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Population ecology and genetic study of gaur (*Bos gaurus*) in a small protected area in Thailand

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Abstract. *Pitimol N, Srikosamatara S. 2023. Population ecology and genetic study of gaur* (Bos gaurus) *in a small protected area in Thailand. Biodiversitas 24: 3355-3363.* Gaur population in Khao Phaeng Ma (KPM) restored forest increased from 6 individuals in 1995 to 1996 in 2006 and more afterward. Data on population sizes, distribution and genetic status are needed for effective management. The aim of this study was to obtain data on gaur population size, structure and broad distribution, and on gaur genetic diversity in KPM. For a one-year period from 2014-2015, data were collected in three areas on gaur number, group size, sex, and age using direct observation. Genetic diversity was examined at 15 cross-species microsatellite loci. The total number of gaurs was at least 184, with these individuals being highly concentrated on the western side of the area. The average group size was 13.15±0.98 (n=356). The proportion of adult males to adult females, juveniles, and calves was 35:100:51:65. From the 10 samples (4 tissue, 6 fecal), the number of alleles per locus ranged from 2 to 6 (average 4.07). The expected and observed heterozygosity were 0.594 and 0.649, respectively. The gaur population is increasing and distributes more at the forest edge. Genetic diversity was lower than gaur populations in Vietnam and India. Habitat management should be done to minimize human-gaur conflicts and increase gene flow between populations.

Keywords: Age-sex composition, forest edge, gaur population, genetic diversity, Khao Phaeng Ma

INTRODUCTION

Global populations of large herbivores have seen declines (Ripple et al. 2015). In the past 30 years, over 70 percent of the global gaur population has been lost (Melletti and Burton 2014). The remaining population is estimated to number 15,000-35,000 worldwide. Gaurs are listed as vulnerable in the International Union for Conservation of Nature's Red List of Threatened Species (IUCN Red List) (Duckworth et al. 2016). Habitat loss and poaching are their main threats (Choudhury 2002; Bhumpakphan and McShea 2011; Melletti and Burton 2014; Duckworth et al. 2016). While gaur can be found both inside and outside of forests, they likely prefer forest edges (Conry 1989). Populations are often shy due to past hunting pressures (Hubback 1937; Srikosamatara and Suteethorn 1995). Improving conservation in Thailand has resulted in more gaur being found near the edge of the forest, for example, in the Huai Kha Khaeng wildlife sanctuary (Srikosamatara 2000). Similar trends have been observed in other protected areas in Thailand since the late 1990s, including Kaeng Krachan National Park, Kui Buri National Park, Ta Phraya National Park and Khao Phaeng Ma Non-hunting area (Srikosamatara 2000; Steinmetz et al. 2010; Bhumpakphan and McShea 2011; Lamb 2011; Duckworth et al. 2016; Tanasarnpaiboon 2016; Sunton et al. unpublished).

Khao Phaeng Ma (KPM) is a small area located on the northeastern edge of Khao Yai National Park (KYNP), Thailand. The gaur population in KPM had seen a continuous increase following forest restoration in the area, beginning in 1995 when six gaurs migrated into the area from elsewhere in KYNP. In 2000, the number of gaurs was approximately 50 (Bidayabha 2001). Ten years after restoration, a direct observation survey was conducted to monitor the gaur population in KPM, covering one-third of the total area. The number of gaurs had nearly doubled, reaching 96 individuals in 2006. This study was carried out primarily by a local community (Sunton et al. unpublished). Gaurs now appear not only inside KPM, but also outside the protected area (Prayong 2014; Pharejaem et al. 2016; the Khao Phaeng Ma Conservation Group: KPMCG, personal communication). The time they spend at the forest edge has also been increasing (KPMCG, personal communication). Aside from KYNP to the south, KPM is otherwise surrounded by agricultural areas, local communities, and resorts. Corn (*Zea mays* L.) and cassava (*Manihot esculenta* Crantz) are major crops grown in this area, and crop raiding along the KPM boundary has been reported. Several gaurs have been killed due to their feeding on food crops (Tangprasert and Chongcharoen 2015; Tangprasert 2017; KPMCG, personal communication).

Management and mitigation efforts to handle this have been carried out by local farmers, local Non-Governmental Organizations (NGOs), forest rangers, as well as researchers. Habitat management by cutting pioneer trees for natural grassland recovery and fencing was undertaken to increase gaur concentrations inside the protected area and minimize the number of gaurs outside (Prayong 2014; Prayong and Srikosamatara 2017). However, exotic Ruzi grass (*Brachiaria ruziziensis* R.Germ. & C.M.Evrard) and legumes were later planted, especially in the western side

of the area, by government officers to attract gaur. The number of gaurs, gaur distribution patterns, and gaur age-sex composition are critical information for better understanding how to effectively manage these gaur populations.

In this study, two methods were used to collect information on the population biology of gaur in KPM without catching and unduly disturbing any individuals. Data on the number of gaurs, group size, and age-sex composition were collected through direct observation. This data collection was carried out with the involvement of KPMCG, government officers and a researcher. Non-invasive genetic sampling was used to obtain genetic information. Cross-species microsatellite markers were tested and standardized. Genetic diversity within the gaur population at KPM was examined. Due to the small area of this study site, genetic diversity might be expected to be small. However, the potential link between the KPM gaur population and those of the nearby and larger protected KYNP raises the possibility that suggests substantial genetic flow may still occur, meaning high levels of genetic diversity might be possible.

MATERIALS AND METHODS

Study site

This study was conducted in restored forest areas of KPM, in Wang Nam Khiao district, Nakhon Ratchasima Province, Thailand (N 14°21´-14°23´, E 101°46´-101°48´). This area is located at the northeastern edge of KYNP, which is part of the Dong Phayayen-Khao Yai Forest Complex (Figure 1). Its area is approximately 800 hectares, with elevation ranging from 400 to 800 meters above sea level.

KPM has been well known not only as one of the famous gaur sighting spots in Thailand but as a successfully restored forest. The dry evergreen forest within KPM was cleared and replaced by cornfields in 1970. The forest was restored by Thai NGOs (Wildlife Fund Thailand, WFT) beginning in 1994. After a few years of restoration, gaurs migrated into this area. The number of gaurs has continually increased. When the restoration project ended in 2002, the area became protected and managed by KPMCG, which was set up by pre-existing NGOs (WFT) and local communities. In 2011, Khao Phaeng Ma was officially designated by the Thai government as a non-hunting area. Habitat management inside protected areas was done by government officers. Exotic Ruzi grasses and legumes were planted inside some open areas.

The climate of this area can be divided into three seasons: winter (November-February), summer (March-June), and the rainy season (July-October). Rainfall records were obtained from the nearest weather station, which was Bann Din Udom weather station no.25971 (Royal Irrigation Department: http://www.hydro-4.com). Based on a 12-year sample, the average annual rainfall is 1,150 mm. During the period of this study, the weather was drier than normal. Total rainfall in 2014 and 2015 was 796 and 774 mm, respectively. Rainfall began late in June and continued until October. Monthly precipitations were especially below average during summer.

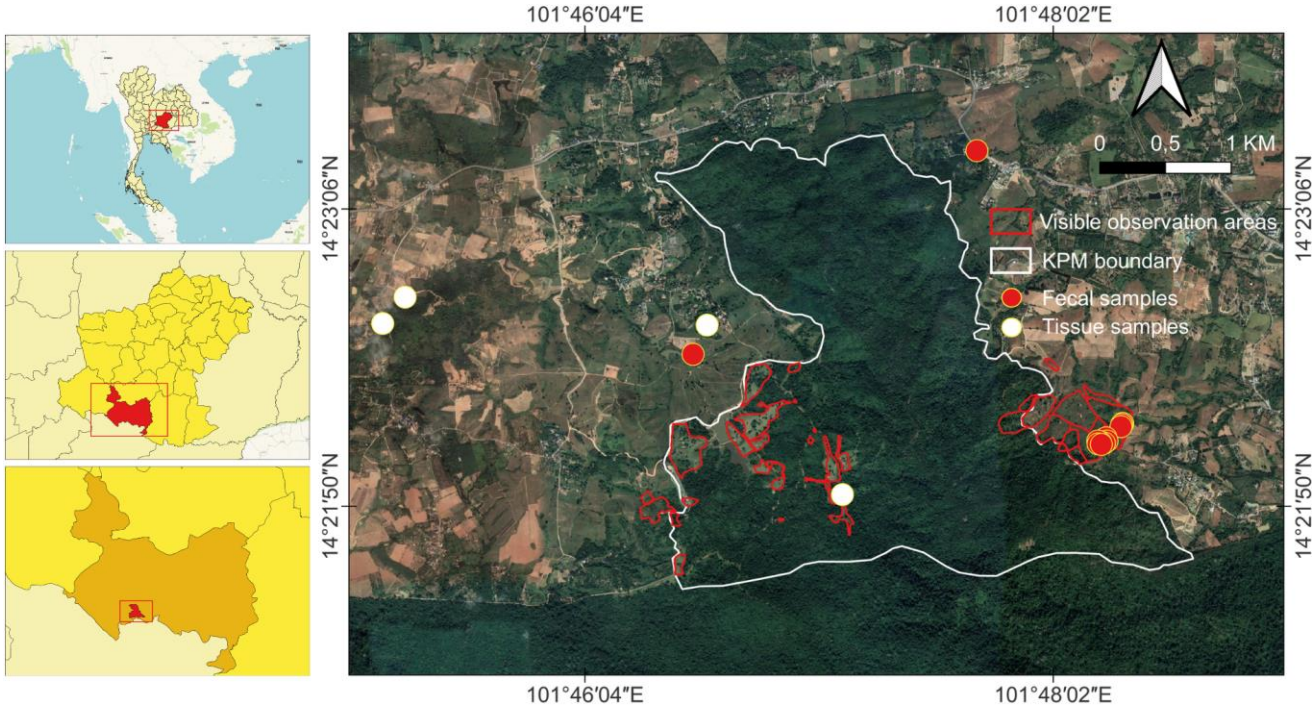

Figure 1. Location of the KPM non-hunting area. Visible observations were carried out in a Western Area (WA), a Middle Area (MA) and an Eastern Area (EA). Sample locations of the 12 fecal samples and the four tissue samples from dead animals are also shown

Direct observation

Direct observations were conducted every month from November 2014 to October 2015. Gaurs can be observed easily in opened areas both inside and outside the 800 hectares protected area. Three open areas where gaurs were regularly sighted were selected for observation, with all heavily used by gaurs and showing the presence of gaur trails and signs. The characteristics of these observation areas are described in Table 1. The observation points were selected based on the highest visibility of the observation area without disturbing animals and the safety of observers. The observation points were on the hill or at the foothill to avoid observation effects on gaur behaviors such as fleeing and avoidance.

Each month, direct observations were performed for four consecutive days. Each observation was conducted in the evening (from 3:30 PM until sunset) and the early morning (before sunrise until 8:00 AM). The duration of each observation was 3.5 and 2.5 hours in the evening and morning, respectively. Data were collected in the evening first to get relevant data for the following morning. The observations were repeated three times, both in the evening and the morning (6 observation sessions in total). Each observation session was divided into three sub-sessions according to the observation area. At least two observers were stationed at each observation point, collecting data at the same time. Local people (KPMCG), government officers and the researcher participated in data and sample collection.

For each sighting, gaurs were counted and their age and sex were identified using binoculars (10x42). The following data were recorded: (1) the location of sightings, time and the direction of gaur movement (2) the number of gaurs found (3) group size, including whether solitary, and (4) age and sex. Gaurs that grouped together and moved in the same direction were defined as being in the same group. Gaurs are defined as belonging to different groups when they move in different directions or forage far away from a potential group. For age and sex determination, gaurs were classified as adult male (>3 years), adult female (>3 years), juvenile (15 months-3 years), large calf (6-14 months), small calf (3-5 months), and newborn calf (0-2 months) based on Ahrestani and Prins (2011) with modifications for observing gaur in the field. Sex was determined only in adults, as there is no morphological sex difference in calves. Determining the sex of juveniles is difficult to apply in the field because there is a small difference in horn shape. Sex determination in adults was characterized based on horn differences (male horn shape curves sideway wider than female), body size (males are larger than females), and prominent features (elevated dorsal ridge, dewlap, and scrotum in males). Juveniles have >80% of the size of adults. Horns begin white from the base up to 20-30% of horn length. Calves were classified into newborn calves (light orange coat, horns show as bumps), small calves (greyish brown coat with 10-15 cm long black horns), and large calves (two-thirds in size of adults with longer black horns). In the case that identification of age and sex was uncertain, e.g. in low light conditions, then only the number of gaurs and groups were recorded. Observations were canceled when weather conditions significantly reduced visibility. A meeting between the local community, government officers and the researcher was held to define the age-sex identification and data collection procedure. All 13 observers participated in training and testing to increase inter-observer reliability. After the observation finished each month, meetings were held to share and discuss collected data.

Data analysis

The total, average, and maximum number was used to describe the number and group size of gaur found in each area and season. Data were tested for whether they were in a normal distribution. If they were not normal, non-parametric Kruskal-Wallis tests were used to compare among observation areas and seasons. Statistical analysis was performed using SPSS version 18.0 and the R program. The age-sex composition was calculated using the data obtained from the month with the largest number of gaurs with a high percentage of age-sex identification.

Genetic analysis

Fecal samples were collected in the morning after a direct observation. The locations and directions of gaur movements as well as fresh trails and marks in the field, helped guide participants to fresh gaur feces. The outer surface of the feces was collected using disposable gloves before being preserved in DETs buffer (20% DMSO, 0.25M EDTA, 100 mM Tris, pH 7.5, and NaCl to saturation) and stored at room temperature. In addition, tissue samples were collected from wild gaurs, which were found dead in the area. This was for cross-species microsatellite test and standardization. Small pieces of muscle tissues (2x2x2 cm) were excised and preserved in absolute ethanol.

Table 1. Description of direct observation areas

Observation area	WA	MA	EA
Size of area (hectares)	40	8	30
Location	The western edge inside the protected area	A more central part of the protected area	An eastern site outside the protected area
Grassland management	Opened areas were maintained using ploughing. Exotic Ruzi grass and legume were planted in some opened areas by protected area officers	Opened areas were maintained using ploughing by protected area officers	Opened areas were maintained using sod cutting, burning, and ploughing by KPMCG
Artificial saltlicks	No	Yes	Yes
Agricultural areas	- Large areas of corn cultivation and cassava - Small areas of vegetables such as pumpkin	No	- small size of agricultural area including corn field, cassava, and tomato
Electric fence	Yes, but it did not work effectively during the study	No	Built and maintained

DNA was extracted from tissue and fecal samples using DNeasy Blood and Tissue kit and QIAamp DNA stool mini kit (Qiagen), respectively. This method followed standard protocols with slight modifications, that is, increasing lysis time and reducing the amount of elution buffer in the final step.

Fifteen informative cross-species microsatellite primers: BM6425, BMS1120, BMS1355, BMS2526, BMS4015, ETH225, ETH3, HEL1, INRA005, INRA032, INRA037, INRA121, TGLA53, TGLA179, and TGLA73 (Nguyen et al. 2007) were selected according to Polymorphic Information Content (PIC). The forward primers were labeled with FAM, NED, PET, or VIC fluorescent tag. All Polymerase Chain Reactions (PCR) were performed in a 10 µL reaction mixture containing 5 µL MasterMix, 2 µL ddH2O, 1 µL each primer (2 µM), and 1 µL extracted DNA. For fecal samples, PCR reactions contained 5 µL MasterMix, 1 µL ddH2O, 1 µL mixed primers (2 µM), and 3 µL extracted DNA. PCR was conducted at 95°C for 15 minutes, followed by denaturing cycles at 95°C for 15 seconds (35 cycles for tissue samples, 50 cycles for fecal samples), annealing at 52-65°C for 45 seconds, and with an extension at 72°C for 1 minute. The final extension was at 72°C for 10 minutes.

The success of PCR reactions was visualized using 2% agarose gel electrophoresis under UV light. PCR products were multiplexed and sent for genotyping. Fragment length was analyzed using software Gene Mapper.

A comparative multi-tube approach was used to obtain reliable consensus for genotypes. Genotyping was performed twice for heterozygotes and three times for homozygotes (Frantz et al. 2003; Hansen et al. 2007). To examine genetic diversity based on microsatellite variation, the number of alleles per locus and expected and observed heterozygosity were calculated. Genotypic Identification with MultiLocus Tags (GIMLET)software was used to construct a consensus genotype and calculate the error rate (Valière 2002). Genetic diversity and divergence from the Hardy-Weinberg equilibrium were tested using ARLEQUIN program (Excoffier and Lisher 2010). In general, genetic diversity is compared over time or among populations. Since there was no microsatellite-based genetic diversity report of other gaur populations in Thailand, the results of our study were compared with the gaur population in Vietnam (Nguyen et al. 2007) and India (Farah et al. 2021; Mukherjee et al. 2022). Microsatellite loci used for genetic diversity study in India varied, some loci were different from our study.

RESULTS AND DISCUSSION

Direct observation

Seventy-one observation sessions (totaling 200 sub-sessions, including 70 sub-sessions in WA, 61 in MA and 69 in EA) were conducted from November 2014 to October 2015 (10 and 6 sub-sessions were canceled and missed, respectively). The average number of gaurs sighted was 65.03±5.07 per observation session (n=71) and 23.46±2.19 per sub-session (n=200). The average number of gaurs per area was 48.64±4.52, 8.38±1.97 and 11.10±1.24 in WA, MA and EA, respectively (Table 2).

More gaurs were found on both edges of KPM forest. Significantly more gaurs were sighted in the WA, where exotic grasses were planted, than in the other observation areas (χ^2=70.00, P=6.30x10^{-16}). Gaur sightings varied across seasons (χ^2=10.76, P=0.0046), with significantly more in the summer than in other seasons.

The largest number of gaurs found in a single observation session was 161 individuals, sighted in March 2015. The largest number of gaurs seen in each observation area (WA, MA, and EA) were 132, 80, and 52, respectively (Table 2). However, gaurs found in MA and WA may be from the same groups, as these two observation areas are close and gaurs were observed moving between these two areas many times. Thus, to calculate the largest number of observed gaurs in this study, only gaurs from WA and EA were counted. This results in a population size of at least 184 individuals.

Group size ranged from 1-108 individuals. The average group size was 13.15±0.98 (n=356), although this significantly differed across the three observation areas (χ^2=34.67, P=2.96x10^{-8}) and across seasons (χ^2 = 13.74, P=0.0010) (Table 2, Table 3). The largest group size of gaurs in WA, MA, and EA were 108, 80 and 34 individuals, respectively (Table 2). There was a high variation in group size in WA. In the winter, the group size was significantly smaller than in the summer and rainy seasons (Table 3). High variation in group size was observed during the summer and rainy seasons. Groups of 1-30 individuals were found most frequently (90%). Groups of around 100 individuals were only occasionally recorded.

This age and sex ratio were derived from observation data collected in March 2015, which was when the largest number of gaurs (N=184) were counted at one time, and which also had the highest percentage of age-sex identification (94.02 %). Out of the 184 gaurs, 173 were classified into age and sex classes. Among these, 24 (13.79%) were adult males, 69 (39.66%) adult females, 35 (20.11%) juveniles, and 45 (25.86%) calves. The number of mature (adult males and females) and immature (juveniles and calves) gaurs were 93 and 80, respectively, giving a ratio of 1:0.86. The adult sex ratio (adult male:adult female) was 1:2.86. The ratio of adult males:adult females:juveniles:calves were 35:100:51:65. The ratio of adult females to calves was 100:65.

Newborn calves were seen throughout the year, with 48 seen over the course of the year. The peak calving season was between March and June, during summer (Figure 2). The number of newborn calves found in WA was higher than in other areas (χ^2 = 33.22, P=6.13x10^{-8}).

Genetic analysis

In total, 4 tissue samples and 12 fecal samples were collected. All tissue samples were collected from different locations. 10 out of the 12 fecal samples were from the same group; the other two were from different groups outside the protected area. Of the 15 microsatellite primers tested, 14 (BM6425, BMS1120, BMS1355, BMS2526, BMS4015, ETH225, ETH3, HEL1, INRA005, INRA037,

INRA121, TGLA53, TGLA179, and TGLA73) were successfully amplified. The annealing temperature for each locus is shown in Table 4.

Of the 12 fecal samples, 6 were successfully amplified; the others were discarded. Overall, ten samples (4 tissue samples and 6 fecal samples) were amplified using the 14 primers. Allele size ranged from 94 to 241 bp. The number of alleles per locus ranged from 2 to 6 (averaging 4.07). The expected and observed heterozygosity were 0.594 and 0.649, respectively (Table 4). One locus (BMS6425) deviated from the Hardy-Weinberg equilibrium. The rate of allelic dropout varied from 0 to 33.60% among loci (averaging 11.80%). The rate of false alleles ranged from 0 to 16.70% (averaging 2.6%). The genotyping error rate is shown in Table 4.

The average number of alleles per locus in this study was equal to that of the study in Vietnam (Nguyen et al. 2007). Some loci, BMS4015, ETH225, HEL1, and TGLA53, were slightly higher. The average expected and observed heterozygosity of gaur in KPM was lower than in Vietnam, while the observed heterozygosity of some loci was higher. The number of alleles was lower than in the studies in India, both samples from wild populations (Farah et al. 2021) and from zoos (Mukherjee et al. 2022). On average, the heterozygosity of gaur in this study was lower than in India.

Table 2. Number of observation sessions and groups observed, the largest number of gaurs and group sightings and the average number of gaurs and group sightings in each observation area

Area	Number of observation sub-sessions	Largest number of gaurs	Average number of gaurs sighted±SE	Number of groups	Largest group size	Average group size±SE
WA	70	132	48.64±4.52*	186	108	18.31±1.65*
MA	61	80	8.38±1.97	55	80	9.29±2.02
EA	69	52	11.10±1.24	115	34	6.66±0.63
Overall	200	132	23.46±2.19	356	108	13.15±0.98

Note: *indicates significant difference among areas at p<0.05

Table 3. Number of observation sessions and groups observed, largest number of gaurs and group sightings and average number of gaur and group sightings in the three different seasons

Season	Number of observation sub-sessions	Largest number of gaurs	Average number of gaur sighting ±SE	Number of groups	Largest group size	Average group size±SE
Winter	70	93	13.77±1.90	131	66	7.36±0.72*
Summer	72	132	35.94±4.66*	145	108	17.85±1.96
Rainy	58	100	19.48±3.55	80	100	14.13±1.98
Overall	200	132	23.46±2.19	356	108	13.15±0.98

Note: *indicates significant difference among season at P<0.05

Table 4. The size range (bp), annealing temperature, frequency of allelic dropout and false alleles among the four tissue and six fecal samples, comparing genetic diversity (Na: number of alleles per locus, He: expected heterozygosity, and Ho: observed heterozygosity) in this study with the study in Vietnam (VN) (Nguyen et al. 2007)

Locus	Size range (bp)	Annealing temperature (°C)		%ADO	%FA	Genetic diversity					
						Na		He		Ho	
		Tissue	Feces			KPM	VN	KPM	VN	KPM	VN
BM6425*	165-179	58	58	0	0	4	6	0.65	0.82	0.90	0.82
BMS1120	121-139	62	58	30.6	0	5	6	0.82	0.84	1.00	0.91
BMS1355	153-161	58	57	16.7	0	4	4	0.49	0.70	0.60	0.82
BMS2526	132-138	61	60	0	11.7	3	4	0.28	0.76	0.10	0.64
BMS4015	132-148	61	57	11.1	16.7	5	4	0.57	0.69	0.60	0.64
ETH225	143-155	63	61	23.3	0	5	3	0.74	0.64	0.80	0.64
ETH3	125-127	63	62	6.7	0	2	3	0.51	0.65	0.60	0.55
HEL1	108-122	56	55	0	0	5	3	0.80	0.63	0.80	0.64
INRA005	239-241	54	55	0	0	2	4	0.27	0.70	0.30	0.82
INRA037	114-124	56	54	11.1	8.3	3	4	0.47	0.73	0.40	0.64
INRA121	112-128	61	58	33.3	0	4	4	0.57	0.71	0.40	0.55
TGLA179	94-104	62	58	13.9	0	3	3	0.63	0.70	0.90	0.64
TGLA53	153-171	56	55	9.7	0	6	5	0.73	0.70	0.80	0.73
TGLA73	121-133	58	57	8.3	0	6	4	0.81	0.75	0.89	0.73
Average				11.80	2.60	4.07	4.07	0.59	0.72	0.65	0.70

Note: * indicated deviation from Hardy-Weinberg equilibrium

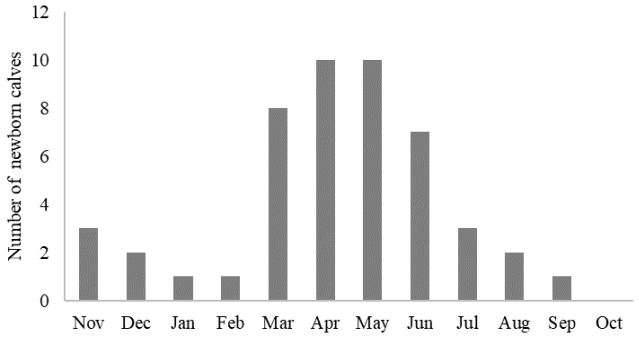

Figure 2. The number of newborn calves observed during each month. The peak of calving was between March and June. The number of newborns may be overestimated due to incomplete changes in newborn coat color during consecutive months of monitoring

Discussion

Most previous works on gaur populations were made within protected areas (Srikosamatara and Suteethorn 1995; Prayurasiddhi 1997; Ashokkumar et al. 2010; Ramesh et al. 2012; Farah et al. 2021). This study however examined a small, restored forest on the edge of a much larger protected area. Design and analysis were done by the researcher. Questions about the reliability of data collection by local-based methods can be overcome by proper design, training and analysis (Danielsen et al. 2005; Burton 2012).

The number of gaurs in KPM nearly doubled between 2006 and 2015 (Sunton et al. unpublished). The number of gaurs counted was lower than an estimate of 253 gaurs calculated using roadside count at night and distance sampling (Pharejaem et al. 2016). While total count methods tend to underestimate population sizes (Sutherland 2006), they provide a minimum count, including for age class and sex ratio. Gaur home ranges vary in size among age-sex classes and seasons. The home range of male, female, and yearling males in Malaysia were 7,018, 5,218 and 2,989 hectares, respectively (Conry 1989). In Thailand, the home range of gaur in Huai Kha Khaeng Wildlife Sanctuary ranges around 3,910 hectares in the wet season and 2,730 hectares in the dry season (Prayurasiddhi 1997). KPM forest of 800 hectares is a part of the larger home range of gaurs.

A large number of gaurs were found on both edges of our study site, while a smaller number of gaurs were found in MA. The larger number of gaur in WA followed a similar trend in the past, which has been confirmed through direct observation in 2006 (Sunton et al. unpublished) and line transects in 2012 (Prayong 2014). During our field observation, we observed the same pattern as Prayong (2014). Gaurs moved from inside the forest to the open areas in the late afternoon. They foraged and waited under tree covers inside the protected area until dark. At night, they crossed the boundary road, sometimes breaking electric fences, and foraged in agricultural or abandoned areas outside.

As expected, a larger number of gaurs were observed during summer. During this period, food availability inside the forest may have been insufficient (Hill 1998). Crop raiding by gaurs in the Mookambika wildlife sanctuary is most intense during the summer (Prashanth et al. 2013). Drier weather during the year of our observations may have caused a scarcity in one or more food sources. Although seasonal crops such as corn were not grown during the summer, there was still grass inside agricultural as well as abandoned areas. Moreover, cassava was also available, being able to grow throughout the year (Nyhus et al. 2000).

The largest group, which was observed in WA, is higher than other populations in many countries. This may be due to the concentration of exotic grasses cultivation in WA. There have been reports of a large group of gaurs (>70 individuals) elsewhere, for example, at Pidaung Wildlife Sanctuary and Jaldapara in India (Johnsingh 1983; Bhattacharya et al. 1997). Based on our observation, the large groups (>60 individuals), which were seen multiple times in WA, were the same group on different occasions. They likely formed a large group while in the open area, and then split into a few smaller groups of 20-40 individuals when they entered a denser forest. The high variation in group size in WA indicated a high rate of group fission and fusion. The average group size in this area (13.15±0.98) was greater than many populations elsewhere but still smaller than that observed in Kui Buri National Park (KBNP) (Tanasarnpaiboon 2016). The large group in this study was found in both the summer and rainy seasons. The aggregation of smaller groups into larger groups was temporary and mostly occurred during the wet season. Foraging for food may be the cause of this aggregation (Srikosamatara and Suteethorn 1995; Ashokkumar et al. 2010; Ramesh et al. 2012). Small groups may also come together to explore an open and agricultural areas, as well as salt licks (Conry 1989). After crop harvesting in December, agricultural areas around KPM are cleared and left empty until the beginning of the rainy season. During this time, grass can grow in agricultural areas, potentially leading to gaur group aggregation. Lastly, gaurs might aggregate for mating during the rutting period.

The age-sex composition reflects fecundity, mortality, reproductive status, and population growth rate. Our adult male:adult female:juvenile:calf ratio showed a similar trend to that of KBNP (Tanasarnpaiboon 2016). The proportion of immature individuals was nearly identical to that of mature individuals. This is higher than the ratio of gaur populations in India, in which the proportion of immature individuals was found to be less than half of that of mature gaurs (Schaller 1967; Karanth and Sunquist 1992; Vairavel 1998; Sankar et al. 2001; Kumar et al. 2004; Ashokkumar et al. 2010; Ramesh et al. 2012). The sex ratio skewed toward females, similar to other gaur populations. Adult males generally disperse from the group when they reach maturity. Males have more competition and higher mortality risk (Ramesh 2012). When out of a social group adult, males often live alone or form groups of bachelors (Melletti and Burton 2014). Solitary individuals and groups of bachelors might be rarely observed because they prefer living in dense vegetation (Steinmetz et al. 2010).

The total number of newborns throughout the year of our observation was 48. However, some of this number

might be due to recounts in consecutive months in cases where the orange-brown body coats of newborn calves do not turn completely brown between observation periods. Newborn calves were seen in every month except October, and calving peaked between March and June. The calving period of gaurs varies between study sites (Melletti and Burton 2014). In some study areas, calves can be found throughout the year (Ahrestani and Prins 2011). Female gaurs can produce a calf once a year given their 9.5 months gestation period (Schaller 1967).

The amplification of half of our fecal samples was unsuccessful, leading to these samples being discarded. This was despite fecal samples being collected within 24 hours of defecation, which should limit DNA degradation. Humidity and temperature at the site negatively correlated with the quantity of extracted DNA (Lucchini et al. 2002; Fernando et al. 2003; Nsubuga et al. 2004). In addition, fecal samples contained PCR inhibitors such as polysaccharides, urea, bile salts, and bacteria that may have affected the success rate of amplification (Monteiro et al. 1997; Beja-Pereira et al. 2009; Schrader 2012). Feces are known to be a low-quality source of DNA (Taberlet et al. 1996). Error rates, including the allelic dropout and false allele, are commonly found. In this study, allelic dropout and false allele were 11.80 and 2.6, respectively. The rate of errors was in the range of other non-invasive genetic studies (Broquet and Petit 2004).

Polymorphism was observed in all 14 loci. The number of alleles per locus ranged from 2 to 6. BMS6425 deviated from the Hardy-Weinberg equilibrium. Observed heterozygosity was higher than expected, which may be due to heterozygosity excess. Expected and observed heterozygosity of gaurs in KPM were lower than those in Vietnam (Nguyen et al. 2007) and India (Farah et al. 2021; Mukherjee et al. 2022). In Vietnam, samples were collected randomly from different populations, whereas in this study, samples were collected from different groups within the same population. Samples in this study are thus much more likely to originate from related individuals. In India, one study analyzed random samples from stable and viable wild gaur populations in a large protected area. The samples from another study were from a zoo. The comparison of our findings with other studies in India, differ partly in the number of samples, number of microsatellite loci and markers, but are useful information (Olech et al. 2023). The genetic diversity of threatened species is mostly lower than that of non-threatened species (Willoughby et al. 2015). The genetic diversity of gaurs in KPM is not as low as that of concerned European bison (*Bison bonasus* Linnaeus, 1758) populations (Tokarska et al. 2015; Olech et al. 2023), and Far Eastern leopards (*Panthera pardus* subsp. *orientalis* Schlegel, 1857) (Sugimoto et al. 2014). Gene flow to nearby populations and gaur behavior may play a key role in maintaining genetics. Gene flow through migration helps maintain and recover genetic diversity in populations with even just one or a few migrants (Jangjoo et al. 2016). However, gaurs are naturally social animals that live in groups of related females, calves, and a few males. The genetic study based on mitochondrial D-loop sequences obtained from 11 wild gaurs in KPM showed low mitochondrial DNA diversity with few maternal lineages and a small number of founder females (Duengkae et al. 2022).

In conclusion, the increasing gaur population at the forest edge and outside of protected areas, especially due to exotic grasses cultivation, creates problems and opportunities for appropriate wildlife management in human-dominated areas. This study suggests that as long as populations of gaurs are maintained as open populations to allow certain interchange of gaurs with nearby subpopulations, the population genetics of gaur will continue to be healthy. At the same time, there is the potential for increasing human-gaur conflicts. Solutions to solve these conflicts should consider areas both inside and outside of protected areas. Currently, extensive exotic grasses cultivation is practiced in only one concentration area at the border of the protected area. This is to encourage a high concentration of gaur to enter that area, which will eventually allow gaurs to disperse to nearby cultivated areas. Management of habitats that generate more food for gaurs should be done in more than one area to allow for large groups to disaggregate and should consider indigenous grassland management, leading to less crop damage if they disperse outside the protected area. At the same time, surrounding areas where gaurs can be found should be managed as buffer zones, including land use planning which minimizes the extensive farming of mono-crops such as maize and cassava.

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