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RESEARCH ARTICLE

The ecological adaptability of cloned sheep to free-grazing in the Tengger Desert of Inner Mongolia, China

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Abstract Since the birth of the first cloned sheep, somatic cell nuclear transfer technology has been successfully used to clone a variety of mammals. Cloned livestock have no apparent health risks, and the quality and safety of the cloned animal products are similar to non-cloned animals. The social behavior and environmental adaptability of postnatal cloned animals, especially when used for grassland farm production purposes, is unknown. In the present study, the cloned Dorper sheep equipped with GPS location devices were free-grazed in a harsh natural environment similar to conditions commonly experienced by Mongolian sheep. The main findings of this research were as follows. (1) Under free-grazing conditions, the cloned sheep showed excellent climatic and ecological adaptability. In extreme temperature conditions ranging from –30 to 40°C, the cloned sheep maintained acceptable body condition and behaved as other sheep. (2) The cloned sheep quickly adapted from a herd feeding strategy to the harsh environment and quickly exhibited a grazing regimen as other free-grazing sheep. (3) The cloned sheep exhibited free-grazing patterns and social behavior as other sheep. (4) The cloned sheep in the harsh environment thrived and produced healthy lambs. Overall, the cloned Dorper sheep exhibited excellent ecological adaptation, which is an important consideration for breeding meat sheep by cloning. The Dorper sheep readily adapted to the free-grazing conditions on the Mongolian plateau grassland, which attests to their ability to withstand harsh environmental conditions.

Keywords somatic cell nuclear transfer, free-grazing synchronization, Dorper sheep, cloned animal ecology

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1 Introduction

Since the birth of the first cloned sheep by somatic cell nuclear transfer (SCNT), a variety of animals have been successfully cloned, but the efficiency of viable offspring is still low [1]. Major problems associated with SCNT are lowered production efficiency resulting from high mortality of clones during pregnancy and low survival rate after birth [2]. Cloned cattle practices over 5 years in Argentina, Brazil and the USA [3] resulted in only 9% of cloned embryos going to term with a resultant calf, and an average mortality rate of 42% in the first 150 days of gestation.

The post-birth development and growth performance and behavior of cloned animals have been extensively studied. In pigs, Shibata et al. [4] and Hu et al. [5] reported that cloned pigs and their offspring were similar to others of the same breed in growth, reproduction and meat production [6]. The reproductive characteristics of cloned boars are highly comparable to naturally produced boars [7,8]. After a series of behavior tests, Archer et al. [9] concluded that food preference, temperament and time budgets of two genetically identified Duroc litters and their naturally bred controls were similar. Gwazdauskas et al. [10] also reported that the behavior of cloned gilts was the same as that of non-cloned gilts.

For cloned cattle, Heyman et al. [11,12] reported that most parameters measured for health and development of the animals as well as evaluation of milk and meat products were within the normal range for the breed. The cloned cows had normal growth, reproductive and lactation characteristics [13,14], which were comparable to the controls. Enright et al. [15] reported the estrous cycle length, ovulatory follicle diameter, number of follicular waves, or profiles of hormonal changes showed no differences between the cloned and control heifers. Ortegon et al. [16] concluded that the heifers sired by the cloned bull had normal chromosomal stability, growth, physical, hematological and reproductive parameters compared to normal heifers. The cloned bulls derived from an aged and infertile bull had normal fertility [17]. Also, nutritional evaluation of milk and meat by rat feeding experiments showed that no differences were found between products derived from clones versus controls [18]. Immune status analysis suggests that cloned cattle present a normal representation of leukocyte subsets and functional immunity was not modified in cloned heifers [19]. These reports indicate that the quality and safety of milk and meat from clinically healthy cloned animals are similar to non-cloned animals [20,21]. Importantly, cloned cattle [22], sheep [23], horses [24] have already been used in commercial breeding and production practices. In China, cloned Dorper rams have been used in commercial production by crossing cloned rams with Gebi sheep ewes with more than 20000 hybrids being produced [25]. Therefore, SCNT has the potential to rapidly expand and contribute significantly to livestock breeding.

The social behavior and intelligence of cloned animals are also important considerations. In mice, Tamashiro et al. [26] did not observe any effect of SCNT on locomotor activity in home cage and on spatial performance in a Morris water task. Savage et al. [27] reported that cloned heifers exhibited higher levels of curiosity, more grooming activities and were more aggressive and dominant than the non-cloned controls. Coulon and coworkers [28] designed a study of the social behavior and kin discrimination of cloned and non-cloned heifers and showed that the cloned heifers from the same donor were more spatially associated and interacted more between themselves than with heifers derived from another donor or artificially inseminated individuals. When the clones were moved to an unfamiliar environment, the cloned cattle exhibited the same level of locomotion and vocalization [28,29]. These results suggest that cloned animals have similar social behavior and intelligence to sexually reproduced animals.

However, the social behavior and environmental adaptability of cloned rangeland animals, especially under harsh conditions, have not been studied. The adaptability of cloned animals to a free-grazing environment is reported for the first time in this paper. Four cloned Dorper sheep equipped with GPS tracking were free-grazed under some of the harshest natural conditions in Central Asia to determine their adaptability by comparing their behavior and body condition to endemic Alashan sheep. This study examined several ecological issues, including climate, social behavior, ecological and feeding adaptation/preference, encountered by the cloned Dorper sheep.

2 Materials and methods

2.1 Ethics statement

All procedures were approved by the Inner Mongolia University Animal Care and Use Committee. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Inner Mongolia University.

This field studies were performed in the Shatetu area (38°34′40″N, 104°58′31″ E) in the Tengger Desert which is state owned and did not involve endangered or protected species, and was permitted and approved by the Bureau of Animal Husbandry of Alashan League, Inner Mongolia.

2.2 Cloned Dorper sheep

The donor cells of the cloned sheep were derived from genetically superior Dorper sheep owned by the Inner Mongolia Sainuo Grassland Sheep Industry Co., Ltd. Small biopsies of ear tissues were collected from donor sheep to establish fibroblast cell cultures. Recipient ewes for embryo transfer were Gebi sheep about 2 years old. The procedures of nuclear transfer, embryo culture, embryo transfer and delivery of the cloned lambs in this study were previously reported [30].

A total of 976 embryos at the 1- to 2-cell stage were transferred to the oviducts of 140 recipient ewes. After transfer for about 50 days, 24 (17%) recipients were pregnant, 19 of them developed to term and delivered naturally or under caesareans with a 14% birth rate. A total of 22 lambs were produced including 3 twins. The birthweight of 14 lambs was from 3 to 5 kg, while that of the other 8 lambs was over 7 kg. Five lambs that were over 8.5 kg died within 6 months after birth. A total of 17 lambs survived and grew well [25]. Four of them (two male, SNY1175 and SNY1054, and two female, SNY1138 and SNY1148) born on the same day and with healthy growth state were selected and used in this study (Fig. 1).

2.3 Grazing lands of the Tengger Desert

The Tengger Desert is one of the four largest deserts of China. It has the most severe of temperatures, ranging from as low as −30°C in winter to over 40°C in summer. The temperature of the birth place (Siziwang area) of the cloned sheep ranges from −20 to 26°C. The vegetation in the Shatetu study site mainly consists of sand sagebrush and *Nitraria schoberi*, with scattered sand bamboo on the dunes.

2.4 Grazing and monitoring

In January 2011, at four months old, the four cloned Dorper sheep were moved to the Shatetu area of the Tengger Desert. During the first week in the desert, the Dorper sheep were fed a maintenance diet to assist in their adaptation to the new environment. They were then released to graze with a local flock of Alashan sheep during daylight hours, and returned to an enclosure in the evening. During the first 30 days in their new environment their diet was supplemented with 600 g per day of concentrated corn to assist in their adaptation to rangeland conditions. One cloned sheep (SNY1175) and a local Alashan sheep were equipped with GPS transmitter collars (Fig. 1C). The GPS collar had a built-in temperature sensor and spherical switch to record temperature and position on an hourly basis. A GPS tracking recorder was also attached between the horns of these sheep to record grazing trajectory information and an animal activity logger installed on a hind leg to monitor movement. After equipping these two sheep, all cloned sheep were allowed to free-graze with a local sheep flock and were no longer confined during the night. The cloned sheep and accompanying local sheep were assembled each month and weighed. A weather station for meteorological monitoring was provided by the Shatetu Experimental Station.

3 Results

3.1 Grazing activity of the tracked Dorper sheep

From March to September, the cloned Dorper sheep gradually expanded their grazing pattern. At the beginning, the daily distance the tracked ram moved was about 2.3 km, which gradually increased to 7.3 km in June. Figure 2 shows the paths of the cloned ram on 16 March traveling a distance of 4.9 km (elevation range 1296–1364 m) and on 17 March traveling a distance of 5.0 km (elevation range 1283–1307 m). The average daily distance the cloned sheep traveled in July and August was about 7.1 km (Fig. 3). From 21 June to 2 July, the distance traveled peaked with a daily average movement of 9.4 km (Fig. 4).

3.2 Activity of the tracked Dorper sheep in relation to ambient temperature

Figure 5 shows the collar temperature and the movement pattern of the tracked sheep. During the test period, the highest temperature occurred on 15 June at 37.2°C. Whenever ambient temperatures peaked during the day, the activity of the tracked sheep significantly decreased (Fig. 6), but as the temperature dropped, the activity level increased. When the ambient temperature dropped to around 30°C, the activity peaked. Peak activity occurred at

Fig. 1 The cloned Dorper sheep free-grazing in the Tengger Desert of Inner Mongolia, China. (a) The cloned sheep on arrival at the sheepfold; (b) the sheep eating nearby the sheepfold during the first month; (c) the sheep equipped with GPS collar (indicated by the arrow); (d) the cloned sheep (indicated by the arrow) free-grazing with a flock of Alashan sheep.

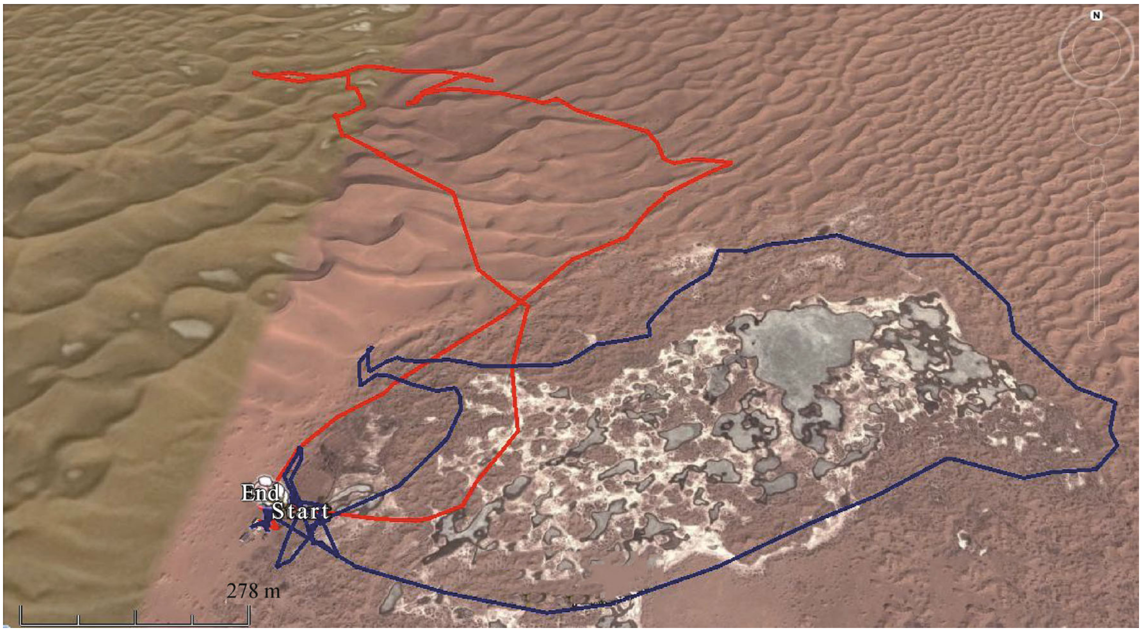

Fig. 2 The paths of the tracked cloned Dorper sheep on 16 March (red line) with traveling distance of 4.9 km (elevation range 1296–1364 m) and on 17 March (blue line) with traveling distance of 5.0 km (elevation range1283 –1307 m)

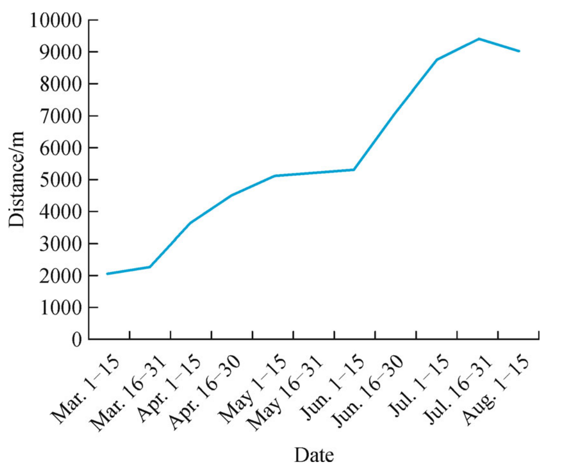

Fig. 3 Average daily movement of the tracked cloned Dorper sheep checked twice per month

about 20:00. The cloned sheep rested for the remainder of the night.

3.3 Grazing activity of the tracked Dorper sheep

Figure 7 shows the grazing profile of both tracked sheep on 28 May. The cloned sheep exhibited a grazing pattern similar to the local sheep. Figure 8 shows the grazing pattern during the day, with the cloned sheep having a similar movement pattern as the local sheep. Figure 9 shows the rhythmic movement patterns of the Dorper and Alashan sheep. The activity patterns of the Dorper and Alashan sheep were very similar, which indicates that the Dorper sheep were adaptable to the grazing patterns of the local Alashan sheep and maintained a companionship within the flock. Also, there was consistent rhythm of social behavior between the cloned Dorper and the local sheep flock. The sheep rested during the highest temperature at 13:00–14:00, and their activity peak occurred at about 20:00.

3.4 Weight changes of the cloned sheep under grazing conditions

The weight of the cloned Dorper sheep during the test period is shown in Table 1. During the free-grazing period the weight of the cloned sheep gradually increased. At about 12 months old, having grazed in the experimental site for 8 months, their weights ranged from 76 to 80 kg. All of the Dorper sheep are strong and healthy.

3.5 Lambing of the cloned Dorper sheep

During January to February 2012, ten hybrid lambs of Dorper rams and Mongolia ewes, and two Dorper lambs were produced. These offspring have subsequently matured and produced their own lambs.

4 Discussion

The Dorper sheep is a South African mutton breed developed in the 1930s from the cross of the Dorset

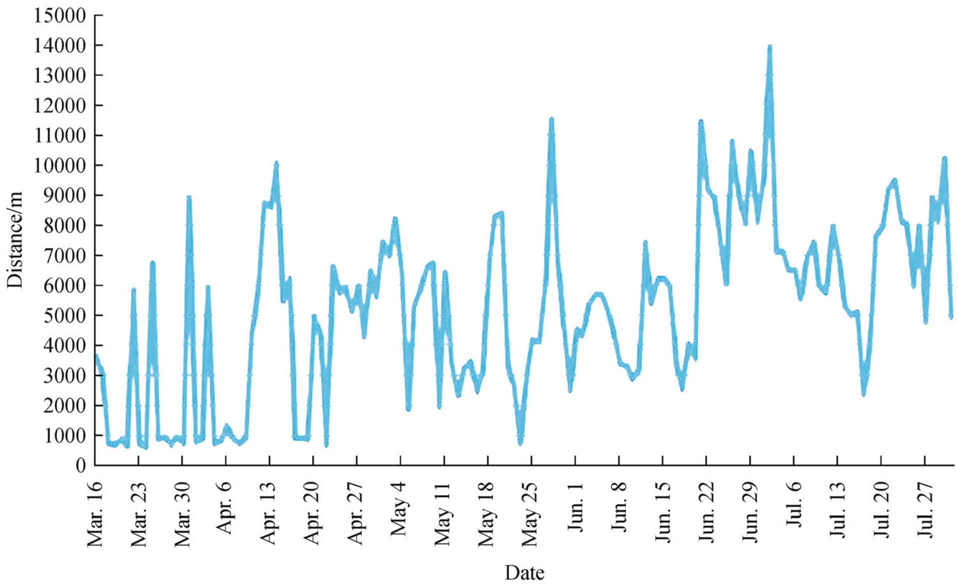

Fig. 4 Daily distance traveled by the tracked cloned Dorper sheep from 16 March to 27 July 2011

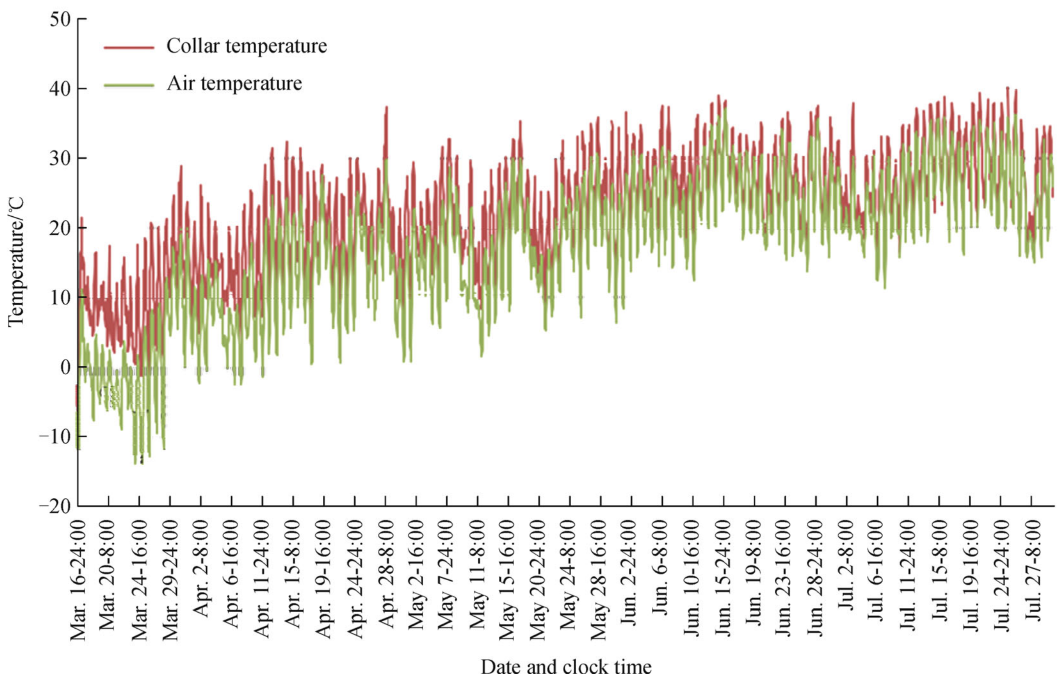

Fig. 5 GPS collar temperature recordings of the tracked cloned sheep and ambient air temperature from 16 March to 27 July 2011

Horn with the Blackheaded Persian breed [31]. The breed was developed to thrive in the arid regions of South Africa. This composite breed has a good body length and a short light covering of hair and wool. The Dorper displays exceptional adaptability to an arid climate, is very hardy and has good reproductive rates and growth potential as well as good mothering abilities [32,33]. Dorper sheep were first imported to China from Australia in 2001, and have been produced in many areas throughout the country [34]. The physiological parameters of body temperature, respiration and heart rate seem to stay constant no matter the environmental conditions the sheep are managed in

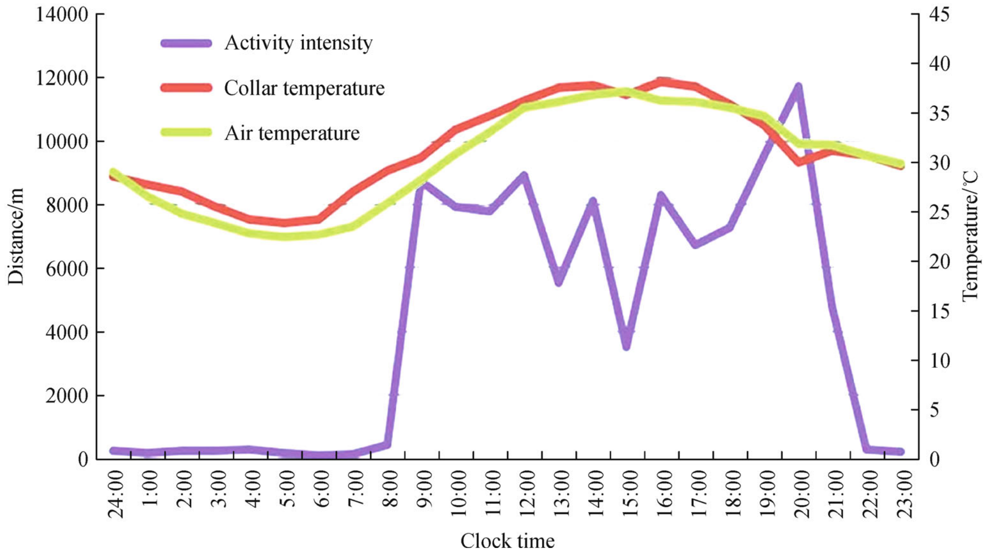

Fig. 6 Relationship between activity and ambient temperature of the tracked cloned sheep during hot weather on 15 June 2011

[35]. The breed does not seem to be affected by climate, altitude or season changes.

Since the birth of the first cloned mammal, there has been controversy, and questions about the use of SCNT relating to health, ethics, reproduction, meat and milk products, intelligence and social behavior of the cloned animals. However, considerable evidence has shown that the health [7,8,11,12], reproduction [12,15], meat and milk quality [18], and intelligence [14–16] of cloned animals are comparable to those from naturally produced animals. The social behavior of cloned animals has also been shown to be similar to non-cloned animals [28]. The present study provides evidence that cloned animals can adapt to an extremely harsh environment. The cloned Dorper sheep were born in Siziwang county, which is located in the middle of the Inner Mongolia Autonomous Region, China (41°10′ to 43°22′ N, 110°20′ to 113° E). It is classified as a mid-temperate continental monsoon climate zone. The annual mean temperature ranges from 1 to 6°C, with the coldest temperatures (–14 to –17°C) in January and hottest (16–24°C) in July. The Tengger Desert, the fourth largest desert in China, has an ecosystem staggered with sand dunes, a lake basin, grassy beaches, hilly areas, monadnocks, and plains areas. The average annual temperature is 7.8°C, the highest yearly temperature is 39°C and the minimum is –29.6°C. The cloned sheep exhibit excellent grazing adaptability and social behavior under the extremely harsh desert conditions. The main findings of this research were as follows. (1) Under free-grazing conditions, the cloned sheep had adapted exceptionally to the prevailing climatic and ecological conditions. Under extreme temperature ranging from –30 to 40°C, the cloned sheep was able to maintain acceptable body condition with what appeared to be normal activity. (2) The cloned sheep were able to adapt from a comfortable feeding regimen to that of a harsh environment and exhibited excellent grazing adaptability. The cloned sheep adapted from a totally artificial feeding regimen to 1:1 feeding:grazing, and then to fully free-grazing, over a period 2 months. This demonstrates that the cloned Dorper sheep can adapt very quickly to arid grassland food conditions. (3) The cloned Dorper sheep had excellent grazing capability as it followed the local flock, synchronizing their grazing patterns and social behavior with those of the flock. Their grazing activity often involved a traveling distance of up to 14 km per day, thus avoiding predators and inclement weather (such as extreme temperatures). The Dorper sheep maintained strong cohesion with the local sheep and did not separate from the flock. (4) The most important finding in this study is that under free-grazing the cloned sheep can adapt to and thrive in harsh environmental conditions, and produce healthy fertile lambs. These findings show that the cloned sheep can be healthy and productive under the harsh rangeland conditions of the Mongolian plateau.

5 Conclusions

This is the first report of an ecological study of cloned animals. The cloned Dorper sheep showed excellent ecological adaptability to the grazing conditions of the local Alashan sheep in harsh environment of the Tengger Desert. Cloning, therefore, could be used to develop excellent meat sheep breeds that are adaptable to ecological and climatic conditions of Mongolia grasslands.

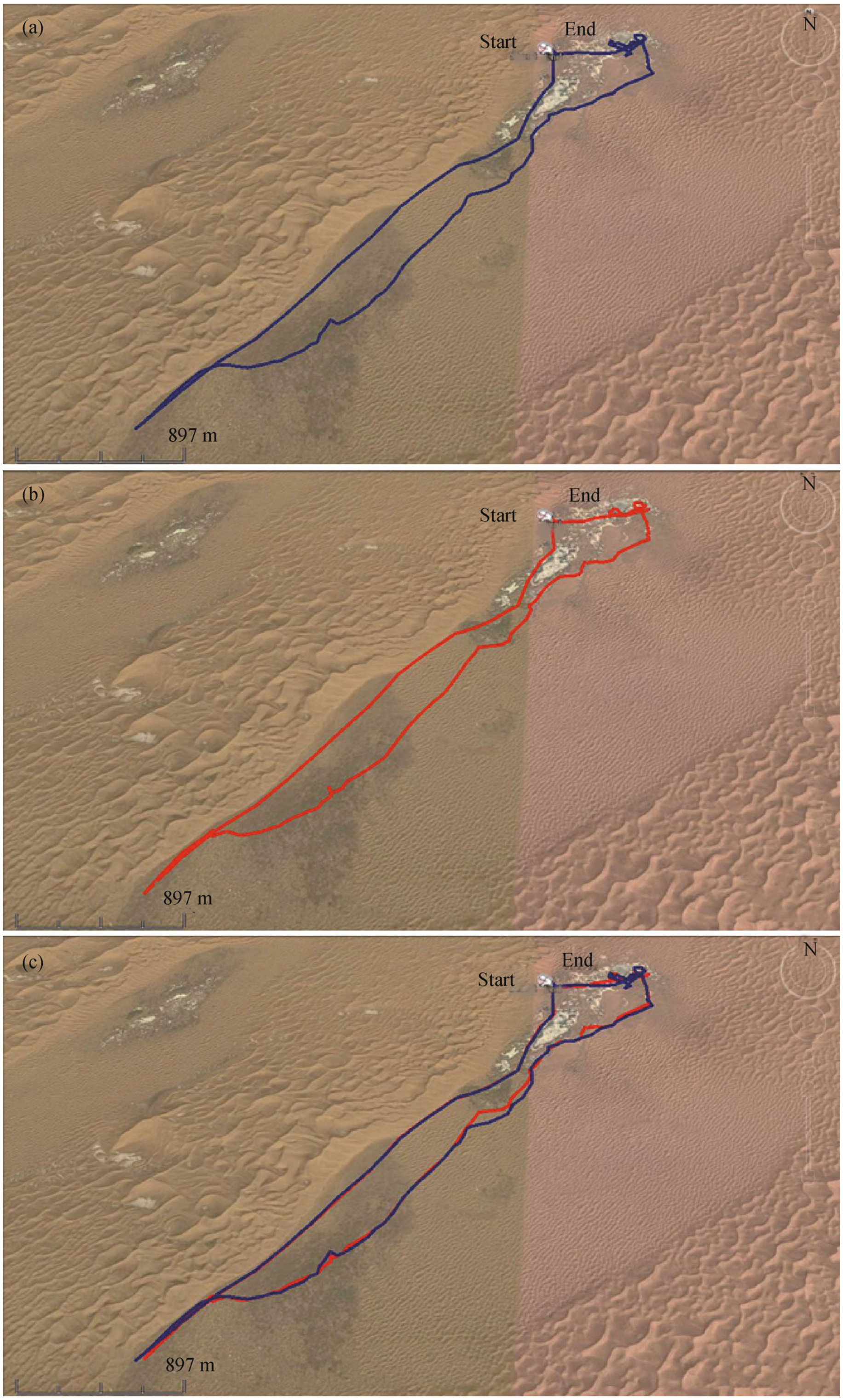

Fig. 7 (a) The paths of the tracked cloned Dorper sheep with traveling distance of 14.7 km (elevation range 1296–1340 m) on 28 May; (b) the paths of the Alashan sheep with traveling distance of 14.4 km (elevation range 1273–1325 m) on 28 May; (c) the merged paths of the cloned Dorper ram (blue line) and the Alashan sheep (red line) traveling on 28 May.

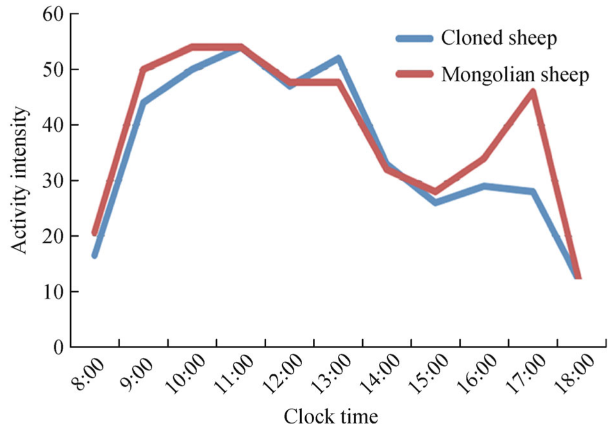

Fig. 8 The activity pattern of the tracked cloned Dorper sheep and Alashan sheep flock grazing on 28 May (from 8:00 to 18:00)

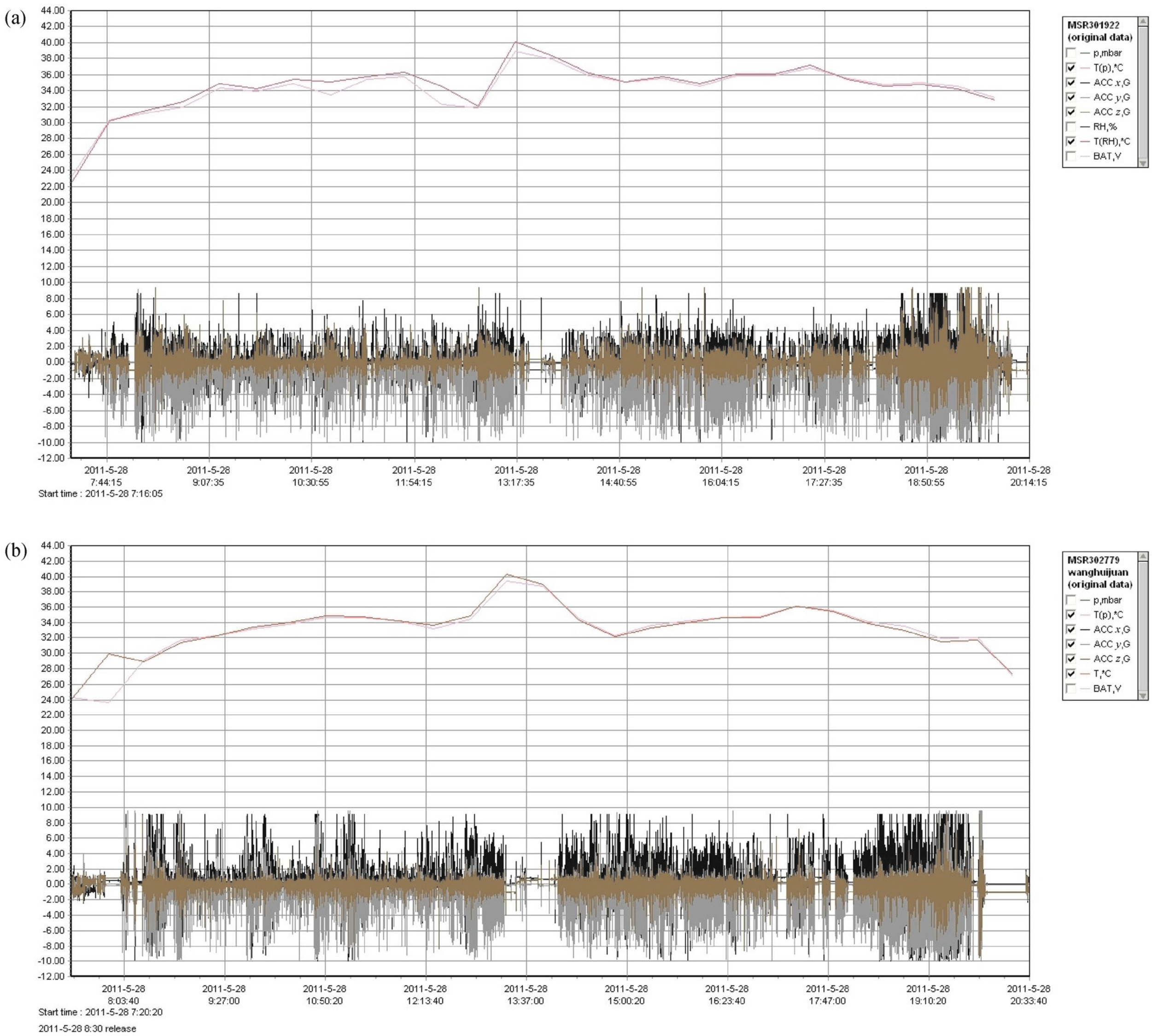

Fig. 9 Comparison of grazing activity of the tracked cloned Dorper sheep (a) and Alashan sheep flock (b). T(p) red curve as temperature (°C) recorded in pressure/temperature sensor, T red curve as temperature (°C) recorded in temperature sensor; ACC *x* and ACC *y* and ACC *z* as acceleration in g recorded by 3-axis acceleration sensors.

Table 1 Bodyweight of four cloned Dorper sheep at various times during a grazing study conducted in the Tengger Desert of Inner Mongolia, China

Sheep no.	Sex	Birthweight/kg	Age/months						
			4	5	6	7	8	10	12
SNY1175	♂	4.0	46.5	47.5	50.0	55.5	63.0	80.5	82.0
SNY1054	♂	4.3	46.0	48.0	51.0	55.0	62.0	79.0	82.5
SNY1138	♀	3.9	44.5	46.5	48.5	54.5	62.5	76.5	78.0
SNY1148	♀	3.2	44.0	46.0	48.0	54.0	62.0	76.0	77.0

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Compliance with ethics guidelines Xinxin Li, Huijuan Wang, Guanghua Su, Zhuying Wei, Chunling Bai, Wuni-Menghe, Yanhui Hou, Changqing Yu, Shorgan Bou and Guangpeng Li declare that they have no conflict of interest or financial conflicts to disclose.

All applicable institutional and national guidelines for the care and use of animals were followed.

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