fernández, 2001). If 15% or more of the geographical range of a species is situated within a climate zone, the species was recorded as present in that climate zone. Since some climatic dominions are small enough to comprise less than 15% of the total distribution ranges of species with large range sizes, a species was also recorded as present in a specific climate zone if it inhabits 50% or more of

Biomes
I. Evergreen tropical rainforest
II. Tropical deciduous woodland
II/III. Savannah
III. Sub-tropical desert
IV. Sclerophyllous woodland and shrubland
V. Temperate evergreen forest
VI. Broad-leaf deciduous forest
VII. Steppe / cold desert
VIII. Boreal coniferous forest (taiga)
IX. Tundra

Table 5.1. Biome typology used in this work (modified from Walter (1970)).

one climatic dominion. Furthermore, since altitudinal gradients represent habitat series analogous to that of biomes, vegetation belts in mountains were also borne in mind when estimating the biome occurrence of species. We define stenobiomic species (biomic specialists) as those species inhabiting only one biome (BSI=1). In turn, eurybiomic species (biomic generalists) are usually defined as those that occupy two or more biomes. Since species inhabiting five or more biomes must face very assorted environment conditions in terms of temperature and rainfall, Hernández Fernández and Vrba (2005b) proposed that this latter category may be subdivided in two other groups: "semi-eurybiomic species" including species with $1 < \text{BSI} < 5$, and "extreme eurybiomic species" with species with $\text{BSI} \geq 5$.

Analyses

Monte Carlo Simulations. In order to test whether a random process may generate significantly more biomic specialists than generalists we conducted Monte Carlo simulations. Randomization is an appropriate method to prove the predictions of the resource-use hypothesis (Hernández Fernández and Vrba, 2005b; Moreno Bofarull et al., 2008), assuming as a null hypothesis that observed presences-absences of each species are to be found randomly among biomes. Nevertheless, each biome particular features have an effect on species richness, in such a way that there is no reason to consider that all the biomes must have the same number of species. Therefore, we conducted a simulation that places species in biomes randomly while constrains the observed species richness in each biome (Hernández Fernández and Vrba, 2005b). BSI null distributions and frequencies were obtained from 10000 random draws. The probability (*p-value*) that a BSI value could obtain by chance a percentage greater than the observed is obtained from the proportion of null values that are above the observed percentage; alternatively,

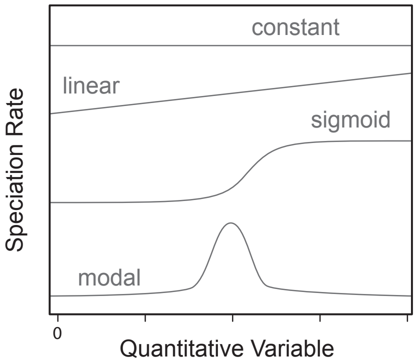


Figure 5.2. Quantitative-trait-dependent speciation models (QuaSSE). Representation of the maximum likelihood speciation rate models compared with QuaSSE, where the rate of speciation changes as a function of the variable under study, BSI in our case.

the fraction of null values below the observed is the probability of obtaining a percentage of species less than the observed value. We used R (R Development Core team, 2008) to perform all the analyses.

BSI-dependent Speciation Models. New likelihood-based methods provide a novel approach for identifying trait-dependent speciation rates (Maddison et al., 2007; FitzJohn, 2010). We explored the influence of biomic specialization on the speciation rates of the ruminants by applying quantitative-state speciation and extinction models (QuaSSE; FitzJohn, 2010), where BSI was treated as a quantitative variable, on the phylogenetic tree of the group (see Fig. 5.1; Hernández Fernández and Vrba, 2005a). Although extinction rates recovered from extant species phylogenies are largely underestimated (Rabosky, 2010) methods as the one used here provide accurate estimations of speciation rates (FitzJohn, 2010). Using QuaSSE we compared different models where BSI affects the speciation rate following constant, linear, sigmoidal and modal functions (Fig. 5.2). QuaSSE also allows to identify a directional or deterministic component in character evolution through the history of the group that may increase the fit of the model. Since QuaSSe requires the tree to be completely resolved, the polytomies were broken and a distribution of 100 fully dichotomous trees was produced following Kuhn et al. (2011). The models were run over each of the 100 trees and compared using their AIC scores (Burnham and Anderson, 2002), which measures the goodness of the fit of a statistical model while penalizing the number of parameters (the complexity) of the model. The best model gets the lower AIC score, and the fit of a model is significantly better than others when the different in their AIC scores is greater than two units. The estimate of parameters was assessed from the 100 trees distributions. We performed QuaSSE in the statistical software R (R Development Core team, 2008) as implemented in the diversitree library (FitzJohn, 2009).

Results

Distribution of the Biomic Specialization Index (BSI)

The frequency distribution of BSI for ruminants is powerfully right-skewed (Fig. 5.3). Mean BSI among ruminants is 2.10, with 79 species (40.1%) inhabiting only one biome and 69 (35.03%) inhabiting two biomes. Conversely, only the 6.10% of the species inhabits five or more different biomes (BSI≥5), being *Odocoileus* the only genus inhabiting eight different biomes. The distribution of the proportions of species with different BSI derived from 10000 randomisations can be seen in detail in Figure 5.4. Our results pinpoint a significantly higher proportion of biomic specialist species (BSI=1) than expected by random draws (Table 5.1). The frequency of species with BSI = 2 is non significantly different than the expected by chance, while the proportion of species inhabiting three (BSI = 3) and four biomes (BSI = 4) is significantly lower than expected. We found non-significant differences in proportions for species with BSI = 5 and 6 between the observed values and those yielded by the 10000 simulations. Nevertheless, the frequency of ruminant species with BSI = 7 is significantly higher than expected by chance. Finally, although the Monte Carlo simulations yielded small percentages of species inhabiting nine and ten biomes, there is no statistically significant difference with the absence of these highly eurybiomic species in our data set (Table 5.1).

The comparison between the real distribution of BSI values in Cervidae and Bovidae and the distribution obtained from 10000 Monte Carlo simulations, are shown in Figure 5.5 and Tables 5.2 and 5.3. Mean BSI in Cervidae is 2.25 and 2.04 in Bovidae. Both families present a right-skewed distribution of BSI and a significantly higher proportion of stenobiomic species (BSI = 1) than the expectations from random draws, which follow the observed trend of Ruminantia. Bovidae present a slightly higher percentage of specialists (42.3%) than Cervidae (36.2%). The proportion of species with BSI = 2 does not differ from what we would expect by chance, while species inhabiting three biomes are more scant than expected in both clades. Bovidae has higher proportion of extreme eurybiomic species (BSI ≥ 5) than Cervidae (7.3% against 4.26%).

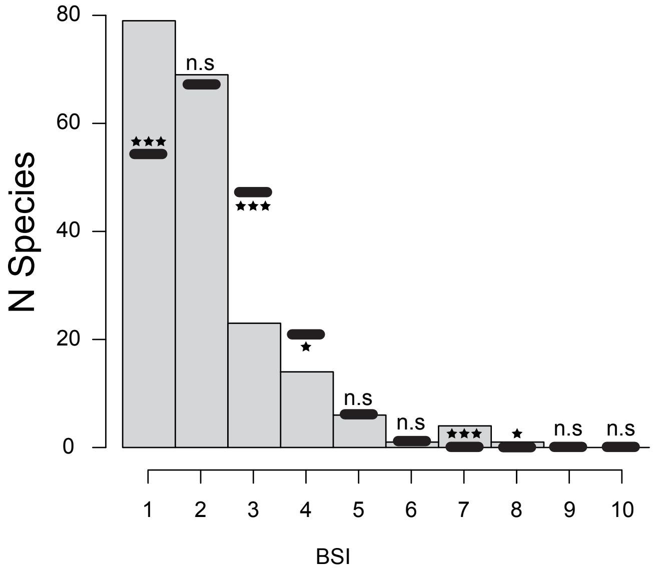


Figure 5.3. Frequency distribution of biomic specialization index (BSI) for ruminants. Bars represent observed distribution of BSI in Ruminantia. Lines show the average number of species from 10000 Monte Carlo Simulations (Table 5.2). ***, $p < 0.001$; **, $0.01 > p > 0.001$; *, $0.05 > p > 0.01$; n.s., not significant.

BSI	%	Monte Carlo Analysis			
		Mean %	Std.dev	Range	*p*
1	40.10	27.47	2.60	18.00 - 38.00	<0.001
2	35.00	34.16	3.50	22.00 - 49.00	0.665
3	11.70	23.94	3.10	13.00 - 36.00	*<0.001*
4	7.10	10.63	2.10	2.80 - 19.00	*0.045*
5	3.05	3.11	1.20	0.00 - 7.90	*0.983*
6	0.51	0.61	0.56	0.00 - 3.40	*0.470*
7	2.03	0.08	0.20	0.00 - 1.70	<0.001
8	0.51	0.01	0.06	0.00 - 1.10	0.011
9	0.00	0.00	0.01	0.00 - 0.57	1.000
10	0.00	0.00	0.01	0.00 - 0.56	1.000

Table 5.2. Observed and simulated BSI values for ruminants. Frequencies of ruminant species in each BSI and comparison with 10000 Monte Carlo simulations. %, proportion of the total number of species (197); p, probability of species in the simulations being greater than or equal to (plain) or lower than or equal to (italics) the observed proportion in ruminants.

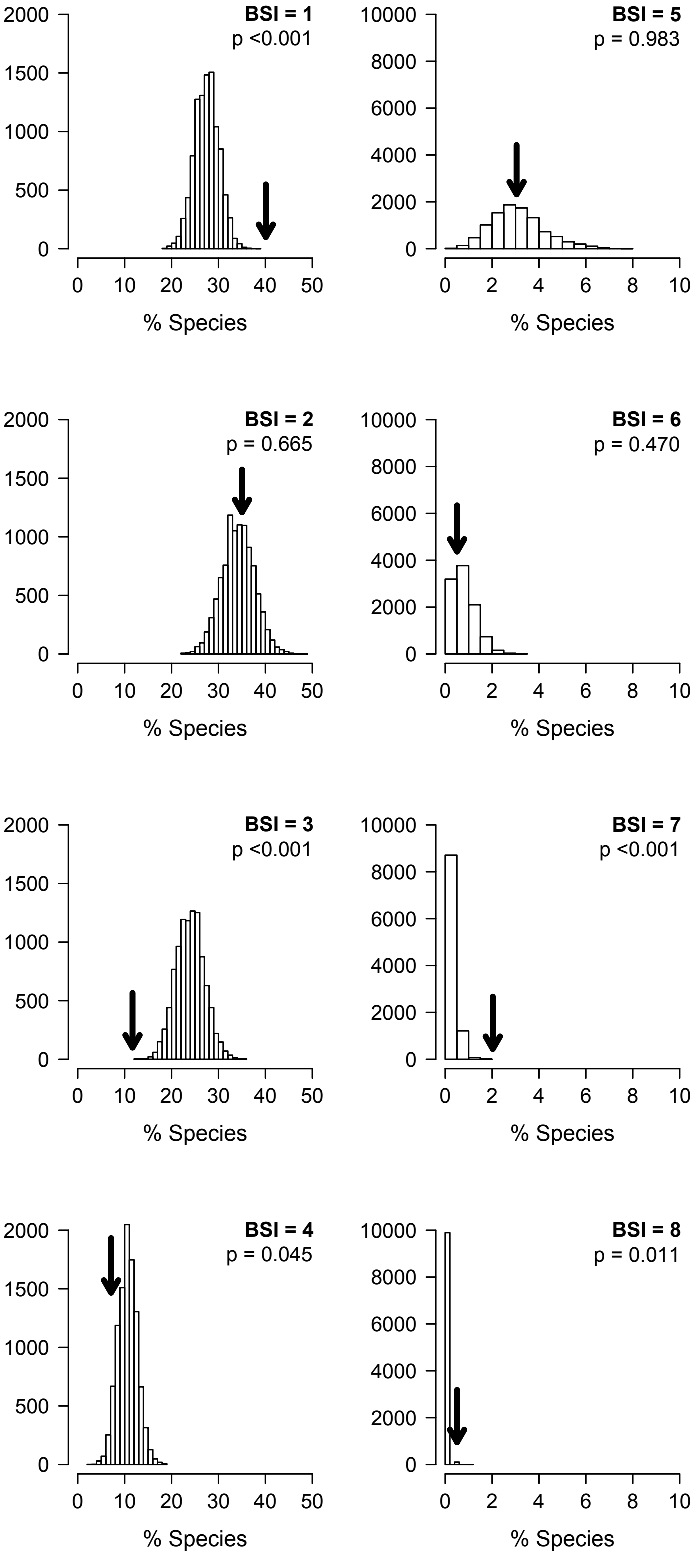


Figure 5.4. Distributions of the proportion of species with different BSI from 10000 Monte Carlo simulations drawn from the data for 197 species of ruminants. Arrows show the observed values in Ruminantia. Note the change of scale in the figures on the right side.

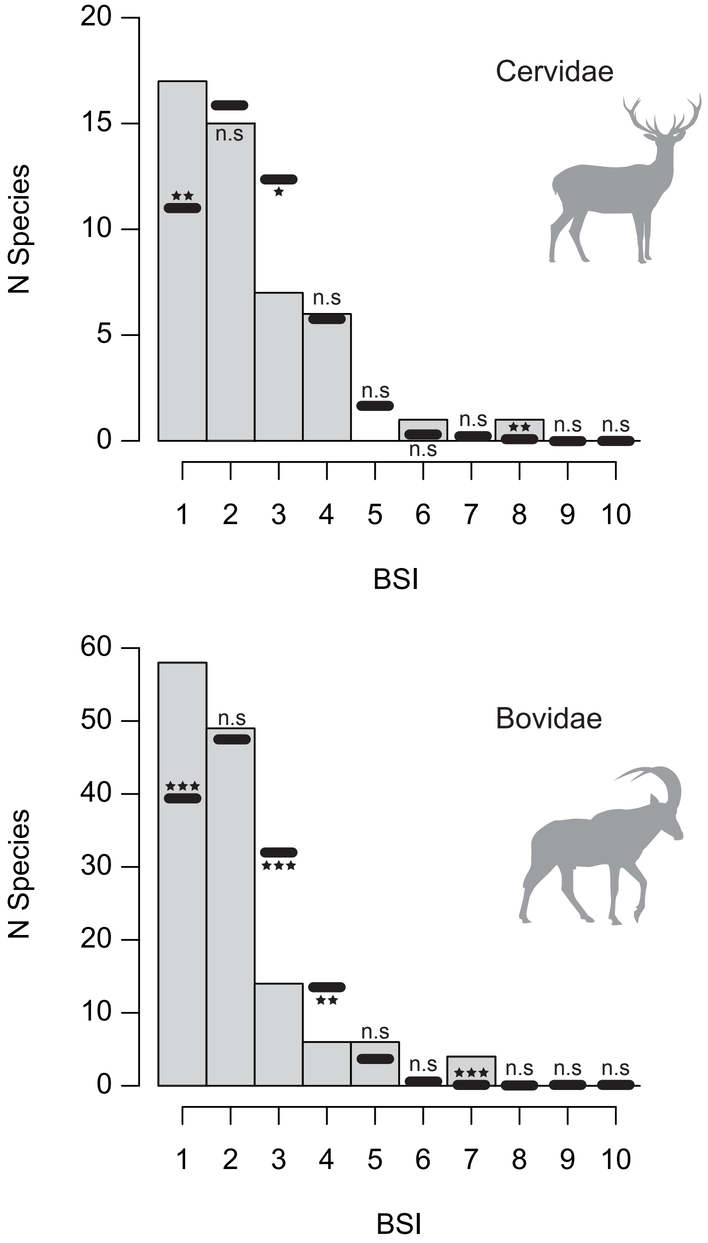


Figure 5.5. Frequency distribution of biomic specialization index (BSI) for Cervidae and Bovidae. Bars represent observed distribution of BSI. Lines show the average number of species from 10000 Monte Carlo Simulations (Table 5.3 and 5.4). ***, p < 0.001; **, 0.01 > p > 0.001; *, 0.05 > p > 0.01; n.s., not significant.

BSI	%	Monte Carlo Analysis			
		Mean %	Std.dev	Range	*p*
1	36.20	23.47	5.00	2.50 - 43.00	0.005
2	31.90	33.73	6.90	11.00 - 63.00	*0.709*
3	14.90	26.28	6.40	4.30 - 54.00	*0.031*
4	12.80	12.23	4.40	0.00 - 32.00	0.832
5	0.00	3.58	2.60	0.00 - 16.00	*0.172*
6	2.13	0.63	1.20	0.00 - 9.50	0.320
7	0.00	0.08	0.42	0.00 - 4.90	*0.968*
8	2.13	0.00	0.09	0.00 - 2.40	0.001
9	0.00	0.00	0.00	0.00 - 0.00	1.000
10	0.00	0.00	0.00	0.00 - 0.00	1.000

Table 5.3. Observed and simulated BSI values for Cervidae. Frequencies of species within Cervidae in each BSI and comparison with 10000 Monte Carlo simulations. %, proportion of the total number of species (47); p, probability of species in the simulations being greater than or equal to (plain) or lower than or equal to (italics) the observed proportion in Cervidae.

BSI	%	Monte Carlo Analysis			
		Mean %	Std.dev	Range	*p*
1	42.30	28.77	3.20	16.00 - 40.00	< 0,001
2	35.80	34.63	4.20	18.00 - 51.00	0.656
3	10.20	23.39	3.60	10.00 - 38.00	*< 0,001*
4	4.38	9.88	2.40	1.50 - 20.00	*0.007*
5	4.38	2.76	1.40	0.00 - 10.00	0.128
6	0.00	0.51	0.62	0.00 - 4.10	*0.520*
7	2.92	0.06	0.22	0.00 - 1.70	< 0,001
8	0.00	0.00	0.06	0.00 - 0.86	*0.994*
9	0.00	0.00	0.01	0.00 - 0.83	*1.000*
10	0.00	0.00	0.00	0.00 - 0.00	1.000

Table 5.4. Observed and simulated BSI values for Bovidae. Frequencies of species within Bovidae in each BSI and comparison with 10000 Monte Carlo simulations. %, proportion of the total number of species (137); p, probability of species in the simulations being greater than or equal to (plain) or lower than or equal to (italics) the observed proportion in Bovidae.

Effect of BSI on speciation rates.

Table 5.4 shows the mean AIC scores for the competing models. Among trait-based speciation and extinction models, there was a strong support for a model in which the speciations rates were inferred to decrease with decreasing biomic specialization (increasing BSI) following a sigmoidal function (Table 5.4). This model obtained the best AIC score. Sigmoid models with a small directional component of character evolution and without such deterministic term were alternatively chosen as best model along the 100 cases (ΔAIC=0.4) and, therefore, does not allow us to determine the presence or absence of directional evolution of biomic specialization through the history of ruminants. The best model depicts a mean speciation rate of 0.17 for lineages with BSI=1 that starts to decrease immediately reaching a inflection point at BSI=4.6 and dropping to speciation rates of around 0.018 in "extreme eurybiomic" lineages (Fig. 5.6).

Proportion of biomic specialists in each biome

There are five biomes with higher proportions of specialist species than expected by chance (Table 5.5). The tropical rainforest (I) houses 44 ruminant species, of which around a 27% are restricted to it. 93 species inhabit the tropical deciduous woodland (II), a biome that present more than 30% of specialist species (29 spp.). The sub-tropical deserts (III) harbour 31.43% of biome specialists, being the biome with higher degree of specialization. We also found more specialists than expected in the sclerophyllous woodland (IV; 23%). The steppes and cold-deserts (VII) represent the only temperate biome housing a significantly high degree of stenobiomic species (20%). Interestingly, we also found that the taiga (VIII) harbours significantly less specialist ruminant species that expected from our Monte Carlo simulations, only a 2.5%.

A significantly high percentage of specialist cervids was found in two biomes (Table 5.6): tropical deciduous woodland (II) and sclerophyllous woodland (IV). On the other hand, more than 30% of the bovids species dwelling in the tropical rain forests (I), the tropical deciduous forests (II) and the sub-tropical deserts (III) are biomic specialists (Table 5.7), and 20% of the bovids inhabiting the steppes and cold-deserts (VII) are exclusive of this biome. We also found that three biomes house no specialist bovid species, which is less than expected under a null distribution (Table 5.7). These biomes are the temperate evergreen forest (V), the broad-leaf deciduous forest (VI) and the taiga (IX).

Model Type	*n*	*lnL*	*lnL Range*	*St.Dev*	*AIC*	*AIC Range*	*St.Dev*	*ΔAIC*
Constant λ	3	-1085.5	-1332.1 – -1076.8	43.8	2241.5	2159.5 – 2670.2	87.6	34.3
Linear λ	4	-1081.7	-1327.5 – -1073.4	43.2	2235.3	2154.8 – 2662.9	86.3	28.1
Sigmoidal λ	6	-1076.6	-1149.4 – -1067.3	18.1	2207.7	2146.6 – 2310.8	36.1	0.4
Modal λ	6	-1084.5	-1158.8 – -1047.1	18.8	2217.5	2106.3 – 2329.5	37.7	10.2
Directional Tendency								
Linear λ	5	-1079.3	-1325.6 – -1073.1	39.0	2226.4	2156.3 – 2661.1	78.0	19.1
Sigmoidal λ	**7**	**-1073.1**	**-1144.4 – -1066.6**	**17.8**	**2207.3**	**2147.2 – 2302.9**	**35.5**	**0.0**
Modal λ	7	-1075.6	-1158.8 – -1046.5	19.2	2213.6	2106.9 – 2331.5	38.4	6.3

Table 5.5. Fits of the quantitative-trait-dependent speciation models (QuaSSE). Summary of model fits for the correlation between biomic specialization index (BSI) and speciation rates for ruminants. lnL, log Likelihood of the fit; n number of parameters; AIC, Akaike Information Criterion; ΔAIC, difference of AIC relative to the best model (sigmoidal λ with directional evolution, in bold). Mean and range of lnL, AIC and ΔAIC from the 100 trees analysed are shown. St.Dev, standard deviation.

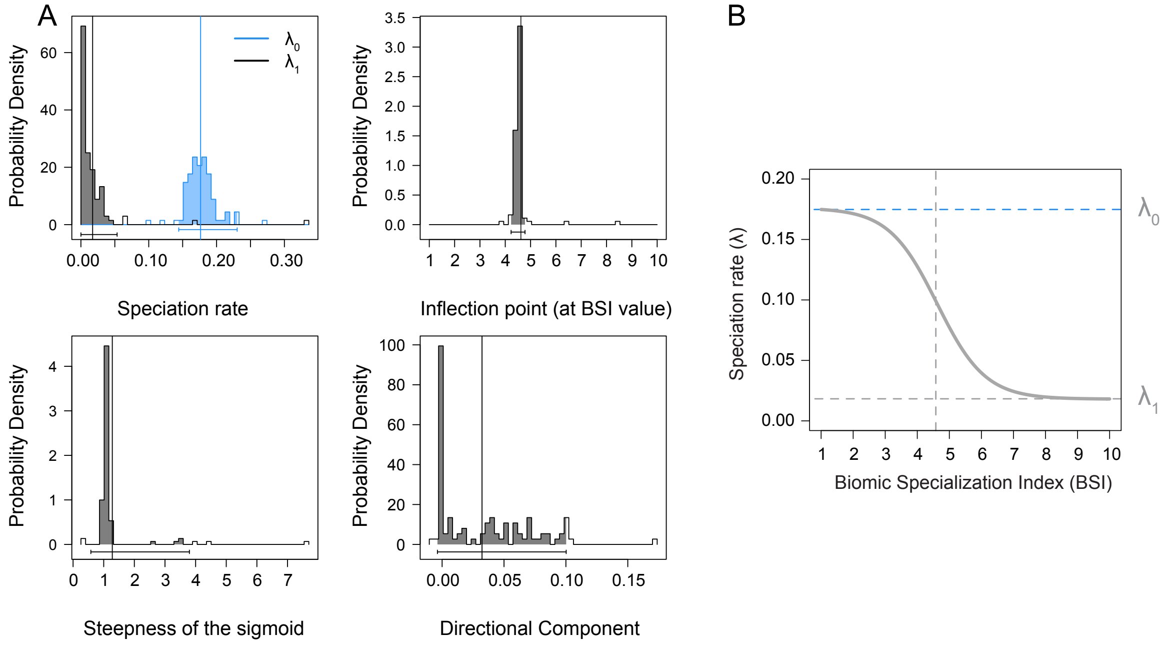


Figure 5.6. Speciation rate sigmoid model fit. Probability distributions of the parameters from the 100 trees analysed (A) and graphic representation of the sigmoid model under QuaSSE (B). λ_0, speciation rate for low values of BSI; λ_1, speciation rate for high values of BSI. Mean values are indicated by the solid vertical lines. Bars at the bottom of the distributions and the shaded areas correspond to the 95% credibility intervals. Under QuaSSE, the speciation rate for low BSI values are almost tenfold the speciation rate for lineages with high BSI (from 0.17 to 0.018), with an inflection point at 4.61.

Biome	Ruminantia			Monte Carlo Analysis			
	sp	sp (BSI=1)	%	Mean %	Std.dev.	Range	*p*
I	44	12	27.27	11.00	4.54	0.00 - 31.82	< 0.001
II	93	29	31.18	16.20	3.42	3.23 - 29.03	< 0.001
II/III	48	5	10.42	11.40	4.40	0.00 - 29.17	*0.907*
III	35	11	31.43	10.50	5.05	0.00 - 31.43	< 0.001
IV	26	6	23.08	9.84	5.63	0.00 - 34.62	0.007
V	44	3	6.82	11.00	4.57	0.00 - 34.09	*0.129*
VI	29	2	6.90	10.00	5.42	0.00 - 34.48	*0.236*
VII	45	9	20.00	11.10	4.49	0.00 - 31.11	0.019
VIII	40	1	2.50	10.70	4.71	0.00 - 30.00	*0.008*
IX	10	1	10.00	8.93	9.03	0.00 - 60.00	0.285

Table 5.6. Observed and simulated distribution of stenobiomic ruminant species (BSI=1) across biomes. sp., number of species; %, proportion of species with BSI = 1 in relation to total number of species in each biome; p, probability in each biome of the proportion of species with BSI = 1 being greater than or equal to (plain) or lower than or equal to (italics) the observed proportion in ruminants.

Biome	Cervidae			Monte Carlo Analysis			
	sp	sp (BSI=1)	%	Mean %	Std.dev.	Range	*p*
I	15	1	6.67	9.26	7.09	0.00 - 40.00	*0.705*
II	25	8	32.00	13.40	6.09	0.00 - 40.00	0.001
II/III	7	0	0.00	7.45	9.80	0.00 - 57.14	*0.732*
III	2	0	0.00	6.46	17.30	0.00 - 100.00	*0.143*
IV	5	2	40.00	6.97	11.30	0.00 - 80.00	0.003
V	18	3	16.67	10.30	6.74	0.00 - 44.44	0.102
VI	11	1	9.09	8.28	8.02	0.00 - 45.45	0.285
VII	10	1	10.00	7.97	8.40	0.00 - 50.00	0.223
VIII	12	1	8.33	8.43	7.79	0.00 - 41.67	*0.373*
IX	1	0	0.00	6.25	24.20	0.00 - 100.00	*0.067*

Table 5.7. Observed and simulated distribution of stenobiomic deer species (BSI=1) across biomes. sp., number of species; %, proportion of species with BSI = 1 in relation to total number of species in each biome; p, probability in each biome of the proportion of species with BSI = 1 being greater than or equal to (plain) or lower than or equal to (italics) the observed proportion in Cervidae.

Biome	Bovidae			Monte Carlo Analysis			
	sp	sp (BSI=1)	%	Mean %	Std.dev.	Range	*p*
I	25	8	32.00	11.10	6.10	0.00 - 44.00	< 0.001
II	65	21	32.31	17.40	4.20	3.08 - 33.85	< 0.001
II/III	38	5	13.16	12.70	5.12	0.00 - 34.21	0.540
III	32	11	34.38	11.90	5.51	0.00 - 40.62	< 0.001
IV	21	4	19.05	10.70	6.55	0.00 - 42.86	0.064
V	25	0	0.00	11.10	6.05	0.00 - 40.00	*< 0.001*
VI	13	0	0.00	10.20	8.26	0.00 - 46.15	*< 0.001*
VII	29	8	27.59	11.60	5.65	0.00 - 37.93	0.002
VIII	23	0	0.00	11.00	6.33	0.00 - 39.13	*< 0.001*
IX	9	1	11.11	9.86	9.84	0.00 - 66.67	0.281

Table 5.8. Observed and simulated distribution of stenobiomic bovid species (BSI=1) across biomes. sp., number of species; %, proportion of species with BSI = 1 in relation to total number of species in each biome; p, probability in each biome of the proportion of species with BSI = 1 being greater than or equal to (plain) or lower than or equal to (italics) the observed proportion in Bovidae.

Discussion

Frequency and speciation rate of biomic specialists.

Our results show a significantly higher proportion of biomic specialist species (BSI=1) than expected by random draws (Table 5.2) which can be interpreted as a direct result of higher net diversification rates in stenobiomic lineages, accordingly to the QuaSSE mocdel (Fig. 5.6). This outcome agrees with the prediction of the resource-use hypothesis (Vrba, 1980a, 1987) and is coherent with the results obtained by Hernández Fernández and Vrba (2005b) for the African large mammals and Moreno Bofarull et al. (2008) for the entire assemblage of South American mammals. While in these previous works conclusions on speciation rates were constructed exclusively on BSI distributions, we here directly tested for higher rates of speciation in stenobiomic lineages and found support for Vrba's hypothesis. We also found that extreme eurybiomic species with BSI = 7 and 8 are significantly more common among ruminants than expected by random simulations (Table 5.2 see Figure 5.3 and 5.5), as previously noticed by Hernández Fernández and Vrba (2005b) and Moreno Bofarull et al. (2008). Nevertheless, under QuaSSE, extreme eurybiomic lineages show low rates of speciation (around a tenth of the speciation rate in specialists; Fig. 5.4). Hence, it should be addressed that these super-generalists possess also low rates of extinction as a result of their high ecological flexibility, which allows them to survive through multiple climatic cycles, as suggested by Hernández Fernández and Vrba (2005b). In any case, no ruminant occupies all the ten biomes, since occupying all extreme biomes requires an unachievable degree of versatility (Hernández Fernández and Vrba, 2005b).

Specialization across biomes.

The resource use hypothesis predicts higher specialization of species inhabiting biomes that underwent a high degree of fragmentation and contraction during climatic cycles (Vrba, 1987, 1992, 1995). At the global scale, these biomes are located at extreme climatic conditions: tropical rain forest (I), subtropical desert (III), steppe (VII) and tundra (IX). As yielded by our Monte Carlo analyses, ruminant biomic specialization in tropical rain forests (I), sub-tropical deserts (III) and steppes (VII) is in concert with such prediction (Table 5.6). Nevertheless, we also found some interesting exceptions. Our analyses revealed that the tropical deciduous forests (II) also present a significantly higher percentage of specialist species than expected under random modeling (31.18%). This biome has already been reported as harbouring a high proportion of specialists. For example, Hernández Fernández and Vrba (2005b) found that a 19.9% of the large mammals in African tropical deciduous woodlands were stenobiomic. Despite not representing a climatic extreme, they argued that this biome, which is in close association with the rainforest (I), did also undergo expansions and retractions of its area during climatic cycles, providing a suitable situation for the creation of patches and refuges where speciation and specialization took place (Vrba, 1992). Furthermore, ruminants are more restricted to a particular vegetation physiognomy and, thus, they are prone to be more stenobiomic than insectivorous, omnivorous or carnivorous clades (Vrba, 1980a, b). The tropical deciduous woodlands are characterised by a seasonal leaf fall, which represents an important decrease in the resources during extremely dry months of the year. Both Cervidae and Bovidae also constitute high percentages of specialists in this biome (Table 5.7 and Table 5.8), which support this trend towards specialization in strongly seasonal deciduous landscapes. This is probably the cause behind the significantly higher proportion of biomic specialist in the sclerophyllous woodland (IV), which shows a 23% of species restricted to it (Table 5.6) and is not located at a climatic extreme either. The specialization in the sclerophyllous woodland (IV) is especially marked in cervids (Table 5.7).

On the other hand, the tundra (IX) does not present as high percentages of ruminant specialist species as predicted by the resource-use hypothesis (Table 5.6). We find a cause for this outcome in the method of codifying the BSI. The percentage of species inhabiting the tundra includes those inhabiting analogous vegetation belts in mountains (see Methods): species of goats and other ruminants that are not constrained to the high mountain landscape, but dwell also in several different altitudinal ranges depending on seasonality and the availability of food (Schaller, 1977; Corbet, 1978; Hall, 1981; Mitchell-Jones et al., 1999). With the purpose of exploring the behaviour of the dwellers of the high latitude tundra we repeated the Monte Carlo simulations and compared our data after excluding all the species inhabiting mountain ranges. We obtained that 50% of the species abiding the tundra (IX) are tundra-specialists, a proportion significantly higher than expected from random modeling ($p= 0.008$) and consistent with the predictions of the resource-use hypothesis.

Noteworthy is the fact that there are significantly less specialist species in the taiga than expected from random draws. The taiga is probably the biome with largest geographical extent today, and its species present extensive distributions (Corbet, 1978; Hall, 1981). In addition, only two main climatic dominions are recognized today and although some fragmentation has been reported for this biome through past climatic cycles (Bigelow et al., 2003), this has been relatively reduced in comparison with other biomes. These facts do not favour vicariance and speciation of its specialist species.

Differential specialization among clades.

The mean value of BSI in Cervidae (2.25) and Bovidae (2.04) is very similar to the mean BSI of Ruminantia (2.10), and the left-skewed distributions of their BSI values largely resemble that of ruminants' (Fig. 5.3 and 5.5). Thus, we can state that the degree of biomic specialization is similar among these clades in terms of BSI distributions, although they differ in their specialization across biomes (Table 5.7 and 5.8). Bovidae presents a significantly higher percentage of specialists than expected in four out from ten biomes (I, II, III and VII), whereas Cervidae only in two (II and IV). It seems that differences in biogeographic history, resource requirements and adaptations have marked the evolution of these two ruminant clades. In Africa the tropical rainforest (I) is widely distributed and is home for a high number of specialist bovids (Table 5.8). Afrotropical ruminant faunas are entirely dominated by bovids whereas tropical cervids, which never entered into the Afrotropics, are found in Asia and the Neotropics. In the Indomalaysian biogeographic region, the tropical rainforest is highly reduced and usually associated with mountainous ranges. In order to maintain genetically viable populations, the species in these areas usually inhabit in several ecosystems (biomes) due to altitudinal zonation. Such is the case of some species of the cervid genus *Rusa*, which are found in islands of the Indomalaysian region where their distributions range from the sea level up to 2000 m, including different vegetation belts. The muntjacs, another group of Indomalaysian cervids, also show distributions that include mountainous ranges. The ruminants of the Neotropics are the result of a radiation of generalists that dispersed into South America when the Panamanian land-bridge appeared c.a. 3.5 Ma (Vrba, 1992; Geist, 1998; Gentry, 2000). Aditionaly, most of the species are also associated to mountains and only a few species of the genus *Mazama* exclusively inhabit the lowlands of the Amazonas. These few species have not proliferated in the Amazon Basin during climate cycles, for their potential niches were already occupied by large species of South American rodents, such as pacas (Agoutidae) and agoutis (Dasyproctidae). In general we could say that tropical rainforests (I) in Africa have capacity to maintain more biomic specialist ruminants than in the Indomalaysian region and the Neotropics owing to their different geographic constraints and evolutionary history. Furthermore, the cladogenesis of bovids has been closely related with the sub-Saharan tropics, and the response of extinct bovids to climatic fluctuations and the appearance of arid environments have been well documented both from fossil and molecular evidence (Vrba, 1997; Hassanin and Douzery, 1999; Bibi et al., 2009).

Our results also reveal that cervids present show specialization in forested biomes with a severe hydric seasonality (tropical deciduous forests and sclerophyllous woodlands). Some authors have argued that the seasonal growth of antlers involves high nutritional requirements for deers and such a demand is hardly covered by eating just grass (Geist, 1998; Moen et al., 1999). As a consequence, cervids would be constrained to rich, concentrated resources that are more abundant in temperate forest-like environments than in deciduous tropical forests or schleropyllous woodlands. These biomes would have selected for specialist cervids that could find enough resources to cover their needs in a context of seasonal availability of resources. The nutritional requirements of cervids would have prevented them from specialising and proliferating in deserts, cold steppes and tundras, the other biome that theoretically favours speciation of specialists.

All these findings strongly support the resource-use hypothesis. While conceding biogeographic history and physiological constraints certain role modulating speciation across biomes, we have demonstrated that, at a global scale, more specialist ruminants are prone to speciate at higher rates. From an empirical perspective, this is the first time that differential speciation rates of specialist lineages has been directly tested in a phylogenetic framework. Testing Vrba's predictions in other mammalian clades (including carnivores and fossil taxa) will shed valuable light on the universality of our conclusions.

Conclusions

Our results agree with the predictions of the resource-use hypothesis proposed by Vrba. We found high frequency of species restricted to a single biome (BSI = 1) as a consequence of high speciation rates in stenobiomic lineages and higher specialization of species inhabiting biomes that underwent a high degree of fragmentation and contraction during climatic cycles: tropical rain forest (I), subtropical desert (III), steppe (VII) and tundra (IX). We also found significantly higher specialization among the species inhabiting tropical deciduous forest (II), which also underwent expansions and retractions of its area during climatic cycles, providing a suitable scenario for speciation. Finally, our findings strongly suggest that the difference in the biogeographic history, resource requirements and adaptations of bovids and cervids have marked their disparate specialization across biomes.

Literature Cited

Answell WFH (1971) Order Artiodactyla. In *The Mammals of Africa: an Identification Manual*, eds Meester J, Setzer HW. Smithsonian Institution Press, Washington. Pp. 1-84.

Bibi F, Bukhsianidze M, Gentry A, Geraads D, Kostopoulos D, Vrba E (2009) The Fossil Record and Evolution of Bovidae: State of the Field. *Palaeontologia Electronica* 12(3).

Bigelow N, Brubaker L, Edwards M, Harrison S, Prentice I, Anderson P, Andreev A, Bartlein P, Christensen T, Cramer W, Kaplan JO, Lozhkin AV, Matveyeva NV, Murray DF, McGuire AD, Razzhivin VY, Ritchie JC, Smith B, Walker DA, Gajewski K, Wolf V, Holmqvist BH, Igarashi Y, Kremenetskii K, Paus A, Pisaric MFJ, Volkova VS (2003) Climate change and Arctic ecosystems: 1. Vegetation changes north of 55 N between the last glacial maximum, mid-Holocene, and present. *J Geophys Res* 108(D19):1-25.

Burnham KP, Anderson GC (2002) Model selection and multimodel inference: a practical information-theoretic approach. Springer-Verlag, New York.

Corbet GB (1978) The mammals of the Palaearctic region: a taxonomic review. Cornell University Press, London.

Corbet GB, Hill JE (1992) The mammals of the Indomalayan Region: a systematic review. Oxford University Press, Oxford.

Doherty D, Harcourt A (2004) Are rare primate taxa specialists or simply less studied? *J Biogeogr* 31(1):57-61.

Eeley H, Foley R (1999) Species richness, species range size and ecological specialisation among African primates: geographical patterns and conservation implications. *Biodivers Conserv* 8(8):1033-1056.

Eisenberg HJF (1989) Mammals of the Neotropics Vol. 1: The Northern Neotropics. University of Chicago Press, Chicago.

Eisenberg HJF, Redford K (2000) Mammals of the Neotropics. Vol. 3: The Central Neotropics. University of Chicago Press, Chicago.

Eronen JT, Alistair RE, Fortelius M, Jernvall J (2009) The impact of regional climate on the evolution of mammals: a case study using fossil horses. *Evolution* 64(2):398-408.

FitzJohn RG (2010) Quantitative traits and diversification. *Syst Biol* 59(6):619-33.

FitzJohn RG (2011) diversitree: comparative phylogenetic tests of diversification. An R package. http://www.zoology.ubc.ca/prog/diversitree.

Geist V (1998) Strategies of deer evolution. In *Deer of the World: Their Evolution Behaivour and Ecology*, ed Geist V. Stackpole Books, Mechanisburg. Pp. 1-18

Gentry AW (2000) The Ruminant radiation. In *Antelopes, Deer, and Relatives: Fossil Record, Behavioral Ecology, Systematics and Conservation*, eds Vrba ES, Schaller GB. Yale University Press, New Haven. Pp. 11-25

Grubb P (1993) Order Artiodactyla. In *Mammal Species of the World: a taxonomic and geographic reference*, eds Wilson DE, Reeder DM. Smithsonian Institution Press, Washington. Pp. 377-414

Hall ER (1981) The mammals of North America. John Wiley & Sons, New York.

Harcourt A (2000) Latitude and latitudinal extent: a global analysis of the Rapoport effect in a tropical mammalian taxon: primates. *J Biogeogr* 27(5):1169-1182.

Harcourt A, Coppeto S, Parks S (2002) Rarity, specialization and extinction in primates. *J Biogeogr* 29(4):445-456.

Hassanin A, Douzery EJP (1999) The tribal radiation of the family Bovidae (Artiodactyla) and the evolution of the mitochondrial cytochrome b gene. *Mol Phylogenet Evol* 13(2):227-243.

Hernández Fernández M (2001) Bioclimatic discriminant capacity of terrestrial mammal faunas. *Glob Ecol Biogeogr* 10(2):189-204.

Hernández Fernández M, Vrba ES (2005a) A complete estimate of the phylogenetic relationships in Ruminantia: a dated species-level supertree of the extant ruminants. *Biol Rev* 80(2):269-302.

Hernández Fernández M, Vrba ES (2005b) Macroevolutionary processes and biomic specialization: testing the resource-use hypothesis. *Evol Ecol* 19(3):199–219.

IUCN. 2008. *Digital distributions maps of the mammals of te world. Version 1.0.* Available at: http://www.iucnredlist.org/initiatives/mammals/description/download_gis_data.

Janis CM (1993) Tertiary mammal evolution in the context of changing climates, vegetation, and tectonic events. *Annu Rev Ecol Syst* 24(1):467-500.

Kingdon J (1997) The Kingdon field guide to African mammals. Academic Press, London.

Kuhn TS, Mooers AØ, Thomas GH (2011) A simple polytomy resolver for dated phylogenies. *Meth Ecol Evol* doi: 10.1111/j.2041-210X.2011.00103.x.

Maddison WP, Midford PE, Otto SP (2007) Estimating a binary character's effect on speciation and extinction. *Syst Biol* 56(5):701-710.

Mitchell-Jones AJ, Amori G, Bogdanowic W, Krystufek B, Reijnders P (1999) The Atlas of European Mammals. T. & A.D. Poyser, London.

Moen R, Pastor J, Cohen Y (1999) Antler growth and extinction of Irish elk. *Evol Ecol Res* 1(2):235-249.

Moreno Bofarull A, Arias Royo A, Hernández Fernández M, Ortiz-Jaurequizar E, Morales J (2008) Influence of continental history on the ecological specialization and macroevolutionary processes in the mammalian assemblage of South America: differences between small and large mammals. *BMC Evol Biol* 8(97):1-18.

Pickford M, Morales J (1994) Biostratigraphy and palaeobiogeography of East Africa and the Iberian Peninsula. *Palaeogeogr Palaeoclimatol Palaeoecol* 112(3-4):297-322.

R Development Core team (2008) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Viena, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rabosky DL (2010) Extinction rates should not be estimated from molecular phylogenies. *Evolution* 64(6):1816-1824.

Redford K, Eisenberg HJF (1992) Mammals of the Neotropics Vol. 2: The Southern Cone. University of Chicago Press, Chicago.

Schaller GB (1977) Distribution. In *Mountain Monarchs: Wild Sheep and Goats of the*

Himalaya., ed Schaller GB. University of Chicago Press, Chicago. Pp. 45-82

van Dam J, Aziz HA, Álvarez Sierra MA, Hilgen F, van den Hoek Ostende L, Lourens L, Mein P, van der Meulen A, Peláez-Campomanes P (2006) Long-period astronomical forcing of mammal turnover. *Nature* 443(7112):687-91.

Vrba ES (1980a) Evolution, species and fossils: how does life evolve? *S Afr J Sci* 76:61-84.

Vrba ES (1980b) The significance of bovid remains as indicators of environment and predation patterns. In *Fossils in the Making. Vertebrate Taphonomy and Paleoecology*, eds Behrensmeyer AK, Hill AP. University of Chicago Press, Chicago. Pp. 247-271

Vrba ES (1987) Ecology in relation to speciation rates: some case histories of Miocene-Recent mammal clades. *Evol Ecol* 1(4):283–300.

Vrba ES (1992) Mammals as a Key to Evolutionary Theory. *J Mammal* 73(1):1-28.

Vrba ES (1993) Turnover-pulses, the Red Queen, and related topics. *Am J Sci* 293A(A):418-452.

Vrba ES (1995) On the connections between paleoclimate and evolution. In *Paleoclimate and Evolution with Emphasis on Human Origins*, eds Vrba ES, Denton GH, Patridge TC, Burcke LH. Yale University Press, New Haven. Pp. 24-45

Vrba ES (1997) New fossil of Alcelaphini and Caprinae (Bovidae: Mammalia) from Awash, Ethiopia, and phylogenetic analysis of Alcelaphini. *Palaeontol Afr* 34:127-198.

Vrba ES (1999) Habita theory in relation to the evolution in African Neogene biota and hominids. In *African Biogeography, Climate Change and Human Evolution*, eds Bromage TG, Schrenk F. Oxford University Press, New York. Pp. 19-39

Vrba ES, Denton GH, Partridge TC, Burckle LH (1995) Paleoclimate and Evolution, with Emphasis on Human Origins. Yale University Press, New Haven.

Walter H (1970) Vegetation and Clima. Eugen Ulmer, Stuttgart.

Wilson DE, Reeder DM (1993) Mammal Species of the World: a taxonomic and geographic reference. Smithsonian Institution Press, Washington.

6

Historical factors of continental and biogeographic regions are responsible for phylogenetic structure in ruminant metacommunities

> “*Protecting our biological resources requires continued commitment to understanding how communities assemble and how they respond to forces of change.*”
>
> *- Jeannine Cavender-Bares et al.-*

Abstract

This work provides a new insight on ruminant evolution, by applying phylogenetic community structure metrics to mammalian assemblages on three different hierarchical levels. While previous studies focussed on local scale and ecological dynamics such as biotic interactions or habitat filtering, here we assessed the signature of assembly processes at a macroevolutionary scale. Phylogenetic relatedness indices were calculated for 79 ruminant assemblages around the planet comparing the global, the continental and the regional phylogenies in order to test for significant phylogenetic clustering or over-dispersion evident in community assembly. In this way we ascertain that processes determining ruminant assemblages at the landscape level are not well-explained solely by biotic interactions. Evolutionary history of each continent, especially temporal and geographic events that manifest at large scales, such as continental drift and climate forcing, appears responsible for great part of the phylogenetic structure of the assemblages under study. There is also an apparent latitudinal gradient of phylogenetic clustering when controlling for both the continental and regional phylogeny, which could be related with the preferential diversification of northern lineages during the Plio-Pleistocene glacial-interglacial cycles. Future studies on community structure addressing biotic interactions at more local scales and encompassing samples all over the planet should avoid these biases by controlling for continental and/or regional pools.

Introduction

The study of how communities are structured is generally pursued to understand the strength of biotic interactions within assemblages of extant species. Traditionally, trends biasing species occurrences in assemblages were assessed using taxonomic approaches, such as species-genus ratios (Elton, 1946; Simberloff, 1970; McFarlane, 1991). More recently, due to the increasing availability of dated phylogenies, researchers have been able to test for phylogenetic clustering (whether species are more phylogenetically related than expected by chance) or dispersion (whether species are less phylogenetically related than expected by chance) through the assemblages under study (Webb, 2000; Webb et al., 2002), helping ecologists to assess the assembly processes involved. The interpretation of trends in community assemblages in some cases has been subject to debate, for very different processes can cause similar patterns. Interspecific interactions can be sometimes difficult to assess, and usually processes such as environmental filtering (on conserved or convergent traits) and gene flow (closely related with vicariance and phylogenetic niche conservatism) make it difficult to untangle the causes of a given pattern (Losos, 2008; Cavender-Bares et al., 2009). Recent efforts concentrate on probing assumptions about trait evolution, niche dynamics, phylogenetic structure test performance and the use of different null models (Gotelli, 2000; Kembel, 2009).

Much of this work has been conducted on plants and microorganisms (Vamosi et al., 2009), with mammals and other vertebrate groups being underrepresented (Cooper et al., 2008; Kamilar and Guidi, 2010; Raia, 2010). The present work focuses on ruminants. With some 200 extant species included in six families (Tragulidae, Giraffidae, Moschidae, Cervidae, Antilocapridae and Bovidae), suborder Ruminantia is considered the most important group of large terrestrial herbivorous mammals (Hernández Fernández and Vrba, 2005a). Ruminants developed a spectacular diversity of ecomorphological specializations, and are distributed widely across many biomes, from the densest tropical forests, to deserts and tundra. This diversity in species, ecology and distribution make ruminants a valuable target for research on evolution and ecology and, in this case, it allows for comparison among localities from all over the globe, with all biomes being represented. Here, we applied phylogenetic relatedness indices in order to assess phylogenetic clustering or overdispersion in ruminant assemblages at three different large scales: landscape (10000km^2), continent and biogeographic region. We then gauge whether the phylogenetic structure in different assemblages is biased by continental species pools and/or environmental-climatic history by testing the phylogenetic clustering or dispersion versus the global, the continental and the regional phylogeny. If the trend remains similar in the three analyses, processes other than those that operate at these very large scales may be causing the observed values at the scale of study. Conversely, if controlling for continental and regional pools reduces the significance of the trend, it is likely that large-scale historic events related to climate and continental drifting, particular for each continent and region, are behind the structure of each assemblage.

Methods

Data

Local, continental and regional assemblages. Several works have highlighted the major influence of geographical scale in phylogenetic community structure (Webb et al., 2002; Heard and Cox, 2007; Cooper et al., 2008; Vamosi et al., 2009; among others). Since it is our purpose to assess the evolutionary pattern involved in the faunal assemblages at landscape scale, in this study the geographic area of each sample is roughly 10000 km^2. This scale is adequate to encompass spatial variation in climate and all possible local habitats. In order to avoid high climate variations imposed by differences in topography (mainly mountain ranges), the selected localities are mostly below 1000 m above sea level. 79 localities from all over the world and from all climates form the database of this study (Fig. 6.1 and Appendix 4.1). Localities were selected in such a way that they represent all major climate zones inside each continent. We assigned each locality to a biome following Walter (1970).We also made the array such that samples were as widely scattered as possible throughout the world. Only localities with two or more ruminant species were included because phylogenetic structure metrics can only be calculated for samples with at least two taxa.

Four continental species pools (North America, South America, Eurasia and Africa) and five regional species pools (Nearctic, Neotropic, Palearctic, Afrotropic and Indomalaysia) were considered. Continental and regional pools are assumed to be the complete list of species whose geographical ranges fall into a determinate continent or biogeographic region. The faunal list of each locality, continental and regional pool was obtained from geographic ranges published in the literature (Answell, 1971; Corbet, 1978; Eisenberg, 1989; Corbet and Hill, 1992; Redford and Eisenberg, 1992; Grubb, 1993; Kingdon, 1997; Mitchell-Jones et al., 1999; Eisenberg and Redford, 2000). These lists encompass living species and those that became extinct in the last two centuries. Species introduced by humans are omitted. For taxonomic consistency, we have followed the species-level taxonomy

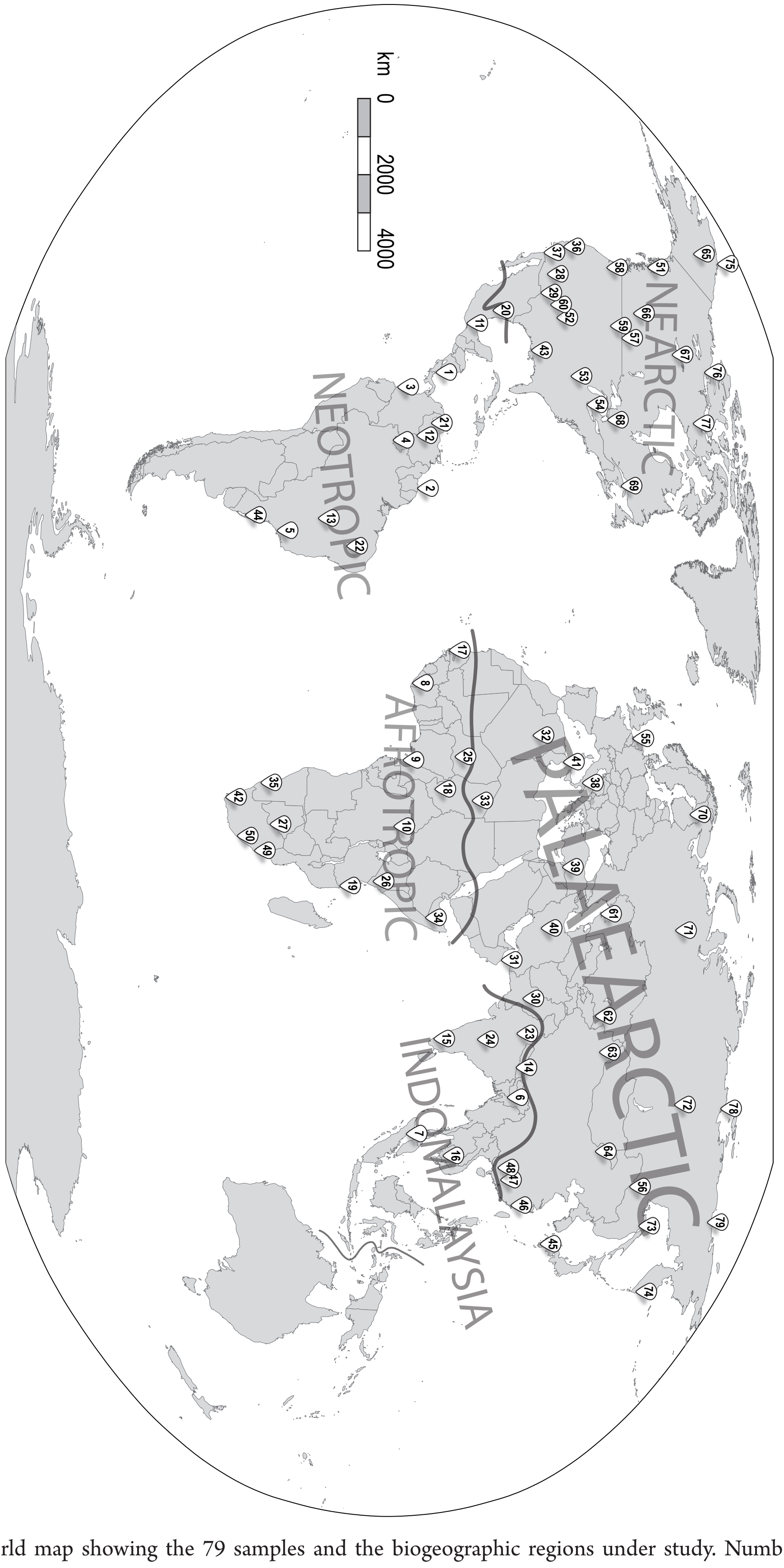


Figure 6.1. World map showing the 79 samples and the biogeographic regions under study. Numbers of localities as in Appendix 4.1.

of Wilson and Reeder (1993).

Phylogeny. The basis of our analysis is a supertree that includes all 197 extant and recently extinct species of the suborder Ruminantia (Hernández Fernández and Vrba, 2005a), following the nomenclature of Wilson and Reeder (1993). This supertree is a consensus from 124 trees published from 1970 to 2003, including morphological, ethological and molecular information. It was constructed using matrix representation with parsimony (MRP). Importantly, 80% of the nodes on the tree are dated using a large compendium of molecular and fossil data, with the remaining 20% of the nodes being interpolated using a pure birth model.

Community Phylogenetic Structure Metrics

Here we use the Net Relatedness Index (NRI) and Nearest Taxon Index (NTI) for measures of relatedness of species occurring in samples. NRI and NTI are standardized values of, respectively, mean pairwise distance (MPD) and mean nearest neighbour distance (MNND) by expectation from random draws (Webb et al., 2002). NRI and NTI describe the difference between average phylogenetic distances in the observed and null localities, standardized by the standard deviation of phylogenetic distances in the null localities (Webb et al., 2008). The indexes are in units of standard deviation and significance can be obtained directly from them. Values of NRI and NTI above 1.96 are considered significantly clustered, while values bellow -1.96 are considered significantly dispersed. For an extensive review of sample-based community phylogenetic structure metrics see Vamosi et al. (2009).

NRI and NTI were calculated applying *Phylocom* software (Webb et al., 2008) to presence-absence matrices of species by locality, which were converted to the proper format using *Picante* (Kembel et al., 2009). Analyses were conducted at several different scales (Heard and Cox, 2007; Kamilar and Guidi, 2010). We tested each locality for phylogenetic clustering or evenness against the complete phylogeny of ruminants. In addition, we tested each locality for phylogenetic clustering or evenness against the phylogenies containing the species of the corresponding continent and biogeographic region (named here as continental and regional phylogenies). These continental and regional phylogenies were pruned out using TreeEdit v1.0a10. By comparing these three tests we may differentiate whether the clustering or dispersion signal of each locality is due to its particular features or influeced by particular historic factors (faunas turnovers and interchanges) controlled by each continent's position, land-bridge connections and biogeographic peculiarities. Correlations between absolute latitude of the localities and their NRI and NTI indexes were also calculated for the 79 samples.

Finally, we carried out tests for phylogenetic structure of the assemblages of each continent and biogeographic region versus the global phylogeny. Different null models can be used in order to estimate NRI and NTI (Webb et al., 2008), and use of the wrong null model can compromise the outcome of an analysis to such extent that conclusions might be fully misleading (Gotelli, 2000; Kembel, 2009; Vamosi et al., 2009). We repeated all the analyses with two different null models. The first (null model 0 as implemented in *Phylocom*) randomizes phylogenetic relationships among the species in a sample by shuffling species labels across the tips of the entire phylogeny of reference. The second null model (null model 2 as implemented in *Phylocom*) maintains the species richness within each sample, but the species occurring in each locality become random draws from the phylogeny pool (Webb et al., 2008). We chose these two null models among different possibilities (Webb et al., 2008) since they are the ones that potentially generate the most divergent outcomes, in such a way that we can test the robustness of our results.

Results

Both null models yielded very similar results (see Appendices 4.1 and 4.2). Only two samples out of 79 demonstrated different significance levels for one of their indices (NRI of Shaoguan against the continental phylogeny and NRI of Fort Smith against the global phylogeny). The significance of the NTI index for the African continental pool also changes (see Appendix 4.2). Below we comment on the results yielded by using the first null model explained above (null model 0).

Continental and Regional clustering

As shown in Fig. 6.2a, African and South American species pools present high values of NRI and NTI. For Eurasia, NTI is higher than NRI, with NTI above the significance level and NRI close to zero. The species pool of North America illustrates a neutral trend (neither clustered, nor dispersed). The pattern observed for the species assemblage of the biogeographic regions (Fig. 6.2b) resemble the trends shown by continental ones, but the regional pools presented more defined trends. Afrotropical, Neotropical and Palearctic regions are clustered as shown by both NRI and NTI values. The Indomalaysian region present clustered values for NTI and NRI values closed to dispersion. Conversely, the Nearctic region does not present any significant trend at all.

Phylogenetic structure of local samples

NRI and NTI values for each of the 79 localities versus the global, continental and regional phylogeny are shown in Appendix 4.1. 20 out of 79 localities presented global NRI values above 1.96 (significantly clustered) and 3 tropical localities from

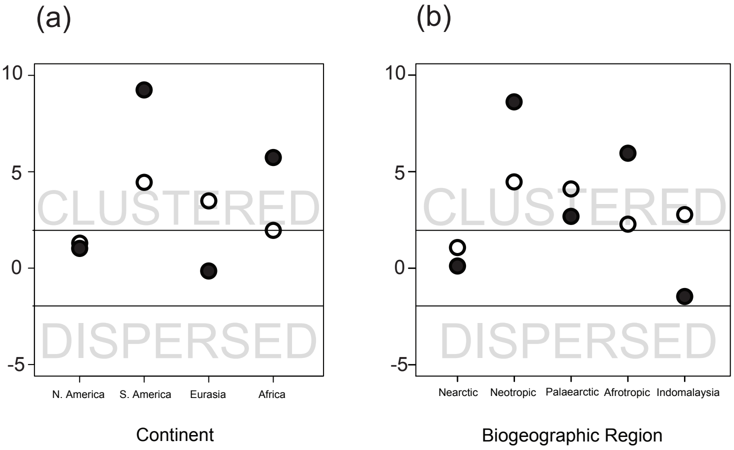


Figure 6.2. Net relatedness index values (NRI, black circles); and nearest taxon index values (NTI, white circles), for species pools of each continent (a) and biogeographic region (a). Horizontal lines at 1.96 and -1.96 represent the boundaries between non-significance (neutral trend), and statistically significant phylogenetic clustering or phylogenetic dispersion.

Asia exhibited values bellow -1.96 (significantly dispersed; Fig. 6.3a). Continental NRI values were more conservative. When controlling for the continental pool no locality was significantly clustered, whereas two localities from south-eastern Asia (Medan and Phnom Phen) remained dispersed (Fig. 6.3b). When controlling for the regional phylogeny, however, only Medan stays dispersed, whereas four localities show clustering: two in the African desert (El-Golea and Faya-Largeao), one in the Chinese broadleaf forests (Shaoguan) and one more in the North American taiga (Fort Smith).

Regarding NTI values against the global phylogeny, 18 localities out of 79 showed significant clustering, but no locality showed dispersion. When continental pool is controlled for, only two localities in the African dessert show clustering (El-Golea and Faya-Largeao). Finally, only the value of NTI for Faya-Largeao (in the chadian desert) remains significantly clustered when compared with the regional phylogeny.

Latitudinal bias

Significance level for the relationships between both indexes, NRI and NTI, and absolute latitude increases from the global to the continental and regional scales, though those relationships are weak (Fig. 6.4). Even when a significant correlation is found, some metacommunities present significant clustering values at medium latitudes, and they are shown as outliers in plots (c) and (f) in Fig. 6.4. These samples correspond to two locations in the Sahara (Faya-Largeao and El-Golea) and one in the broadleaf forests of southern China (Shaoguan).

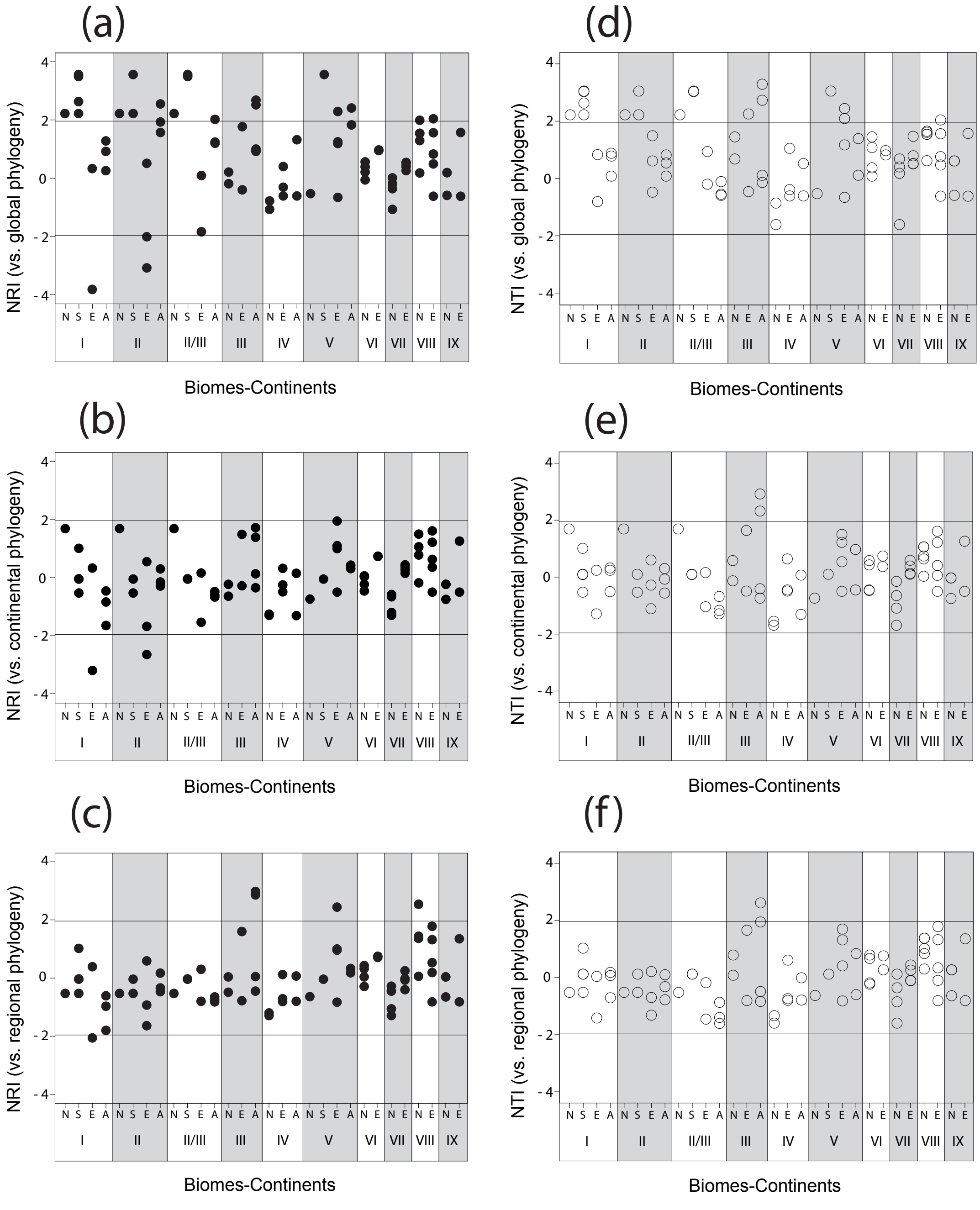


Figure 6.3. Net relatedness index values (NRI, black circles); and nearest taxon index values (NTI, white circles), for each of the 79 metacommunities under study ordered by biome and continent. a) NRI against the global phylogeny; b) NRI against the continental phylogeny; c) NRI against the regional phylogeny; d) NTI against the global phylogeny; e) NTI against the continental phylogeny; f) NTI against the regional phylogeny. Horizontal lines at 1.96 and -1.96 represent the boundaries between non-significance (neutral trend), and statistically significant phylogenetic clustering or phylogenetic dispersion. Biomes: I, evergreen tropical rain forest; II, tropical deciduous woodland; II/III, savanna; III, subtropical desert; IV, sclerophylus woodland-shrubland; V, temperate evergreen forest; VI, nemoral broadleaf deciduous forest; VII, steppe to cold desert; VIII, boreal coniferous forest; IX, tundra. Continents: N, North America; S, South America; E, Eurasia; A, Africa.

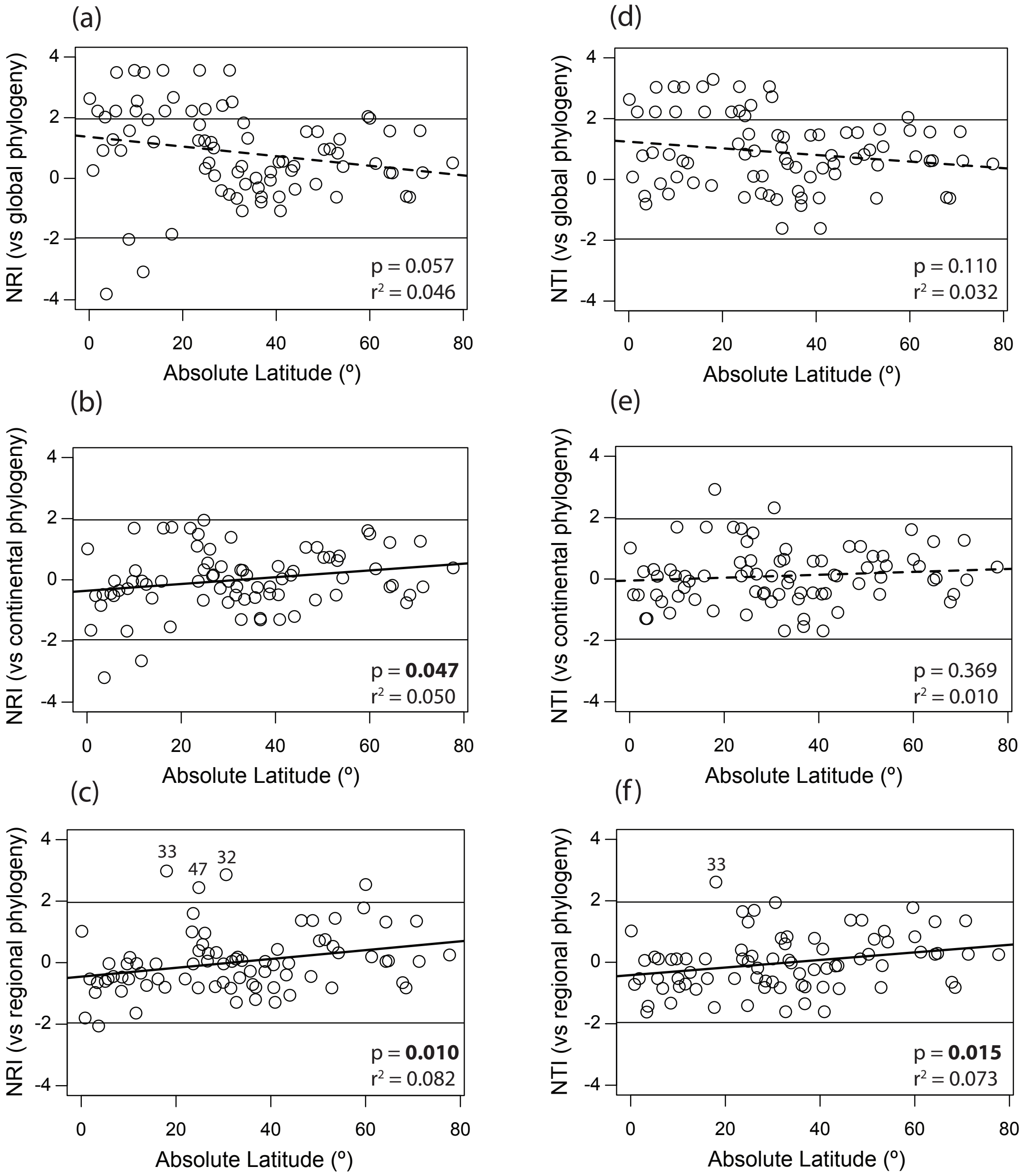


Figure 6.4. Relationship between absolute Latitude of each locality and: a) NRI against the global phylogeny; b) NRI against the continental phylogeny; c) NRI against the regional phylogeny; d) NTI against the global phylogeny; e) NTI against the continental phylogeny; f) NTI against the regional phylogeny. Horizontal lines at 1.96 and -1.96 represents the boundaries between non-significance (neutral trend), phylogenetic clustering and phylogenetic dispersion. Linear regressions are shown with dashed lines when their p-value is non-significant. The numbers of outlaying localities are only shown when correlation is significant. Numbers of outlying localities mentioned in the text as in Appendix S1.

Discussion

The influence of continental and regional phylogenetic history

Our results clearly indicate that the phylogenetic structure of the samples under study is mainly due to the continental and regional species pools from which they have been assembled (Fig. 6.3). Points in the Fig.3b and Fig.3c get closer to zero (they lose statistical significance) when controlling for both sets of species. From the original 23 communities with significant clustering or dispersion, 18 and 15 changed when NRI is calculated against the continental pool and the regional pool respectively, which affects every continent and biome in a similar way.

South America represents a clear case. All nine ruminant metacommunities in this continent are clustered, but none of them remain significantly clustered when the continental and regional effect is removed. Only 13 species of cervids are present in the continent, all belonging to a single clade (Geist, 1998; Hernández Fernández and Vrba, 2005a) as result of an explosive radiation of the tribe Odocoileini from North America into the Neotropics during the late Pliocene (Gentry, 2000). South American samples never contain more than three species, but because of this very special continental prearrangement, the community structure metrics values indicate high clustering. Furthermore, this extends through the whole neotropic region. The communities in North America falling inside this region show a similar pattern, because they present clear Neotropical features. The values of NRI and NTI for those North American tropical communities are closer to a neutral trend (closer to zero) when we control for the regional pool than when the continental pool is taken into account. Hence, in this case, the faunas found in each biogeographic region are well defined and their phylogenetic history is responsible in a higher degree for the phylogenetic trend that we found at landscape scale than the continental species pool.

The Nearctic region presents a very different evolutionary history. The first American cervids, Rangiferini and Odocoileini, entered from Asia ca. 5 my ago, and became well established two million years later (Janis et al., 1998). *Alces* and *Cervus* immigrated from Asia in a later pulse (Gentry, 2000). The Nearctic Region also preserves some northern bovid lineages that immigrated from Eurasia through the Beringian connection during different Plio-Pleistocene glacial episodes (Lundelius et al., 1988); the appearance of *Bison* marks the end of the Irvingtonian (ca. 0.3 Ma), and *Ovibos*, *Oreamnos* and *Ovis* entered North America during the Rancholabrean (Middle-Late Pleistocene). Furthermore, the last extant antilocaprid (*Antilocapra americana*) is also found here, representing a long lineage with an estimated origin around 28 m.a. (Hernández Fernández and Vrba, 2005a) and which formed a major part of North American ungulate faunas from the Middle to Late Miocene (Janis et al., 2000; Janis et al., 2002; Semprebon et al., 2004). All this exceptional phylogenetic diversity, as result of successive of faunal turnovers and interchanges, configure a phylogenetically "balanced" pool (Figs. 2a and 2b).

Similar to South America, the large-scale historical biogeographic history of Africa is responsible for the high clustering of the continental and regional pool (Fig. 6.2a and 6.2b). Today, 81 out of 85 ruminant species living in Africa are bovids (Kingdon, 1997). Africa has been the cradle for several large cladogenetic events during the Neogene giving rise to most of the existing tribes in Bovidae. During the Late Miocene and Pliocene, a period marked by a global increase of aridity and the appearance of open grasslands (Cerling et al., 1993), a range of bovid tribes adapted to savannah-like environments emerged (Aepycerotini, Alcelaphini, Hippotragini, Reduncini and Tragelaphini), and Antilopini expanded through all the continent, reaching Eurasia. Subsequent radiations during the Plio-Pleistocene, coincident with massive cooling pulses, gave rise to some of the most successful bovid genera we see today (Vrba, 1997; Hassanin and Douzery, 1999; Bibi et al., 2009). The richest communities included in this work are in sub-Saharan Africa and many of them show high global NRI and NTI values (phylogenetic clustering). This would be an impossible if local ecological signature and biotic interactions like interespecific competition and exclusion were the main forces working at the scale of study. Furthermore, this trend vanishes when controlling for the continental pool and the biogeographic region. These results strongly imply that the African bovid cladogenesis explained above and the physical changes spurring them are the main cause for the phylogenetic pattern exhibited by African metacommunities. A phylogenetic skewness due to historical circumstances of the continent has major impact on species assemblages at smaller scale (Webb et al., 2002).

Significant clustering of Eurasian localities was lost when controlling for the continental pool. However, some localities with significant dispersion keep their significant NRI values when controlling for the continental pool (Medan and Phnom Phen). Moreover, Medan remains significant for NRI after controlling for the regional phylogeny. This location, situated in the island of Sumatra, presents a very diverse ruminant fauna, including two species of tragulids, two cervids, one species of goat and the water buffalo. The succession of glacial and interglacial episodes during the Plio-Pleistocene and the subsequent fluctuations of the sea level resulted in intermittent connections among some Southeast Asian islands and mainland (Esselstyn et al., 2009). Thus, these isolated faunas have experienced interchanges with continental faunas followed by shrinkages of their geographic range. In this scenario of complete isolation and reduced distribution areas, extinction pressures may select for more "threat tolerant" lineages and favour the evolution of new species and island endemics (Corbet and Hill, 1992; Waldron). Such selection may explain the diverse lineages present in these islands, and the consequent phylogenetic dispersion observed.

On the other hand, the species pool of Eurasia presents high NTI values and relatively low NRI values (Fig. 6.2a). The phylogeny containing the species in Eurasia shows several clusters connected by relatively long branches. This trend is something close to the "clumping of clustering" mentioned by Vamosi et al. (2009). The role of Eurasia in the biogeography of ruminants is the main reason behind this pattern. The position of Eurasia in relation to other continents makes it a central bridge articulating connections, allowing faunal interchanges between Africa and North America. Similarly, the phylogenetic clustering observed in the Indomalaysian region presents "clumping of clustering" (Fig. 6.2b), and perhaps explains some of the trend observed for the continent of Eurasia. The Asian tropics are refuge for three of the four living species of tragulids, a very ancient groups and a clade basal to ruminants. This entails long branches linking tragulids with the rest of the clades present in this region: cervids and bovids. Because these three families are separated by millions of years of evolutionary history, NRI values are close to the significant dispersion. Indomalayian species inside the three groups, however, are phylogeneticaly close, which makes the NTI values significantly clustered (Fig. 6.2b).

Nevertheless, the species present in a given biogeographic region are not always the best explanation for the phylogenetic structure of the samples under study, as shown for four samples. El-Golea and Faya-Largeao in the Sahara show neutral trends when compared with the continental species phylogeny, but present higher NRI values (significant clustering) when controlling for the regional species pool (Fig. 6.3b and 6.3c). They also show high continental and regional NTI (Fig. 6.3e and 6.3f). These two localities fall inside the Palaeartic region, but they have taxa phylogenetically closer to Afrotropical faunas. The Sahara represents an extreme environment of relatively recent development (Leroy and Dupont, 1994) and a zone of biogeographic transition. It probably was occupied by afrotropical lineages with special pre-adaptations to the long arid periods of the tropical savannas that gave rise to the very specialised faunas we find today in this desert (Hernández Fernández and Vrba, 2005c). Shaoguan, a locality from the south-eastern Chinese broadleaf evergreen forests, likewise is phylogenetically clustered when compared to the regional pool, but it does not show significant trend when compared with the species pool of Eurasia. Shaoguan is located in an ecotone, a zone of transition between temperate evergreen forests and tropical deciduous forests, close to the boundary between the Palaearctic and Indomalaysian regions, and houses species from both biogeographic influences. Finally, Forth Smith, in the Canadian taiga, presents significant clustered NTI values when estimated from the global and regional pool, but not when estimated from the continental pool. The only difference between the North American species pool and the Nearctic pool is the presence of two species of *Mazama*. The absence of *Mazama* in the regional pool suggests that four out of five species in Forth Smith belong to a whole clade in the regional phylogeny (*Alces alces, Rangifer tarandus, Odocoileus hemionus* and *Odocoileus virginianus*).

These data are consistent with previous works that highlight that global climate changes, tectonic activity and sea level fluctuations are responsible for global faunal turnover pulses by triggering vicariance events through creating geographic and climatic barriers, and conversely allowing faunal interchanges by the creation of land bridges and shifting climate zones (Vrba, 1992, 1993; Hernández Fernández and Vrba, 2005c; Hernández Fernández and Vrba, 2005b; Moreno Bofarull et al., 2008).

Latitudinal gradient and diversification across biomes

Once the effect of the species pool available in each continent and biogeographic region has been removed, the NRIs and NTIs of the samples (against the continental and regional pools) show phylogenetic trends at the local scale of the sample itself. There is a positive but weak correlation between the latitudinal position of each locality (plots b, c and f in Fig. 6.5) and these indices, which points out that species in localities at higher latitudes may be more phylogenetically related than expected by chance independently of the particular historical factors influencing the species pools of their continent or region. Among available species from which samples can be assembled, the species in higher latitudes are found with closer relatives. On the contrary, although assemblages closer to the Equator are highly clustered when are studied in a global context, they are not when related to their continental and biogeographic features.

Increasing latitude is correlated with increasing seasonality and productivity, and decreasing mean temperature. Hostile environments and extreme conditions select for more pre-adapted or biomic specialised lineages (Hernández Fernández and Vrba, 2005c). When a lineage gets specialised in an extreme landscape, it may occupy empty niches and may then diversify. The positive correlation between NTI controlled for the regional phylogeny and latitude points in this direction. Samples close to the Equator have more dispersed NTI values than assemblages in higher latitudes, meaning that branches linking one species to their nearest neighbour are on average longer in the tropics. This appears to be in agreement with the suggestions of Weir and Schluter (2007) on the preponderance of recent radiations in higher latitudes, which have probably outpaced those in the tropics, where diversification may have approached a carrying capacity limit. Thus, the tropics-as-cradle hypothesis proposed by some authors for other groups (Arita and Vazquez-Dominguez, 2008; Tobias et al., 2008) does not seem to match with the most recent evolutionary history of ruminants. Interestingly, some samples appeared as outliers (Fig. 6.4c and 6.4f). These correspond to two metacommunities in the Sahara (Faya-Largeao and El-Golea) and one in the broadleaf forests of southern China (Shaoguan). Deserts, like steppes and tundras, are extreme biomes of recent development, although they are located in subtropical latitudes. Localities in the Sahara desert show phylogenetic clustering even when controlling for the species set of the continent or the biogeographic region due to recent radiations of specialised lineages (see above). Particular biogeographic features of Shaoguan were also discussed above. The fact that these samples are in low latitudes makes them fall outside the general trend.

Conclusions

Broad-scale habitat changes due to climatic forcing and continental connections are the primary drivers of lineage evolution and distribution at the planetary scale. Their history can be read in the rocks, but because past events are so important in assembly processes we can also look for footprints of the past in present faunas. This work shows that the particular historical factors of each continent and biogeographic region have moulded species assemblages at the landscape scale (roughly 10000 km^2). Our results also point to a latitudinal gradient of phylogenetic clustering when controlling for both the continental and regional phylogeny. Nearest taxon indexes (NTI) show higher phylogenetic clustering for temperate and cold biomes than for the tropical ones, suggesting recent higher diversification rates for lineages inhabiting the extreme biomes at medium and high latitudes. Those species and environments appeared during the Plio-Pleistocene as a consequence of climatic cooling and the establishment of the glacial cycles. Future studies on community structure encompassing samples from throughout the planet should bear in mind the influence of the species pool in each continent and/or biogeographic region. Controlling for this factor should yield more accurate and standardized results whatever the scale of study.

Literature Cited

Answell WFH (1971) Order Artiodactyla. In *The Mammals of Africa: an Identification Manual*, eds Meester J, Setzer HW. Smithsonian Institution Press, Washington. Pp. 1-84

Arita HT, Vazquez-Dominguez E (2008) The tropics: cradle, museum or casino? A dynamic null model for latitudinal gradients of species diversity. *Ecol Lett* 11(7):653-663.

Bibi F, Bukhsianidze M, Gentry A, Geraads D, Kostopoulos D, Vrba E (2009) The Fossil Record and Evolution of Bovidae: State of the Field. *Palaeontologia Electronica* 12(3).

Cavender-Bares J, Kozak KH, Fine PVA, Kembel SW (2009) The merging of community ecology and phylogenetic biology. *Ecol Lett* 12(7):693-715.

Cerling TE, Wang Y, Quade J (1993) Expansion of C4 ecosystems as indicator of global ecological change in the late Miocene. *Nature* 361(6410):344-345.

Chapman JA, Feldhammer GA (1982) Wild mammals of North America: Biology, Management, and Conservation. The Johns Hopkins Univiversity Press, Baltimore.

Cooper N, Rodriguez J, Purvis A (2008) A common tendency for phylogenetic overdispersion in mammalian assemblages. *Proc R Soc B* 275(1646):2031-7.

Corbet GB (1978) The mammals of the Palaearctic region: a taxonomic review. Cornell University Press, London.

Corbet GB, Hill JE (1992) The mammals of the Indomalayan Region: a systematic review. Oxford University Press, Oxford.

Eisenberg HJF (1989) Mammals of the Neotropics Vol. 1: The Northern Neotropics. University of Chicago Press, Chicago.

Eisenberg HJF, Redford K (2000) Mammals of the Neotropics. Vol. 3: The Central Neotropics. University of Chicago Press, Chicago.

Elton C (1946) Competition and the Structure of Ecological Communities. *J Anim Ecol* 15(1):54-68.

Esselstyn JA, Timm RM, Brown RM (2009) Do geological or climatic processes drive speciation in dynamic archipelagos? The tempo and mode of diversification in Southeast Asian shrews. *Evolution* 63(10):2595-610.

Geist V (1998) Strategies of deer evolution. In *Deer of the World: Their Evolution Behaivour and Ecology*, ed Geist V. Stackpole Books, Mechanisburg. Pp. 1-18

Gentry AW (2000) The Ruminant radiation. In *Antelopes, Deer, and Relatives: Fossil Record, Behavioral Ecology, Systematics and Conservation*, eds Vrba ES, Schaller GB. Yale University Press, New Haven. Pp. 11-25

Gotelli NJ (2000) Null model analysis of species co-occurrence patterns. *Ecology* 81(9):2606-2621.

Grubb P (1993) Order Artiodactyla. In *Mammal Species of the World: a taxonomic and geographic reference*, eds Wilson DE, Reeder DM. Smithsonian Institution Press, Washington. Pp. 377-414

Hassanin A, Douzery EJP (1999) The tribal radiation of the family Bovidae (Artiodactyla) and the evolution of the mitochondrial cytochrome b gene. *Mol Phylogenet Evol* 13(2):227-243.

Heard SB, Cox GH (2007) The shapes of phylogenetic trees of clades, faunas, and local assemblages: Exploring spatial pattern in differential diversification. *Am Nat* 169(5):E107-E118.

Hernández Fernández M, Vrba ES (2005a) A complete estimate of the phylogenetic relationships in Ruminantia: a dated species-level supertree of the extant ruminants. *Biol Rev* 80(2):269-302.

Hernández Fernández M, Vrba ES (2005b) Macroevolutionary processes and biomic specialization: testing the resource-use hypothesis. *Evol Ecol* 19(3):199–219.

Hernández Fernández M, Vrba ES (2005c) Rapoport effect and biomic specialization in African mammals: revisiting the climatic variability hypothesis. *J Biogeogr* 32(5):903-918.

Janis CM, Damuth J, Theodor JM (2000) Miocene ungulates and terrestrial primary productivity: where have all the browsers gone? *Proc Natl Acad Sci U S A* 97(14):7899-7904.

Janis CM, Damuth J, Theodora JM (2002) The origins and evolution of the North American grassland biome: the story from the hoofed mammals. *Palaeogeogr Palaeoclimatol Palaeoecol* 177(1-2):183-198.

Janis CM, Scott KM, Jacobs LL (1998) Evolution of Tertiary Mammals of North America. Volume 1: Terrestrial Carnivores, Ungulates, and Ungulatelike Mammals. Cambridge University Press, Cambridge.

Kamilar JM, Guidi LM (2010) The phylogenetic structure of primate communities: variation within and across continents. *J Biogeogr* 37(5):801-813.

Kembel SW (2009) Disentangling niche and neutral influences on community assembly: assessing the performance of community phylogenetic structure tests. *Ecol Lett* 12(9):949-960.

Kembel SW, Ackerly DD, Blomberg SP, Cowan PD, Helmus MR, Morlon H, Webb CO (2009) picante: R tools for integrating phylogenies and ecology. R package version 0.7-1. http://picante.r-forge.r-project.org.

Kingdon J (1997) The Kingdon field guide to African mammals. Academic Press, London.

Leroy S, Dupont L (1994) Development of vegetation and continental aridity in northwestern Africa during the Late Pliocene: the pollen record of ODP Site 658. *Palaeogeogr Palaeoclimatol Palaeoecol* 109(2-4):295-316.

Losos JB (2008) Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. *Ecol Lett* 11(10):995-1003.

Lundelius EL, Downs T, Lindsay EH, Semken HA, Zakrzewiski RJ, Churcher CS, Harington CR, Schultz GE, Webb D (1988) The North American quaternary sequence. In *The Cenozoic Mammals of North America: Geochronology and Biostratigraphy*, ed Woodburne MO. University of California Press, Berkeley and Los Angeles. Pp. 211-235

McFarlane DA (1991) The species-genus relationship in Antillean bat communities. *Mammalia* 55(3):363-370.

Mitchell-Jones AJ, Amori G, Bogdanowic W, Krystufek B, Reijnders P (1999) The Atlas of European Mammals. T. & A.D. Poyser, London.

Moreno Bofarull A, Arias Royo A, Hernández Fernández M, Ortiz-Jaurequizar E, Morales J (2008) Influence of continental history on the ecological specialization and macroevolutionary processes in the mammalian assemblage of South America: differences between small and large mammals. *BMC Evol Biol* 8(97):1-18.

Redford K, Eisenberg HJF (1992) Mammals of the Neotropics Vol. 2: The Southern Cone. University of Chicago Press, Chicago.

Semprebon G, Janis C, Solounias N (2004) The diets of the Dromomerycidae (Mammalia: Artiodactyla) and their response to Miocene vegetational change. *J Vertebr Paleontol* 24(2):427-444.

Simberloff DS (1970) Taxonomic Diversity of Island Biotas. *Evolution* 24(1):23-47.

Tobias JA, Bates JM, Hackett SJ, Seddon N (2008) Comment on "The latitudinal gradient in recent speciation and extinction rates of birds and mammals". *Science* 319(5865):901.

Vamosi SM, Heard SB, Vamosi JC, Webb CO (2009) Emerging patterns in the comparative analysis of phylogenetic community structure. *Mol Ecol* 18(4):572-592.

Vrba ES (1992) Mammals as a Key to Evolutionary Theory. *J Mammal* 73(1):1-28.

Vrba ES (1993) Turnover-pulses, the Red Queen, and related topics. *Am J Sci* 293A(A):418-452.

Vrba ES (1997) New fossil of Alcelaphini and Caprinae (Bovidae: Mammalia) from Awash, Ethiopia, and phylogenetic analysis of Alcelaphini. *Palaeontol Afr* 34:127-198.

Waldron A (2010) Lineages that cheat death: surviving the squeeze on range size. *Evolution* 64(8):2278-2292.

Walter H (1970) Vegetation and Clima. Eugen Ulmer, Stuttgart.

Webb CO (2000) Exploring the phylogenetic structure of ecological communities: An example for rain forest trees. *Am Nat* 156(2):145-155.

Webb CO, Ackerly DD, Kembel SW (2008) Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics* 24(18):2098-2100.

Webb CO, Ackerly DD, McPeek MA, Donoghue MJ (2002) Phylogenies and Community Ecology. *Annu Rev Ecol Evol Syst* 33:475-505.

Weir JT, Schluter D (2007) The latitudinal gradient in recent speciation and extinction rates of birds and mammals. *Science* 315(5818):1574-1576.

Wilson DE, Reeder DM (1993) Mammal Species of the World: a taxonomic and geographic reference. Smithsonian Institution Press, Washington.

Appendix 6.1

List of the 79 localities studied in this work, classified by biome and ordered by biogeographic region and continent. Phylogenetic relativeness indexes are shown for all of them: net relatedness index values (NRI) and nearest taxon index values (NTI) against the whole phylogeny of ruminants and against the continental and regional phylogeny (see Material and Methods). Results yield by both using null model 0 and 2 (as implemented in Phylocom) are included. Bold-italic is used for values over 1.96 (phylogenetically clustered) and below -1.96 (phylogenetically dispersed; see Material and Methods).

Biomes:

I: Evergreen Tropical Rain Forest

II: Tropical Deciduous Forest

II/III: Savannah

III:Sub-Tropcial Desert

IV: Sclerophylus Woody Plants

V: Temperate Evergreen Forest

VI: Broad-leaf Dciduous Forest

VII: Stepe / Cold Desert

VIII: Boreal Coniferous Forest (taiga)

IX: Tundra

null model 0 randomizes phylogenetic relations among the species in a sample by shuffling species labels across the tips of the entire phylogeny of reference (Webb et al., 2008).

null model 2 maintains the species richness within each sample, but the species occurring in each locality become random draws from the phylogeny pool (Webb et al., 2008).

Biome	Nº	Locality	Country	Continent	Sp Richness	Region	NULL MODEL 0						NULL MODEL 2					
							NRI vs GLOBAL	NRI vs CONT	NRI vs REG	NTI vs GLOBAL	NTI vs CONT	NTI vs REG	NRI vs GLOBAL	NRI vs CONT	NRI vs REG	NTI vs GLOBAL	NTI vs CONT	NTI vs REG
I	1	Puerto Limón	Costa Rica	North America	2	Neotropic	***2.220***	1.694	-0.528	***2.220***	1.694	-0.528	***2.2284***	1.7193	-0.5383	***2.2284***	1.7193	-0.5383
	2	Paramaribo	Surinam	South America	3	Neotropic	***3.486***	-0.037	-0.032	***3.032***	0.094	0.109	***3.4903***	-0.064	-0.0623	***3.0855***	0.0741	0.0778
	3	Tumaco	Colombia	South America	2	Neotropic	***2.220***	-0.525	-0.528	***2.220***	-0.525	-0.528	***2.26***	-0.5413	-0.5368	***2.26***	-0.5413	-0.5368
	4	Uapes	Brasil	South America	2	Neotropic	***2.629***	1.014	1.016	***2.629***	1.014	1.016	***2.6324***	0.9666	0.9825	***2.6324***	0.9666	0.9825
	5	Sao Paulo	Brasil	South America	3	Neotropic	***3.558***	-0.047	-0.038	***3.045***	0.095	0.105	***3.4285***	-0.0657	-0.052	***3.0117***	0.0735	0.0924
	6	Silchar	India	Eurasia	5	Indomalaysia	0.327	0.333	0.390	0.832	0.243	0.027	0.3488	0.3219	0.419	0.8249	0.2518	0.0534
	7	Medán	Indonesia	Eurasia	6	Indomalaysia	***-3.815***	***-3.198***	***-2.060***	-0.809	-1.289	-1.433	***-3.8563***	***-3.1551***	***-2.0165***	-0.8158	-1.2874	-1.3715
	8	Greenville	Liberia	Africa	12	Afrotropic	1.276	-0.455	-0.610	0.880	0.315	0.168	1.3135	-0.4398	-0.5751	0.8584	0.3338	0.1582
	9	Kribi	Cameroon	Africa	12	Afrotropic	0.919	-0.835	-0.974	0.777	0.243	0.059	0.978	-0.8452	-0.9655	0.7945	0.2366	0.0807
	10	Yangambi	Zaire	Africa	13	Afrotropic	0.262	-1.654	-1.799	0.081	-0.505	-0.717	0.3209	-1.6476	-1.8026	0.1062	-0.5142	-0.7158
II	11	Salina Cruz	Mexico	North America	2	Neotropic	***2.220***	1.694	-0.528	***2.220***	1.694	-0.528	***2.2476***	1.7461	-0.5308	***2.2476***	1.7461	-0.5308
	12	Puerto Ayacucho	Venezuela	South America	2	Neotropic	***2.220***	-0.525	-0.528	***2.220***	-0.525	-0.528	***2.2356***	-0.5407	-0.5361	***2.2356***	-0.5407	-0.5361
	13	Brasilia	Brasil	South America	3	Neotropic	***3.558***	-0.047	-0.038	***3.045***	0.095	0.105	***3.4197***	-0.0701	-0.0669	***3.0542***	0.0689	0.0702
	14	Patna	India	Eurasia	8	Indomalaysia	0.508	0.546	0.595	1.486	0.599	0.201	0.5907	0.5563	0.6519	1.5298	0.5957	0.2526
	15	Trivandrum	India	Eurasia	7	Indomalaysia	***-2.014***	-1.678	-0.934	-0.480	-1.113	-1.330	***-1.9735***	-1.6787	-0.95	-0.4714	-1.139	-1.3386
	16	Phnom Phen	Camboya	Eurasia	9	Indomalaysia	***-3.082***	***-2.651***	-1.645	0.609	-0.277	-0.708	***-3.0773***	***-2.676***	-1.6043	0.5962	-0.3277	-0.6996
	17	Ziguinchor	Senegal	Africa	17	Afrotropic	1.930	-0.150	-0.341	0.542	-0.068	-0.328	1.9577	-0.1733	-0.3339	0.5378	-0.0874	-0.3474
	18	Moundou	Chad	Africa	14	Afrotropic	1.567	-0.294	-0.474	0.823	0.304	0.089	1.6203	-0.3372	-0.43	0.8517	0.2818	0.0986
	19	Mtwara	Tanzania	Africa	18	Afrotropic	***2.550***	0.302	0.175	0.075	-0.563	-0.793	***2.4482***	0.2768	0.1324	0.1085	-0.5443	-0.8051
II/III	20	Río Verde	Mexico	North America	2	Neotropic	***2.220***	1.694	-0.528	***2.220***	1.694	-0.528	***2.2629***	1.7204	-0.5328	***2.2629***	1.7204	-0.5328
	21	Las Piedras	Venezuela	South America	3	Neotropic	***3.486***	-0.037	-0.032	***3.032***	0.094	0.109	***3.5095***	-0.0705	-0.0639	***3.1036***	0.0689	0.076
	22	Remanso	Brasil	South America	3	Neotropic	***3.558***	-0.047	-0.038	***3.045***	0.095	0.105	***3.4911***	-0.0633	-0.0592	***3.1083***	0.0777	0.0789
	23	Jaipur	India	Eurasia	7	Indomalaysia	0.089	0.164	0.295	0.937	0.155	-0.189	0.1605	0.1707	0.3293	0.9548	0.1404	-0.1548
	24	Sholapur	India	Eurasia	9	Indomalaysia	-1.836	-1.537	-0.799	-0.201	-1.042	-1.469	-1.7597	-1.4855	-0.7992	-0.1882	-1.0606	-1.4585
	25	Zinder	Niger	Africa	12	Afrotropic	1.199	-0.600	-0.743	-0.108	-0.675	-0.878	1.1807	-0.6056	-0.7407	-0.0952	-0.6868	-0.8709
	26	Voi	Kenia	Africa	21	Afrotropic	***2.020***	-0.482	-0.652	-0.554	-1.290	-1.621	***1.965***	-0.4941	-0.6729	-0.5294	-1.2887	-1.6222
	27	Gaberone	Botswana	Africa	13	Afrotropic	1.243	-0.673	-0.822	-0.593	-1.174	-1.409	1.1879	-0.7118	-0.8566	-0.5766	-1.1816	-1.3984
III	28	Phoenix	USA	North America	4	Nearctic	-0.187	-0.642	-0.494	0.677	-0.125	0.072	-0.2366	-0.6674	-0.4687	0.6911	-0.1725	0.088
	29	El Paso	USA	North America	4	Nearctic	0.206	-0.233	0.041	1.449	0.576	0.784	0.1592	-0.2427	0.028	1.4318	0.5533	0.7903
	30	Jacobabad	Pakistan	Eurasia	3	Palaearctic	-0.403	-0.283	-0.777	-0.463	-0.488	-0.816	-0.3715	-0.2976	-0.7404	-0.4432	-0.4983	-0.7798
	31	Mascate	Oman	Eurasia	3	Palaearctic	1.771	1.486	1.600	***2.254***	1.644	1.650	1.7424	1.4606	1.5794	***2.2342***	1.6101	1.6603
	32	El-Golea	Argelia	Africa	6	Palaearctic	***2.517***	1.391	***2.860***	***2.725***	***2.322***	1.943	***2.464***	1.4278	***2.7033***	***2.691***	***2.3521***	1.9373
	33	Faya-Largeao	Chad	Africa	5	Palaearctic	***2.675***	1.721	***2.979***	***3.288***	***2.917***	***2.605***	***2.5985***	1.7433	***2.8659***	***3.2451***	***2.9298***	***2.5719***
	34	Galcayo	Somalia	Africa	7	Afrotropic	0.915	-0.354	-0.453	-0.139	-0.740	-0.848	0.9619	-0.3193	-0.4341	-0.1756	-0.6959	-0.8217
	35	Lüderitz Bay	Namibia	Africa	4	Afrotropic	0.998	0.132	0.052	0.105	-0.411	-0.500	0.9962	0.1143	0.0713	0.0904	-0.434	-0.4701
IV	36	Fresno	USA	North America	4	Nearctic	-0.781	-1.261	-1.202	-0.856	-1.554	-1.346	-0.7861	-1.2302	-1.1853	-0.8352	-1.5843	-1.3487
	37	San Diego	USA	North America	3	Nearctic	-1.066	-1.298	-1.291	-1.610	-1.694	-1.613	-1.0343	-1.3147	-1.2742	-1.5807	-1.7622	-1.5794
	38	Potenza	Italia	Eurasia	2	Palaearctic	-0.610	-0.493	-0.812	-0.610	-0.493	-0.812	-0.6331	-0.483	-0.823	-0.6331	-0.483	-0.823
	39	Aleppo	Siria	Eurasia	3	Palaearctic	-0.306	-0.246	-0.708	-0.386	-0.453	-0.746	-0.3739	-0.2922	-0.7582	-0.4393	-0.4927	-0.7969
	40	Esfahan	Iran	Eurasia	3	Palaearctic	0.404	0.316	0.120	1.047	0.636	0.601	0.3516	0.3182	0.0781	1.0258	0.6758	0.581
	41	Tunisia	Tunisia	Africa	2	Palaearctic	-0.609	-1.311	-0.802	-0.609	-1.311	-0.802	-0.6414	-1.3008	-0.8006	-0.6414	-1.3008	-0.8006
	42	El Cabo	South Africa	Africa	6	Afrotropic	1.317	0.146	0.067	0.524	0.072	-0.022	1.284	0.1423	0.0833	0.5537	0.0703	-0.0033
V	43	Nueva Orleans	USA	North America	2	Nearctic	-0.534	-0.740	-0.640	-0.534	-0.740	-0.640	-0.6447	-0.7175	-0.6356	-0.6447	-0.7175	-0.6356
	44	Puerto Alegre	Brasil	South America	3	Neotropic	***3.558***	-0.047	-0.038	***3.045***	0.095	0.105	***3.3846***	-0.0705	-0.0556	***3.0133***	0.0643	0.0838
	45	Kagoshima	Japón	Eurasia	2	Palaearctic	-0.658	-0.496	-0.827	-0.658	-0.496	-0.827	-0.6251	-0.4841	-0.7917	-0.6251	-0.4841	-0.7917
	46	Foochow	China	Eurasia	6	Palaearctic	1.191	1.004	0.960	***2.444***	1.495	1.685	1.1921	1.0642	0.9964	***2.3922***	1.5363	1.636
	47	Shaoguan	China	Eurasia	6	Palaearctic	***2.285***	1.946	***2.438***	***2.093***	1.217	1.310	***2.3445***	***2.0292***	***2.472***	***2.0718***	1.2694	1.2645
	48	Pingnan	China	Eurasia	5	Palaearctic	1.251	1.096	0.999	1.173	0.540	0.400	1.2714	1.1245	1.0761	1.1724	0.5453	0.3944
	49	Cape StLucia	South Africa	Africa	14	Afrotropic	***2.405***	0.426	0.334	0.110	-0.448	-0.610	***2.2748***	0.4024	0.2469	0.1741	-0.4154	-0.6481
	50	East London	South Africa	Africa	9	Afrotropic	1.826	0.308	0.182	1.391	0.970	0.833	1.7258	0.2764	0.1697	1.4232	0.9693	0.8286

VI	51	Prince Rupert	Canada	North America	3	Nearctic	0.393	0.063	0.319	1.079	0.429	0.664	0.3849	0.0498	0.2867	1.0905	0.4193	0.6315
	52	Colorado Springs	USA	North America	4	Nearctic	0.206	-0.233	0.041	1.449	0.576	0.784	0.1484	-0.2457	0.03	1.4458	0.5501	0.7858
	53	St Louis	USA	North America	3	Nearctic	-0.059	-0.459	-0.285	0.075	-0.451	-0.243	-0.14	-0.4358	-0.2612	0.0298	-0.427	-0.2151
	54	Cleveland	USA	North America	4	Nearctic	0.558	0.027	0.430	0.367	-0.473	-0.202	0.463	0.0882	0.4595	0.3601	-0.455	-0.1884
	55	Vlissingen	Holanda	Eurasia	2	Palaearctic	0.972	0.744	0.748	0.972	0.744	0.748	0.9558	0.8055	0.7158	0.9558	0.8055	0.7158
	56	Blagoveschenks	Rusia	Eurasia	4	Palaearctic	0.949	0.733	0.714	0.819	0.370	0.258	0.8974	0.7667	0.6674	0.8826	0.4203	0.2575
VII	57	Medicine Lake	USA	North America	4	Nearctic	-0.187	-0.662	-0.446	0.677	-0.151	0.111	-0.243	-0.6728	-0.4698	0.6855	-0.1865	0.0956
	58	Winnemucca	USA	North America	3	Nearctic	-1.066	-1.298	-1.291	-1.610	-1.694	-1.613	-1.0524	-1.3121	-1.2819	-1.5804	-1.7562	-1.5987
	59	Dakota	USA	North America	6	Nearctic	-0.360	-1.197	-1.062	0.176	-1.086	-0.864	-0.4366	-1.201	-1.0325	0.1978	-1.1037	-0.8413
	60	Santa Fé	USA	North America	5	Nearctic	0.005	-0.581	-0.278	0.399	-0.648	-0.371	-0.0703	-0.5995	-0.3293	0.4122	-0.6732	-0.4091
	61	Caspio	Kazajastan	Eurasia	2	Palaearctic	0.511	0.387	0.250	0.511	0.387	0.250	0.4881	0.3831	0.2525	0.4881	0.3831	0.2525
	62	Alma-ata	Kazajastan	Eurasia	6	Palaearctic	0.257	0.151	-0.397	0.785	0.129	-0.117	0.1575	0.1599	-0.4634	0.8195	0.1538	-0.1184
	63	Urumchi	China	Eurasia	4	Palaearctic	0.397	0.278	-0.019	0.515	0.100	-0.106	0.3159	0.2992	-0.0764	0.516	0.1248	-0.1005
	64	Gobi	China	Eurasia	7	Palaearctic	0.536	0.438	-0.073	1.471	0.593	0.429	0.5025	0.4515	-0.0427	1.4598	0.6278	0.4394
VIII	65	Fairbanks	USA	North America	3	Nearctic	0.179	-0.176	0.063	0.619	0.028	0.288	0.157	-0.1682	0.0389	0.6251	0.0441	0.2525
	66	Edmonton	Canada	North America	4	Nearctic	1.286	0.778	1.438	1.651	0.741	1.009	1.2013	0.9148	1.3775	1.6418	0.781	1.0013
	67	Fort Smith	Canada	North America	5	Nearctic	***1.985***	1.501	***2.535***	1.614	0.639	0.827	1.8796	1.6949	***2.5767***	1.6567	0.6476	0.8324
	68	Smooky falls	Canada	North America	2	Nearctic	1.541	1.057	1.366	1.541	1.057	1.366	1.535	1.1493	1.3359	1.535	1.1493	1.3359
	69	Gaspé	Canada	North America	2	Nearctic	1.541	1.057	1.366	1.541	1.057	1.366	1.5658	1.1363	1.3493	1.5658	1.1363	1.3493
	70	Kajaani	Finlandia	Eurasia	2	Palaearctic	1.562	1.216	1.317	1.562	1.216	1.317	1.5755	1.2386	1.377	1.5755	1.2386	1.377
	71	Serov	Rusia	Eurasia	2	Palaearctic	***2.044***	1.608	1.779	***2.044***	1.608	1.779	***2.0219***	1.6321	1.7723	***2.0219***	1.6321	1.7723
	72	Erbogachen	Rusia	Eurasia	3	Palaearctic	0.494	0.363	0.185	0.755	0.412	0.326	0.457	0.393	0.1846	0.7709	0.453	0.3404
	73	Nikolaevsk-na-Amure	Rusia	Eurasia	4	Palaearctic	0.826	0.631	0.528	0.468	0.057	-0.115	0.7548	0.659	0.4665	0.5108	0.0995	-0.114
	74	Petropavlosk	Rusia	Eurasia	2	Palaearctic	-0.623	-0.502	-0.820	-0.623	-0.502	-0.820	-0.6208	-0.4879	-0.8186	-0.6208	-0.4879	-0.8186
IX	75	Barrow	USA	North America	3	Nearctic	0.187	-0.232	0.037	0.608	-0.032	0.260	0.1569	-0.1619	0.0442	0.6204	0.0421	0.2665
	76	Coopermine	Canada	North America	2	Nearctic	-0.593	-0.749	-0.646	-0.593	-0.749	-0.646	-0.6227	-0.7057	-0.6424	-0.6227	-0.7057	-0.6424
	77	Baker Lake	Canada	North America	3	Nearctic	0.187	-0.232	0.037	0.608	-0.032	0.260	0.1563	-0.1697	0.0622	0.6114	0.0319	0.279
	78	Bulun	Rusia	Eurasia	2	Palaearctic	1.570	1.257	1.354	1.570	1.257	1.354	1.5679	1.2855	1.3385	1.5679	1.2855	1.3385
	79	Nizhne-Kolmsk	Rusia	Eurasia	2	Palaearctic	-0.623	-0.502	-0.820	-0.623	-0.502	-0.820	-0.6241	-0.4755	-0.8152	-0.6241	-0.4755	-0.8152

Appendix 6.2

Table showing the net relatedness index values (NRI) and nearest taxon index values (NTI) of the continental (A) and regional (B) species pools. Results yield by both using null model 0 and 2 (as implemented in Phylocom) are included. Bold-italic is used for values over 1.96 (phylogenetically clustered) and below -1.96 (phylogenetically dispersed; see Material and Methods).

(a)

Continent	***Sp Richness***	***Null Model 0***		***Null Model 2***		***Null Model 0***		***Null Model 2***	
		NRI	***NTI***	***NRI***	***NTI***	***NRI***	***NTI***	***NRI***	***NTI***
North America	13	1.0124	1.2771	0.7839	1.3792	-	-	-	-
South Americarica	13	***9.2296***	***4.5001***	***8.9258***	***4.4844***	CLUSTERED	CLUSTERED	CLUSTERED	CLUSTERED
Eurasia	95	-0.1594	***3.5127***	-0.2074	***3.48***	-	CLUSTERED	-	CLUSTERED
Africa	85	***5.7542***	1.9556	***6.0963***	***1.9914***	CLUSTERED	-	CLUSTERED	CLUSTERED

(b)

Region	***Sp Richness***	***Null Model 0***		***Null Model 2***		***Null Model 0***		***Null Model 2***	
		NRI	***NTI***	***NRI***	***NTI***	***NRI***	***NTI***	***NRI***	***NTI***
Indomalaysia	34	-1.3776	***2.8603***	-1.3127	***2.8572***	-	CLUSTERED	-	CLUSTERED
Nearctic	11	0.199	1.1454	0.2168	1.1274	-	-	-	-
Neotropic	13	***8.699***	***4.535***	***8.7673***	***4.4723***	CLUSTERED	CLUSTERED	CLUSTERED	CLUSTERED
Afrotropic	82	***6.044***	***2.3855***	***6.2361***	***2.4253***	CLUSTERED	CLUSTERED	CLUSTERED	CLUSTERED
Palaearctic	73	***2.7361***	***4.2026***	***2.4639***	***4.1505***	CLUSTERED	CLUSTERED	CLUSTERED	CLUSTERED

CONCLUSIONES

Breves conclusiones

Dado que la presente tesis está organizada en diferentes capítulos y cada uno trata sobre un aspecto diferente de la evolución de los rumiantes, creemos oportuno empezar por destacar los principales hallazgos y aportaciones de cada sección. Posteriormente añadiré un par de reflexiones personales intentando sintetizar ideas y opiniones.

Linajes fantasma y su correlación con variables ecológicas (Capítulo 2).

Se ha desarrollado una nueva metodología para correlacionar una serie de variables ecológicas con la bondad del registro fósil relacionado con los linajes que han dado lugar a las especies actuales. Nuestros resultados indican que el desfase temporal entre la información presentada por el registro fósil y la inferida filogenéticamente es menor en linajes de rumiantes con amplias distribuciones o que habitan ecosistemas más propensos a la formación de yacimientos, como aquellos desarrollados en mayor proporción sobre cuencas sedimentarias. La realización de aproximaciones similares para datos de otros grupos supondrá un gran paso para comprender los sesgos que presenta nuestro conocimiento del registro y acercar posiciones entre aproximaciones paleontológicas y moleculares.

Tasas de diversificación en los linajes de rumiantes (Capítulo 3).

La tasa de diversificación en los linajes de rumiantes ha cambiado significativamente en repetidas ocasiones a lo largo de su historia evolutiva. El periodo de mayor diversificación comienza cerca de los 20 millones de años y llega hasta los 17, coincidiendo con el Óptimo Climático del Mioceno y el establecimiento de climas más estacionales. Este periodo se corresponde con tasas de diversificación muy altas tanto en Bovidae como en Cervidae. Tras un breve periodo con tasas de diversificación reducidas, éstas parecen volver a incrementarse alrededor de los 15-14 Ma. Este máximo parece ser corto en Cervidae, mientras que en Bovidae las altas tasas se mantienen hasta hace 10 millones de años aproximadamente. Muy probablemente este segundo pico se corresponde con el incremento en la aridez y la estacionalidad más severa que caracterizó a los ecosistemas a partir del Mioceno Medio debido al establecimiento definitivo de la placa de hielo del este de la Antártida. También hemos encontrado evidencia que apoya un pico de diversificación importante en los linajes de cérvidos hace unos 5 millones de años y que coincide con radiaciones en linajes eurasiáticos y americanos. En general, los eventos señalados por nuestro análisis están en sincronía con cambios físicos en los ecosistemas a nivel global y con los cambios faunísticos asociados que afectaron a los rumiantes así como a otros grupos animales.

Influencia de la dieta en la diversificación de los rumiantes (Capítulo 4).

Por primera vez se contrasta de manera directa el efecto de la dieta en la especiación de los rumiantes. De esta forma hemos podido comprobar que, tal y como habían sugerido anteriormente otros autores, las dietas facultativas promueven la especiación de los linajes en este grupo, por encima de las especializadas (pastadoras y ramoneadoras). Además, nuestros resultados refutan el modelo clásico en el que la dieta de los rumiantes (y de los artiodáctilos en general) evolucionó de ramoneadora a pastadora pasando por dietas mixtas como un mero paso intermedio. En su lugar proponemos un modelo en el que existe una gran tasa de transición desde dietas pastadoras, más especializadas, de nuevo a dietas mixtas seguramente para acomodar momentos de cambio ambiental. Pese a la inclusión de información climática en algunos modelos no se obtuvo ninguna mejora significativa posiblemente porque el clima global tiene una heterogeneidad regional en su influencia sobre los aspectos evolutivos debido a diversos factores (topografía, corrientes oceánicas...). Según nuestra reconstrucción ancestral de las dietas, los nodos basales de Ruminantia, Tragulidae, Giraffidae y Moschidae se muestran como ramoneadores, mientras que el linaje ancestral de Bovidae sería mixto. La reconstrucción propone un ancestro de Cervidae mixto, aunque en algunos casos un ancestro ramoneador podría también ser consistente con los datos.

Especiación biómica y la hipótesis del uso de los recursos (Capítulo 5).

En este capítulo hemos puesto a prueba las predicciones de la hipótesis del uso de los recursos de Vrba. Hemos encontrado una mayor proporción de especies que habitan sólo un bioma de lo esperado por azar, como resultado de una mayor tasa de especiación en los linajes de especialistas de bioma. También es más alto el número de especies especialistas en los biomas más propensos a sufrir fragmentación y contracción durante ciclos climáticos, tales como la pluvisilva, el bosque tropical deciduo, el desierto subtropical, la estepa y la tundra. Las diferencias observadas entre cérvidos y bóvidos radicarían en una historia biogeográfica y unos requerimientos nutricionales y fisiológicos muy diferentes. Todos estos resultados apoyan la mencionada hipótesis macroevolutiva sobre el uso de los recursos de Vrba.

Estructura filogenética de comunidades de rumiantes (Capítulo 6).

En este trabajo, a una escala de muestreo de 10.000 Km^2, los resultados obtenidos apuntan a que los factores responsables de la estructura filogenética observada en las asociaciones estudiadas estarían relacionados con patrones históricos y biogeográficos. Encontramos comunidades donde la estructura es muy agrupada, principalmente relacionadas con ambientes extremos (por ejemplo desiertos) o eventos de dispersión muy localizados como en el caso de los cérvidos sudamericanos. También se han observado ciertas tendencias latitudinales en los patrones de las estructuras de comunidades, posiblemente asociadas con diversificaciones recientes en biomas fríos en latitudes altas. Concluimos que los trabajos sobre estructura filogenética de comunidades deberán controlar para los factores históricos de los continentes y las regiones biogeográficas cuando intenten identificar dinámicas locales en localidades repartidas por todo el planeta.

Síntesis

Al plantearse la realización de esta tesis sobre cuestiones evolutivas en rumiantes, teníamos muchas preguntas en mente acerca de la evolución de estos particulares artiodáctilos. Mientras que algunas de las cuestiones no han podido ser abordadas por no existir un contexto metodológico adecuado, el desarrollo de nuevas metodologías en los últimos años nos ha permitido complementar nuestro trabajo con otras herramientas adicionales a las inicialmente planteadas.

Hay una problemática que ha suscitado muchísimas preguntas tanto a biólogos como a paleontólogos. En numerosas ocasiones la elaboración de filogenias moleculares arroja fechas de aparición de linajes que preceden significativamente a sus primeras apariciones fósiles. Esto representa uno de los grandes interrogantes pendientes en el campo de la paleontología y la evolución. Sin embargo, nuestros resultados parecen indicar que ese desfase temporal entre la información fósil y molecular para los rumiantes podría estar, en parte, relacionado con las características ecológicas de las especies. Por tanto, casar las dos estimas no depende exclusivamente de mejorar nuestro conocimiento del registro, o de refinar las técnicas de análisis molecular, de mejorar los relojes moleculares y las calibraciones, sino que existe la posibilidad de que puntos concretos de la historia evolutiva de los rumiantes nunca lleguen a ser plenamente esclarecidos. En cualquier caso, el avance en estas cuestiones ha de pasar por alcanzar una mayor integración de las aproximaciones paleontológicas y neontológicas. La tarea es ardua y nuestra correcta interpretación del pasado depende de ello.

Como mencionaba en la introducción, uno de los avances más importantes en las aproximaciones filogenéticas es nuestra creciente capacidad para la interpretación de los patrones evolutivos a partir de las huellas que dejan en los árboles filogenéticos. En el capítulo 3 hemos explorado estas huellas y hemos podido comprobar que en la mayor parte de los casos los fósiles y los datos filogenéticos nos están contando lo mismo. Por ejemplo, todas las evidencias señalan que el final del Mioceno Inferior, hace entre 20 y 17 millones de años, fue un momento clave para la evolución de los rumiantes. Los resultados de nuestros análisis para los linajes que dan lugar a las familias actuales así lo reflejan, y los fósiles lo corroboran además para otros grupos de rumiantes hoy extintos. Existe consenso entre los paleontólogos en señalar que este primer gran pulso en la radiación de los rumiantes está íntimamente relacionado con la aparición de astas y cuernos de manera independiente en varios linajes. Éste y otros momentos importantes en la historia del grupo parecen estar relacionados con varios factores. En primer lugar responden a cambios en las reglas del juego macroevolutivo, los cuales afectan a la vez a diferentes grupos faunísticos. Eventos como el establecimiento de intercambios faunísticos entre continentes o el cambio hacia climas más áridos y el aumento de la heterogeneidad climática debido al paulatino enfriamiento global del Mioceno Medio y Superior, por poner algunos ejemplos, parecen marcar pautas claras en los patrones evolutivos de los rumiantes. El otro factor tiene que ver con la ecología del grupo. En la presente tesis hemos explorado dos factores ecológicos que parecen ser determinantes en la diversificación de los rumiantes y que están íntimamente relacionados con esos cambios de los que hablaba anteriormente. Uno de ellos es la especialización biómica, que favorece los procesos de vicarianza y la subsiguiente especiación. El otro es la flexibilidad en la dieta, que seguramente opera en consonancia con la especialización biómica. En un momento de cambio físico a gran escala los dominios climáticos y las poblaciones de taxones más especialistas de cada bioma se fragmentan. Entra en juego en ese momento la capacidad de las poblaciones para sobrevivir a la escasez de recursos y en este momento los taxones que, en palabras de Waldron, "engañan a la muerte" son aquellos que pueden obtener alimento de una mayor variedad de recursos dentro de la limitación climática a la que están sujetos por ser especialistas del bioma que habitan. Este hecho no sólo se traduce en una mayor proliferación (mayor diversificación) de linajes con dietas facultativas, sino en una enorme capacidad de los linajes para volver a dichas dietas desde dietas pastadoras más especializadas.

Sin embargo, también hemos podido comprobar que los efectos de los cambios climáticos a nivel global se hacen sentir de manera muy heterogénea en la evolución de los rumiantes. Las curvas climáticas nos pueden dar una idea de tendencias globales, pero los procesos evolutivos que desencadenan no son unidireccionales, sino que están modulados regional y localmente por multitud de factores. Estos mismos procesos físicos tienen tal alcance en sus influencias sobre las faunas que han condicionado la historia biogeográfica del grupo hasta el punto que su huella puede todavía leerse incluso a la escala de comunidad en las asociaciones faunísticas actuales. A día de hoy, sin embargo, hay pocos trabajos de este tipo para comunidades de mamíferos. Como hemos visto, la mejor comprensión de los procesos macroevolutivos y macroecológicos que moldean dichas asociaciones pasa por estudiar estas cuestiones a diferentes escalas espaciales, así como por trasladar este tipo de estudios a muchos otros grupos. Uno de los mayores retos de los próximos años es esclarecer precisamente cómo los factores bióticos y abióticos van sustituyéndose en importancia a medida que variamos la escala de estudio. Sólo así lograremos una comprensión global y sintética de la biogeografía de los mamíferos y de cómo se modelan sus comunidades tanto en la actualidad como en el pasado.

Reflexión final

Como todos los trabajos científicos, éste envejecerá. Mejores datos de partida y mejores métodos analíticos estarán disponibles de aquí a poco tiempo. Espero tener algo de culpa en ello; sería muy buena señal. Los avances nos podrán dar la razón, como nosotros hemos hecho con otros trabajos. O podrán quitárnosla. La gran aportación de este trabajo es la demostración de que con una gran variedad de aproximaciones, todas ellas basadas en datos filogenéticos, podemos contrastar diferentes hipótesis macroevolutivas para el conjunto de todas las especies de un grupo. Retomando hipótesis construidas inicialmente sobre datos fósiles o sobre información de las especies actuales que no incluían información sobre sus relaciones evolutivas, se han añadido nuevos datos y puntos de vista que han permitido apoyarlas o complementarlas. Allí donde nuestras conclusiones entran en conflicto con lo previamente demostrado, lejos de la imposición de postulados, contribuimos con el más sano ingrediente de la ciencia: la duda. Así avanzaremos, ensayo y error, paso a paso, puliendo nuestras herramientas y en continuo diálogo interdisciplinar, a hombros de gigantes.

En definitiva, hay dos maneras de mirar al pasado. Una de ellas es mirar el registro fósil, la evidencia directa que los seres vivos de épocas pasadas han dejado en las rocas sedimentarias. La otra, estudiar la información contenida en los árboles filogenéticos, representaciones sintéticas de las relaciones evolutivas entre taxones a partir de información molecular y/o morfológica (a veces incluso etológica) que con la ayuda de las dataciones proporcionadas por los fósiles pueden ser calibradas de manera que los momentos de ramificación puedan quedar emplazados en una escala temporal. Históricamente ha habido, y en cierto modo hay, poca permeabilidad entre estas dos visiones a la hora de asomarse a la evolución de la vida en la tierra. Por un lado los biólogos evolutivos emplean los fósiles para datar los árboles filogenéticos moleculares y posteriormente parecen olvidarse de ellos, dando la impresión de que el árbol que acaban de construir contiene toda la información evolutiva del clado representado. Por el otro, los paleontólogos miran con recelo los árboles filogenéticos que no incorporan más que especies actuales y dan poca credibilidad a reconstrucciones de caracteres o aproximaciones similares, basadas en *cosas que se inventa el ordenador*. En vez de preocuparse por las limitaciones de cada una de sus aproximaciones y buscar en la otra vía la información que las complemente o subsane, parece que en los últimos años las dos corrientes han compartido más bien poca dialéctica. Pensemos en los físicos. ¿Acaso pueden los físicos experimentales rechazar las conclusiones de los teóricos simplemente porque lo que les cuentan todavía no lo han visto? Los agujeros negros, por ejemplo, fueron descritos matemáticamente antes de descubrirse. Y, al contrario, ¿se pueden permitir los físicos teóricos desacreditar a los primeros porque incluso suponiendo una precisión infinita de sus instrumentos de medida nunca podrán medir la realidad hasta el ultimo de los (supuestamente) infinitos universos?

En los años de realización de esta tesis he tenido la oportunidad de trabajar a caballo entre la paleontología y la biología evolutiva y la experiencia de conexión entre los dos mundos ha sido muy positiva, no sólo para mí sino para los equipos de investigadores que han entrado en juego. Ambas disciplinas han salido beneficiadas y enriquecidas por las aportaciones de la otra. Ha habido momentos en los que ambas aproximaciones han concluido en el mismo punto, reforzando ideas anteriores. En otras ocasiones los resultados no han coincidido, poniendo de manifiesto que ambas metodologías pueden reflejar diferentes procesos. Finalmente, hay que admitir que también existen limitaciones. Sí, ambas metodologías tienen limitaciones. Los datos de partida, su medición, su categorización, su interpretación, su síntesis, todos ellos pasos necesarios en la investigación y todos ellos sujetos al error humano. Mi punto de vista es que ambas miradas tienen que convertirse en una. Y mi impresión es que desde hace unos pocos años el telón de acero ha comenzado a rasgarse. Un ejemplo es el taller de metodología filogenética que hay anunciado para el 71º encuentro de la *Society of Vertebrate Paleontology* que se celebra este año (2011) en Las Vegas. En él se abordan temas como el método comparativo, métodos de máxima probabilidad y bayesianos, incertidumbre filogenética y evolución correlacionada de caracteres. La dirección de las dos corrientes parece verdaderamente empezar a confluir y el tren ha comenzado a andar. Subirse a él depende de dos cosas. Una es el aperturismo mental y el abandono de ideas preconcebidas. La otra será el desarrollo de un marco real en el que unificar toda la información. Este segundo punto requerirá la creación de metodologías potentes que nos permitan conjugar la información contenida en los fósiles con la que nos proporcionan las especies actuales. Algunos pasos ya se están dando. Un ejemplo es la construcción de filogenias que contengan taxones fósiles y actuales, tanto a partir de supermatrices que aúnen caracteres moleculares y morfológicos de todos los taxones posibles como mediante la realización de superárboles del conjunto de especies que han existido a lo largo de la historia evolutiva de cada grupo. De esta manera se podrá incorporar información más precisa a las filogenias y se conseguirán estimaciones más ajustadas de los cambios de diversidad de los grupos a lo largo del tiempo. Pronto también será posible incluir, junto a los datos para las especies actuales, los patrones temporales y las variables ecológicas disponibles para taxones fósiles. De esta forma, análisis como los presentados en esta tesis podrán hacerse empleando bases de datos combinadas de especies actuales y fósiles. Este tipo de aproximaciones harán posible análisis a amplia escala como nunca antes se habían podido llevar a cabo y, pese a que este nuevo escenario no estará exento de retos, todo apunta a que el estudio de la evolución será mucho más integral en el futuro de lo que ha sido hasta ahora. Cuestiones como la paleobiogeografía se beneficiarán enormemente de la incorporación de toda esta información, y la paleoecología y la diversificación de taxones extintos podrán estudiarse en un contexto integrador teniendo en cuenta la totalidad del grupo. Hoy más que nunca tienen sentido las palabras de Fredrik Ronquist:

> *"Es el comienzo de una nueva y excitante era para la*
> *reconstrucción de la evolución"*