

Did the expansion of C₄ plants drive extinction and massive range contraction of micromammals? Inferences from food preference and historical biogeography of pikas

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ABSTRACT

The expansion of C₄ plants is thought to have profound effects on the faunal turnover of large mammals during the latest Miocene. However, it is unclear whether this event also induced extinction and range contraction of small mammals. Pikas (Ochotonidae) are highly adapted to arctic or alpine environments, and are considered ideal indicators in biochronology. Previous studies have demonstrated that large numbers of ochotonids were widely geographically distributed in the middle Miocene, although most went extinct in the late Miocene. It is uncertain whether their extinction was induced by the accelerated expansion of C₄ plants. A comprehensive review of the worldwide fossil occurrence in Ochotonidae and a detailed analysis of its historical biogeography are also lacking. Here, we categorize plants that are selected by extant pikas for food and hay piles according to their photosynthesis pathway. Fossil records of Ochotonidae are also reviewed. The divergence time within *Ochotona* is estimated using multiple fossil calibrators and the combined matrix of two mitochondrial genes. We also infer the historical biogeography of *Ochotona* using event based methods (S-DIVA, Bayes-DIVA and Maximum Parsimony). The expansion of C₄ plants in the late Miocene may have resulted in the extinction and range contraction of pikas. This hypothesis is supported by the prominent preference of C₃ plants in extant pikas. The Qinghai–Tibet Plateau is identified as the most likely ancestral center or origin of extant *Ochotona*. Evolution of the pikas' geographical range shows a strong concordance with the global climate and paleoecological change. Their historical extinction and recent range contraction, as well as future survival prospects, appear to be closely associated with climate-driven vegetation changes in their habitats.

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1. Introduction

Plants using the C₄ photosynthetic pathway comprise only 4% of the vascular plant species, however, they dominate today's savanna ecosystem and account for 20%–30% of terrestrial carbon fixation (Osborne, 2008; Edwards et al., 2010). The origin of C₄ plants was dated at 25–32 Ma (Oligocene), while they reached dominance during a period of grassland expansion in the tropical and temperate regions during the late Miocene to early Pliocene (Barry et al., 2002). It has been hypothesized that the expansion of C₄ biomass was accelerated by carbon dioxide starvation of C₃ plants during 8–6 Ma (Quade et al., 1989; Cerling et al., 1997) or possibly a tectonic-induced low-latitude aridity and seasonal imbalance in global rainfall (Pagani et al., 1999). Most C₄ plants are flammable, although they are highly adapted to periodic fires (through rapid re-occupation [Simon et al.,

2009]). In addition, C₄ plants are dominated by a high C/N ratio, but are low in protein. This reduction in food quality resulted in faunal turnover of large mammals in the late Miocene (MacFadden and Cerling, 1994; Cerling et al., 1997, 1998; Ehleringer et al., 2002; Osborne and Beerling, 2006; Osborne, 2008). It is uncertain whether small mammals that live with less energy requirements have been affected by the global expansion of C₄ plants.

Carbon and oxygen isotope analyses of fossil and extant deers, elephants, rhinos, horses and giraffes tooth enamel indicate forage changes that include the dominance of pure C₃ plants or a mixed C₃ and C₄ plants, that occurred in the late Miocene to Pliocene (Zhang et al., 2009; Muhlbachler et al., 2011; Uno et al., 2011). Although the diverse and geographically cosmopolitan of small mammals ranking them as key components for understanding the evolutionary history of mammalian diversity, there are uncertainties regarding this kind of food preferences in these animals, because individual specimens of these small-sized animals do not provide sufficient material required by current carbon and oxygen isotope analysis methods (Grimes et al., 2008). A synthesis of the physiology of food plants with the evolution of small mammals (within geological time scales

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and geographic ranges) will improve our understanding of the paleoecological changes across a large region of the earth.

Within lagomorphs (hare-shaped small to medium-sized terrestrial herbivorous mammals), pikas (Ochotonidae) are generally considered ideal models for studying the climate-driven expiration of animals because of their highly specialized thermal biology and adaptation to alpine or arctic environments (MacArthur and Wang, 1974; Smith, 1974; Hafner, 1994; Beever et al., 2003, 2010, 2011; Wang et al., 2006; Morrison and Hik, 2007; Yang et al., 2008; Galbreath et al., 2009, 2010; Rodhouse et al., 2010). They are also considered ideal indicators for biochronology, the dating of biological events using biostratigraphic remains and palaeontological methods (Angelone, 2008). The oldest fossils in the family Ochotonidae were reported from Asia and North America, dated to the Eocene epoch (Rose et al., 2008). Pikas were widely distributed and highly diversified in the Holarctic during the Early and Middle Miocene. There is a large volume of literature referring to their fossil occurrences and diversification in different regions and epochs (Weston, 1982; Agadjanian and Erbajeva, 1983; Erbajeva, 1988, 1994; Mead and Spaulding, 1995; Grayson, 2005; Bair, 2006; Lopatin and Averianov, 2006; Jass, 2009; Hordijk, 2010; Erbajeva et al., 2011), while a comprehensive review of the worldwide fossil records is still lacking. It is noteworthy that the extant species of pikas are currently confined to a single genus, *Ochotona* (pikas), with 28 species (*O. nigrizia* and *O. gaoligongensis* were considered as synonyms of *O. forresti* in Ge et al., 2012) (Hoffmann and Smith, 2005). Fortunately, the forage selection of several extant pikas has been well studied (Smith et al., 1990; Sokolov et al., 1994). This provides more straightforward information for identifying whether these species prefer C₃ plants or C₄ plants, and thereby allow inferences about the effects of C₄ plant expansion on the biogeographical range evolution of pikas.

The phylogenetic relationships among extant pikas (*Ochotona*) have been investigated using molecular systematic approaches, with particular attention paid to the identification of different ecotype groups (Yu et al., 2000; Niu et al., 2004). Divergence times within *Ochotona* were recently calculated using a relaxed molecular clock technique and suggested a single invasion of *Ochotona* into North America, and major diversification of this genus within the late Miocene (Lanier and Olson, 2009). *O. pusilla*, a late Pliocene relic in this genus (Erbajeva, 1994), was the particular focus of attention in the discussion, although not included in the dated tree (Lanier and Olson, 2009), while *O. huangensis* and *O. erythrotis* were suggested to represent the earliest divergent species within this genus. The divergence time between Leporidae and Ochotonidae was estimated at 31 Ma, 37 Ma, and 65 Ma, using three models. However, the newly discovered fossil in India indicated that the split of Leporidae and Ochotonidae could probably date back to the early Eocene (about 53 Ma) (Rose et al., 2008), rather than the date suggested by each of these three models. Molecular trees of extant mammals based on multiple genes or the entire mitochondrial genome approximately coincided with these fossil records in dating the split of Leporidae and Ochotonidae to 51–52 Ma (Springer et al., 2003; Bininda-Emonds et al., 2007). The deduced divergence time within *Ochotona* was probably delayed due to the use of 31 and 37 Ma as time priors for the split of Leporidae and Ochotonidae. The 65 Ma model dated the split of Leporidae and Ochotonidae at the K/T boundary, and inferred the divergence of *Ochotona* beginning around 20.253 Ma (16.013–24.857 Ma), while fossil evidence for this model is lacking, since the earliest *Ochotona* were dated to the earliest late Miocene (not in the early Miocene) (Fostowicz-Frelik et al., 2010). Dawson (1961) considered the early to middle Miocene *Bellatona* as the likely ancestor of *Ochotona*. This point of view was generally accepted because a series of transitional morph-types between these two genera were discovered in Inner Mongolia, China. The minor differences in the occlusal surface of P₃ made it difficult to classify several specimens into *Bellatona* or *Ochotona* (Zhou, 1988; Qiu, 1996).

These arguments suggest a date of 50 million years for the split of Leporidae and Ochotonidae and that the presence of *Ochotona* was probably accompanied by the disappearance of *Bellatona* in the earliest Late Miocene. Therefore, the divergence time within *Ochotona* needs to be recalibrated and a comprehensive analysis of its historical biogeography is required.

The present study aims to review the fossil occurrences of Ochotonidae and to identify the photosynthetic pathway of the plants selected by *Ochotona*, as well as to infer the historical biogeography of this family based on the occurrence of fossils and the molecular data of *Ochotona*. Based on these analyses, we aim to identify the main ecological factors affecting the massive extinction and range contraction of Ochotonidae and to infer or deduce any possible concordance between C₄ plants expansion and the evolutionary history of pikas.

2. Material and methods

2.1. Discerning food preference

In order to test whether the expansion of C₄ plants affected the food availability of pikas, the possible food plants of extant species were obtained from the available literatures. The main literatures available in the Chinese Academy of Sciences include JSTOR (<http://www.jstor.org/>), Blackwell (<http://onlinelibrary.wiley.com/>), BioOne (<http://www.bioone.org/>), Elsevier ScienceDirect (<http://www.sciencedirect.com/>), Google Scholar (<http://scholar.google.com/>), the China Knowledge Resource Integrated Database (<http://www.cnki.net/>) and several journals preserved in the National Science Library of Chinese Academy of Sciences (<http://english.las.cas.cn/>). “Forage selection” or “food plants” alternatively, together with “pika” or “*Ochotona*” were used as keywords in search requests. The resulting literature included information about plants that were directly consumed and plants selected by pikas to construct haypiles.

We examined the photosynthetic pathway of these plants based on the reported C₄ plant lists (Li, 1993; Yin and Li, 1997; Sage et al., 1999; Tang et al., 1999) and categorized the dietary composition of pikas using the family level status of those species. There were controversial points of view regarding the photosynthetic pathway of several plants, for example, *Achnatherum splendens*, *Artemisia sieversiana*, *Potentilla anserine*, and *Elymus nutans* were reported as C₄ plants (Wang, 2002a, b), however, Li et al. (2009) reported that these plants should be identified as C₃ according to their δ¹³C values. Here, we followed the study of stable carbon isotope analysis by Li et al. (2009) and categorized these plants as C₃.

We also compared the studies of Fan et al. (1995), Jiang and Xia (1985), and Millar and Zwickel (1972), as these studies not only investigated the utilization of different plant species by different pikas, but also considered the relative abundance of those species in their habitats. The top ten plants selected by 4 wild populations of 3 *Ochotona* species were compared. In addition, several literatures referring to the food plants of pikas were published in Russian, for example, Andrushko (1952), and Sapragel'dyev (1987) in Sokolov et al. (1994), and many of the English-language studies on the forage selection of pikas are unpublished or in doctoral dissertations. Information from these sources was unavailable for detailed analysis.

2.2. Tracking fossil records

Data regarding the fossil records of Ochotonidae was downloaded from the Paleobiology database (<http://paleodb.org/cgi-bin/bridge.pl>), the Neogene of Old World Database of fossil mammals (<http://www.helsinki.fi/science/now/>), the Miocene Mammal Mapping Project of west United States (<http://www.ucmp.berkeley.edu/miomap/>) and the National Infrastructure of Mineral Rock and Fossil Resources for Science and Technology of China (<http://www.nimrf.net.cn/>). The fossil records of each genus were also checked by

searching the Zoological Record from 1864 to 2011, which was provided by the ISI Web of Knowledge (<http://apps.webofknowledge.com/>). We also checked the fossil occurrences of Ochotonidae in different epochs. “Ochotonidae” together with the name of different epochs were used as key words in searches. Synonyms were excluded in the analysis. Geographic coordinate information was obtained from the original database, the original records in the literature or with the help of Google Earth (<http://www.google.com/earth/index.html>). These records were mapped on the world map by three layers: the first layer included fossil records from the Eocene and Oligocene epochs, the second layer included fossils from the Miocene, and the third layer included fossils from Pliocene to the recently extinguished populations. The current distribution of pikas was also illustrated based on the data provided by the IUCN Red List of threatened species (<http://www.iucnredlist.org/news/iucn-red-list-site-made-easy-guide>).

2.3. Recalibrating divergence time within *Ochotona*

Molecular dating of the origin and divergence time of *Ochotona* was based on two mitochondrial genes, *cytb* and ND4, which were downloaded from Genbank (<http://www.ncbi.nlm.nih.gov/>). It was suggested that molecular dating using large amount of data and multiple fossil calibrators could improve the accuracy in inferring divergence time (Conroy and van Tuinen, 2003; Renner, 2005; Yang and Rannala, 2006). Here, 28 species, representing all species of *Ochotona* (Hoffmann and Smith, 2005) were included as the in-group, and nine genera of Leporidae, four representative genera of Rodentia, Primates (2 genera), Scandentia (one genus) and Carnivora (one genus) were used as out-group taxa (SI Table 1). Accession numbers for *Cytb* and ND4 of these taxa are given in SI Table 1. 50.2 Ma for the split of Ochotonidae and Leporidae and 69.0 Ma for the crown age of Rodentia were used as time priors. These time priors were based on the most recent study, which combined 26 gene fragments and 82 fossil constraints by using likelihood-based methods and relaxed molecular clocks (Meredith et al., 2011). A relaxed molecular phylogenetics technique was implemented in the program BEAST (Drummond and Rambaut, 2007). The GTR substitution model was used with the dataset being partitioned by codon and substitution models unlinked. The Yule speciation hypothesis was used. MCMC chains were run for 800 million generations for three times and sampled every 1000 generations. The first 25% of trees of each run were discarded as burn-in phase. The results were examined in Tracer 1.5.0 (Rambaut and Drummond, 2004) to confirm the effective sample size for each parameter exceeded 200. The final tree was annotated in TreeAnnotator v1.6.1 (<http://beast.bio.ed.ac.uk/TreeAnnotator>). Figtree v 1.2.2 (<http://tree.bio.ed.ac.uk/>) and Treeview (<http://taxonomy.zoology.gla.ac.uk/rod/treeview.html>) was used to view the topology of trees and generate graphics files.

2.4. Inferring historical biogeography of *Ochotona*

In the past ten years, several different methods for inferring patterns of historical biogeography had been proposed and widely used in relation to two general kinds of tasks (Hovenkamp, 1997; Fattorini, 2008). The first aim is to discern area relationships, and the second is for inferring geographical history of the lineage. Most of these methods are based on the phylogenies. For example, the event base method, Dispersal–Vicariance Analysis (DIVA) has frequently been used both for plants and animals (Ronquist, 1997; Eick et al., 2005; Nylander et al., 2008; Barbero et al., 2009). The newly proposed Bayesian approach of Dispersal–Vicariance analysis (Bayes-DIVA) and statistic analysis of Dispersal–Vicariance (S-DIVA) have resulted in the substantial improvement of DIVA, by taking uncertainties inherent in phylogenies into account (Nylander et al., 2008; Harris and Xiang, 2009). The program S-DIVA (Yu et al., 2010) and its newly released version RASP (<http://mnh.scu.edu.cn/>

S-DIVA/) have been developed to implement these two methods together with the original function of DIVA.

The current distribution of *Ochotona* was divided into central Asia (A), Northeast Eurasia (B), the QHTP and its vicinity (C). We also included North America (D), where *O. collaris* and *O. princeps* are found and Europe (E), which have rich fossil records of *O. pusilla*. Widespread species were coded as present in multiple regions. Analysis of the potential ancestral distribution of *Ochotona* was implemented in S-DIVA version 2 (PASP) (Yu et al., 2010). Trees obtained from the above Bayesian MCMC analysis in BEAST were used as phylogenetic uncertainties. Statistic Dispersal–Variate Analysis, Bayesian MCMC analysis and maximum parsimony analysis were performed to test the accuracy and stability of our inferences.

3. Results

3.1. Food preference of pikas

Among 312 plant species either directly selected as food or used in the construction of hay piles by pikas (SI Table 2), Asteraceae (19%), Rosaceae (9%), Fabaceae (9%), Poaceae (9%) and Ranunculaceae (5%) comprised more than half of the total species (SI Table 2). These plants included about 273 species that had been identified to species and another 39 of them were only listed with genus names (Fig. 1 and SI Table 2). Additionally, different pikas showed prominent preferences for Poaceae, Asteraceae, Fabaceae and Rosaceae. Within all these plants that had been identified to species, *Artemisia dracunculus*, *Cleistogene squarrosa*, *Salsola ruthenica*, 3 C₄ species, were reported as selected by pikas (Bannikov, 1954; Fan et al., 1995; Borisova et al., 2001). A caespitose C₄ grass, *Blepharoneuron tricholepis*, was recorded as abundant in the habitats of *O. princeps*, but it was rarely grazed by this herbivore (Huntly, 1987). A table of detailed information for these plants is given in supplementary documents (SI Table 2).

Comparing the studies of Fan et al. (1995), Jiang and Xia (1985) and Millar and Zwickel (1972) indicated that the grasses (*Elymus*, *Stipa*, *Poa*) and sedges (*Carex*) comprised nearly one half of the food plants in different species, and herbs in Asteraceae, Fabaceae and Rosaceae provided another major choice (Fig. 2). Among these species, *Artemisia dracunculus*, a C₄ plant, was recorded as relatively abundant in the habitats of *O. curzoniae* and *O. dauurica* (Fig. 2a, b), but it was conservatively selected by *O. curzoniae* and much less consumed by *O. dauurica* (Fan et al., 1995). The utilization of the first three most preferred plant species or groups (all belong to C₃ plants) prominently exceeded their relative abundance in these regions. Besides these angiosperms that comprised the major food resources of pikas, fern, pine, mushrooms and feces of marmots were also recorded as occasionally selected by pikas (Rausch, 1962; Elliott, 1980; Su et al., 2004).

3.2. Fossil records of *Ochotonidae*

In total, there are about 31 genera and more than 150 species in Ochotonidae that have been reported. The fossil records of Ochotonidae show some marked regional differences and a strong concordance with global climate change, with most species concentrated in Europe, central North America, northern China, southern Russia, Mongolia, and mostly along the latitude of 40° in the northern hemisphere (Fig. 3). The distribution of *Ochotona* fossils shows a distribution trend northward in latitude and upward in altitude in comparison with the distribution of the ancient genera. The earliest genus *Desmatolagus* appeared in China and North America from the late Eocene to the early Oligocene (Fig. 3b, c). *Sinolagomys*, *Amphilagus*, *Piezodus*, *Bohlinotona*, and *Ochotonolagus* in Eurasia and *Cuyamalagus* and *Oreolagus* in North America appeared from Oligocene to the early Miocene. *Austrolagomys* and *Kenyalagomys* occurred in Africa during the early Miocene. Large numbers of genera diversified

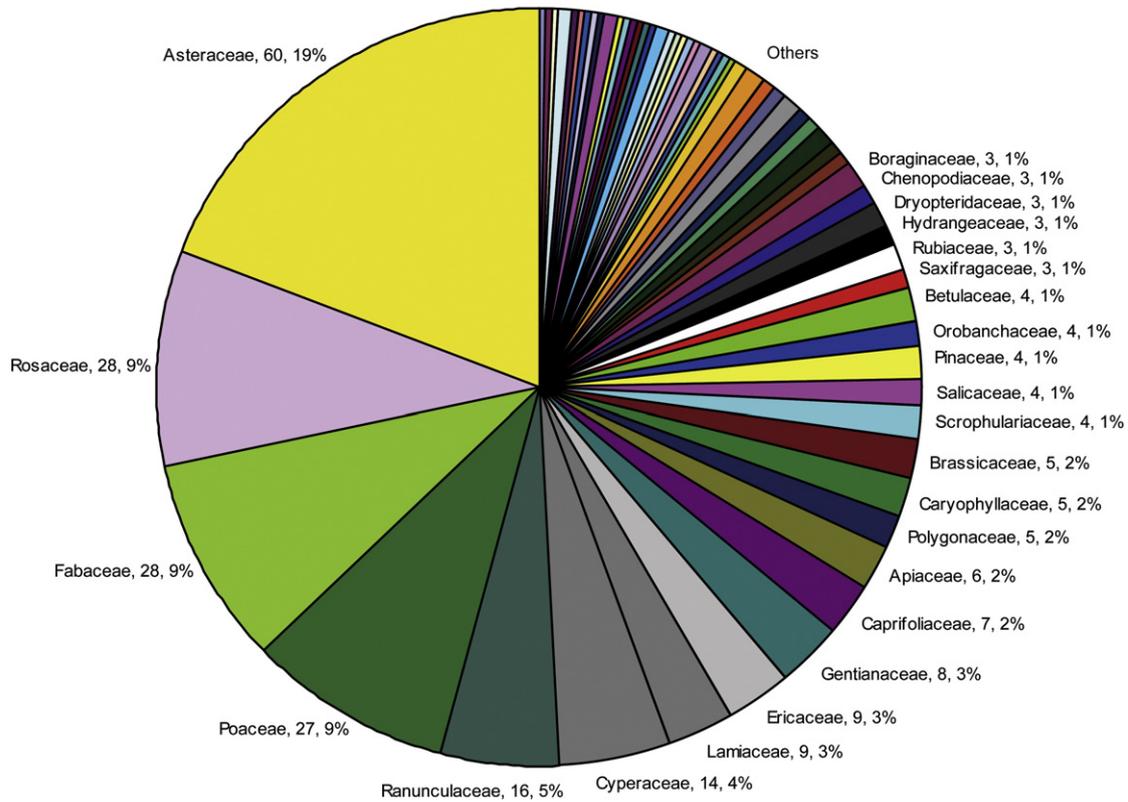


Fig. 1. Proportions of different plant families selected by extant pikas. The plant information was summarized from 29 references that related to forage selection of 11 extant *Ochotona* species. The number and percentage of species in each family were given beside the pie. Species within Asteraceae, Rosaceae, Fabaceae and Poaceae comprised 19%, 9%, 9% and 9% of the total species that was selected by extant pikas.

synchronously in Eurasia and North America in the middle Miocene. Eurasia housed the most diversified fauna in the middle Miocene, while only *Heterologus*, *Prolagus*, *Pliolagomys* and the extant genus *Ochotona* survived the transition of Miocene/Pliocene (Fig. 3b, d, e). The extant pikas remain in fragmented populations in the Middle East and northwestern Russia with most of them confined to alpine and arctic areas of Asia (Fig. 3f). Central North America was also occupied by various genera from the early Miocene to the late Miocene, while only *Ochotona* survived until the Pliocene and Pleistocene (Fig. 3e). *Ochotona* even reached the eastern United States, but populations currently remain fragmented in mountainous areas of the western United States and western Canada (Fig. 3f). The time sequence of each genus is illustrated in Fig. 3b, which shows two times of transcontinental dispersals between Eurasia and North America: one in the late Oligocene and the other in the middle Miocene. This is demonstrated by the existence of *Desmatolagus* and *Ochotona* in these two regions. One transcontinental dispersal can be observed between Europe and Africa, which was indicated by the presence of *Kenyalagomys* and *Australagomys* in Africa (Fig. 3b, c, d). A table that included all reported genera in Ochotonidae is given in appendix (SI Table 3).

3.3. Divergence time and historical biogeography of *Ochotona*

Molecular dating using multiple fossil calibrations indicated the divergence of *Ochotona* probably occurred around 14.65 Ma, which was approximately the time of the fossil records of *Bellatona* and earlier than the earliest occurrence of *Ochotona* in Northern China. The phylogenetic relationships reconstructed here recognize three well supported phylogenetic branches, the first branch includes *O. erythrotis*, *O. muliensis*, *O. gloveri*, *O. forresti*, *O. rufescens*, *O. ladacensis*, *O. koslowi*, *O. illiensis*, *O. rutila*, *O. himalayana*, *O. macrotis* and *O. roylei*; the second branch includes *O. princeps*, *O. collaris*, *O. argentata*, *O. pallasi*,

O. hyperborea, *O. hoffmanni*, *O. turuchanensis* and *O. alpina*; and the third branch includes *O. huangensis*, *O. pusilla*, *O. thomasi*, *O. thibetana*, *O. dauurica*, *O. cansus*, *O. curzoniae* and *O. nubrica*. The crown age for each of the above three lineages was 12.39, 9.35 and 11.41 Ma, respectively. The split of *O. princeps* and *O. collaris* was dated around 5.35 Ma (Fig. 4).

S-DIVA, Bayes-DIVA and maximum parsimony analysis in RASP both indicated the QHTP (C) as the most possible ancestral range of extant *Ochotona* (98.99% in S-DIVA, 98.45% in Bayes-DIVA, 99.84% in maximum parsimony analysis) (Fig. 5). These three lineages established here all originated from QHTP (C), in which the first branch dispersed along the Himalayan Mountains (C) and reached the Middle East and central Asia (A). The second branch expanded to North America (D) and diversified in North Asia (B), and the third branch apparently speciated on the QHTP (C) with one species expanding to Europe (E).

4. Discussion

4.1. Food preferences and evolutionary dynamic of Ochotonidae

After a period of diversification which occurred during the early to middle Miocene, pikas experienced rapid extinction and massive range contraction with only ~6 genera (down from about 20 in the middle Miocene) surviving into the Pliocene (Fig. 3b). In addition to global climate change, causal factors might include more direct ecosystem changes, specifically, the replacement of C_3 grasses by C_4 grasses in tropical and temperate areas. C_4 plants have a reduced CO_2 compensatory point, using water and nitrogen more efficiently than C_3 species (Ehleringer and Monson, 1993). Their adaptations include the ability to rapidly reoccupy fire swept areas (Simon et al., 2009), more so than C_3 plants, giving selective advantage in habitats prone to such periodic disruptions. These differences in morphological and biochemical characteristics of C_3 and C_4 plants have led to

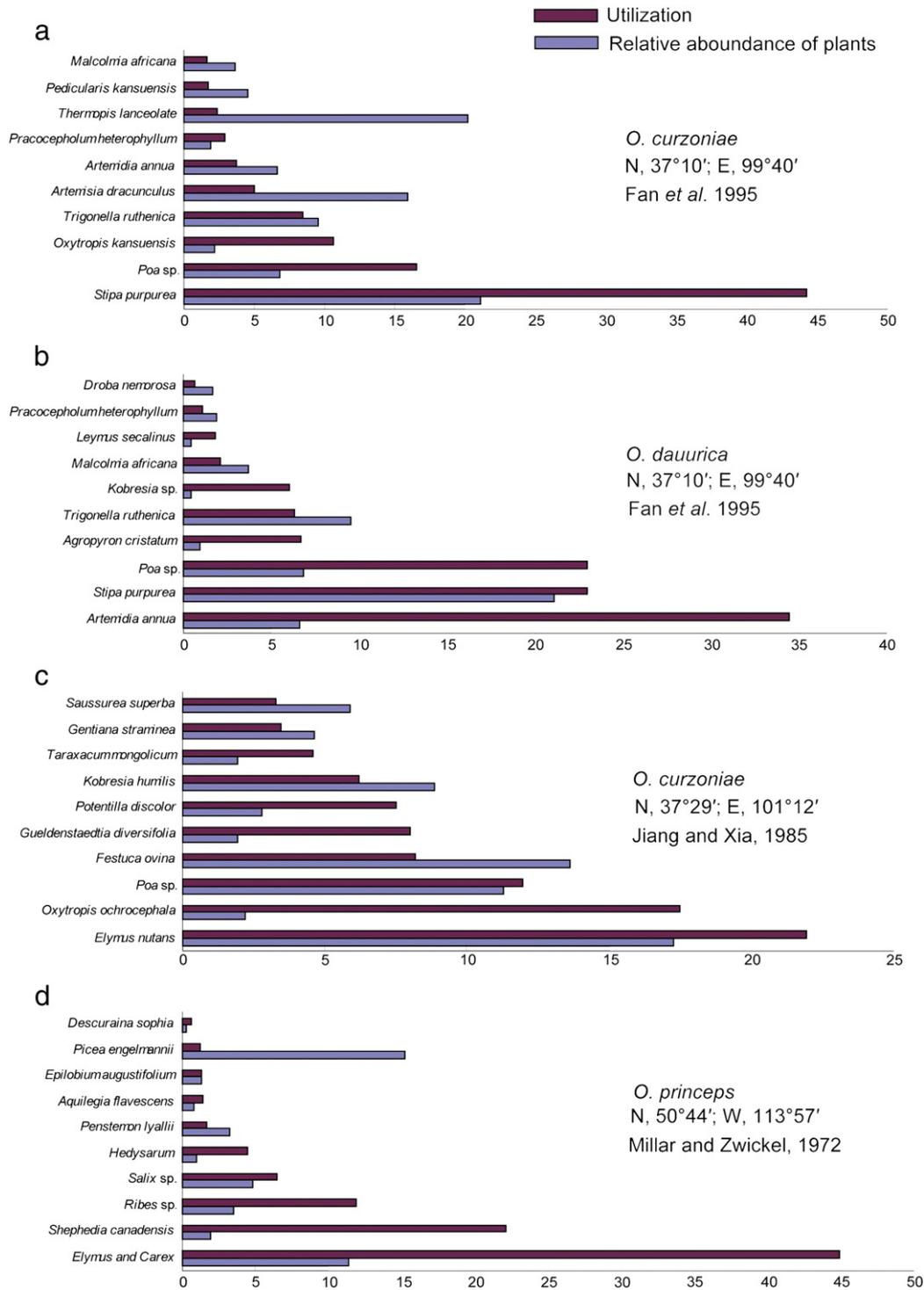


Fig. 2. Comparing the relative abundance and the top ten most preferred plants of pikas. a. Forage selection of *O. curzoniae* in Gangcha county, Qinghai, China (Fan et al., 1995); b. forage selection of *O. dauurica* in Gangcha county, Qinghai, China (Fan et al., 1995); c. forage selection of *O. curzoniae* in Haibei county, Qinghai, China (Jiang and Xia, 1985); d. Plants selected for hay piles by *O. princeps* near Sheep river, Bow forest in Canada (Millar and Zwickel, 1972).

various adaptations in behavior, anatomy and physiology in herbivores. The diversification of mammals is driven by diversification to fill different ecological niches (Luo, 2007; Smith et al., 2010). Their abilities in metabolizing C₄ or C₃ plants have resulted from a long period of historical adaptations. For example, several herbivorous mammals are currently accustomed to a mixture of C₃ and C₄ plants, their body sizes and ecological niches are also extended (Luo, 2007; Smith et al., 2010; Muhlbachler et al., 2011). Amongst these mammals, ancient Artiodactyla (forest-dwelling omnivores) developed a rumen

at nearly 50 million years ago when they were still small in size (<5.0 kg) (Metais et al., 2001). This specialization contributes greatly to digestion of large amounts of C₄ plants that are low in protein but rich in fibers (Hackmann and Spain, 2010). Pikas are small in size and occupy fairly narrow niches. Their food plants have a significantly higher caloric, protein, lipid and water content (Smith and Weston, 1990).

Even pikas show prominent territory behavior, they also have the ability to disperse among ecological patches within a short or long

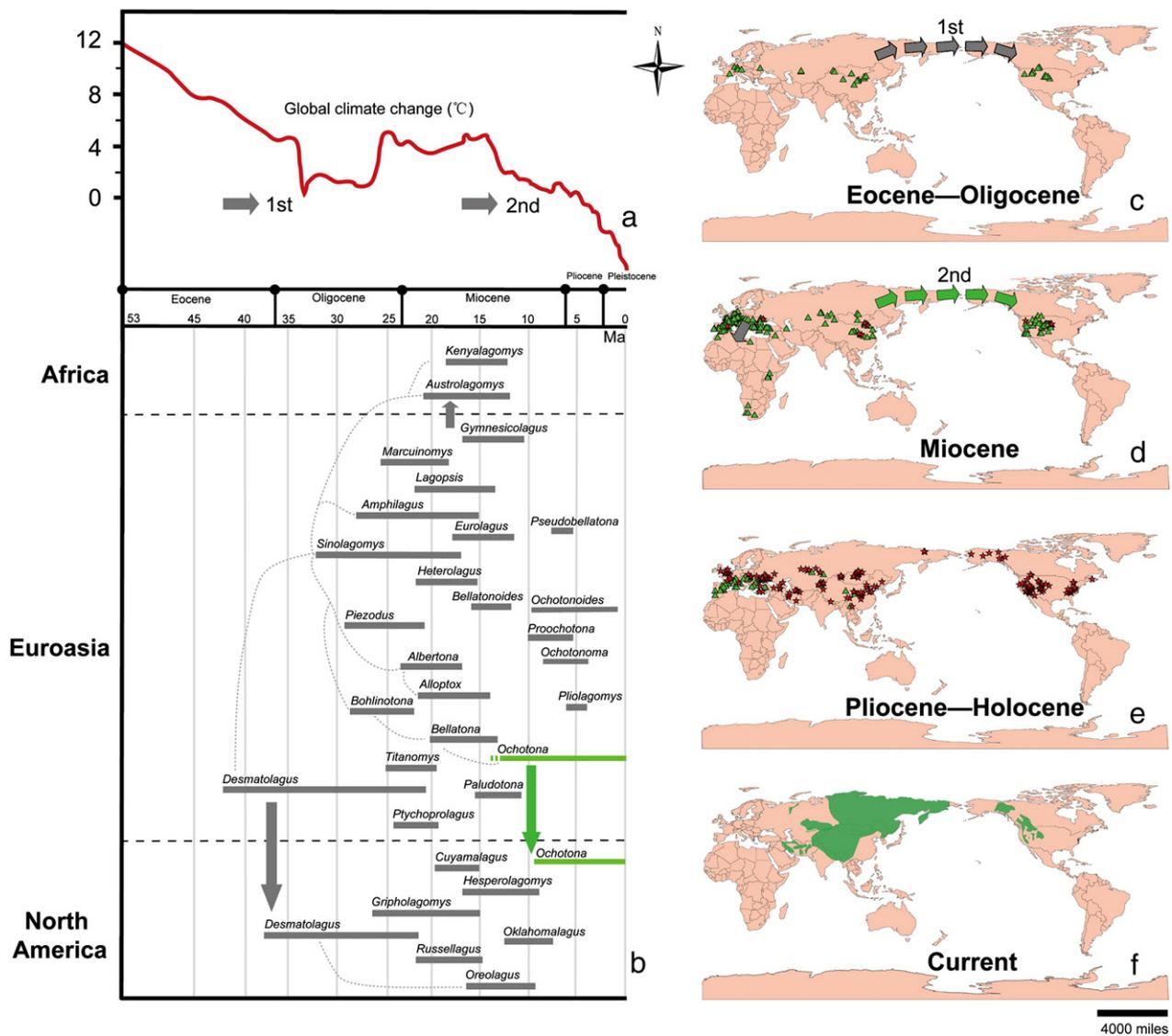


Fig. 3. Transcontinental dispersal and evolution of Ochotonidae. a. The global climate change trend (Fig. 2 in Zachos et al., 2001); b. presence of Ochotonidae in different epochs, gray bars show the ancient genera, green bars indicate extant genera; c–e. The occurrence of fossil Ochotonidae on earth: c. Eocene to Oligocene; d. Miocene; e. Pliocene to Pleistocene; f. the current distribution of *Ochotona*. Triangles show the ancient genera of Ochotonidae, stars show the occurrence of *Ochotona*.

distance, especially, their juveniles show a higher proportion of inter-patch movements (Franken and Hik, 2004). Li et al. (2005; 2009) reported that there are about 52 C_4 plant species (32 genera and 9 families) found in the alpine localities of QHTP, consisting about 1.49% of total plant species in these regions. *Pennisetum centrasiatum*, *Arundinella yunnanensis* and *Orinus thoroldii* are notable in expanding above 4000 m (Luo et al., 2004). However, these plants are not present in the food or hay piles of pikas (SI Table 2) Stable carbon and nitrogen isotope analysis has demonstrated the plateau pikas have less ability in digesting C_4 foods in controlled feeding experiments (Yi et al., 2004). Based on the forage selection of extant species (SI Table 2), the pikas seem to mainly insist on C_3 plants that are rich in protein and secondary components. Ingestion of secondary components is a physiological and behavioral challenge for herbivores, and several species containing these components are avoided by large herbivorous mammals (Sorensen et al., 2005). However, pikas are well adapted in consumption of these toxic components (Dearing, 1997a,b; Dearing et al., 2005; Gliwicz et al., 2006). Several species also harvest food in the late summer and autumn (Hirakawa, 2001). Additionally, pikas show coprophagic behavior, extracting additional

nutrition by giving food a second pass through the gut (Pshennikov et al., 1990), behaviors less reported in ruminants.

The worldwide expansion of C_4 plants in the late Miocene induced extensive replacement of forest by C_4 grassland, a process that was completed in a relatively short historical period (Edwards et al., 2010). The change from C_3 dominated plants to C_4 plants within a short period was probably an evolutionary challenge for mammals such as pikas. Large numbers of ancient pikas went extinct in savanna and temperate areas during this period of global ecosystem change, which resulted in food shortage and habitat losses. These inferences are demonstrated by the extinction of pikas in Africa, Europe and their prominent range contraction in Asia and North America from the late Miocene to the Pliocene. Alpine areas in Asia and Europe probably functioned as refugia for pikas in the late Miocene, particularly the rising of QHTP plateau, on which C_3 plants still maintain their dominance to date (Yi et al., 2003; Edwards et al., 2010). This area likely also harbored the ancestors of extant pikas (*Ochotona*). More open-adapted rhizomyids and hares occupy large areas of tropical and temperate habitats (Cerling et al., 1997), which are dominated by C_4 plants.



Fig. 4. Phylogenetic relationship and divergence time of *Ochotona*. This tree was derived from maximum clade credibility tree of Bayesian inference analysis in Beast. Blue bars at each node shown the 95% confidence interval for the divergence time. Posterior probabilities of each node were shown above the nodes. The divergence time of each node were given near the blue bars. Color of the braches indicating three ecotype groups recognized in the analysis: the Shrub-steppe group, the Northern group and the Mountain group.

Despite few reports referring to the occurrence of America pika (*O. princeps*) at atypical low-elevation localities (Beever et al., 2008; Simpson, 2009; Millar and Westfall, 2010; Manning and Hagar, 2011), it is generally accepted that the average elevation of *O. princeps* in the great basin raised prominently in recent years, and several of its lower latitude populations is susceptible to contraction or localized extirpations (Beever et al., 2003, 2010, 2011; Grayson, 2005; Beever and Smith, 2011; Wilkening et al., 2011).

Besides, several endemic species in Eurasia are also under the threat of range contraction, not least due to continuing global warming. *O. argentata* is critically endangered, *O. iliensis*, *O. koslowi*, and *O. hoffmanni* are now ranked as endangered while another four species are also under the trend of decreasing (Li and Smith, 2005; Smith and Johnston, 2008a,b,c,d). This is being driven by a combined set of forcing factors, such as regionally heterogeneous atmospheric concentration caused by anthropogenic or natural events, land use

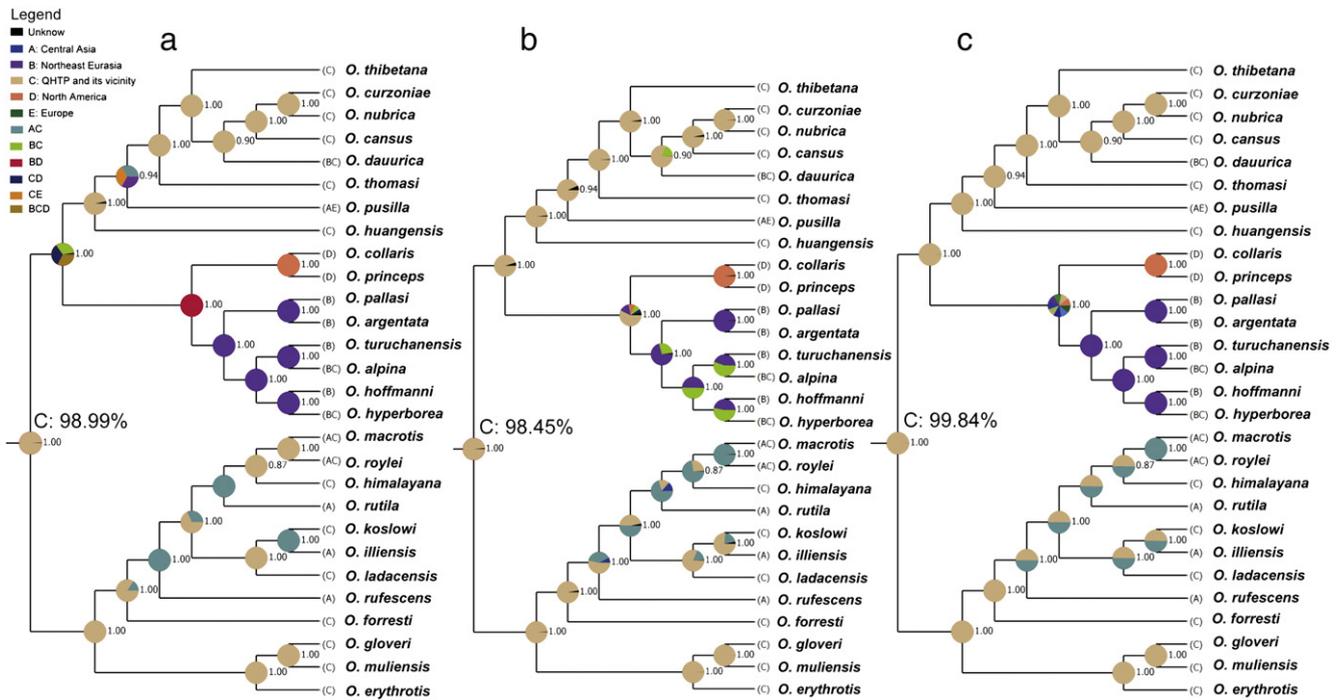


Fig. 5. Ancient distributions of extant *Ochotona* inferred using different algorithms. a. Statistic Dispersal–Vicariance Analysis; b. Bayesian MCMC analysis; c. Maximum parsimony analysis. The phylogeny was based on the Majority consensus trees derived from Bayesian inference analysis of two genes (outgroups were excluded in this analysis). Posterior probability were shown beside each node. Biogeographical regions used in the analysis including: A. Central Asia; B. Northeast Eurasia; C. QHTP and its vicinity; D. North America; E. Europe. Pie charts at internal nodes represent the marginal probabilities for each alternative ancestral area. For example, the pie charts at the root of *Ochotona* indicating QHTP and its vicinity as the most common ancestral area of this genus was 98.99% in the Statistic Dispersal–Vicariance Analysis (a), 98.45% in the Bayesian MCMC analysis (b) and 99.84% in the maximum parsimony analysis (c).

changes due to the increasing size of the human population, as well as global-scale influences from solar variability and transient increases in human-produced greenhouse gases (Schneider, 1994; Knohl and Veldkamp, 2011; Wolff, 2011). On the account of C_4 grasses that prosper in a warmer, CO_2 -enriched world (Baldochi, 2011), the extant pikas probably will experience a challenge similar to their ancestors. Besides undertaking range shift into the new habitats of cooler regions (moving towards the North Pole or to higher altitudes), some endemic species are likely to risk extinction and range contraction due to habitat loss and food shortage.

4.2. Origination, transcontinental dispersal of Ochotonidae and the global climate change

Desmatolagus, possibly the earliest genus of Ochotonidae to date, were reported in the late Eocene during a period of global warming. It survived global cooling in the transition from the Eocene to Oligocene. In the cooling period of the early Oligocene, the pikas diversified slowly. They flourished in the “global climate optimum” from late Oligocene to the middle Miocene, and even reached southern Africa, when the global average temperature was about 0.7 K warmer than present-day (Krapp and Jungclaus, 2011). After the global climate optimum in the middle Miocene, there was a trend of gradual global cooling from the late Miocene to Pleistocene. The generic diversity of Ochotonidae greatly decreased during this period. It disappeared in Africa and dramatically declined in Eurasia and North America. The only extant genus, *Ochotona*, is now confined to cooler habitats in alpine and arctic regions. However, large number of other herbivores, for example, ruminants, leporids and several species in Rodentia, were still expanding their ranges after the late Miocene (Chivers, 1989).

DIVA analysis identified QHTP and its vicinity (C) as the most likely ancestral distribution area of pikas. This result is in conflict with the fossil record, which considered the earliest occurrence of *Ochotona* as

northern China (Fostowicz-Frelik et al., 2010). However, this result is generally in agreement with the “tropical conservatism hypothesis”, which claims the tropical origin of primitive taxa in several different animals (Hawkins and DeVries, 2009). The historical biogeographic analysis of *Ochotona* indicated that the ecological conditions of QHTP was largely different from other regions of the world during the transition period of Miocene to Pliocene. The QHTP probably offered refugia for some extant mammals in these global “green revolution” events, particular for mammals that prefer C_3 plants as food resources (Osborne and Beerling, 2006).

Two dispersal events of pikas between Eurasia and North America both coincided with periods of global cooling (Fig. 3a), as indicated by the presence of *Desmatolagus* and *Ochotona*, the common genera in these regions. The first transcontinental dispersal event occurred within the Eocene–Oligocene transition, which was associated with a prominent decrease in temperature. The second transition probably occurred in the late Miocene, as the earliest fossils of ancient *Ochotona* were already present in North America in the late Miocene (Oregon, Nebraska) (Shotwell, 1956; Erbjajeva et al., 2011). The global climate during the second transcontinental dispersion was similar to the transition during the Eocene–Oligocene, being characterized by lower temperatures. The Bering Land Bridge probably provided connectivity between Asia and North America during those two periods. These inferences could be further supported by the expansion of *O. princeps* in the glacial period and their extinction and range contraction caused by interglacial retraction (Galbreath et al., 2009). The ‘Gomphotherium Landbridge’ connecting Africa and Eurasia in the early Miocene (Harrison and Gu, 1999; Harzhauser et al., 2007), probably also facilitated the migration of pikas to Africa during this period.

4.3. The phylogeny and biogeographic history of *Ochotona*

Though fossil records of Ochotonidae are abundant, the phylogenetic relationships among primitive ochotonids have been scarcely

studied and remain poorly known. Convergent evolution of rootless genera with hypselodont genera on different continents presents pikas as a very confusing family in taxonomy. The oldest genus, *Desmatolagus*, appeared in Northern China and central North America during the late Eocene, possibly representing the most primitive genus of this family (Qiu, 1996). However, this genus had been considered a Leporidae by several researchers (Matthew and Granger, 1923; Martin, 2004). We followed Qiu (1996) and Dawson (2008) in considering this genus as a primitive taxon within Ochotonidae. The biogeographical distribution of this genus indicates the close relationship of the Asian fauna with the North American fauna, and hence its importance in Ochotonidae should probably not be neglected. It is possible that the lack of fossil records of Ochotonidae in the eastern Russia and central Canada might be due to insufficient sampling.

The difference among the phylogenetic relationships within *Ochotona* that were reconstructed in the current study and previous publications may be due to differences in the numbers of species included in analysis and different strategies in rooting of trees. The present analysis includes multiple outgroup taxa, while previous studies only included one or two species of Leporidae as the outgroup (Yu et al., 2000; Niu et al., 2004; Lanier and Olson, 2009). It has been pointed out that rooting with multiple taxa may greatly improve the accuracy of phylogenetic inference, particularly when using Cytochrome *b* to reconstruct the phylogeny of mammals (May-Collado and Agnarsson, 2006). We believe the current strategies for reconstructing the phylogeny and dating the divergence time of *Ochotona* are more reliable. The phylogeny reconstructed here generally agrees with Yu et al. (2000) in recognizing three ecotype groups: the shrub-steppe group, the northern group and the mountain group. While *O. huangensis* was found as the earliest diverging species within the shrub-steppe group, instead of forming a separate basal lineage as in Yu's study. *O. pusilla* appears younger than *O. huangensis*, instead of being the earliest diverged within the extant species. Interestingly, the morphology of *O. huangensis* shows multiple primitive characteristics (Fostowicz-Freluk et al., 2010), which are extremely similar to *O. pusilla*. For example, the skull roof of *O. huangensis* is almost flat dorsally, the fenestration below the maxillary is dense and well-developed, and the posterior region of the zygomatic arch is prominently strengthened. Identifying *O. huangensis* as the earliest taxon within shrub-steppe group also indicated that the climate in Central China was probably similar to Northern China around 12 Ma, and that the elevation of QHTP probably had not reached a point resulting in separation of this species from other shrub-steppe species.

The three ecosystems currently harboring extant pikas coincide with global climate change events in the northern hemisphere from the Late Miocene to Pleistocene. The mountain group diverged from the ancestral species in the southwest of QHTP, and dispersed along the Gaoligong Mountain. One lineage branched into the Middle East (*O. rufescens*) and the other dispersed along the Himalayan Mountains. Seven species then arose in the northwest of QHTP and in central Asia. Species within this group were mainly adapted to a rocky environment, and speciation was probably induced by uplift of the QHTP in South Asia and the Pamir Mountainous region in Central Asia. The Northern group also separated from its ancestor in the QHTP, one lineage expanded to North America around 9 Ma, and the other survived in Northeast Asia. Species within the Northern group mainly live in forest areas of Asia and North America, the former mainly diversified within the Pliocene. Their speciation probably resulted from the formation of East Asia monsoon climate, which heavily influenced vegetation in those areas. The shrub-steppe group was mainly adapted for a burrowing existence, consistent with the strengthened zygomatic arch in *O. huangensis* and *O. pusilla*. The latter evolved around 11 Ma and expanded into Europe in the late Miocene, but currently survives as fragmented populations in central Asia. The other species within the shrub-steppe group are mainly

concentrated in the central region of QHTP, and their speciation was probably connected with ecological differentiation. For example, *O. thomasi* and *O. cansus* are usually observed in shrub areas, *O. thibetana* are mainly present in forest, while *O. curzoniae* and *O. nubrica* dominate more open habitats. These inferences indicate the ecological differentiation of extant *Ochotona* probably occurred before the diversification within this genus. The evolution within each of these three branches is possibly correlated with their specialized habitat preferences.

5. Conclusions

The expansion of C_4 plants in the late Miocene fundamentally altered the global ecosystem. This event not only induced faunal turnover of large mammals, it was also a likely causal factor in the extinction and range contraction of small mammals that were adapted to C_3 plants. The data presented here indicates the prominent preference of C_3 plants in extant pikas. The extinction of their ancestors in late Miocene was probably a result of the “accelerated replacement” of C_3 plants by C_4 plants in the ecosystem. The recent extinction and range contraction of their wild populations in the semi-arid area was probably also accompanied by continuing expansion of C_4 plants as a result of global warming and increased aridity (Williams et al., 2010; Morgan et al., 2011). It is reasonable to suggest their future distribution will be driven by the range dynamics of C_3 plants within their habitats. The geographical distribution and dispersal events of ochotonids coincided with change in the global climate and ecosystem, with two episodes of transcontinental dispersal events both occurring within a period of global cooling. The extant *Ochotona* appear to be derived from QHTP, but the extent and hierarchy of Ochotonidae can only be determined with a more comprehensive sampling of fossils in different regions, particularly in central Canada, eastern Russia and the Southwest of China.

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Appendix A. Supplementary data

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