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First Stable Isotope Analysis of Asiatic Wild Ass Tail Hair from the Mongolian Gobi

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First stable isotope analysis of Asiatic wild ass tail hair from the Mongolian Gobi

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Abstract

Stable isotope analysis has become a powerful tool to study feeding ecology, water use or movement pattern in contemporary, historic and ancient species. Certain hair and teeth grow continuously, and when sampled longitudinally can provide temporally explicit information on dietary regime and movement pattern. In an initial trial, we analysed a tail sample of an Asiatic wild ass (*Equus hemionus*) from the Mongolian Gobi. We found seasonal variations in H, C and N isotope patterns, likely being the result of temporal variations in available feeds, water supply and possibly physiological status. Thus stable isotope analysis shows promise to study the comparative ecology of the three autochthonous equid species in the Mongolian Gobi.

Key words: isotope analysis, *Equus hemionus*, hair, feeding conditions, Mongolia

Introduction

Equid species are very similar in size and body shape and seem to occupy very similar ecological niches (JANIS 1976, RUBENSTEIN 1989, BAUER et al. 1994, MOEHLMAN 2002). Overlap zones among species are mostly small and little work has been done to understand resource use and physical adaptations that explains species distribution (BAUER et al. 1994). In the Dzungarian Gobi of Mongolia three equid species, the Asiatic wild ass (*Equus hemionus*, fig. 1), the reintroduced Przewalski's horse (*Equus ferus przewalskii*, fig. 3) and the domestic horse (*Equus caballus*, fig. 4) share the same habitat and thus provide a unique opportunity for comparative ecological studies (KACZENSKY et al. 2008). Continuous observations of free-ranging equid species are almost impossible to conduct in this remote and harsh environment; a problem compounded by the large expanse of their home ranges. Physiological measurements either require highly invasive techniques and/or confinement to a captive or semi-captive environment (e.g. ARNOLD et al. 2006, KUNTZ et al. 2006). This requires the adoption of novel approaches.

Stable isotope analysis has become a powerful tool to study feeding ecology, water use and infer movement pattern in contemporary, historic and ancient species (Hobson 1999, Sponheimer et al. 2009). The basic principle is that the isotopic composition of animal tissues reflects the isotopic structure of its food, water and ambient environmental conditions. The isotopic structure of its food varies with season, location and species. Based on their isotopic signature and their turnover rate, different tissues allow different inferences to be drawn over different time spans. Certain hair and teeth, grow continuously and when sampled longitudinally can provide temporally explicit information on dietary regime and movement pattern (SCHWERTL et al. 2003, HOPPE et al. 2004a, CERLING et al. 2006a,b, 2008; SCHNYDER et al. 2006, WITTMER et al. 2010).

Browsers and grazers in African savannah systems have been traditionally separated using the ratio of C_3 to C_4 plants in their diet (CODRON et al. 2007, CERLING et al. 2008, CODRON & CODRON 2009). This is possible because in tropical and subtropical grasslands the majority of grasses show the C_4 pathway, while the majority of shrubs have a C_3 pathway (EHLERINGER et al. 2005). In the cold-tempered grasslands of Asia, on the other hand, grasses primarily use the C_3 pathway (EDWARDS & SMITH 2010), while a multitude of annuals and perennials, particularly of the family Chenopodiaceae, and utilize the C_4 pathway (PYANKOV et al. 2000, EHLERINGER et al. 2005). Consequently, in central Asian drylands the relationship of C_3 to C_4 plants in grazers and browsers is reversed (MAKAREWICZ & TUROSS 2006).

Fig. 1: Stallion and mare Asiatic wild ass at Chonin us oasis in the center of Great Gobi B SPA (photo: P. KACZENSKY).

Fig. 2: Tail of an Asiatic wild ass chemically immobilized for radiocollaring in Great Gobi B SPA (photo: P. KACZENSKY).

Isotopic analysis of hydrogen and oxygen isotopes gives information about the water ingested by the animal. The isotopic composition of winter precipitation usually has lower hydrogen and oxygen isotope values than summer precipitation, due to its dependency on temperature. Different water sources may also have different isotope values, e.g. springs fed largely by water from snow melt and experiencing little evaporation will possess low isotope ratios (depleted), whereas large stagnant ponds will have high δ^2H and $\delta^{18}O$ ratios (enriched; DANSGAARD 1964, GAT & GONFIANTINI 1981, HOBSON et al. 1999).

Isotopic analysis of nitrogen isotopes can allow inferences about the physiological status of an organism. N isotopes in animals are incorporated in animal tissue from the animal feed with a positive offset (enrichment). If starvation during winter time is a relevant factor, an increase in N isotopes should be reported in animal tissue (MARTINEZ DEL RIO et al. 2009, MCCUE & POLLOCK 2008). However, the plant material consumed by the animals can also vary in N isotope values and thus changes in N isotope values of animal tissue do not necessarily only reflect physiological changes but may also be caused by dietary changes.

The mane and tail hair of domestic horses (*Equus caballus*) grows continuously at a rather constant rate of 0.792 mm/day or 28.9 cm/year (DUNNETT 2002, DUNNETT & LEES 2003). Consequently, incrementally sampled horse tail hair can provide seasonal information on dietary and water use changes which in turn permits inferences about movement pattern and physiological processes (CERLING et al. 2006a,b; MARTINEZ DEL RIO et al. 2009).

To test for potential seasonal patterns in free-ranging wild equids, we analysed a tail sample of an Asiatic wild ass from the Mongolian Gobi and discuss the potential of stable isotope analysis to study the comparative ecology of the three autochthonous equid species in the Mongolian Gobi.

Materials and Methods

We obtained a 38 cm long tail hair sample of a 4-year old Asiatic wild ass mare (fig. 2) captured in July 2009 (KACZENSKY & WALZER 2009). The tail hair was stored at room temperature in a dry paper envelope. Prior to analysis we thoroughly cleaned the hair sample by washing it with detergent and ethanol to remove any dirt and grease. We subsequently cut the tail sample in 38 one centimetre long pieces.

We weighted aliquots of the samples into tin and silver capsules. Hydrogen isotope analysis was performed with a thermal combustion elemental analyzer (TC/EA, Finnigan, Bremen/Germany) connected via a Conflo (Finnigan, Bremen/Germany) to a Delta xp isotope ratio mass spectrometer (IRMS, Finnigan, Bremen/Germany). For carbon and nitrogen isotope measurements samples were introduced into an elemental analyzer (Vario, Elementar, Hanau/Germany) and the evolving gases were flushed via a ConFlo (Finnigan, Bremen/Germany) into an IRMS (Finnigan, Bremen/Germany).

We present isotope values as ‰ deviation with respect to internationally accepted standards: hydrogen isotopes versus V-SMOW (Vienna Standard Mean Ocean Water), carbon isotopes versus V-PDB (Vienna PeeDee Belemnite), and nitrogen isotopes versus N-air. Standard deviations were better than 3 ‰ for H isotopes (1 δ) and better than 0.3 ‰ for C and N isotopes. For visualization of potential seasonal patterns we assumed a constant hair growth of rate of 0.792 mm/day or 28.9 cm/year as described for domestic horses (DUNNETT 2002, DUNNETT & LEES 2003).

We also collected eight common food plant samples near the Takhin Tal research station (45°32’19’’ N, 93°39’5’’ E) at the NE corner of the Great Gobi B Strictly Protected Area in July 2009. We dried the samples and stored them in paper envelopes. For analysis we homogenized each individual sample using a ball mill. Aliquots of the plant samples were analyzed in the same way as the hair samples, but only for C and N isotopes.

We obtained data on average daily temperatures and precipitation from a temperature datalogger and rain gauge (Hobo, Onset Computer Corporation, Inc., MA, USA) at the Takhin Tal research station.

Fig. 3: Przewalski horse mare and foal along the Bij river in the Great Gobi B SPA (photo: P. Kaczensky).

Fig. 4: Children riding domestic horses during a Nadam celebration near the village of Bij at the edge of the Great Gobi B SPA (photo: P. Kaczensky).

Results

Isotope values varied between -122 and -80 ‰ V-SMOW for hydrogen, 7.5 and 9.7 ‰ N_{Air} for nitrogen, and -22.6 and -18.6 ‰ V-PDB for carbon. Whereas hydrogen and carbon isotopes seemed to show negatively correlated oscillation pattern, nitrogen isotopes seemed to follow a different pattern (fig. 5).

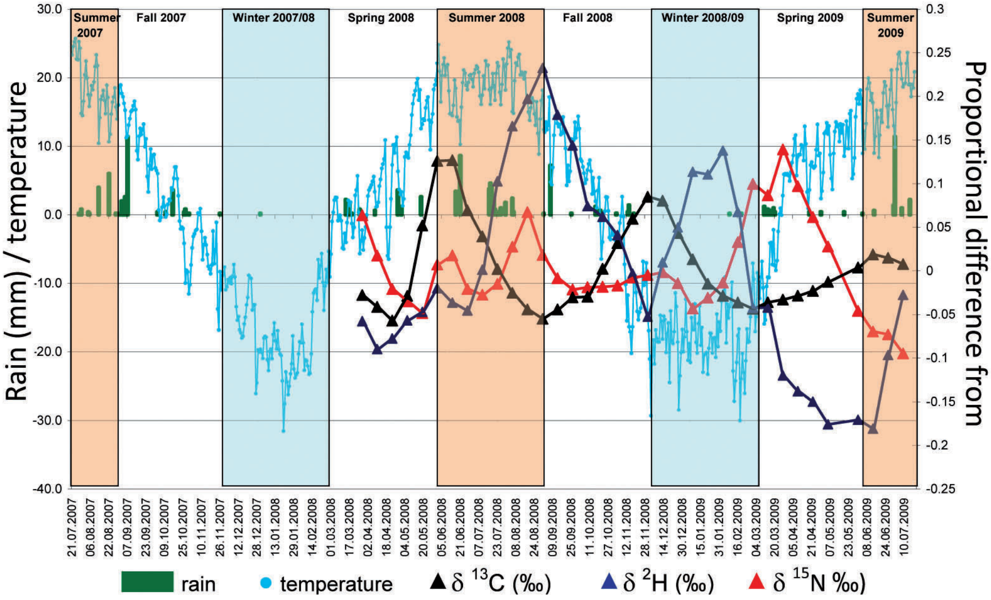

Fig. 5: Sequential isotope signature of a 38 cm long tail hair sample of an Asiatic wild ass mare plucked during live capture in July 2009. The time line is based on assuming a constant tail hair growth of 0.792 mm/day or 28.9 cm/year as described for domestic horses (DUNNETT 2002, DUNNETT & LEES 2003).

The plant samples show nitrogen isotope values varying widely from -0.2 ‰ for *Zygophyllum neglectum* to 12.0 ‰ for *Ephedra przwalskii.* Saxaul (*Haloxylon ammodendron*), a C_4 plant with a $\delta^{13}C$ of -13.5 ‰, had a moderate $\delta^{15}N$ value of 8 ‰, whereas the other plant samples are all C_3 metabolizers with C isotope values ranging from -26.2 to -23.0 ‰ (table 1).

Table 1: Isotope signature of eight common food plants collected in the Great Gobi B SPA in SW Mongolia in July 2009

Plant species	$\delta^{15}N$	$\delta^{13}C$
Achnatherum splendens	8.2	-25.8
Allium mongolicum	4.9	-24.4
Artemisia spec.	11.4	-25.0
Caragana leucophloea	1.5	-24.7
Ephedra przwalskii	12.0	-23.0
Haloxylon ammodendrum	8.0	-13.5
Stipo glareosae	3.6	-24.1
Zygophyllum neglectum	-0.2	-26.2

Discussion

Using the constant tail growth rate from domestic horses (DUNNETT 2002, DUNNETT & LEES 2003) the 38 cm wild ass tail would date back ~16 months and thus covered the period between mid March 2008 and mid July 2009. On this time line the highest H isotope values coincide with late summer and the height of winter, suggesting that the animal covered its water demand largely from enriched water sources (e.g plant material or water sources with high evaporation) and in spring and at the end of fall from depleted sources (e.g. precipitation fed springs).

Carbon isotopes suggest that with fresh vegetation becoming available the wild ass switched from a shrub-dominated C_4 diet in spring to a grass-dominated C_3 diet in early summer, back to a shrub-dominated diet in fall but back to a grass-dominated diet in mid-winter. The N-isotope pattern is more difficult to interpret but points towards enriched values in late winter / early spring, the time when wild and domestic horses tend to be in the worst body condition.

Our preliminary results suggest that stable isotope analysis shows promise to study the comparative ecology of free-ranging equids in the Mongolian Gobi. In a next phase we plan to analyse multiple tail samples from all of the three sympatric equids. Furthermore, tail growth rates in wild asses and Przewalski's horses needs to be validated and water sources and key food plants analysed in respect to their spatial and temporal variability. Ultimately we also hope to be able to draw conclusions about habitat use between recent, re-introduced Przewalski's horses and historic, autochthonous Przewalski's horses by additionally analysing museum samples.

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Participants of the symposium, from left: Prof. Dr. Martin Pfeiffer (Ulaanbaatar), Karl-Heinz Schindlatz (Lohberg), Dr. Petra Kaczensky (Wien).