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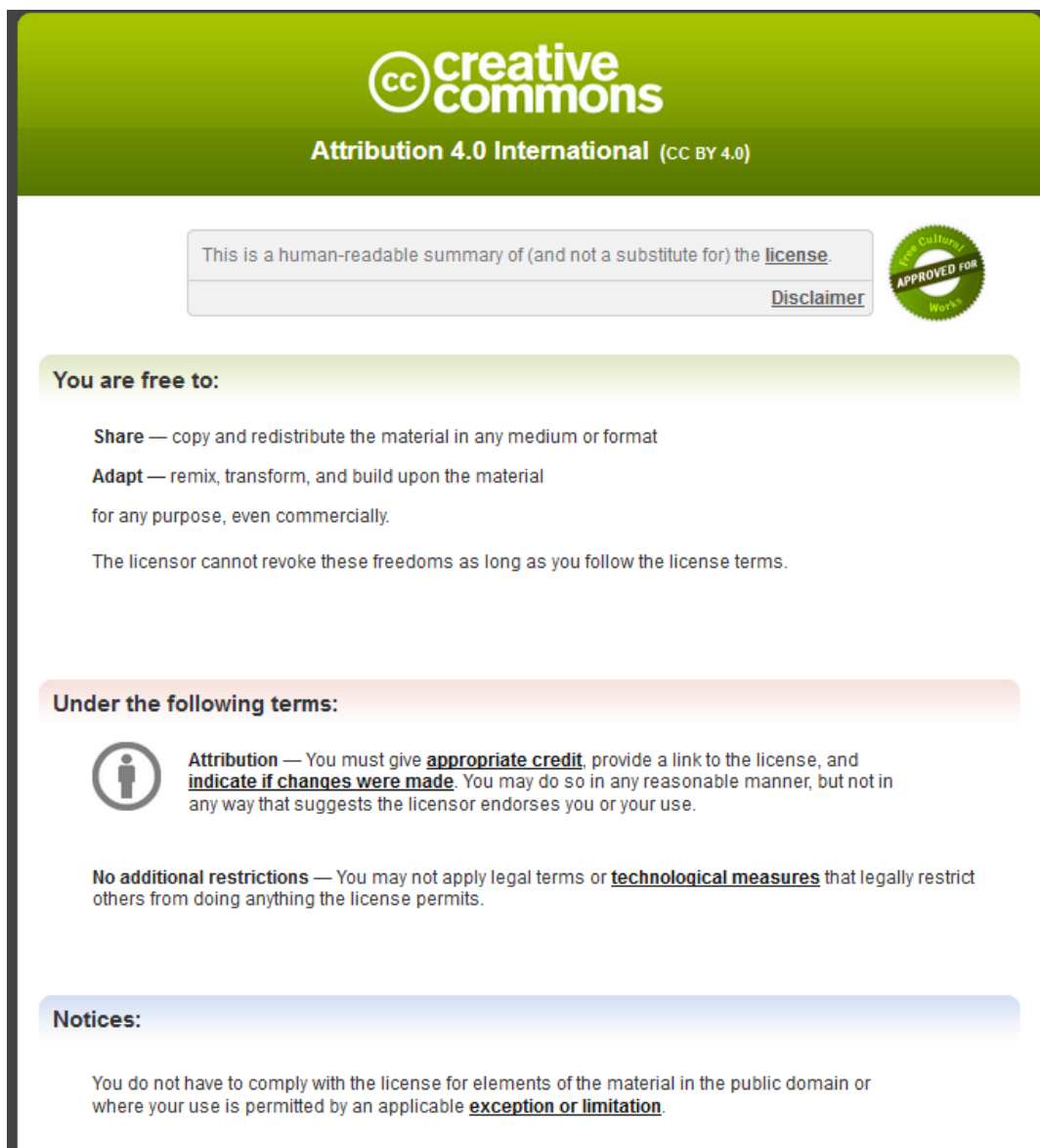
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The moose, purine degradation, and environmental adaptation

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Abstract It is accepted that allantoin is the end-product of purine degradation in mammals, except that uricase activity has been lost during the evolution of humans in which uric acid protects the brain from oxidative damage. However, we have found that the moose *Alces americanus* excretes extremely low urinary concentrations of allantoin and high concentrations of uric acid very similar to those of humans. Exposure to extreme cold is known to cause oxidative damage, and we suggest that the retention of uric acid by the moose represents an adaptation enabling the species to survive at high latitudes.

Keywords *Alces americanus* · China · Purine degradation · Uric acid

Introduction

It is generally accepted that low concentrations of plasma uric acid occur in non-primate mammalian species (Cutler 1984). However, in 1959 it was pointed out that the conclusion that all mammals other than primates have uricase capability is based on studies of a limited number of species (Keilin 1959). The seminal data for concentrations of purine derivatives in ungulate urine

was provided by Hunter and Givens (1914), who examined the major domestic ungulates, and found that purine derivatives were mostly to almost always excreted as allantoin. Since then, all published studies of mammals other than primates have confirmed the primacy of allantoin as the end-product of purine degradation, including additional studies in ruminants such as sheep (Bristow et al. 1992; Surra et al. 1997), goats (Belenguer et al. 2002; Bristow et al. 1992), cattle (Bristow et al. 1992), water buffalo (Chen et al. 1996), yak (Long et al. 1999), camel (Mohamed 2006; Guerouali et al. 2004), llama (Bakker et al. 1996), red deer (Vagnoni et al. 1996; Garrott et al. 1996; Christianson and Creel 2010; Zhang and Zhang 2012), and white-tailed deer (DelGiudice et al. 2000; Cabanac et al. 2005). Nevertheless, only a small portion of ruminant species have been investigated, and many environments to which ruminants have adapted do not have a representative species.

We undertook a study intended to determine whether the nutritional status of moose *Alces americanus* in northeast China was influencing winter survival. We used analyses of purine derivatives in urine, which have been used as indices for evaluation of nutritional condition in various species of domestic (Moorby et al. 2006) and free-ranging (White et al. 1997) ruminants. In the course of that study, we failed to find allantoin in the urine of any Chinese moose. We obtained confirmation of our finding through samples collected on our behalf from North American moose and sent directly to an independent North American metabolomics laboratory for analysis.

The aim of this paper is to report that all non-primate mammals are committed to extending the process of purine degradation to the conversion of uric acid to allantoin, and to suggest that the loss of uricase capability by the moose is adaptive.

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Methods

Field study

During January in the winters of 2006–2010, we collected 106 frozen urine samples from the surface of the snow at Erkehe Forest, Heilongjiang Province, China (48° 39' 30"–48° 48' 21" N; 127° 59' 05"–128° 15' 19" E). January wind chill (WC) ranged from −15.8 to −50.9 °C, and mean snow depth from 174 to 413 mm. We searched for moose bedding sites, and then followed hoof prints until we found urine deposits. We used hoof-print locations and trajectories to ensure that individual moose were represented no more than once each year. We collected samples into containers, added sulphuric acid to maintain pH below 3, and stored them at −20 °C. For comparison with well-fed moose free of cold stress, during 2012, we arranged for urine from ten well-fed captive moose in Alaska (mean WC on the day of collection 4.1 °C) and one in Colorado (WC 15.5 °C) to be sent directly to the Metabolomics Laboratory, University of California Davis (UC Davis) Genome Center, for analysis.

Laboratory analysis

We used HPLC to detect hypoxanthine, xanthine, urate, and allantoin in urine following George et al. (2006). Creatinine was used to standardize against dilution effects. Separation was performed at 25 °C on a Thermo-Fisher Scientific C₁₈ reversed-phase column (250×4.60 mm, 0.5 µm particle size). The mobile phase was a 0.01 mol/L potassium dihydrogen phosphate solution. Standard samples were used to identify PD. Peak areas were calculated using Waters Corporation Empower software. Calibration curves were calculated ranging from 1.0 to 200.0 µg/ml for the four PD. Analysis at the Metabolomics Laboratory, UC Davis, of the urine of captive moose was undertaken using liquid chromatographic and gas chromatographic mass spectroscopy following Robertson et al. (2011). All data were converted to molar values to facilitate comparison.

Results

No allantoin was detected in the samples from free-living moose, but it was unambiguously identified in extremely low concentrations representing a very small proportion of PD (Table 1) in samples from captive moose. In those moose, the molar ratio of allantoin to creatinine was 0.0037±0.0019, whereas the normal human molar ratio is 0.0099 (Tolun et al. 2012). By human standards (hyperuricuria >7 µg/ml) (Campo et al. 2003), about a quarter of free-living moose in our sample had hyperuricuria. Hence, we conclude that the moose, like the human, has negligible levels of uricase activity.

Table 1 Molar proportions of purine derivatives in the urine of moose (%)

Status	<i>n</i>	Allantoin	Uric acid	Xanthine	Hypoxanthine
Captive	11	0.56±0.29	61.0±21.2	5.7±3.2	32.8±19.3
Free living	106	ND ¹	41.3±23.8	16.3±16.6	40.6±23.9

ND not detected

Discussion

The urinary concentrations of purine derivatives reported here for moose are comparable to those in the human, and imply that the moose, like the human and some other primates, is deficient in uricase activity. Human deficiency in uricase was once described as an “inherited genetic defect”, but a number of benefits arising from high blood levels of uric acid, mostly based on its antioxidative capacity, have been postulated, particularly related to protection of the nervous system (Álvarez-Lario and Macarrón-Vicente 2011), and the loss of uricase is coming to be regarded as an evolutionary adaptation.

The question, then, is what adaptive function might the loss of uricase activity perform in the moose? We note that exposure to cold disposes to oxidative damage (Siems et al. 1994), and we considered that extreme cold was likely the most significant physiological stressor that the moose is exposed to in its natural environment and one to which it must have adapted. The oxidative damage occurring as a consequence of exposure to cold occurs as a side effect of increased thermogenesis (Venditti et al. 2009) and is also associated with ischemia (Proctor 2008), but uric acid has antioxidative capacity (Álvarez-Lario and Macarrón-Vicente 2011) and there are indications of physiological increases in body uric acid levels in response to exposure to cold, including in humans (Hawkins and Zipkin 1964). Associations have been made between increased blood levels of uric acid and high altitudes in several species including cattle (Ramirez et al. 1992) which were attributed at the time to hypoxia or diet, but those studies were not controlled for temperature, yet a significant and substantial contribution by cold at high altitudes has been demonstrated (Sinha et al. 2010). Further, it has been suggested that high blood uric acid concentrations is an adaptation by llamas to protect against the oxidative damage associated with high altitude cold (Bakker et al. 1996). The moose reduces aerobic and increases anaerobic respiration during winter (Kochan 2007), which suggests a need for a mechanism to reduce oxidative damage. We posit that the moose has adapted to protect itself against the oxidative damage associated with high latitude winter cold by losing uricase activity, the consequence of which would be high levels of uric acid in its blood and hence the high levels that we observed in its urine.

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