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Latrine ecology of nilgai antelope

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The use of scent for communication is widespread in mammals, yet the role of scent-marking in the social system of many species is poorly understood. Nilgai antelope (*Boselaphus tragocamelus*) are native to India, Nepal, and Pakistan. They were introduced to Texas rangelands in the United States during the 1920s to 1940s, and have since expanded into much of coastal South Texas and northern Mexico. The nilgai social system includes the use of latrines or repeated defecation at a localized site. We quantified and described physical and behavioral characteristics of nilgai latrine ecology to investigate drivers of latrine use at three sites in South Texas, during April 2018 to March 2019. Latrines were abundant (2.6–8.7 latrines/ha on unpaved roads, 0.4–0.9 latrines/ha off-roads), with no evidence for selection as to vegetation communities; latrines were dynamic in persistence and visitation rates. We found higher densities of latrines in Spring surveys, just after the peak of nilgai breeding activity, compared to Autumn surveys. Density of nilgai latrines was 3–10 times greater than estimated population densities, indicating individual nilgai must use multiple latrines. Camera traps and fecal DNA analysis revealed latrines were mainly (70%) visited by bulls and defecated on by bulls (92% in photos, 89% for DNA samples). The greatest frequency of visits occurred during the peak in the nilgai breeding season, from December–February; latrines were visited every 2–3 days on average. Body characteristics of photographed individuals and genetic analysis of feces indicated repeated visits from the same individuals. Nilgai cows occasionally used latrines; their use was sometimes followed by bulls showing flehmen responses after a female defecated or urinated on the latrine. We propose that dominant bulls use latrines for territory demarcation to display social dominance to both cows in estrus and subordinate bulls. Cows likely use latrines to communicate reproductive status. This study is the first intensive assessment focused on latrine ecology in nilgai. Our results directly contradict anecdotal descriptions of latrine use and behavior in nilgai but are consistent with predictions of antelope social systems based on body size, feeding type, and group dynamics.

Key words: behavior, *Boselaphus tragocamelus*, dung piles, Indian antelope, latrines, nilgai antelope, olfactory communication, social structure

Scent-marking through defecation and urination is a common form of communication among mammals (Ralls 1971; Wyatt 2003). Species and individual-specific pheromones allow recognition of conspecifics and individuals and convey information about an individual's health and social status (Ralls 1971; Gosling and Roberts 2001; Espírito-Santos et al. 2007). Proposed drivers of scent-marking for communication include kin recognition (Ramsay and Giller 1996), communication of reproductive status (Walls et al. 1989; Palanza et al. 1994), maintenance of social hierarchies (Gosling and Wright 1994; Gosling et al. 1996), intrasexual information exchange (Wronski et al. 2013), and delineation of home ranges or territories (Gorman 1984; Clapperton et al. 1988; Gosling 1990; Simons et al. 1994; Lenti Boero 1995).

Many animals use latrines for chemical communication (Brown and MacDonald 1985), including species of antelope, gazelle, rhinoceros, monkeys, and mesocarnivores. Repeated defecation on a localized site forms a latrine, also referred to as dung piles, dung heaps, or middens. Placement of latrines is nonrandom and is influenced by the intended function. For instance, some species place latrines in the center of their home range (Hendrichs and Hendrichs 1971; Dunbar and Dunbar 1974), facilitating social group communication (Jordan et al. 2007; Dröscher and Kappeler 2014). Other species place latrines to mark territorial boundaries (Kruuk 1978; Brashares and Arcese 1999). Placement of scent-marks for territorial demarcation occurs in areas with a high probability of detection by conspecifics (Gosling 1981; Gosling and Roberts 2001), such as boundaries or trails (Gosling 1981, 1982). Animal group size, home-range size, time-energy budget, and territory size can influence where scent-marking is focused in relation to home-range and territory centers (Mills and Gorman 1987; Brashares and Arcese 1999; Gosling and Roberts 2001; Wronski and Plath 2010). The size of latrines also may relate to use or social function; large latrines may be from long-term use or use by large groups of animals (Walther 1984; Gosling 1985).

Marking territories could serve as a display of territory ownership, presence, rank, and ability to fend off intruders (Gosling 1982; Sillero-Zubiri and McDonald 1998; Gosling and Roberts 2001; Lewis 2005), and is common among male mammals as a primary function of intrasexual competition (Gosling and Roberts 2001). In some species, females may defend territories along with a mate or against conspecifics (Jarman 1974; Hendrichs 1975; Dunbar and Dunbar 1980). The physical location of defecation within a latrine also may convey meaning to conspecifics. For instance, territorial males may defecate in the center of the latrine, whereas subordinates defecate on the outer edge (Marneweck et al. 2018). In monogamous species, males scrape female feces and over-mark as a means of mate-guarding (Hendrichs 1975; Brotherton 1994; Brotherton et al. 1997).

Rates of scent-marking and latrine defecations vary depending on the function and social dynamics of the animal. If latrine function serves for territorial demarcation in males, one would predict that scent-marks are placed on boundaries more frequently (Dröscher and Kappeler 2014), and males would scent-mark more often than females (Wronski et al. 2006). The number of neighboring males or defecations by an intruder may influence the rate of scent-marking (Brashares and Arcese 1999; Black-Decima and Santana 2011). Females may use latrines for advertising reproductive receptivity to males (Blüm 1985; Rodgers et al. 2015), which may lead to increased rates of scent-marking by one or both sexes during breeding seasons (Dunbar and Dunbar 1974; Novellie et al. 1984; Kranz 1991; Roberts and Dunbar 2000; Jordan et al. 2007).

Although latrine function is directly integrated into the animal's behavior and movements, latrine behaviors are often complex and lack research attention. This is the case for nilgai antelope (*Boselaphus tragocamelus*), which are native to India, Nepal (Dinerstein 1980), and Pakistan (Mirza and Khan 1975). Nilgai were introduced to South Texas during 1924–1949 (Leslie 2008), and free-ranging nilgai populations now extend from northeast Mexico through much of coastal South Texas. Nilgai are abundant in South Texas, with estimates of over 36,700 animals (Traweek and Welch 1992). Nilgai can breed year-round, but the peak in nilgai breeding occurs between December–March (Fall 1972; Sheffield et al. 1983). Nilgai provide a significant economic benefit as a huntable resource in Texas but may compete for forage with native herbivores and livestock (Sheffield 1983; Kahn 2014), serve as a host for cattle fever ticks (Teel et al. 1996), and damage fences (Sheffield et al. 1983; Zoromski 2019).

Little information is available on the basic ecology of nilgai. Knowledge of nilgai social behavior is limited to observational studies, some from unpublished thesis or dissertations (Texas: Sheffield et al. 1971; Fall 1972; Sheffield 1983; Pakistan: Mirza and Khan 1975; India: Singh 1995). One poorly understood aspect of nilgai social behavior is their use of latrines, especially the role of latrines in social communication. Studies in native and introduced ranges have noted latrine presence and predict the importance of latrines (Sharma et al. 2009; Bayani and Watve 2016; Goolsby et al. 2017). Leslie's (2008) review concluded that "…both sexes and all age classes defecate repeatedly in conspicuous fecal piles…." (Leslie 2008:10). However, this pattern is inconsistent with predictions based on social behaviors and group organization of African antelopes (Jarman 1974).

Jarman (1974) reviewed ecological, social, and behavioral characteristics of African antelope species and classified them into five social classes (A, B, C, D, E). Classes are not mutually exclusive but provide similarities and comparisons between antelope species. Although nilgai antelope were not included in Jarman's (1974) classification, the text provides working hypotheses for social organization in nilgai based on species of antelope with similar physical and behavioral characteristics. After review of available research on nilgai ecology, we suggest that nilgai fit into class C. This includes "waterbucks (*Kobus ellipsiprymnus*), kob (*Kobus kob*), puku (*Kobus vardonii*), lechwe (*Kobus leche*), springbok (*Antidorcas marsupialis*), gazelles (*Gazella* sp.), impala (*Aepyceros melampus*), nyala (*Tragelaphus angasii*), and greater kudu (*Tragelaphus strepsiceros*). Although adult males may be found singly, young males and females very rarely occur singly…most individuals find themselves in groups of between 6 and 60 animals…group size can vary seasonably and regionally" (Jarman 1974:232–233). Scent-marking is important for class C species, and often territorial males urinate and defecate while patrolling territories (Jarman 1974). Latrine use is not exclusive to class C, as many antelope species form latrines. However, species in class C tend to exhibit visual displays with marking, unlike species in class A, where latrines remain inconspicuous due to high predation risk (Jarman 1974). Class C species are sexually dimorphic in size and coloration, and only males have horns in most species. Class C species may seasonally occupy a range of vegetation types and feed on grasses and browse (Jarman 1974).

Nilgai show sexual dimorphism, with differences in coloration and absence of horns in females (Sheffield et al 1983). Nilgai males can be found singularly, or in bachelor groups, and the presence of cow–calf groups is common (Fall 1972; Singh 1995). Some males have small home ranges but others are much larger (Qureshi 1991; Foley et al. 2017). Nilgai also form latrines (Sharma et al. 2009; Bayani and Watve 2016; Goolsby et al. 2017) and are intermediate feeders (Sheffield 1983; Singh 1995; Hines 2016). These characteristics align with Jarman's

(1974) class C. Jarman (1974) observed that antelope species in this class often have a "…proportion of adult males that obtain exclusive mating rights by possession of a demarcated piece of ground without having permanent ownership of females" (Jarman 1974:249). A proportion of adult males in class C will hold territories for at least the duration of the main mating period, within which the male holds exclusive mating rights (Jarman 1974). The female herd composition may be more related to a physiological state (i.e. state of pregnancy) than relatedness (Jarman 1974). Therefore, nilgai latrines should serve important roles in territorial demarcation instead of general communication among age and sex classes. Bagchi et al. (2008) evaluated social organization and population structure of nilgai in India, and suggested that nilgai should be territorial based on antelopes with similar social structures and behaviors. Conversely, Khan (1992) observed no dominance behaviors toward other males when crop raiding in India. Overall, contradictory reports among studies and poor understanding of nilgai social behavior in general do not allow definitive statements about territoriality in nilgai.

There has never been a formal study focused on nilgai latrine behavior, so the function of latrine use in nilgai is unknown. Latrine use is an important aspect of their biology that, with further understanding, will help uncover valuable knowledge on nilgai movements and social behaviors. We evaluated the hypothesis that nilgai would exhibit behaviors consistent with Jarman's (1974) class C antelopes. Strong conclusions cannot be made about nilgai latrine function until latrine placement, use, and behaviors at latrine sites are described. Our overall goal was to document and analyze descriptive and quantitative factors of nilgai latrines to lead the investigation of social function of latrines for nilgai. Specific objectives were to determine: (i) density, area, and placement of latrines; (ii) seasonal frequency, sex, and age-classes of nilgai that use latrines; and (iii) individual nilgai use of latrines via DNA analysis and photos.

MATERIALS AND METHODS

Study area.— This study took place at three sites in South Texas: the El Sauz, Santa Rosa, and Russell ranches (Fig. 1). The East Foundation, an Agricultural Research Organization that manages over 87,000 ha of rangeland across South Texas (https://www.eastfoundation.net), manages the El Sauz and Santa Rosa ranches. East Foundation properties are maintained as native rangeland and working cattle ranches to promote land stewardship advancement through ranching, science, and education.

The El Sauz Ranch is 10,984 ha, and borders the community of Port Mansfield, in Willacy County (26°40′N, 97°35′W). It is located in the Coastal Sand Plains, Lower Rio Grande Valley, and Laguna Madre Coastal Marshes ecoregions (Bailey et al. 1994). Common vegetation communities include grasslands composed of gulf cordgrass (*Spartina spartinae*), marshhay cordgrass (*Spartina patens*), and seacoast bluestem (*Schizachyrium scoparium*) interspersed with live oak (*Quercus virginiana*) and mesquite (*Prosopis glandulosa*) woodlands. Minor communities for El Sauz included: arrowfeather threeawn (*Aristida purpurascens*), bermudagrass, King Ranch bluestem (*Bothriochloa ischaemum*), saltgrass (*Distichlis spicata*), and woolly doveweed early-seral grasslands (*Croton capitatus*), and Colima (*Zanthoxylum fagara*), ebano (*Ebenopsis ebano*), spiny hackberry or granjeno (*Celtis pallida*), and sea oxeye shrublands (*Borrichia frutescens*), and saltwort wetlands (*Batis maritima*).

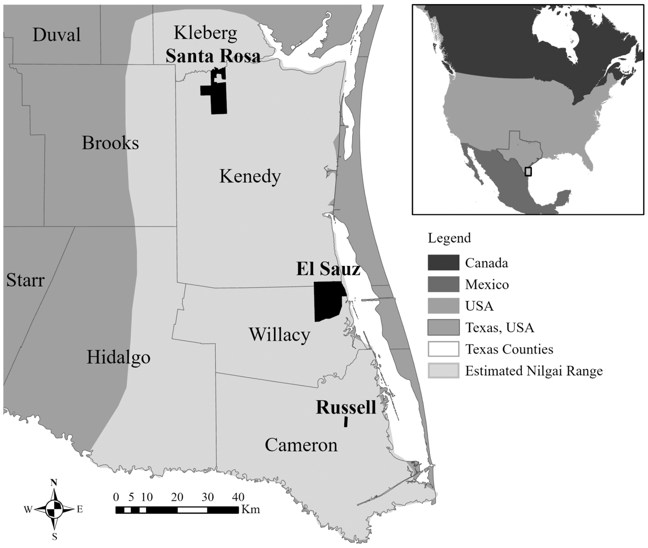

Fig. 1.—The distribution of free-ranging nilgai antelope in South Texas, United States and study sites on the Santa Rosa (27°13′N, 97°51′W), El Sauz (26°40′N, 97°35′W), and Russell ranches (27°13′N, 97°51′W). Camera traps were deployed on 10 randomly selected latrines at Santa Rosa and El Sauz, and 4 latrines at Russell during April 2018 to March 2019.

The Santa Rosa Ranch is 7,545 ha, located near the community of Riviera, in Kenedy County (27°13′N, 97°51′W). It is located in the Coastal Sand Plain ecoregion (Bailey et al. 1994). Dominant vegetation communities include huisache (*Acacia farnesiana*) woodlands, live oak woodlands, mesquite woodlands, and spiny aster (*Leucosyris spinosa*) wetlands. Minor vegetation communities for Santa Rosa included bermudagrass (*Cynodon dactylon*), buffelgrass (*Cenchrus ciliaris*, or *Pennisetum ciliare*), doveweed early-seral (*Murdannia nudiflora*), mixed grasses, gulf cordgrass, seacoast bluestem, thin paspalum grasslands (*Paspalum setaceum*), and spiny aster wetland.

The Russell Ranch is 289 ha near the community of Rio Hondo, Cameron County (26°12′N, 97°26′W). Dominant vegetation includes Guineagrass (*Megathyrsus maximus*), honey mesquite thornscrub, and prickly pear cacti (*Opuntia engelmannii*). The Russell Ranch is located in the Lower Rio Grande Alluvial Floodplain ecoregion, the most subtropical ecoregion of Texas (Bailey et al. 1994).

The East Foundation conducts annual aerial surveys for nilgai on each of their properties. These surveys involve flying transects via helicopter and using distance sampling (Thomas et al. 2002; Peterson et al. 2020) to estimate population size and sex ratios. During February 2017, aerial helicopter surveys indicated about 0.09 and 0.08 nilgai per ha, on El Sauz and Santa Rosa, respectively (East Foundation, unpublished data). From 2014–2019, nilgai sex ratios were 1 bull:1.44–2.98 cows at El Sauz, and 1 bull:1.57–2.56 cows at Santa Rosa (East Foundation, unpublished data). No population estimates are available for the Russell due to the small size and closed canopy; however, nilgai sightings and signs were abundant (Zoromski L.D., Texas A&M University–Kingsville, Kingsville, Texas, personal observation, September 2017). All three study sites allowed harvest of nilgai.

Latrine definition.— We defined a latrine as a group of ≥5 piles of fecal deposits within a 1-m^2 area (Fig. 2). Latrines are often distinctive, where repeated defecation results in a large, oval-shaped mound. Individual pellet groups may be difficult to distinguish within latrine mounds unless freshly deposited (<1 week). Therefore, we categorized latrines by freshness based on the freshest feces on a latrine. Fresh latrines with feces dark brown to black in color were considered to be used recently (<2 weeks). Intermediate latrines with light brown feces were considered to be >2 weeks old, but the timing of the most recent defecation may be difficult to determine. Feces in old latrines were weathered (~40 days), with a white and tan coloration, indicating latrines were not used for many weeks.

Density, area, and placement of latrines.— Latrines are common on unpaved ranch roads (crushed stone or sand 2-track) in South Texas. We drove a utility vehicle (UTV) on all unpaved ranch roads (131 km) on the 3 sites to survey for latrines. Surveys occurred during Autumn 2017, 2018, and Spring 2018, 2019. Autumn surveys were October–November, except the Russell Autumn 2018 survey was conducted in January because the site was inaccessible due to flooding. Spring surveys were late April–early June. We recorded area (maximum length and perpendicular width), maximum height above ground, GPS location, and usage (fresh, intermediate, or old feces) on every latrine observed. Single fecal groups that did not form latrines were initially recorded in Autumn 2017, but not in the subsequent surveys because single piles were abundant (25 groups at El Sauz, 24 Santa Rosa, 22 Russell) and easily scattered on the road, making measurements difficult. Although single piles may be substantial and require further research attention, we focused our survey time on established latrines. We digitized roads (scale 1:1,000) and latrine locations using ArcGIS ArcMap 10.5.1 (ESRI, Redlands, California), and determined average Euclidean distance between latrines, and location relative to road intersections and nearest vegetation communities. An average road width of 5 m was used for each site to estimate density of on-road latrines, assuming all latrines on roads were detected. We used a vegetation classification shape file (East Foundation, unpublished data) to classify latrines into vegetation community classes on Santa Rosa and El Sauz, into vegetation community classes presented in the study area descriptions, and a combined "other" category for minor vegetation communities (17% vegetation available at El Sauz and 7% at Santa Rosa). There was no quantitative vegetation classification for Russell. However, most of the site is thick mesquite thornscrub and grassland pastures, so we categorized Russell latrines into general thornscrub and grassland classes. If a road divided two vegetation communities, the road was classified as "Transitional."

Fig. 2.—Nilgai antelope bull defecating on a latrine at the East Foundation's El Sauz Ranch near Port Mansfield, Willacy County, Texas, United States, during October 2018.

To estimate density of off-road latrines, we assigned stratified random generated transect groups (linear North–South orientation) in major vegetation communities using ArcGIS, which equates to 49.8 km of Euclidean distance. We walked these transects, or drove a UTV when able, to record latrine area, GPS location, and usage in Spring 2018. Although it would be valuable to assess off-road latrines in each season, we conducted a one-time intensive survey with the intent to increase sample size. It was not practical to repeat this survey because of the time investment for researchers navigating difficult terrain. At El Sauz, we assigned a group of 10 transects, 50 m apart, and 500 m in length in each of the five major vegetation communities (Supplementary Data SD1). For Santa Rosa, we assigned groups of 10 transects, 50 m apart, and 500 m in length for each of the four major vegetation communities (Supplementary Data SD2). At Russell, we randomly assigned four groups of six transects, 200 m in length, spaced 50 m apart, stratified to have two within grasslands and two within mesquite thornscrub communities (Supplementary Data SD3). Transects were shorter for the Russell due to the smaller property size. We used distance sampling (Thomas et al. 2002) to estimate abundance of off-road latrines on each site. For each latrine encountered, we recorded the perpendicular distance from the transect to each latrine. Single nilgai fecal piles that did not form latrines were recorded, but not used in the estimation of latrine abundance. We estimated density of off-road latrines using distance sampling analysis in the R software package Distance (R Core Team 2013; Miller 2017) with truncation = 20 m.

Seasonal frequency, sex, and age-classes of nilgai that use latrines.— We randomly selected 10 latrines at El Sauz, 10 at Santa Rosa, and 4 at Russell from latrines identified through the on-road surveys to monitor with camera traps (Reconyx HyperFire HC500 or XR6 UltraFire, Reconyx, Holmen, Wisconsin; Moultrie A-5 Gen2 MCG-12688 Moultrie feeders, Alabaster, Alabama). On-road latrines were selected over off-road because of the ease of access, ability for continued monitoring in various weather conditions, and minimal disturbance. Half of the cameras were placed on randomly selected large latrines (>6 m^2) and half on small latrines (<6 m^2) at each ranch, under the expectation that larger latrines receive more visitations than small latrines. We placed cameras 39–125 cm above the ground and 210–536 cm from the latrine, depending on the latrine's location and area such that the entire latrine was included in the photo. The cameras were programmed to take a 3-photograph burst with a 10-s delay (Moultrie) or 15-s delay (Reconyx), with high detector sensitivity. We deployed cameras from April 2018 to March 2019, and classified the first 2 weeks of photos per month (336 h per month). Every 2 weeks, we checked cameras to replace memory cards, measure latrines, and assess freshness of fecal deposits on latrines (fresh, intermediate, or old). If we classified a latrine as "old" for more than 4 weeks, we moved the camera to a new randomly chosen latrine. All animals captured in the photographs were recorded as "on," "near," or "away" from the latrines. Animals "on" the latrine either had a foot on the latrine, or their head over the latrine. Animals "near" latrines did not have a foot on the latrine but either acknowledged the latrine via sniffing or walking toward it. Animals "away" were either far off in the background in photographs or did not appear to acknowledge the latrine (i.e., browsing nearby). Both animals on and near latrines were included in analyses as "visits." We also recorded whether animals "defecated" (displayed posture to defecate or urinate), sniffed, or displayed other behavioral responses (i.e., flehmen, mating, fighting) at latrines.

We cataloged photographs using Mapview Professional Software (Reconyx). Nilgai were classified into age-classes: old adult (bulls only), adult, subadult, and calf, based on physical attributes described by Singh (1995). Pelage of nilgai bulls changes as they age, from brown when young, to a steel gray-black as they mature. For bulls, "old adult" refers to steel gray in color, fully developed horns, barrel-shaped chest, and thick neck and body. Many old adult bulls have scars from previous fights. The age and social rank of "old adult" bulls is unknown, but this classification may be important if future studies find connections between those factors. "Adult bull" is similar to "old adult," but less physically robust, and relatively smaller barrel chest. "Subadult" males have full-grown horns, and are beginning to develop steel gray pelage, but still, have brown hair. Male and female calves were classified together in the "calf" category for analysis. It is difficult to categorize female nilgai into multiple adult age-classes, unlike for males. We categorized females as subadult and adult. Females are without horns and brown, with some having interspersed light gray fur. Subadult females have a relatively thin head and body compared to adult females.

To understand the time of day when nilgai visited or defecated on latrines, we categorized time data into eight parts of the day. The first 3-h category started with 0500–0759 h because the 0500 h best encompassed dawn or the first hour of light during the study period. Through photo analysis we estimated animal visits, % of nilgai visits, visitation rates, % and frequency of visits by nilgai age and sex class, frequency of behavioral responses (defecations, sniffing, etc.), and proportion of nilgai visits and defecations by time categories.

Individual nilgai use of latrines.— We obtained nilgai tissue (tongue) samples from harvested nilgai as a source of high-quality DNA. We extracted DNA from samples using a commercial kit (DNeasy Blood and Tissue Kit), with protocols recommended by the manufacturer (Qiagen Inc., Hilden, Germany). DNA microsatellite loci amplified in other species of bovids and cervids were used (Anderson et al. 2002; DeYoung et al. 2003). We screened 20 markers and 2 sex determination markers (Table 1). We amplified selected loci individually, pooled the resulting polymerase chain reaction (PCR) products, and loaded onto an ABI 3130*xl* DNA sequencer (Applied Biosystems, Foster City, California) for separation and detection of alleles. Fragment sizes and allele bins were quantified using the software GeneMapper 4.0 (Applied Biosystems). We estimated the probability of identity using the computer program Cervus 3.0 (Kalinowski et al. 2007) to ensure that the markers had sufficient resolution to identify individuals. After successfully genotyping nilgai through high-quality DNA, we evaluated the markers for use with fecal DNA to study latrine ecology.

Fresh fecal samples were collected from the 24 camera-monitored latrines every 7–8 days for 5 weeks (9 January 2019 through 9 February 2019) at all sites. We classified fecal samples by "freshness" (1 = wet coating, very soft, light brown, 2 = wet coating, soft, dark brown, 3 = dry coating, hard, dark brown).

Table 1.—Microsatellite DNA loci and sex determination markers optimized for nilgai antelope fecal samples from South Texas, United States. Thermocycling conditions were 94°C for 10 min, 10 cycles of 94°C for 30 s, within-cycle decreasing annealing temperature (T_M [°C]), extension of 72°C for 60 s, 40 cycles at 94°C for 30 s, annealing temperature (T_M [°C]) for 90 s (except S4B for 45 s), and 72°C for 60 s, and final extension of 72°C for 30 min. The resulting polymerase chain reaction (PCR) products of primers were pooled into two panels for fragment separation and detection.

Panel	Primer	Decreasing T_M (°C)			T_M (°C)	T_M (s)
1	BM415	62	61	60	54	90
1	BM4208	62	61	60	54	90
1	BM848	61	60	59	54	90
1	ETH152	62	61	60	58	90
2	BovPRL	62	61	60	54	90
2	ILSTS	59	58	57	54	90
2	OCAM	60	59	58	54	90
2	S4B	62	61	60	52	45

Photographs of each visit were taken to ensure the collection of only new samples each week. Each latrine received a trail camera for an independent assessment of usage and gauge time since fecal samples deposition. Images of nilgai that defecated were classified to individuals if nilgai had distinctive physical characteristics. We handled the samples with latex gloves and changed gloves in between each sample to reduce potential for contamination. A top pellet was collected from fresh groups of newly distinguishable piles, using the weekly photographs for reference. Fecal samples were placed in 2-ml screw-top tubes containing DETs buffer (DMSO/EDTA/Tris/salt; Frantzen et al. 1998). The buffer included 20% dimethyl sulfoxide, 0.25 M sodium-EDTA, 100 mM Tris, pH 7.5, and NaCl to saturation (Seutin et al. 1991). Samples were stored in shaded containers on ice and transported to a −20°C freezer within 10 h of the time of collection. Samples were maintained at this temperature until DNA extraction. We extracted DNA from fecal samples using the QIAamp DNA Stool Mini Kit with manufacturer protocols (Qiagen Inc.), but for the final elution step, we used 120 µl of buffer after a 5-min incubation. Every extraction set consisted of 12–13 samples and had a negative control. The negative controls contained only reagents for quality control, to guard against cross-contamination (Waits and Paetkau 2005). Extracts were then stored at 4°C until genotyping.

We identified seven genetic markers that amplified in nilgai and were sufficiently variable to identify individuals: BM415, BM4208, ETH152, BovPRL, BM848, ILSTS, OCAM, and sex determination marker S4B (Supplementary Data SD4). The sexing marker was included to determine if males or females deposited feces; the X- and Y-chromosome-specific alleles of the amelogenin gene can be detected via small fragment sizes, and are a good candidate for noninvasive samples (Kageyama et al. 2004; Rivière-Dobigny et al. 2009). We used a 16-µl PCR volume for each sample, including 8.0 µl AmpliTaq Gold 360 PCR master mix (thermal stable DNA polymerase, $MgCl_2$, and dNTPs), 3.5 µl double-deionized H_2O, 1.0 µl bovine serum albumin (2 mg/ml; ThermoFisher Scientific, Waltham, Massachusetts), 10 pmol for each forward and reverse primer, and 1.5 µl DNA extract. We included negative controls during extraction and PCR to verify the absence of contamination. Selected markers were amplified after the empirical optimization of the PCR protocols (Table 1). We pooled markers into two panels before loading onto an ABI 3130*xl* DNA sequencer for separation and detection (Table 1). Panel 1 included BM415, BM4208, BM848, and ETH152; we mixed 2 µl of each PCR product and combined 1 µl of the mixture with formamide and an internal size standard, as recommended by the manufacturer. Panel 2 consisted of BovPRL, ILSTS, OCAM, and S4B; we prepared the mixture for loading onto the sequencer in the same manner. Fragment sizes and allele bins were quantified using the software GeneMapper 4.0. We genotyped all heterozygotes ≥2 times and all homozygotes ≥3 times to account for the potential of allelic dropout, failed amplification, and false alleles (Taberlet et al. 1999; Broquet et al. 2007).

Results

Density, area, and placement of latrines.— Latrines were abundant in all road surveys, with higher densities during five of six Spring surveys than Autumn surveys over all sites (Fig. 3). During the four road surveys, we identified 192 latrine locations at El Sauz, 112 at Santa Rosa, and 97 at Russell. Of the 401 latrine locations detected, 15% were detected in all four surveys (October 2017 to May 2019). Excluding latrines that were only present in the last survey, 32% (*n* = 113) of the latrines persisted for <6 months.

Average area (± *SD*) of road latrines was similar between sites; 5.5 ± 4.0 m^2 at El Sauz, 4.7 ± 3.0 m^2 at Santa Rosa, and 4.2 ± 3.6 m^2 at Russell, and latrines detected during Spring tended to be larger than latrines detected the previous Autumn. However, variation was high (Fig. 4). Latrines ranged from 0.2–42.4 m^2 in size. The greatest maximum height was 18 cm and averaged (± *SD*) 5.13 ± 3.0 cm. Some latrines classified as old in Autumn 2017 to Autumn 2018 (29 at El Sauz, 3 at Santa Rosa, and 16 at Russell) were active in subsequent surveys (90% at El Sauz, 67% at Santa Rosa, and 56% at Russell). The average distance between road latrines (± *SE*) was 180 ± 10 m (range 3–2,043 m) at El Sauz, 352 ± 23 m (1–2,583 m) at Santa Rosa, and 108 ± 6 m (range 1–431 m) at Russell.

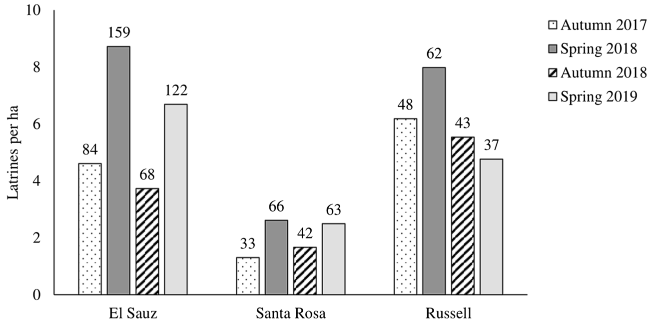

Fig. 3.—Density of nilgai latrines on unpaved roads at the El Sauz, Santa Rosa, and Russell ranches in South Texas, United States, during Autumn 2017, 2018, and Spring 2018, 2019. Surveys were complete counts of latrines assuming a fixed 5-m width. The number of latrines identified are labeled above bars.

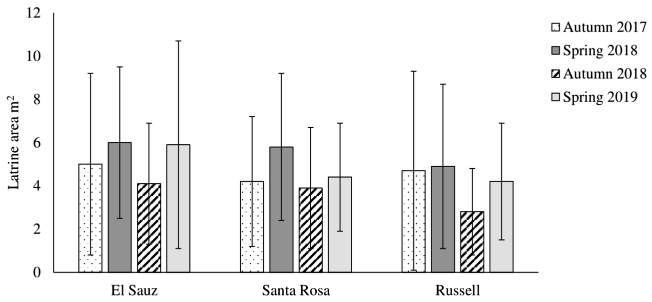

Fig. 4.—Spatial area (length × width) of nilgai antelope latrines on unpaved roads (m^2 ± *SD*) at the El Sauz, Santa Rosa, and Russell ranches, South Texas, United States, calculated from surveys during Autumn 2017, 2018, and Spring 2018, 2019.

Overall, 28% of road intersections (*n* = 139) had latrines with 24% at El Sauz (*n* = 42), 20% at Santa Rosa (*n* = 60), and 46% at Russell (*n* = 37). Road latrines occurred in 16 vegetation classes, with highest % use in vegetation classes with highest % available (Fig. 5). Densities of on-road latrines were >6 times greater than off-road for all three sites (Table 2). The average off-road mean latrine area (± *SD*) of 6.0 ± 2.9 m^2 (*n* = 68) was similar to on-road latrine mean area (± *SD*) of 5.7 ± 3.5 m^2 (*n* = 287) during Spring 2018. For the off-road surveys, we recorded 68 latrines and 1,039 fecal piles (nonlatrines; Table 2). All transect groups had single nilgai fecal piles observed, but not all had latrines. The spiny aster wetland community at Santa Rosa, and the grassland community at Russell had no latrine detections. Because the number of latrine detections was not sufficient to generate community-specific estimates, we pooled all site detections to estimate detection functions. Off-road latrines per ha (± *SE*) were lowest at Santa Rosa, but total off-road latrines estimated per site via distance sampling were lowest at Russell (Table 2).

Seasonal frequency, sex, and age-classes of nilgai that use latrines.— Some latrines became inactive over the course of monitoring (*n* = 13), so we moved cameras to active latrines. Thus, in total we monitored 37 latrines with 17 at El Sauz, 11 at Santa Rosa, and 9 at Russell. Some monitored latrines remained active throughout the entire study period from April 2018 to March 2019, including 4 at El Sauz, 9 at Santa Rosa, and 2 at Russell. We monitored El Sauz for 36,024 h, Santa Rosa for 33,749 h, and Russell for 13,983 h.

We recorded 10,101 animal visits (on or near latrines) at on-road latrines, including 1,544 nilgai visits (15%). El Sauz had 5,723 animal visits to latrines (635 nilgai, 12% of total visits), Santa Rosa had 3,749 visits (709 nilgai, 19%), and Russell had 629 visits (200 nilgai, 32%). Sixteen species besides nilgai were observed, including nine-banded armadillo (*Dasypus novemcinctus*), badger (*Taxidea taxus*), bobcat (*Lynx rufus*), domestic cattle, coyote (*Canis latrans*), white-tailed deer (*Odocoileus virginianus*), domestic dog (*Canis familiaris*), feral swine (*Sus scrofa*), collared peccary (*Tayassu tajacu*), red lechwe (*K. leche*), ocelot (*Leopardus pardalis*; only observed on El Sauz), eastern cottontail (*Sylvilagus floridanus*), raccoon (*Procyon lotor*), striped skunk (*Mephitis mephitis*), turkey (*Meleagris gallopavo*), and other birds. Besides nilgai, we observed defecations on latrines by cattle (*n* = 3), coyote (*n* = 19), and deer (*n* = 1). We recorded sniffing events from cattle (*n* = 48), coyote (*n* = 44), deer (*n* = 27), feral swine (*n* = 12), collared peccary (*n* = 4), and raccoon (*n* = 3).

On average, a given latrine had a nilgai visitation rate of 0.42 visits/day at El Sauz, 0.50 visits/day at Santa Rosa, and 0.34 visits/day at Russell. The observed frequency of latrine visits by latrine area was not consistent among sites. El Sauz had higher

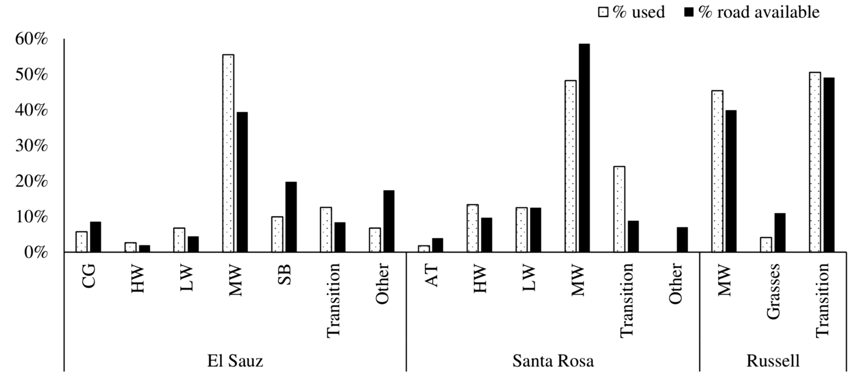

Fig. 5.—Location of nilgai antelope latrines encountered on unpaved roads by vegetation communities at the El Sauz, Santa Rosa, and Russell ranches in South Texas, United States, during Autumn 2017 to Spring 2019. Surveys were complete counts of all latrines on roads, and included 192 latrine locations at El Sauz, 112 at Santa Rosa, and 97 at Russell. Vegetation communities included: gulf cordgrass grassland (CG), huisache woodland (HW), live oak woodland (LW), mesquite woodland (MW), seacoast bluestem (SB), arrowfeather threeawn (AT), grassy areas dominated by guinea grass (Grasses), and roads that separated vegetation communities (Transition).

Table 2.—Densities of nilgai antelope latrines on and off-road (± *SE*) for the El Sauz, Santa Rosa, and Russell ranches, South Texas, United States, during Spring 2018.

	Sites		
Location	El Sauz	Santa Rosa	Russell
On-road[a] latrines/ha	8.7	2.6	8.0
Off-road[b] latrines/ha	0.9 ± 0.2	0.4 ± 0.1	0.8 ± 0.3
Off-road[b] ha surveyed	100	80	19.2
Latrine estimate[c]	10,206 ± 1,906	2,655 ± 621	244 ± 97

[a] Complete count of latrines encountered assuming a fixed 5-m road width per site.
[b] Identified via walking transects in major vegetation communities on each site.
[c] Total off-road number of latrines estimated per site via distance sampling.

frequencies of visits to small latrines (0.53 visits/day vs. 0.33). Russell had similar visits between small and large latrines (0.38 vs. 0.33), whereas Santa Rosa had a higher frequency of visits to large latrines (0.57 vs. 0.40). Overall, small latrines (*n* = 19) which averaged 0.46 visits per day (*n* = 641) were similar to large latrines (*n* = 18) which averaged 0.43 visits per day (*n* = 903). The latrine with the highest visitation frequency varied month to month; some latrines had the highest visitation rates during 1 month and no visits the next.

We observed peaks in visitation frequencies for El Sauz during April–June and December–February, Santa Rosa had peaks during August and December–March, and Russell had peaks during April–June and January–March (Fig. 6). Bulls contributed the highest proportion of nilgai latrine visits overall (Table 3), with 74% and 75% of visits at El Sauz and Santa Rosa, respectively. Russell had similar cow to bull proportions (42% cows vs. 38% bulls, remainder unknown or calves; Table 3). Subadult bulls were rarely detected and contributed to 4% of visits at El Sauz, 3% at Santa Rosa, and 7% at Russell (Table 3).

All age and sex classes defecated on latrines based on camera data, although frequency varied dramatically by sex and age. Nilgai latrine defecations averaged once every 10 days at El Sauz, 12 days at Santa Rosa, 20 days at Russell, and 12 days overall. We recorded 356 defecations with 147 nilgai defecation events at El Sauz (23% of visits resulted in defecation), 180 at Santa Rosa (25% of visits), and 29 at Russell (15% of visits). Overall, we recorded defecations from 329 bulls (92%, only one subadult bull), 20 cows (6%), 6 calves, and 1 unknown nilgai. Of the 20 cow defecation events, 13 (65%) had bulls visiting by the following day, 9 of which bulls visited within 15 h of the defecation. There were five cow defecations on latrines that received a defecation from a bull by the next day. For two of these events, the cow was with a bull when she defecated, and one bull defecated immediately following the cow. Sniffing events occurred from all sex and age-classes, with 178 events at El Sauz, 348 at Santa Rosa, and 37 at Russell. Bulls contributed to 81% of the sniffing events at El Sauz, 87% at Santa Rosa, and 37% at Russell. Nilgai sniffing events occurred at similar frequencies to defecations; sniffing events averaged every 8 days El Sauz (*n* = 178), 6 days at Santa Rosa (*n* = 348), 16 days Russell (*n* = 37), and 7 days overall (*n* = 563).

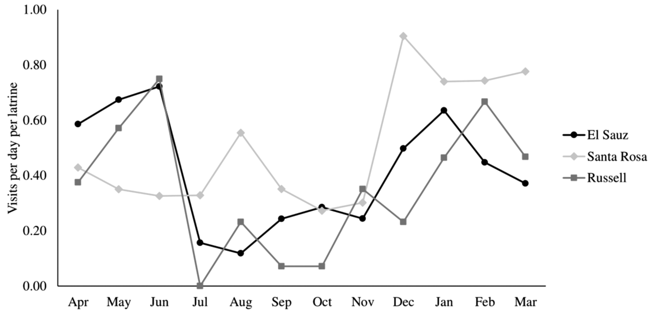

Fig. 6.—Average visits per day at nilgai antelope latrines at the El Sauz, Santa Rosa, and Russell ranches in South Texas, United States, during April 2018 to March 2019. Visits were recorded by trail cameras for 2 weeks of each month (10 cameras at El Sauz, 10 at Santa Rosa, and 4 at Russell).

The lowest proportion of the nilgai latrine visitations was midday and midafternoon (1400–1659 h, 12%, Fig. 7). Nilgai cows had higher proportions of visitation from early morning to midday (0500–1359 h), whereas bulls had higher proportions of visitations during twilight–night (1700–0459 h). Most (75%) of the nilgai bull defecations occurred during the twilight–night (1700–0459 h).

We noticed other behavioral responses of nilgai at latrine sites besides defecation and sniffing. These behavioral responses included the following: five cases of nilgai bulls showing flehmen responses (Fig. 8), two cases of bulls displacing other bulls, four cases of bulls appearing to tend cows (court an estrous female), three cases of bulls rubbing their head in vegetation near latrines, one cow urinating on a latrine as a bull licked her urine and displayed a flehmen response, one active mating, and two possible matings with both sexes showing erect tails at latrines. A fight occurred around a latrine; both bulls circled the latrine, and one urinated and defecated during the dispute. Cows and calves were documented defecating on that latrine 4 days earlier. Both bulls were photographed previously defecating at that latrine, and after the fight, both males still defecated on the latrine, but at different times.

Table 3.—Number and proportion of nilgai antelope latrine visits by age–sex class during April 2018 to March 2019, at the El Sauz, Santa Rosa, and Russell ranches in South Texas, United States. Data were collected from 37 latrines monitored with camera traps.

	Visits (% of total)			
Age-class	El Sauz	Santa Rosa	Russell	Total
Adult females	98 (15%)	102 (14%)	57 (29%)	257 (17%)
Subadult females	29 (5%)	17 (2%)	27 (14%)	73 (5%)
Old adult males	247 (39%)	337 (48%)	27 (14%)	611 (40%)
Adult males	193 (30%)	177 (25%)	35 (18%)	405 (26%)
Subadult males	27 (4%)	18 (3%)	14 (7%)	59 (4%)
Calves	15 (2%)	43 (6%)	29 (15%)	87 (6%)
Unknown	26 (4%)	15 (2%)	11 (6%)	52 (3%)
Total	635	709	200	1,544

Individual nilgai use of latrines.— Few nilgai bulls were individually recognizable, though physical characters such as horns, scars, or pelage could identify some individuals. Therefore, genetic analyses were needed to confirm identity of latrine visitations. We visited 24 monitored latrines and collected 114 samples from 15 latrines (El Sauz had 45

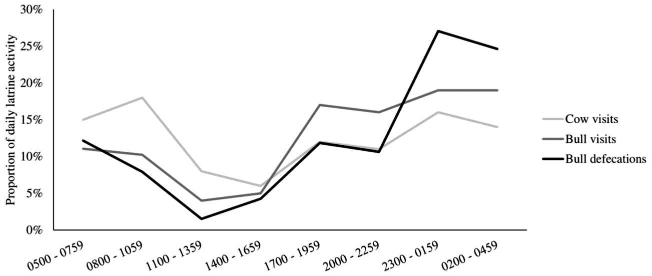

Fig. 7.—Proportion of daily activity of nilgai antelope by sex and number of defecations at latrine sites at the El Sauz, Santa Rosa, and Russell ranches in South Texas, United States, during April 2018 to March 2019. Camera traps revealed 330 cow visits, 1,075 bull visits, and 329 bull defecations. Cow defecations were rare ($n = 20$) and are not included. Times were categorized into 3-h segments of the day.

Fig. 8.—Nilgai antelope bull displaying flehmen behavior in response to nilgai cow urination on a latrine at the East Foundation's El Sauz Ranch, near Port Mansfield, Willacy County, Texas, United States, February 2019.

samples from six latrines; Santa Rosa had 69 samples from nine latrines). Some latrines ($n = 9$) were inactive during the sampling period. Initial data analysis revealed low genotyping success for freshness class 3 (dry coating, hard, dark brown samples). We excluded 39 samples from latrines either in class 3 and some in class 2 for latrines with an abundance of class 1 samples. We extracted 75 samples from 15 latrines that had >2 defecation events.

Only 25 samples were genotyped to individuals at ≥4 loci from nine different latrines and one latrine had only one successful genotyped sample. Genotyping success was low in part because defecations were relatively infrequent (about 1 per 10 days). Of the genotyped samples, 10 were only a few hours postdefecation (classified as #1 freshness), 14 were classified as #2 fresh, and 1 was #3 fresh. The S4B sexing marker amplified well, and we were able to sex 57 samples (76% of extracts); 51 were males (89%) and 6 were females (11%) across 15 latrines. For the 25 successfully genotyped samples, 20 were males and 5 were females. The five female samples were from two latrines at the El Sauz site. Photographs of defecation events indicated several repeated defecations from the same individuals (Table 4). We identified six individual bulls with 2–13 repeated defecations on latrines from January to February 2019, five were the only recognizable bulls to defecate on the latrine. In one case, a latrine received repeated defecations by two old adult bull individuals, one distinguishable with an ear tag from a previous study.

Discussion

This study, the first focused directly on latrine behavior by nilgai antelope, yielded insights into the abundance, distribution, and use of latrines. Latrine densities were 3–10 times higher than estimated nilgai densities at El Sauz and Santa Rosa. This indicates individual nilgai must use multiple latrines. Piles from single defecations were also abundant, showing defecations were not restricted to latrines. There was no qualitative evidence of selection or avoidance of vegetation communities. The lack of preference may be consistent with nilgai foraging ecology. Nilgai are intermediate feeders that use both grasses and browse (Sheffield 1983; Singh 1995; Hines 2016), and Jarman (1974) also observed antelope with similar behavioral characteristics to occupy a range of vegetation types that may vary seasonally. The only evidence of avoidance of a land cover type was that nilgai latrines in their native range, India, were found exclusively in forested areas, but were not detected in agricultural fields (Bayani and Watve 2016). Within vegetation communities in our study, nilgai appeared to prefer areas of high movement and visibility, which included the intersection of unpaved roads and fence lines, consistent with Fall (1972). Nilgai did not seem to avoid anthropogenic features (roads, intersections, fence lines), but latrine communication networks can be complex and there is a lack of overall understanding how ecological factors (Dröscher and Kappeler 2014) or anthropogenic influences (Ziege et al. 2016) may influence these networks. Jarman (1974) discussed how class C antelope tend to show visual displays with scent-marking, and while nilgai place latrines in highly visible areas, most defecations are at night by a single bull. Roads and especially intersections may be used frequently by nilgai; thus, the function of this placement may be to increase conspecific encounters to latrines.

Use of individual latrines was dynamic and persistent at specific locations; there was not a strong relationship between size of latrine and frequency of latrine visitation, and latrine sites may go unused, but nilgai often reformed latrines at the same locations. This suggests that the physical location of latrines is important. The most comprehensive review of nilgai biology implied regular use of latrines by both sexes and all age-classes (Leslie 2008) with reference to Blanford (1888), Brander (1923), Dharmakumar-sinhji (1959), Schaller (1967), Fall (1972), Prater (1980), Sheffield et al. (1983). Most of these studies are based on summaries of casual observations, with the exception of Sheffield et al. (1983) which documented latrine abundance but not usage by age–sex class, and Fall (1972) who monitored fresh defecations on latrines but noted observations of individual defecations observed in the field. While we documented all age and sex classes defecated on latrines, our results revealed that latrine usage differed by

Table 4.—Defecations on latrines by individual nilgai antelope at the El Sauz, Santa Rosa, and Russell ranches, Texas, United States, from 1 January 2019 through 28 February 2019. Data were collected from 24 latrines monitored with camera traps; latrines with no defecations photographed were excluded.

Site	Latrine ID	Total defecations[a]	Individual descriptions	% defecations[b]
El Sauz	E5	19	Old adult bull—ear tagged	68%
			Old adult bull—no ear tag	11%
			Unidentifiable old adult bulls	21%
	E25	6	Old adult bull	100%
	E96	12	Old adult bull—foot mark	67%
			Unidentifiable old adult bulls	17%
			Cow	8%
			Calf	8%
	E100	2	Cow	100%
	L152	3	Unidentifiable old adult bulls	100%
Santa Rosa	S18	2	Unidentifiable old adult bulls	100%
	S19	1	Old adult bull—odd shoulder	100%
	S28	9	Old adult bull—lightning scar	22%
			Unidentifiable old adult bulls	78%
Santa Rosa	S32	12	Old adult bull—lightning scar	67%
			Unidentifiable old adult bulls	8%
			Cow	8%
			Male calf	8%
			Unknown calf	8%
	S40	3	Unidentifiable old adult bulls	100%
	S59	3	Unidentifiable old adult bulls	100%
Russell	R50	2	Old adult bull—sharp horns	50%
			Cow	50%

[a] Number of defecations recorded at the latrine monitored with a camera trap.

[b] Proportion individual contributed to overall latrine defecations.

nilgai sex and age-classes. Adult bulls contributed to most of the photos of latrine visits and defecations. We documented only one photograph of a subadult bull that defecated on a latrine. Subadult bulls would often sniff latrines but not step foot on the latrine. Cows would occasionally just walk over the latrine with no acknowledgment of it, but males always reacted to latrines by sniffing or defecating. The few cow visits to latrines elicited behavioral responses by bulls such as flehmen, mating, tending, and fighting with another adult bull.

Our fecal DNA analysis produced mixed results due to the low quality and quantity of DNA, whereby we were unable to identify individuals consistently. This was due mainly to the long interval between defecations, where we could not consistently collect fresh fecal samples from all latrines. We elected not to increase collection frequency to minimize human disturbance at latrine sites and reduce the risk of altering nilgai behavior. In hindsight, disturbance may not have been an issue, as nilgai readily placed latrines near anthropogenic features such as unpaved roads and fences. Nonetheless, the genetic sexing results were more robust to sample quality and did support the camera data in that most defecations were from males (89% of fecal samples). The subset of samples identifiable to individuals indicated repeated visits from the same males.

Consistent with Jarman's (1974) predictions, our observations support the hypothesis that latrines serve as a dominance or territorial demarcation function for adult bulls and a means for advertisement of reproductive status and intersexual information exchange for cows. Territoriality is common among antelope species, especially those that scent-mark through urination and defecation (Dasmann and Mossman 1962; Estes 1967; Jarman 1979). Adult bulls mainly used latrines, and the one bull fight at a latrine involved defecations before, during, and after the fight that were characteristics for territorial demarcation (Wronski et al. 2006). Typical photographs of defecations from adult bulls showed individuals with all 4 feet on the latrine defecating at the center of the latrine, a behavior that indicates dominance status in some species (Schaller 1967; Marneweck et al. 2018). Territorial male animals tend to be larger, better fighters, and are typically the dominant males of social groups (Jarman 1974). These "old adult" nilgai bulls have thick necks and thick skin on the neck, which could be beneficial since nilgai are the only species of bovid where males display neck-fighting (Walther 1974; Jarman 1979; Sharma and Rahmani 2004). Territorial male antelope will also evict young males from female herds, causing male bachelor groups to form (Jarman 1974), as observed in nilgai (Fall 1972). Cows increased latrine visitation rates during the typical peak in the nilgai breeding season, from December to March (Fall 1972; Sheffield et al. 1983), a behavior seen in other species of antelopes (Dunbar and Dunbar 1974; Novellie et al. 1984; Kranz 1991; Roberts and Dunbar 2000). For klipspringer (*Oreotragus oreotragus*), females may increase marking rates when they are receptive, which is thought to be a strategy of females to promote male competition (Roberts and Dunbar 2000). The bull fight we documented around a latrine, which was 4 days after a cow–calf group defecated on it, provides some anecdotal support for a female-driven male competition for mating rights. The elicited behavioral responses by bulls to cow visits are consistent with the use of latrines for intersexual communication, of likely reproductive status (Wronski et al. 2006).

Territoriality in nilgai was considered unlikely by some previous studies. However, arguments against territorial behavior were based on behavioral observations (reviewed in Leslie [2008]), that were unable to elucidate relationships between latrine use and territoriality. For instance, Fall (1972) contended it was unlikely nilgai used latrines to maintain territories because the observed interval of 4 days between fecal deposits on latrines was considered too low for regularly maintained territorial markers. However, he did not benefit from remote camera technology to assess frequency of visitations that did not involve defecation. Nonetheless, the assumption about defecation frequency is arbitrary and Fall (1972) was unable to monitor individual movements or social interactions on latrine sites.

Our study provides insights into the role of latrines in the behavioral ecology of nilgai, yet the relationship between latrine placement, dynamics, and nilgai social behavior requires further study. A change in available food resources, water, or cover may influence latrine density (Jarman 1974, 1983). Conversely, a shift in latrine dynamics might result from dominance interactions or even harvest of territorial males. Nilgai tend to move through roads and fence crossings often. These areas generally serve as common locations for territorial animals to place latrines (Wronski et al. 2013; Zoromski 2019), since conspecifics may be more likely to encounter the latrines (Black-Decima and Santana 2011). Also, if latrines are visited more frequently at borders to other territorial rivals, then this combination of placement to increase encounters with neighbors and increased visitation rates would signify nilgai latrines function for maintenance of borders for territorial defense (Gosling 1986; Smith et al. 1989; Johansson and Liberg 1996). Male and female nilgai have large annual home ranges ($\bar{x}$ > 4,600 ha and >1,600 ha for males and females, respectively; Qureshi 1991; Moczygemba et al. 2012; Foley et al. 2017). However, some individual adult bulls had comparatively small (<1,000 ha) home ranges (Qureshi 1991; Foley et al. 2017). Furthermore, the relationship of social rank of the nilgai bulls to home ranges is not well known and requires further attention.

Our study on nilgai latrine ecology helps illustrate the role latrines serve for communication and provides support for the hypothesis of territorial behaviors. Nilgai–human conflicts are increasing in their native range because of extensive crop damage (Kahn 2014) and in their introduced range in Texas due to their ability to spread cattle fever ticks (Pérez de León et al. 2012). Insights into nilgai behavioral ecology may prove useful in their monitoring or management strategies. Further research is needed on nilgai latrine use and dynamics associated with social status, mating success, home ranges, and territories.

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SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Mammalogy* online.

Supplementary Data SD1.—Location of transects used to estimate nilgai antelope latrines off-road at the East Foundation's East El Sauz Ranch (10,984 ha) near Port Mansfield, Willacy County, Texas, during Spring 2018. Each of the five major vegetation communities received 10 500-m transects.

Supplementary Data SD2.—Location of transects used to estimate nilgai antelope latrines off-road at the East Foundation's Santa Rosa Ranch (9,000-ha) near Riviera, Kenedy County, Texas, during Spring 2018. Each of the four major vegetation communities received 10 500-m transects.

Supplementary Data SD3.—Location of transects used to estimate nilgai antelope latrines off-road at Russell Ranch (289 ha) near Rio Hondo, Cameron County, Texas, during Spring 2018. Twelve 200-m transects were located in both grassland and mesquite thornscrub communities.

Supplementary Data SD4.—Microsatellite DNA loci and sex determination markers optimized for nilgai antelope tissue samples. MgCl concentrations (in mM), primer concentrations (μM), and annealing temperature (T_M [°C]) used in each polymerase chain reaction (PCR) are provided. The loci with sufficient variability to identify individuals were pooled into two panels for separation and detection.

LITERATURE CITED

Anderson J.D., Honeycutt R.L., Gonzales R.A., Gee K.L., Skow L.C., Gallagher R.L., Honeycutt D.A., DeYoung R.W. 2002. Development of microsatellite DNA markers for the automated genetic characterization of white-tailed deer populations. Journal of Wildlife Management 66:67–74.

Bagchi S., Goyal S.P., Shankar K. 2008. Social organization and population structure of ungulates in a dry tropical forest in Western India (Mammalia, Artiodactyla). Mammalia 72:44–49.

Bailey R.G., Avers P.E., King T., McNab W.H. 1994. Ecoregions and subregions of the United States (map; scale 1:7,5000,000). United States Department of Agriculture Forest Service, Washington, District of Columbia, USA.

Bayani A., Watve M. 2016. Differences in behaviour of the nilgai (*Boselaphus tragocamelus*) during foraging in forest versus in agricultural land. Journal of Tropical Ecology 32:469–481.

Black-Decima P., Santana M. 2011. Olfactory communication and counter-marking in brown brocket deer *Mazama gouazoubira*. Acta Theriologica 56:179–187.

Blanford W.T. 1888. The fauna of British India, including Ceylonand Burma. Mammalia. Taylor and Fransis, London, United Kingdom.

Blüm V. 1985. Vergleichende Reproduktionsbiologie der Wirbeltiere. Springer, Berlin, Heidelberg, New York, USA.

Brander A.A.D. 1923. Wild animals in Central India. Edward Arnold and Company, London, United Kingdom.

Brashares J.S., Arcese P. 1999. Scent marking in a territorial African antelope: the maintenance of borders between male oribi. Animal Behavior 57:1–10.

Broquet T., Ménard N., Petit E. 2007. Noninvasive population genetics: a review of sample source, diet, fragment length and microsatellite motif effects on amplification success and genotyping error rates. Conservation Genetics 8:249–260.

Brotherton P.N.M. 1994. The evolution of monogamy in the dik-dik. Dissertation, Cambridge University, Cambridge, United Kingdom.

Brotherton P.N.M., Pemberton J.M., Malarkey G., Komers P.E. 1997. Genetic and behavioural evidence of monogamy in a mammal, Kirk's dik-dik (*Madoqua kirkii*). Proceedings of the Royal Society of London, B: Biological Sciences 264:675–681.

Brown R.E., MacDonald D.W. 1985. Social odours in mammals. Oxford University Press, Oxford, United Kingdom.

Clapperton B.K., Minot E.O., Crump D.R. 1988. An olfactory recognition system in the ferret *Mustela furo* L. (Carnivora: Mustelidae). Animal Behaviour 36:541–553.

Dharmakumarsinhji K.S. 1959. A field guide to big game census in India. Indian Board for Wild Life, New Delhi, India.

Dasmann R.F., Mossman A.S. 1962. Population studies of impala in southern Rhodesia. Journal of Mammalogy 43:375533–375395.

DeYoung R.W., Demarais S., Honeycutt R.L., Gonzales R.A., Gee K.L., Anderson J.D. 2003. Evaluation of a DNA microsatellite panel useful for genetic exclusion studies in white-tailed deer. Wildlife Society Bulletin 32:220–232.

Dinerstein E. 1980. An ecological survey of the Royal Karnali-Bardia Wildlife Reserve, Nepal. Biological Conservation 18:5–37.

Dröscher I., Kappeler P.M. 2014. Maintenance of familiarity and social bonding via communal latrine use in a solitary primate (*Lepilemur leucopus*). Behavioral Ecology and Sociobiology 68:2043–2058.

Dunbar R.I.M., Dunbar E.P. 1974. Social organization and ecology of the klipspringer (*Oreotragus oreotragus*) in Ethiopia. Zeitschrift für Tierpsychologie 35:481–493.

Dunbar R.I.M., Dunbar E.P. 1980. The pair bond in klipspringer. Animal Behavior 28:219–229.

Espírito-Santos C., Rosalino L.M., Santos-Reis M. 2007. Factors affecting the placement of common genet latrine sites in a Mediterranean landscape in Portugal. Journal of Mammalogy 88:201–207.

Estes R. 1967. Territorial behavior of the wildebeest (*Connochaetes taurinus* Burchell, 1823). Zeitschrift für Tierpsychologie 26:284–370.

Fall B.A. 1972. On social organization and behavior of nilgai antelope, *Boselaphus tragocamelus* (Pallas), in South Texas. Dissertation, Texas A&M University, College Station, Texas, USA.

Foley A.M., Goolsby J.A., Ortega-S. A. Jr., Ortega-S. J.A., Pérez de León A., Singh N.K., Schwartz A., Ellis D., Hewitt D.G., Campbell T.A. 2017. Movement patterns of nilgai antelope in South Texas: implications for cattle fever tick management. Preventive Veterinary Medicine 146:166–172.

Frantzen M.A.J., Silk J.B., Ferguson J.W., Wayne R.K., Kohn M.H. 1998. Empirical evaluation of preservation methods for fecal DNA. Molecular Ecology 7:1423–1428.

Goolsby J.A., Singh N.K., Ortega-S. A. Jr., Hewitt D.G., Campbell T.A., Wester D., Pérez de León A.A. 2017. Comparison of natural and artificial odor lures for nilgai (*Boselaphus tragocamelus*) and white-tailed deer (*Odocoileus virginianus*) in South Texas: developing treatment for cattle fever tick eradication. International Journal for Parasitology 6:100–107.

Gorman M.L. 1984. Scent-marking and territoriality. Acta Zoologica 171:49–53.

Gosling L.M. 1981. Demarkation in a gerenuk territory: an economic approach. Zeitschrift für Tierpsychologie 56:305–322.

Gosling L.M. 1982. A reassessment of the function of scent-marking in territories. Zeitschrift für Tierpsychologie 60:89–118.

Gosling L.M. 1985. The even-toed ungulates: Order Artiodactyla. In: Brown R.E., MacDonald D., editors. Social odours in mammals. Oxford University Press, Oxford, United Kingdom; p. 550–618.

Gosling L.M. 1986. Economic consequences of scent marking in mammalian territoriality. In: Duvall D., Müller-Schwarze D., Silverstein R.M., editors. Chemical signals in vertebrates. Vol. 4. Plenum Press, New York City, New York, USA; p. 385–395.

Gosling L.M. 1990. Scent marking by resource holder: alternative mechanisms for advertising the costs of competition. In: MacDonald D., Müller-Scharze D., Natynczuk S.E., editors. Chemical signals in vertebrates. Vol. 5. Oxford University Press, Oxford, United Kingdom; p. 315–328.

Gosling L.M., Atkinson N.W., Dunn S., Collins S.A. 1996. The response of subordinate male mice to scent marks varies in relation to their own competitive ability. Animal Behavior 52:1185–1191.

Gosling L.M., Roberts S.C. 2001. Scent-marking by male mammals: cheat-proof signals to competitors and mates. Advances in the Study of Behavior 30:169–217.

Gosling L.M., Wright K.H.M. 1994. Scent marking and resource defence by male coypus (*Myocastor coypus*). Journal of Zoology 234:423–436.

Hendrichs H. 1975. Changes in a population of dik-dik, *Madoqua kirkii*. Zeitschrift für Tierpsychologie 38:55–69.

Hendrichs H., Hendrichs U. 1971. Freilanduntersuchungen zur Ökologie und Ethologie der Zwerg-Antilope Madoqua (*Rhynchotragus kirki*) (Günther, 1880). In: Hendrichs H., Hendrichs U., editors. Dik-dik und elefanten. Piper, Munich, Germany; p. 9–75.

Hines S.L. 2016. Cattle, deer, and nilgai interactions. Dissertation, Texas A&M University–Kingsville, Kingsville, Texas, USA.

Jarman M.V. 1979. Impala social behaviour: territory, hierarchy, mating, and the use of space. Advances in Ethology 21:1–93.

Jarman P. 1983. Mating system and sexual dimorphism in large, terrestrial, mammalian herbivores. Biological Reviews 58:485–520.

Jarman P.J. 1974. The social organisation of antelope in relation to their ecology. Behaviour 48:215–267.

Johansson A., Liberg O. 1996. Functional aspects of marking behaviour by male roe deer (*Capreolus capreolus*). Journal of Mammalogy 77:558–567.

Jordan N.R., Cherry M.I., Manser M.B. 2007. Latrine distribution and patterns of use by wild meerkats: implications for territory and mate defence. Animal Behavior 73:613–622.

Kageyama S., Yoshida I., Kawakura K., Chikuni K. 2004. A novel repeated sequence located on the bovine Y chromosome: its application to rapid and precise embryo sexing by PCR. Journal of Veterinary Medical Science 66:509–514.

Kahn K.A. 2014. Current status and distribution of nilgai (*Boselaphus tragocamelus*) in Aligarh District. Master's thesis, Aligarh Muslim University, Aligarh, Uttar Pradesh, India.

Kalinowski S.T., Taper M.L., Marshall T.C. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16:1099–1106.

Khan I.A. 1992. Distribution and crop raiding behaviour of nilgai in Western U.P. (in the villages of Aligarh District). Master's thesis, Aligarh Muslim University, Aligarh, Uttar Pradesh, India.

Kranz K.R. 1991. Monogamy in the dik-dik. Applied Animal Behaviour Science 29:87–105.

Kruuk H. 1978. Spatial organization and territorial behavior of the European badger, *Meles meles*. Journal of Zoology 184:1–19.

Lenti Boero D. 1995. Scent-deposition behavior in alpine marmots (*Marmota marmot* L.): its role in territorial defence and social communication. International Journal of Behavioural Biology 100:26–38.

Leslie D.M. 2008. *Boselaphus tragocamelus* (Artiodactyla: Bovidae). Mammalian Species 813:1–16.

Lewis R.J. 2005. Sex differences in scent-marking in sifaka: mating conflict or male services?. American Journal of Physical Anthropology 128:389–398.

Marneweck C., Jürgens A., Shrader A.M. 2018. The role of middens in white rhino olfactory communication. Animal Behaviour 140:7–18.

Miller D.L. 2017. Distance: distance sampling detection function and abundance estimation. R package version 0.9.7.

Mills M.G.L., Gorman M.L. 1987. The scent-marking behaviour of the spotted hyaena *Crocuta craocuta* in the southern Kalahari. Journal of Zoology 212:483–497.

Mirza Z.B., Khan M.A. 1975. Study of distribution, habitat and food of nilgai *Boselaphus tragocamelus* in Punjab. Pakistan Journal of Zoology 7:209–214.

Moczygemba J.D., Hewitt D.G., Campbell T.A., Ortega-S J.A., Feild J., Hellickson M.W. 2012. Home ranges of the nilgai antelope (*Boselaphus tragocamelus*) in Texas. Southwestern Naturalist 57:26–30.

Novellie P.A., Manson J., Bigalke R.C. 1984. Behavioural ecology and communication in the Cape grysbok. South African Journal of Zoology 19:22–30.

Palanza P., Parmigiana S., vom Saal F.S. 1994. Male urinary cues stimulate intra-sexual aggression and urine-marking in wild female mice, *Mus musculus domesticus*. Animal Behaviour 48:245–247.

Pérez de León A.A., Teel P.D., Auclair A.N., Messenger M.T., Guerrero F.D., Schuster G., Miller R.J. 2012. Integrated strategy for sustainable cattle fever tick eradication in the USA is required to mitigate the impact of global change. Frontiers in Physiology 3:1–17.

Peterson M.K., Foley A.M., Tri A.N., Hewitt D.G., DeYoung R.W., DeYoung C.A., Campbell T.A. 2020. Mark-recapture distance sampling for aerial surveys of ungulates on rangelands. Wildlife Society Bulletin 44:713–723.

Prater S. H. 1980. The book of Indian animals. Bombay Natural History Society, Bombay, India.

Qureshi M.Q. 1991. Population status and movement of nilgai around the village Ghursikaran near Aligarh University. Master's thesis, Aligarh Muslim University, Aligarh, Uttar Pradesh, India.

Ralls K. 1971. Mammalian scent marking. Science 171:443–449.

Ramsay N.F., Giller P.S. 1996. Scent-marking in ring-tailed lemurs: responses to the introduction of foreign scent in the home range. Primates 37:13–23.

R Core Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www.R-project.org/.

Rivière-Dobigny T., Pham Doan L., Le Quang N., Maillard J., Michaux J. 2009. Species identification, molecular sexing and genotyping using non-invasive approaches in two wild bovid species: *Bos guarus* and *Bos javanicus*. Zoo Biology 28:127–136.

Roberts S.C., Dunbar R.L.M. 2000. Female territoriality and the function of scent-marking in a monogamous antelope (*Oreotragus oreotragus*). Behavioral Ecology and Sociobiology 47:417–423.

Rodgers T.W., Giacalone J., Heske E.J., Pawlikowski N.C., Schooley R.L. 2015. Communal latrines act as potentially important communication centers in ocelots *Leopardus pardalis*. Mammalian Biology 80:380–384.

Schaller G.B. 1967. The deer and the tiger. University of Chicago Press, Chicago, Illinois, USA.

Seutin G., White B.N., Boag P.T. 1991. Preservation of avian blood and tissue samples for DNA analysis. Canadian Journal of Zoology 69:82–90.

Sharma K., Rahmani A.R. 2004. Blue bull or nilgai. In: Sankar K., Goyal S.P., editors. Ungulates of India. ENVIS Bulletin: wildlife and protected areas. Vol. 7. Wildlife Institute of India, Dehradun, Uttarakhand, India; p. 29–40.

Sharma K., Rahmani A.R., Singh Chundawat R. 2009. Natural history observations of the four-horned antelope *Tetracerus quadricornis*. Journal of the Bombay Natural History Society 106:72–82.

Sheffield W.J. Jr. 1983. Food habits of nilgai antelope in Texas. Journal of Range Management 36:316–322.

Sheffield W.J. Jr., Ables E.D., Fall B.A. 1971. Geographic and ecologic distribution of nilgai antelope in Texas. Journal of Wildlife Management 35:250–257.

Sheffield W.J. Jr., Fall B.A., Brown B.A. 1983. The nilgai antelope in Texas. Texas Agricultural Experiment Station, Texas A&M University Press, College Station, Texas, USA.

Sillero-Zubiri C., Macdonald D.W. 1998. Scent-marking and territorial behaviour of Ethiopian wolves *Canis simensis*. Journal of Zoology 245:351–361.

Simons R.R., Felgenhauer B.E., Jaeger R.G. 1994. Salamander scent marks: site of production and their role in territorial defence. Animal Behaviour 48:97–103.

Singh R.V. 1995. Some studies on the ecology and behavior of nilgai (*Boselaphus tragocamelus* Pallas) with an assessment of damage to agricultural crops and development of strategy for damage control in southwestern Haryana. Dissertation, Centre of Wildlife and Ornithology, Aligarh Muslim University, Aligarh, Uttar Pradesh, India.

Smith J.L.D., McDougal C., Miquelle D. 1989. Scent marking in free range tigers, *Panthera tigris*. Animal Behaviour 37:1–10.

Taberlet P., Waits L.P., Luikart G. 1999. Noninvasive genetic sampling: look before you leap. Trends in Ecology and Evolution 14:323–327.

Teel P.D., Marin S.L., Grant W.E. 1996. Simulation of host-parasite-landscape interactions: influence of season and habitat on cattle fever tick (*Boophilus* sp.) population dynamics. Ecological Modelling 84:19–30.

Thomas L., Buckland S.T., Burnham K.P., Anderson D.R., Laake J.L., Borchers D.L., Strinberg S. 2002. Distance sampling. In: Harrawi A.H.S., Piegorsch W.W., editors. Encyclopedia of environmetrics. John Wiley & Sons, Ltd., Chichester, New York, USA; p. 544–552.

Traweek M., Welch R. 1992. Exotics in Texas. Texas Parks and Wildlife Department, Austin, Texas, USA.

Waits L.P., Paetkau D. 2005. Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. Journal of Wildlife Management 69:1419–1433.

Walls S.C., Mathis A., Jaeger R.G., Gergits W.F. 1989. Male salamanders with high quality diets have faeces attractive to females. Animal Behaviour 38:546–548.

Walther E.P. 1974. Some reflections on expressive behaviour in combats and courtship of certain horned ungulates. In: Geist V., Walther F., editors. The behaviour of ungulates and its relation to management—a symposium. IUCN Publication 24, Morges, Switzerland; p. 56–106.

Walther F.R. 1984. Communication and expression of hoofed animals. Indiana University Press, Bloomington, Indiana, USA.

Wronski T., Apio A., Plath M. 2006. The communicatory significance of localised defecation sites in bushbuck (*Tragelaphus scriptus*). Behavioral Ecology and Sociobiology 60:368–378.

Wronski T., Apio A., Plath M. 2013. Sex difference in communicatory significance in localized defecation sites in Arabian gazelles (*Gazella arabica*). Journal of Ethology 31:129–140.

Wronski T., Plath M. 2010. Characterization of the spatial distribution of latrines in reintroduced mountain gazelles: do latrines demarcate female group home ranges?. Journal of Zoology 280:92–101.

Wyatt T.D. 2003. Pheromones and animal behavior, communication by smell and taste. Cambridge University Press, Cambridge, United Kingdom.

Ziege M., Bierbach D., Bischoff A., Brandt A., Brix M., Greshake B., Merker S., Wenninger S., Wronski T., Plath M. 2016. Importance of latrine communication in European rabbits shifts along a rural-to-urban gradient. BMC Ecology 16:29.

Zoromski L.D. 2019. Social behavior and movement ecology of nilgai antelope. Master's thesis, Texas A&M University–Kingsville, Kingsville, Texas, USA.

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