Genetic diversity of Eld's deer *Rucervus eldii siamensis* populations captive-bred at Phnom Tamao Wildlife Rescue Centre, Takeo, Cambodia

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មូលន័យសង្ខេប

វិបុលកម្ម (ការបង្កាត់ពូជ) ជាយុទ្ធសាស្ត្រអភិរក្សត្រូវបានស្នើឡើងជាទូទៅសម្រាប់ប្រភេទដែលមានប៉ូពុយឡេសិុងធ្លាក់ចុះខ្លាំងពេក ដើម្បីឲ្យមាននានាភាពនៃសេនេទិចក្នុងព្រៃ គួយ៉ាងដូចជាប្រភេទរងគ្រោះសត្វម៉ាង ក្នុងប្រភេទរង *Rucervus eldii siamensis*។ កម្មវិធីទាំងនេះទាមទារការគ្រប់គ្រង និងការថែរក្សាយ៉ាងលទ្ធិលាយនុភាពចម្រុះសេនេទិចខ្ពស់ក្នុងប៉ូពុយឡេសិុងដែលបង្កាត់ក្នុងដែនកំណត់។ មជ្ឈមណ្ឌលសង្រ្គោះសត្វព្រៃភ្នំតាម៉ៅ និងព្រៃឈើលើទទ្ធិជុំវិញក្នុងខេត្តតាកែវនៃប្រទេសកម្ពុជា ជាកន្លែងបង្ហាង និងប្រលែងប៉ូពុយឡេសិុងសត្វម៉ាងបង្កាត់ ដែលជាកូនចៅរបស់សត្វម៉ាងពីរក្បាលបានរើបអូសពីការជួញដូរសត្វព្រៃខុសច្បាប់នាក៏ឡុង ឆ្នាំ២០០១។ ពេលដែលប៉ូពុយឡេសិុងបានកើនឡើង វាត្រូវបានបំបែកជាពីរហ្គុងដែលដាក់នៅដាច់ដោយឡែកពីគ្នាហ្គុងដល់ក្រុម គួចៗត្រូវបានប្រលែងនៅឆ្នាំ២០១៨។ យើងបានធ្វើការវាយតម្លៃទៅលើកម្រិតនៃការបង្កាត់ជិត និងភាពសម្បូរបែបនៃសេនេទិចរបស់ប៉ូពុយឡេសិុង (សត្វ២៧ ក្បាល) ដោយការធ្វើតេស្តមើលភាពចម្រុះនៃសេណូមរបស់វាឡើងវិញ (re-sequencing) លើសំណាកឈាមសត្វម៉ាងចំនួនប្រាំបីក្បាល។ កម្រិតទាបនៃភាពចម្រុះសេនេទិចត្រូវបានរកឃើញក្នុងសត្វទាំងនេះ។ អគ្រាអេតុរ៉ុស្ស៊ីកុតេមចម្រម និងភាពចម្រុះនៃនុយក្លេអូទីត (π) គឺ $5.538 \times 10^{-6} \pm 1.854 \times 10^{-6}$ និង 5.475×10^{-5}។ តម្លៃមេគុណនៃការបង្កាត់ជិតជាមធ្យម (F_{ROH}) គឺ 0.026 ± 0.060 ហើយឯកត្ត:ភាគច្រើនជាប់សាច់ញាតិជំនាន់ទី២។ កំណត់ត្រាប្រជាសាស្ត្រសត្វម៉ាងពីខែមករា ឆ្នាំ២០០៩ ដល់ខែមេសា ឆ្នាំ២០២២ ចំពោះប៉ូពុយឡេសិុសត្វដែលបង្ហាងទុកបានបង្ហាញថាអគ្រាស្លាប់នៃកូនទើបនឹងកើតជាមធ្យមគឺ26.85% ដែលមាននិយថាប៉ូពុយឡេសិុងចូលហ្ស៊ុន្ធមិនរងគ្រោះខ្លាំងពីការបង្កាត់ជិតទេ។ លទ្ធផលនៃការសិក្សាបង្ហាញថា ហ្គុងសត្វបង្ហាង និងហ្គុងសត្វពាក់កណ្ដាលព្រៃ (semi-wild) ដែលបានប្រលែងនៅមជ្ឈមណ្ឌលសង្រ្គោះសត្វព្រៃភ្នំតាម៉ៅអាចជាប្រភពប៉ូពុយឡេសិុង សមស្របសម្រាប់ការបង្កើនចំនួនសត្វម៉ាងឡើងវិញនាពេលអនាគត បើទោះបីជាពួកវាអាចនឹងទទួលបានអត្ថប្រយោជន៍ពីការបន្ថែមឯកត្ត:ថ្មីដើម្បីការពារការថយចុះនូវនានាភាពនៃសេនេទិចក៏ដោយ។

Abstract

Captive-breeding is a commonly proposed conservation strategy for species whose populations have become too small to be genetically viable in the wild, such as the Endangered Eld's deer subspecies *Rucervus eldii siamensis*. These programmes require extensive management and maintenance of high genetic diversity within the captive population. The Phnom Tamao Wildlife Rescue Centre and surrounding forests in Takeo, Cambodia, are home to a captive and a released breeding population of Eld's deer that are descended from two founders confiscated from the illegal wildlife trade in 2001. As the captive population grew, it was separated into two herds which remained isolated until small groups were released in 2018. We assessed the level of inbreeding and genetic diversity of the population (27 animals) by re-sequencing genome wide variants using blood samples from eight captive individuals. A low level of genetic diversity was found in the animals. The average heterozygosity rate and nucleotide diversity (π) were 5.538×10^{-6} $\pm$ 1.854×10^{-6} and 5.475×10^{-5}, respectively. The mean inbreeding coefficient (F_{ROH}) was 0.026 ± 0.060, and most individuals were in second degree kinship. Demographic records from January 2009 to April 2022 for the captive population revealed average neonatal mortality was 26.85%, which suggests the population does not currently suffer striking hazards from inbreeding. Our results suggest that the captive and released semi-wild herds at the centre may be a suitable source population for future reintroductions, although they would benefit from the addition of new individuals to protect against genetic erosion.

Keywords Captive-breeding, inbreeding, kinship coefficient, reintroduction, whole-genome sequencing.

Introduction

Captive-breeding has become a well-established strategy in conservation plans to safeguard against species extinction (Wilson & Stanley Price, 1994; IUCN/SSC, 2008; Bowkett, 2009; Leus, 2011). The ultimate aim of these programmes is to create genetically diverse and resilient captive source populations for future reintroduction into native habitats (Beck *et al.*, 1994; IUCN/SCC, 2013; Ralls & Ballou, 2013). Reintroduction can re-establish species in areas where they have been extirpated and provide new genetic lineages to small wild subpopulations suffering inbreeding depression and Allee effects after prolonged isolation (Deredec & Courchamp, 2007; IUCN/SCC, 2013). Captive-breeding programmes for reintroduction complement *in situ* conservation initiatives, which can be as equally resource intensive (Ralls & Ballou, 1992). These programmes require complicated collaborations amongst various stakeholders, decades of captive management (to mitigate the original causes of wild population decline before release becomes feasible and maintain a captive source population post-release), and prolonged monitoring of the reintroduced population (Wilson & Stanley Price, 1994; Spalton *et al.*, 1999; Ralls & Ballou, 2013). Despite the challenges, captive-breeding and reintroduction initiatives have led to re-establishment of extirpated species including golden lion tamarins *Leontopithecus rosalia* (Kierulff *et al.*, 2012); California condors *Gymnogyps californianus* (Toone & Wallace, 1994), Arabian oryx *Oryx leucoryx* (Spalton *et al.*, 1999); Père David's deer *Elaphurus davidianus* (Jiang *et al.*,

1973) and Persian fallow deer *Dama dama mesopotamica* (Bar-David *et al.*, 2005).

Maintaining genetic diversity in captive populations has long been a goal of breeding programmes (El Alqamy *et al.*, 2012; Ralls & Ballou, 1992, 2013; Chen *et al.*, 2019). To mitigate inbreeding depression, loss of genetic variability and minimize adaptation to captivity, these programmes aim to start with as large and diverse a number of founders as realistically possible and limit the number of generations in captivity (McPhee, 2004; Frankham, 2008; Robert, 2009; Purohit *et al.*, 2021). To support the reintroduction success of individuals and encourage appropriate behaviours for survival, programmes attempt to provide captive environments that mimic wild habitats (Bremner-Harrison *et al.*, 2004; McPhee, 2004). In practice, this can be challenging when captive populations are descended from a small number of founders, when few individuals remain in the wild for capture or when the pressures on wild populations causing declines limit the options for release (Ahmad Zafir *et al.*, 2011; Ralls & Ballou, 2013). Inbreeding depression has been commonly associated with lower fecundity and increased neonatal mortality within populations (Ralls *et al.*, 1979; Ralls & Ballou, 1986; Lacy *et al.*, 1993). However, species-specific studies have found less definitive links between highly inbred populations suffering lower juvenile survivorship (Kalinowski & Hedrick, 2001; Zeng *et al.*, 2013). Even if high levels of inbreeding do not affect a population in such a way, it may leave them more vulnerable to successive stochastic events, an important consideration for

reintroduction programmes based on a limited number of founders (Thévenon & Couvet, 2002).

Southeast Asia supports a large number of threatened species that have experienced population declines due to habitat loss and illegal hunting (Sodhi *et al.*, 2010; Gray *et al.*, 2018). Eld's deer *Rucervus eldii* is an Endangered tropical cervid that historically occurred across the region but has suffered population fragmentation and declines throughout its range (Gray *et al.*, 2015). Three subspecies are traditionally recognised, *R. e. eldii*, *R. e. thamin* and *R. e. siamensis* (Gray *et al.*, 2015), although individuals from Hainan Island (China) are now considered to be a fourth subspecies, *R. e. hainanus* (Wong *et al.*, 2021) and are recognized as such in this study. *Rucervus e. siamensis* has been extirpated from Vietnam and Thailand, although a reintroduction programme has begun in the latter (Wong *et al.*, 2018). Wild populations remain in Laos and Cambodia and Cambodia is considered to be the last stronghold for the subspecies despite suffering a 90% population reduction in the early 2000's (Gray *et al.*, 2015). Less than 400 individuals are currently estimated to remain in fragmented populations across nine protected areas in the country, which are suspected to be declining (Ladd *et al.*, 2022).

Genetic studies of the four subspecies have found that *hainanus* and *eldii* have likely suffered bottleneck effects in wild and captive populations (Balakrishnan *et al.*, 2003; Pang *et al.*, 2003; Angoma & Hussain, 2013; Zheng *et al.*, unpubl. data). At the time of testing, Balakrishnan *et al.* (2003) concluded that populations of *thamin* and *siamensis* remained genetically diverse. Since wild populations face a risk of genetic erosion, captive populations may provide fresh genetic lineages that can contribute to genetic recovery (Theodorou & Couvet, 2004; Hedrick & Fredrickson, 2008). Efforts are underway to breed and reintroduce Eld's deer subspecies in their native ranges, including *eldii* in India (Singh & Dookia, 2017), *siamensis* and *thamin* in Thailand (Wong *et al.*, 2018) and *hainanus* in China (Wong *et al.*, 2021). As suitable natural habitats still remain in Cambodia, similar actions for *siamensis* have been recommended in the country (Gray *et al.*, 2015; Ladd *et al.*, 2022). At present, few captive populations of *siamensis* are recorded in facilities in Thailand, France and the USA. Additionally, the genetic purity of the subspecies is unknown and could be hybridized with *thamin* (Hartley, M. pers. comm.). As such, one pure population of *siamensis* may remain at present, namely breeding herds in natural enclosures or semi-wild animals in the forests surrounding the Phnom Tamao Wildlife Rescue Centre (PTWRC or centre) in Takeo Province, Cambodia.

As of May 2022, populations of Eld's deer at the PTWRC comprised 15 individuals in captivity and an estimated 40 free-roaming animals that were released from enclosures or born in the forests surrounding the site. The population began with two unrelated individuals which were rescued as fawns from the illegal wildlife trade in 2001. Although the herd was not managed for captive-breeding and reintroduction, it was split into two sub-herds as it grew and these remained separate until they were mixed into small groups for release efforts in 2018. In 2017, blood samples were taken by Kadoorie Farm Botanic Garden to study the taxonomic status of *hainanus* in relation to *siamensis* using low coverage next-generation re-sequencing data. The level of inbreeding within the PTWRC's captive herd was examined by re-sequencing genome wide variants of these individuals. This paper documents the genetic indices of the Phnom Tamao population in terms of inbreeding and genetic diversity. At the time of testing, no *siamensis* had been released, but all deer released into the forest since then are descendants of this herd. Details of captive-care and release protocols are described. Metrics of population health are included to argue the viability of the Phnom Tamao herds as a source population for future reintroduction initiatives in Cambodia or genetic exchanges with other fragmented populations.

Methods

Study site

The PTWRC was established in 1995 by the Cambodian Forestry Administration and has been supported technically and financially by Wildlife Alliance (WA) since 2001. The centre is set within a large area of regenerated deciduous dipterocarp forest which covered 2,025 ha in July 2022. Enclosures are spread across 400+ ha of this area, which was enclosed by a chain link fence in 2016. The centre and surrounding forests are protected by a community anti-poaching unit, which patrols the forest to confiscate hunting equipment, apprehend offenders and remove snares, traps and dogs. The site is a safe location and now holds healthy populations of reintroduced wild boar *Sus scrofa*, red muntjac *Muntiacus muntjak*, sambar deer *Rusa unicolor* and Eld's deer (WA, unpubl. data).

Captive-care

The two captive Eld's deer sub-herds are currently housed in outdoor enclosures measuring 60 m x 60 m. These are located in a public area where animals are on display for visitors. Both enclosures include a pool and an additional roofed section measuring 10 m x 10 m. The first enclosure was built in 2004 after the two original

arrivals had two fawns. The second was built in 2009 and joined to the first with a gate, to accommodate the growing herds and create two sub-herds which remained separate until 2018. Originally constructed around a section of forest and filled with natural vegetation that provided good cover for fawns, these areas have since become over-grazed and so grasses no longer regenerate.

A third enclosure was built in a remote area of forest in the PTWRC as a pre-release enclosure in 2018. This is made of two separate sections measuring 60 m x 60 m each, connected by a central holding area which measures 10 m x 10 m. These is not accessible to the public and contain natural vegetation to encourage wild behaviours and disassociation with humans. Groups moved into the pre-release enclosure were formed by mixing deer from the two sub-herds, with one stag and at least two hinds included in each group.

The herds in the display and pre-release enclosures are left to select their own mates and breed at will. The offspring are mother-raised and hand-rearing only occurs when fawns have been neglected and are visibly weakened. All of the herds are breeding, including the smaller groups moved to the pre-release enclosure for acclimatization.

Release

Small groups of deer were first released from the pre-release enclosure and subsequently from the original enclosures within the centre. Deer were released in groups of two to four animals. Single stags were released when there was an imbalance in sexes within the herd to reduce pressure and fighting. When numbers increased sufficiently, the enclosure door was opened and selected deer were allowed to leave at will. Supplemental food was provided twice daily (potatoes and bananas in the morning and water greens in the evening) in the three locations where the semi-wild herds were consistently observed. This was done to encourage the deer to remain in the area and enable *ad hoc* monitoring of their health, movements and births. Not all deer returned for the supplementary feeding, although wilder populations in remoter forest areas of Phnom Tamao have been seen occasionally.

Genetic sample collection

Deer were sedated by veterinary staff at the centre by administering Xylazine (0.25 mg/kg) which was injected intramuscularly with a dart gun, and were revived with Atepamezole (0.250.5mg/kg) which was injected intravenously. To reduce the possibility of capture myopathy and stress on the herds (totalling 27 animals),

the easiest adults to catch in the display enclosures that met our requirements were targeted. 100–200 μl of blood was collected from each of these (with five males and five females sampled) and kept in Eppendorf tubes containing 500μl of 95–100% ethanol. The samples were stored on site in a freezer (at -20 °C) prior to transport in a cooler box to laboratory facilities in Phnom Penh.

Whole-genome re-sequencing

Total genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Germany) following the manufacturer's protocol. DNA quality was quantified with a NanoDrop ND-2000 (Thermo Fisher Scientific, USA). Qualified DNA was sequenced on the Illumina HiSeq Xten platform (Illumina, USA) with PE150 using standard library preparation protocols and an insert size of 350 bp. Raw data from these individuals was processed by removing low-quality bases with Phred-quality scores < 20 and adapter sequences. The sequencing and filtering were performed by default pipelines by the Beijing Genomics Institute company. Clean data was aligned to the draft genome assembly of red deer *Cervus elaphus* (GCA_910594005.1) using the Burrows-Wheeler Aligner (v0.5.17) with default parameters (Li & Durbin, 2009). Sequence depth and coverage were obtained using Bamdst (https://github.com/shiquan/bamdst). After PCR duplicate removal using Picard v1.91 (Broad Institute, 2019), genetic variants as single nucleotide polymorphisms (SNPs) were called using GATK v4.0.2 (McKenna *et al.*, 2010). We filtered the low-quality SNPs with sequencing depths lower than three, base-missing rates higher than 10% and minimum quality values lower than 20 using VCFtools v0.1.14 (Danecek *et al.*, 2011) and removed the sites on the sex chromosomes. We also removed the SNPs that deviated significantly from the Hardy-Weinberg expectation ($p<0.001$). As spurious clustering may generate during the process of population structure analysis due to the background linkage disequilibrium (LD), we filtered the LD-based SNPs (--indep-pairwise 50 10 0.2) using Plink v1.91 (Purcell *et al.*, 2007) to calculate kinship coefficients.

Genetic diversity analysis

To evaluate levels of genetic diversity, we estimated the genome-wide heterozygosity for all sequenced individuals using filtering SNPs without LD pruning. Genome-wide heterozygosity is calculated as the total number of heterozygotes divided by genome effective length. The number of heterozygotes and the nucleotide diversity (π) were calculated using VCFtools v0.1.14 (Danecek *et al.*, 2011) based on a sliding window approach (window size: 50 kb). We also estimated the level of inbreeding

through inbreeding coefficients (F_{ROH}). First, the runs of homozygosity (ROH) segments were identified using Plink v1.91 (Purcell *et al.*, 2007) with adjusted parameters (--homozyg-window-snp 50 --homozyg-snp 50 --homozyg-window-missing 3 --homozyg-kb 100 --homozyg-density 1000) based on the filtering SNPs without LD pruning (Meyermans *et al.*, 2020). Following this, F_{ROH} was computed via the ratio of the total length of ROH to genome effective length in the individual's genome (Mcquillan *et al.*, 2008). The genomic effective length of each individual was defined as the reference genome length multiplied by the coverage of genome alignment.

We calculated pairwise kinship values among all re-sequenced individuals using KING v2.1.3 (Manichaikul *et al.*, 2010) based on the unlinked SNPs. This method estimates the kinship coefficient by accurately calculating the genetic distance between a pair of individuals as a function of their allele frequencies. Based on Manichaikul *et al.* (2010), a pair of individuals (a dyad) possess a full-sibling or parent-offspring relationship (first degree relative) when their kinship coefficient values range between 0.177 and 0.354 and to a half-sibling relationship (second degree relative) if these values fall between 0.088 and 0.177. A negative kinship coefficient value or a value lower than 0.044 means individuals are not closely related genetically.

Demographic analysis

Manual records were kept at the centre during its early years and tabulated in Excel from 2009 onwards. The records for 2009–2022 analysed in this paper include two sets of monthly stock lists (one of which is organized by enclosure and the other by species) and a report of all arrivals to and departures from the centre each month. The latter includes new arrivals of rescued animals, animals that are born or die and animals that are released from the centre. As hundreds of rescued animal of dozens of species move through the centre each month, records are organized in terms of gross numbers of species and not by individual animals.

In analysis, annual numbers of births, deaths and releases of captive Eld's deer were compiled based on data from the monthly reports on arrivals and departures and checked against both stock lists. All fatalities of captive animals in the same month as a birth were considered to be of fawns unless noted otherwise in the reports. The percentage of neonatal death was calculated each year and averaged between January 2009 and April 2022. The relative birth rate was calculated by dividing the total number of recorded births in a year by the total number of captive animals recorded in the December stock lists of that year, except for 2022 which was calculated from April. As inconsistencies between the stock lists and reports on arrivals and departures suggested births and deaths were under-reported, a minimum count per year was used.

No systematic census has been undertaken as yet of captive-bred Eld's deer released into the forest surrounding the PTWRC and offspring subsequently born. In some cases however, species stocklists included notes on new births of semi-wild fawns within the released groups that returned for supplementary food.

Results

Genetic diversity

Among the ten deer sampled, samples from two individuals (05A & 09A) yielded a low volume of highly degraded DNA and so were not used to construct a whole genome library (Table 1). The eight remaining individuals were successfully sequenced. We obtained an average number of 51,171,950 mapped reads and an average mapping rate of 97.37%. This gave an average sequencing depth of 2.5×. We obtained 48,505 autosomal SNPs and 3,086 unlinked autosomal SNPs after filtering for further analysis.

We obtained an average heterozygosity site of 15,955 ± 5,343 (mean ± SD, the same as below), accounting for a heterozygosity of $5.538 \times 10^{-6} \pm 1.854 \times 10^{-6}$ (Table 1). The mean nucleotide diversity (π) of the sample individuals was 5.475×10^{-5}. Values of F_{ROH} ranged from 0.00013 to 0.177, with a mean F_{ROH} of 0.026 ± 0.060.

Genetic relatedness

From the eight individuals sequenced, we obtained a matrix of pairwise kinship coefficients among 28 dyads with a minimum allele frequency of ≥0.05. The number of SNPs for each pair was 2274. The largest value of kinship was 0.133 and the lowest value was -0.066, and most dyads were second degree relative pairs (Table 2).

Captive management

Five subgroups of Eld's deer were released between 2018 and 2021. As of August 2022, herds observed appear to be adapting well, with no known deaths and at least five births recorded in the forest in December 2020 and December 2021. Wild births and deaths are under-recorded, as observations occur *ad hoc* when the herds

Table 1 Summary of blood samples, DNA concentration, sequencing coverage and depth, homozygotes (HOZ), heterozygotes (HEZ) and heterozygosity rate (HR) of Eld's deer *Rucervus eldii siamensis* studied at the Phnom Tamao Wildlife Rescue Centre, Takeo, Cambodia.

Sample Codes	♀ / ♂	DNA Concentration (ng/µL)	Total Mass (µg)	No. of Mapped Reads	Mapping Rate	Depth	Cover (%)	HOZ	HEZ	HR (×10⁻⁶)	Inbreeding Coefficient (F$_{ROH}$)
01A	♀	29.4	0.735	41,409,279	0.9759	2.5291	99.83	28936	17459	6.059	0.00296
02A	♂	19.5	0.4875	37,006,729	0.9709	2.2981	99.82	28855	17540	6.087	0.00323
03A	♂	18.7	0.4675	9,160,690	0.9741	2.5179	99.70	43447	2948	1.023	0.17516
04A	♂	23	0.575	40,677,926	0.9740	2.5266	99.83	26913	19482	6.761	0.00230
05A	♀	0.8	0.0184	/	/	/	/	/	/	/	/
06A	♂	16.2	0.405	40,191,601	0.9724	2.5178	99.82	27915	18480	6.414	0.177
07A	♀	34.8	0.87	40,192,742	0.9727	2.5203	99.79	28300	18095	6.282	0.00782
08A	♀	23.2	0.58	40,717,672	0.9765	2.5354	99.83	28863	17532	6.084	0.00806
09A	♀	0.7	0.0161	/	/	/	/	/	/	/	/
10A	♂	51	1.275	37,677474	0.9731	2.5351	99.77	30289	16106	5.592	0.00013

Table 2 Relative kindship values calculated between pairs of Eld's deer *Rucervus eldii siamensis* sampled at the Phnom Tamao Wildlife Rescue Centre, Takeo, Cambodia.
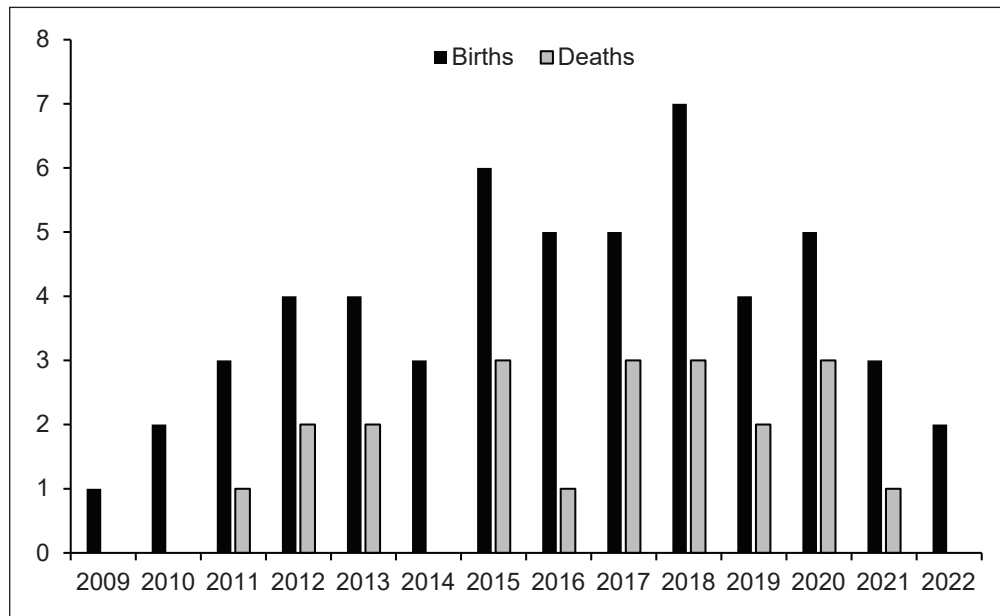
ID1	ID2	Kinship	ID1	ID2	Kinship
06A	10A	0.0271	03A	06A	0.1653
01A	03A	-0.3521	03A	08A	0.1777
04A	08A	0.1234	01A	07A	0.1148
08A	10A	-0.0281	03A	10A	0.2304
01A	02A	0.0832	03A	04A	-0.3026
01A	08A	0.0985	02A	03A	-0.4122
06A	08A	0.1062	07A	10A	0.0167
04A	07A	0.1273	03A	07A	-0.3662
04A	10A	0.0230	06A	07A	0.1019
07A	08A	0.1058	02A	04A	0.1023
01A	10A	-0.0192	02A	08A	0.0697
01A	06A	0.0910	02A	10A	-0.0375
01A	04A	0.1023	02A	07A	0.0811
04A	06A	0.1332	02A	06A	0.0781

come for supplementary food. The only intervention in the semi-wild herd to date occurred in January 2022, when a juvenile with a broken leg was recaptured for treatment and released back into the forest in April 2022. The average percentage of neonatal death in captivity was 26.85% (SD ± 21.58) (Table 3, Fig. 1). The herd has grown from two unrelated individuals (which arrived at the centre in 2001) to 15 captive animals and 19 released into the forest, giving a minimum of 36 deer (Table 3, Fig. 2). Based on our observations however, we estimate the semi-wild herds have actually grown to between 30 and 40 individuals.

Table 3 Demographics of captive Eld's deer *Rucervus eldii siamensis* from January 2009 to April 2022 at the Phnom Tamao Wildlife Rescue Centre, Takeo, Cambodia.

Year	No. of Births	Relative Birth Rate (%)	No. of Fawn Deaths	Neonatal Death (%)	No. of Deaths	No. of Releases	No. of Animals in Captivity[1]
2009	1	16.7	0	0	0	0	6
2010	2	28.6	0	0	0	0	7
2011	3	33.3	1	33.3	1	0	9
2012	4	36.4	2	50.0	2	0	11
2013	4	33.3	2	50.0	2	0	12
2014	3	17.7	0	0	0	0	17
2015	6	27.3	3	50.0	3	0	22
2016	5	20.0	1	20.0	1	0	25
2017	5	18.5	2	40.0	3	0	27
2018	7	26.9	1	14.3	3	3	26
2019	4	14.8	1	25.0	2	0	27
2020	5	27.8	3	60.0	3	10	18
2021	3	18.8	1	33.3	1	6	16
2022	2	13.3	0	0	0	0	15

Fig. 1 Births and deaths of captive Eld's deer *Rucervus eldii siamensis* from January 2009 to April 2022 at the Phnom Tamao Wildlife Rescue Centre, Takeo, Cambodia.
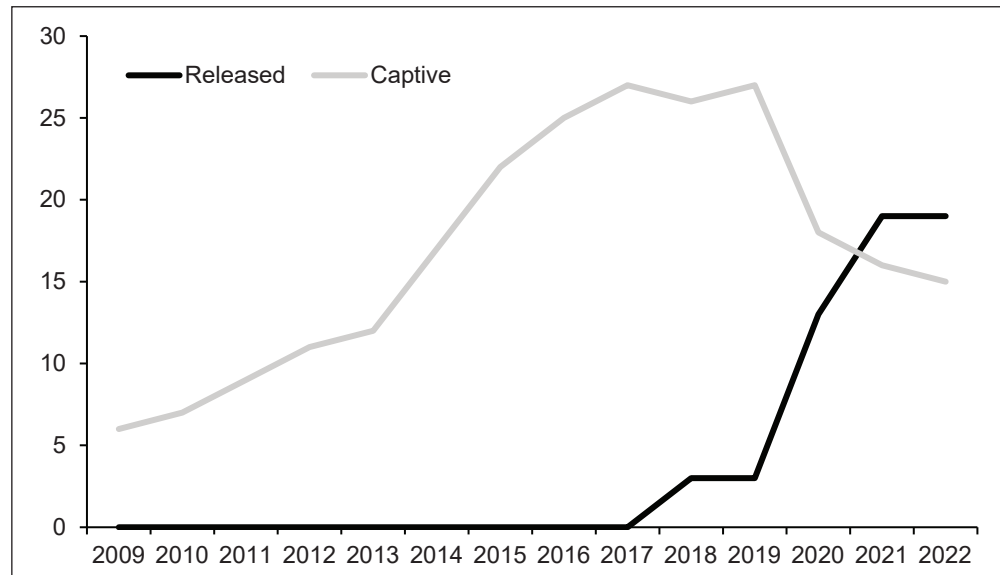
Fig. 2 Total number of Eld's deer *Rucervus eldii siamensis* in captivity and released into the surrounding forest from January 2009 to April 2022 at the Phnom Tamao Wildlife Rescue Centre, Takeo, Cambodia.

Discussion

As of August 2022, the captive herds in the PTWRC and semi-wild herds released into the surrounding forests appear to form a healthy and viable breeding population. These may represent the only increasing subpopulation of Eld's deer in Cambodia (Ladd *et al.*, 2022). As the PTWRC population originated from just two founders and most dyads in our analysis were second degree relatives, there is a risk of inbreeding depression. Our genetic analysis of eight individuals showed levels of inbreeding were not particularly high (F_{ROH} 0.026). This result is encouraging, although it may not be representative of the entire population. Sample collection from more individuals within the herds is recommended to increase the accuracy of the results. Future testing of other *siamensis* populations (captive or wild) for comparative purposes would inform future management of the Phnom Tamao population.

An extremely low level of genetic diversity i.e., heterozygosity and nucleotide diversity, was observed during the study. The value of genome-wide heterozygosity (5.538×10^{-6}) was lower than most Endangered species, such as crested ibis *Nipponia nippon* (430×10^{-6}) (Li *et al.*, 2014), mountain gorilla *Gorilla beringei* (640×10^{-6}) (Xue *et al.*, 2015) and island fox *Urocyon littoralis* ($1.33 \pm 30 \times 10^{-6}$) (Robinson *et al.*, 2018). As genetic diversity determines the adaptive potential of a species to environmental change, it plays a key role in their long-term survival (Booy *et al.*, 2000; Supple & Shapiro, 2018). While

strong deleterious mutations can be removed through genetic purifying to mitigate inbreeding depression in small populations, moderate deleterious mutations can still accumulate during prolonged bottlenecks (Xie *et al.*, 2022), reducing population viability. As such, estimation of deleterious mutations using high-quality sequencing data should be undertaken to illuminate the effects of inbreeding and subsequent extinction risk to the study population.

High neonatal deaths and decreased fecundity in adults of various species, including Eld's deer, have long been attributed to inbreeding depression (Prescott, 1897; Ralls *et al.*, 1979; Thévenon & Couvet, 2002). The continued breeding of the free-roaming and captive deer with relatively high infant survivorship suggests the PWTRC population does not suffer severe negative effects of inbreeding. Since 2009, the average percentage of neonatal deaths among the captive herds has been 26.85%, although this figure must be taken as a minimum count as records are incomplete. The survivorship of infants in the semi-wild herds is unknown although the herds continue to grow. Although the forests surrounding the centre contain predators such as jackals *Canis aureus*, the semi-wild deer herds are not subject to the same level of predation as wild herds, so infant survivorship could be higher compared to these (Linnell *et al.*, 1995; Jarnemo, 2004). However, Dion *et al.* (2020) found similar rates of neonatal mortality in populations of white-tailed deer *Odocoileus virginianus* in areas with and without natural predators. Nevertheless, levels of infant mortality in the

captive population at Phnom Tamao are still substantially smaller than the mortality rate of up to 90% recorded for captive Eld's deer in France, where only 21% of offspring made it to 18 months (Prescott, 1987). High neonatal mortality and reduced fecundity in captive Eld's in Thailand has also long been a concern, although the exact rates are not available for comparison (Siriaroonrat, 2003). The survival of fawns and relative stability of the birth rates at PTWRC over the 12 years analysed could be partly due to allowing the deer to select their mates, which is recommended as a way of increasing reproductive output (Asa *et al.*, 2011; Martin-Wintle *et al.*, 2018). Although free mate selection can increase offspring survival, there is a risk of decreased genetic diversity as an uneven distribution of individuals will breed (Haig *et al.*, 1990; Gooley *et al.*, 2018). However, several studies of captive and reintroduced populations of ungulates with inbreeding coefficients above 0.2 have found low genetic variability did not affect herd demographics, breeding rates or neonate mortality (Kalinowski & Hedrick, 2001; Sternicki *et al.*, 2003; Zeng *et al.*, 2013; Moreno *et al.*, 2020). As such, the benefits of allowing free mate choice among animals kept in their natural social configurations may outweigh the risks of reduced genetic diversity.

To safeguard against genetic erosion within the captive herd, it would be beneficial to introduce new and unrelated individuals of pure *siamensis* from wild populations that continue to decline. While it would be preferable to protect habitats of threatened species and ensure connectivity between fragmented populations, should the wild population decline dramatically, it may not be able to recover naturally without intervention (Phumanee *et al.*, 2020). As with all subspecies of Eld's deer, the population of *siamensis* is fragmented in the wild and therefore risks losing genetic variability, leaving it more susceptible to environmental stochasticity (Song, 1996; Thévenon & Couvet, 2002; Angom & Hussain, 2013). Should a species breed faster in captivity, Tenhumberg *et al.* (2004) recommended capturing an entire wild population containing less than 20 females. However, it would be more realistic to exchange a few stags between the fragmented wild and captive populations, provided adequate protection can be ensured for the former. Depending on the relative genetics of these, this could support genetic rescue in both (Theodorou & Couvet, 2004). This has been undertaken successfully with captive Mexican wolves *Canis lupus baileyi*, whereby one population descended from three founders was mixed with unrelated individuals from two separate lineages each originating from two founders, increasing genetic diversity among the three populations (Hedrick & Fredrickson, 2008). The addition of new lineages to the captive populations of Eld's Deer at the PTWRC should

begin as soon as possible to safeguard their genetic diversity until such time as reintroduction becomes a responsible option. Many captive-breeding initiatives begin too late to acquire sufficient founders to ensure a genetically diverse and sustainable population, thus risking the success of the overall programme (Ahmad Zafir *et al.*, 2011; Ralls & Ballou, 2013).

Captive-breeding programmes must consider that captive environments can select for adaptations that are inappropriate for survival in the wild (Bremner-Harrison *et al.*, 2004; McPhee, 2004; Frankham, 2005). Continued release of Eld's deer in the forests of Phnom Tamao may mitigate the effect of captivity on the genome, allowing herds to live and breed within a safe setting. Exposure to a wild environment has been shown to increase the survival of offspring of released animals (Evans *et al.*, 2014). This has also been achieved through enclosures that mimic wild habitats (Beck *et al.*, 1991; Frankham, 2008). The Eld's deer enclosures at the centre are located in the on-display section and have lost much of their vegetation due to over-grazing. However, fawns are still born despite the lack of undergrowth, and once deer were moved into the remote forest enclosure, they became wary of humans (Marx, N. pers. obs.). Some of the deer released from the centre display tolerance for people, but continue to forage naturally. The survival of released individuals and their offspring could be partly due to the minimal occurrence of natural predators and hunting in the forest. Should release become an option in future, only animals expressing predator avoidance and sufficient fear of humans should be selected. Further, candidates for release should be monitored to ensure they exhibit appropriate behaviours in acclimatization enclosures prior to release (IUCN/SCC, 2013).

There are many complimentary actions that can be taken to conserve wild populations of *R. e. siamensis*. The herds at PWTRC should not be overlooked as a potential source of animals that could be used in future to re-establish the subspecies within its historical range. Successful programmes exist that originated from a few founders, such as one for black-footed ferrets *Mustela nigripes* that was based on ten individuals, though only five were successfully breeding (Ralls & Ballou, 2013). Further, Moreno *et al.* (2020) found that rates of births and infant survival were similar between reintroduced and captive groups of Cuvier's gazelles *Gazella cuvieri* with high inbreeding coefficients, despite the released animals experiencing greater stress in the wild. As such, we believe it would be incorrect to dismiss the Eld's deer herds at the PWTRC as a source for future reintroduction efforts due to the small number of animals the population descended from.

The forests surrounding Phnom Tamao are an undervalued resource for conserving certain species. These include sambars, which were also bred in captivity and released at the centre. Although these are currently listed as Vulnerable, captive populations will be increasingly important to survival of the species as the wild populations continue to decline throughout their range (Gray *et al.*, 2012; Timmins *et al.*, 2015). The forests of Phnom Tamao are now a breeding ground for this and other species, some of which could be translocated to other forests once these sites are known to be safe. If properly managed following IUCN guidelines, with animals translocated to carefully selected sites, sambar or Eld's deer from PTWRC may be used to repopulate other forests.

Given that forests and wildlife in Cambodia are declining throughout the country, the PTWRC and other captive populations could ensure the survival of some species. Successful captive-breeding and monitored release efforts for other species, such as the reintroduction of captive-born pileated gibbons *Hylobates pileatus* to the Angkor Archaeological Park (Leroux *et al.*, 2019) and release of binturongs *Arctictis binturong* into Tatai Wildlife Sanctuary (Marx & Roth, 2014), demonstrate that this approach works. Despite a small sample size, our study suggests that herds of Eld's deer at Phnom Tamao have been managed in a way that will allow similar releases in future provided appropriate areas of safe habitat can be found. Provided the centre is professionally managed and the surrounding forests are protected, Phnom Tamao can contribute greatly to conservation in Cambodia by rehabilitating, captive-breeding and releasing rescued animals to bolster dwindling populations in the wild.

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