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Original investigation

Pleistocene diversification of Afghan pikas *Ochotona rufescens* (Gray, 1842) (Lagomorpha; Ochotonidae) in Western Asia

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ABSTRACT

Phylogeography and taxonomy of Afghan pikas *Ochotona rufescens* in Western Asia were investigated based on a combination of mitochondrial cyt *b* gene sequences and morphometric analyses. We measured 200 specimens from the whole range of *O. rufescens* in Western Asia, including the holotypes of *O. r. regina*, *O. r. shukurovi*, *O. r. vulturna*, *O. r. vizier* and samples from vicinity of the type locality of *O. r. rufescens*. Principal Component and Factor analyses did not reveal any notable geographical variations. The phylogenetic analysis of mitochondrial cyt *b* sequence data from 72 specimens of Afghan pikas included six topotypes of three of the recognized subspecies and revealed that divergence between most populations is shallow. The major genetic divergence lay between the samples from Afghanistan and other populations. Previously unrecognized lineages of Afghan pikas were identified in Central Alborz and central east Iran. We demonstrated minor divergence between *O. r. regina* from Kopet Dagh Mountains, and *O. r. shukurovi* from the Great Balkhan Mountains, indicating a recent divergence between these two populations. We hypothesize that Pleistocene climate fluctuations in Western Asia are responsible for the diversification in *O. rufescens*, but that adverse local edaphic conditions in the lowlands may have largely prevented dispersal during glacial periods, thus making dispersal events more infrequent than for comparable populations of pikas in North America.

Introduction

Pleistocene cooling is often thought of as causing populations to contract to refugia (Darlington, 1957; Hewitt, 1999, 2004a) but particularly for Alpine and high mountain dwellers population expansions have also been demonstrated (Galbreath and Cook, 2004; Guralnick, 2007; Galbreath et al., 2009). The impacts of cyclic glacial and interglacial Pleistocene events (in approximately 100000-year cycles; Rutherford and D'Hondt, 2000; Shackleton, 2000; Lisiecki and Raymo, 2005a) on demographic features and the distributional patterns of temperate species have been investigated to a higher degree for the Nearctic region (Hewitt, 2000; Beever et al., 2003; Galbreath et al., 2009; Galbreath et al., 2010; Zgurski, 2011), and the European part of Palaearctic (Taberlet et al., 1998; Hewitt, 1996, 1999, 2000, 2004b; Pilot et al., 2010) than for most other areas. Nevertheless, there are also studies investigating the effect of Pleistocene climate changes on the genetic structure and geographical pattern of distribution of taxa in lower latitudes of the Western Asia (Michaux et al., 2004; Kasapidis et al., 2005; Dubey et al., 2006; Stöck et al., 2006; Graham et al., 2012). Slight to moderate (less severe) impact of Pleistocene climate change on lower latitudes (Bobek, 1937; Wright, 1962; Shroder and Bishop, 2010) resulted in regression of the Caspian Sea (Boomer et al., 2000) and increasing continentally corroborated expansion of steppe habitats, increased seasonality (van Zeist and Bottema, 1991; Djamali et al., 2011; Wasylikowa, 2005; Manafzadeh et al., 2017; Wang et al., 2016) and retreat of forest habitats in Western and Central Asia during Quaternary (El-Moslimany, 1986, 1987; Mirzaie Ataabadi, 2007; Djamali, 2008; Manafzadeh et al., 2017). The concomitant effect of these oscillations was cyclic contraction and expansion of fauna and flora and displacement or fragmentation of their ranges

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in Central and Western Asia. However, the response appears to have been different between lowland and alpine adapted forms (Smith et al., 1990; Grayson, 2005; Albach et al., 2006). During cold periods of Pleistocene Alpine dwellers such as pikas could expand their populations to the lower altitudes and could also occupy new low land habitats owing to decline in temperature (Galbreath et al., 2009, 2010). On the other hand, formation of Pleistocene deserts in Western Asia (Kehl, 2010; Wang et al., 2016) and expansion of C4-grass lands in response to climate change, as a limiting factor against increasing population size of pikas stopped gene flow between different populations and corroborated vicariance events (Ge et al., 2012, 2013).

Afghan pika *Ochotona rufescens* (Gray, 1842) is the only species of pika inhabiting mountains of the Western Asia. It was described by Gray as *Lagomys rufescens* from the hills near "Baker's Tomb" (Kabul, Afghanistan). Afghan pika is a rock dwelling species in arid areas; its occurrence is patchy in the region, occupying mainly high altitudes mountains above 1200–3600 m (a. s. l.) (Hassinger, 1973; Roberts, 1977). However, it has occasionally been reported from farm-lands and open fields at lower elevations (Hassinger, 1973; Puget, 1976; Roberts, 1977; Fulk and Khokhar, 1980). The species' distribution in Western Asia encompasses Zagros and Central Alborz Mountains extending to the east through Kopet Dagh Mountains in the northeast Iran to southwest Turkmenistan. It also occurs from Paropamisus Mountains along the northern part of Afghanistan to the western mountains of Pakistan (Ellerman and Morrison-Scott, 1951; Lay, 1967; Hassinger, 1973; Corbet, 1978; Weston, 1982; Smith et al., 1990; Gromov and Erbajeva, 1995; Ziaie, 1996; Firouz, 1999; Hoffmann and Smith, 2005; Čermák et al., 2006; Fostowicz-Frelik et al., 2010; Lissovsky, 2016).

Five nominal taxa associated with this species have been described, but authors differ in their treatment of these. Ellerman and Morrison-Scott (1951) list three subspecies: *O. r. rufescens*, from Afghanistan; *O. r. regina*, Thomas, 1911 from Kopet Dagh Mountains; and *O. r. vizier* Thomas, 1911 from Kohrud, central west Iran. Additionally, Smith et al. (1990) added *O. r. shukurovi* Heptner, 1961 from the Great Balkhan Mountains, Turkmenistan. Hoffmann and Smith (2005) recognized three subspecies and assigned *O. r. vizier* into synonymy with *O. r. rufescens*. All cited authors (Ellerman and Morrison-Scott, 1951; Smith et al., 1990) considered the subspecies *O. r. vulturna* Thomas, 1920 from Chiltan Mountains in Quetta, Pakistan invalid; because the description was based on an aberrant specimen (incisive and palatal foramina are separated). However, Afghan pikas display notable variation in palatal foramen structure. There is a degree of variation of closure between palatal and incisive foramen. Incisive foramina and the palatal foramina are completely or partially distinct from each other in some specimens and are confluent in some others. Additionally, the presence of narrow gap between maxillary and premaxillary bones in some Afghan pikas was mentioned (Lissovsky, 2014). The holotype of *O. r. vulturna* is similar to other Afghan pikas in cranial morphology (Lissovsky, 2014) and the holotype of *vulturna* falls within normal intraspecific morphometric and morphological variations. The taxon *seiana* Thomas, 1922 was placed in *O. rufescens* by many authors (Smith et al., 1990; Hoffmann and Smith, 2005), but is now considered a junior synonym of *O. curzoniae* Hodgson, 1858 (Corbet, 1978; Lissovsky, 2014)

To date, studies on *O. rufescens* have mainly focused on morphometrics (Weston, 1982; Angelici and Corti, 1990; Aliabadian, 1995; Lissovsky, 2014), morphology (Aliabadian, 1995; Fostowicz-Frelik et al., 2010) and karyology (Nadler et al., 1969; Vorontsov and Ivanitskaya, 1973; Aliabadian, 1995). Molecular analyses have only covered parts of the species' range (Khalilipour et al., 2014). No taxonomic revisions of Afghan pika based on quantitative data across the entire range have so far been undertaken. In this study we investigate i) the genetic divergence of *O. rufescens* in Western Asia and ii) the historical phylogeography of *O. rufescens* in Western Asia.

Material and methods

Samples for morphometric and genetic study

For morphometric analyses, 200 skulls of *O. rufescens* were examined from the collections of the Zoological Museum of Moscow University (ZMMU, Moscow), Zoological Institute of Russian Academy of Sciences (ZIN, Saint-Petersburg), Natural History Museum (BMNH, London), Field Museum of Natural History (FMNH, Chicago), Smithsonian Institution's National Museum of Natural History (USNM, Washington, D.C.), Museum of Vertebrate Zoology (MVZ, Berkeley), American Museum of Natural History (AMNH, New York), Slezské zemské museum (SZM, Czech), Zoological Survey of India (ZSI, Kolkata) and Zoology Museum of Ferdowsi University of Mashhad (ZMFUM Mashhad, Iran; Fig. 1 and Supplementary Table 1).

For molecular and population genetic analyses, a total of 32 pikas were collected by live traps from different localities of the northeast and central east Iran and the central Afghanistan. In addition, three skin samples from Kopet Dagh and two skin samples from the Great Balkhan, Turkmenistan were obtained from the collections of the ZMMU (Supplementary Table 1). Moreover, we included mitochondrial cytochrome *b* (cyt *b*) sequences of 35 *O. rufescens* and 28 other pika species from GenBank. Additionally, *Lepus europaeus*, *L. capensis*, *L. granatensis* and *Oryctolagus cuniculus* were used as outgroups (Supplementary Table 2).

Morphometric analyses

In total, 19 variables from 200 skulls of *O. rufescens* including the holotypes of *O. r. regina*, *O. r. shukurovi*, *O. r. vulturna*, *O. r. vizier* and samples from the vicinity of the type locality of *O. r. rufescens* were measured using Vernier caliper of an accuracy of 0.01 mm (Lissovsky, 2014). The abbreviations of morphometric variables are as follows: condylobasal length (CBL), upper diastemal length (UDL), alveolar length of maxillary toothrow (MTL), rostral length (from the anterior edge of premaxillary bones to the posterior edge of maxillary toothrow alveoli) (RL), length of auditory bulla (ABL), distance between auditory bullae (ABD), length of the suture between parietal bones (PSL), length of the suture between frontal bones (FSL), maximal length of orbit (OL), maximal width of orbit (OW), interorbital constriction (IC), width of the rostrum between maxillary foramina (RW), zygomatic breadth (ZB), postorbital constriction (PC), maximal width between lateral edges of auditory bullae (ABW), general skull height (SH), skull height on the level of auditory bullae (ABH), general height of mandible (MH), height of mandible behind the toothrow (MBH). The cranial and mandibular measurements are illustrated in Supplementary Fig. 1.

For morphometric analysis the following approach was performed: specimens were categorized to three age groups: (1) obvious juvenile, (2) subadults and (3) adults (Lissovsky, 2004, 2014). To exclude age bias, an orthogonal projection of log-transformed data along the vector of age variation (Burnaby, 1966) was used. A modified Factor analysis was applied as an ordination method: first, the vector of age variation was calculated as the first eigenvector of the between-group covariance matrix computed with nested two-factor MANOVA, in which the variable containing age classes 1 and 3 (we excluded age class 2 in order to minimize errors arising from the inaccuracy in the determination of the age), as well as the identifier of the one-species geographical sample were used as the grouping variables. The age factor was nested in geographical sample. Therefore, the dataset with reduced age was obtained calculating the eigenvectors of the within-group covari-