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Commentary

Conservation-focused biobanks: A valuable resource for wildlife DNA forensics

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ABSTRACT

Wildlife crime continues to pose a great threat to animal and plant populations and their environments. Among the different disciplines striving to halt the current biodiversity crisis, wildlife forensics has become instrumental in combating wildlife crime, with the application of DNA forensics methods to the gathering of evidence and intelligence for investigations gaining increasing importance. The wide range of services offered by wildlife DNA forensics laboratories requires the development of diagnostic genetic markers and robust molecular genetics protocols to ensure that any evidence gathered can withstand scrutiny in court. Appropriately vouchered reference samples are crucial for the generation of robust wildlife forensics DNA data; however, access to these reference samples for rare, endangered species, or those inhabiting remote areas, can be challenging. Here, we highlight the importance of conservation and research-focused biobanks as a source to obtain adequate reference samples for wildlife DNA forensics. Furthermore, we encourage collaboration between wildlife forensic geneticists and conservation and research-focused biobanks, as well as the sharing of digital DNA among the wildlife forensics community, as strategies to overcome the challenges associated with sample and DNA data acquisition and hence accelerate the successful implementation of law enforcement for combating wildlife crime.

Wildlife forensics

Wildlife forensics as an instrument of law enforcement for combating wildlife crime has become increasingly important [1,2]. Wildlife crime continues to increase worldwide through wildlife trade, poaching, illegal logging, and the fraudulent use of wild derivatives in food, traditional medicine, artefacts and cosmetics [3,4]. This range of activities poses a great threat to wildlife populations and their environments and significantly hinders conservation actions aimed at halting the current biodiversity crisis [5,6]. In addition, public health concerns over the emergence and transmission of zoonotic diseases are increasing globally [7], with wildlife trade being identified as one of the main drivers [7–9]. Wildlife forensics thus plays a pivotal role not only in wildlife conservation and law enforcement but also in the well-being of our societies [4].

Wildlife DNA forensics

Wildlife DNA forensics has developed as a key discipline to aid investigations linked to wildlife-related crime and law enforcement, in particular when samples representing the available evidence are incomplete, degraded, highly processed and/or morphologically unidentifiable [3,10–12]. Wildlife DNA forensics also plays a key role in the monitoring of the implementation of national and international agreements regulating wildlife harvest and trade, to ensure sustainable use of natural resources and biodiversity conservation, and offers a powerful approach to gather evidence and intelligence [10,13,14].

The most frequent services provided by forensic geneticists to wildlife officers are related to species identifications of unidentified samples, species detection in mixed-DNA samples, assignment of an unidentified sample to a particular individual or individualisation, sexing of samples, and assignment of geographic origin [12,15,16]. Wildlife DNA forensics has proved successful in many cases, but the discipline still faces a number of challenges [11,12,16,17].

DNA analyses as part of wildlife forensic investigations provide information to support prosecution and law enforcement and, therefore, confidence in the results of the investigations is crucial [10,12,18]. Similar to human forensic analyses, DNA analyses in wildlife forensics

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need to be conducted following internationally recognised quality assurance standards, as the genetic results provided will need to be robust to withstand scrutiny in court [10,19]. Therefore, access to reference samples is essential to develop adequate genetic markers and molecular genetics protocols applicable to evidence samples and to ensure their reliable validation [3,4,14]. This validation is particularly important for species that have not yet been studied in a wildlife forensics context [14], and for endangered species for which genetic studies, and, therefore, availability of genetic markers, are still scarce [20].

The need for reference samples in wildlife DNA forensics

One of the main challenges encountered in wildlife DNA forensics is access to appropriately vouchered reference samples to build a reference database that can be confidently interrogated for the identification of species, geographic origin of samples, or the individualisation of samples [10,15]. Wildlife forensics laboratories often build and curate their in-house sample collections and databases of sequences or genotypes [18]. However, representative reference samples for certain species, in particular for rare, endangered species or those inhabiting remote areas might often be challenging to obtain for many laboratories [14,18], although there are continuing efforts to build large repositories of DNA data from wildlife species generated with the validation and quality standards required for wildlife forensics (e.g., ForCyt, [21]; RhoDis®, [22]). Wildlife forensics laboratories can also benefit from the use of the comprehensive DNA sequences or genotypes available from publicly available repositories [4,23]. However, genetic data available in these repositories will need to undergo an additional process of validation (examination, vetting, scrutiny) to meet the quality standards in forensic investigations [10,18]. Considering the current biodiversity crisis and the increasing levels of endangerment that many species face, the need for acquiring further reference samples for wildlife DNA forensics will only increase. Therefore, it would be prudent to plan the gathering of appropriate reference samples, as well as to formalise data sharing of established forensic DNA databases between wildlife forensic practitioners, to anticipate future genetic testing that might be required by investigators and prosecutors [18].

Biobanks as a source of reference samples for wildlife DNA forensics

Since the implementation of the Nagoya Protocol in 2014 by the Convention on Biological Diversity [24], an international agreement calling for fair and equitable access and benefit-sharing from the use of genetic resources (https://www.cbd.int/abs/), biobanks are increasingly considered valuable national assets for conducting research and contributing to wildlife conservation [25,26]. The Nagoya Protocol has generated opportunities for countries to govern access to the use of their genetic resources and traditional knowledge, and has encouraged collaboration between scientists and stakeholders from different countries [27,28]; such outcomes are starting to address the long tradition of "parachute science" or "colonial science" [29,30]. To this end, a Prior Informed Consent (PIC) under Mutually Agreed Terms (MAT) between researchers and relevant government agencies from different countries are required. Processes to obtain PIC and MAT require time, first to establish the collaborations and agreements, then to complete the necessary paperwork, as well as to seek additional funding to cover any potential monetary benefits (e.g., fees for access to samples, support to trust funds dedicated to the conservation and sustainable use of biodiversity, research funding, joint ownership of intellectual property rights). Therefore, impediments associated with compliance with the Nagoya Protocol for non-commercial research and the potential negative effects of its implementation have generated concern among the research and conservation communities [31–33]. Similar concerns would be expected in wildlife DNA forensics given the need for reference samples, particularly within the constraints of the limited turnaround times to provide results to prosecutors or investigators.

In addition to the need to obtain PIC and MAT between researchers or practitioners and governmental agencies from those countries that have ratified the Nagoya Protocol (see CBD website for details of the ratifying countries; https://www.cbd.int/abs/nagoya-protocol/signatories/) and collecting permits required by local legislation, CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) import and export permits would in many instances required for obtaining samples from endangered species. As an international agreement between governments, CITES oversees the international trade in specimens of species covered by the Convention through a licensing system for import, export, re-export and introduction from the seas (https://www.cites.org). Depending on which CITES Appendix the species from which the specimen is required is listed, an export (or re-export), as well as an import permit, might need to be expedited by one of the management authorities of each Party (country) signatory to the Convention. The CITES website provides extensive information regarding the procedures to trade specimens or products derived from listed species as well as exemptions to the normal procedures and other special provisions.

Wildlife samples for building forensic reference databases and/or development of genetic markers are now considered by CITES research material, after the acknowledgement of CITES Parties of the need to facilitate access to samples for wildlife forensic testing. This led to the amendment of the Resolution Conf. 11.15 (Rev. CoP 18) in which similar to specimens held in museums and herbariums, the movement of CITES specimens between registered scientific institutions or wildlife forensics research institutions does not require CITES permits or certificates. However, this exemption does not apply to marine biological samples (introduced from the sea) which will always require either a permit or certificate (https://cites.org/sites/default/files/document/E-Res-11-15-R18.pdf). Resolution Conf. 11.15 (Rev. CoP 18) also provides detailed information on standards required for scientific and forensic research institutions registration; a list of registered institutions, management authorities and enforcement location points in each Party can be found on the CITES website.

Biobanks can help overcome some of the challenges associated with reference sample collection and, therefore, are an excellent resource to be exploited for wildlife DNA forensics. Cryopreserved biological collections of animal and plant biological samples and/or DNA, and associated metadata, are stored and curated in biobanks according to professional standards [34]. Different professional societies provide best-practice guidelines to ensure that biobanks guarantee a set of quality standards for the acquisition (including evidence of legal provenance), storage and curation of samples as well associated metadata; for example, ISBER (International Society for Biological and Environmental Repositories, www.isber.org) and ESBB (European, Middle Eastern & African Society for Biopreservation and Biobanking, www.esbb.org).

The importance of biobanks as a resource for research and commercial development has long been recognised, in particular in the human health sector, followed by the livestock and crop production and health sectors [35–37]. Long-term biobanking initiatives have proliferated in the last decade, with interest beyond the cryopreservation of human, livestock and crop samples. In addition to long-established biobanks in museums and botanical gardens, increasing awareness of the biodiversity crisis and the increasing risk of extinction of wild and captive populations has led to the establishment of new biobanking initiatives aiming to contribute to wildlife conservation [26,38–41]. Some of these conservation-focused biobanking initiatives include the Frozen Zoo ([42]; https://science.sandiegozoo.org/resources/frozen-zoo®), The Frozen Ark ([43]; www. frozenark.org), the European Association of Zoos and Aquaria Biobank (EAZA; https://www.eaza.net/conservation/research/eaza-biobank/), the Global Genome Biodiversity Network ([44]; GGBN, www.ggbn.org), and more recently CryoArks

(www.cryoarks.org, see Box 1) and Nature's Safe (https://www.natures-safe.com/).

The contribution to research by collections held in museums, zoological and botanical gardens, and academic biobanks is widely acknowledged [45,46], but their potential value for wildlife DNA forensic investigations is often less publicised, except for museums in which sample acquisition from voucher specimens, and in some instances complete wildlife forensic analysis, are integral services provided by these institutions (e.g., The Natural History Museum of London, The Australian Museum). Biobank consortiums that include academic/research partners, in addition to museums, zoological and botanical gardens partners, can offer extra resources beyond reference samples for species identification. Conservation genetics research tends to include population-level sampling and in many instances, the sampling is conducted across different geographic regions [47,48]. Research collections, therefore, are valuable resources to develop DNA sequence and allele frequency reference databases and DNA registers to improve sample localization (geographical origin) and sample individualisation (linkage of a carcass to DNA evidence from a suspect, linkage of a trophy to a carcass, determining the number of individuals in mixture samples, reproductive origin); other main interrogations in addition to species identification conducted by wildlife forensic geneticists [4,12,14,16]. Furthermore, research collections associated with conservation genetics studies also provide opportunities to overcome current challenges encountered in wildlife DNA forensics, such as the limited availability of appropriate samples to develop molecular genetics protocols to confidently identify subspecies and hybrids [15,18]. Standard procedures for the donation of samples to conservation and research biobanks include submission of any legal documentation associated with the samples donated, including sample collection permits, CITES permits, and for samples collected after October 2014, when the Nagoya Protocol was implemented, documentation indicating authorisation for access to genetic resources and requirements established for benefit-sharing by the ratifying country where the sample(s) originated [49].

However, it is important to acknowledge that not all the samples held in biobanks might be optimal for the use in wildlife forensics. Known source is crucial for use of a reference sample; therefore, only samples with adequate metadata could be used for wildlife forensics [19,21]. To increase the visibility of such samples, databases in biobanks could incorporate a field to indicate samples in the repository suitable for wildlife forensics.

Conservation and research biobanks challenges

Conservation and research-focused biobanks face similar challenges to other biobanks. These challenges include facilities to cryopreserve samples, implementing procedures and employing personnel to process samples and associated metadata, and, importantly, achieving the financial capacity to guarantee the long-term sustainability of the repository [41,50]. Public and end-user support is crucial to increase awareness of the value of these biobanks, not only in terms of their research utility and contribution to wildlife conservation but also in terms of the current and potential socioeconomic impacts of the collections they contain [50]. The value of biobanks also needs to be further recognised by relevant funding bodies if long-term sustainability is to be attained. Collaboration of biobanks with end-users, such as wildlife forensic geneticists and practitioners, in funding proposals could help secure the provision of funds for sample procurement and curation [50].

Interoperability is also key for the sustainability of biobanks and consortiums such as The Frozen Ark, the EAZA Biobank and CryoArks (see Box 1) in the UK. Fig. 1 illustrates how multicentre collaborations at the national level in the UK can facilitate cost-effective and optimal usage of samples and associated metadata. Further collaboration and interoperability at the international level are also crucial for biobanks and consortiums aiming to contribute to wildlife conservation and combating wildlife crime. International biobanking networks similar to those established for human samples and associated clinical data (see [35]) could be adopted by conservation-focused biobanks to seek funding and sharing of operational resources to maintain the long-term sustainability of collections, as well as to benefit end-users by providing access to a wider range of samples legally compliant with current international treaties and agreements [25].

Digital DNA data sharing in wildlife forensics

Reference material for wildlife forensics is often in sample form but the use of digital DNA data as reference standards can help overcome potential challenges on sample acquisition, and to encourage more effective use of reference samples among the wildlife forensics community [51]. Initiatives for sharing DNA data between wildlife forensics practitioners already exist (e.g., ForCyt; [21]; e.g., RhODis®; [22]; e.g., Puma Genetic Database; [52]) but further comprehensive and more widely accessible DNA databases are needed to effectively combat wildlife crime [53]. For the establishment of new initiatives promoting

Box 1
The CryoArks biobank

CryoArks is a UK-based animal biobanking consortium for research and conservation (www.cryoarks.org). Funded by the Biotechnology and Biological Sciences Research Council (BBSRC), this initiative is coordinated by Cardiff University in partnership with the Natural History Museum of London, National Museums of Scotland, the Royal Zoological Society of Scotland, The Frozen Ark, University of Nottingham and The University of Edinburgh. The main aim of CryoArks is to increase the visibility and availability of cryopreserved samples held in museums, zoos, research institutes and universities across the UK to facilitate their access by the country's research and conservation communities. CryoArks has also established partnerships with the Frozen Ark, the European Association of Zoos and Aquaria (EAZA) and the Global Genome Biobanking Network (GGBN), and collaborates with other biobank-related initiatives such as CRYOSOCIETIES (https://cryosocieties.uni-frankfurt.de/) and Otlet (https://otlet.io/) to accelerate the expansion of genetic collections for conservation-focused research and the discoverability of samples by end-users.

Interested parties can either donate samples to be stored and curated in one of the CryoArks hubs, or make their collection accessible by uploading details of samples and associated metadata on the CryoArks online database. The database has been developed using the open-source software Specify (The University of Kansas Biodiversity Institute), which also supports a user-friendly web environment for sample requests and the monitoring of sample use. To increase awareness of the consortium's activities and the inherent value of the CryoArks biobank for research and conservation, the team has dedicated significant effort in communications and the preparation of promotional materials to reach potential end-users and the general public through their website and social media channels (Twitter, Facebook, LinkedIn, YouTube, – details can be found in the CryoArks website; www.cryoarks.org) as well as through their presence at relevant events (conferences and workshops on conservation, biobanking or genetic resources) and the media (e.g., broadcasted interviews, social media).

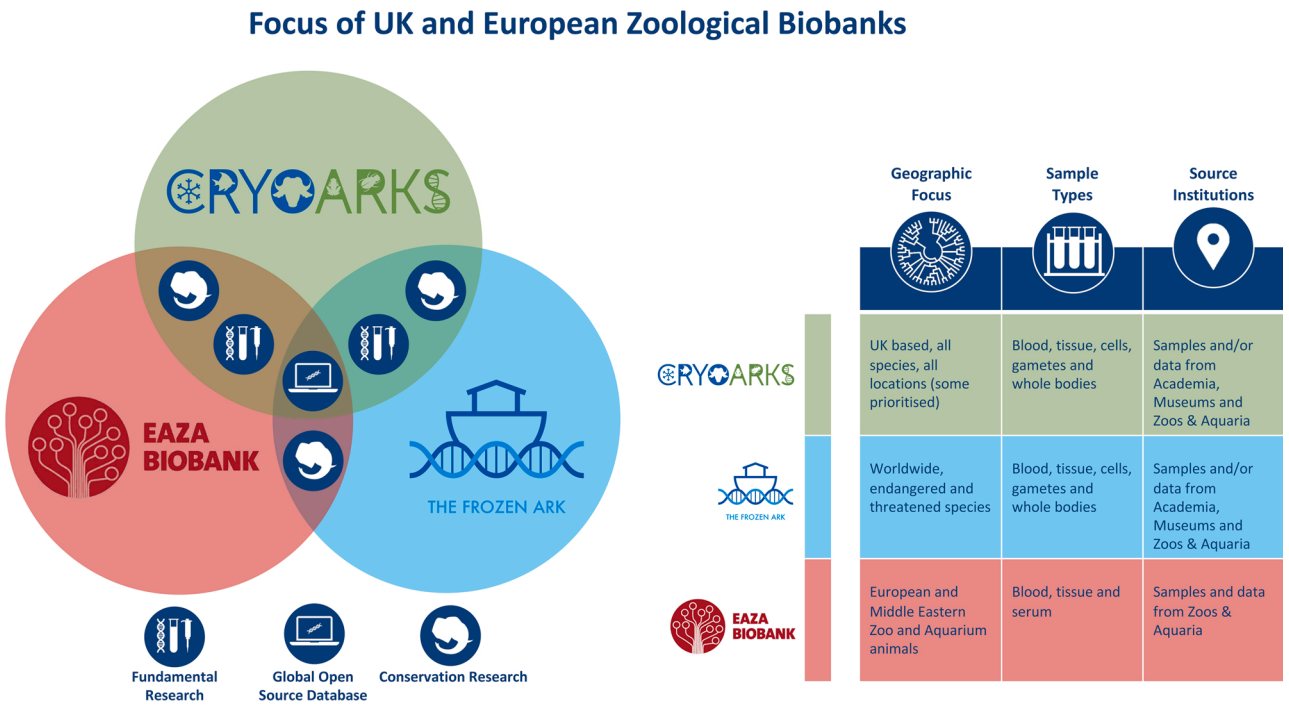

Fig. 1. Diagram highlighting the focus and interactions between animal samples biobanks with conservation and research focus operating in the UK. Diagram from CryoArks modified from the original version of The Frozen Ark by David Kirkham.

DNA data sharing between wildlife forensics practitioners, beyond the already publicly available repositories, it is important to consider legal and regulatory compliance for the sharing of such data. Data sharing agreements (DSAs; [54]) conditions for the use or sharing of data, protection of sensitive metadata associated with the DNA data, and intellectual property statements will be required among the parties (and potentially third parties) using the data [55]. These initiatives will also need to implement measures to ensure the correct storage, management and security of the data records as well as to provide information about the quality assurance, quality controls and forensic validations that were undertaken for the generation of the genetic data for its accredited use [10,21]. It is important to note that similarly to samples deposited to a biobank, permits (e.g, collection, CITES, Nagoya Protocol) for any samples used to generate the DNA data might be required [56,57].

Summary

Wildlife DNA forensics is increasingly important for gathering evidence and intelligence in wildlife crime investigations by providing identification of unidentified samples, species detection in mixed-DNA samples, assignment of unidentified samples to a particular individual, sexing of samples, and assignment of geographical origin to samples, among other services. Reference samples for the development of genetic markers and molecular genetic protocols to provide robust results to withstand scrutiny in court are essential in wildlife DNA forensics. However, access to appropriately vouchered reference samples can be challenging, in particular for rare, endangered species, or those inhabiting remote areas.

Conservation and research-focused biobanks, in addition to those collections held in museums, zoological and botanic gardens biobanks, are an excellent resource to obtain reference samples for wildlife DNA forensics, in particular since the implementation of the Nagoya Protocol in 2014 by the Convention on Biological Diversity. These biobanks' collections cannot only provide single reference samples for species identification but, due to the population-level sampling conducted in conservation genetics research, can also offer sets of samples to develop DNA sequence and allele frequency references databases, as well as to help improve DNA registers for sample localization. However, the use of the biobank samples for wildlife forensics will be conditioned by the quantity and quality of metadata associated with the samples. Particular attention will also need to be paid to the associated collection and import and export permits, as well as any stipulation regarding access and benefit-sharing, associated with any of the samples.

Conservation and research-focused biobanks face several challenges including the procurement and maintenance of cryopreservation facilities, securing staff to implement procedures to process, curate and dispatch samples. Public and end-user support, as well as collaborations between biobanks and practitioners such as wildlife forensic geneticists, therefore, is crucial for the long-term sustainability of conservation and research-focused biobanks. In addition to potential collaborations between biobanks and wildlife forensics practitioners, further initiatives encouraging the sharing of digital DNA data as reference standards among the wildlife forensics community will also help to overcome the challenges associated with sample acquisition.

Declaration of Competing Interest

No conflict of interest to declare.

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