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Arai, K., H. Qi and M. Inoue-Murayama (2023). "Age estimation of captive Asian elephants (*Elephas maximus*) based on DNA methylation: An exploratory analysis using methylation-sensitive high-resolution melting (MS-HRM)." *PLoS ONE* **18**(12): e0294994.

Age is an important parameter for bettering the understanding of biodemographic trends-development, survival, reproduction and environmental effects-critical for conservation. However, current age estimation methods are challenging to apply to many species, and no standardised technique has been adopted yet. This study examined the potential use of methylation-sensitive high-resolution melting (MS-HRM), a labour-, time-, and cost-effective method to estimate chronological age from DNA methylation in Asian elephants (*Elephas maximus*). The objective of this study was to investigate the accuracy and validation of MS-HRM use for age determination in long-lived species, such as Asian elephants. The average lifespan of Asian elephants is between 50-70 years but some have been known to survive for more than 80 years. DNA was extracted from 53 blood samples of captive Asian elephants across 11 zoos in Japan, with known ages ranging from a few months to 65 years. Methylation rates of two candidate age-related epigenetic genes, RALYL and TET2, were significantly correlated with chronological age. Finally, we established a linear, unisex age estimation model with a mean absolute error (MAE) of 7.36 years. This exploratory study suggests an avenue to further explore MS-HRM as an alternative method to estimate the chronological age of Asian elephants.

Bader, C., A. Delapr e and A. Houssaye (2023). "Shape variation in the limb long bones of modern elephants reveals adaptations to body mass and habitat." *J Anat* **242**(5): 806-830.

During evolution, several vertebrate lineages have shown trends towards an increase in mass. Such a trend is associated with physiological and musculoskeletal changes necessary to carry and move an increasingly heavy body. Due to their prominent role in the support and movement of the body, limb long bones are highly affected by these shifts in body mass. Elephants are the heaviest living terrestrial mammals, displaying unique features allowing them to withstand their massive weight, such as the columnarity of their limbs, and as such are crucial to understand the evolution towards high body mass in land mammals. In this study, we investigate the shape variation of the six limb long bones among the modern elephants, *Elephas maximus* and *Loxodonta africana*, to understand the effect of body mass and habitat on the external anatomy of the bones. To do so, we use three-dimensional geometric morphometrics (GMMs) and qualitative comparisons to describe the shape variation, at both the intraspecific and interspecific levels. Our results reveal that the two species share similar negative ontogenetic allometric patterns (i.e. becoming stouter with increased length) in their humerus and femur, but not in the other bones: the proximal epiphyses of the stylopod bones develop considerably during growth, while the distal epiphyses, which are involved in load distribution in the elbow and knee joints, are already massive in juveniles. We attribute this pattern to a weight-bearing adaptation already present in young specimens. Among adults of the same species, bone robustness increases with body mass, so that heavier specimens display stouter bones allowing for a better mechanical load distribution. While this robustness variation is significant for the humerus only, all the other bones appear to follow the same pattern. This is particularly visible in the

ulna and tibia, but less so in the femur, which suggests that the forelimb and hindlimb adapted differently to high body mass support. Robustness analyses, while significant for the humerus only, suggest more robust long bones in Asian elephants than in African savanna elephants. More specifically, GMMs and qualitative comparisons indicate that three bones are clearly distinct when comparing the two species: in *E. maximus* the humerus, the ulna and the tibia display enlarged areas of muscular insertions for muscles involved in joint and limb stabilization, as well as in limb rotation. These results suggest a higher limb compliance in Asian elephants, associated with a higher dexterity, which could be linked to their habitat and foraging habits.

Berzaghi, F., F. Bretagnolle, C. Durand-Bessart and S. Blake (2023). "Megaherbivores modify forest structure and increase carbon stocks through multiple pathways." Proc Natl Acad Sci U S A **120**(5): e2201832120.

Megaherbivores have pervasive ecological effects. In African rainforests, elephants can increase aboveground carbon, though the mechanisms are unclear. Here, we combine a large unpublished dataset of forest elephant feeding with published browsing preferences totaling nearly 200,000 records covering >800 plant species and with nutritional data for 145 species. Elephants increase carbon stocks by: 1) promoting high wood density trees via preferential browsing on leaves from low wood density species, which are more palatable and digestible; and 2) dispersing seeds of trees that are relatively large and have the highest average wood density among tree guilds based on dispersal mode. Loss of forest elephants could cause an increase in abundance of fast-growing low wood density trees and a 6% to 9% decline in aboveground carbon stocks due to regeneration failure of elephant-dispersed trees. These results demonstrate the importance of megaherbivores for maintaining diverse, high-carbon tropical forests. Successful elephant conservation will contribute to climate mitigation at a globally-relevant scale.

Bester, T., M. H. Schmitt and A. M. Shrader (2023). "The deterrent effects of individual monoterpenes odours on the dietary decisions of African elephants." Anim Cogn **26**(3): 1049-1063.

African savanna elephants use pre-ingestive olfactory cues when making dietary choices, and previous research has observed that elephant diet choice is negatively correlated with vegetation species that contain high concentrations of monoterpenes. However, the frequency and concentration of monoterpenes can vary dramatically across plant species. Thus, we aimed to explore the effects that the odours of individual monoterpenes have on elephant diet choice and how these effects vary with concentration. To do this, we conducted three odour-based choice experiments focusing on eight common monoterpenes found in the woody plants in Southern African savannas. In the first experiment, we tested whether elephant diet choice for a frequently consumed plant (*Euclea crispa*) was influenced by the addition of the odour of an individual monoterpene at a set concentration. In the second experiment, we explored the relative deterrence of each monoterpene. Lastly, we tested how elephant diet choice varied as a function of the addition of individual monoterpene odours at 5%, 10%, and 20% concentrations. We found that the elephants avoided most individual monoterpenes at high concentrations, with the exception being  $\alpha$ -pinene. Furthermore, we found that the odours of some individual monoterpenes were, in fact, more deterrent than others. In the third experiment, we found that the elephants avoided  $\beta$ -pinene, limonene, ocimene,  $\gamma$ -terpinene, and terpinolene across all concentrations, but only avoided sabinene and linalool at high concentrations. Ultimately, our results show that the odour of individual monoterpenes may deter elephant consumption, but that this deterrent effect depends on both the monoterpene and its concentration.

Bo, T., H. Liu, M. Liu, Q. Liu, Q. Li, Y. Cong, Y. Luo, Y. Wang, B. Yu, T. Pu, L. Wang, Z. Wang and D. Wang (2023). "Mechanism of inulin in colic and gut microbiota of captive Asian elephant." Microbiome **11**(1): 148.

**BACKGROUND:** Gut microbiota have a complex role on the survivability, digestive physiology, production, and growth performance in animals. Recent studies have emphasized the effects of prebiotics therapy on the gut disease, but the relationship between elephant gut-related diseases and prebiotics remains elusive. Here, a case study was undertaken to evaluate the mechanism of inulin treatment in colic in Asian elephant (*Elephas maximus* Linnaeus). **METHODS:** Fecal samples were collected from a sick elephant and four healthy elephants. Analysis of microbial profile was carried out by 16S rRNA sequencing, and the short chain fatty acids were tested by gas chromatography. The physiological function of "inulin-microbiota" of elephant was verified in mice by fecal microbial transplantation (FMT). The expression of related proteins was determined by Western blotting and qPCR. **RESULTS:** (1) Eating inulin can cure gut colic of the sick elephant and changed gut microbiota. (2) It was found that "inulin microbiota" from the post-treatment elephants can promote the proliferation of intestinal cells, increase the utilization of short chain fatty acids (SCFAs), maintain intestinal barrier, and reduce the inflammation in mice. (3) The mechanism was inulin-gut microbiota-SCFAs-immune barrier. **CONCLUSIONS:** Inulin contributed to rehabilitate the gut microbiota and gut immune barrier of the elephant with colic. This provides reasonable verification for using prebiotics to treat the colic in captive elephants. Prebiotics will surely play an increasingly important role in disease prevention and treatment of captive animals in the future. Video Abstract.

Bowman, J., D. Enard and V. J. Lynch (2023). "Phylogenomics reveals an almost perfect polytomy among the almost ungulates (Paenungulata)." bioRxiv.

Phylogenetic studies have resolved most relationships among Eutherian Orders. However, the branching order of elephants (Proboscidea), hyraxes (Hyracoidea), and sea cows (Sirenia) (i.e., the Paenungulata) has remained uncertain since at least 1758, when Linnaeus grouped elephants and manatees into a single Order (Bruta) to the exclusion of hyraxes. Subsequent morphological, molecular, and large-scale phylogenomic datasets have reached conflicting conclusions on the branching order within Paenungulates. We use a phylogenomic dataset of alignments from 13,388 protein-coding genes across 261 Eutherian mammals to infer phylogenetic relationships within Paenungulates. We find that gene trees almost equally support the three alternative resolutions of Paenungulate relationships and that despite strong support for a Proboscidea+Hyracoidea split in the multispecies coalescent (MSC) tree, there is significant evidence for gene tree uncertainty, incomplete lineage sorting, and introgression among Proboscidea, Hyracoidea, and Sirenia. Indeed, only 8-10% of genes have statistically significant phylogenetic signal to reject the hypothesis of a Paenungulate polytomy. These data indicate little support for any resolution for the branching order Proboscidea, Hyracoidea, and Sirenia within Paenungulata and suggest that Paenungulata may be as close to a real, or at least unresolvable, polytomy as possible.

Brickson, L., L. Zhang, F. Vollrath, I. Douglas-Hamilton and A. J. Titus (2023). "Elephants and algorithms: a review of the current and future role of AI in elephant monitoring." J R Soc Interface **20**(208): 20230367.

Artificial intelligence (AI) and machine learning (ML) present revolutionary opportunities to enhance our understanding of animal behaviour and conservation strategies. Using elephants, a crucial species in Africa and Asia's protected areas, as our focal point, we delve into the role of AI and ML in their conservation. Given the increasing amounts of data

gathered from a variety of sensors like cameras, microphones, geophones, drones and satellites, the challenge lies in managing and interpreting this vast data. New AI and ML techniques offer solutions to streamline this process, helping us extract vital information that might otherwise be overlooked. This paper focuses on the different AI-driven monitoring methods and their potential for improving elephant conservation. Collaborative efforts between AI experts and ecological researchers are essential in leveraging these innovative technologies for enhanced wildlife conservation, setting a precedent for numerous other species.

Budd, K. (2023). "Correction to "Effects of a hydropower project on a high-value Asian elephant population"." Ecol Evol **13**(11): e10631.

[This corrects the article DOI: 10.1002/ece3.10353.]

Budd, K., D. Suddychan, M. Tyson, C. N. Z. Coudrat, A. McWilliam, C. D. Hallam, A. Johnson and L. S. Eggert (2023). "Effects of a hydropower project on a high-value Asian elephant population." Ecol Evol **13**(7): e10353.

Habitat loss and fragmentation are leading contributors to the endangered status of species. In 2006, the Nakai Plateau contained the largest known Asian elephant (*Elephas maximus*) population in the Lao People's Democratic Republic (Lao PDR), and the population was among those with the highest genetic diversity reported for Asian elephants. In 2008, completion of the Nam Theun 2 hydroelectric dam inundated much of the Plateau, resulting in the loss of 40% of elephant habitat. We studied elephant presence, movements, and the incidence of human-elephant conflict (HEC) on the Nakai Plateau and surrounding areas from 2004 to 2020, before and for 12 years after dam completion. To examine contemporary population dynamics in the Nakai elephants, we used genetic sampling to compare minimum population numbers, demography, and levels of genetic diversity from the wet and dry seasons in 2018/2019, 10 years after dam completion, with those reported in a pre-dam-completion genetic survey. After dam completion, we found a major increase in HEC locally and the creation of new, serious, and persistent HEC problems as far as 100 km away. While we were unable to compare estimated population sizes before and after dam completion, our data revealed a decrease in genetic diversity, a male-biased sex ratio, and evidence of dispersal from the Plateau by breeding-age females. Our results raise concerns about the long-term viability of this important population as well as that of other species in this region. Given that hydropower projects are of economic importance throughout Laos and elsewhere in southeast Asia, this study has important implications for understanding and mitigating their impact.

Cabral de Mel, S. J., S. Seneweera, A. Dangolla, D. K. Weerakoon, T. Maraseni and B. L. Allen (2023). "Attitudes towards the Potential Use of Aversive Geofencing Devices to Manage Wild Elephant Movement." Animals (Basel) **13**(16).

Aversive geofencing devices (AGDs) or animal-borne satellite-linked shock collars might become a useful tool to mitigate human-elephant conflict (HEC). AGDs have the potential to condition problem elephants to avoid human-dominated landscapes by associating mild electric shocks with preceding audio warnings given as they approach virtual boundaries. We assessed the opinions of different stakeholders (experts, farmers, and others who have and have not experienced HEC; n = 611) on the potential use of AGDs on Asian elephants. Most respondents expressed positive opinions on the potential effectiveness of AGDs in managing elephant movement (62.2%). About 62.8% respondents also provided positive responses for the acceptability of AGDs if pilot studies with captive elephants have been successful in managing their movements. Some respondents perceived AGDs to be

unacceptable because they are unethical or harmful and would be unsuccessful given wild elephants may respond differently to AGDs than captive elephants. Respondents identified acceptability, support and awareness of stakeholders, safety and wellbeing of elephants, logistical difficulties, durability and reliable functionality of AGDs, and uncertainties in elephants' responses to AGDs as potential challenges for implementing AGDs. These issues need attention when developing AGDs to increase support from stakeholders and to effectively reduce HEC incidents in the future.

Campbell, K. M., J. A. Wilson and K. A. Morfeld (2023). "Predictors of testosterone in zoo-managed male African elephants (*Loxodonta Africana*)."  
*Zoo Biol* **42**(2): 268-282.

Reproductive complications for both male and female zoo-managed African elephants (*Loxodonta africana*) contribute to the rapidly declining population. In zoo-managed bull elephants, few studies have explored the potential physiological, physical, social, and environmental factors that influence bull fertility, particularly, androgen production. Testosterone is the essential steroid hormone for male sexual maturation and inadequate concentrations can be detrimental for spermatogenesis. Testosterone, fecal glucocorticoid metabolites, leptin, glucose, insulin, and triglycerides were analyzed from weekly fecal and blood serum samples taken over 6 months from six zoo-managed African elephant bulls (10-19 years of age). Testosterone levels were compared to endocrine factors, weekly social and environmental variables, daily musth signs, and body condition scores (BCS). The glucose-to-insulin ratio (G:I) was the only physiological biomarker found to be positively associated with testosterone. Predictive physical variables included Musth Score (+) and Moderate Exercise (+). Bulls with BCS signifying overweight (BCS 4) had lower testosterone ( $36.6 \pm 1.6$  ng/g fecal extraction [FE]) than bulls with healthy BCS 3;  $51.2 \pm 4.9$  ng/g FE). Numerous social variables influenced testosterone concentrations, including Total Contact Day (+), Female Interaction Day (+), Indirect Contact Day (+), Indirect Contact Night (+) and Total No Contact (-). Both percentage of Time Outdoor and Time Mixed positively influenced testosterone, whereas testosterone decreased for percentage of Time Indoors. Each additional daily browse opportunity increased testosterone by approximately 7 ng/g FE. In managed care, the emphasis should be placed on optimizing these predictors of testosterone production to promote bull reproductive health.

Cao, K., Y. Wang, X. Bai, J. Wang, L. Zhang, Y. Tang, R. C. Thuku, W. Hou, G. Mo, F. Chen and L. Jin (2023). "Comparison of Fecal Antimicrobial Resistance Genes in Captive and Wild Asian Elephants."  
*Antibiotics (Basel)* **12**(5).

The Asian elephant (*Elephas maximus*) is a flagship species of tropical rainforests, and it has generated much concern. In this case, the gut bacterial communities of captive and wild Asian elephants are particularly noteworthy. We aim to compare the differences in bacterial diversity and antibiotic resistance gene (ARG) subtypes in fecal samples of Asian elephants from different habitats, which may affect host health. Analyses reveal that differences in the dominant species of gut bacteria between captive and wild Asian elephants may result in significant differences in ARGs. Network analysis of bacterial communities in captive Asian elephants has identified potentially pathogenic species. Many negative correlations in network analysis suggest that different food sources may lead to differences in bacterial communities and ARGs. Results also indicate that the ARG levels in local captive breeding of Asian elephants are close to those of the wild type. However, we found that local captive elephants carry fewer ARG types than their wild counterparts. This study reveals the profile and relationship between bacterial communities and ARGs in different sources of Asian elephant feces, providing primary data for captive breeding and rescuing wild Asian elephants.

Cernohorska, H., S. Kubickova, P. Musilova, M. Vozdova, R. Vodicka and J. Rubes (2023). "Supernumerary Marker Chromosome Identified in Asian Elephant (*Elephas maximus*)." *Animals (Basel)* **13**(4).

We identified a small, supernumerary marker chromosome (sSMC) in two phenotypically normal Asian elephants (*Elephas maximus*): a female ( $2n = 57,XX,+mar$ ) and her male offspring ( $2n = 57,XY,+mar$ ). sSMCs are defined as structurally abnormal chromosomes that cannot be identified by conventional banding analysis since they are usually small and often lack distinct banding patterns. Although current molecular techniques can reveal their origin, the mechanism of their formation is not yet fully understood. We determined the origin of the marker using a suite of conventional and molecular cytogenetic approaches that included (a) G- and C-banding, (b) AgNOR staining, (c) preparation of a DNA clone using laser microdissection of the marker chromosome, (d) FISH with commercially available human painting and telomeric probes, and (e) FISH with centromeric DNA derived from the centromeric regions of a marker-free Asian elephant. Moreover, we present new information on the location and number of NORs in Asian and savanna elephants. We show that the metacentric marker was composed of heterochromatin with NORs at the terminal ends, originating most likely from the heterochromatic region of chromosome 27. In this context, we discuss the possible mechanism of marker formation. We also discuss the similarities between sSMCs and B chromosomes and whether the marker chromosome presented here could evolve into a B chromosome in the future.

Chen, Y., Y. Sun, M. Hua, K. Shi and D. Dudgeon (2023). "Using genetic tools to inform conservation of fragmented populations of Asian elephants (*Elephas maximus*) across their range in China." *Integr Zool* **18**(3): 453-468.

A herd of 15 Chinese elephants attracted international attention during their 2021 northward trek, motivating the government to propose establishment of an Asian elephant national park. However, planning is hampered by a lack of genetic information on the remaining populations in China. We collected DNA from 497 dung samples from all 5 populations encompassing the entire range of elephants in China and used mitochondrial and microsatellite markers to investigate their genetic and demographic structure. We identified 237 unique genotypes (153 females, 84 males), representing 81% of the known population. However, the effective population size was small (28, range 25-32). Historic demographic contraction appeared to account for low haplotype diversity ( $H(d) = 0.235$ ), but moderate nucleotide and nuclear diversity ( $\pi = 0.6\%$ ,  $H(e) = 0.55$ ) was attributable to post-bottleneck recovery involving recent population expansion plus historical gene exchange with elephants in Myanmar, Lao PDR, and Vietnam. The 5 populations fell into 3 clusters, with Nangunhe elephants differing consistently from the other 4 populations ( $F(ST) = 0.23$ ); elephants from Mengyang, Simao, and Jiangcheng belonged to a single population (henceforth, MSJ), and differed from the Shangyong population ( $F(ST) = 0.11$ ). Interpopulation genetic variation reflected isolation by distance and female-biased dispersal. Chinese elephants should be managed as 2 distinct units: Nangunhe and another combining Shangyong and MSJ; their long-term viability will require restoring gene flow between Shangyong and MSJ, and between elephants in China and neighboring countries. Our results have the potential to inform conservation planning for an iconic megafaunal species.

Cherney, M. D., D. C. Fisher, R. J. Auchus, A. N. Rountrey, P. Selcer, E. A. Shirley, S. G. Beld, B. Buigues, D. Mol, G. G. Boeskorov, S. L. Vartanyan and A. N. Tikhonov (2023). "Testosterone histories from tusks reveal woolly mammoth musth episodes." *Nature* **617**(7961): 533-539.

Hormones in biological media reveal endocrine activity related to development, reproduction, disease and stress on different timescales(1). Serum provides immediate circulating concentrations(2), whereas various tissues record steroid hormones accumulated over time(3,4). Hormones have been studied in keratin, bones and teeth in modern(5-8) and ancient contexts(9-12); however, the biological significance of such records is subject to ongoing debate(10,13-16), and the utility of tooth-associated hormones has not previously been demonstrated. Here we use liquid chromatography with tandem mass spectrometry paired with fine-scale serial sampling to measure steroid hormone concentrations in modern and fossil tusk dentin. An adult male African elephant (*Loxodonta africana*) tusk shows periodic increases in testosterone that reveal episodes of musth(17-19), an annually recurring period of behavioural and physiological changes that enhance mating success(20-23). Parallel assessments of a male woolly mammoth (*Mammuthus primigenius*) tusk show that mammoths also experienced musth. These results set the stage for wide-ranging studies using steroids preserved in dentin to investigate development, reproduction and stress in modern and extinct mammals. Because dentin grows by apposition, resists degradation, and often contains growth lines, teeth have advantages over other tissues that are used as records of endocrine data. Given the low mass of dentin powder required for analytical precision, we anticipate dentin-hormone studies to extend to smaller animals. Thus, in addition to broad applications in zoology and palaeontology, tooth hormone records could support medical, forensic, veterinary and archaeological studies.

Childs-Sanford, S. E., A. J. Makowski, R. L. Hilliard and J. J. Wakshlag (2023). "EXPERIMENTAL CHOLECALCIFEROL SUPPLEMENTATION IN A HERD OF MANAGED ASIAN ELEPHANTS (*ELEPHAS MAXIMUS*)."  
*J Zoo Wildl Med* **54**(2): 219-230.

Vitamin D supplementation may pose a significant health risk in species where levels of deficiency, sufficiency, and toxicity have not been clearly established, and species-specific research on vitamin D supplementation should be performed. This study documented the effect of vitamin D supplementation on serum vitamin D metabolites and other analytes of Ca homeostasis in Asian elephants (*Elephas maximus*). Six adult Asian elephants received PO supplementation with cholecalciferol at 300 IU/kg of body weight (BW) once a week for 24 wk. Serum was analyzed every 4 wk for 25-hydroxyvitamin D(2)/D(3) [25(OH)D]; 24,25-dihydroxyvitamin D(2)/D(3) [24,25(OH)(2)D]; 1,25-dihydroxyvitamin D [1,25(OH)(2)D]; parathyroid hormone (PTH); total Ca; ionized Ca (iCa); P; and Mg. After the supplement was discontinued, serum 25(OH)D(2)/D(3) was measured every 4 wk until levels returned to baseline. At the start of the study, the average serum 25(OH)D(3) was nondetectable (<1.5 ng/ml). With cholecalciferol supplementation, 25(OH)D(3) increased at an average rate of 2.26 ng/ml per month and reached an average concentration of 12.9 ± 3.46 ng/ml at 24 wk. Both 24,25(OH)(2)D(3) and 1,25(OH)(2)D increased over time with supplementation from an average of <1.5 to 12.9 ng/ml and from 9.67 to 36.4 pg/ml, respectively. PTH, iCa, Ca, P, and Mg remained within reported normal ranges throughout supplementation. After the supplement was discontinued, serum 25(OH)D(3) demonstrated a slow decline to baseline, taking an average of 48 wk. Elephants demonstrated significant individual variation in response to supplementation and subsequent return to baseline. Supplementation of Asian elephants with a weekly dose of 300 IU/kg BW cholecalciferol for 24 wk appears to be effective and safe. Additional clinical studies would be necessary to investigate the safety of other routes of administration, dosages, and duration of vitamin D supplementation, as well as associated health benefits.

Christensen, M. M., O. Hallikas, R. Das Roy, V. Väänänen, O. E. Stenberg, T. J. Häkkinen, J. C.

François, R. J. Asher, O. D. Klein, M. Holzenberger and J. Jernvall (2023). "The developmental basis for scaling of mammalian tooth size." Proc Natl Acad Sci U S A **120**(25): e2300374120.

When evolution leads to differences in body size, organs generally scale along. A well-known example of the tight relationship between organ and body size is the scaling of mammalian molar teeth. To investigate how teeth scale during development and evolution, we compared molar development from initiation through final size in the mouse and the rat. Whereas the linear dimensions of the rat molars are twice that of the mouse molars, their shapes are largely the same. Here, we focus on the first lower molars that are considered the most reliable dental proxy for size-related patterns due to their low within-species variability. We found that scaling of the molars starts early, and that the rat molar is patterned equally as fast but in a larger size than the mouse molar. Using transcriptomics, we discovered that a known regulator of body size, insulin-like growth factor 1 (Igf1), is more highly expressed in the rat molars compared to the mouse molars. Ex vivo and in vivo mouse models demonstrated that modulation of the IGF pathway reproduces several aspects of the observed scaling process. Furthermore, analysis of IGF1-treated mouse molars and computational modeling indicate that IGF signaling scales teeth by simultaneously enhancing growth and by inhibiting the cusp-patterning program, thereby providing a relatively simple mechanism for scaling teeth during development and evolution. Finally, comparative data from shrews to elephants suggest that this scaling mechanism regulates the minimum tooth size possible, as well as the patterning potential of large teeth.

Chu, P. C., K. Wierucka, D. Murphy, H. B. Tilley and H. S. Mumby (2023). "Human interventions in a behavioural experiment for Asian Elephants (*Elephas maximus*)." Anim Cogn **26**(2): 393-404.

Experiments are widely used to investigate the behaviour and cognition of animals. While the automation of experiments to avoid potential experimenter bias is sometimes possible, not all experiments can be conducted without human presence. This is particularly true for large animals in captivity, which are often managed by professional handlers. For the safety of the animals and experimenters, a handler must be present during behavioural studies with certain species. It is not always clear to what extent cues provided by handlers affect the animals, and therefore the experimental results. In this study, we investigate handler interventions during the training process for a behavioural experiment with Asian elephants (*Elephas maximus*) in Nepal. We show that elephant handlers (mahouts) intervened to guide elephants in performing the learning task using vocal and behavioural cues, despite experimenters requesting minimal intervention. We found that although the frequency of mahout interventions did not decrease as the training progressed, the nature of their interventions changed. We also found more non-verbal than verbal cues across the training. Our results suggest that guidance from handlers may be common in behavioural studies, and continued consideration should be put into experimental design to reduce or account for cues that animals may receive from humans. This study also emphasises the need to take into account the presence of humans in interpreting the results of animal behavioural experiments, which not only presents challenges to behavioural research, but also represents opportunities for further study.

Chusyd, D. E., J. L. Brown, L. Golzarri-Arroyo, S. L. Dickinson, V. B. Kraus, J. Siegal-Willott, T. M. Griffin, J. L. Huebner, K. L. Edwards, D. B. Allison and S. N. Austad (2023). "RELATIONSHIP BETWEEN REPRODUCTIVE AND BONE BIOMARKERS AND OSTEOARTHRITIS IN ZOO ASIAN (*ELEPHAS MAXIMUS*) AND AFRICAN (*LOXODONTA AFRICANA*) ELEPHANTS." J Zoo Wildl Med **53**(4): 801-810.

Osteoarthritis (OA) is common in zoo Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants. This study investigated the relationship between confirmed or



suspected OA with ovarian cyclicity, gonadotropins, progestagens, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and collagen type I (CTX-I) in zoo elephants. In Asian elephants, odds of having confirmed or suspected OA decreased with cycling (OR = 0.22, P = 0.016; OR = 0.29, P = 0.020, respectively), however, not when adjusted for age (odds ratio [OR] = 0.31, P = 0.112; OR = 0.58, P = 0.369, respectively). In African elephants, none of the models between confirmed OA and cycling status were significant (P > 0.060), while the odds of having suspected OA decreased with cycling (OR = 0.12, P = 0.001), even after adjusting for age (OR = 0.15, P = 0.005). Progestagens (Asian elephants P > 0.096; African elephants P > 0.415), LH (Asian P > 0.129; African P > 0.359), and FSH (Asian P > 0.738; African P > 0.231) did not differ with confirmed or suspected OA status, unadjusted. CTX-I concentrations were not related to OA status (P > 0.655). This study concluded hormonal changes may not have a strong impact on OA, so additional investigation into other serologic biomarkers is warranted.

Clemente, C. J. and T. J. M. Dick (2023). "How scaling approaches can reveal fundamental principles in physiology and biomechanics." *J Exp Biol* **226**(7).

Among terrestrial mammals, the largest, the 3 tonne African elephant, is one-million times heavier than the smallest, the 3 g pygmy shrew. Body mass is the most obvious and arguably the most fundamental characteristic of an animal, impacting many important attributes of its life history and biology. Although evolution may guide animals to different sizes, shapes, energetic profiles or ecological niches, it is the laws of physics that limit biological processes and, in turn, affect how animals interact with their environment. Consideration of scaling helps us to understand why elephants are not merely scaled-up shrews, but rather have modified body proportions, posture and locomotor style to mitigate the consequences of their large size. Scaling offers a quantitative lens into how biological features vary compared with predictions based on physical laws. In this Review, we provide an introduction to scaling and its historical context, focusing on two fields that are strongly represented in experimental biology: physiology and biomechanics. We show how scaling has been used to explore metabolic energy use with changes in body size. We discuss the musculoskeletal and biomechanical adaptations that animals use to mitigate the consequences of size, and provide insights into the scaling of mechanical and energetic demands of animal locomotion. For each field, we discuss empirical measurements, fundamental scaling theories and the importance of considering phylogenetic relationships when performing scaling analyses. Finally, we provide forward-looking perspectives focused on improving our understanding of the diversity of form and function in relation to size.

Compton, Z., V. Harris, W. Mellon, S. Rupp, D. Mallo, S. Kapsetaki, M. Wilmot, R. Kennington, K. Noble, C. Baciú, L. Ramirez, A. Peraza, B. Martins, S. Sudhakar, S. Aksoy, G. Furukawa, O. Vincze, M. Giraudeau, E. Duke, S. Spiro, E. Flach, H. Davidson, A. Zehnder, T. Graham, B. Troan, T. Harrison, M. Tollis, J. Schiffman, A. Aktipis, L. Abegglen, C. Maley and A. Boddy (2023). "Cancer Prevalence Across Vertebrates." *Res Sq*.

Cancer is pervasive across multicellular species, but what explains differences in cancer prevalence across species? Using 16,049 necropsy records for 292 species spanning three clades (amphibians, sauropsids and mammals) we found that neoplasia and malignancy prevalence increases with adult weight (contrary to Peto's Paradox) and somatic mutation rate, but decreases with gestation time. Evolution of cancer susceptibility appears to have undergone sudden shifts followed by stabilizing selection. Outliers for neoplasia prevalence include the common porpoise (<1.3%), the Rodrigues fruit bat (<1.6%) the black-footed penguin (<0.4%), ferrets (63%) and opossums (35%). Discovering why some species have particularly high or low levels of cancer may lead to a better understanding of cancer

syndromes and novel strategies for the management and prevention of cancer.

Cook, K. A., P. D. Ling, K. A. Terio, W. A. Baumgartner, L. L. Howard and J. A. Landolfi (2023). "DETECTION OF ELEPHANT ENDOTHELIO TROPIC HERPESVIRUS 1A IN ARCHIVAL TISSUE USING RNASCOPE(®) IN SITU HYBRIDIZATION." *J Zoo Wildl Med* **53**(4): 661-669.

Hemorrhagic disease due to elephant endotheliotropic herpesvirus infection (EEHV-HD) is an important cause of calf mortality in managed and free-ranging Asian (*Elephas maximus*) and African elephant (*Loxodonta* spp.) populations. Consequently, infection has profound implications for elephant population growth and sustainability. The mechanisms of disease caused by EEHV (i.e., infection, dissemination, shedding, latency) are relatively undefined, in part because of a lack of robust validated assays for detecting viral gene products in relevant samples. To address this issue, we used RNAscope(®) in situ hybridization (ISH) based on EEHV1A DNA polymerase and terminase genes to detect EEHV1A RNA in archival formalin-fixed, paraffin-embedded Asian elephant heart and tongue from PCR-confirmed cases (n = 4) of EEHV-HD and Asian elephants (n = 2) that died from other causes. EEHV1A-positive cases had positive hybridization signal in endothelial cell nuclei of both tissues for both DNA polymerase and terminase. EEHV-negative cases lacked signal. In positive cases, the number of positive nuclei was manually assessed to provide an estimate of the viral load and compare sensitivity of the two probes. In all cases, heart had greater signal than tongue for both probes (Wilcoxon rank test;  $P \leq 0.01$ ). Overall, terminase hybridization signal was greater than DNA polymerase signal (Wilcoxon rank test;  $P \leq 0.01$ ). Results indicate RNAscope ISH is a valuable tool for detection of EEHV in archival samples and for confirming infection. Additionally, the terminase gene is the optimal target and heart is preferable to tongue for detection in cases of EEHV-HD. Results will inform future investigations of viral tropism in EEHV-HD cases due to EEHV1A.

Curry, A. (2023). "Neanderthals lived in groups big enough to eat giant elephants." *Science* **379**(6631): 428.

Meat from the butchered beasts would have fed hundreds.

Davidar, P., R. Sharma, S. de Silva, A. Campos-Arceiz, B. Goossens, J. P. Puyravaud, B. Habib, R. De, E. P. Wong, D. Neupane, N. B. Othman and S. P. Goyal (2023). "Connect elephant habitats in Asia." *Science* **379**(6634): 765.

de Silva, S., T. Wu, P. Nyhus, A. Weaver, A. Thieme, J. Johnson, J. Wadey, A. Mossbrucker, T. Vu, T. Neang, B. S. Chen, M. Songer and P. Leimgruber (2023). "Land-use change is associated with multi-century loss of elephant ecosystems in Asia." *Sci Rep* **13**(1): 5996.

Understanding historic patterns of land use and land cover change across large temporal and spatial scales is critical for developing effective biodiversity conservation management and policy. We quantify the extent and fragmentation of suitable habitat across the continental range of Asian elephants (*Elephas maximus*) based on present-day occurrence data and land-use variables between 850 and 2015 A.D. We found that following centuries of relative stability, over 64% (3.36 million km<sup>2</sup>) of suitable elephant habitat across Asia was lost since the year 1700, coincident with colonial-era land-use practices in South Asia and subsequent agricultural intensification in Southeast Asia. Average patch size dropped 83% from approximately 99,000-16,000 km<sup>2</sup> and the area occupied by the largest patch decreased 83% from ~4 million km<sup>2</sup> (45% of area) to 54,000 km<sup>2</sup> (~7.5% of area). Whereas 100% of the area within 100 km of the current elephant range could have been considered suitable habitat in the year 1700, over half was unsuitable by 2015, driving potential conflict with people. These losses reflect long-term decline of non-forested

ecosystems, exceeding estimates of deforestation within this century. Societies must consider ecological histories in addition to proximate threats to develop more just and sustainable land-use and conservation strategies.

Deiringer, N., U. Schneeweiß, L. V. Kaufmann, L. Eigen, C. Speissegger, B. Gerhardt, S. Holtze, G. Fritsch, F. Göritz, R. Becker, A. Ochs, T. Hildebrandt and M. Brecht (2023). "The functional anatomy of elephant trunk whiskers." Commun Biol **6**(1): 591.

Behavior and innervation suggest a high tactile sensitivity of elephant trunks. To clarify the tactile trunk periphery we studied whiskers with the following findings. Whisker density is high at the trunk tip and African savanna elephants have more trunk tip whiskers than Asian elephants. Adult elephants show striking lateralized whisker abrasion caused by lateralized trunk behavior. Elephant whiskers are thick and show little tapering. Whisker follicles are large, lack a ring sinus and their organization varies across the trunk. Follicles are innervated by ~90 axons from multiple nerves. Because elephants don't whisk, trunk movements determine whisker contacts. Whisker-arrays on the ventral trunk-ridge contact objects balanced on the ventral trunk. Trunk whiskers differ from the mobile, thin and tapered facial whiskers that sample peri-rostrum space symmetrically in many mammals. We suggest their distinctive features-being thick, non-tapered, lateralized and arranged in specific high-density arrays-evolved along with the manipulative capacities of the trunk.

Dettmer, A. M. and D. E. Chusyd (2023). "Early life adversities and lifelong health outcomes: A review of the literature on large, social, long-lived nonhuman mammals." Neurosci Biobehav Rev **152**: 105297.

Social nonhuman animals are powerful models for studying underlying factors related to lifelong health outcomes following early life adversities (ELAs). ELAs can be linked to lifelong health outcomes depending on the species, system, sensitive developmental periods, and biological pathways. This review focuses on the literature surrounding ELAs and lifelong health outcomes in large, social, relatively long-lived nonhuman mammals including nonhuman primates, canids, hyenas, elephants, ungulates, and cetaceans. These mammals, like humans but unlike the most-studied rodent models, have longer life histories, complex social structures, larger brains, and comparable stress and reproductive physiology. Collectively, these features make them compelling models for comparative aging research. We review studies of caregiver, social, and ecological ELAs, often in tandem, in these mammals. We consider experimental and observational studies and what each has contributed to our knowledge of health across the lifespan. We demonstrate the continued and expanded need for comparative research to inform about the social determinants of health and aging in both humans and nonhuman animals.

Docter-Loeb, H. (2023). "Helping to protect elephants - using software." Nature **618**(7967): 1102.

Downs, C. J., L. A. Schoenle, E. W. Goolsby, S. J. Oakey, R. Ball, R. H. Y. Jiang and L. B. Martin (2023). "Large Mammals Have More Powerful Antibacterial Defenses Than Expected from Their Metabolic Rates." Am Nat **201**(2): 287-301.

Abstract Terrestrial mammals span seven orders of magnitude in body size, ranging from the <2-g Etruscan pygmy shrew (*Suncus etruscus*) to the >3,900-kg African elephant (*Loxodonta africana*). Although body size profoundly affects the behavior, physiology, ecology, and evolution of species, how investment in functional immune defenses changes with body size across species is unknown. Here, we (1) developed a novel 12-point dilution curve approach to describe and compare antibacterial capacity against three bacterial species among >160 terrestrial species of mammals and (2) tested published predictions

about the scaling of immune defenses. Our study focused on the safety factor hypothesis, which predicts that broad, early-acting immune defenses should scale hypermetrically with body mass. However, our three statistical approaches demonstrated that antibacterial activity in sera across mammals exhibits isometry; killing capacity did not change with body size across species. Intriguingly, this result indicates that the serum of a large mammal is less hospitable to bacteria than would be predicted by its metabolic rates. In other words, if metabolic rates underlie the rates of physiological reactions as postulated by the metabolic theory of ecology, large species should have disproportionately lower antibacterial capacity than small species, but they do not. These results have direct implications for effectively modeling the evolution of immune defenses and identifying potential reservoir hosts of pathogens.

Duan, Y., Z. Wang, J. Li, M. Zhang and Z. Bi (2023). "An adolescent female African elephant (*Loxodonta africana*) gave birth to an abnormal calf." Vet Med Sci.

Maternal nutrition and the uterine environment can influence placental development in mammals, leading to the birth of abnormal infants who often experience difficulties in independent standing. This article documents an adolescent female African elephant (*Loxodonta africana*) giving birth to an African elephant calf with a shoulder height below the mean, and its inability to stand for the first 10 h after birth, a time span that significantly exceeds the average duration for independent standing. Through the implementation of assisted feeding and assisted standing measures, the calf eventually achieved independent standing and nursing from its mother after 27 h and subsequent catch-up growth at 5 months of age. This study presents the first case report on the growth parameters during pregnancy of an adolescent African elephant, the birth and care (feeding, assisted standing) of a small African elephant calf with the inability to stand alone and nurse independently for several hours, highlighting pregnancy may influence the growth of cows during their ongoing growth and development phase, the calf's survival and subsequent catch-up growth at 5 months of age. These findings offer valuable insights into the care of abnormal African elephant calves.

Durham, S. D., Z. Wei, D. G. Lemay, M. C. Lange and D. Barile (2023). "Creation of a milk oligosaccharide database, MilkOligoDB, reveals common structural motifs and extensive diversity across mammals." Sci Rep **13**(1): 10345.

The carbohydrate fraction of most mammalian milks contains a variety of oligosaccharides that encompass a range of structures and monosaccharide compositions. Human milk oligosaccharides have received considerable attention due to their biological roles in neonatal gut microbiota, immunomodulation, and brain development. However, a major challenge in understanding the biology of milk oligosaccharides across other mammals is that reports span more than 5 decades of publications with varying data reporting methods. In the present study, publications on milk oligosaccharide profiles were identified and harmonized into a standardized format to create a comprehensive, machine-readable database of milk oligosaccharides across mammalian species. The resulting database, MilkOligoDB, includes 3193 entries for 783 unique oligosaccharide structures from the milk of 77 different species harvested from 113 publications. Cross-species and cross-publication comparisons of milk oligosaccharide profiles reveal common structural motifs within mammalian orders. Of the species studied, only chimpanzees, bonobos, and Asian elephants share the specific combination of fucosylation, sialylation, and core structures that are characteristic of human milk oligosaccharides. However, agriculturally important species do produce diverse oligosaccharides that may be valuable for human supplementation. Overall, MilkOligoDB facilitates cross-species and cross-publication comparisons of milk

oligosaccharide profiles and the generation of new data-driven hypotheses for future research.

Fletcher, R. J., Jr., A. O'Brien, T. F. Hall, M. Jones, A. D. Potash, L. Kruger, P. Simelane, K. Roques, A. Monadjem and R. A. McCleery (2023). "Frightened of giants: fear responses to elephants approach that of predators." Biol Lett **19**(10): 20230202.

Animals are faced with a variety of dangers or threats, which are increasing in frequency with ongoing environmental change. While our understanding of fearfulness of such dangers is growing in the context of predation and parasitism risk, the extent to which non-trophic, interspecific dangers elicit fear in animals remains less appreciated. We provide an experimental test for fear responses of savannah ungulates to a dominant and aggressive megaherbivore, the African bush elephant (*Loxodonta africana*), and contrast responses to an apex predator known to elicit fear in this system. Using an automated behavioural response system, we contrast vigilance and run responses of ungulates to elephant, leopard (*Panthera pardus*), and control (red-chested cuckoo *Cuculus solitarius*) vocalizations. Overall, we find that ungulates responded to elephant calls, both in terms of an increase in run and vigilance responses relative to controls. The magnitude of most behavioural responses (four of six considered) to elephant vocalizations were not significantly different than responses to leopards. These results suggest that megaherbivores can elicit strong non-trophic fear responses by ungulates and call to broaden frameworks on fear to consider dominant species, such as megaherbivores, as key modifiers of fear-induced interactions.

Foggin, C. M., L. E. Rosen, M. M. Henton, A. Buys, T. Floyd, A. D. Turner, J. Tarbin, A. S. Lloyd, C. Chaitezvi, R. J. Ellis, H. C. Roberts, A. Dastjerdi, A. Nunez, A. H. M. van Vliet and F. Steinbach (2023). "Pasteurella sp. associated with fatal septicaemia in six African elephants." Nat Commun **14**(1): 6398.

The sudden mortality of African elephants (*Loxodonta africana*) in Botswana and Zimbabwe in 2020 provoked considerable public interest and speculation. Poaching and malicious poisoning were excluded early on in the investigation. Other potential causes included environmental intoxication, infectious diseases, and increased habitat stress due to ongoing drought. Here we show evidence of the mortalities in Zimbabwe as fatal septicaemia associated with Bisgaard taxon 45, an unnamed close relative of *Pasteurella multocida*. We analyse elephant carcasses and environmental samples, and fail to find evidence of cyanobacterial or other intoxication. Post-mortem and histological findings suggest a bacterial septicaemia similar to haemorrhagic septicaemia caused by *P. multocida*. Biochemical tests and 16S rDNA analysis of six samples and genomic analysis of one sample confirm the presence of Bisgaard taxon 45. The genome sequence contains many of the canonical *P. multocida* virulence factors associated with a range of human and animal diseases, including the pmHAS gene for hyaluronidase associated with bovine haemorrhagic septicaemia. Our results demonstrate that Bisgaard taxon 45 is associated with a generalised, lethal infection and that African elephants are susceptible to opportunistically pathogenic *Pasteurella* species. This represents an important conservation concern for elephants in the largest remaining metapopulation of this endangered species.

Fomsgaard, A. S., S. A. Tahas, K. Spiess, C. Polacek, J. Fonager and G. J. Belsham (2023). "Unbiased Virus Detection in a Danish Zoo Using a Portable Metagenomic Sequencing System." Viruses **15**(6).

Metagenomic next-generation sequencing (mNGS) is receiving increased attention for the detection of new viruses and infections occurring at the human-animal interface. The ability to actively transport and relocate this technology enables in situ virus identification, which

could reduce response time and enhance disease management. In a previous study, we developed a straightforward mNGS procedure that greatly enhances the detection of RNA and DNA viruses in human clinical samples. In this study, we improved the mNGS protocol with transportable battery-driven equipment for the portable, non-targeted detection of RNA and DNA viruses in animals from a large zoological facility, to simulate a field setting for point-of-incidence virus detection. From the resulting metagenomic data, we detected 13 vertebrate viruses from four major virus groups: (+)ssRNA, (+)ssRNA-RT, dsDNA and (+)ssDNA, including avian leukosis virus in domestic chickens (*Gallus gallus*), enzootic nasal tumour virus in goats (*Capra hircus*) and several small, circular, Rep-encoding, ssDNA (CRESS DNA) viruses in several mammal species. More significantly, we demonstrate that the mNGS method is able to detect potentially lethal animal viruses, such as elephant endotheliotropic herpesvirus in Asian elephants (*Elephas maximus*) and the newly described human-associated gemykibivirus 2, a human-to-animal cross-species virus, in a Linnaeus two-toed sloth (*Choloepus didactylus*) and its enclosure, for the first time.

Gallini, S. H., P. M. DiGeronimo, E. Ward, W. Thepapichaikul, K. Tachampa, N. D. Girolamo and J. Brandão (2023). "EVALUATION OF PLASMA CARDIAC TROPONIN I IN ASIAN ELEPHANTS (*ELEPHAS MAXIMUS*) USING TWO CLINICAL ANALYZERS." *J Zoo Wildl Med* **53**(4): 654-660.

Cardiac troponin I (cTnI) is specific to myocardial tissue, highly conserved across taxa, and a reliable indicator of myocardial disease in human and veterinary medicine. Biomarkers, like cTnI, may be useful for cardiac evaluation of elephants because the application of other modalities is complicated by the size of the animal. The goal of this study was to establish observed ranges for plasma cTnI in Asian elephants (*Elephas maximus*) measured by two point-of-care analyzers. Blood was collected from captive juvenile ( $\leq 15$  yr;  $n = 9$ ), adult (16-50 yr;  $n = 42$ ), and geriatric ( $> 50$  yr;  $n = 16$ ) elephants. Following centrifugation, heparinized plasma was stored at 5°C prior to and in between analyses on iSTAT (Abbott Point of Care Inc, Princeton, NJ 08540, USA) and HUBI-QUANpro (Humiasis Co, Ltd, Anyang-si 14042, South Korea) analyzers. With the exception of two results, plasma concentrations of cTnI were below the limit of quantification (LOQ  $< 0.05$  ng/ml) for the HUBI-QUANpro ( $n = 64$ ), which prohibited comparison between the two analyzers. Observed ranges were determined for plasma cTnI concentrations reported by the iSTAT for the entire population sampled ( $n = 58$ ; mean 0.011 ng/ml; SD  $\pm 0.013$  ng/ml; range 0.00-0.07 ng/ml; 95% CI 0.008-0.015 ng/ml; median 0.01 ng/ml) and with outliers excluded ( $n = 50$ ; mean 0.007 ng/ml; SD  $\pm 0.007$  ng/ml; range 0.00-0.02 ng/ml; 95% CI 0.005-0.009 ng/ml; median 0.01 ng/ml). No significant differences were observed between age classes ( $P = 0.70$ ) or sexes ( $P = 0.34$ ). Higher cTnI concentrations were significantly correlated with increasing age (Pearson's  $r = 0.426$ ;  $P = 0.002$ ). Future studies are warranted to investigate the diagnostic potential of plasma cTnI in Asian elephants.

Gao, Y., Y. Liu, Y. Luo, D. Biggs, W. Zhao and S. G. Clark (2023). "Tracking Chinese newspaper coverage of elephant ivory through topic modeling." *Conserv Biol*: e14072.

Mass media worldwide has contributed to increasing awareness of the illegal wildlife trade and its significant impact on wildlife conservation. We used mass media coverage as a proxy for macro-level public opinion to analyze the media framing of elephant ivory in 6394 Chinese newspaper articles published from 2000 to 2021 and thus determine the effects of wildlife policies on public opinion. We focused on 2 events: the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) approval of China as a trading partner in the purchase and import of ivory stockpiles from Africa in July 2008 and the Chinese government's announcement of a domestic ivory ban in December 2016. Using latent Dirichlet allocation topic modeling, we identified 8 topics about elephant ivory and

grouped them into 3 frames: ivory arts and culture, ivory crimes, and elephant conservation. Over the last 2 decades, topics related to ivory crimes remained the most prevalent in news articles. Topics about ivory arts and culture showed a significant shift in media salience before and after the 2 events (from 0.44 to 0.19 and from 0.08 to 0.15, respectively,  $p < 0.05$ ), whereas the other 2 frames did not change significantly. Contrary to popular belief, our results indicated that Chinese macro-level public opinion on ivory had become more negative following the CITES approval of ivory importation and less negative after the ivory ban announcement, at least for certain periods. The relationship between mass media, public opinion, and wildlife trade policies is complex and requires further examination of the sociopolitical dynamics that influence media narratives. Our results showed the value of topic modeling in monitoring and assessing media representations of wildlife issues in the era of big data. Conservationists should remain vigilant of mass media coverage and collaborate with media practitioners to produce comprehensive narratives on wildlife issues if resources permit.

Gao, Y., Y. Liu, Y. Luo, D. Biggs, W. Zhao and S. G. Clark (2023). "Tracking Chinese newspaper coverage of elephant ivory through topic modeling." *Conserv Biol* **37**(5): e14072.

Mass media worldwide has contributed to increasing awareness of the illegal wildlife trade and its significant impact on wildlife conservation. We used mass media coverage as a proxy for macro-level public opinion to analyze the media framing of elephant ivory in 6394 Chinese newspaper articles published from 2000 to 2021 and thus determine the effects of wildlife policies on public opinion. We focused on 2 events: the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) approval of China as a trading partner in the purchase and import of ivory stockpiles from Africa in July 2008 and the Chinese government's announcement of a domestic ivory ban in December 2016. Using latent Dirichlet allocation topic modeling, we identified 8 topics about elephant ivory and grouped them into 3 frames: ivory arts and culture, ivory crimes, and elephant conservation. Over the last 2 decades, topics related to ivory crimes remained the most prevalent in news articles. Topics about ivory arts and culture showed a significant shift in media salience before and after the 2 events (from 0.44 to 0.19 and from 0.08 to 0.15, respectively,  $p < 0.05$ ), whereas the other 2 frames did not change significantly. Contrary to popular belief, our results indicated that Chinese macro-level public opinion on ivory had become more negative following the CITES approval of ivory importation and less negative after the ivory ban announcement, at least for certain periods. The relationship between mass media, public opinion, and wildlife trade policies is complex and requires further examination of the sociopolitical dynamics that influence media narratives. Our results showed the value of topic modeling in monitoring and assessing media representations of wildlife issues in the era of big data. Conservationists should remain vigilant of mass media coverage and collaborate with media practitioners to produce comprehensive narratives on wildlife issues if resources permit.

Garaï, M. E., V. L. Boulton and H. R. Zitzer (2023). "Identifying the Effects of Social Disruption through Translocation on African Elephants (*Loxodonta africana*), with Specifics on the Social and Ecological Impacts of Orphaning." *Animals (Basel)* **13**(3).

African elephants (*Loxodonta africana*) exhibit a long developmental period during which they acquire complex social and ecological knowledge through social networks. Central to this is that matriarchs and older individuals play an important role as repositories of information gained through experience. Anthropogenic interventions-including poaching, culling, translocation, and hunting-can disrupt elephants' social networks, with implications for individual fitness and potential long-term population viability. Here, we draw on a unique

long-running, individual-based dataset to examine the impacts of translocation on a population of elephants in South Africa, taking into consideration demographic rates, social dynamics, and ecological decision-making. Specifically, we compared two translocated groups: a group of unrelated culling Orphans and a family herd. We found that the Orphan group experienced accelerated reproductive rates when compared with the family herd. The Orphan group also fissioned more frequently and for longer periods of time, suggesting lower cohesiveness, and were less decisive in their large-scale movement decisions. These results add to the growing body of literature on the downstream impacts of social disruption for elephants. Whilst the translocation of culling Orphans is no longer practised in South Africa, we encourage careful consideration of any elephant translocation and the resulting social disruption.

Gaudzinski-Windheuser, S., L. Kindler, K. MacDonald and W. Roebroeks (2023). "Hunting and processing of straight-tusked elephants 125,000 years ago: Implications for Neanderthal behavior." Sci Adv **9**(5): eadd8186.

Straight-tusked elephants (*Palaeoloxodon antiquus*) were the largest terrestrial mammals of the Pleistocene, present in Eurasian landscapes between 800,000 and 100,000 years ago. The occasional co-occurrence of their skeletal remains with stone tools has generated rich speculation about the nature of interactions between these elephants and Pleistocene humans: Did hominins scavenge on elephants that died a natural death or maybe even hunt some individuals? Our archaeozoological study of the largest *P. antiquus* assemblage known, excavated from 125,000-year-old lake deposits in Germany, shows that hunting of elephants weighing up to 13 metric tons was part of the cultural repertoire of Last Interglacial Neanderthals there, over >2000 years, many dozens of generations. The intensity and nutritional yields of these well-documented butchering activities, combined with previously reported data from this Neumark-Nord site complex, suggest that Neanderthals were less mobile and operated within social units substantially larger than commonly envisaged.

Gaudzinski-Windheuser, S., L. Kindler and W. Roebroeks (2023). "Widespread evidence for elephant exploitation by Last Interglacial Neanderthals on the North European plain." Proc Natl Acad Sci U S A **120**(50): e2309427120.

Neanderthals hunted and butchered straight-tusked elephants, the largest terrestrial mammals of the Pleistocene, in a lake landscape on the North European plain, 125,000 years ago, as recently shown by a study of the Last Interglacial elephant assemblage from Neumark-Nord (Germany). With evidence for a remarkable focus on adult males and on their extended utilization, the data from this location are thus far without parallel in the archaeological record. Given their relevance for our knowledge of the Neanderthal niche, we investigated whether the Neumark-Nord subsistence practices were more than a local phenomenon, possibly determined by local characteristics. Analyzing elephant remains from two other Last Interglacial archaeological sites on the North European plain, Gröbern and Taubach, we identified in both assemblages similar butchering patterns as at Neumark-Nord, demonstrating that extended elephant exploitation was a widespread Neanderthal practice during the (early part of the) Last Interglacial. The substantial efforts needed to process these animals, weighing up to 13 metric tons, and the large amounts of food generated suggest that Neanderthals either had ways of storing vast amounts of meat and fat and/or temporarily aggregated in larger groups than commonly acknowledged. The data do not allow us to rule out one of the two explanations, and furthermore both factors, short-term larger group sizes as well as some form of food preservation, may have played a role. What the data do show is that exploitation of large straight-tusked elephants was a



widespread and recurring phenomenon amongst Last Interglacial Neanderthals on the North European plain.

Gautam, H. and T. N. C. Vidya (2023). "Do food distribution and competitor density affect agonistic behaviour within and between clans in a high fission-fusion species?" *R Soc Open Sci* **10**(12): 230990.

According to the ecological model of female social relationships (EMFSR), within-group competition and between-group competition in female-bonded species are shaped by food distribution. Strong between-group contests are expected over large, monopolizable resources and high population density, but not when low-quality food is distributed across large, undefended home ranges. Within-group contests are expected to be more frequent with increasing heterogeneity among feeding sites and with group size. We tested these predictions in female Asian elephants, which show traits associated with infrequent contests-graminivory, high fission-fusion and overlapping home ranges. We examined how food distribution and competitor density affected agonistic interactions within and between female elephant clans (social groupings) in the Kabini grassland, southern India. We found stronger between-clan contest in the grassland than that known from neighbouring forests, and more frequent agonism between females between clans than within clans. Such strong between-clan contest was attributable to the grassland being a food-rich habitat patch, thus supporting the EMFSR. Within-clan agonism was also frequent, but did not increase with food heterogeneity, contradicting the EMFSR. Contrary to recent claims, increasing within-clan agonism with group size suggested ecological constraints on large groups despite high fission-fusion. High population density may explain such frequent contests despite graminivory and fission-fusion.

Giliba, R. A., C. Kiffner, P. Fust and J. Loos (2023). "Modelling elephant corridors over two decades reveals opportunities for conserving connectivity across a large protected area network." *PLoS ONE* **18**(10): e0292918.

Protected area (PA) connectivity is pivotal for the persistence of wide-ranging wildlife species, but is challenged by habitat loss and fragmentation. We analyzed habitat suitability and connectivity for the African elephant (*Loxodonta africana*) across PAs in south-western Tanzania in 2000, 2010, and 2019. We quantified land-use changes through remote sensing data; estimated habitat suitability through aerial survey data, remotely sensed variables and ensemble species distribution models; modelled least-cost corridors; identified the relative importance of each corridor for the connectivity of the PA network and potential bottlenecks over time through circuit theory; and validated corridors through local ecological knowledge and ground wildlife surveys. From 2000 to 2019, cropland increased from 7% to 13% in the region, with an average expansion of 634 km<sup>2</sup> per year. Distance from cropland influenced elephant distribution models the most. Despite cropland expansion, the locations of the modelled elephant corridors (n = 10) remained similar throughout the survey period. Based on local ecological knowledge, nine of the modelled corridors were active, whereas one modelled corridor had been inactive since the 1970s. Based on circuit theory, we prioritize three corridors for PA connectivity. Key indicators of corridor quality varied over time, whereas elephant movement through some corridors appears to have become costlier over time. Our results suggest that, over the past two decades, functional connectivity across the surveyed landscape has largely persisted. Beyond providing crucial information for spatial prioritization of conservation actions, our approach highlights the importance of modeling functional connectivity over time and verifying corridor models with ground-truthed data.

Gill, B. A., G. Wittemyer, T. E. Cerling, P. M. Musili and T. R. Kartzinel (2023). "Foraging history of individual elephants using DNA metabarcoding." R Soc Open Sci **10**(7): 230337.

Individual animals should adjust diets according to food availability. We used DNA metabarcoding to construct individual-level dietary timeseries for elephants from two family groups in Kenya varying in habitat use, social position and reproductive status. We detected at least 367 dietary plant taxa, with up to 137 unique plant sequences in one fecal sample. Results matched well-established trends: elephants tended to eat more grass when it rained and other plants when dry. Nested within these switches from 'grazing' to 'browsing' strategies, dietary DNA revealed seasonal shifts in food richness, composition and overlap between individuals. Elephants of both families converged on relatively cohesive diets in dry seasons but varied in their maintenance of cohesion during wet seasons. Dietary cohesion throughout the timeseries of the subdominant 'Artists' family was stronger and more consistently positive compared to the dominant 'Royals' family. The greater degree of individuality within the dominant family's timeseries could reflect more divergent nutritional requirements associated with calf dependency and/or priority access to preferred habitats. Whereas theory predicts that individuals should specialize on different foods under resource scarcity, our data suggest family bonds may promote cohesion and foster the emergence of diverse feeding cultures reflecting links between social behaviour and nutrition.

Gordon, C. E., M. Greve, M. Henley, A. Bedetti, P. Allin and J. C. Svenning (2023). "Elephant rewilding affects landscape openness and fauna habitat across a 92-year period." Ecol Appl **33**(3): e2810.

Trophic rewilding aims to promote biodiverse self-sustaining ecosystems through the restoration of ecologically important taxa and the trophic interactions and cascades they propagate. How rewilding effects manifest across broad temporal scales will determine ecosystem states; however, our understanding of post-rewilding dynamics across longer time periods is limited. Here we show that the restoration of a megaherbivore, the African savannah elephant (*Loxodonta africana*), promotes landscape openness (i.e., various measures of vegetation composition/complexity) and modifies fauna habitat and that these effects continue to manifest up to 92 years after reintroduction. We conducted a space-for-time floristic survey and assessment of 17 habitat attributes (e.g., floristic diversity and cover, ground wood, tree hollows) across five comparable nature reserves in South African savannah, where elephants were reintroduced between 1927 and 2003, finding that elephant reintroduction time was positively correlated with landscape openness and some habitat attributes (e.g., large-sized tree hollows) but negatively associated with others (e.g., large-sized coarse woody debris). We then indexed elephant site occurrence between 2006 and 2018 using telemetry data and found positive associations between site occurrence and woody plant densities. Taken alongside the longer-term space-for-time survey, this suggests that elephants are attracted to dense vegetation in the short term and that this behavior increases landscape openness in the long term. Our results suggest that trophic rewilding with elephants helps promote a semi-open ecosystem structure of high importance for African biodiversity. More generally, our results suggest that megafauna restoration represents a promising tool to curb Earth's recent ecological losses and highlights the importance of considering long-term ecological responses when designing and managing rewilding projects.

Greenspoon, L., E. Krieger, R. Sender, Y. Rosenberg, Y. M. Bar-On, U. Moran, T. Antman, S. Meiri, U. Roll, E. Noor and R. Milo (2023). "The global biomass of wild mammals." Proc Natl Acad Sci U S A **120**(10): e2204892120.

Wild mammals are icons of conservation efforts, yet there is no rigorous estimate available

for their overall global biomass. Biomass as a metric allows us to compare species with very different body sizes, and can serve as an indicator of wild mammal presence, trends, and impacts, on a global scale. Here, we compiled estimates of the total abundance (i.e., the number of individuals) of several hundred mammal species from the available data, and used these to build a model that infers the total biomass of terrestrial mammal species for which the global abundance is unknown. We present a detailed assessment, arriving at a total wet biomass of  $\approx 20$  million tonnes (Mt) for all terrestrial wild mammals (95% CI 13-38 Mt), i.e.,  $\approx 3$  kg per person on earth. The primary contributors to the biomass of wild land mammals are large herbivores such as the white-tailed deer, wild boar, and African elephant. We find that even-hoofed mammals (artiodactyls, such as deer and boars) represent about half of the combined mass of terrestrial wild mammals. In addition, we estimated the total biomass of wild marine mammals at  $\approx 40$  Mt (95% CI 20-80 Mt), with baleen whales comprising more than half of this mass. In order to put wild mammal biomass into perspective, we additionally estimate the biomass of the remaining members of the class Mammalia. The total mammal biomass is overwhelmingly dominated by livestock ( $\approx 630$  Mt) and humans ( $\approx 390$  Mt). This work is a provisional census of wild mammal biomass on Earth and can serve as a benchmark for human impacts.

Gübert, J., G. Schneider, M. Hahn-Klimroth and P. W. Dierkes (2023). "Nocturnal behavioral patterns of African ungulates in zoos." *Ecol Evol* **13**(12): e10777.

Currently, most studies on ungulates' behavior are conducted during the daylight hours, but their nocturnal behavior patterns differ from those shown during day. Therefore, it is necessary to observe ungulates' behavior also overnight. Detailed analyses of nocturnal behavior have only been conducted for very prominent ungulates such as Giraffes (*Giraffa camelopardalis*), African Elephants (*Loxodonta africana*), or livestock (e.g., domesticated cattle, sheep, or pigs), and the nocturnal rhythms exhibited by many ungulates remain unknown. In the present study, the nocturnal rhythms of 192 individuals of 18 ungulate species from 20 European zoos are studied with respect to the behavioral positions standing, lying-head up, and lying-head down (the typical REM sleep position). Differences between individuals of different age were found, but no differences with respect to the sex were seen. Most species showed a significant increase in the proportion of lying during the night. In addition, the time between two events of "lying down" was studied in detail. A high degree of rhythmicity with respect to this quantity was found in all species. The proportion of lying in such a period was greater in Artiodactyla than in Perissodactyla, and greater in juveniles than in adults.

Harrington, L. A., A. Elwin and N. D'Cruze (2023). "Elephant 'selfies': Evaluating the effectiveness of Instagram's warning of the potential negative impacts of photo opportunities with wild animals." *PLoS ONE* **18**(4): e0283858.

Wildlife tourist attractions offering opportunities to observe, touch, and interact with wild animals, are visited by millions of people every year. Wildlife tourism has considerable economic value in many countries and can have positive impacts on wild animal populations (e.g. through habitat protection); it can also have negative impacts on population conservation and individual welfare (due to, e.g. habitat encroachment, disturbance, or disease). The recent phenomenon of 'wildlife selfies' shared on social media may seem harmless but can involve animals illegally or unsustainably captured from the wild, kept in poor conditions, or subject to cruel treatment. To address this issue, Instagram introduced a pop-up alert system that is triggered when users search for wild animal selfie hashtags (e.g. #elephantselfie), warning of the potential negative impacts of wildlife selfies on wild animals. Using elephant selfies as a case study, we found that Instagram's alert was

triggered by only 2% of 244 elephant selfie-related hashtags tested. By comparing three pairs of similar hashtags (one of each pair that triggered the warning and one that did not), we were unable to detect a consistent difference in the type of post using each of the hashtags, the popularity of posts, or the sentiment of viewer comments. The warning is not shown when posting an image, or if a post is viewed directly by a follower, only if the post is encountered via a hashtag search. Currently, what is portrayed on social media appears to be inconsistent with apparent recent shifts in social acceptability regarding tourism, particularly as concerns direct contact between tourists and elephants. Instagram's wildlife selfie initiative was commendable but given its apparent lack of effect, we urge Instagram and other social platforms to do more to prevent harmful content from being posted on their platforms and to promote fair, ethical and sustainable interactions between wild animals and people.

Hoerner, F., A. Lawrenz, A. K. Oerke, D. W. H. Müller, I. Azogu-Sepe, M. Roller, K. Damerou and A. Preisfeld (2023). "Long-Term Olfactory Memory in African Elephants." *Animals (Basel)* **13**(4).

African elephants are capable of discriminating scents up to a single changed molecule and show the largest reported repertoire of olfactory receptor genes. Olfaction plays an important role in family bonding. However, to the best of our knowledge, no empirical data exist on their ability to remember familiar scents long-term. In an ethological experiment, two mother-daughter pairs were presented with feces of absent kin, absent non-kin, and present non-kin. Video recordings showed reactions of elephants recognizing kin after long-term separation but only minor reactions to non-kin. Results give the empirical implication that elephants have an olfactory memory longer than 1 year and up to 12 years and can distinguish between kin and non-kin just by scent. These findings confirm the significance of scent for family bonds in African elephants.

Hoerner, F., J. Rendle-Worthington, A. Lawrenz, A. K. Oerke, K. Damerou, S. Borragán Santos, T. Hard and G. Preisfeld (2023). "Differences in Mother-Infant Bond and Social Behavior of African Elephant Calves Living In Situ and Ex Situ." *Animals (Basel)* **13**(19).

African zoo elephants live in safe environments with sufficient resources, are protected from threats, and have their health and body conditions cared for. Calves ex situ undergo the same developmental stages as in situ and are raised by the whole family unit. However, due to environmental differences, there might be behavioral modifications between calves in situ and ex situ. We hypothesize that these differences increase with ongoing generations. This ethological study compares social and general behavior and the distance calves kept to their mothers' between calves of the first (F1) and second (F2) zoo generation and the wild. Using ethological methods, data were collected for ~90 in situ calves and 16 ex situ (8 F1, 8 F2) between the ages of 0.5 to 4 years (120 observation hours per group). Results showed that in situ calves spent significantly more time close to mothers than the F1 and the F2 zoo generations (F1/in situ:  $p = <0.001$ ; F2/in situ:  $p = 0.007$ ). The behaviors of eating, drinking, trunk movement, washing, and affiliative behaviors showed significant differences between in situ and ex situ calves. The amount and distribution of affiliative and agonistic behavior initiated and received by calves was displayed with a greater variety ex situ. Ex situ calves not only performed affiliative but, in contrast to the in situ, also agonistic behavior (F1/in situ: initiated  $p = 0.002$ , received  $p = 0.010$ ; F2/in situ: initiated  $p = 0.050$ , received  $p = 0.037$ ). The comparison of zoo generations suggests that differences did not increase with the generation. The more casual bonding between mothers and offspring in zoos and the age-dependent improvement of social behavior of zoo-born calves are seen as a result of elephants' adaptation to secure zoo conditions. The results of this study agree with the faster development of ex situ African elephants, like earlier puberty and more

frequent breeding patterns, as known from the literature.

Imron, M. A., D. M. Glass, M. Tafrichan, R. D. Crego, J. A. Stabach and P. Leimgruber (2023). "Beyond protected areas: The importance of mixed-use landscapes for the conservation of Sumatran elephants (*Elephas maximus sumatranus*)." *Ecol Evol* **13**(10): e10560.

Elephants were once widely distributed across the Indonesian island of Sumatra but now exist in small, isolated populations. Using the best data available on elephant occurrence, we aimed to (a) predict potential habitat suitability for elephants (*Elephas maximus sumatranus*) across the island of Sumatra and (b) model landscape connectivity among the extant elephant populations. We used direct sightings and indirect observations of elephant signs, as well as six remotely sensed proxies of surface ruggedness, vegetation productivity and structure, and human land use and disturbance, to model habitat suitability in a Google Earth Engine (GEE) environment. We validated the habitat suitability prediction using 10-fold spatial block cross validation and by calculating the area under the precision-recall curve (AUC-PR), sensitivity, and specificity for each model iteration. We also used a geolocation dataset collected from global positioning system (GPS) collars fitted on elephants as an independent validation dataset. Models showed good predictive performance with a mean AUC-PR of 0.73, sensitivity of 0.76, and specificity of 0.68. Greater than 83% of the independent GPS collar geolocations were located in predicted suitable habitat. We found human modification, surface ruggedness, and normalized difference vegetation index to be the most important variables for predicting suitable elephant habitat. Thirty-two percent, or 135,646 km<sup>2</sup>, of Sumatra's land area was predicted to be suitable habitat, with 43 patches of suitable habitat located across Sumatra. Areas with high connectivity were concentrated in the Riau and North Sumatra provinces. Though our analysis highlights the need to improve the quality of data collected on Sumatran elephants, more suitable habitat remains on Sumatra than is used by known populations. Targeted habitat conservation, especially of the suitable habitat in and around the Lamno, Balai Raja, Tesso Tenggara, Tesso Utara, Bukit Tigapuluh, Seblat, Padang Sugihan, and Bukit Barisan Selatan ranges, may improve the long-term viability of this critically endangered species.

Jansen van Vuuren, A., J. Bolcaen, M. Engelbrecht, W. Burger, M. De Kock, M. Durante, R. Fisher, W. Martínez-López, X. Miles, F. Rahiman, W. Tinganelli and C. Vandevoorde (2023). "Establishment of Primary Adult Skin Fibroblast Cell Lines from African Savanna Elephants (*Loxodonta africana*)." *Animals (Basel)* **13**(14).

Following population declines of the African savanna elephant (*Loxodonta africana*) across the African continent, the establishment of primary cell lines of endangered wildlife species is paramount for the preservation of their genetic resources. In addition, it allows molecular and functional studies on the cancer suppression mechanisms of elephants, which have previously been linked to a redundancy of tumor suppressor gene TP53. This methodology describes the establishment of primary elephant dermal fibroblast (EDF) cell lines from skin punch biopsy samples (diameter:  $\pm 4$  mm) of African savanna elephants ( $n = 4$ , 14-35 years). The applied tissue collection technique is minimally invasive and paves the way for future remote biopsy darting. On average, the first explant outgrowth was observed after  $15.75 \pm 6.30$  days. The average doubling time ( $T_d$ ) was  $93.02 \pm 16.94$  h and  $52.39 \pm 0.46$  h at passage 1 and 4, respectively. Metaphase spreads confirmed the diploid number of 56 chromosomes. The successful establishment of EDF cell lines allows for future elephant cell characterization studies and for research on the cancer resistance mechanisms of elephants, which can be harnessed for human cancer prevention and treatment and contributes to the conservation of their genetic material.

Jiang, X., H. J. Liu, Z. Y. Jiang and R. P. Ni (2023). "Identifying Migration Routes of Wild Asian Elephants in China Based on Ecological Networks Constructed by Circuit Theory Model." Animals (Basel) **13**(16).

Humans overlap with Asian elephants, resulting in frequent costly human-elephant conflicts, which disturb and even threaten local residents. In this study, we treat provincial and national nature reserves where Asian elephants still exist and other alternative habitats suitable for Asian elephants in southern Yunnan, China, as ecological patches. By using this approach, we can treat the terrain and surface state factors that hinder the migration of Asian elephants as a form of ecological resistance surface. We can then use a circuit theory model and remote sensing data to construct an ecological network, which allows us to identify ecological corridors and ecological pinch points. Herein, the possible migration routes of wild Asian elephants were identified. The main results are as follows: (1) In the study area, dense forests with steep slopes and high altitudes, cultivated land, and building land have greater migration resistance, while the gently undulating shrubs, bamboo forests, and grasslands far away from the city have less migration resistance. (2) There are three ecological corridor groups in the study area, mainly composed of shrub and grassland. The ecological corridors identified in this paper are the most likely migration routes of wild Asian elephants in China, and areas with higher simulated current densities reflect a higher probability of Asian elephants passing through. (3) According to the analysis, the ecological pinch points in the study area are 602 km<sup>2</sup> in total, and woodland and grassland account for 89.2% of the total ecological pinch area. The areas where the pinch points are located have a high probability of Asian elephants passing through and a narrow space. Our findings can provide suggestions and solutions for the current conservation of wild Asian elephant species, alleviate human-elephant conflicts, promote the harmonious coexistence between humans and nature, and provide reference for biological protection and biological reserve planning.

Kaufmann, L. V., R. Becker, A. Ochs and M. Brecht (2023). "Elephant banana peeling." Curr Biol **33**(7): R257-r258.

Video abstract.

Kelly, S., Y. Dong, W. Wang, S. Matthee, J. M. Wentzel, L. A. Durden and R. Shao (2023). "Mitochondrial genome sequence comparisons indicate that the elephant louse *Haematomyzus elephantis* (Piaget, 1869) contains cryptic species." Med Vet Entomol.

The parvorder Rhynchophthirina contains three currently recognised species of lice that parasitize elephants (both African savanna elephant *Loxodonta africana* and Asian elephant *Elephas maximus*), desert warthogs (*Phacochoerus aethiopicus*) and Red River hogs (*Potamochoerus porcus*), respectively. The Asian elephant lice and the African savanna elephant lice are currently treated as the same species, *Haematomyzus elephantis* (Piaget, 1869), based on morphology despite the fact that their hosts diverged 8.4 million years ago. In the current study, we sequenced 23 mitochondrial (mt) genes of African savanna elephant lice collected in South Africa and analysed the sequence divergence between African savanna elephant lice and previously sequenced Asian elephant lice. Sequence comparisons revealed >23% divergence for the 23 mt genes as a whole and ~17% divergence for *cox1* gene between African savanna and Asian elephant lice, which were far higher than the divergence expected within a species. Furthermore, the mt gene sequence divergences between these lice are 3.76-4.6 times higher than that between their hosts, the African savanna and Asian elephants, which are expected for the co-divergence and co-evolution between lice and their elephant hosts. We conclude that (1) *H. elephantis* (Piaget,

1869) contains cryptic species and (2) African savanna and Asian elephant lice are different species genetically that may have co-diverged and co-evolved with their hosts.

Kerr, T. J., J. van Heerden, W. J. Goosen, L. Kleynhans, P. E. Buss, E. Latimer and M. A. Miller (2023). "DETECTION OF ELEPHANT ENDOTHELIOTROPIC HERPESVIRUS (EEHV) IN FREE-RANGING AFRICAN ELEPHANTS (*LOXODONTA AFRICANA*) IN THE KRUGER NATIONAL PARK, SOUTH AFRICA." *J Wildl Dis* **59**(1): 128-137.

Elephant endotheliotropic herpesvirus (EEHV) infection can cause acute, often fatal, EEHV hemorrhagic disease in free-ranging and human-managed Asian elephants (*Elephas maximus*) and human-managed African elephants (*Loxodonta africana*). However, significant knowledge gaps exist pertaining to the presence of EEHV in free-ranging African elephant populations. We retrospectively screened 142 opportunistically collected samples (blood, n=98; bronchoalveolar lavage (BAL) fluid, n=21; trunk wash (TW) fluid, n=23) obtained between 2010 and 2020 from 98 free-ranging African elephants in the Kruger National Park, South Africa, for the presence of different EEHVs, as well as determining the real-time quantitative PCR positivity rate in this population. With the use of validated, previously published DNA extraction and real-time quantitative PCR protocols provided by the National Elephant Herpesvirus Laboratory (Washington, DC, USA), EEHV was detected in nine male African elephants from samples collected in 2011 (n=1), 2013 (n=1), 2018 (n=2), 2019 (n=4), and 2020 (n=1). Viral detection was more common in respiratory compared with blood samples. Six elephants tested positive for EEHV2 subtype (blood, n=2; BAL, n=3; TW, n=2), including one individual that tested positive on matched respiratory samples (BAL and TW). Four elephants tested positive for EEHV3-4-7 (blood, n=1; BAL, n=2; TW, n=1), whereas EEHV6 was not detected in any of the study animals. One elephant tested positive for both EEHV2 and EEHV3-4-7 in the same BAL sample. Even though the levels of viremia varied between 158 and 1,292 viral genome equivalents/mL blood and viral shedding of EEHV2 and EEHV3-4-7 was detected in respiratory samples, no clinical signs were observed in these apparently healthy elephants. These findings are consistent with reports of asymptomatic EEHV infection in human-managed African elephants.

Kilburn, J. J., D. Schmitt, W. Kiso, M. G. Papich and K. A. Backues (2023). "PHARMACOKINETICS OF RECTALLY AND ORALLY ADMINISTERED LEVOFLOXACIN IN ASIAN ELEPHANTS (*ELEPHAS MAXIMUS*)."  
*J Zoo Wildl Med* **53**(4): 670-678.

Appropriate and effective antibiotic use is a critical component of veterinary medicine, but there are variations across species regarding dosage and administration of these drugs. Oral or rectal routes of administration are typically used in elephants, but not all medications can achieve adequate concentrations rectally. The fluoroquinolone antimicrobials are used in elephants because of their favorable antimicrobial spectrum and pharmacokinetics compared with other oral agents. They are commonly used as part of multiple antibiotic regimens for the treatment of tuberculosis (*Mycobacterium tuberculosis*). The objective of this study was to determine the pharmacokinetic profile of levofloxacin after oral and rectal administration in Asian elephants (*Elephas maximus*). Dosages of 5 mg/kg orally and 15 mg/kg rectally were evaluated in 13 Asian elephants. Blood was collected at various time points from 0 to 72 h for pharmacokinetic analysis. Pharmacokinetic parameters were determined and reached concentrations above minimum inhibitory concentrations of various bacterial organisms via both routes. A pharmacokinetic-pharmacodynamic assessment was used to estimate appropriate minimal inhibitory concentrations for bacteria that could be potentially treated with this antimicrobial. Based on these findings, levofloxacin may be a consideration for administration orally (5 mg/kg) and rectally (15 mg/kg) in Asian elephants. Antimicrobial stewardship principles, culture and susceptibility of suspected

pathogens, and blood level monitoring should be used to tailor administration of levofloxacin in this species.

Klinhom, S., S. Sriwichaiin, S. Kerdphoo, J. Khonmee, N. Chattipakorn, S. C. Chattipakorn and C. Thitaram (2023). "Characteristics of gut microbiota in captive Asian elephants (*Elephas maximus*) from infant to elderly." *Sci Rep* **13**(1): 23027.

Gut microbiota play an important role in the health and disease of Asian elephants, however, its characteristics at each stage of life have not been thoroughly investigated in maintaining and regulating health of elephants. This study, therefore, aimed to characterize the profiles of the gut microbiota of captive Asian elephants from infants to the elderly. Gut microbiota were identified by 16S rRNA sequencing from the feces of captive Asian elephants with varying age groups, including infant calves, suckling calves, weaned calves, subadult and adult elephants, and geriatric elephants. The diversity of the gut microbiota was lowest in infants, stable during adulthood, and slightly decreased in the geriatric period. The gut microbiota of the infant elephants was dominated by milk-fermenting taxa including genus *Bifidobacterium* of family Bifidobacteriaceae together with genus *Akkermansia*. The fiber-fermenting taxa such as Lachnospiraceae\_NK3A20\_group were found to be increased in suckling elephants in differential abundance analysis by Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC). The gut microbiota profiles after weaning until the adult period has been uniform as indicated by no significant differences in beta diversity between groups. However, the composition of the gut microbiota was found to change again in geriatric elephants. Understanding of the composition of the gut microbiota of captive Asian elephants at various life stages could be beneficial for promoting good health throughout their lifespan, as well as ensuring the welfare of captive elephants.

Klinsawat, W., P. Uthapaisanwong, P. Jenjaroenpun, S. Sripiboon, T. Wongsurawat and K. Kusonmano (2023). "Microbiome variations among age classes and diets of captive Asian elephants (*Elephas maximus*) in Thailand using full-length 16S rRNA nanopore sequencing." *Sci Rep* **13**(1): 17685.

Asian elephant (*Elephas maximus*) is the national symbol of Thailand and linked to Thai history and culture for centuries. The elephant welfare improvement is one of the major components to achieve sustainable captive management. Microbiome inhabiting digestive tracts have been shown with symbiotic relations to host health. This work provided high-resolution microbiome profiles of 32 captive elephants at a species level by utilizing full-length 16S rRNA gene nanopore sequencing. Eleven common uncultured bacterial species were found across elephants fed with solid food including uncultured bacterium Rikenellaceae RC9 gut group, Kiritimatiellae WCHB1-41, Phascolarctobacterium, Oscillospiraceae NK4A214 group, Christensenellaceae R-7 group, Oribacterium, Oscillospirales UCG-010, Lachnospiraceae, Bacteroidales F082, uncultured rumen Rikenellaceae RC9 gut group, and Lachnospiraceae AC2044 group. We observed microbiome shifts along the age classes of baby (0-2 years), juvenile (2-10 years), and adult (> 10 years). Interestingly, we found distinct microbiome profiles among adult elephants fed with a local palm, *Caryota urens*, as a supplement. Potential beneficial microbes have been revealed according to the age classes and feed diets. The retrieved microbiome data could be provided as good baseline microbial profiles for monitoring elephant health, suggesting further studies towards dietary selection suitable for each age class and the use of local supplementary diets.

Kongsawasdi, S., B. Chuatrakoon, T. Angkawanish, C. Thitaram, W. Langkaphin, K. Namwongprom, P. Prupetkaew and K. Wantanajittikul (2023). "Variability of gait characteristics in



lameness elephant." *J Vet Med Sci* **85**(2): 226-231.

Lameness has a significant impact not only on the economy but also on elephant welfare. Several gait characteristics are altered to compensate for the discomfort. The traditional approach to detecting lameness has relied on mahout and veterinarian observation. The study aimed to determine how lameness affected the variability of an elephant's gait by using a three-dimensional inertial measurement unit (IMU) with Wi-Fi sensors. Five elephants with lameness, as determined by an experienced veterinarian and two, non-lamed elephants, aged between 58-80 years were included in the study. Gait biomechanics including limb segment motion, obtained from individually gyrometric- and accelero-based parameters and demonstrated as a graphical pattern showing changes in absolute rotation angle over time. The result revealed some character changes in gait kinematics parameters, but it was heterogeneity with an inconclusive pattern. The interlimb coordination could be a part of maintaining the actual locomotion pattern, or it could be a result of the mild degree of lameness for which all of the clients have compensated. This study introduces a new objective method for quantifying gait changes caused by lameness, additional research is required to make this objective more clinically applicable.

Kosaruk, W., J. L. Brown, P. Towiboon, K. Pringproa, V. Punyapornwithaya, P. Tankaew, N. Kittisirikul, W. Toonrongchang, T. Janyamathakul, P. Muanghong and C. Thitaram (2023). "Seasonal patterns of oxidative stress markers in captive Asian elephants in Thailand and relationships to elephant endotheliotropic herpesvirus shedding." *Front Vet Sci* **10**: 1263775.

**INTRODUCTION:** Oxidative stress refers to an imbalance between oxidant and antioxidant activity and accumulation of reactive oxygen species, which can have detrimental effects on animal health. Annual fluctuations in oxidative stress status can occur, increasing disease susceptibility during certain time periods. However, a full understanding of factors related to oxidative stress in Asian elephants and how to mitigate the negative consequences is lacking. **METHODS:** This study measured six serum oxidative stress markers [reactive oxygen species (ROS), malondialdehyde (MDA), 8-hydroxydeoxyguanosine (8-OHdG), albumin, glutathione peroxidase (GPx), and catalase] and two stress markers [serum cortisol and fecal glucocorticoid metabolites (fGCM)] in 23 captive Asian elephants in Thailand over a 12 months period to examine relationships with age and season. **RESULTS:** Seasonal variations were observed, with several markers exhibiting significantly higher concentrations in the summer (ROS, MDA, 8-OHdG, albumin) and lower values during the rainy/winter seasons (MDA, 8-OHdG, albumin, catalase). By contrast, GPx was the only marker to be highest during the rainy season. For the stress markers, higher fGCM concentrations were noted during the rainy season, which contrasts with earlier studies showing more activity in the winter (tourist season). Positive correlations were found between the temperature-humidity index and ROS, GPx, and fGCM, while a negative correlation was observed with serum albumin. Elephant endotheliotropic herpesvirus (EEHV) shedding events were associated with higher concentrations of ROS and MDA. A moderate negative correlation was observed between 8-OHdG and the PCR threshold cycle of EEHV shedding (Ct), indicating DNA damage may be involved in EEHV shedding in elephants. **DISCUSSION:** Results revealed significant age and seasonal effects on several oxidative stress markers, indicating those factors should be considered in study design and data interpretation. There also may be physiological adaptations in oxidative stress conditions in relation to environmental changes that could impact health outcomes.

Kosaruk, W., J. L. Brown, P. Towiboon, V. Punyapornwithaya, K. Pringproa and C. Thitaram (2023). "Measures of Oxidative Status Markers in Relation to Age, Sex, and Season in Sick and Healthy Captive Asian Elephants in Thailand." *Animals (Basel)* **13**(9).

Oxidative stress is a pathological condition that can have adverse effects on animal health, although little research has been conducted on wildlife species. In this study, blood was collected from captive Asian elephants for the assessment of five serum oxidative status markers (reactive oxygen species (ROS) concentrations; malondialdehyde, MDA; albumin; glutathione peroxidase, GPx; and catalase) in healthy (n = 137) and sick (n = 20) animals. Health problems consisted of weakness, puncture wounds, gastrointestinal distress, eye and musculoskeletal problems, and elephant endotheliotropic herpesvirus hemorrhagic disease (EEHV-HD). Fecal samples were also collected to assess glucocorticoid metabolites (fGCMs) as a measure of stress. All data were analyzed in relation to age, sex, sampling season, and their interactions using generalized linear models, and a correlation matrix was constructed. ROS and serum albumin concentrations exhibited the highest concentrations in aged elephants (>45 years). No sex differences were found for any biomarker. Interactions were observed for age groups and seasons for ROS and catalase, while GPx displayed a significant interaction between sex and season. In pairwise comparisons, significant increases in ROS and catalase were observed in summer, with higher ROS concentrations observed only in the adult female group. Lower catalase activity was exhibited in juvenile males, subadult males, adult females, and aged females compared to subadult and adult elephants (males and females) in winter and the rainy season. There was a positive association between catalase activity and fGCMs ( $r = 0.23$ ,  $p < 0.05$ ), and a number of red blood cell parameters were positively associated with several of these biomarkers, suggesting high oxidative and antioxidative activity covary in red cells ( $p < 0.05$ ). According to health status, elephants with EEHV-HD showed the most significant changes in oxidative stress markers, with MDA, GPx, and catalase being higher and albumin being lower than in healthy elephants. This study provides an analysis of understudied health biomarkers in Asian elephants, which can be used as additional tools for assessing the health condition of this species and suggests age and season may be important factors in data interpretation.

Kottwitz, J., U. Bechert, C. Cruz-Espindola, J. M. Christensen and D. Boothe (2023). "SERUM DISPOSITION OF A SINGLE DOSE OF ORALLY ADMINISTERED FIROCOXIB IN AFRICAN ELEPHANTS (LOXODONTA AFRICANA)." Journal of zoo and wildlife medicine : official publication of the American Association of Zoo Veterinarians **54**(2): 350-359.

The time course of serum firocoxib concentrations was described after administration of two single oral doses (0.01 and 0.1 mg/kg) of commercially available firocoxib tablet (n = 4) and paste (n = 2) formulations to six healthy adult female African (*Loxodonta africana*) elephants. Firocoxib was quantitated by high-performance liquid chromatography. Firocoxib serum concentrations were below detectable levels after administration of 0.01 mg/kg of both formulations. A dose of 0.1 mg/kg (n = 4) of the tablet formulation had the following mean  $\pm$  SD of pharmacokinetic parameters: area under the curve (AUC)  $1,588 \pm 362$  h \* ng/ml, maximum plasma concentration (C<sub>max</sub>)  $31 \pm 6.6$  ng/ml at  $6.4 \pm 1.8$  h, and disappearance half-life (T<sub>1/2</sub>)  $66 \pm 59$  h. Elephant compliance to oral administration of the paste formulation was challenging, with only two elephants accepting administration of the paste at 0.1 mg/kg. Pharmacokinetic parameters determined included AUC of  $814$  h \* ng/ml, C<sub>max</sub> of  $44$  ng/ml at T<sub>max</sub> of  $7.0$  h, and T<sub>1/2</sub> of  $36.4$  h. Based on mean AUC, the relative bioavailability of paste compared to tablet formulations was 50%. Limitations of this study were the small number of participants and elephant compliance with the paste formulation. This study supports an oral dose of 0.1 mg/kg every 24 h. Multidose and IV trials are indicated to confirm firocoxib dosing requirements for African elephants.

Kuiper, T., R. Altwegg, C. Beale, T. Carroll, H. T. Dublin, S. Hauenstein, M. Kshatriya, C. Schwarz, C. R. Thouless, A. Royle and E. J. Milner-Gulland (2023). "Drivers and facilitators of the illegal

killing of elephants across 64 African sites." *Proc Biol Sci* **290**(1990): 20222270.

Ivory poaching continues to threaten African elephants. We (1) used criminology theory and literature evidence to generate hypotheses about factors that may drive, facilitate or motivate poaching, (2) identified datasets representing these factors, and (3) tested those factors with strong hypotheses and sufficient data quality for empirical associations with poaching. We advance on previous analyses of correlates of elephant poaching by using additional poaching data and leveraging new datasets for previously untested explanatory variables. Using data on 10 286 illegally killed elephants detected at 64 sites in 30 African countries (2002-2020), we found strong evidence to support the hypotheses that the illegal killing of elephants is associated with poor national governance, low law enforcement capacity, low household wealth and health, and global elephant ivory prices. Forest elephant populations suffered higher rates of illegal killing than savannah elephants. We found only weak evidence that armed conflicts may increase the illegal killing of elephants, and no evidence for effects of site accessibility, vegetation density, elephant population density, precipitation or site area. Results suggest that addressing wider systemic challenges of human development, corruption and consumer demand would help reduce poaching, corroborating broader work highlighting these more ultimate drivers of the global illegal wildlife trade.

Kurz, M. J. and J. R. Hutchinson (2023). "Visual feedback influences the consistency of the locomotor pattern in Asian elephants (*Elephas maximus*)." *Biol Lett* **19**(9): 20230260.

Elephants are atypical of most quadrupeds in that they maintain the same lateral sequence footfall pattern across all locomotor speeds. It has been speculated that the preservation of the footfall patterns is necessary to maintain a statically stable support polygon. This should be a particularly important constraint in large, relatively slow animals. This suggests that elephants must rely on available sensory feedback mechanisms to actively control their massive pillar-like limbs for proper foot placement and sequencing. How the nervous system of elephants integrates the available sensory information for a stable gait is unknown. Here we explored the role that visual feedback plays in the control of the locomotor pattern in Asian elephants. Four Asian elephants (*Elephas maximus*) walked with and without a blindfold as we measured their stride time intervals. Coefficient of variation was used to assess changes in the overall variability of the stride time intervals, while approximate entropy was used to measure the stride-to-stride consistency of the time intervals. We show that visual feedback plays a role in the stride-to-stride consistency of the locomotor pattern in Asian elephants. These results suggest that elephants use visual feedback to correct and maintain proper sequencing of the limbs during locomotion.

Lacomme, L., C. Guerbois, H. Fritz, A. Ganswindt and B. Rey (2023). "Validation of a field-friendly faeces drying and storage method for quantifying faecal glucocorticoid metabolites in African elephants (*Loxodonta africana*) opens up new perspectives for conservationists." *Conserv Physiol* **11**(1): coad053.

Faecal glucocorticoid metabolites (fGCMs) are a relevant means of non-invasively assessing adrenocortical activity and thus, a key physiological stress response in wildlife populations. However, the widespread use of fGCMs as a stress-related biomarker in conservation biology is often hampered by the logistical challenge of storing collected faecal material frozen until it reaches the laboratory for analysis. Although alternative approaches to minimize potential alteration of fGCM composition post-defecation have been recently identified, there is to our knowledge, no satisfactory alternative method established for the preservation of elephant dung. In this study, we validated a field-friendly protocol for dehydrating African elephant faeces samples using a food dehydrator with desiccant and

investigated the stability of fGCM concentrations in the dehydrated faeces when stored at ambient temperature. We collected 40 faecal samples from African elephants and compared fGCM concentrations of freeze-dried and dehydrated sample sub-sets. Samples dried in the field showed a slight but significant overall -6% reduction in fGCM concentration compared with frozen control samples. However, fGCM concentrations following field dehydration protocol match those of control samples with high accuracy, as evidenced by the low bias and strong coefficient of determination between the two approaches ( $R(2) = 0.88$ ). In addition, over nearly 2 months, storage time at ambient temperature of the dehydrated samples had no effect on the fGCM concentrations compared with those measured in the control samples (F-statistic = 1.82;  $P = 0.18$ ). Dehydrating the samples in the field thus provides an easy and cost-effective alternative to freezing, especially when working in remote areas with unstable electrical supply. Our results encourage the widespread use of fGCMs by conservationists as non-invasive means of steroid monitoring of African elephants in the current context of a general increase in wildlife welfare research. Future studies are needed to extend the use of this protocol to other species and to other steroid classes.

LaDue, C. A., K. E. Hunt, W. K. Kiso and E. W. Freeman (2023). "Hormonal variation and temporal dynamics of musth in Asian elephants (*Elephas maximus*) are associated with age, body condition and the social environment." *Conserv Physiol* **11**(1): coad019.

The sustainability of endangered Asian elephants in human care is threatened in part by low breeding success and concerns over individual animal wellbeing. Male elephants have received less research attention compared to females, yet males deserve special consideration due to their unique reproductive biology (particularly the sexual state of "musth") and the complex interaction of physiological, environmental, and social pressures they face. We measured fecal androgen metabolites (FAMs), fecal glucocorticoid metabolites (FGMs), and fecal triiodothyronine metabolites (FT3s) collected weekly over approximately 12 months from 26 male Asian elephants housed in zoos across the US, hypothesizing that FAM, FGM, and FT3 concentrations would be associated with temporal correlates of musth and would vary further with intrinsic (musth status, age, body condition) and extrinsic (social environment) factors. The duration of each musth episode was positively associated with exposure to male conspecifics and negatively associated with body condition. Further, elevated FAM concentrations were associated with social exposure, age, and body condition, and FGM concentrations also varied with age and body condition. FT3 concentrations were not associated with any factor we measured. We also identified periods of lower FAM concentration than confirmed musth episodes (but still higher than baseline FAM concentrations) that we termed "elevated FAM episodes." The durations of these episodes were negatively correlated with exposure to other male elephants. Together, these results provide evidence that hormone profiles (including those that are predicted to change around musth) vary significantly between male Asian elephants in a way that may be attributed to intrinsic and extrinsic factors. Studies like these serve to enhance the sustainability of ex-situ populations by providing wildlife managers with information to enhance the health, welfare, and reproduction of threatened species like Asian elephants.

LaDue, C. A. and R. J. Snyder (2023). "Asian elephants distinguish sexual status and identity of unfamiliar elephants using urinary odours." *Biol Lett* **19**(12): 20230491.

Despite the ubiquity of odours in mammals, few studies have documented the natural olfactory abilities of many 'non-model' species such as the Asian elephant. As Asian elephants are endangered, we may apply odours to more effectively manage threatened populations. We implemented a habituation-discrimination paradigm for the first time in Asian elephants to test the ability of elephants to discriminate between unfamiliar male

elephant urine, hypothesizing that elephants would successfully distinguish non-musth from musth urine and also distinguish identity between two closely related individuals. We conducted two bioassay series, exposing three female and three male zoo-housed elephants to the same urine sample (non-musth urine in the first series, and urine from an unfamiliar individual in the second) over 5 days. On the sixth day, we simultaneously presented each elephant with a novel sample (either musth urine or urine from a second unfamiliar individual) alongside the habituated urine sample, comparing rates of chemosensory response to each sample to indicate discrimination. All elephants successfully discriminated non-musth from musth urine, and also urine from two unfamiliar half-brothers. Our results further demonstrate the remarkable olfactory abilities of elephants with promising implications for conservation and management.

Li, X., J. Chen, C. Zhang, S. Zhang, Q. Shen, B. Wang, M. Bao, B. Xu, Q. Wu, N. Han and Z. Huang (2023). "Fecal Metagenomics Study Reveals That a Low-Fiber Diet Drives the Migration of Wild Asian Elephants in Xishuangbanna, China." *Animals (Basel)* **13**(20).

The rare northward migration of wild Asian elephants in Xishuangbanna, China, has attracted global attention. Elephant migration is a complex ecological process, and the factors driving this long-distance migration remain elusive. In this study, fresh fecal samples were collected from both captive and wild Asian elephants, along with breastfed calves residing within the Wild Elephant Valley of Xishuangbanna. Our aim was to investigate the relationship between diet, gut microbiota, and migration patterns in Asian elephants through comprehensive metagenomic sequencing analyses. Among the breastfed Asian elephant group, Bacteroidales and Escherichia emerged as the dominant bacterial taxa, while the primary carbohydrate-active enzymes (CAZymes) enriched in this group were GH2, GH20, GH92, GH97, GH38, GH23, and GH43, aligning with their dietary source, namely breast milk. The bacterial taxa enriched in captive Asian elephants (CAEs) were mainly *Butyrivibrio*, *Treponema*, and *Fibrobacter*, and the enriched lignocellulose-degrading enzymes mainly included GH25, GH10, GH9, and cellulase (EC 3.2.1.4). These findings are consistent with the high-fiber diet of captive elephants. In contrast, the main bacterial taxa enriched in wild Asian elephants (WAEs) were *Ruminococcus* and *Eubacterium*, and the enriched CAZymes included GH109, GH20, GH33, GH28, GH106, and GH39. The abundance of lignocellulose-degrading bacteria and CAZyme content was low in WAEs, indicating challenges in processing high-fiber foods and explaining the low-fiber diet in this group. These findings suggest that wild elephant herds migrate in search of nutritionally suitable, low-fiber food sources.

Li, X., P. Wang, Q. Pan, G. Liu, W. Liu, O. Omotoso, J. Du, Z. Li, Y. Yu, Y. Huang, P. Zhu, M. Li and X. Zhou (2023). "Chromosome-level Asian elephant genome assembly and comparative genomics of long-lived mammals reveal the common substitutions for cancer resistance." *Aging Cell*: e13917.

The naked mole rat (*Heterocephalus glaber*), bats (e.g., genus *Myotis*), and elephants (family Elephantidae) are known as long-lived mammals and are assumed to be excellent cancer antagonists. However, whether there are common genetic changes underpinning cancer resistance in these long-lived species is yet to be fully established. Here, we newly generated a high-quality chromosome-level Asian elephant (*Elephas maximus*) genome and identified that the expanded gene families in elephants are involved in Ras-associated and base excision repair pathways. Moreover, we performed comparative genomic analyses of 12 mammals and examined genes with signatures of positive selection in elephants, naked mole rat, and greater horseshoe bat. Residues at positively selected sites of CDR2L and ALDH6A1 in these long-lived mammals enhanced the inhibition of tumor cell migration

compared to those in short-lived relatives. Overall, our study provides a new genome resource and a preliminary survey of common genetic changes in long-lived mammals.

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Lin, H., J. Hu, S. Baleka, J. Yuan, X. Chen, B. Xiao, S. Song, Z. Du, X. Lai, M. Hofreiter and G. Sheng (2023). "A genetic glimpse of the Chinese straight-tusked elephants." *Biol Lett* **19**(7): 20230078.

Straight-tusked elephants (genus: *Palaeoloxodon*) including their island dwarf forms are extinct enigmatic members of the Pleistocene megafauna and the most common Pleistocene elephants after the mammoths. Their taxonomic placement has been revised several times. Using palaeogenomic evidence, previous studies suggested that the European *P. antiquus* has a hybrid origin, but no molecular data have been retrieved from their Asian counterparts, leaving a gap in our knowledge of the global phylogeography and population dynamics of *Palaeoloxodon*. Here, we captured a high-quality complete mitogenome from a Pleistocene Elephantidae molar (CADG841) from Northern China, which was previously morphologically assigned to the genus *Elephas* (Asian elephant), and partial mitochondrial sequences (838 bp) of another *Palaeoloxodon* sp. specimen (CADG1074) from Northeastern China. We found that both Chinese specimens cluster with a 244 000-year-old *P. antiquus* (specimen name: WE) from Western Europe, suggesting that this clade may represent a population with a large spatial span across Eurasia. Based on the fossil record and the molecular dating of both the divergences of different *Palaeoloxodon* mitochondrial clades and previously determined hybridization events, we propose that this Eurasian-wide WE clade provides evidence for an earlier migration and/or another hybridization event that happened in the evolutionary history of straight-tusked elephants.

Lo Preti, M. and L. Beccai (2023). "Sensorized objects used to quantitatively study distal grasping in the African elephant." *iScience* **26**(9): 107657.

Nature evolved many ways to grasp objects without using hands: elephants, octopuses, and monkeys use highly dexterous appendices. From a roboticist's perspective, the elephant trunk is a fascinating manipulator, which strategies can empower robots' interaction capabilities. However, quantifying prehensile forces in such large animals in a safe, ethical, and reproducible manner is challenging. We developed two sensorized objects to investigate the grasping of an adult African elephant with deliberately occluded vision. A cylinder and a handle provided a distributed force (80 and 6 taxels) and inertial measurements in real-

time, resisting dirt and shocks. The animal curled the distal portion of the trunk to grasp the tools. Using force and contact area data of the cylinder revealed the animal's ability to finely modulate pressure. The handle data provided insights into the energy-efficient behavior of the animal, with no significant grasping force changes despite variations imposed on both weight (5-15 kg) and initial position of the object.

Longren, L. L., L. Eigen, A. Shubitidze, O. Lieschnegg, D. Baum, J. A. Nyakatura, T. Hildebrandt and M. Brecht (2023). "Dense reconstruction of elephant trunk musculature." *Curr Biol* **33**(21): 4713-4720.e4713.

The elephant trunk operates as a muscular hydrostat(1)(,)(2) and is actuated by the most complex musculature known in animals.(3)(,)(4) Because the number of trunk muscles is unclear,(5) we performed dense reconstructions of trunk muscle fascicles, elementary muscle units, from microCT scans of an Asian baby elephant trunk. Muscle architecture changes markedly across the trunk. Trunk tip and finger consist of about 8,000 extraordinarily filigree fascicles. The dexterous finger consists exclusively of microscopic radial fascicles pointing to a role of muscle miniaturization in elephant dexterity. Radial fascicles also predominate (at 82% volume) the remainder of the trunk tip, and we wonder if radial muscle fascicles are of particular significance for fine motor control of the dexterous trunk tip. By volume, trunk-shaft muscles(6) comprise one-third of the numerous, small radial muscle fascicles; two-thirds of the three subtypes of large longitudinal fascicles (dorsal longitudinals, ventral outer obliques, and ventral inner obliques);(7)(,)(8)(,)(9) and a small fraction of transversal fascicles. Shaft musculature is laterally, but not radially, symmetric. A predominance of dorsal over ventral radial muscles and of ventral over dorsal longitudinal muscles may result in a larger ability of the shaft to extend dorsally than ventrally(10) and to bend inward rather than outward. There are around 90,000 trunk muscle fascicles. While primate hand control is based on fine control of contraction by the convergence of many motor neurons on a small set of relatively large muscles, evolution of elephant grasping has led to thousands of microscopic fascicles, which probably outnumber facial motor neurons.

Macías-Rioseco, M., J. Ochoa, J. Asín, R. B. Moeller and F. A. Uzal (2023). "Salmonellosis in elephants in managed care: report of 2 cases and literature review." *J Vet Diagn Invest* **35**(3): 295-299.

In animals, salmonellosis is seen typically as enteritis and/or septicemia. Subclinical infection also occurs, and outwardly healthy animals can serve as reservoirs of infection. Reports of salmonellosis in elephants are rare, limited to a few serovars, and the gross and microscopic lesions of enteric salmonellosis in this species have not been described in detail. We present here, in 2 elephants in managed care settings, cases of salmonellosis that resulted from infection by *Salmonella enterica* serovar Muenchen and *S. enterica* serovar Montevideo, serovars that have not been described previously as the cause of salmonellosis in elephants, to our knowledge. We also review the literature on salmonellosis in elephants. Animal A, an adult Asian elephant that was euthanized because of gastrointestinal hemorrhage, had multifocal, necrotizing, suppurative enterocolitis, and necrotizing gastritis. Animal B, an adult African elephant with chronic, recurrent colic, followed by death, had necrotizing typhlocolitis. The origin of infection was not determined in either case. The animals came from different facilities and did not have a common feed source. Previously reported cases of salmonellosis in elephants were caused by *Salmonella* Dublin, *Salmonella* Typhimurium, or *Salmonella* Enteritidis. The definitive diagnosis of salmonellosis is made based on compatible gross and microscopic lesions, coupled with the detection of *Salmonella* spp. in the affected tissues. Effective biosecurity should be adopted to minimize the risk of

salmonellosis in elephants in managed care.

Makgabo, S. M., K. A. Brayton, M. C. Oosthuizen and N. E. Collins (2023). "Unravelling the diversity of Anaplasma species circulating in selected African wildlife hosts by targeted 16S microbiome analysis." *Curr Res Microb Sci* **5**: 100198.

Organisms in the genus *Anaplasma* are obligate intracellular alphaproteobacteria. Bovine anaplasmosis, predominantly caused by *Anaplasma marginale*, is the most prevalent tick-borne disease (TBD) of cattle worldwide. Other *Anaplasma* species are known to cause disease; these include *A. ovis*, *A. platys* in dogs, *A. capra* in goats and humans, and *A. phagocytophilum* in humans. The rapid advancement of next-generation sequencing technologies has led to the discovery of many novel sequences ascribed to the genus *Anaplasma*, with over 20 putative new species being proposed since the last formal organization of the genus. Most 16S rRNA gene surveys for *Anaplasma* were conducted on cattle and to a lesser extent on rodents, dogs, and ticks. Little is known about the occurrence, diversity, or impact of *Anaplasma* species circulating in wildlife species. Therefore, we conducted a 16S rRNA gene survey with the goal of identifying *Anaplasma* species in a variety of wildlife species in the Kruger National Park and neighbouring game reserves, using an unbiased 16S rRNA gene microbiome approach. An *Anaplasma*/*Ehrlichia*-group specific quantitative real-time PCR (qPCR) assay revealed the presence of *Anaplasma* and/or *Ehrlichia* species in 70.0% (21/30) of African buffalo, 86.7% (26/30) of impala, 36.7% (11/30) of greater kudu, 3.2% (1/31) of African wild dog, 40.6% (13/32) of Burchell's zebra, 43.3% (13/30) of warthog, 22.6% (7/31) of spotted hyena, 40.0% (12/30) of leopard, 17.6% (6/34) of lion, 16.7% (5/30) of African elephant and 8.6% (3/35) of white rhinoceros samples. Microbiome sequencing data from the qPCR positive samples revealed four 16S rRNA sequences identical to previously published *Anaplasma* sequences, as well as nine novel *Anaplasma* 16S genotypes. Our results reveal a greater diversity of putative *Anaplasma* species circulating in wildlife than currently classified within the genus. Our findings highlight a potential expansion of the *Anaplasma* host range and the need for more genetic information from other important genes or genome sequencing of putative novel species for correct classification and further assessment of their occurrence in wildlife, livestock and companion animals.

Makopa, T. P., G. Modikwe, U. Vrhovsek, C. Lotti, J. P. Sampaio and N. Zhou (2023). "The marula and elephant intoxication myth: assessing the biodiversity of fermenting yeasts associated with marula fruits (*Sclerocarya birrea*)." *FEMS Microbes* **4**: xtad018.

The inebriation of wild African elephants from eating the ripened and rotting fruit of the marula tree is a persistent myth in Southern Africa. However, the yeasts responsible for alcoholic fermentation to intoxicate the elephants remain poorly documented. In this study, we considered Botswana, a country with the world's largest population of wild elephants, and where the marula tree is indigenous, abundant and protected, to assess the occurrence and biodiversity of yeasts with a potential to ferment and subsequently inebriate the wild elephants. We collected marula fruits from over a stretch of 800 km in Botswana and isolated 106 yeast strains representing 24 yeast species. Over 93% of these isolates, typically known to ferment simple sugars and produce ethanol comprising of high ethanol producers belonging to *Saccharomyces*, *Brettanomyces*, and *Pichia*, and intermediate ethanol producers *Wickerhamomyces*, *Zygorhynchus*, *Candida*, *Hanseniaspora*, and *Kluyveromyces*. Fermentation of marula juice revealed convincing fermentative and aromatic bouquet credentials to suggest the potential to influence foraging behaviour and inebriate elephants in nature. There is insufficient evidence to refute the aforementioned myth. This work serves as the first work towards understanding the biodiversity marula



associated yeasts to debunk the myth or approve the facts.

Meuffels-Barkas, J., S. Wilsher, W. R. T. Allen, C. Ververs and I. Lueders (2023). "Comparative reproduction of the female horse, elephant and rhinoceros: implications for advancing Assisted Reproductive Technologies (ART)." Reprod Fertil.

Recent loss of rhinoceros subspecies has renewed interest in using more advanced assisted reproductive technologies (ART) in rhinoceroses and elephants. Currently, only semen collection, semen preservation and artificial insemination (AI) have been used repeatedly with success in these species. Although ovum pick-up (OPU) and intra-cytoplasmic sperm injection (ICSI) have been reported in rhinoceroses, the techniques are not yet optimised. In contrast, multiple ART applications are routinely used in the horse. Since elephant and rhinoceroses share some reproductive features with equids, we postulate that procedures such as OPU, ICSI, in vitro fertilisation (IVF) and embryo transfer (ET), which are well established in the horse, may represent a basis to develop protocols for endangered pachyderms. In this review, we summarize current knowledge on reproductive physiology relevant to ART. We discuss the current state of ART in all three families and the requirements for the successful implementation of OPU, ICSI, IVF and ET in these species.

Mills, G. (2023). "How eyesight helps elephants move steadily." Vet Rec **193**(7): 264-265.

Georgina Mills discusses new research looking at how visual feedback helps the world's largest quadrupeds stay stable.

Min, J., P. Kim, S. Yun, M. Hong and W. Park (2023). "Zoo animal manure as an overlooked reservoir of antibiotic resistance genes and multidrug-resistant bacteria." Environ Sci Pollut Res Int **30**(1): 710-726.

Animal fecal samples collected in the summer and winter from 11 herbivorous animals, including sable antelope (SA), long-tailed goral (LTG), and common eland (CE), at a public zoo were examined for the presence of antibiotic resistance genes (ARGs). Seven antibiotics, including meropenem and azithromycin, were used to isolate culturable multidrug-resistant (MDR) strains. The manures from three animals (SA, LTG, and CE) contained 10(4)-fold higher culturable MDR bacteria, including *Chryseobacterium*, *Sphingobacterium*, and *Stenotrophomonas* species, while fewer MDR bacteria were isolated from manure from water buffalo, rhinoceros, and elephant against all tested antibiotics. Three MDR bacteria-rich samples along with composite samples were further analyzed using nanopore-based technology. ARGs including *lnu*(C), *tet*(Q), and *mef*(A) were common and often associated with transposons in all tested samples, suggesting that transposons carrying ARGs may play an important role for the dissemination of ARGs in our tested animals. Although several copies of ARGs such as *aph*(3')-IIc, *bla*(L1), *bla*(IND-3), and *tet*(42) were found in the sequenced genomes of the nine MDR bacteria, the numbers and types of ARGs appeared to be less than expected in zoo animal manure, suggesting that MDR bacteria in the gut of the tested animals had intrinsic resistant phenotypes in the absence of ARGs.

Montero-De La Torre, S., S. L. Jacobson, M. Chodorow, M. Yindee and J. M. Plotnik (2023). "Day and night camera trap videos are effective for identifying individual wild Asian elephants." PeerJ **11**: e15130.

Regular monitoring of wild animal populations through the collection of behavioral and demographic data is critical for the conservation of endangered species. Identifying individual Asian elephants (*Elephas maximus*), for example, can contribute to our understanding of their social dynamics and foraging behavior, as well as to human-elephant

conflict mitigation strategies that account for the behavior of specific individuals involved in the conflict. Wild elephants can be distinguished using a variety of different morphological traits-e.g., variations in ear and tail morphology, body scars and tumors, and tusk presence, shape, and length-with previous studies identifying elephants via direct observation or photographs taken from vehicles. When elephants live in dense forests like in Thailand, remote sensing photography can be a productive approach to capturing anatomical and behavioral information about local elephant populations. While camera trapping has been used previously to identify elephants, here we present a detailed methodology for systematic, experimenter differentiation of individual elephants using data captured from remote sensing video camera traps. In this study, we used day and night video footage collected remotely in the Salakpra Wildlife Sanctuary in Thailand and identified 24 morphological characteristics that can be used to recognize individual elephants. A total of 34 camera traps were installed within the sanctuary as well as crop fields along its periphery, and 107 Asian elephants were identified: 72 adults, 11 sub-adults, 20 juveniles, and four infants. We predicted that camera traps would provide enough information such that classified morphological traits would aid in reliably identifying the adult individuals with a low probability of misidentification. The results indicated that there were low probabilities of misidentification between adult elephants in the population using camera traps, similar to probabilities obtained by other researchers using handheld cameras. This study suggests that the use of day and night video camera trapping can be an important tool for the long-term monitoring of wild Asian elephant behavior, especially in habitats where direct observations may be difficult.

Nguyen, T. D., H. Li, Y. Zhuang, B. Chen, K. Kinoshita, M. A. Jamal, K. Xu, J. Guo, D. Jiao, K. Tanabe, Y. Wei, Z. Li, W. Cheng, Y. Qing, H. Y. Zhao and H. J. Wei (2023). "In vitro and in vivo development of interspecies Asian elephant embryos reconstructed with pig enucleated oocytes." Anim Biotechnol **34**(6): 1909-1918.

Interspecies somatic cell nuclear transfer (iSCNT) has an immense potential to rescue endangered animals and extinct species like mammoths. In this study, we successfully established an Asian elephant's fibroblast cell lines from ear tissues, performed iSCNT with porcine oocytes and evaluated the in vitro and in vivo development of reconstructed embryos. A total of 7780 elephant-pig iSCNT embryos were successfully reconstructed and showed in vitro development with cleavage rate, 4-cell, 8-cell and blastocyst rate of 73.01, 30.48, 5.64, and 4.73%, respectively. The total number of elephant-pig blastocyte cells and diameter of hatched blastocyte was 38.67 and 252.75  $\mu\text{m}$ , respectively. Next, we designed species-specific markers targeting EDNRB, AGRP and TYR genes to verify the genome of reconstructed embryos with donor nucleus/species. The results indicated that 53.2, 60.8, and 60.8% of reconstructed embryos ( $n = 235$ ) contained elephant genome at 1-cell, 2-cell and 4-cell stages, respectively. However, the percentages decreased to 32.3 and 32.7% at 8-cell and blastocyst stages, respectively. Furthermore, we also evaluated the in vivo development of elephant-pig iSCNT cloned embryos and transferred 2260 reconstructed embryos into two surrogate gilts that successfully became pregnant and a total of 11 (1 and 10) fetuses were surgically recovered after 17 and 19 days of gestation, respectively. The crown-rump length and width of elephant-pig cloned fetuses were smaller than the control group. Unfortunately, none of these fetuses contained elephant genomes, which suggested that elephant embryos failed to develop in vivo. In conclusion, we successfully obtained elephant-pig reconstructed embryos for the first time and these embryos are able to develop to blastocyst, but the in vivo developmental failure needs further investigated.

O'Connell-Rodwell, C. E., J. L. Berezin, A. Dharmarajan, M. E. Ravicz, Y. Hu, X. Guan, K. N.

O'Connor and S. Puria (2023). "The impact of size on middle-ear sound transmission in elephants, the largest terrestrial mammal." [bioRxiv](#).

Elephants have a unique auditory system that is larger than any other terrestrial mammal. To quantify the impact of larger middle ear (ME) structures, we measured 3D ossicular motion and ME sound transmission in cadaveric temporal bones from both African and Asian elephants in response to air-conducted (AC) tonal pressure stimuli presented in the ear canal (P (EC) ). Results were compared to similar measurements in humans. Velocities of the umbo (V (U) ) and stapes (V (ST) ) were measured using a 3D laser Doppler vibrometer in the 7-13,000 Hz frequency range, stapes velocity serving as a measure of energy entering the cochlea—a proxy for hearing sensitivity. Below the elephant ME resonance frequency of about 300 Hz, the magnitude of V (U) /P (EC) was an order of magnitude greater than in human, and the magnitude of V (ST) /P (EC) was 5x greater. Phase of V (ST) /P (EC) above ME resonance indicated that the group delay in elephant was approximately double that of human, which may be related to the unexpectedly high magnitudes at high frequencies. A boost in sound transmission across the incus long process and stapes near 9 kHz was also observed. We discuss factors that contribute to differences in sound transmission between these two large mammals.

Olson, W., L. Zhang, D. H. O'Connor and D. Kleinfeld (2023). "Elephant trunks: Strength and dexterity from mini-fascicles." [Curr Biol](#) **33**(22): R1203-r1205.

Muscular hydrostats, such as the elephant trunk, can perform precise motor actions. A new study has revealed that the elephant trunk contains a dense network of tiny muscle fascicles, suggesting that muscle miniaturization may be a key toward understanding how soft organs achieve both strength and dexterity.

Osorio, S., J. Soto, D. Schmitt, W. Kiso and C. Cray (2023). "Preliminary assessment of serum capillary zone electrophoresis in the Asian elephant (*Elephas maximus*)." [Front Vet Sci](#) **10**: 1204880.

Serum protein electrophoresis has been demonstrated to have utility in diagnostic workup, wellness exams, and prognosis. Agarose gel electrophoresis (AGE) has previously been described for use with serum from Asian elephants (*Elephas maximus*). As the newer method of capillary zone electrophoresis (CZE) is becoming more commonplace in veterinary diagnostic laboratories, serum samples from Asian elephants were examined using this method. CZE allowed for a reproducible definition of two beta fractions and, overall, showed a low coefficient of variation for fraction quantitation. Preliminary reference intervals were generated using samples primarily from an older population of 22 female elephants. Albumin levels determined by CZE were also compared with those determined by the bromocresol green method on a chemistry analyzer. It was found that the latter method overestimated the level of albumin with a mean positive bias of 11.6% or 0.38 g/dL, thus method-specific reference intervals should be used. Significant negative correlations were observed between A/G ratio determined by CZE and serum amyloid A levels ( $r = -0.47$ ,  $p < 0.0001$ ) and haptoglobin ( $r = -0.52$ ,  $p < 0.0001$ ); both APP were significantly correlated with the alpha 2 globulin fraction ( $p < 0.0001$ ). CZE reflects an overall picture of changes in acute phase proteins and immunoglobulins and accurate quantitation of albumin and thus should be considered as an adjunct tool to the use of other measures of the acute phase response in patient monitoring.

Osthoff, G., I. Wiese and F. Deacon (2023). "African Elephant Milk Short Saccharide and Metabolite Composition and Their Changes over Lactation." [Animals \(Basel\)](#) **13**(3).

Elephant milk composition is unique, as are its changes over lactation. Presented here is

the milk non-dedicated metabolite composition of three African elephants. Their lactation times are overlapping and span day one to thirty months. Metabolites were identified and quantified by (1)H nuclear magnetic resonance spectroscopy. Lactose and short oligosaccharides are a large component of the metabolites, with lacto-N-difucohexaose I as the major oligosaccharide. These were followed by metabolites of lipids, amino acids, and the citric acid cycle. The content of lactose, lacto-N-difucohexaose I, 2'-fucosyllactose, and some unidentified oligosaccharides decrease over lactation, while that of difucosyllactose and other unidentified ones increase. The high content of glutamate, as a glucogenic amino acid, supported the uprated synthesis of saccharides by the milk gland cells. The content of succinate and choline increase over lactation, indicating higher energy expenditure and phospholipid synthesis during later lactation.

Pant, B., H. P. Sharma, B. R. Dahal, S. Regmi and J. L. Belant (2023). "Spatio-temporal patterns of human-wildlife conflicts and effectiveness of mitigation in Shuklaphanta National Park, Nepal." PLoS ONE **18**(4): e0282654.

Human-wildlife interactions occur where human and wildlife coexist and share common resources including food or shelter. Increasing wildlife populations within protected areas also can increase interactions with humans living adjacent to these areas, resulting in conflicts including human casualty, livestock depredation, crop damage, and property loss. We analyzed six years human-wildlife conflict data from 2016-2021 in the buffer zone of Shuklaphanta National Park and conducted questionnaire survey to investigate factors influencing human-wildlife conflicts. Nineteen people were attacked by wildlife, primarily wild boar (*Sus scrofa*). Ninety-two livestock were killed by leopard (*Panthera pardus*), and among these most were sheep or goats killed near ShNP during summer. Crops were most frequently damaged by Asian elephants (*Elephas maximus*), followed by wild boar. Greatest economic losses were from damage to rice, followed by sugarcane and wheat. Asian elephant was the only reported species to cause structural damage to property (e.g., homes). Majority of respondents (83%) considered that the mitigation techniques that are currently in practice are effective to reduce the conflicts. However, the effectiveness of the mitigation techniques are the species specific, we recommend use of more efficacious deterrents (e.g., electric fencing) for large herbivores and mesh wire fencing with partially buried in the ground. Effective collaboration among different tiers of government, non-governmental organizations, civil societies and affected communities are important to share the best practices and continue to apply innovative methods for impactful mitigation of human-wildlife conflicts in the region.

Pardo, M. C. (2023). "Legal Personhood for Animals: Has Science Made Its Case?" Animals (Basel) **13**(14).

The use of Latin in identifying an organism's genus and species is likely familiar to scientists and zoological professionals, but a traditional legal doctrine, known as habeas corpus (meaning "you have the body") may not have obvious applicability to nonhumans in the animal kingdom. In recent years, animal rights organizations have utilized the habeas corpus doctrine as a basis to bring legal challenges on behalf of nonhuman animals to expand "legal personhood" to them. These lawsuits, which have focused on species such as nonhuman primates and elephants, seek to challenge the "confinement" of animals in zoological institutions and by private owners, much like a prisoner or other detainee. The small but vocal animal legal personhood movement bases its argument on the fact that elephants and nonhuman primates are highly sentient and have complex cognitive characteristics. Proponents of legal personhood for animals have argued that the common law has progressed and expanded over the years as societal norms and conditions have

changed and, much like the law has expanded to afford women and persons of color legal rights and protections, so should the law expand to treat animals the same as humans. Despite these efforts, to date, no court in the United States has accepted this invitation. This article summarizes key legal challenges and decisions to date in the United States, examines how science and societal conditions have influenced the law, and analyzes the reasons why legal personhood for animals so far has been viewed as a "bridge too far" in the American legal system.

Petneházy, Ö., S. Rück, E. Sós and L. Z. Reinitz (2023). "3D Reconstruction of the Blood Supply in an Elephant's Forefoot Using Fused CT and MRI Sequences." *Animals (Basel)* **13**(11).

Being the largest still-living terrestrial mammal on earth, an elephant's feet play an important role in its health status. The musculoskeletal structures in the forefoot are well described in the literature, but information about vascularization is limited. The novel aim of this work is to provide anatomical guidance to structures found in the forefoot, focusing on the arterial system. Initially, native CT and MRI sequences were taken of the left forefoot of a deceased 6-year-old female Asian elephant; the foot was then filled with an iodine-containing contrast medium through the a. mediana and the CT scans were repeated in the same position. The images obtained were processed with 3D Slicer software for the 3D reconstruction of the bones and arteries. The results clearly showed the palmar blood supply of the forefoot. A so far undescribed vessel was revealed, stemming from the a. metacarpea, supplying the first digit and the digital cushion. The course of the deep palmar arch's terminal section was also established. This paper provides the first description of the exact disposition of the arteries in the palmar aspect of an elephant's forefoot and may be used in planning surgeries in clinically affected animals.

Pongma, C., S. Songthammanuphap, S. Puthong, A. Buakeaw, T. Prammananan, S. Warit, W. Tipkantha, E. Kaewkhunjob, W. Jairak, P. Kongmakee, C. Pabutta, S. Sripiboon, W. Yindeeyoungyeon and T. Palaga (2023). "Using whole blood cultures in interferon gamma release assays to detect Mycobacterium tuberculosis complex infection in Asian elephants (*Elephas maximus*)." *PLoS ONE* **18**(7): e0288161.

Elephants are susceptible to Mycobacterium tuberculosis (*M. tb*) complex (MTBC) infections. Diagnosis of tuberculosis (TB) in elephants is difficult, and most approaches used for human TB diagnosis are not applicable. An interferon gamma release assay (IGRA) to diagnose TB in Asian elephants (*Elephas maximus*) using peripheral blood mononuclear cells (PBMCs) has been previously developed. Although the assay is shown to be valid in determining MTBC infection status, the laborious PBMC isolation process makes it difficult to use. In this study, we simplified the method by using whole blood cultures (WC) as the starting material. Using PBMC cultures for IGRA, the MTBC infection status of 15 elephants was first confirmed. Among these animals, one has been previously confirmed for *M. tb* infection by both TB culture and PCR and the other was confirmed for MTBC infection in this study by droplet digital PCR (ddPCR) method. WC for IGRA consisted of an unstimulated sample, a mitogen stimulated sample, and sample stimulated with recombinant *M. tb* antigens, ESAT6 and CFP10. Using WC for IGRA in the 15 enrolled elephants, the results showed that 7 out of 15 samples yielded MTBC infection positive status that were completely concordant with those from the results using PBMCs. To test this method, WC for IGRA were applied in another elephant cohort of 9 elephants. The results from this cohort revealed a perfect match between the results from PBMC and WC. Responses to ESAT6 or CFP10 by PBMC and WC were not completely concordant, arguing for the use of at least two *M. tb* antigens for stimulation. Given the ease of sample handling, smaller blood sample volumes and equivalent efficacy relative to the PBMC approach, using WC for IGRA provides a novel,

rapid, and user-friendly TB diagnostic method for determining the MTBC infection in elephants.

Prado, N. A., E. E. Armstrong, J. L. Brown, S. Z. Goldenberg, P. Leimgruber, V. R. Pearson, J. E. Maldonado and M. G. Campana (2023). "Genomic Resources for Asian (*Elephas maximus*) and African Savannah Elephant (*Loxodonta africana*) Conservation and Health Research." J Hered.

We provide novel genomic resources to help understand the genomic traits involved in elephant health and to aid conservation efforts. We sequence 11 elephant genomes (5 African savannah, 6 Asian) from North American zoos, including 9 de novo assemblies. We estimate elephant germline mutation rates and reconstruct demographic histories. Finally, we provide an in-solution capture assay to genotype Asian elephants. This assay is suitable for analyzing degraded museum and non-invasive samples, such as feces and hair. The elephant genomic resources we present here should allow for more detailed and uniform studies in the future to aid elephant conservation efforts and disease research.

Prado, N. A., E. E. Armstrong, J. L. Brown, S. Z. Goldenberg, P. Leimgruber, V. R. Pearson, J. E. Maldonado and M. G. Campana (2023). "Genomic resources for Asian (*Elephas maximus*) and African savannah elephant (*Loxodonta africana*) conservation and health research." J Hered **114**(5): 529-538.

We provide novel genomic resources to help understand the genomic traits involved in elephant health and to aid conservation efforts. We sequence 11 elephant genomes (5 African savannah, 6 Asian) from North American zoos, including 9 de novo assemblies. We estimate elephant germline mutation rates and reconstruct demographic histories. Finally, we provide an in-solution capture assay to genotype Asian elephants. This assay is suitable for analyzing degraded museum and noninvasive samples, such as feces and hair. The elephant genomic resources we present here should allow for more detailed and uniform studies in the future to aid elephant conservation efforts and disease research.

Preston, A. J., A. Rogers, M. Sharp, G. Mitchell, C. Toruno, B. B. Barney, L. N. Donovan, J. Bly, R. Kennington, E. Payne, A. Iovino, G. Furukawa, R. Robinson, B. Shamloo, M. Buccilli, R. Anders, S. Eckstein, E. A. Fedak, T. Wright, C. C. Maley, W. K. Kiso, D. Schmitt, D. Malkin, J. D. Schiffman and L. M. Abegglen (2023). "Elephant TP53-RETROGENE 9 induces transcription-independent apoptosis at the mitochondria." Cell Death Discov **9**(1): 66.

Approximately 20 TP53 retrogenes exist in the African and Asian elephant genomes (*Loxodonta Africana*, *Elephas Maximus*) in addition to a conserved TP53 gene that encodes a full-length protein. Elephant TP53-RETROGENE 9 (TP53-R9) encodes a p53 protein (p53-R9) that is truncated in the middle of the canonical DNA binding domain. This C-terminally truncated p53 retrogene protein lacks the nuclear localization signals and oligomerization domain of its full-length counterpart. When expressed in human osteosarcoma cells (U2OS), p53-R9 binds to Tid1, the chaperone protein responsible for mitochondrial translocation of human p53 in response to cellular stress. Tid1 expression is required for p53-R9-induced apoptosis. At the mitochondria, p53-R9 binds to the pro-apoptotic BCL-2 family member Bax, which leads to caspase activation, cytochrome c release, and cell death. Our data show, for the first time, that expression of this truncated elephant p53 retrogene protein induces apoptosis in human cancer cells. Understanding the molecular mechanism by which the additional elephant TP53 retrogenes function may provide evolutionary insight that can be utilized for the development of therapeutics to treat human cancers.

Pretorius, Y., T. Eggeling and A. Ganswindt (2023). "Identifying potential measures of stress and disturbance during a captive to wild African elephant reintegration." PLoS ONE **18**(10): e0291293.

There is increasing evidence of compromised welfare for elephants managed in captivity. Should such facilities eventually close, more elephants will need to be rehabilitated and reintegrated into the wild. The goal of such reintegration would be to restore any physical or psychological aspects of the elephant that may have been compromised in captivity, followed by introduction into a free-roaming system where they can interact with other elephants. However, to achieve this goal, the reintegration methods implemented need to be assessed to ensure that welfare remains the priority. The objective of this study was to test whether parameters generally associated with stress and disturbance in African elephants, respond to changes in potentially stressful environmental conditions, assessed at multiple temporal scales ranging from minutes to months. The main changes in environmental conditions that were investigated included the different phases of reintegration of a group of elephants from captivity into the wild. Stress and disturbance related parameters used for comparisons included physiological responses, namely the extent of temporal gland secretions (eTGS) and faecal glucocorticoid metabolite (fGCM) levels as well as behavioural responses, namely the display of stereotype and stress-related behaviours. Results showed that eTGS significantly increased during the initial release of the elephants compared to when in captivity. Stereotypic behaviours were only recorded during the captive phase and immediately ceased after release. Faecal GCM levels spiked in the first year after release before decreasing back to pre-reintegration levels during the third year. These findings indicate that fGCM levels, the eTGS and disturbance related behaviours all proved effective in explaining the changes in stress and disturbance experienced by elephants during the initial years after being reintegrated from captivity into the wild.

Qurratul-Saadah, Z., A. Che-Amat, S. S. Syed-Hussain, J. Kamaludden, S. M. Z. Ariffin, N. H. Basripuzi and A. A. Nor-Azlina (2023). "Gastrointestinal Parasites in Asian and African Elephants: A Systematic Review." *Trop Biomed* **40**(1): 55-64.

Gastrointestinal parasites (GIPs) in elephants have been reported in several studies over the last decades. Nonetheless, comprehensive data on clinicopathology of elephant GIPs, parasite burden threshold value, and the effectiveness of conventional anthelmintic drugs are still lacking. Herein, we have systematically reviewed the available knowledge on elephant GIPs identified among different parts of the world based on their prevalence, epidemiology, pathology, diagnosis, treatment, and control. Two electronic databases were searched for publications that met the inclusion criteria. About 19 English journal articles published between year of 2011- 2021 were included. The main GIPs reported in elephants were Cyathostomidae (at least 14 species), Ancylostomidae, *Haemonchus contortus*, *Trichostrongylus colubriformis*, *Oesophagostomum columbianum*, *Oesophagostomum aceleatum*, Ascarids, Trichurids, Strongyloides, Anophlocephalidae, flukes, and Coccidia across different parts of the world, including Malaysia, Indonesia, Thailand, Myanmar, Sri Lanka, India, Kenya, Nigeria, and South Africa. Most elephants show no clinical signs until the equilibrium between parasite and host is disturbed. The common diagnostic methods for GIPs are traditional direct smear, faecal floatation, sedimentation, and McMaster egg counting technique, all involving morphological identification. However, some articles described the use of molecular detection to characterise common GIPs of elephants. Although benzimidazoles and macrocyclic lactones group of anthelmintic are the most conventional GIPs treatment and control for captive and semi-captive elephants, there is limited data on the threshold value of faecal egg count as the baseline for treatment decision. Over the last decades, various studies regarding elephant GIPs have been conducted. However, more focused and systematic studies are required to enhance our knowledge in multiple aspects of elephant parasitology to find effective solutions and

improve elephant health.

Rahman, D. A., R. Herliansyah, B. Subhan, D. Hutasoit, M. A. Imron, D. B. Kurniawan, T. Sriyanto, R. D. Wijayanto, M. H. Fikriansyah, A. F. Siregar and N. Santoso (2023). "The first use of a photogrammetry drone to estimate population abundance and predict age structure of threatened Sumatran elephants." *Sci Rep* **13**(1): 21311.

Wildlife monitoring in tropical rainforests poses additional challenges due to species often being elusive, cryptic, faintly colored, and preferring concealable, or difficult to access habitats. Unmanned aerial vehicles (UAVs) prove promising for wildlife surveys in different ecosystems in tropical forests and can be crucial in conserving inaccessible biodiverse areas and their associated species. Traditional surveys that involve infiltrating animal habitats could adversely affect the habits and behavior of elusive and cryptic species in response to human presence. Moreover, collecting data through traditional surveys to simultaneously estimate the abundance and demographic rates of communities of species is often prohibitively time-intensive and expensive. This study assesses the scope of drones to non-invasively access the Bukit Tigapuluh Landscape (BTL) in Riau-Jambi, Indonesia, and detect individual elephants of interest. A rotary-wing quadcopter with a vision-based sensor was tested to estimate the elephant population size and age structure. We developed hierarchical modeling and deep learning CNN to estimate elephant abundance and age structure. Drones successfully observed 96 distinct individuals at 8 locations out of 11 sampling areas. We obtained an estimate of the elephant population of 151 individuals (95% CI [124, 179]) within the study area and predicted more adult animals than subadults and juvenile individuals in the population. Our calculations may serve as a vital spark for innovation for future UAV survey designs in large areas with complex topographies while reducing operational effort.

Rajbhandari, R. M., R. Napit, P. Manandhar, R. Raut, A. Gurung, A. Poudel, N. Shrestha, A. Sadaula, D. Karmacharya, C. Gortázar, P. C. Alves, J. de la Fuente and J. Queirós (2023). "Phylogenomic analysis supports Mycobacterium tuberculosis transmission between humans and elephants." *Front Vet Sci* **10**: 1133823.

**INTRODUCTION:** Tuberculosis is an infectious disease caused by a group of acid-fast bacilli known as Mycobacterium tuberculosis complex (MTC), which has a major impact on humans. Transmission of MTC across the human-animal interface has been demonstrated by several studies. However, the reverse zoonotic transmission from humans to animals (zooanthroponosis) has often been neglected. **METHODS:** In this study, we used Nanopore MinION and Illumina MiSeq approaches to sequence the whole genome of M. tuberculosis strains isolated from two deceased Asian elephants (*Elephas maximus*) and one human in Chitwan, Nepal. The evolutionary relationships and drug resistance capacity of these strains were assessed using the whole genome data generated by the stand-alone tool Tb-Profiler. Phylogenomic trees were also constructed using a non-synonymous SNP alignment of 2,596 bp, including 94 whole genome sequences representative of the previously described M. tuberculosis lineages from elephants worldwide (lineages 1 and 4) and from humans in Nepal (lineages 1, 2 and 3). **RESULTS AND DISCUSSION:** The new genomes achieved an average coverage of 99.6%, with an average depth of 55.67x. These M. tuberculosis strains belong to lineage 1 (elephant DG), lineage 2 (elephant PK) and lineage 4 (human), and none of them were found to have drug-resistant variants. The elephant-derived isolates were evolutionarily closely related to human-derived isolates previously described in Nepal, both in lineages 1 and 2, providing additional support for zooanthroponosis or bidirectional transmission between humans and elephants. The human-derived isolate clustered together with other published human isolates from Argentina, Russia and the United Kingdom in the



lineage 4 clade. This complex multi-pathogen, multi-host system is challenging and highlights the need for a One Health approach to tuberculosis prevention and control at human-animal interface, particularly in regions where human tuberculosis is highly endemic.

Raviv, L., S. L. Jacobson, J. M. Plotnik, J. Bowman, V. Lynch and A. Benítez-Burraco (2023). "Elephants as an animal model for self-domestication." Proc Natl Acad Sci U S A **120**(15): e2208607120.

Humans are unique in their sophisticated culture and societal structures, their complex languages, and their extensive tool use. According to the human self-domestication hypothesis, this unique set of traits may be the result of an evolutionary process of self-induced domestication, in which humans evolved to be less aggressive and more cooperative. However, the only other species that has been argued to be self-domesticated besides humans so far is bonobos, resulting in a narrow scope for investigating this theory limited to the primate order. Here, we propose an animal model for studying self-domestication: the elephant. First, we support our hypothesis with an extensive cross-species comparison, which suggests that elephants indeed exhibit many of the features associated with self-domestication (e.g., reduced aggression, increased prosociality, extended juvenile period, increased playfulness, socially regulated cortisol levels, and complex vocal behavior). Next, we present genetic evidence to reinforce our proposal, showing that genes positively selected in elephants are enriched in pathways associated with domestication traits and include several candidate genes previously associated with domestication. We also discuss several explanations for what may have triggered a self-domestication process in the elephant lineage. Our findings support the idea that elephants, like humans and bonobos, may be self-domesticated. Since the most recent common ancestor of humans and elephants is likely the most recent common ancestor of all placental mammals, our findings have important implications for convergent evolution beyond the primate taxa, and constitute an important advance toward understanding how and why self-domestication shaped humans' unique cultural niche.

Robertson, M. R., L. J. Olivier, J. Roberts, L. Yonthantham, C. Banda, B. N'Gombwa I, R. Dale and L. N. Tiller (2023). "Testing the Effectiveness of the "Smelly" Elephant Repellent in Controlled Experiments in Semi-Captive Asian and African Savanna Elephants." Animals (Basel) **13**(21).

Crop-raiding by elephants is one of the most prevalent forms of human-elephant conflict and is increasing with the spread of agriculture into wildlife range areas. As the magnitude of conflicts between people and elephants increases across Africa and Asia, mitigating and reducing the impacts of elephant crop-raiding has become a major focus of conservation intervention. In this study, we tested the responses of semi-captive elephants to the "smelly" elephant repellent, a novel olfactory crop-raiding mitigation method. At two trial sites, in Zambia and Thailand, African elephants (*Loxodonta africana*) and Asian elephants (*Elephas maximus*) were exposed to the repellent, in order to test whether or not they entered an area protected by the repellent and whether they ate the food provided. The repellent elicited clear reactions from both study groups of elephants compared to control conditions. Generalised linear models revealed that the elephants were more alert, sniffed more, and vocalised more when they encountered the repellent. Although the repellent triggered a response, it did not prevent elephants from entering plots protected by the repellent or from eating crops, unlike in trials conducted with wild elephants. Personality played a role in responses towards the repellent, as the elephants that entered the experimental plots were bolder and more curious individuals. We conclude that, although captive environments provide controlled settings for experimental testing, the ecological

validity of testing human-elephant conflict mitigation methods with captive wildlife should be strongly considered. This study also shows that understanding animal behaviour is essential for improving human-elephant coexistence and for designing deterrence mechanisms. Appreciating personality traits in elephants, especially amongst "problem" elephants who have a greater propensity to crop raid, could lead to the design of new mitigation methods designed to target these individuals.

Roca, A. L. (2023). "Evolution: Untangling the woolly mammoth." *Curr Biol* **33**(16): R870-r872. Twenty-two woolly mammoth genomes have been compared to those of living elephants, identifying genes under strong evolutionary pressure in mammoths, including genes associated with curly, wiry, thick, bushy, coarse, uncombable and (of course) woolly hair.

Rogers, A., A. Treves, R. Karamagi, M. Nyakoojo and L. Naughton-Treves (2023). "Trenches reduce crop foraging by elephants: Lessons from Kibale National Park, Uganda for elephant conservation in densely settled rural landscapes." *PLoS ONE* **18**(7): e0288115.

Crop loss to wildlife, particularly elephants, threatens livelihoods and support for conservation around many protected areas in Africa and Asia. Low-cost elephant barriers have been successfully deployed in savannas but seldom tested around isolated forest parks where the stakes are high for local farmers and isolated elephant populations. We measured the effectiveness of a series of  $\geq 3$  m deep trenches dug by farmers neighboring Kibale National Park, Uganda. We monitored trench quality and crop loss to elephants weekly for a year across 47 transects in four park-adjacent communities, and conducted controlled, before-and-after comparisons of verified damage. Elephants damaged or destroyed  $>4$  ha of crops during 48 independent foraging events, the majority  $<220$ m from the forest boundary. The frequency of damage varied significantly between and within communities. The majority of trenches were not breached by elephants but five suffered  $\geq 4$  breaches. Elephant-breached trenches and their neighboring trenches were lower quality than those not breached in the same week (Wilcoxon test:  $p < 0.001$ ). Trenches were also more likely to be breached where people had planted more crops favored by elephants (Wilcoxon test:  $p = 0.014$ ). Thus, trench quality and the draw of palatable crops both appeared to influence elephant damage. Although trenches may deter elephants, challenges include heavy labor and difficulties of digging in rocky and swampy areas. Trenches alone will not prevent conflict but this strategy holds promise for hot-spots of conflict at forest edges. Given the stakes for farmers and biodiversity, we call for systematic assessment of crop losses and offer recommendations on monitoring and analysis. Such data will allow for stronger inference about effectiveness before investment of effort and resources in interventions.

Saengsawang, P., M. Desquesnes, S. Yangtara, P. Chalermwong, N. Thongtip, S. Jittapalapong and T. Inpankaew (2023). "Molecular detection of *Loxodonto-filaria* spp. in Asian elephants (*Elephas maximus*) from elephant training camps in Thailand." *Comp Immunol Microbiol Infect Dis* **92**: 101910.

Filarial infection is an important disease in human and animal medicine. Several filarial worms are of importance, especially nematodes in the Onchocercidae. The Asian elephant (*Elephas maximus*) is an endangered animal and is very important from several socio-economic and ecological aspects in Thailand. Various parasites can be found in elephants; however, data related to filarial infections in elephants is limited. The objective of this study was to detect filaria in the blood of Asian elephants in Thailand, based on a polymerase chain reaction (PCR) technique. Blood samples were collected from 208 Asian elephants and detected for filaria using PCR, targeting the region of the internal transcribed spacer 2 (ITS2), the cytochrome c oxidase subunit 1 (cox1), and the RNA polymerase II large subunit

(rbp1). In total, 4.33% (9 out of 208) of the sampled elephants had *Loxodontofilaria* spp. DNA with 100% query coverage. In addition, the obtained *cox1* and *rbp1* sequences matched with *Loxodontofilaria* sp., *Onchocerca* sp., and *Dirofilaria* sp. There were no identified risk factors (sex, age, location, and packed cell volume) related to *Loxodontofilaria* infection in elephants. The analyses of the phylogeny of ITS2 sequences demonstrated that the *Loxodontofilaria*-positive sequences were closely related to *Onchocerca dewittei japonica* and *Onchocerca dewittei dewittei* with 100% query coverage. Notably, the concatenated phylogenetic trees of ITS2 and the *cox1* and *rbp1* genes were closely similar to *Loxodontofilaria* sp. To describe in detail the genomic DNA of *Loxodontofilaria* spp., other genes should be additionally studied using a more discriminatory technique, such as DNA barcoding or whole genome sequencing.

Sarma, R. K., A. Gohain, T. H. Ahmed, A. Yadav and R. Saikia (2023). "An environment-benign approach of bamboo pulp bleaching using extracellular xylanase of strain *Bacillus stratosphericus* EB-11 isolated from elephant dung." *Folia Microbiol (Praha)* **68**(1): 135-149.

The use of microbial enzymes is highly encouraged in paper and pulp industries to reduce the excessive use of hazardous chemicals. During the study, xylanase of *Bacillus stratosphericus* EB-11 was characterized for pulp bleaching applications. The extracellular xylanase was produced under submerged fermentation using bamboo waste as a natural carbon source. There was fast cell division and enzyme production under optimized fermentation conditions in the bioreactor. The highest activity was 91,200U after 30 h of growth with  $K_m$  and  $V_{max}$  of 3.52 mg/mL and 391.5  $\mu\text{mol}/\text{min}$  per mg respectively. The purified enzyme with molecular mass  $\sim 60$  kDa had conferred positive activity on native PAGE. The strong inhibition by ethylenediaminetetraacetate and SDS showed the metallo-xylanase nature of the purified enzyme. The bacterial xylanase reduces the use of hydrogen peroxide by 0.4%. Similarly, biological oxygen demand and chemical oxygen demand were reduced by 42.6 and 35.2%. The xylanase-hydrogen peroxide combined treatment and conventional chlorine dioxide-alkaline (CDE(1)D(1)D(2)) bleaching showed almost similar improvement in physicochemical properties of bamboo pulp. Xylanase-peroxide bleaching reduces the lignin content to 4.95% from 13.32% unbleached pulp. This content after CDE(1)D(1)D(2) treatment was 4.21%. The kappa number decreased from 15.2 to 9.46 with increasing the burst factor (15.51), crystallinity index (60.25%), viscosity (20.1 cp), and brightness (65.4%). The overall finding will encourage the development of new cleaner methods of bleaching in the paper and pulp industry.

Scherer, L., L. Bingaman Lackey, M. Clauss, K. Gries, D. Hagan, A. Lawrenz, D. W. H. Müller, M. Roller, C. Schiffmann and A. K. Oerke (2023). "The historical development of zoo elephant survivorship." *Zoo Biol* **42**(2): 328-338.

In the discussion about zoo elephant husbandry, the report of Clubb et al. (2008, *Science* 322: 1649) that zoo elephants had a "compromised survivorship" compared to certain non-zoo populations is a grave argument, and was possibly one of the triggers of a large variety of investigations into zoo elephant welfare, and changes in zoo elephant management. A side observation of that report was that whereas survivorship in African elephants (*Loxodonta africana*) improved since 1960, this was not the case in Asian elephants (*Elephas maximus*). We used historical data (based on the Species360 database) to revisit this aspect, including recent developments since 2008. Assessing the North American and European populations from 1910 until today, there were significant improvements of adult ( $\geq 10$  years) survivorship in both species. For the period from 1960 until today, survivorship improvement was significant for African elephants and close to a significant improvement in Asian elephants; Asian elephants generally had a higher survivorship than Africans.

Juvenile (<10 years) survivorship did not change significantly since 1960 and was higher in African elephants, most likely due to the effect of elephant herpes virus on Asian elephants. Current zoo elephant survivorship is higher than some, and lower than some other non-zoo populations. We discuss that in our view, the shape of the survivorship curve, and its change over time, are more relevant than comparisons with specific populations. Zoo elephant survivorship should be monitored continuously, and the expectation of a continuous trend towards improvement should be met.

Schiffmann, C., L. Hellriegel, M. Clauss, S. Brother, K. Knibbs, C. Wenker, T. Hård and C. Galeffi (2023). "From left to right all through the night: Characteristics of lying rest in zoo elephants." *Zoo Biol* **42**(1): 17-25.

Despite increased research during the past years, many characteristics of resting behavior in elephants are still unknown. For example, there is only limited data suggesting elephants express longer lying bouts and increased total nightly lying durations on soft substrates as compared to hard surfaces. Additionally, it has not been investigated how frequently elephants change body sides between lying bouts. Here we present these characteristics based on observations of nighttime lying behavior in 10 zoo elephants (5 African *Loxodonta africana* and 5 Asian *Elephas maximus* elephants) living in five different European facilities. We found that elephants housed on soft substrates have significantly increased total lying durations per night and longer average lying bouts. Furthermore, at 70%-85% of all bouts, a consistently higher frequency of side change between lying bouts occurred on soft substrates, leading to an overall equal laterality in resting behavior. Deviations from this pattern became evident in elephants living on nonsand flooring or/and in nondominant individuals of nonfamily groups, respectively. Based on our findings, we consider elephants to normally have several lying bouts per night with frequent side changes, given an appropriate substrate and healthy social environment. We encourage elephant-keeping facilities to monitor these characteristics in their elephants' nighttime behavior to determine opportunities for further improvements and detect alterations putatively indicating social or health problems in individual elephants at an early stage.

Sooriyabandara, M. G. C., J. Jayasundara, M. Marasinghe, H. Hathurusinghe, A. U. Bandaranayake, K. Jayawardane, R. M. R. Nilanthi, R. C. Rajapakse and P. C. G. Bandaranayake (2023). "Genetic features of Sri Lankan elephant, *Elephas maximus maximus* Linnaeus revealed by high throughput sequencing of mitogenome and ddRAD-seq." *PLoS ONE* **18**(6): e0285572.

*Elephas maximus maximus* Linnaeus, the Sri Lankan subspecies is the largest and the darkest among Asian elephants. Patches of depigmented areas with no skin color on the ears, face, trunk, and belly morphologically differentiate it from the others. The elephant population in Sri Lanka is now limited to smaller areas and protected under Sri Lankan law. Despite its ecological and evolutionary importance, the relationship between Sri Lankan elephants and their phylogenetic position among Asian elephants remains controversial. While identifying genetic diversity is the key to any conservation and management strategies, limited data is currently available. To address such issues, we analyzed 24 elephants with known parental lineages with high throughput ddRAD-seq. The mitogenome suggested the coalescence time of the Sri Lankan elephant at ~0.2 million years, and sister to Myanmar elephants supporting the hypothesis of the movement of elephants in Eurasia. The ddRAD-seq approach identified 50,490 genome-wide SNPs among Sri Lankan elephants. The genetic diversity within Sri Lankan elephants assessed with identified SNPs suggests a geographical differentiation resulting in three main clusters; north-eastern, mid-latitude, and southern regions. Interestingly, though it was believed that elephants from the Sinharaja rainforest are of an isolated population, the ddRAD-based genetic analysis

clustered it with the north-eastern elephants. The effect of habitat fragmentation on genetic diversity could be further assessed with more samples with specific SNPs identified in the current study.

Sree Lakshmi, P., M. Karikalan, G. K. Sharma, K. Sharma, S. Chandra Mohan, K. Rajesh Kumar, K. Miachio, A. Kumar, M. K. Gupta, R. K. Verma, N. Sahoo, G. Saikumar and A. M. Pawde (2023). "Pathological and molecular studies on elephant endotheliotropic herpesvirus haemorrhagic disease among captive and free-range Asian elephants in India." *Microb Pathog* **175**: 105972.

In the present research pathology and molecular diagnosis of elephant endotheliotropic herpes virus-haemorrhagic disease (EEHV-HD) among Asian elephants was studied. Out of 76 cases, 20 were positive for EEHV infection in PANPOL and POL1 based semi-nested PCR. Out of 20 samples, 10 samples were fatal cases of EEHV-HD while 10 were of either subclinical or latent infection. Acute onset haemorrhagic disease with EEHV-HD had anorexia, facial and neck swelling, cyanotic buccal mucosa and tongue, nasal and ocular discharge, and colic. The hallmark of gross finding in all cases were severe haemorrhagic lesions in the internal organs viz. cyanosis of tongue with multifocal petechial haemorrhages, diffuse epicardial and endocardial haemorrhages, swollen liver (rounded edges) with parenchymal haemorrhages, serosal and mucosal haemorrhages in gastrointestinal tract, congested kidneys with corticomedullary haemorrhages, highly congested meninges, and brain capillaries with haemorrhages. Microscopic findings in all the cases had severe vascular changes in the visceral organs. Microthrombi was present in the vasculature of tongue, heart, lung, liver, kidney, and brain. The endothelial lining of most of the blood vessels were swollen with apoptotic changes. Amphophilic to basophilic intranuclear inclusion bodies were observed in the endothelial cells. Immunostaining using anti-EEHV DNAPOL hyperimmune sera revealed intense positive signals in the endothelium of blood vessels and their walls. Quantification of viral load in necropsy tissue samples revealed highest in the heart ( $7.4 \times 10(6)/\mu\text{g}$  of sample) and least in the brain ( $9 \times 10(3)/\mu\text{g}$  of sample). The PCR amplicons from EEHV1 specific genes (POL1(U38) and TER) were subjected to partial genome sequencing which had 99.9% similarity with the EEHV1A subtype. It was concluded that Asian elephants in India are latently infected for EEHV1 and in all the fatal EEHV-HD cases, EEHV1A subtype was the causative agent with characteristic pathomorphological changes in visceral organs.

Steyrer, C., M. Miller, J. Hewlett, P. Buss and E. H. Hooijberg (2023). "Markers of inflammation in free-living African elephants (*Loxodonta africana*): Reference intervals and diagnostic performance of acute phase reactants." *Vet Clin Pathol* **52 Suppl 1**: 75-86.

**INTRODUCTION:** Acute phase reactants (APRs) have not been investigated in free-living African elephants (*Loxodonta africana*), and there is little information about negative APRs albumin and serum iron in elephants. **OBJECTIVES:** We aimed to generate reference intervals (RIs) for APRs for free-living African elephants, and to determine the diagnostic performance of APRs in apparently healthy elephants and elephants with inflammatory lesions. **METHODS:** Stored serum samples from 49 apparently healthy and 16 injured free-living elephants were used. The following APRs and methods were included: albumin, bromocresol green; haptoglobin, colorimetric assay; serum amyloid A (SAA), multispecies immunoturbidometric assay, and serum iron with ferrozine method. Reference intervals were generated using the nonparametric method. Indices of diagnostic accuracy were determined by receiver-operator characteristic (ROC) curve analysis. **RESULTS:** Reference intervals were: albumin 41-55 g/L, haptoglobin 0.16-3.51 g/L, SAA < 10 mg/L, and serum iron 8.60-16.99  $\mu\text{mol/L}$ . Serum iron and albumin concentrations were lower and haptoglobin and SAA concentrations were higher in the injured group. Serum iron had the best ability

to predict health or inflammation, followed by haptoglobin, SAA, and albumin, with the area under the ROC curve ranging from 0.88-0.93. CONCLUSIONS: SAA concentrations were lower in healthy African vs Asian elephants, and species-specific RIs should be used. Serum iron was determined to be a diagnostically useful negative APR which should be added to APR panels for elephants.

Supanta, J., J. L. Brown, P. Bansiddhi, C. Thitaram, V. Punyapornwithaya, K. Punturee, N. Somboon, P. Towiboon and J. Khonmee (2023). "Physiological changes in captive elephants in Northern Thailand as a result of the COVID-19 tourism ban - muscle, liver, metabolic function, and body condition." *Front Vet Sci* **10**: 1303537.

The international travel ban initiated in March 2020 due to the COVID-19 pandemic greatly affected how captive elephants were managed in Thailand. A lack of tourists and associated income meant elephants were chained longer with reduced food provisions, had fewer mahouts, and limited exercise like riding, which likely affected health and welfare. Fifty-eight elephants from six tourist camps were assigned a body condition score (BCS) and blood samples were collected monthly for 2 years during the travel ban to measure: (1) muscle enzymes [creatinine kinase (CK), aspartate aminotransferase (AST)]; (2) liver enzymes [aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT)]; (3) lipids [total cholesterol (TC), triglycerides (TG), low (LDL) and high (HDL) density lipoproteins]; and metabolic function [glucose, insulin, fructosamine]. Serum CK concentrations were lower at the end of the study, possibly due to no tourist activities like riding. Changes in liver function included increased AST and ALP, also possibly due to physical inactivity. Feeding less bananas and sugar cane was associated with fewer elephants in the obese category and lower TG concentrations. However, increases in glucose, insulin and fructosamine were observed as local people returned to feed elephants after lifting travel restrictions. In sum, changes in several health biomarkers were observed in association with restricted activities and food resources. Camps need better plans to meet the health and welfare needs of elephants during any future disruptions to the tourism industry, although reduced feeding of sweet treats appeared to have positive effects on body condition and metabolic function.

Takehana, K., M. Adachi, S. Ishikawa and N. Yamagishi (2023). "The serum activities of alkaline phosphatase isoenzymes measured using two approved methods in zoo-managed Asian elephants (*Elephas maximus*)." *J Vet Med Sci* **85**(2): 232-235.

The approved Japanese measurement method of circulating alkaline phosphatase (ALP) has changed from that of the Japan Society of Clinical Chemistry (JSCC) to that of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). We measured the serum levels of total ALP (t-ALP) and those of the isoenzymes ALP2 and ALP3 in 50 Asian elephant (*Elephas maximus*) specimens using both methods. The activities determined by the IFCC method were roughly one-third lower than those determined by the JSCC method. We present conversion formulae. Our results enable comparisons of historical and current data on serum ALP activities in endangered, zoo-managed Asian elephants.

Takehana, K., M. Adachi, S. Ishikawa and N. Yamagishi (2023). "Agarose gel electrophoresis pattern of serum creatine kinase and lactate dehydrogenase isoenzymes in zoo-managed Asian elephants (*Elephas maximus*)." *J Vet Med Sci* **85**(5): 578-583.

Serum levels of creatine kinase (CK) and lactate dehydrogenase (LDH) isoenzymes were evaluated in nine zoo-managed Asian elephants (*Elephas maximus*) using a commercial agarose gel electrophoresis (AGE) kit. CK was separated into two major fractions, CK-BB

and CK-MM, along with a small fraction of macroenzyme-CK type 2 (mCK2); CK-MM was the largest fraction. LDH was separated into five fractions (LDH1-5); LDH3 was the largest fraction. Age was negatively and positively correlated with the percentages of CK-BB and CK-MM, respectively, and negatively correlated with CK-BB and mCK2 activities. These results indicate that an AGE kit can be used to evaluate CK and LDH isoenzymes. Routine isoenzyme testing may enable early detection of disease and physiological changes.

Teodoro, T. G. W., F. A. Uzal, N. Streitenberger, M. A. Samol, E. E. Henderson and J. Asin (2023). "Colonic sand impaction with cecal rupture and peritonitis in an adult African savanna elephant, and review of noninfectious causes of gastrointestinal disease in elephants." J Vet Diagn Invest **35**(1): 47-52.

Gastrointestinal disorders are among the most common disease processes in captive elephants. Colic is a frequent clinical presentation and may have several infectious and noninfectious causes. Ingestion of sand has been reported in elephants living in enclosures with loose sandy soils. Similar to the situation in horses, sand ingestion can cause intestinal impaction and colic in elephants. Here we describe a case of colonic sand impaction with cecal rupture and peritonitis in an African savanna elephant from a zoologic collection that died after several days of colic. On autopsy, abundant, gritty, sandy material was found within a segment of colon immediately aboral to the cecum. There was a full-thickness tear in the cecal wall, free intestinal contents within the abdominal cavity, and peritonitis. To our knowledge, the postmortem examination of an elephant with sand impaction and cecal rupture has not been reported previously; this condition should be included among the differential diagnoses in elephants with colic. We review the reports of noninfectious causes of gastrointestinal disease in elephants, which include cases of small intestinal and colonic torsion and of intestinal obstruction by fecal boluses.

Thorel, M., D. Obregon, B. Mulot, A. Maitre, L. Mateos-Hernandez, P. Y. Moalic, A. Wu-Chuang, A. Cabezas-Cruz and A. Leclerc (2023). "Conserved core microbiota in managed and free-ranging *Loxodonta africana* elephants." Front Microbiol **14**: 1247719.

The gut microbiota plays a crucial role in animal health and homeostasis, particularly in endangered species conservation. This study investigated the fecal microbiota composition of European captive-bred African savanna elephants (*Loxodonta africana*) housed in French zoos, and compared it with wild African savanna elephants. Fecal samples were collected and processed for DNA extraction and amplicon sequencing of the 16S rRNA gene. The analysis of  $\alpha$  and  $\beta$  diversity revealed significant effects of factors such as diet, daily activity, and institution on microbiota composition. Specifically, provision of branches as part of the diet positively impacted microbiota diversity. Comparative analyses demonstrated distinct differences between captive and wild elephant microbiomes, characterized by lower bacterial diversity and altered co-occurrence patterns in the captive population. Notably, specific taxa were differentially abundant in captive and wild elephants, suggesting the influence of the environment on microbiota composition. Furthermore, the study identified a core association network shared by both captive and wild elephants, emphasizing the importance of certain taxa in maintaining microbial interactions. These findings underscore the impact of environment and husbandry factors on elephant gut microbiota, highlighting the benefits of dietary enrichment strategies in zoos to promote microbiome diversity and health. The study contributes to the broader understanding of host-microbiota interactions and provides insights applicable to conservation medicine and captive animal management.

Tsegaye, A., A. Bekele and A. Atikem (2023). "Local's attitude towards African elephant conservation in and around Chebra Churchura National Park, Ethiopia." PLoS ONE **18**(10):

e0292641.

Economic growth and development in developing countries often involves land-use changes which fragment natural areas, bring humans and wildlife into closer proximity and escalating human-wildlife conflicts. Human-wildlife conflicts impose huge costs on local people and their livelihoods. Balancing developmental activities with the conservation of mega fauna such as the African and Asian elephants (*Loxodonta africana*, *Elephas maximus*; respectively) remains problematic. Understanding the reasoning upon which perceived risks and level of human- elephant conflict laid is critical to address societal or cultural beliefs in order to develop effective mitigation strategies. The perceived risks and level of conflict have to be properly addressed for effective planning and implementation of appropriate mitigation strategies. We studied human- elephant interactions in Chebra Churchura National Park Ethiopia (CCNP) from September 8 to October 28, 2022 and collected baseline data on human perceptions of conflicts in an area where elephant populations are increasing. To complete our study, we surveyed 800 household from 20 villages adjacent to the CCNP. The purpose of this investigation was to identify the relevance of the existing human-elephant conflict (HEC) with the attitude of local communities towards elephant conservation, the park management and perceived effective mitigation techniques. The local communities trust in the implementation of different traditional mitigation techniques is generally weak. The households interviewed were less positive towards the effectiveness of most of the traditional techniques in chasing elephants away from their farm lands. They believed that elephants had already adapted and do not respond to most of these techniques. Against the above perception in exception of their usual absence and late arrival, perception of local communities about shooting warning gun by park scouts is among the most accepted effective methods in chasing elephants from their farm lands. The majority of respondents believe that separation of elephants and humans by constricting barriers is the best solution to the problem. The idea of constructing barriers such as electric fence; ditch or concrete wall and blocking corridors between the Park boundary and the villages have become the most popular idea of local communities followed by relocating people to other safer places, as the best protection method against the elephant attack irrespective of the associated initial and maintenance costs.

Turner, A., N. Masters, T. Pfau, J. R. Hutchinson and R. Weller (2023). "DEVELOPMENT AND EVALUATION OF A STANDARDIZED SYSTEM FOR THE ASSESSMENT OF LOCOMOTOR HEALTH IN ELEPHANTS UNDER HUMAN CARE." *J Zoo Wildl Med* **54**(3): 529-537.

Although lameness is a common problem in elephants (Asian elephant [*Elephas maximus*] and African elephants *Loxodonta africana* and *Loxodonta cyclotis*) under human care, there has not been a standardized lameness assessment system to date. This study developed and evaluated a standardized system for the assessment of locomotion in elephants under human care regardless of husbandry system. In total, 72 elephants out of a possible 73 in the United Kingdom and Ireland were filmed from behind, from in front, and from both sides. Using a questionnaire and a select panel of elephant specialists, a zoo veterinarian, and a locomotion expert, a numerical rating scoring (NRS) system was proposed. Locomotion was scored on a 4-point scale with numerical values 0-4 corresponding to specific criteria as follows: 0 = clinically sound, 1 = stiffness, 2 = abnormal tracking, and 4 = reluctance to bear weight. The intra- and interobserver repeatability of five veterinary surgeons using this system was determined and compared with a visual analog scale (VAS) expressed as a 100-mm line. Overall intraobserver reliability was moderate (Cohen's kappa [ $\kappa$ ] = 0.676) and interobserver reliability was fair ( $\kappa$  = 0.37) for the presence of lameness. Interobserver agreement improved from the first scoring to second scoring from slight agreement to fair agreement for stiffness and reluctance to bear weight. Abnormal tracking



had moderate intraobserver agreement for both scoring sessions. There were wide widths of agreement for the VAS interobserver (67 mm); however, they were narrower for the intraobserver (33 mm). The developed NRS can be used on freely moving elephants to evaluate elephant locomotion, regardless of husbandry methods, and has been shown to be more reliable than a VAS.

Ukonaho, S., V. Berger, D. J. Franco Dos Santos, W. Htut, H. H. Aung, U. K. Nyeing, S. Reichert and V. Lummaa (2023). "Seasonal variation in molecular and physiological stress markers in Asian elephants." *Conserv Physiol* **11**(1): coad029.

Free-living species exhibit seasonal variation in various life history traits, including vital rates such as birth and death patterns. Different physiological mechanisms are thought to underlie the expression of life history traits that contribute to lifetime fitness. However, although the broad impacts of seasonality on life history traits and trade-offs is well established in many systems, the exact physiological mechanisms responsible for driving differences within and between individuals are poorly understood. Among them, molecular and physiological stress pathways, such as stress hypothalamic-pituitary-adrenal axis and oxidative stress, have potential to mediate relationships between individual survival, reproduction and environmental seasonality. Here, we determine how different physiological markers of stress including faecal cortisol metabolites (FCMs), heterophils/lymphocytes (H/L) ratio, two markers indicating oxidative balance including a marker of oxidative damage (reactive oxygen metabolites, ROM) and a marker of antioxidant defences (superoxide dismutase, SOD) and body weight vary in a large semi-captive population of Asian elephants (*Elephas maximus*) exposed to extreme seasonality (e.g. elevated temperatures). Individuals showed higher FCM levels and H/L ratios during cold season, indicating increased stress, and the lowest FCM levels during monsoon season and H/L ratios during hot and dry season, but we found no pattern in oxidative stress (ROM and SOD) levels. Hot season also associated with a decline in body weight. The present study shows how different physiological parameters (FCM levels and H/L ratio), molecular (oxidative stress) and body condition vary with seasonal changes, and how these parameters might allow individuals to adapt to such variations. Our results on an endangered long-lived species are crucial in indicating the most productive timing for conservation efforts, predicting how individuals cope with environmental changes, and allow for a more accurate representation of how animal physiology operates in nature.

van Zyl, C. W., M. van Reenen, G. Osthoff and I. du Preez (2023). "Evaluation of BAYESIL for automated annotation of (1)H NMR data using limited sample volumes: application to African elephant serum." *Metabolomics* **19**(4): 31.

INTRODUCTION: Technological advancements enabled the analyses of limited sample volumes on (1)H NMR. Manual spectral profiling of the data is, however, complex, and timely. OBJECTIVE: To evaluate the performance of BAYESIL for automated identification and quantification of (1)H NMR spectra of limited volume samples. METHOD: Aliquots of a pooled African elephant serum sample were analyzed using standard and reduced volumes. Performance was evaluated on confidence scores, non-detects and laboratory CV. RESULTS: Of the 47 compounds detected, 28 had favorable performances. The approach could differentiate samples based on biological variation. CONCLUSIONS: BAYESIL is valuable for limited sample (1)H NMR data analyses.

Vasudev, D., R. J. Fletcher, Jr., N. Srinivas, A. J. Marx and V. R. Goswami (2023). "Mapping the connectivity-conflict interface to inform conservation." *Proc Natl Acad Sci U S A* **120**(1): e2211482119.

Balancing the competing, and often conflicting, needs of people and wildlife in shared landscapes is a major challenge for conservation science and policy worldwide. Connectivity is critical for wildlife persistence, but dispersing animals may come into conflict with people, leading to severe costs for humans and animals and impeding connectivity. Thus, conflict mitigation and connectivity present an apparent dilemma for conservation. We present a framework to address this dilemma and disentangle the effects of barriers to animal movement and conflict-induced mortality of dispersers on connectivity. We extend random-walk theory to map the connectivity-conflict interface, or areas where frequent animal movement may lead to conflict and conflict in turn impedes connectivity. We illustrate this framework with the endangered Asian elephant *Elephas maximus*, a species that frequently disperses out of protected areas and comes into conflict with humans. We mapped expected movement across a human-dominated landscape over the short- and long-term, accounting for conflict mortality. Natural and conflict-induced mortality together reduced expected movement and connectivity among populations. Based on model validation, our conflict predictions that explicitly captured animal movement better explained observed conflict than a model that considered distribution alone. Our work highlights the interaction between connectivity and conflict and enables identification of location-specific conflict mitigation strategies that minimize losses to people, while ensuring critical wildlife movement between habitats. By predicting where animal movement and humans collide, we provide a basis to plan for broad-scale conservation and the mutual well-being of wildlife and people in shared landscapes.

Verma-Kumar, S., D. Abraham, N. Dendukuri, J. V. Cheeran, R. Sukumar and K. N. Balaji (2023). "Correction: Serodiagnosis of Tuberculosis in Asian Elephants (*Elephas maximus*) in Southern India: A Latent Class Analysis." *PLoS ONE* **18**(11): e0294550.  
[This corrects the article DOI: 10.1371/journal.pone.0049548].

Vollrath, F. (2023). "Uncoupling elephant TP53 and cancer." *Trends Ecol Evol* **38**(8): 705-707.  
Elephant testicles do not descend, with implications for sperm production being hot enough to compromise germline DNA replication/repair. Uniquely, elephants also possess 20 copies of a gene encoding for the p53 protein. Did elephants evolve multiplication of the TP53 gene complex to protect their germline rather than to fight cancer?

Von Hagen, L., C. A. LaDue and B. A. Schulte (2023). "Elephant Scar Prevalence in the Kasigau Wildlife Corridor, Kenya: Echoes of Human-Elephant Conflict." *Animals (Basel)* **13**(4).  
Human-elephant conflict (HEC) compromises crop security and threatens elephant conservation. Most commonly, HEC manifests as crop-foraging as elephants modify natural foraging strategies to incorporate crops. Farmers may retaliate by frightening or harming elephants, leaving scars from inflicted wounds. We assessed the prevalence and distribution of scars on the bodies of African savanna elephants (*Loxodonta africana*) observed in the Kasigau Wildlife Corridor (KWC), part of the Greater Tsavo Ecosystem of Kenya, where conflict is prevalent. We surmised that scars on the body are largely a result of HEC as opposed to scars on the rump or head, which we attributed primarily to elephant-elephant conflict. We hypothesized that: (1) male elephants would have more scars than females; (2) older males would be more likely to have scars than younger males; and (3) most scars would be located on the bodies of elephants. We assessed scars from a photographic catalogue of elephants from the KWC. In line with our hypotheses, male elephants were more likely to have scars than females (32% of males compared to 6% of females); older males had significantly more scars than younger males (61% compared to 24%); and the majority of scars (89%) were located on the body. Scar presence may be useful as an

animal-centered indicator to estimate the prevalence and demographic patterns of HEC.

Voskarides, K. and N. Giannopoulou (2023). "The Role of TP53 in Adaptation and Evolution." Cells **12**(3).

The TP53 gene is a major player in cancer formation, and it is considered the most important tumor suppressor gene. The p53 protein acts as a transcription factor, and it is involved in DNA repair, senescence, cell-cycle control, autophagy, and apoptosis. Beyond cancer, there is evidence that TP53 is associated with fertility, aging, and longevity. Additionally, more evidence exists that genetic variants in TP53 are associated with environmental adaptation. Special TP53 amino-acid residues or pathogenic TP53 mutations seem to be adaptive for animals living in hypoxic and cold environments or having been exposed to starvation, respectively. At the somatic level, it has recently been proven that multiple cancer genes, including TP53, are under positive selection in healthy human tissues. It is not clear why these driver mutations do not transform these tissues into cancerous ones. Other studies have shown that elephants have multiple TP53 copies, probably this being the reason for the very low cancer incidence in these large animals. This may explain the famous Peto's paradox. This review discusses in detail the multilevel role of TP53 in adaptation, according to the published evidence. This role is complicated, and it extends from cells to individuals and to populations.

Watanabe, K., M. Kondo, Y. Ikenaka, S. M. M. Nakayama and M. Ishizuka (2023). "A Comparative Genomic and Phylogenetic Investigation of the Xenobiotic Metabolism Enzymes of Cytochrome P450 in Elephants Shows Loss in CYP2E and CYP4A." Animals (Basel) **13**(12).

Cytochrome P450 is an important enzyme that metabolizes a variety of chemicals, including exogenous substances, such as drugs and environmental chemicals, and endogenous substances, such as steroids, fatty acids, and cholesterol. Some CYPs show interspecific differences in terms of genetic variation. As little is known about the mechanisms of elephant metabolism, we carried out a comparative genomic and phylogenetic analysis of CYP in elephants. Our results suggest that elephant CYP genes have undergone independent duplication, particularly in the CYP2A, CYP2C, and CYP3A genes, a unique cluster specific to elephant species. However, while CYP2E and CYP4A were conserved in other Afrotheria taxa, their decay in elephants resulted in genetic dysfunction (pseudogene). These findings outline several remarkable characteristics of elephant CYP1-4 genes and provide new insights into elephant xenobiotic metabolism. Further functional investigations are necessary to characterize elephant CYP, including expression patterns and interactions with drugs and sensitivities to other chemicals.

Wattanani, P., Y. Yingchutrakul, K. Kornkaewrat, S. Mahasawangkul, S. Roytrakul and A. Pinyopummin (2023). "Non-targeted proteomic analysis of Asian elephant (*Elephas maximus*) seminal plasma using an in-solution digestion technique and liquid chromatography tandem-mass spectrometry." Front Vet Sci **10**: 1174078.

Seminal plasma proteins have recently been reported to play a significant role as valuable materials for understanding male reproductive biology, identifying causes of fertility problems, and developing reproductive biomarkers. Proteomic analysis of seminal plasma holds promise in advancing the understanding of male Asian elephant reproductive biology. This study aims to explore seminal plasma proteins of Asian elephants and their probable functions to provide fundamental information about male reproduction in this species. The protein solution from pooled seminal plasma from 10 bulls (a total of 33 ejaculates) was digested into peptides and identified using LC-MS/MS. Out of 986 proteins, 597 were mapped and matched with 58 species in UniProt databases, including *Elephas maximus*.

These mapped proteins were mostly involved in binding function, catalytic activity, cellular process, and metabolic process. Only 29 mapped proteins were recognized to be related in reproductive process, mainly associated in spermatogenesis and sperm capacitation. Additionally, several seminal plasma proteins related to fertility or semen quality in other mammals were also found in Asian elephant semen, such as keratin type I, aldose reductase, thrombospondon-1, fibronectin 1, platelet-activating factor acetyl hydrolase, mannosidase, and semenogelin-2. This discovery clearly reveals the beneficial protein profile in seminal plasma of the Asian elephant and serves as a crucial step in investigating infertility and poor semen quality in this valuable species.

Wettasin, M., R. Chaiyarat, N. Youngpoy, N. Jieychien, R. Sukmasuang and P. Tanhan (2023). "Environmental factors induced crop raiding by wild Asian elephant (*Elephas maximus*) in the Eastern Economic Corridor, Thailand." Sci Rep **13**(1): 13388.

Crop raiding are an increasing concern in wildlife conservation. This study identified the environmental factors that cause wild Asian elephants (*Elephas maximus*) to enter suburban and rural areas and share resources with humans in the Eastern Economic Corridor (EEC) in the eastern part of Thailand. The snowball method was used to interview villagers that had crop raiding experienced in seven provinces of the EEC and adjacent provinces in the eastern part of Thailand in 2020, and data from 183 households indicated that crop raiding had increased continuously from 2000 to 2020, especially in Chonburi, Chachoengsao, and Prachinburi provinces, which have seen increases in damaged agricultural areas. MaxEnt analysis showed an increase in incidents of crop raiding, while the elephants distribution area decreased from 9534 km<sup>2</sup> in 2000 to 5199 km<sup>2</sup> in 2010 and 4850 km<sup>2</sup> in 2020. The study area has had land use changes in the low elevations from croplands of cassava and sugar cane to eucalyptus, para rubber, and fruits. These mixed crop plantations provide a pseudo-habitat for wild Asian elephants. The results from this study provide evidence that changes in land use and reduction of suitable habitat are factors that influenced the movement of wild Asian elephants to the rural agricultural areas and increased the incidents of crop raiding.

Yang, J., Y. Chen, Z. Dong, W. Zhang, L. Liu, W. Meng, Q. Li, K. Fu, Z. Zhou, H. Liu, Z. Zhong, X. Xiao, J. Zhu and G. Peng (2023). "Distribution and association of antimicrobial resistance and virulence characteristics in *Enterococcus* spp. isolates from captive Asian elephants in China." Front Microbiol **14**: 1277221.

*Enterococcus* spp., as an opportunistic pathogen, are widely distributed in the environment and the gastrointestinal tracts of both humans and animals. Captive Asian elephants, popular animals at tourist attractions, have frequent contact with humans. However, there is limited information on whether captive Asian elephants can serve as a reservoir of antimicrobial resistance (AMR). The aim of this study was to characterize AMR, antibiotic resistance genes (ARGs), virulence-associated genes (VAGs), gelatinase activity, hemolysis activity, and biofilm formation of *Enterococcus* spp. isolated from captive Asian elephants, and to analyze the potential correlations among these factors. A total of 62 *Enterococcus* spp. strains were isolated from fecal samples of captive Asian elephants, comprising 17 *Enterococcus hirae* (27.4%), 12 *Enterococcus faecalis* (19.4%), 8 *Enterococcus faecium* (12.9%), 7 *Enterococcus avium* (11.3%), 7 *Enterococcus mundtii* (11.3%), and 11 other *Enterococcus* spp. (17.7%). Isolates exhibited high resistance to rifampin (51.6%) and streptomycin (37.1%). 50% of *Enterococcus* spp. isolates exhibited multidrug resistance (MDR), with all *E. faecium* strains demonstrating MDR. Additionally, nine ARGs were identified, with tet(M) (51.6%), erm(B) (24.2%), and cfr (21.0%) showing relatively higher detection rates. Biofilm formation, gelatinase activity, and  $\alpha$ -hemolysin activity were

observed in 79.0, 24.2, and 14.5% of the isolates, respectively. A total of 18 VAGs were detected, with gelE being the most prevalent (69.4%). Correlation analysis revealed 229 significant positive correlations and 12 significant negative correlations. The strongest intra-group correlations were observed among VAGs. Notably, we found that vancomycin resistance showed a significant positive correlation with ciprofloxacin resistance, cfr, and gelatinase activity, respectively. In conclusion, captive Asian elephants could serve as significant reservoirs for the dissemination of AMR to humans.

Yang, Y., P. Tummaruk, T. Angkawanish, W. Langkaphin and K. Chatdarong (2023). "Seasonal Effects on Body Condition and Characteristics of the Estrous Cycle in Captive Asian Elephants (*Elephas maximus*) in Thailand: A Retrospective Study." *Animals (Basel)* **13**(7).

The aim of this study was to investigate the effects of season on the body condition score (BCS), the characteristics of the estrous cycle (luteal phase [LPL], follicular phase [FPL], estrous cycle [ECL] lengths, and the start of the luteal phase [SLP] and follicular phase [SFP]), and progesterone levels (baseline and peak) of eight captive Asian elephants (*Elephas maximus*) in Thailand. From 2014 to 2019, blood samples were collected weekly for serum progesterone enzyme immunoassays (EIAs). Estrous cycles (n = 70), including the luteal and follicular phases, and BCS (n = 70) were recorded. Based on the BCS, the LPL, FPL, and ECL were assigned to the following two groups: normal (BCS = 3.0-4.0, n = 38) and overweight (BCS = 4.5-5.0, n = 32). The findings demonstrated that there was no difference in LPL between the groups. However, in the normal group, the ECL was one week longer ( $14.9 \pm 1.7$  vs.  $13.9 \pm 1.7$  weeks;  $p < 0.05$ ), and the FPL also tended to be one week longer ( $7.2 \pm 1.7$  vs.  $6.4 \pm 1.5$  weeks;  $p = 0.06$ ) than in the overweight group. The mean progesterone level during the rainy, hot, and cool seasons was not statistically different. Based on the yearly averaged BCS from three seasons, the baseline and peak levels of progesterone were classified into the normal (n = 16) and overweight (n = 12) groups. Females with a normal BCS tended to exhibit higher progesterone peak levels ( $p = 0.08$ ). The majority of peaks appeared during the rainy season (53.57%). The BCS was highest during the hot (4.47) and rainy (4.38) seasons, but not during the cool (4.12) season. The LPL, FPL, and ECL were not affected by the season in which the luteal phase occurred. On the other hand, the rainy season had a significant effect on the SFP, resulting in a longer LPL ( $p < 0.05$ ) and ECL ( $p = 0.01$ ); both were the longest during the rainy season. In conclusion, the effects of season on BCS may be related to characteristics of the estrous cycle and peak progesterone levels. Ultimately, these findings provide ground knowledge to assist elephant managers and owners in planning breeding activities using seasonal effects and BCS measurements in tropical climates.

Zhang, C., Z. Lian, B. Xu, Q. Shen, M. Bao, Z. Huang, H. Jiang and W. Li (2023). "Gut Microbiome Variation Along A Lifestyle Gradient Reveals Threats Faced by Asian Elephants." *Genomics Proteomics Bioinformatics*.

The gut microbiome is closely related to host nutrition and health. However, the relationships between gut microorganisms and host lifestyle are not well characterized. In the absence of confounding geographic variation, we defined clear patterns of variation in the gut microbiomes of Asian elephants (AEs) in the Wild Elephant Valley, Xishuangbanna, China, along a lifestyle gradient (completely captive, semicaptive, semiwild, and completely wild). A phylogenetic analysis using the 16S rRNA gene sequences highlighted that the microbial diversity decreased as the degree of captivity increased. Furthermore, the results showed that the bacterial taxon WCHB1-41\_c was substantially affected by lifestyle variations. qRT-PCR analysis revealed a paucity of genes related to butyrate production in the gut microbiome of AEs with a completely wild lifestyle, which may be due to the

increased environmental unfavorable factors. Overall, these results demonstrate the distinct gut microbiome characteristics among AEs with a gradient of lifestyles and provide a basis for designing strategies to improve the well-being or conservation of this important animal species.

Zheng, B., X. Lin, D. Yin and X. Qi (2023). "Does Tobler's first law of geography apply to internet attention? A case study of the Asian elephant northern migration event." PLoS ONE **18**(3): e0282474.

One of the basic assumptions of spatial theory is formulated in Waldo Tobler's first law of geography: "everything is related to everything else, but near things are more related than distant things." However, as internet space is a complex virtual space independent of the real world, whether this law is applicable to things in the internet space remains to be explored in depth. Therefore, this study takes the event of Asian elephant northern migration as an example, attempts to investigate the issue of the applicability of Tobler's first law of geography to internet attention by integrating geographic methods such as spatial visualization, spatial correlation analysis, and Geo-detector. The results show that Tobler's first law of geography does not fully apply to internet attention, which does not decay with increasing distance. Geographical distance, within certain boundaries, is influenced by "identity" and "relevance", and still plays a large role in internet attention. However, once the boundaries are exceeded, the impact of geographic distance on internet attention is weakened by the intervention of influencing factors such as the degree of information technology, population, and the strength of news media publicity. Overall, the strength of news media publicity has the greatest impact on internet attention. And when it interacts with geographic proximity, it has the most significant effect on internet attention.