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ORIGINAL PAPER

Forensic science, genetics and wildlife biology: getting the right mix for a wildlife DNA forensics lab

Rob Ogden

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Abstract Wildlife DNA forensics is receiving increasing coverage in the popular press and has begun to appear in the scientific literature in relation to several different fields. Recognized as an applied subject, it rests on top of very diverse scientific pillars ranging from biochemistry through to evolutionary genetics, all embedded within the context of modern forensic science. This breadth of scope, combined with typically limited resources, has often left wildlife DNA forensics hanging precariously between human DNA forensics and academics keen to seek novel applications for biological research. How best to bridge this gap is a matter for regular debate among the relatively few full-time practitioners in the field. The decisions involved in establishing forensic genetic services to investigate wildlife crime can be complex, particularly where crimes involve a wide range of species and evidential questions. This paper examines some of the issues relevant to setting up a wildlife DNA forensics laboratory based on experiences of working in this area over the past 7 years. It includes a discussion of various models for operating individual laboratories as well as options for organizing forensic testing at higher national and international levels.

Keywords Wildlife DNA · Laboratory · Forensic identification · Validation · Illegal trade

R. Ogden (✉)
TRACE Wildlife Forensics Network, Royal Zoological Society of Scotland, Edinburgh EH12 6TS, UK
e-mail: rob.ogden@tracenetwork.org

R. Ogden
LGC Forensics, Queen's Road, Teddington, Middlesex TW11 0LY, UK

Introduction

Wildlife DNA forensics is becoming established as an applied scientific field incorporating disciplines ranging from biochemistry to evolutionary genetics to support the development and application of forensic analytical methods. Despite tracing its origins back to the late 1980's, researchers and practitioners have only recently begun to draw together the multiple strands of work underway around the world, under the umbrella of organisations such as the Society for Wildlife Forensic Science (est. 2009) and the TRACE Wildlife Forensics Network (est. 2006). One of the many issues to emerge from such endeavours is the collective need to share experiences and discuss best practice with respect to capacity building. The aim of this paper is to examine one area of particular interest: setting up a laboratory facility for wildlife DNA forensics.

The application of DNA forensic techniques to wildlife crime investigation is complicated by a number of factors. The potential range of target species for which methods require development, validation and reference data is large; the frequency with which any single analysis is employed may be low and the priority given to resourcing wildlife forensic work is often lower still, resulting in wildlife DNA forensic services being viewed as expensive to maintain. With notable exceptions, the majority of wildlife DNA forensic work has traditionally taken place in academic institutions where scientists with particular expertise undertake forensic analysis on an ad-hoc basis. The involvement of academic scientists is essential to drive the development of new genetic identification techniques and generate comparative data, however, the performance of forensic analysis by scientists who may lack forensic training and operate in research-grade laboratories can

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seriously compromise the integrity of analytical evidence [1].

This paper briefly describes how the field has developed and assesses the different laboratory models under which wildlife DNA forensics has been performed, highlighting examples of good practice and situations to avoid. It then explores the practical options available to authorities or organizations wishing to develop capacity in this area.

History

As with most scientific applications, wildlife DNA forensics began with the discovery of a technique, followed by some thoughts on what could be done with it. The two core analytical approaches, DNA sequencing and fragment analysis, were developed in the mid to late 1980's and their potential applications to sample identification and legal enforcement were rapidly demonstrated. The first publication describing hypervariable minisatellite markers in 1985 [2] led to their recognition as human forensic markers [3], then as non-human markers [4], then as wildlife forensic markers [5], in the space of 4 years. At the same time, the use of DNA sequencing for species identification and its subsequent application to wildlife crime investigations was also underway [6].

With the support of government resources, the field of human DNA forensics expanded rapidly and techniques were transferred to dedicated, accredited forensic laboratory facilities, while wildlife DNA forensics remained a very specialist field, practiced by few scientists. The relative lack of support for wildlife applications, together with the breadth of biological identification issues that need to be addressed have been key constraints to the transfer of casework from research laboratories to the type of human DNA forensic facilities found in many countries today. Despite steadily increasing awareness of how DNA methods can provide intelligence to support wildlife crime investigations, for example through work on the illegal trade in whale meat [7], caviar [8] and ivory [9], wildlife DNA forensics remained a niche subject and this has often dictated the laboratory environment in which the work takes place.

Over the past 5 years, interest in wildlife DNA forensics has increased. Public and scientific concern over the loss of biodiversity has prompted governments to strengthen enforcement of legislation regulating hunting and trade in wildlife products and derivatives. At the same time the growing suspicion that organized crime is involved in illegal trade has helped to raise the status of wildlife crime among enforcement agencies. The potential for forensic genetic approaches to investigate wildlife crime is gradually being realized, resulting in steadily increasing demand for wildlife DNA forensic services.

The effects of these developments are that more countries are seeking to establish capacity in this area while at the same time the performance of forensic identification is coming under increasing scrutiny. These are both welcome developments to the field of wildlife DNA forensics, but they also raise a number of challenges to new and existing practitioners alike. From an analytical perspective, it is important to define the end-use of data and clearly distinguish between forensic casework and intelligence applications. While the genetic techniques used and the results generated by a research laboratory will invariably be identical to those of a forensics lab, they fundamentally differ in the process used to achieve those results. One key consideration when aiming to develop a true forensic analytical service is the type of laboratory facility involved. The optimum solution will almost certainly vary on a case-by-case basis; an assessment of the key options and some lesson learnt is presented here.

Models for laboratory services

It is important to recognize the distinction between the laboratory facilities used to undertake research and development and those used for casework. The research underpinning wildlife DNA forensic applications almost always includes work undertaken in a non-forensic laboratory environment; this is perfectly acceptable and should be encouraged in order to increase the number of identification methods available. Validation studies, essential to demonstrate that a method is fit for the purpose, can also theoretically be undertaken in a non-forensic environment, however a well-planned study will be based on considerable experience of quality assurance issues and their performance and outputs are usually of little interest to academic scientists, resulting in validation work typically being carried out by forensic scientists. The level of validation required for wildlife DNA forensic methods has been the subject of some debate [10, 11] and is worthy of further discussion. The focus of this paper, however, is on the laboratory facilities used to undertake casework in order to provide legal evidence in a criminal investigation.

Laboratory facilities used for wildlife DNA forensic work range from non-forensic research labs to specialist wildlife DNA forensics facilities operating under externally accredited quality assurance (QA) schemes (Fig. 1). In reality most labs fall somewhere between these two extremes and attempt to balance the need for forensic rigour with the fundamental limit of service cost. The two key issues affecting this balance are: what level of QA is required to produce evidential data, and how is the analysis being funded?

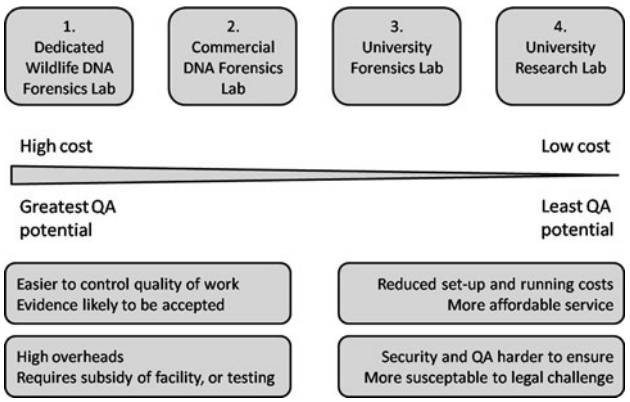

Fig. 1 Summary of four possible laboratory models for undertaking wildlife DNA forensic analysis, describing their key characteristics

Quality

The purpose of QA is to prevent errors in data production, to ensure that any errors are identified prior to data release and to feedback improvements into the analytical process. To achieve this, laboratories can implement and be accredited under a number of quality systems, such as ISO17025 or Good Laboratory Practice (GLP). These systems will address issues such as continuity of evidence, equipment calibration and maintenance, correct use of analytical controls, staff training, data storage and reporting procedures. While clearly seen as a gold standard, and often a requirement for human forensic labs, the cost of accreditation for a wildlife DNA forensic laboratory may be prohibitively expensive, particularly given the range of tests performed. Nevertheless, the principles of QA schemes such as ISO17025 are fundamental to producing accurate, reproducible and secure results and therefore any laboratory undertaking wildlife DNA forensic analysis must adhere to a QA system, regardless of whether or not formal accreditation has been gained. The capacity for a laboratory to implement a QA scheme and the commitment of its staff and management to follow it, are vital considerations when establishing a forensic laboratory service.

Funding

The second consideration when balancing forensic quality requirements with service cost is the way in which the analysis is funded. Any analytical service, whether commercial or governmental, must operate within a budget and there are a number of ways to achieve this. A government or university facility may receive core funding to subsidize a service, enabling end-users to have samples analysed at no charge, or for a fraction of the real cost. In many respects, this is an ideal system as it enables the laboratory to maintain a range of genetic analysis techniques that individually may be used too infrequently to justify providing commercially. However, such government subsidies are not available in many countries, where insufficient funds are available for wildlife forensics, or where there is an ethos of full cost recovery within non-commercial institutions.

The second option is for a laboratory to operate either commercially, or on the basis of full cost recovery and charge a commercial rate. This has been the situation in the UK for the past 10 years and provides a useful example of this approach. Following a decision not to use university research laboratories for forensic DNA testing, the only alternative for wildlife crime enforcers was to submit samples to the UK Forensic Science Service (FSS). However, at the time, the FSS was being converted from a government laboratory service into a government company operating full cost recovery. The fee charged for techniques such DNA species identification was prohibitively expensive ($\sim$3,000 GBP per sample), owing to the occasional nature of the work and the cost model applied. This effectively resulted in no casework being processed. In 2003 a commercial operation began offering forensic services at a much reduced rate. While this enabled some investigations to use forensic DNA analysis, many UK enforcement agencies still struggled to find sufficient money to pay for it. This prompted the UK government and several non-governmental organisations to establish a forensic analysis fund to provide 50% match funding to the cost of any wildlife forensic test required by the police or customs authorities. This initiative has further increased the use of wildlife DNA forensics by UK enforcement agencies, but ultimately still limits both the number and range of tests that can be undertaken.

The method of funding wildlife DNA forensic testing affects the feasibility of the laboratory model. The UK experience serves to demonstrate that wildlife DNA forensic testing, unlike its human equivalent, is not a commercially viable service. Some form of government subsidy is essential; the level of support dictates the type of service that can be provided (Fig. 1).

Comparative laboratory systems

For the purposes of broad comparison, the following section describes the typical properties of four laboratory models: (1) A government-funded dedicated wildlife DNA forensic facility; (2) a private forensic genetic facility offering wildlife DNA services; (3) a university or institutional research facility incorporating dedicated forensic laboratory space; (4) a multi-use research laboratory (Fig. 1). It is stressed that there will always be exceptions to such categorization and support or criticism of individual laboratories is not implied.

Government-funded dedicated wildlife DNA forensics facility

A core facility for processing all casework together with the capacity to undertake method specific validation studies represents an ideal scenario in most respects. Such a system should enable all enforcement agencies to access services for a nominal cost, which in turn will help promote the use of forensic genetic techniques in wildlife crime enforcement. This positive feedback is maintained by the economies of scale associated with a high throughput of casework. The implementation of a rigorous QA system is not compromised by the demand for profit, or alternate uses of the same laboratory space. A pool of forensic expertise can be developed able to address a very wide range of questions.

The major drawback of this system is cost. Most individual countries cannot justify the cost of developing and maintaining a facility based on the limited number of samples processed per year. Associated with this issue is the length of time it may take to establish a dedicated facility. While the conversion of an existing laboratory, staff training and internal validation may be possible within 12 months, the decision process and construction of a new laboratory is likely take many more years. Regional core facilities servicing multiple countries under an international agreement offer a potential solution to these issues, but such models are difficult to implement, as discussed in subsequent sections.

Private forensic genetic facility offering wildlife DNA services

Organisations operating for profit (or at least full cost recovery) may be interested in providing services, however financial controls will limit the forensic methods supported to those used most frequently, restricting the skill base and reducing the range of services available. The laboratory facility is likely to offer quality assured analysis, however, the recovery of cost in relation to the QA system is likely to make the per sample analysis fee very high. In most countries, such a model will only function if the enforcement agencies receive specific additional support to fund forensic analysis.

It should be noted that there may be other drawbacks to using private forensic services that have been experienced in the UK. Without the necessary security clearance, it may be impossible for enforcement officers to fully discuss ongoing casework with the forensic scientist. This hampers an investigation by increasing the risk of undertaking inappropriate analysis, or missing important avenues of investigation.

University or institutional research facility with dedicated forensic laboratory space

This model encompasses a broad range of institutional laboratory facilities, from correctly designed, properly controlled, professional forensic units, to academic experts in their field with little understanding of forensic processes who are, for a variety of reasons, offering a forensic service. There is no doubt that at one extreme, a number of universities have dedicated sufficient laboratory space, staff and resources to provide first class forensic genetic services. However, if a department has funded the facility itself, it will be looking for a return on investment, increasing the service price. A department making a smaller investment may rely on other sources of income to support the facility, reducing the service price but potentially limiting the level of quality assurance. Direct government support of a university-run facility can be an economic way to deliver wildlife DNA forensic services, although a university-based service may be limited by the skill base and time commitments of the resident academics involved in running the unit.

Multi-use research laboratory

Research laboratories typically operate with extremely broad access to buildings, rooms, storage units and computer systems. Pre- and post-PCR liquid handling often occurs at the same bench, equipment is rarely fully calibrated, quality systems are not in place, and neither staff nor students have undergone any forensic training. The routine use of research laboratories to provide forensic evidence in wildlife crime investigations should therefore not occur. Nevertheless, it does and it is very important that the enforcement agencies and scientists involved are made aware of the potential failings of such systems and are encouraged to either transfer the work elsewhere, or move towards the creation of a dedicated forensic unit within the laboratory facility, as described in the third model, above.

As a rare exception to the general rule preventing forensic work from taking place in research laboratories, it is recognized that the breadth of potential investigative questions may sometimes require expertise not available within an existing forensic laboratory. In these circumstances it may be necessary to approach a research expert for assistance with a case. Such work is fraught with difficulties from a forensic perspective, including issues of laboratory security, analytical QA, data interpretation and reporting. The technique involved is unlikely to be validated which may prevent acceptance of evidence in court, limiting the information gained to the level of investigative intelligence. Despite these problems, it is possible to generate forensic genetic data from a research laboratory, but

work should be well-planned, undertaken in isolation, fully recorded at the time and under the direct and constant observation of a forensic scientist who is able to witness and confirm every stage of the work.

Comparing these four model systems it is easy to conclude that the fully-funded government model is the ideal and that a research laboratory model is not acceptable. In reality many countries already using wildlife DNA forensic analysis, or that are seeking to develop capacity, are restricted to operating under the second and third models. In order for these models to work, it is essential to have the correct resources in place, not only in terms of laboratory facilities, but also in relation to personnel. The following section will briefly discuss options for developing expertise in wildlife DNA forensics.

Building expertise

What does it take to become a wildlife DNA forensic scientist? Who should a laboratory try to recruit? There are currently no academic or vocational qualifications that will train people specifically to work in this field and staff will probably require training in post. The primary recruitment decision is often between a human DNA forensic candidate who will require training in wildlife genetics and a wildlife geneticist who will require training in forensics. In a large laboratory with multiple staff, a blend of backgrounds is probably preferable, allowing for knowledge transfer in a common environment. However, most countries building capacity in this area appear to begin the process by nominating a single individual per facility to be responsible for wildlife DNA forensics.

The main consideration in choosing between an established forensic scientist and a wildlife geneticist should be the duration and level of training required to obtain the other's skills. From personal experience based on training scientists from a dozen different countries, it is generally easier to teach a wildlife geneticist to do forensic casework than it is to convert a human forensic DNA specialist into a wildlife DNA forensic scientist. The reason for this is primarily that the consistency of approach employed in forensic analysis allows general rules and guidelines to be applied to a wide range of laboratory processes and genetic markers, addressing species, geographic and individual identification questions. The reverse is not the case; a human forensic scientist attempting to learn the range of scientific techniques and underlying biological assumptions involved in different wildlife identification enquiries is faced with a very large, diverse body of knowledge to attain.

The recruitment and training of staff is vital to establishing a viable wildlife DNA forensic laboratory. Although the selection of candidates may be limited for many different reasons, an experienced wildlife geneticist with an interest in forensic methods and an appreciation of the need for quality assurance will often prove to be the best choice. The scarcity of such expertise becomes an important consideration when building capacity in countries without existing wildlife DNA forensic laboratories. The next section considers the possible approaches for delivering services within such regions.

Developing capacity in new countries

The challenges and compromises in choosing how to establish an individual wildlife DNA forensics laboratory have been discussed. Where insufficient capacity, resources or demand are present to create laboratory facilities within national regions, or entire countries, coordinating resources at a national or international level may provide the most effective solution. While a collaborative approach often makes sense in principle, in reality it can be very hard to achieve. This section will briefly consider the issues restricting the development of single national wildlife DNA forensics labs, before examining several models for international collaboration based on current and past experience.

Establishing a single national facility

Many countries that are beginning to examine their national capacity for undertaking wildlife DNA forensic analysis find that services are currently provided by a number of different universities or institutions with a variable degree of forensic quality assurance. Casework is typically assigned to an expert working in the same taxonomic field as the species under investigation and in some cases the laboratory may develop a routine service for a specific investigative issue, for example, bird parentage testing. Where a country is seeking to formalize its wildlife DNA forensic work, it often makes sense to bring casework together under one roof, via one of the first three models discussed earlier. The advantages to such a strategy are obvious: increased economies of scale, the opportunity for a single forensic-grade service and a single point of contact for enforcement authorities, among others. However, such a move requires the transfer of expertise, data and probably reference samples from a personal research environment to a shared national resource and there can be reluctance on the part of the researcher to engage and commit time to this process, when they often receive no benefit.

A second consideration is that additional validation studies will always need to be undertaken when transferring techniques to a single central facility. While this

should ideally be limited to an internal validation exercise, it is likely that a degree of additional developmental validation would also be preferable [12]. This requires time and money that must be factored into plans to rationalize wildlife DNA forensic services.

While neither of these issues should necessarily prevent the development of a single national facility, awareness of them is important to prevent stagnation of projects that are aiming to improve the coordination and delivery of wildlife DNA forensic services.

International collaborations

Many of the arguments for and against a single national facility can be extended up to an international level. In many parts of the world, it simply does not appear to make sense to spend time and money to train staff and build capacity in every country, where this might result in over-capacity and over-stretched resources within individual states. The European Union (EU) provides a good example of this situation. There are currently 27 member states within the EU; the entire area is treated as a single customs zone in terms of implementing CITES regulations (i.e. there is free trade across borders) and legislation relating to national wildlife protection is broadly similar. The results of a recent survey of member states indicated that approximately half have no current forensic DNA capacity for wildlife crime investigation, while the other half use a range of services from university research labs through to accredited national forensic facilities. Enforcement officers collaborate at a European level and the demand for forensic services is reasonably high. In many respects, the best solution would be to create a single European facility for validation and casework analysis, but where would such a service be based and who would run it?

Three possible models for an international collaborative approach are considered here: (a) A network of independent national laboratories, (b) A single international laboratory, and (c) A central regional laboratory with national satellite laboratories (Fig. 2).

A network of independent national laboratories

The development of independent laboratories in every country within a region has disadvantages, principally relating to overall cost, efficiency of analysis and the practicalities of training sufficient wildlife DNA forensic scientists. However in some regions, it is the only politically viable option and does bring benefits relating to capacity building and national ownership within each country (Fig. 2a). In such cases it is worth considering how to optimize the development of services.

As an example, the UK government is currently funding a wildlife forensics network in the ASEAN region (Association of Southeast Asian Nations; Box 1). The first aim of the project is to offer training and support to scientists in the areas of research and development, laboratory organization, forensic protocols and casework approaches.

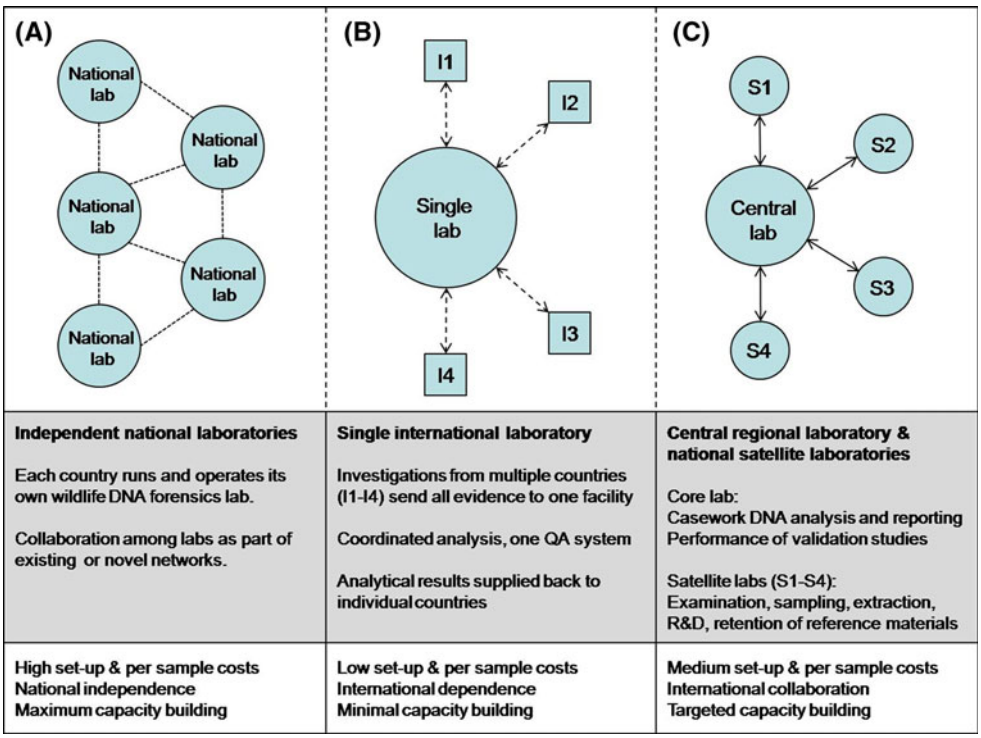

Fig. 2 Three models for providing wildlife DNA forensic laboratory services across a number of regions or countries. *Model A* describes a loosely linked system of independent laboratories, *Model B* describes a single laboratory system and *Model C* describes an intermediate solution, incorporating one central laboratory and multiple satellite laboratories

Box 1 Developing wildlife forensic capacity for ASEAN biodiversity conservation—a Darwin initiative

Countries in the ASEAN region have been coordinating action against wildlife crime since 2005 through the ASEAN Wildlife Enforcement Network. As part of this approach, individual nations recognized the need to increase their capacity for undertaking forensic analysis to support wildlife crime investigations. The regional laboratory facilities for undertaking forensic DNA work vary widely, as does the experience of scientists in each country. The Darwin project began in 2009 with an evaluation of existing capacity and an assessment of wildlife DNA forensic needs at a national level. This included identifying key scientists and wildlife enforcement managers with responsibility and enthusiasm for developing laboratory services

The project has since proceeded on the basis of selecting the most appropriate laboratory model for each country, based on local facilities, expertise, administrative structures and enforcement systems. At an international level, training workshops have been run to address common issues in laboratory set-up and operation, as well as to develop a network of practitioners facing similar wildlife DNA forensic challenges

In the ASEAN region at this time, the independent laboratory network model is considered to be the only viable political option. However it is hoped that ongoing communications will lead to regional collaborations among institutions and wildlife DNA forensic scientists, enabling laboratories to exchange and implement best practice

The project is managed by TRACE Wildlife Forensics Network in partnership with TRAFFIC Southeast Asia and ASEAN-WEN

The second aim is link together scientists and enforcement authorities within and among countries in order to provide a platform for exchanging techniques, data and common enforcement issues.

Working with governments and scientists in the ASEAN region takes time, but there are clear advantages to engaging at this level, which ensures that the laboratory facilities and processes that are developed are tailored to meet national needs. By stressing the benefits of international collaboration and providing a forum for communication, it is hoped that scientists will have regional access to advice and facilities as necessary.

A single international laboratory

The opposite approach to a system of national laboratories is to have a single, quality assured, internationally recognized laboratory offering affordable services to all nations (Fig. 2b). This is what the US Fish and Wildlife Service's Forensic Laboratory (USFWS-FL) set out to provide when it offered forensic analysis of wildlife crime samples relating to CITES enforcement free of charge, over 10 years ago. The laboratory is not restricted to DNA analysis and offers a wide range of modern forensic services. The single laboratory model should be the most cost-effective solution, providing analysis to a very high standard; however this type of system requires effective international collaboration and is not suitable for individual nations that wish to build capacity for solving wildlife crime issues themselves.

The service provided by the USFWS-FL has resulted in many notable successes, including for example, the investigation of illegal ivory shipments from Africa to east Asia. In many cases, the capacity to undertake the analysis in the range state simply did not exist, demonstrating the value of such a model. Despite the existence and global promotion of this free service, however, it has been observed that the opportunity to access forensic analysis is often not taken. Certain countries may be unwilling to engage with such a service due to issues of data exchange, investigative process or higher political disagreement. Others may not want to rely on an external agency at the perceived expense of developing their own capacity. Until this situation changes, the single international laboratory model, however attractive, is not a total solution to the provision of wildlife DNA forensic laboratory analysis.

A central regional laboratory with national satellite laboratories

In regions where international collaboration is highly developed and legislation allows, a third model is proposed that seeks to rationalize the use of resources while maintaining national involvement in casework and ownership of wildlife DNA forensic research (Fig. 2c). Under this model, each country is responsible for coordinating the forensic analysis of their own samples, including submission to a 'satellite' laboratory, examination of the item, recovery of evidential samples and, as appropriate, extraction of DNA. In addition to these processes, countries would be encouraged to undertake research and development to support new wildlife forensic techniques. The satellite laboratory would then submit the sample to a single central wildlife DNA forensic laboratory that maintains a full range analytical tests operating under an accredited quality system. The central laboratory would be fully equipped and staffed by wildlife forensic scientists experienced in performing casework analysis and the developmental validation of methods developed by the research community.

The advantages and disadvantages of this model relate to those already discussed for the national laboratory network and the single international laboratory models. However the intention is to mitigate the disadvantages and retain the advantages of each. It is envisaged that this approach may be suitable at a European level, or even within a single large country.

Summary

The issues involved in setting up a wildlife DNA forensics laboratory are complex. The opposing influences of restrictive budgets and the need for forensic rigour are complicated by the breath of analytical techniques, variable casework demand and access to expertise. While ideal and unacceptable models for a laboratory facility are moderately simple to define, intermediate solutions will inevitably be required, necessitating careful consideration of how to ensure the implementation of quality systems while preventing analytical costs from becoming prohibitively high.

Although the engagement of individual researchers and links to academia will always be an integral part of this applied field, it is essential that correct forensic laboratory practices are followed, not only with respect to individual cases, but also to maintain the reputation of wildlife DNA analysis in the wider forensic community. Where solutions at a local level fail to offer a suitable environment for undertaking wildlife DNA forensic analysis, opportunities should be sought through collaboration and cooperation at a wider national or international level.

Different approaches will be appropriate in different regions and the question of how to establish a wildlife forensic DNA laboratory will always be answered on a case by case basis. However as an overall strategy, it is recommended to explore to what extent it is feasible to collaborate and coordinate resources from the local to the national to the international level. Placing a laboratory as far along this path as is practicable should help to provide the most cost-effective, quality-assured, broad range of wildlife DNA forensic services possible.

Key points

1. Forensic genetic techniques are increasingly used to identify non-human evidence in wildlife crime investigations.
2. Wildlife DNA forensic applications require a synthesis of wildlife genetics and forensic expertise which is rarely available at a single facility.
3. The development of new facilities can follow several alternative models, all of which are based upon performing DNA analysis to rigorous forensic standards.
4. The casework requirements and available resources to support wildlife DNA forensic services may favour solutions at a national or international level, where the correct levels of expertise and quality assurance can be most effectively combined.

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