RAPID COMMUNICATION

TESTOSTERONE IN ANCIENT HAIR FROM AN EXTINCT SPECIES

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Abstract: Testosterone is a key regulator in vertebrate development, physiology and behaviour. Whereas technology allows extraction of a wealth of genetic information from extant as well as extinct species, complementary information on steroid hormone levels may add a social, sexual and environmental context. Hair shafts have been previously used to sequence DNA from >50 000 ^{14}C years old Siberian woolly mammoths (*Mammuthus primigenius*). Hair-testing has also been used to measure endogenous steroids in multiple extant species. Here we use small quantities of woolly mammoth hair samples to measure testosterone, and a genomics-based approach to determine sex, in permafrost-preserved mammoths dated to *c.* 10 000–60 000 ^{14}C years. Our validated method opens up exciting opportunities to measure multiple steroids in keratinized tissues from extinct populations of mammals. This may be specifically applied to investigating life histories, including the extinct Quaternary megafauna populations whose remains are preserved in the permafrost throughout the northern hemisphere.

Key words: testosterone, endogenous steroids, hair-testing, woolly mammoth.

STEROID hormones play a fundamental role in the regulation of development, physiology, reproduction and behaviour. For example, progesterone and testosterone can provide information on reproductive state, such as a pregnancy or an age class. Testosterone is also involved in social processes, including parental behaviour, social status formation, risk-taking behaviour, sexual displays and mating (Ketterson & Nolan 1999; Muller 2017). When elevated systemically, testosterone can have profound effects on fitness, often increasing reproductive success at the expense of survival and parental care (Dufty 1989; Paternostro 1994; Reed *et al.* 2006). Thus, testosterone levels may provide an insight on socio-environmental conditions that shape outcomes for individuals, groups and populations.

Traditionally, endogenous testosterone has been measured in the circulation through blood samples. However, this matrix is not available for extinct species. Hair, on the other hand, is more resilient in withstanding the wear and tear of time. Hair samples have been used for genetic analysis, with DNA sequenced from >50 000 ^{14}C year old Siberian mammoths (*Mammuthus primigenius*) (Gilbert *et al.* 2007, 2008). In the same study, DNA was also extracted from hair of a specimen that had been kept in room temperature for over 200 years. Hair-testing for steroids has been used in extant species (e.g. Koren *et al.* 2002, 2008; Koren & Geffen 2009; Malcolm *et al.* 2013). Routes of entry into the hair may be the blood vessel that nourishes the hair as it grows, sweat, sebum, conspecifics, and local (i.e. follicular) synthesis (Cone 1996). Hair steroids have been shown to be associated with blood, saliva, urine and fecal steroids levels and their metabolites (e.g. Tian *et al.* 2015). Since hair integrates endogenous pounds as it grows, it reflects gross patterns, al inter-individual and population comparisons (Bryan 2015). Ancient human cortisol has been measured hair of archaeological mummies (AD550–1532) (*et al.* 2010) and ancient sterols have been detected in fecal samples >500 years old (up to 1000–2000 years old) (Lin

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