Elephant reproduction: Improvement of breeding efficiency and development of a breeding strategy

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Introduction

The efficiency of reproduction of the Asian elephant (Elephas maximus) in captivity has become of major concern over the past decades. The low birth rate and high mortality cause the captive population to decline rapidly [c.f. 39]. Knowledge on breeding elephants is lacking, however, and research in this area would be of high significance to alleviate this problem. The study of reproduction in elephants is restricted, due to ethical considerations and hampered by the large body size, the length of the reproductive cycle, and concerns of safety when handling animals. Hence, the number of papers published on reproduction in elephants is much lower than for other exotic species, e.g. deer, non-domestic felids and canids.

Self-sustaining populations in tourist elephant camps, timber elephant camps, circus or zoos [5] [13] would be essential to prevent a drain of wild populations and to reduce illegal wild capture [36]. Unfortunately, in many zoos and elephant facilities bulls are not available or not allowed to breed. Moreover, some proven bulls are used to father many calves in their area, or even across the country, by natural or artificial breeding. In addition, female elephants have a relatively short reproductive life span, i.e. approximately 15 years after the onset of ovarian cyclicity. When animals do not get pregnant, the incidence of reproductive pathologies does increase [19] [c.f. 39]. Captive breeding programs worldwide have met with limited success and few ex situ elephant populations are self-sustaining. Therefore, our understanding of elephant reproductive biology needs to be improved. For instance, a reliable and practical estrus detection method would enable to breed a cow at the right time and in that way improve the reproductive success. In addition, for conservation on the long term, genetic monitoring and management should be considered. This applies also to small wild populations. The current status of the Asian elephant breeding management, the female reproductive physiology and mating behavior are described in more detail. Subsequently, genetic management and the contribution of a molecular-genetic approach to improve elephant reproduction are discussed.

Status of Asian elephants

Range countries

The number of Asian elephants in the world decreases at an alarming rate. The wild population with an estimated size of 30,000 - 50,000 animals (2006) in 13 countries of South and Southeast Asia has declined over the last decades [c.f. 39], primarily due to habitat destruction. These estimates were based on unreliable or nonstandard methods [3]. The size of the captive elephant populations has been estimated to be about 16,000 in 11 Asian countries in 1995 and has declined also in parallel with the wild species [c.f. 39]. For instance, in Myanmar, the largest captive elephant population (~6,000 individuals) is not self-sustainable, and may become extinct in the long run [c.f. 39]. Hence, Asian elephants have been in the appendix 1 category of the Convention for International Trade in Endangered Species of Wild Fauna and Flora (CITES) since 1973 (http://www.cites.org/), and in the endangered species Red list of the International Union of Conservation for Nature (IUCN) since 1986 (Asian Elephant Specialist Group, 1996) (http://www.iucn.org/). It is, therefore, crucial to support breeding of Asian elephants in captivity and thereby maintain or increase both the population size and the genetic diversity.

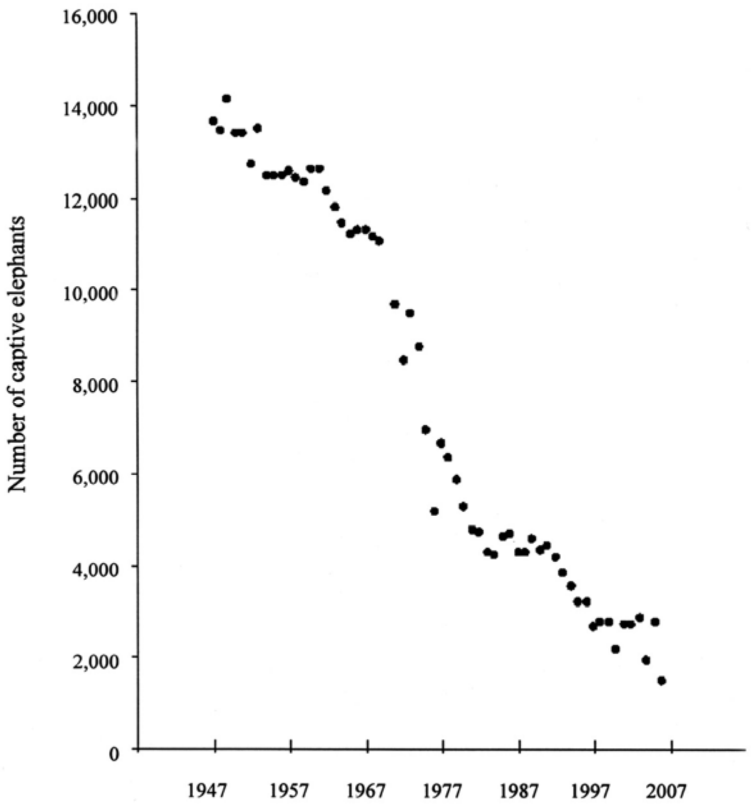

Figure 1. Decline of the captive elephant population in Thailand (after Wildlife conservation section, National Park Wildlife and Plant Conservation Department, 2003).

Thailand

The Asian elephant has been a symbol of Thailand since ancient times, and is considered part of the national identity. Historically, they have been used extensively during wars, in the forestry industry, and as an animal of burden. Since the government closed down commercial forestry in Thailand in 1989, many of these elephants and their handlers have found employment in the tourist industry, performing shows and giving rides at commercial camps instigated to attract local and international tourists. During the early twentieth century, there were approximately 100,000 captive elephants in Thailand. By 1965 there were only 11,000 captive elephants, a number that had declined to 3,500 in 2007 [c.f. 39]. The number of wild elephants also decreased and is now estimated to be 2,000. The demographic changes of the captive Thai elephant population are depicted in Figure 1. At the current rate of decline, the elephants in Thailand may reach critically low numbers in only a few decades and then will not be self-sustainable as a population.

Western zoos

There are approximately 350-400 elephants in zoos in the USA, and 500 elephants in Europe, a number that declines gradually [c.f. 39]. The captive elephant populations in the USA have not been self-sustaining over the last 50 years, and are predicted to be unviable [c.f. 39], unless the fecundity increases significantly. The decline of the populations is caused by e.g. a too low number of breeding bulls, separation of males and females due to the aggression of the bull, female reproductive senescence, a low birth rate and a high infant and adult mortality [20] [c.f. 39].

Breeding management of Asian elephants

Range countries

Procedures for breeding captive Asian elephants differ across range countries and are influenced by e.g. work obligations and religion. In Northern and Southern India, elephants that belong to Hindu institutions may be prohibited to breed [24]. Traditionally, Asian elephant cows were bred to wild bulls [36] [c.f. 39]. In Myanmar timber elephants, mating of captive bulls and cows resulted in an average birth rate of 7.1% per year per mature female during 1991-1995 [24] [38]. Similarly, free contacts during the grazing period and bathing time in the Sri Lankan elephant facilities [c.f. 39] resulted in a yearly birth rate of 20% [38]. In Southern India forest camps, captive elephant groups consisting of adult cows, calves and bulls of various ages, were allowed to forage in the forest at night, so cows were bred with either captive or wild bulls [37]. This resulted in the birth rate of 13% [38]. In wild elephants in South India the birth rate is 21% or ~1 calf born per mature cow every 5 years [c.f. 39]. So it appears that the major limiting factor in captive breeding is the frequency of male-female contact in a favorable environment that supports estrus detection and mating.

Thailand

Significant changes in elephant husbandry have occurred in Thailand due to the shift in elephant use from commercial forestry to tourism. This has had an impact on many aspects of elephant management, most particular, on the management of elephant reproduction. In the past, after a 9-month period of work, both male and female timber elephants had a resting period from March to May, the so-called “Pang Ram”. Then foraging in the forest allowed sexual contacts and the production of calves. However, since the logging industry was banned, this breeding technique is not practiced anymore and elephants are now working the year around, particularly in the tourist industry. Managers thus also do avoid the risk of females being injured by unrestrained males, which can occur in the forest. In addition to the logistical concerns related to tourist camp operations, male elephants with long tusks must be kept under close observation to prevent illegal cutting and theft.

Currently, one of the breeding strategies is that a single female elephant, considered to be in estrus on the basis of subjective observations, is coupled with the breeding bull for a period of several hours. If the bull shows interest and tries to copulate, this is allowed. When there is no interest, the animals are brought back to work. Another breeding method, applied in some camps, uses the flehmen response of male elephants as they smell urine from female elephants to determine if the female is in estrus. The implementation of this approach, commonly referred to as the “urine test”, varies among camps. One breeding strategy is to place 6 to 8 female elephants in a row every morning and let 2 or 3 bulls walk behind the females to “test” their urine. If a female evokes interest by more than one bull based on flehmen or other courtship responses (e.g. penis protrusion, erection, mounting attempts), she is presumed to be in estrus, and will be brought in the forest or breeding ground to stay with a breeding bull during the daytime, where they are observed. Mahouts have reported copulations up to 13 times during a mating period, which typically lasts several days but sometimes weeks. If mating is not observed within a couple of days or the bull becomes too aggressive during breeding attempts, breeding will be terminated and the female will be returned to routine activities.

In several tourist elephant camps, mahouts and owners often feel that breeding their elephants will decrease their income when they trade working or earning time for mating, pregnancy, parturition rest and care for the newborn. Furthermore, the cow owner has to pay the bull owner for mating without the guarantee of pregnancy [c.f. 39]. Unlike the elephant facilities in Myanmar (Myanmar Timber Enterprise) and Sri Lanka (Pinnawala Elephant Orphanage, government), most elephants in India and Thailand belong to private owners which makes breeding of elephants dependent on commercial considerations. However, at least two large elephant camps in Chiang Mai and Ayutthaya (Thai provinces) have instituted a breeding program and dedicated a few bulls to mating which resulted in a satisfactory calving rate.

Western zoos

Captive breeding has now become most urgent for western zoos, as import of animals from range countries has become increasingly difficult since the 80’s and sustaining the populations fully depends on animals born in captivity [c.f. 39]. The major problems with regard to breeding are on the one hand, a failure of conception and on the other hand, a high calf mortality by stillbirth and maternal infanticide [38]. One of the plausible causes of the low birth rate in zoos is the problem of handling male aggressiveness, particularly during the musth period. Therefore, zoos tend to keep only a few breeding bulls or none at all. Social ranking also may cause poor quality semen and suppress libido [c.f. 39]. Furthermore, nulliparous females older than 30 years show a relatively high incidence of reproductive pathologies [19], and in cows over 35 years parturition disorders become more frequent [20]. In addition, aged cows may exhibit irregular cycling or no cycling at all [8] [c.f. 39]. These factors have a high impact on the fecundity rate and sustainability of the population of Asian elephants in western countries. Presently, artificial insemination (AI) is a successful breeding method in elephants [7] [c.f. 39]. Shipment of semen can help to minimize the bull-handling problems in zoos with less contact between both sexes and help to sustain the population in a long term.

Female reproduction

Ovarian cycle

The normal estrous cycle of the female Asian elephant is between 14 and 18 weeks in length with pregnancy lasting 20-22 months. The non-pregnant luteal phase, characterized by high circulating progestagen concentrations, ranges between 10-14 weeks, while the interluteal phase (or follicular phase) lasts between 3 and 6 weeks. During the follicular phases, 2 surges (peaks) of luteinizing hormone (LH) occur. The first LH surge is anovulatory (anLH), whereas ovulation occurs three weeks later around 24 hours after the second LH (ovLH) surge [6] [c.f. 39]. A scheme of an ovarian cycle in the elephant is shown in Figure 2. A female only has three chances per year to conceive. Within each cycle, the fertile period can be considered to be from 2 days before, until shortly after the ovulation. Therefore, identification of this brief period is most critical to ensure that males breed females at the proper time.

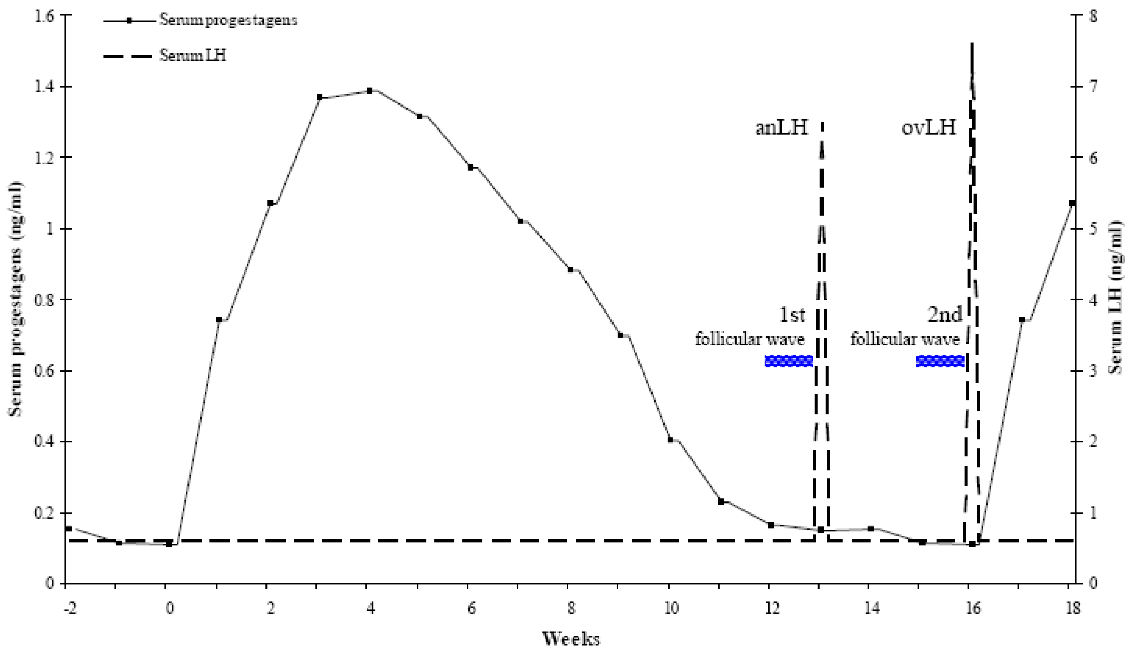

Figure 2. Estrous cycle of the elephant. Elevated progestagens indicate the luteal phase. When progestagens return to baseline, the follicular phase begins. Two follicular waves occur with high estradiol concentrations associated with non-ovulated multiple follicles prior to the anLH surge and, 3 weeks later, one Graafian follicle prior to the ovLH surge are observed. Ovulation occurs approximately 24 hours after the ovLH surge, with a subsequent increase of progestagen concentration due to the steroidogenic activity of the corpus luteum.

Several methods have been developed to characterize the estrous cycle in Asian elephants; for instance, by observation of reproductive behaviors or the monitoring of serum and urinary estrogen and progesterone or their derivatives. A list of studies on the estrous cycle in both Asian and African (Loxodonta Africana) elephants is published [39]. Most studies were performed in Western zoos where climate, nutritional status and management are different from those in the natural habitat, while only few were done in range countries. These factors might influence the estrous cycle. Furthermore, hormonal monitoring generally involves radioimmunoassays and only rarely enzyme immunoassays are used. From a logistic standpoint, it is important to eliminate potential problems associated with waste disposal and environmental contamination by radiolabelled assay reagents. Most of the reproductive cycle studies in elephants include estimations of progestagens, the major one being 5α-pregnane-3,20-dione (5α-DHP) [5] [21]. Progestagen profiles were used to determine the onset of the luteal phase, which approximately indicates the ovulation period [c.f. 39], as progestagens do increase 2-3 days before the ovLH surge, and ovulation will occur 24 hours after the ovLH

[7] [25]. Progestagen profiles could also be used for pregnancy diagnosis characterized by continuous elevated concentrations of progestagens over a 3-5 months period at twice higher concentrations than during the normal luteal phase of the ovarian cycle [5] [21]. Thus, it is important to identify appropriate hormone assay procedures to evaluate luteal function during the estrous cycle and pregnancy in elephants.

Double LH surge

Two follicular waves have been identified during the follicular phase just before each of the LH surges by ovarian ultrasonography [c.f. 39] and by characteristic profiles of urinary estradiol [10]. Although the changes in reproductive hormones during the cycle have been described, the unique double LH surge mechanism is still not understood. Particularly the function of the anLH surge is puzzling, while the ovLH surge is known to induce ovulation [20]. This double LH surge has not been observed in any other mammalian species. LH surges in elephants have been studied for several decades [6] [c.f. 39], which gave more insight in this unique phenomenon of reproductive physiology and ovulation in the proboscis species. Later, a few studies focused on the association of LH surges with reproductive behaviors [27] and vocal communication [c.f. 39]. The knowledge of the double LH surge and ovulation has been used in the assisted reproduction by checking for the anLH surge. Then 18-22 days later semen was deposited in the genital tract to fertilize the oocyte [7] [c.f. 39]. Thus, monitoring endocrine changes is necessary for determining ovulation time [21].

Environmental influence

In many species, environmental variation i.e. availability of nutrients and length of photoperiod do influence reproductive cyclicity and behaviors e.g in sheep, goats, horses, deer and elk [c.f. 39]. In general, mammalian species give birth and rear their offspring during the time when nutritional resources are randomly available to be able to cope with the high energy requirement during lactation [c.f. 39]. In elephants, few studies on the influence of seasonal and environmental factors on reproduction have been reported, and then mainly related to African species. It was demonstrated that photoperiod did not influence the hormonal profiles in that species under zoo conditions in countries with a temperate climate [2]. Also, a seasonal effect on fetal sex ratio in the savannah subspecies was not observed [c.f. 39]. However, fecal progesterone metabolite concentrations were lower during the dry season than during the wet season in wild African elephants, while fecal cortisol metabolites were higher during the dry season than during the wet season [c.f. 39]. Furthermore, reproductive phenology, a study of periodical events affected by environment, in a wild elephant population indicated that conception and fecundity were positively influenced by season, in a such a way that parturition coincides with the period of most abundant vegetation [c.f. 39]. Thus, the study of the influence of seasonal and ecological factors on elephant reproduction both in captivity as well as in their natural habitat could improve the understanding of the reproductive physiology and lead to improvements in breeding and environmental management.

Estrous behavior

Unlike other domestic mammals, the elephant cow does not show clear and distinct morphological genital changes or obvious behavioral changes characteristic for estrus. For instance, cattle show vulva swelling, clear mucous discharge, and mounting or standing behavior, while mares show tail deflection and clitoris exposure by labial eversion. Bitches show vulva swelling and tail deflection and cats show head rubbing and rolling, and vulva presentation [c.f. 39]. The fact that estrous behavior in the female elephant is not very obvious may be due to the long urogenital tract, 3 meters from vestibule to ovary [20] [c.f. 39], and the location of the vulva, which is at the ventral part of the body between two hind legs, while only a small amount of discharge is expelled. However, some specific behaviors, like increased clitoris-directed, under-body tail flicking have occasionally been observed in estrous cows [c.f. 39] [41], while it was observed that the tail flicking behavior was significantly higher in the follicular phase than in the luteal phase [c.f. 39]. Furthermore, low frequency vocalization was higher in the anovulatory follicular phase than in other phases of the ovarian cycle, to signal a distant bull to come and be available for mating some weeks later [c.f. 39].

Pheromones

Pheromones, intraspecific interaction substances, are used by females to attract males in many species [29] [c.f. 39]. In elephants, pheromones are secreted in the urine during the estrous period, which can be interpreted as a specific communication signal [c.f. 39]. The major one [(Z)-7-dodecynyl acetate (Z7-12:Ac)] is present in urine at the end of the luteal phase and gradually increases during the long follicular phase to peak in concentration just prior to ovulation [29] [c.f. 39]. Higher frequencies of the flehmen responses, penis erections and premating behaviors from male Asian elephant are observed when this pheromone is excreted [28]. Thus, the presence of pheromone at this specific period facilitates efficient reproduction in this large pachyderm.

Estrus detection

Several methods for estrus detection in elephants i.e. behavioral observation, endocrine monitoring, and reproductive organ visualization by rectal ultrasonography have been reported [5] [c.f. 39] [41].

Few studies have used a combination of laboratory analyses in conjunction with behavioral observations in elephants. However, this approach has a high potential for improving the practical management of breeding programs. A summary of behavioral studies associated with analysis of reproductive hormones and pheromones is published [39]. In brief, a bull shows interest in an estrous cow by increasing the rate of trunk exploration of the female's urogenital area (Figure 3), or smelling her urine (Figure 4a) and feces followed by placing the trunk tip in the mouth directed to the vomeronasal organ (Figure 4b) to check for pheromones. These behaviors are defined in four steps and described [c.f. 39] (Figure 5). Other pre-mating behaviors as penis erections and mounting also occur [30]. These observations indicate that male elephants can detect the preovulatory pheromone and then by changes in behavior, pinpoint the receptive and ovulatory period [c.f. 39]. Further studies are required to develop techniques that reliably detect estrus in captive elephants [38].

Mating behaviors

In their natural habitat, male elephants which live isolated or in small herds are always searching for female elephants in estrus. Bulls use olfactory detection by elevation of the trunk and pointing the tip in the direction of females. Approaching a selected cow is followed by the gently touch the area of the vulva by the bull, which results in urination. During courtship, the bull touches face, eyes, ears, hind legs and vulva of the cow with the trunk and also raises his head and trunk to reach over her shoulder or flank before trying to mount and copulate. Copulation takes around 30-60 seconds for mounting and 10-15 seconds for intromission [c.f. 39].

Figure 3. Trunk exploration: one of the standard premating behaviors of the bull directed to the female urogenital area to check the reproductive status and for the presence of pheromones.

Figure 4. Flehmen responses: behaviors where the elephant uses the trunk tip to touch urine (a) and later places the tip in the mouth directly to the vomeronasal organ (b).

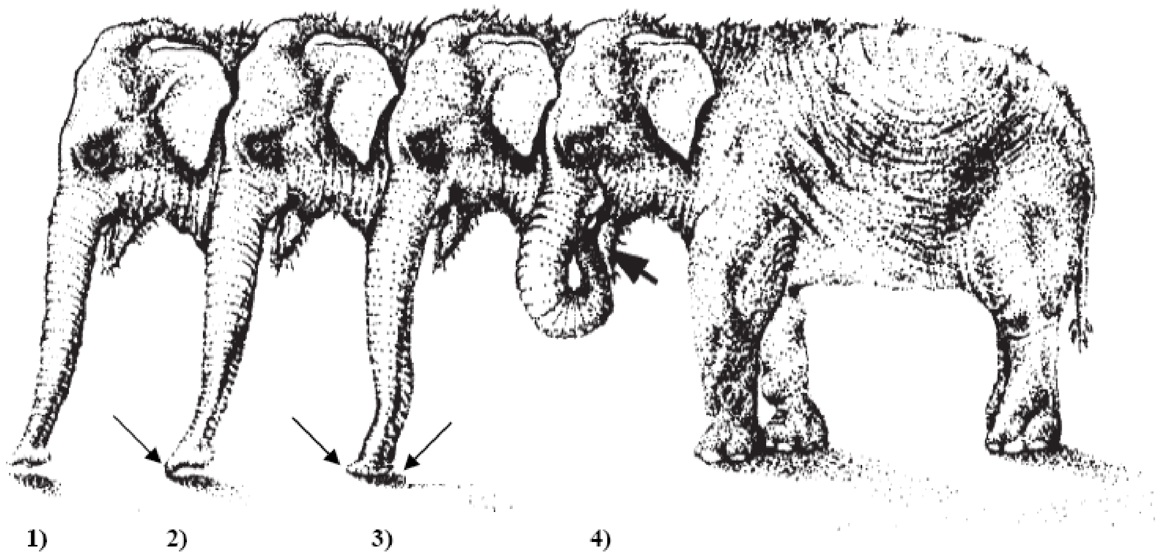

Figure 5. Urine test behaviors displayed by the male using the trunk: 1) Sniff (olfactory): hovering of the trunk over the sample without contact; 2) Check (tactolfactory): touching the sample with the dorsal trunk tip finger; 3) Place (tactolfactory): flattening of the end of the trunk onto the urine sample; 4) Flehmen (vomerolfactory): touching of the trunk tip from the sample to the openings of ducts in the roof of the mouth that lead to the vomeronasal organ (after [39]).

Genetic management

General considerations

In large natural populations, inbreeding and loss of genetic variation does not occur because males are driven away from the natal herd in their juvenile period (Asian, [c.f. 39] and African, [1]). However, the fragmented habitat leads to genetic isolation of small populations. In wild and captive populations, preference of cows for tuskless bulls may further contribute to non-random mating [35] [39]. Captive populations are also vulnerable, because within a camp, only a single or a few bulls are used for breeding without exchange between camps [c.f. 39].

As a consequence, inbreeding and loss of genetic variation occurs both in captivity and in the wild, particularly in small populations [17] [18]. Deleterious alleles, kept at a low level in the wild due to natural selection, can reach a high frequency in captive populations due to non-random mating and small founder numbers [c.f. 39]. The resulting inbreeding depression may decrease reproductive fitness [c.f. 39] and disease resistance. In the effective captive breeding program, genetic management should maintain 90% of the genetic diversity for 100 years [18]. Hence, the genotypic and phenotypic information, phylogenetic and systemic identity of individuals and population should be recorded [c.f. 39]. On the other hand, crossbreeding different subspecies or high distant geographic populations, e.g. mainland versus island, may lead to outbreeding depression by insufficient environmental adaptation of the first generation offspring [18] [c.f. 39]. An additional risk factor is the exposure to virulent pathogens near human settlements because of the presence of domestic animals [c.f. 39]. Also in western zoos, the captive Asian elephant populations have declined because of a lack of breeding bulls and the aging of the population [20] [c.f. 39]. Inbreeding effects can be minimized by breeding non-related animals [26], but pedigree information is often incomplete.

Molecular approaches

Molecular methods can contribute to genetic management by estimation of relevant parameters as effective population size, bottlenecks, sex-specific gene flow and founder contributions and also allow to infer the historical and geographical relationships between groups [c.f. 39]. This creates valuable tools for policy makers [c.f. 39] in order to make decisions on ecological conservation. Several molecular markers have been used in biological conservation e.g. Amplified- Fragment-Length Polymorphism, (AFLP), DNA sequencing, single nucleotide nuclear polymorphism (SNPs) and microsatellites [11]. So far, microsatellites have been the most popular marker of choice in conservation genetics study [32], but eventually high-density SNP screens will be more informative [c.f. 39]. However, this requires information about the presence of allelic variation of singe nucleotide positions in the genome [32], which is not yet available for most endangered species, including Asian elephants. For this reason, microsatellites marker evaluation presently is a preferred method for evaluation.

Microsatellites, also known as short tandem repeats (STRs) or simple sequence repeats (SSRs), consist of a tandem array of repeat units of 2-6 base pairs (bp) (Figure 6) [12] [9] [34]. Microsatellites have a high degree of heterozygosity (up to 90%, [c.f. 39]) and a mutation rate (10-3-10-4 per locus per generation, [c.f. 39]) that is higher than the rate of mitochondrial DNA (mtDNA), and much higher (102-103) than the rate of single copy nuclear DNA [c.f. 39]. The variability of microsatellites is partially understood and is assumed to be caused by replication slippage during lagging strand synthesis. This slippage creates a temporary bulge which during DNA repair will lead to deletion or insertion of repeat units (see [12] [c.f. 39]).

Because their high heterozygosity and mutation rate, microsatellites are the marker of choice for genetic mapping [c.f. 39], and linkage or associated analysis [c.f. 39]. In addition, microsatellites are valuable for parentage and kinship analysis [4] [22], individual identification tests [9] [c.f. 39] and genetic relatedness evaluation [c.f. 39], and forensic sciences for both human and wildlife [c.f. 39]. Furthermore, on the level of populations, microsatellites are now the most popular marker for genetic diversity [c.f. 39]. For instance, microsatellite variation has been used to detect hybridization between closely related species [c.f. 39] population subdivision structure, migration between subpopulations [c.f. 39] and genetic relationships between subpopulations [c.f. 39].

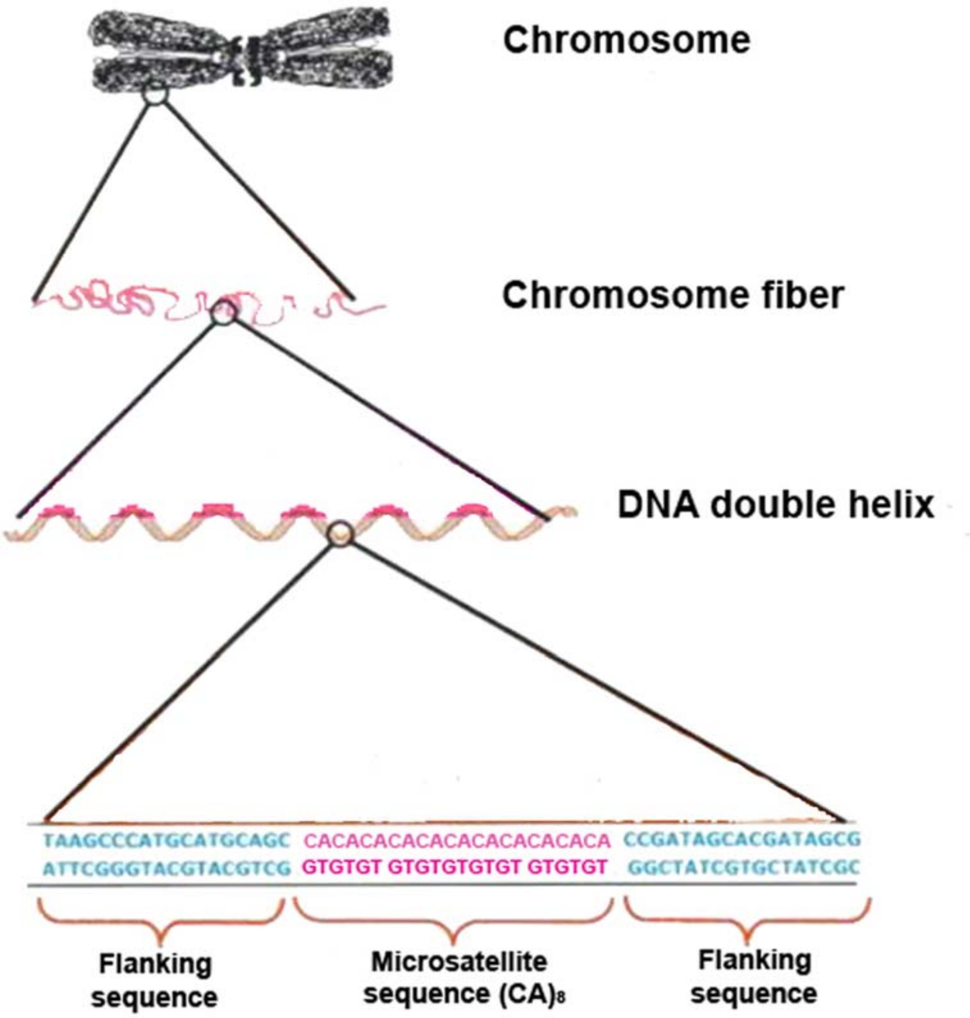

Figure 6. Diagram that shows the microsatellites or variable number of tandem repeats (VNTRs) at a specific locus on the chromosomal nuclear DNA. Di-nucleotide repeats $(CA)_n$ between two flanking regions are widely distributed in eukaryote genomes with high mutation.

Conservation genetics of the Asian elephant

Several reports have described contributions of molecular genetics to the conservation of the Asian elephant. Classical DNA fingerprinting by Southern blotting was used for the estimation of relatedness [c.f. 39] and for testing paternity [c.f. 39] in captive elephants. Behavior in combination with genetic analysis was performed in wild elephants from Sri Lanka and indicated that the social organization coincides with maternal lineage as revealed by mtDNA [14]. Moreover, relatedness analysis by microsatellite loci revealed a close relationships (mother-daughter, full sisters) between adult females, but no significant relatedness between adult cows and sub-adult or adult bulls. This suggested an absence of inter-group transfer of female elephants, while in the natural situation male dispersal takes place [c.f. 39]. These applications are useful for understanding the natural social and reproductive behaviors, which can be utilized in developing the captive breeding and reintroduction programs.

Population genetics studies have been conducted in several elephant populations from different Asian countries or western zoos. Thirty-three polymorphic proteins were used to study genetic variation in zoo elephants originating from Sri Lanka, South India, Thailand and Nepal [c.f. 39]. This revealed a low degree of polymorphism and heterozygosity, and was confirmed by the low genetic variation [c.f. 39] on the basis of 44 blood protein and enzyme coding loci in 26 elephants from German and Swiss zoos. DNA-based studies of Asian elephants have mainly focused on the maternally transmitted mitochondrial DNA. The amplification of mtDNA sequence targets the cytochrome b (Cyt b) gene and a control region. Seventeen mitochondrial haplotypes were divided in 2 clades or haplogroups (α and β), and were found in 118 free-ranging and captive elephant dung samples from Sri Lanka, Bhutan, India, Laos and Vietnam [14]. Cyt b and the control region were also studied in 57 captive elephants with known origin from India, Sri Lankan Nepal, Burmar, Thailand, Malaysia and Indonesia [16], and two major clades were identified, one of which carried by all animals from Indonesia and Malaysia. Two mitochondrial genes were sequenced [c.f. 39], the NADH dehydrogenase subunit 5 (ND5) and cytochrome b in captive and wild Sri Lankan elephants. Seven haplotypes were observed, but this study did not confirm a postulated subspecies status of Sri Lankan elephants, Elephas maximus maximus. However, elephants from Borneo, Elephas maximus borneensis, were found to be a new subspecies with a specific mitochondrial D-loop sequence not seen elsewhere in Asia. Furthermore, a relatively low genetic diversity was observed in the Bornean population by nuclear microsatellite analysis [15].

The Y-chromosome, which is transmitted along the paternal lineage, was studied by sequencing a 438-bp fragment of the DBY gene (438 bp), and revealed no variation in a panel of 22 Thai elephants [c.f. 39]. Diversity of nuclear DNA, generally more informative than variation in mitochondrial DNA [35], has been studied more extensively in African elephants than in Asian elephants. However, microsatellite markers, developed for Asian elephants [23] [c.f. 39], were used for the elephant population genetic structure across Asia. Microsatellites and mtDNA were studied in southern India, and showed that Nilgiris-Eastren Ghats elephants, worldwide the largest Asian elephant population, carry one mtDNA haplotype and have a low microsatellite diversity [40]. Later, both methods were implemented on elephants across India, and showed that northern-northeastern, central, and two areas in southern India harbor four demographically autonomous populations, which should be managed separately [41]. However, only 5 to 7 microsatellite markers were used in those studies. Additional loci may very well give more accurate information and are also required for parentage and relationship analysis [c.f. 39]. Improved insight in the genetic and geographic variation in the Asian elephant may be accomplished by standardization of microsatellite genotyping across laboratories and countries.

Discussion

The Asian elephant is the national symbol of Thailand. It is also a keystone species in the ecology of the South-East Asian forest by dispersing plant species and clearing dense jungle for other wildlife. Thus, this mega herbivore is most important for the maintenance of biodiversity. To sustain the wild population, its habitat should be conserved while minimizing poaching and conflicts between human and elephant. In parallel with the wild populations, the captive relatives should also be conserved and studied as representative of the in situ species. So far captive breeding propagation in Asian elephants has been implemented with a low success rate both in western zoos and in many elephant facilities in range countries. The discussion presents the main achievements of the study [39], focusing on reproduction and breeding strategies, and genetics. Finally, recommendations and applications for a breeding program, and concluding remarks are formulated.

Female reproductive endocrinology: a general perspective

Previous research has revealed a remarkable peculiarity of reproduction of elephants: a long ovarian cycle with a unique double LH surge. The length of the follicular and luteal phase varies among individuals and across seasons. It appears that many factors influence the ovarian cycle, which implies that each breeding female should be monitored and evaluated for her reproductive status. In our study [39], 31.8% (n=7) of the elephants were non-cycling or had irregular cycles, which was especially prominent in aged elephants (>35 years). Thus, when a population gets older, this incidence may increase. Most reproductive studies on Asian elephants were conducted in western zoos and only a few in range countries. In spite of a different climate, environment and management, similar endocrine patterns were observed across these studies. However, the interval between “progestagens declining to baseline” and the first LH surge (anLH surge) in a cycle, varied among seasons in Thailand, which indicated that the environment influences the reproductive physiology in this species. By contrast, the interval between the anLH and the second LH surge (ovLH) is not affected by ecological factors. These observations instigated the investigation [39], which revealed a constant 3-week interval between the two LH surges and resulted in the hypothesis that timing of the ovLH surge is controlled by the anLH. This study does not only generate a new physiological explanation of the unique double LH surges, but also offers a therapeutic option to solve the acyclicity or “flatliner” problems [4] [5].

Two follicular waves, two LH surges, one ovulation: a proposed mechanism

The ovarian cycle in elephants is characterized by two follicular waves, two LH surges and one ovulation [6] [10] [c.f. 39]. A proposed mechanism to explain this phenomenon is based on the literature and observations [39]. Follicular stimulating hormone (FSH), an important glycoprotein hormone for follicular development, is regulated through GnRH, which is controlled by two centers in the hypothalamus [c.f. 39]. The tonic center, responsible for the basal hormone level, controls the pulse secretion and gradually raises the GnRH secretion during the luteal phase for the regulation of FSH for follicular development. Later, during the follicular phase, the hypothalamic surge center will release a specific quantity of GnRH to stimulate LH secretion. The ratio between FSH and LH secreted by gonadotrophs in the pituitary gland is regulated by the frequency and amplitude of GnRH pulses from the hypothalamus [c.f. 39]. In domestic mammals, FSH is highest just before or around the time that LH is highest for optimal follicular growth and ovulation. By contrast, in elephants, FSH is highest at the beginning of the follicular phase for development of multiple follicles and then gradually decreases to a nadir shortly after the ovLH surge [6]. High estradiol concentrations during the first follicular wave stimulate the GnRH receptor in the pituitary gland to enhance LH secretion, but reduce the FSH secretion [c.f. 39]. The interaction between estradiol concentrations during the first follicular wave and GnRH administration for LH surge induction as described [39] should be further studied to elucidate the endocrine mechanism. The first LH surge may functionally stimulate recruitment of the follicles and development of multiple corpora lutea [c.f. 39] which contribute to the luteal phase and early pregnancy [c.f. 39], as well as maturation of the Graafian follicle, and timing of the ovLH surge required for the ovulation. However, the explanation for the fixed 3-week period between the 2 LH surges is still unclear, and further studies are warranted.

Reproduction and environment: the interaction with breeding management

The seasonal effect on the estrous cycle in Thai elephants was investigated. The follicular phase proved to be shorter in the second half of the winter (mid December-mid February) and the summer (mid February-mid May), when the anLH surge occurred sooner after the progesterone decline than in the remaining part of the year. The aforementioned period coincided with the resting period of logging elephants, when both bulls and cows were released in the forest for foraging and traditional breeding, which resulted in the high fecundity rate in elephants (Myanmar, [c.f. 39]; Southern India, [37]) and birth in a period when there is abundant availability of food.

A delay of the anLH surge may result in ovarian inactivity. Such inactivity does occur in African elephants in North American zoos during the winter time when animals spent less time outside than in the summer period [33]. Environment has a major influence on the timing of the anLH surge. Stress from the management (i.e. restricted exercise in zoos in wintertime in temperate countries) or environment (i.e. heat stress in range countries) activates the hypothalamo-pituitary-adrenal (HPA) axis and causes the release of corticotropic releasing hormone (CRH) and vasopressin. These two peptide hormones play a role in the inhibition of the hypothalamic-pituitary-ovarian (HPO) axis, resulting in decreased GnRH secretion, which affects the secretion of FSH and LH, and may cause a prolonged follicular phase [c.f.39] or result in ovarian inactivity [5]. A prolonged follicular phase and temporary ovarian inactivity can lead to permanent ovarian inactivity or non-cycling, and a loss of fertility, if not diagnosed and treated. Thus endocrine monitoring, at least of progestagens, is recommended for evaluation of the reproductive status, (cycling, non-cycling or irregular cycling). The ovLH surge induction protocol [39] has a therapeutic potential in reducing the follicular phase length by inducing the anLH surge, followed by the ovLH surge and the luteal phase and could be applied in a captive breeding program.

Reproductive behavior and mating

Sustaining the captive population depends on successful breeding and subsequent calving. Unquestionably, monitoring of reproductive hormones is an excellent way to identify the estrous period. However, when an endocrine laboratory is not available, a reliable and economical method of estrus detection as developed and described [39] is suitable for zoos as well as elephant camps and facilities.

False estrus, eliciting interest of the bull during the non-estrous period, was reported to occur before the receptive period [20] [27] [c.f. 39] and also in pregnant elephants [c.f. 39], as well as in our study [39]. False estrus affects the accuracy of estrus detection methods. More distinct behavior, e.g. tail flicking, was reported to be significantly higher in the follicular phase than in the luteal phase, based on progestagen profiles without LH surge data. However, the highest number of tail flicking behavior was considered to be around the anLH surge, not the ovLH surge, and thus would predict ovulation to occur 3 weeks later [c.f. 39].

During the long follicular phase, pheromones are excreted in the urine to solicit the wandering bull to come and be present during the receptive or estrous period. Males may be interested in cows both during estrus as well as during the non-estrous follicular phase. Thus, copulation without conception could occur at the non-receptive period [38] [c.f. 39]. Successful mating resulting in conception is mainly instigated by the female. Therefore, morphological characteristics and female behaviors specific for estrus, as well as the interaction between bull and cow during estrus should be further investigated to identify the true estrous period.

Aspects of breeding

The importance of elephant captive breeding programs was mentioned in western zoos, which can also be applied for in situ conservation [c.f. 39]. In range countries, genetic exchange is recommended between captive breeding groups and wild populations [38]. Although the demography of ex situ elephant populations is not adequately analyzed in most countries [37] [c.f. 39] a captive elephants breeding program is still important in order to 1) sustain the captive population, either for the tourist industry or legal logging in specific areas 2) improve education and strengthen the human-elephant relation 3) reintroduce offspring to the wild to protect the natural habitat.

Disease transmission

One of the critical aspects in an elephant breeding program is disease transmission, especially manifest in the last two decades. Endotheliotropic elephant herpes virus (EEHV) has caused sudden death in many calves, probably resided in the cow and was transmitted through direct contact [31] [c.f. 39]. It is of major concern for the captive breeding program [c.f. 39]. This viral disease was first confirmed by PCR in a fatal case in a range country in 2006 [c.f. 39]. Thus, breeders should be aware of this disease, when exchanging breeding bulls or cows between elephant facilities, as well as when introducing wild elephants. The etiology and epidemiology of this disease should be further investigated. Moreover, other infections that can be acquired through direct contact i.e. tuberculosis, brucellosis should be monitored and infected animals quarantined to prevent spreading of specific pathogens through the breeding program.

Mating the right partner at the right time

To breed elephant efficiently, it is necessary to investigate the relationship between individuals. The “minimizing kinship” method, pairing individuals with low kinship, is strongly suggested for a captive elephant breeding program in zoos and range countries in order to maintain genetic variation [c.f. 39]. However, the success of this method, as well as calculation of kinship values depends on insight in the population’s pedigree, where the relationships among individuals are known [c.f. 39]. In fact, the pedigree is generally available in zoo elephants, but not in tourist and logging elephants, many of which are wild caught and information on the family background is lacking. Furthermore, the elephant translocation from one region to another region is influenced by the tourist industry. Therefore, reliable and informative pedigree data preferably through individual genetic analysis, and on variation in conformation [c.f. 39] are required to obtain insight in the genetic relatedness, and establish an effective breeding program [c.f. 39]. Our results [39] can be utilized as a tool for the genetic relatedness and pedigree analysis. Mating in the receptive period ensures a higher chance of pregnancy, which can be achieved through the estrus detection method as the genital inspection or urine test [39] or the ovLH surge induction procedure [39].

Genetic aspects

Monitoring the genetic variation of elephant populations in captivity requires a panel of informative genetic markers. A limited number of microsatellite markers for Asian elephants was published in 2001 [c.f. 39], with more markers developed for the African species [15] [c.f. 39]. In order to increase the number of available microsatellite markers for Asian elephants, markers developed for African elephants were tested in the Asian species and evaluated [39]. These results may very well be significant for future conservation and population genetics studies in the Asian elephant [c.f. 39]. Recently, other microsatellite markers for the Asian elephant were reported [23]. In a preliminary analysis, we observed that 14 of these markers were indeed informative for genetic analysis. So now tools are available for individual and parentage analysis [22], detection of inbreeding [c.f. 39], and molecular comparison of populations. The microsatellite marker quality is very important for correct information particularly for forensic cases. Evaluation of genetic relatedness between individuals, parentage analysis and individual identification can be seriously impacted by genotyping errors [c.f. 39], hence it is crucial to establish rigorous procedures for genotyping individuals.

Biodiversity of Thai elephants: genetic information required for a breeding program

In order to develop elephant breeding programs, general information on the elephant populations is required [c.f. 39]. From the results obtained [39], it is concluded that there is an appreciable genetic diversity in the captive Thai elephant population. This indicates a long history of random mating over the whole country. Moreover, the long generation interval by itself is expected to slow down the inbreeding process when compared to other species. Factors such as habitat loss could cause a rapid decline in population size and result in loss of genetic variation [c.f. 39]. Fortunately, due to the interaction between wild and captive populations in the past, the genetics of the wild population is extensively represented in the captive population [13]. In the captive population, animals were selected for breeding on the basis of traits as tameness, good characteristics and tusk formation. The effective population size (Ne) of animals of the reproductive age and of equal sex ratio that are needed to conserve 90% of the existent genetic diversity for a period of 100 years can be calculated from the following equation [18]: Ne = 475/ L where L is the generation length in years. With L = 20 [c.f. 39], Ne becomes 24 captive elephants. This can be achieved in large scale tourist or timber elephant camps, but is not always feasible for zoos where the space, facilities, management and financial supports are limited.

Implications for wild elephant genetics

The need of captive born calves' importation to western zoos to sustain their population [c.f. 39] provokes illegal capture of wild calves [24]. Indeed in Thailand, a "suspected" wild caught calf was placed with a captive foster cow [c.f. 39]. Our parentage analysis [39] could expose such cases by DNA analysis [22], and in this way contribute to the protection of wild populations. Information on gene exchange between wild and captive elephants, due to traditional mating of wild bulls and captive cows [39], remains essential for the conservation management of the Thai elephant. Moreover, more detailed data on the diversity in captive populations will be useful, while the genetic diversity of wild populations in Thailand has not been investigated yet. Comparison of mitochondrial, Y-chromosomal and autosomal DNA of wild and captive populations, will give insight in the gene flow between these two groups.

Proposed strategy for a captive breeding program

One of the aims of the research [39] is to increase the elephant population by improving breeding efficiency. An easy and economical way is to convince owners and mahouts that having a newborn calf not only attracts the tourist and raises their income, but also sustains the population and contributes to the conservation of elephants. Indeed, these stakeholders should be more aware of the importance of reproduction. For optimal breeding, individual elephants should have adequate facilities and sufficient physical comfort: enough food, relaxation time, space and health care for quality of life in order to reduce stress. These are prerequisites for a successful breeding program. For females, breeding individuals during the optimal reproductive stage of life (15-30 years) is recommended to reduce reproductive disorders and pathological problems. The use of a proven bull is advised for covering the female in estrus. Detection of the estrous period can be optimized by applying the procedures as developed [39]. A consistent and intensive method for estrus detection can help to achieve the breeding success i.e., pregnancy and calving. The alternative way of ovLH surge induction to pinpoint the ovulation time [39] is useful in natural and artificial mating. Monitoring reproductive hormones is quite expensive, but may be valuable in case of AI or restricted availability of semen or a breeding bull. In range countries, however, the most effective breeding strategy still is to breed the elephants the natural way.

To maintain the genetic diversity of captive elephant populations, DNA analysis is required. Fortunately, the diversity appears still to be high in Thai captive populations, and will remain so for a period of time [39]. Thus, an intensive breeding management in order to maintain the biodiversity is not of high importance; on the other hand, still pairing of related individuals should be prevented. If cows are allowed to mate wild bulls near the camps, paternity testing of calves would be useful in order to determine how many different bulls contribute to the next generation. Genetic analysis of elephants of Dutch zoos does not yet indicate inbreeding (unpublished data). However, inbreeding can easily occur when mating solely within this population, particularly when only one breeding bull is available. Hence, also in zoo elephants, breeding close relatives, or closely genetic related individuals should be avoided. Breeding individuals with a genetic relatedness less than 0.125 is recommended.

Conclusion

The aim of Asian elephant long term conservation is to maintain a viable population. The elephant population in captivity should be self-sustaining and this calls for an integrated approach to maintain wild and captive stocks [36]. Increasing the birth rate via an efficient captive breeding program should be paralleled by a reduction in mortality rate through promoting health care, e.g. by providing animal hospitals and mobile clinics. The traditional objective of a captive breeding program is the preservation of species as "living museum" [18] and sometimes also the increase in animal numbers in order to release groups into the wild. The intention is not the construction of a wild population on the short term, but instead the protection of the natural biotope in which the elephant functions as the key stone species. Although an elephant reintroduction program was considered impossible [3], it may be the most sustainable strategy for both elephant conservation and forest preservation. On the long term (100 -200 years), it could also result in the establishment of an in situ population. Thus, there is the mutual benefit for both owners and conservationists to increase elephant numbers and to maintain a viable captive population. Efficient breeding strategies, considering both the reproductive process and a broad genetic basis, may very well serve to increase and maintain the Asian elephant population on the long term.

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