

Research Article

Behavioral Determinants of Mating Success in Blackbuck under Semienclosed Condition: A Cross-Sectional Study in Shuklaphanta National Park, Nepal

Puja Saud ¹, Juna Neupane ¹, and Mukesh Kumar Chalise ²

¹Central Department of Zoology, Institute of Science and Technology, Tribhuvan University, Kathmandu, Nepal

²Nepal Biodiversity Research Society (NEBORS), Lalitpur, Nepal

Correspondence should be addressed to Juna Neupane; zunaneupane@gmail.com

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Knowing reproductive behavior is important for establishing a viable population of nationally endangered blackbuck in Nepal. This study explored the behavioral events and states associated with copulatory mountings in blackbuck by focal animal sampling method under semienclosed conditions of Hirapurphanta within Shuklaphanta National Park, Nepal. We recorded different behavioral initiations for the mating reproductive activities and the behaviors followed after copulatory mounting. Four dominant adult males were used as a subject. The highest number of copulatory mountings was observed during the months of February–March. Among the diurnal observational phases, the highest number of copulatory mountings was recorded during 9.00–12.00 hours followed by 15.00 hours onwards. Copulatory mounting was significantly preceded by sniffing the vulva of female and showing flehmen response, whereas copulatory mounting was followed by walking.

1. Introduction

Blackbucks (*Antelope cervicapra*, Bovidae, Artiodactyla) were formerly widespread across the Indian subcontinent but are now limited to only small, scattered herds in Nepal, India, and Pakistan due to excessive hunting and reduced habitat range [1]. Blackbuck is one of the highly threatened and nationally protected species in Nepal [2]. They were thought almost extinct until a small herd of nine individuals was discovered in Khairapur of Bardiya district in 1975 which initiated their conservation efforts [3]. The last remaining wild population of the species is less than 250 individuals and localized in the small area of the Blackbuck Conservation Area (BCA) in the Bardiya district of the western lowland, Nepal [4]. In Nepal, the wild habitat of the species is under tremendous pressure from anthropogenic activities and livestock grazing [2]. The population of Blackbuck in the Blackbuck Conservation Area is unstable and highly confined, and therefore, it is susceptible to stochastic deleterious events. In addition to the conservation

efforts in the wild condition, ex-situ breeding in the captivity and introduction to the semiwild habitats are undergoing.

Captive breeding is one of the best ways to ensure the future existence of any plant or animal. However, the captive environment can be affecting the animal behavior mainly in four ways: the regular presence of large numbers of unfamiliar and behaviorally diverse (passive-active) humans, restricted and unchanging space, forced proximity to other animal species, and human management [5]. Effects of captivity on the behavior and survival vary among animal species depending on their behavioral flexibility, that is, the ability of animals to modify behavior in adaptive ways over time and space [6, 7]. Through captive breeding practices, efforts are made to breed endangered or threatened animal species so that they could be reintroduced in their natural habitats [8]. Captive breeding can play a crucial role in the recovery of some species for which effective alternatives are unavailable in the short term [9]. The success of captive breeding and reintroduction depends upon a clear understanding of the reproductive behavior of the species [10].

In ungulates, males and females have contrasting life histories, as usually, only the females raise the young, and only a handful of territorial males contribute the genetic material [11]. Females become sexually mature at the age of eight months but mate no earlier than two years of age. Males mature later, at 1.5 years. Mating takes place throughout the year. Gestation is typically six months long, after which a single calf is born. Sexual selection theory predicts that the opportunity for sexual selection is strong when reproductive success varies widely among males, with a few males highly successful at mating and many others males failing to mate. An association exists between strong sexual selection, high mating polygyny, and high variance in male reproductive success [12, 13]. Therefore, it is important to know the environmental and behavioral determinants of successful mating between the male and estrous female.

Knowing reproductive behavior is important for establishing a viable population of Blackbuck in Nepal. Male blackbucks engage in rutting throughout the year whereas the peak rutting season for females is from July to August and from February to March [14]. The blackbuck territories have reproductive connotations and are held by adult males only [15]. During the heat, the blackbucks mark their territory by means of urination, defecation, and rubbing preorbital glands against the hard objects. The scent of the fluid from preorbital glands warns the other males [16]. Excessive fighting also occurs between the males to attract the females during their rutting period [17]. It is important to understand the reproductive behavior of the endangered species in order to multiply its population ex-situ.

A total of 28 blackbucks (22 from Nepalgunj Mini Zoo and 6 from Central Zoo, Lalitpur) were reintroduced in Shuklaphanta National Park (SNP) in 2012 as a part of a national effort to establish a second viable population in Nepal (site-specific conservation action plan for blackbuck in Shuklaphanta National Park, Nepal 2016–2020). Currently, 70 individuals of the blackbuck reside in the semi-captive condition of the Hirapurphanta within the SNP [18]. The success in the re-establishment of the species depends on how the animals can cope with the environment and maintain their behavior. Being territorial in nature, the reproductive success of blackbuck depends on the adult breeding dominant males, referred as the alpha males. Therefore, we aimed to understand reproductive behavior and major pre and postcopulatory activities of the breeding males in the semiwild condition of the Hirapurphanta. We employed focal animal sampling methods for behavioral sampling and analyzed the association of behavioral states of males with successful mounting events.

2. Materials and Methods

2.1. Study Area. This study was conducted in the blackbuck enclosure of Hirapurphanta in Shuklaphanta National Park (80.095–80.361°N, 28.763–29.047°E), Kanchanpur, Nepal (Figure 1). Hirapurphanta has a total area of about 150 hectares, but the area of the blackbuck enclosure is 17 hectares only. The blackbuck enclosure lies in the central part of the Hirapurphanta with 95% of the area covered by

grassland, 03% riverine forest, 01% mango orchard, and 01% wetland (site-specific conservation action plan for blackbuck in Shuklaphanta Wildlife Reserve, Nepal 2016–2020).

2.2. Study Animals. In the beginning of the study period (November 2018), the blackbuck enclosure contained a total population of 70 animals. The group was classified into 24 adult males, 28 adult females, five subadult males, four subadult females, and nine yearlings. By the end of May 2019, the population reached to 86. The reproductive behavior was observed in four adult males only which were in a high rut (Figure 2). Each individual was recognized by the variation in the shape of the horn and the color of the body. The following morphological features were used to identify the focal male blackbucks among groups [19] (Table 1).

2.3. Behavioral Observation. The diurnal activities of blackbucks were observed daily from 9:00 AM to 5:00 PM for 28 days. The focus on the reproductive behavior was performed with well-identified animals for an observational shift of three hours (9.00–12.00, 12.00–15.00, and after 15.00 hours, respectively) by focal sampling method [20]. An ethogram was developed for recording the reproductive behavior of blackbuck (Table 2).

2.4. Data Analysis. The association between the behaviors and copulatory mounting was determined by cross-tabulation followed by the Chi-square test. Microsoft Excel was used to analyze the data statistically. Tables were used to present the data in simplified form. The magnitude of the effect of factors on behavior for both preceding and succeeding the copulatory mounting was measured on the basis of phi value for 2×2 cross-tabulation. The following guidelines were used to determine the magnitude of the effect size at a 5% level of association of behavioral events with the copulatory mounting [21] (Table 3).

3. Results

3.1. Temporal Association of Successful Mounting. Blackbucks were in high rut during February–March than during July–August. The higher number of newly born infants was observed in the month of June/July indicating the higher conception during February–March. A total of 149 copulatory mounting events were documented during the study period, of which 44.3% were recorded during the observational phase of 09.00–12.00 hours, and the least (21.47%) were observed during the afternoon phase (12.00–15.00) (Figure 3).

3.2. Precopulatory and Courtship Activities. There were various behaviors such as walking, feeding, fighting, chasing, resting, and sniffing the vulva of female, showing flehmen response, drinking, urinating, rubbing, and defecating which preceded copulatory mounting. There was a significant association between walking, feeding, fighting, chasing, resting, and sniffing the vulva of female, showing flehmen

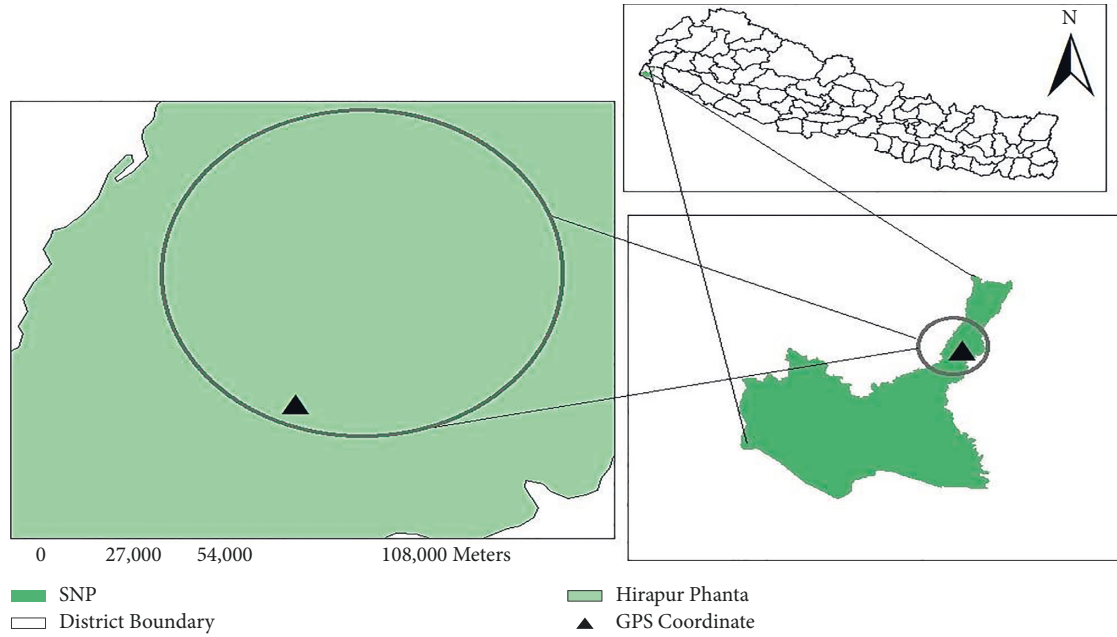


FIGURE 1: Map of Shuklaphanta National Park indicating Hirasurphanta.

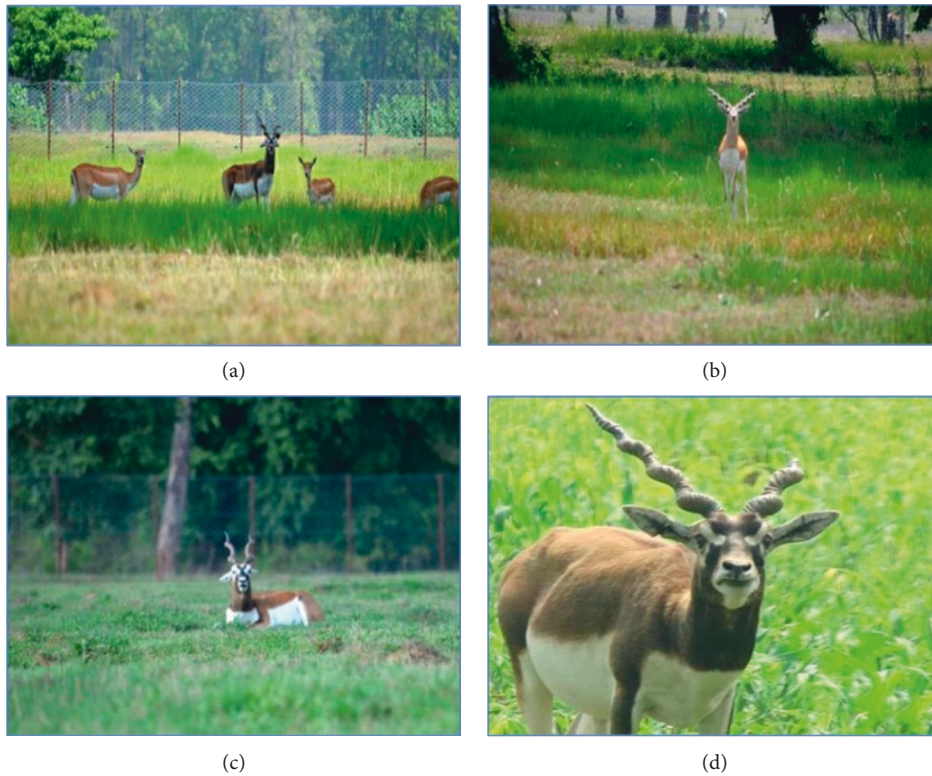


FIGURE 2: Adult males (a–d) observed for focal animal sampling.

TABLE 1: Identification features of adult male blackbucks.

Animal	Number of twists in horn	Identification characters
A	3	One horn was long and pointed, and the other was small and twisted outward.
B	4	Tip of the horns bent inward, long horns, and very broad like a triangle, and body was brown in color.
C	4	Thick and parallel horns, most parts of the body were black, and little was brown in color.
D	2	Broad and thick horns. One horn was broken during fighting. Body was black in color.

TABLE 2: Ethogram for monitoring reproductive behaviors of blackbucks inside the captivity of Hirapurphanta.

S.N.	Behavior	Description
1	Sexual display	Highly rutted males walked with deliberate steps. Head raised and nose-up and horns consequently laid back.
2	Territorial behavior	Males marked their territory by rubbing preorbital glands on the ground or by urination and defecation (depositing their pellets at same place), aggressive calls, fighting, and chasing other males visiting their territory.
3	Reproductive behavior	Sniffing the vulva of female and showing flehmen response. Attracting the females through fighting with other males who visited their territory, showing dominancy, copulatory mounting.
4	Other behaviors	Walking, feeding, resting, drinking.

TABLE 3: Phi value and magnitude of effect.

Magnitude of effect	Phi value (ϕ -value)
Strong negative association	-1.0 to -0.7
Weak negative association	-0.7 to -0.3
Little or no association	-0.3 to +0.3
Weak positive association	+0.3 to +0.7
Strong positive association	+0.7 to +1.0

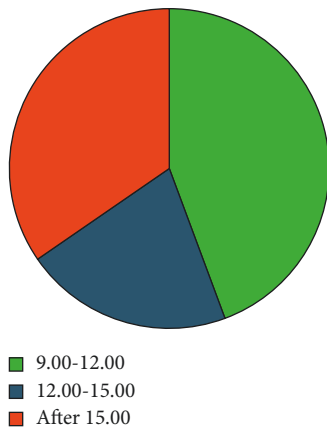


FIGURE 3: Number of copulatory mountings of blackbuck during three phases of behavioral sampling.

response and urinating preceding copulatory mounting, that is, $p < 0.05$ (Table 4). There was a strong positive association between sniffing the vulva of female (ϕ -value = 0.713) and showing Flehmen response preceding copulatory mounting (ϕ -value = 0.799). Little or no association as reflected by phi value was observed between walking, feeding, fighting, chasing, urinating, and resting preceding the copulatory mounting. There was no significant association between the behaviors such as rubbing preorbital glands, defecating, and drinking preceding copulatory mounting, that is, $p > 0.05$.

3.3. Postcopulatory Activities. Among the behavioral events observed succeeding the copulatory mountings, a weak positive association between walking followed after copulatory mounting (ϕ -value = 0.347). There is a little or no association between sniffing the vulva of female (ϕ -value = 0.278) and showing flehmen response (ϕ -value = 0.279) followed after copulatory mounting. Conversely, a weak negative association was recorded between resting and copulatory mounting. There was no

significant association ($p > 0.05$) between feeding, fighting, drinking, urinating, rubbing preorbital glands, and defecating followed after copulatory mounting (Table 5).

4. Discussion

4.1. Mating Behavior and Its Seasonality in Blackbuck. The mating system of blackbuck appears to be flexible. The basic pattern is territorial defense by males [14, 22, 23]. They have two peaks in their rutting season, one from March and April, and the other from August to October. During the study period, the blackbucks were found to be in high estrus in the month of March and April. Blackbucks breed throughout the year, but newly born infants were seen more in the month of June. It is well known that environmental conditions may importantly influence the reproductive strategies of ungulates.

Courtship was initiated by the adult males who searched and followed the female in heat. Fighting occurred when other dominant male visits its territory. Polygynous resource territories, typically held by a single male, generally encompass important resources such as food, water, or particular types of habitat, and are the most common form of territoriality in polygynous species [24]. During this time, the females got attracted by the competitive behavior of the dominant male and chose the strongest male for copulation. The dominant male in the rutting period sniffed the vulva of the female, and it gave a head-up with twisting lips. This gesture had been termed as "Flehmen." During excessive heat, the males became more excited and chased the females continuously. During this study, one female died because of forceful mating and fighting with another dominant male on 1st June 2019. A dominant male followed two or three females and copulated with one which reached the state of conceiving.

The female showed receptivity by waving her tail and thumping the hind legs on the ground, and the buck followed the female every time. Mounting was attempted by holding the doe, and the copulation occurred at that stage. After copulation, the doe stood for a while and then started grazing. The doe sometimes urinated. The buck took rest due to loss of energy while copulating. Buck sometimes mated with another doe. It followed the other doe in his territory and tried to attempt mounting with them. That was due to the excitement of the copulation.

Vats and Bhardwaj [25] observed that the strongest individual dominated other members of the group. The encounters happened when one male entered the territory of

TABLE 4: Behavioral events preceding copulatory mounting among the dominant males.

Behavioral events	Observed frequency	Expected frequency	X^2 -value	p value	ϕ -value
Walking	47	38.87	3.932	0.047	0.076
Feeding	3	23.7	29.498	<0.0001	-0.210
Fighting	3	11.8	8.802	0.003	-0.0114
Chasing	25	11.5	21.071	<0.0001	0.177
Resting	0	11.8	15.791	<0.0001	-0.153
Sniffing the vulva	57	8.7	341.184	<0.0001	0.713
Flehmen response	69	10.4	428.714	<0.0001	0.799
Drinking	0	2.0	2.290	0.130	-0.058
Urinating	8	3.5	6.917	0.008	0.102
Rubbing preorbital gland	6	3.3	2.747	0.097	0.064
Defecating	0	2.1	2.429	0.119	-0.060

TABLE 5: Behavioral events succeeding copulatory mounting among the dominant males.

Behavioral events	Observed frequency	Expected frequency	X^2 -value	p value	ϕ -value
Walking	28	49.7	31.532	<0.0001	0.347
Feeding	5	7.5	1.905	0.168	-0.085
Fighting	2	3.2	1.015	0.314	-0.062
Chasing	34	23.1	13.199	0.0002	0.224
Resting	2	18.1	35.244	<0.0001	-0.367
Sniffing the vulva	27	15.6	20.250	<0.0001	0.278
Flehmen response	27	15.5	20.440	<0.0001	0.279
Drinking	1	0.5	0.869	0.351	0.057
Urinating	17	15.3	0.430	0.512	0.041
Rubbing preorbital gland	5	5.9	0.307	0.579	-0.034
Defecating	1	2.7	2.315	0.128	-0.094

another male or when the male is in excessive heat. The buck took 5–6 hours in courting the female and finally mounting her copulation terminated courtship. During the copulatory mount position of the body of the buck, movements of the feet are exhibited in perfection to complete the mounting.

Nutritional requirements vary throughout the reproductive cycle, and strategic feed supplementation can also be an important tool to improve reproductive efficiency [26]. The nutritional limitations affect the reproductive process in both genders. The high energy intake boosts spermatogenesis before fertilization and provides energy reserves to support sexual and aggressive behavior during mating [27]. When energy intake is maintained and protein intake is reduced, there happens a significant reduction in libido and semen volume and a trend for reduced sperm output [28]. Male ungulates incur a substantial energetic cost for engaging in reproduction, and they trade off reproductive success with reduced body condition during winter, which may lower survival [29]. The Shuklaphanta National Park used to grow maize and lentils during the dry season. Lentils provide protein which improves the reproductive status whereas maize is necessary because it provides carbohydrates which provide energy to the blackbuck. Additionally, the blackbucks are supplemented with wheat, barley, maize, soybean, etc.

4.2. Precopulatory and Postcopulatory Behaviors. Among 11 behavioral states and events observed preceding or succeeding the copulatory mount in blackbuck males, flehmen

response, sniffing the vulva, and walking beside the estrous female were the most frequent behaviors associated with the copulatory mounting. The flehmen or lip-curl behavior is a response of male mammals primarily to female urine, performed primarily during the reproductive season [30]. It is displayed by most ungulate species and is a sexually dimorphic behavior seen most frequently when males investigate the genital region of females or freshly voided urine from females. Functionally, flehmen appears to be related to the transport of nonvolatile chemosensory materials from the oral cavity to the vomeronasal organ [31]. However, the relative frequency of flehmen in different contexts may not reflect its biological significance [32]. While comparing with reproductive importance, males show higher rates and duration of flehmen response to estrous urine than to nonestrous urine [33–35] suggesting its importance in the assessment of females' reproductive condition.

The second most frequent behavioral state associated with the copulatory mounting in blackbuck was sniffing the vulva of estrous female. Almost similar observation has been described in Reeve's deer where the highest amount of time is invested by the reproductive male in sniffing or licking the vulva of female [36]. Olfactory and gustatory stimuli received by the male when the sniffs and licks the vulva of an estrous female evoke sexual excitement expressed by the male's flehmen response [37].

Females presumably limit the number of mating bouts with a given male, but the time between successive bouts is determined by the dominant male [36]. For determining the

dominance, the relationship between the behaviors, fecal testosterone levels, and glandular volatiles play an important role in male blackbuck. It was reported that the frequency of aggressive behavior, preorbital gland scent-marking behavior, and fecal testosterone level was significantly higher ($p < 0.001$) in the dominant males than in the other males [38]. His result suggested that the male blackbuck's preorbital gland marking behavior was meant to defend the home range or territorial region, and the scent odor was meant to suppress aggression, scent marking, scent production, and territorial patrolling of subordinate males. The male, which maintained their dominance over a long period of time, mated with number of females than other dominant males.

5. Conclusion

Although there was a weak association between chasing preceding copulatory mounting, chasing played an important role in copulatory mounting. We found that the females which were in the verge of rut got more heated when chased by male, and immediately, copulatory mounting took place. Therefore, it is concluded that copulatory mounting in blackbucks is preceded by sniffing the vulva of female, showing flehmen response, and sometimes chasing the female.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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