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Wildlife forensic science: A review of genetic geographic origin assignment

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ABSTRACT

Wildlife forensic science has become a key means of enforcing legislation surrounding the illegal trade in protected and endangered species. A relatively new dimension to this area of forensic science is to determine the geographic origin of a seized sample. This review focuses on DNA testing, which relies on assignment of an unknown sample to its genetic population of origin. Key examples of this are the trade in timber, fish and ivory and these are used only to illustrate the large number of species for which this type of testing is potentially available. The role of mitochondrial and nuclear DNA markers is discussed, alongside a comparison of neutral markers with those exhibiting signatures of selection, which potentially offer much higher levels of assignment power to address specific questions. A review of assignment tests is presented along with detailed methods for evaluating error rates and considerations for marker selection. The availability and quality of reference data are of paramount importance to support assignment applications and ensure reliability of any conclusions drawn. The genetic methods discussed have been developed initially as investigative tools but comment is made regarding their use in courts. The potential to compliment DNA markers with elemental assays for greater assignment power is considered and finally recommendations are made for the future of this type of testing.

1. Wildlife DNA forensics

The illegal wildlife trade (IWT) is leading to the destruction of habitats and the unsustainable exploitation of many animal and plant species. This has negative socio-economic impacts at local and global scales, as well as ultimately leading to the extinction of species and the consequent loss of biodiversity and ecosystem services. Prevention and investigation of alleged wildlife crime is an essential requirement of any judicial system that not only values their biodiversity but also brings to justice those organised syndicates that gain financially from this pernicious trade. The scale of IWT is extremely hard to quantify as so much goes undetected, but estimates in the region of $20 billion per year are widely cited [1,2]. Difficulties in assessing IWT are compounded by the fact that a large proportion of traded products originate from under-developed countries where wildlife trade monitoring is limited, as is the ability of the enforcement agencies to act. Despite increased awareness of the problem, wildlife law enforcement remains chronically under-resourced and wildlife forensic science falls a long way down the list of priorities of almost all forensic science laboratories that focus primarily on crimes against humans and their property. Nevertheless, the field of wildlife forensic science (wildlife forensics) is widely recognised as having an extremely important role in wildlife law enforcement and has become established as a forensic discipline in its own right [3].

The application of forensic analysis to wildlife law enforcement dates back several decades, but the area has seen increasing interest and rapid development during the past five years. A case in point is the request for a 'Wildlife Forensic Science' paper in this special edition and the inclusion of non-human DNA testing within the remit of the ISFG [4]. These developments sit alongside the establishment of the Society for Wildlife Forensic Science in September 2009 [5] and the publication of international standards and guidelines by the US Scientific Working Group on Wildlife Forensic Science (SWGWILD) [6].

In its broadest sense, wildlife forensics encompasses all of the available analytical techniques that may be employed to investigate crime against wildlife, including ballistics, fibre analysis, toxicology and veterinary pathology [7]. However what often

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makes wildlife forensics unique is the need to identify the biological (wildlife) evidence in order to demonstrate that a crime has been committed. Morphological, isotopic and DNA analysis are all used to characterise evidence items, with DNA analysis being most widely employed for the identification of wildlife parts and derivatives [8,9]. This paper focuses on the application of DNA forensics to wildlife crime, however, complementary non-genetic methods are also briefly discussed.

The genetic identification of biological evidence typically seeks to address up to four investigative questions:

I Is this sample from a legally protected species?
II Is this sample from a specific member of a legally protected species?
III Is this sample from an individual that was captive bred or wild caught?
IV Is this sample from a specific population or geographical region?

Previous reviews have addressed the use of DNA in species testing, individualisation and parentage testing [9–13]. Geographic assignment is less well-developed but is becoming more commonly requested by investigators of alleged wildlife crime and it is this area of research and practise that will be discussed in this review.

Demonstrating where an animal or plant originated may be necessary to assess its legal status. For example, when illegally sourced timber or fish are laundered into a legal commercial trade, geographic origin may be the primary point to prove in an investigation (see Box 1). In other situations, for species where all trade is banned, geographic assignment may not be central to a prosecution, but may have an important role to play in identifying poaching 'hotspots', understanding criminal networks or repatriating live seizures.

2. Principles of genetic geographic origin assignment

Species are usually discrete evolutionary genetic units within a defined geographic range, enabling some assessment of origin to be made simply via species identification. For example, within Thailand, the carving and sale of ivory from domestic Asian elephants, *Elephas maximus*, is currently legal, whereas the sale of ivory from African elephants, *Loxodonta africana*, is not. Thus a relatively simple species identification test may result in clear evidence of broad geographic origin that is sufficient to ascertain whether ivory that is being traded in Thailand is, or is not, legal. However, from a forensic genetic perspective, identifying the geographic origin of a sample is usually equivalent to identifying its reproductive population of origin. In this regard, it is identical to the issue of identifying the breed or variety of an animal or plant for food product authentication [14]. Biological populations include many different levels of genetic variation, from extended families to subspecies, making them difficult to define. Populations are often capable of sharing genetic material, therefore compared to species identification, DNA markers are less likely to show discrete differences amongst groups (Fig. 1). Geographic origin identification is based on our ability to simultaneously assign a sample to a particular population, while excluding it from others, requiring the source population to be sufficiently genetically distinct from other candidate populations and usually relying on the existence of population data from multiple geographic areas. As with all wildlife DNA forensic applications, the availability and selection of genetic markers and reference data influence the power of assignment techniques. What makes geographic origin identification particularly challenging is the selection of appropriate assignment methods, evaluation of their accuracy, and interpretation of the subsequent results in a forensic context, all of which are discussed below.

In addition to population genetic considerations, forensic applications must also take into account the need for correspondence between the genetic population signal and the geographic boundaries relevant to law enforcement. The distribution of populations and the spatial resolution at which they can be detected can further complicate the use of DNA analysis for origin assignment. Consequently, unlike DNA species identification, for which universal tests are available within large taxonomic groups (e.g. mammals, fish), geographic assignment techniques are specific to both a single species and a defined investigative question, for example, ascertaining the geographic origin of cod within northern European waters (Box 2).

3. DNA markers and methods for geographic origin assignment

3.1. Lineage markers

Loci on the mitochondrial and chloroplast genomes have been used extensively in species identification, focussing on the mitochondrial cytochrome b [15] and cytochrome oxidase 1 genes for animals [16] and rbcL and matK chloroplast genes, in plants [17–20]. These traditional loci do not typically exhibit the finer resolution needed for within-species population assignment and hence interest lies in the hypervariable mitochondrial DNA D-loop (or control region). As with humans [21], D-loop haplotypes in wildlife species can distinguish mitochondrial lineages that

Box 1. Legal requirements for timber origin identification

Determining the origin of timber and wood products is necessary in order to assess whether or not they have been legally sourced. Illegal logging is a threat to both biodiversity and the sustainability of the legal timber trade. A raft of recent legislation across Australia, the USA and Europe has tightened regulations controlling the timber trade and placed the burden of proof of legality on the importer. For instance, the Australian Government passed the Illegal Logging Prohibition Act 2012, which stipulates as an offence the importation into Australia of illegally logged timber and derived products, with a subsequent 2013 amendment requiring importers and domestic processors to have a 'complying due diligence system' (DDS) in place. Similar DDS requirements are also found within the USA (Lacy Act 2008 amendment) and within the European Union Timber Regulation (EUTR), which affects companies inside and outside the EU along the entire length of the supply chain. Such legislation paves the way for the development and application of timber verification techniques, such as assessing country of origin claims using DNA testing [55]. One example of such timber traceability is provided by work on Central and South American mahogany (*Swietenia macrophylla*) where microsatellite DNA typing has been used to assign wood samples to their correct country of origin [59] from Mexico to Bolivia. An alternative approach is employed in Southeast Asia to verify the integrity of timber supply chains via DNA testing, based on a one-up, one-down approach, whereby laundering of illegal timber is detected via exclusion, as opposed to assignment [57]. These types of application are increasingly recognised as potential authenticity tools in the fight against the illegal trade in natural resources [60].

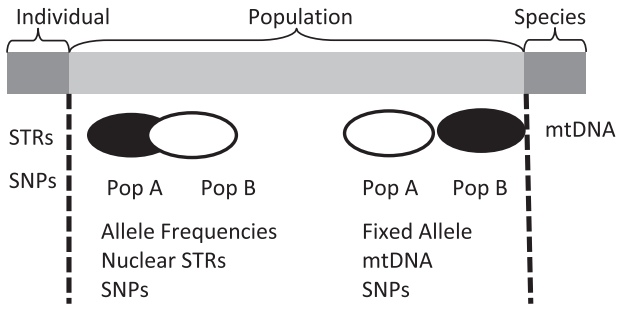

Fig. 1. The level of genetic diversity between populations exhibits continuous variation, from individualisation, through populations to species. The degree of population divergence dictates the selection of genetic markers and subsequent analytical methods used to assign a sample to its geographic origin.

correspond to specific geographic ranges and hence allow origin to be determined. Wildlife examples of D-loop geographic assignment to population of origin include the testing of seized blue-and-yellow macaws (*Ara ararauna*) [22], blue marlin [23], African elephant ivory [24] and cannabis [25]. Despite these examples, mitochondrial and chloroplast DNA often fail to display sufficient variation amongst geographically distributed populations and nuclear DNA markers must be employed.

3.2. Microsatellites

The use of nuclear DNA markers to differentiate populations and link a sample to a particular origin relies on variation in marker allele frequencies amongst populations. The high levels of polymorphism observed at many STR markers (microsatellites, or SSRs), alongside their establishment in human DNA forensics, has led to their use in determining geographic origin in a range of wildlife species including African ivory [26,27], bobcats in the USA [28], Sardinian mouflon [29], tortoise [30] and bears [31], as well as fish [32,33], molluscs [34] and plants [35]. In well differentiated populations ($F_{ST} > 0.1$), a relatively low number of microsatellite markers ($n = 10$) would likely provide sufficient assignment power for identification of population origin, however in more weakly structured populations, the number of markers required to assign unknown samples with confidence rises rapidly. In such situations, the technical difficulties associated with applying or transferring large numbers of microsatellite markers start to become prohibitive and alternative marker types may be preferable.

3.3. Single nucleotide polymorphisms (SNPs)

The use of genome-wide analyses in wildlife research is allowing large numbers of SNP markers to be isolated and characterised in an increasing number of species. Such approaches have paved the way for the development of SNP panels in wildlife forensics [36]. As with microsatellite markers, SNP markers not under selection may exhibit allele frequency differences between populations due to random genetic drift. Due to their lower allelic variability, a greater number of neutral SNP markers is typically required to generate the same assignment power as microsatellite markers. However in comparison to the relatively unrefined microsatellite panels available to wildlife forensic practitioners, SNP markers can facilitate more accurate genotyping and considerably simpler inter-laboratory method and data transfer. Large numbers of SNP markers can also be analysed simultaneously for a single sample. In humans, SNP analysis has fast become the main approach for genetic ancestry assignment using 52 [37,38] or 96 [39] separate loci [40], and these approaches have been transferred into wildlife assignment applications [41].

Where SNP markers offer a significant advantage for geographic origin assignment is the ability to choose markers that are associated with genetic regions under selection for local geographic conditions. These non-neutral markers may exhibit very high inter-population divergence across small spatial scales, thus increasing the resolution of geographic assignment while reducing the number of markers required. A good example of this concept is provided by methods developed for the traceability of European commercial fish, in which small panels of SNP markers ($n = 10$–30) for cod, herring, hake and sole were selected from a much larger pool of candidate SNPs. In many cases, the high assignment power of individual markers was subsequently explained through association with specific gene regions involved in traits under local environmental selection (Box 2). While such selection of minimum marker panels with maximum power can significantly aid population assignment, it is important to avoid upward bias in predicted assignment accuracy through the use of a training data set of reference populations for marker panel selection and a separate test data set for power estimation [42].

4. Data analysis

There are multiple approaches published for the assignment of individuals to their genetic population of origin, however, as yet there is little consensus as to which to use for forensic geographic origin identification. This is in part due to the number of different possible scenarios being addressed and variation in the level of existing information relating to population structure. For example,

Box 2. Use of non-neutral DNA markers for geographic origin analysis

The development of genetic traceability systems for marine fish in Europe provides a good example of how small panels of DNA markers with high assignment power can be identified and applied to help enforce fisheries regulations. Under research funded by the European Union (FP7 FishPopTrace project), genome wide SNP discovery and genotyping was employed to characterise genetic population structure in cod, hake, herring and sole. The geographic clusters identified using up to 1000 SNPs provided a much greater degree of spatial resolution than had ever been previously achieved using conventional mtDNA or nuclear microsatellite markers. Locus specific F-statistics were employed to assess the power of individual SNP markers [61] and to create custom marker panels for addressing specific assignment questions between geographic regions. For example, a panel of 15 SNPs was identified to differentiate Baltic Sea from North Sea cod, while a panel of 10 SNPs was developed to assign hake to the Mediterranean or the Atlantic [62]. Using Bayesian outlier analysis, the majority of high power SNPs were found to exhibit non-neutral variation and subsequent annotation using available genome sequence data indicated association with gene regions considered likely candidates for being under selective pressure for local environmental variables such as salinity or temperature (e.g. cod [63]). The resulting assignment tests were subjected to a comprehensive validation process and some have subsequently been transferred into UK wildlife forensics laboratories. While developmental issues such as marker selection bias [42] and temporal stability of allele frequencies [62] need to be accounted for, the potential power of genome wide approaches for geographic origin assignment represents an exciting opportunity for wildlife forensics.

where the candidate populations of origin are already clearly defined, a straight assignment test may be applied based on the multilocus genotype of an individual and the expected probabilities of that genotype occurring in each of the potential source populations. However in other situations it may first be necessary to implement a clustering method to identify the component populations within a mixture, prior to identifying from which cluster a test sample is most likely to originate (see Manel et al., 2005 for review [43]). In a development on standard assignment methods, the assignment of African elephant ivory samples to their likely geographic origin has been addressed by smoothing allele frequency estimates for reference locations using data from neighbouring localities [44]. This was found to not only improve assignment accuracy, but also allows for interpolation between reference populations in order to assign test samples to geographic localities lacking reference data [27]. Using this approach it was possible to associate 37 tusks from a single ivory seizure to an area centred on southern Zambia [44]. While the adopted method for linking ivory seizures to a geographic location has yet to be fully validated, the test has been successfully used as an investigative tool to indicate hot-spots of poaching and is detailed in UNODC guidelines on ivory analysis in law enforcement [45].

The assessment of different genetic assignment approaches to forensic investigation of geographic origin in wildlife has not often been explicitly considered. Manel et al. [46] provide a notable exception through a study that empirically tests the performance of two of the most commonly used software programmes for assignment, GeneClass [47] and STRUCTURE [48], using population genetic data for ten wildlife species. STRUCTURE employs a Bayesian approach to calculate the posterior probability that a sample genotype originates from a population amongst the available candidate populations. By considering all populations simultaneously, the result can be interpreted as the likelihood that a sample originates from a claimed population, rather than one or all alternate populations. Importantly, under this approach, a test sample will always be assigned to one of the reference populations, even when reference data from the true origin is absent. GeneClass provides a range of assignment calculations, but based on a previous comparative study [49], Manel et al. focussed on the evaluation of the partially-Bayesian exclusion method [50], which evaluates the likelihood of a test sample belonging to each candidate population based on observed genotype frequencies, and subsequently calculates an exclusion probability for the test sample from each candidate population. This approach accounts for the possibility that the test sample did not originate from any of the candidate source populations and therefore allows the origin of a test sample to be evaluated to some extent when reference data are only available from the claimed population. Manel et al. devised thresholds for assignment (STRUCTURE) and exclusion (GeneClass) that they considered to be sufficiently stringent to be informative in the case of a wildlife crime investigation. The results of the study indicated that STRUCTURE outperformed GeneClass with both higher assignment rates and lower assignment error, however the imposition of a threshold led to relatively low assignment rates using either approach (61% and 36%, respectively). While this does ensure very high levels of accuracy, it also highlights the risk of discarding potentially informative evidence based on an essentially arbitrary acceptance threshold.

The use of likelihood ratios, now standard practise for the evaluation of individual DNA profile matches, circumvents this issue by providing a quantitative comparison for the likelihood of observing the genetic evidence under two competing scenarios (usually constructed as the defence and prosecution hypotheses). This approach has the dual advantages of enabling the defence claim of an alternate geographic origin to be directly assessed (in addition to simply identifying the most likely source population), while removing the need for a specific threshold or test statistic. It also allows the inclusion of prior (non-genetic) information regarding the origin of a sample. A likelihood ratio will indicate both the direction and relative strength of the evidence, enabling the judiciary to interpret the significance of the findings in the context of the case.

Likelihood ratios can be derived from the output of both STRUCTURE and GeneClass, but it is important to recognise that the point estimate for the likelihood that a sample genotype belongs to a specific population masks the genetic variation within that population. For populations with low genetic variability, this may have little effect, but in highly variable candidate source populations, the distribution of individual likelihoods may be very broad and the degree of error around the likelihood estimate should be considered (Fig. 2). This issue has been investigated in breed assignment of cattle [51], where a method was presented for evaluating the distribution of likelihood ratios and calculating a positive predictive probability statistic that incorporates the potential errors associated with incorrect assignment of a sample to an alternate population, and incorrect inclusion of a sample from an alternate population. As the distribution of likelihood ratios for reciprocal scenarios is not symmetrical, these two types of error need to be calculated independently in the first instance (Fig. 2).

The analysis of geographic origin through population genetic assignment should then, where possible, combine a likelihood ratio calculation (and an associated estimate of error) with an exclusion test to account for the possibility that the true population of origin has not been sampled. The combination of assignment and exclusion testing, as proposed for wildlife forensic applications [46], has been demonstrated in concert with an estimate of positive predictive probability in the area of food authenticity, where the approach was used to demonstrate the power of SNP marker panels for breed assignment in British pork products [52]. A similar approach has been used to assign marine fish to their geographic origin within European waters, developed as a tool for authenticating eco-labelling and investigating illegal, unreported and unregulated (IUU) fishing [53].

The approaches reviewed here provide examples of how population assignment methods from the research community can be used in combination to address investigative questions. Other approaches may be more appropriate in certain situations. One of the challenges for the future is to develop a consensus approach (or set of approaches) to data analysis for forensic geographic origin assignment.

5. Reference data

DNA data are only useful if there is something with which to compare them and this is particularly true for population assignment methods. Like all forensic science, the quality of the reference data needs to be unquestionable, however unlike most human DNA applications, obtaining sufficient geographic reference material for wildlife species can be almost impossible and is often the largest practical restriction to the development of geographic assignment tests. Wildlife law enforcement normally focuses on the protection of species that are already rare in the wild and often very difficult to sample. This impacts on both the type and number of reference biological samples available for a given location, which subsequently affects the analytical options and subsequent assignment power. Depending on marker type, a minimum of 30–50 samples per locality should ideally be collected to allow accurate estimates of population allele frequency. The vast majority of existing reference materials have been collected for research purposes and may not be accompanied by detailed provenance documentation, compromising their utility as forensic

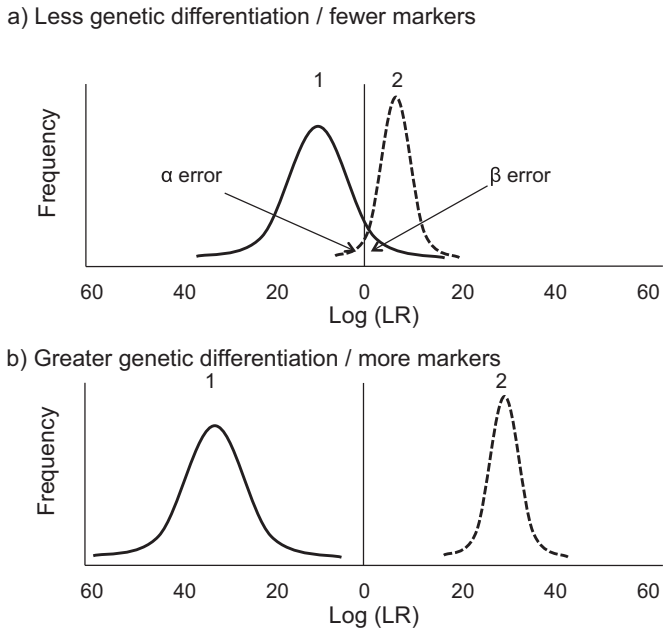

Fig. 2. Assessment of power (positive predictive probability) associated with the assignment of test samples to their genetic population of origin using likelihood ratios (LR). Solid line represents the distribution of log (LR) for a sample belonging to Population 1, given an alternative claim of belonging to Population 2. Dashed line represents the distribution of log (LR) for a sample belonging to Population 2, given an alternative of Population 1. As the distribution passes log (LR) = 0, misassignment occurs. Under scenario (a) the positive predictive probability for the test can be calculated by combining α, the error of assigning a Population 2 individual to Population 1, with β, the error of assigning a Population 1 individual to Population 2 (after [51]). Scenario (b) demonstrates log (LR) distributions with very low risk of missassignment, consistent with high population differentiation and/or a large number of informative genetic markers.

reference standards. This is particularly problematic where the costs of sampling specifically for the development of forensic tests, for example in marine fisheries, can be huge. Last, and by no means least, existing reference samples and data, which may of huge potential help to wildlife crime investigations, are often inaccessible to the forensic genetic community, primarily because there is limited knowledge of their existence.

This rather challenging list of issues is gradually being addressed to some extent in the scientific community. In terms of reference samples, museums and biobanks represent repositories where quality control is applied to sample collection and curation, ensuring verification of the sample to a particular species and location. Modern museum collections increasingly accommodate the need for DNA-friendly storage of biological material and digitisation of collection catalogues is starting to make the search for reference material less arduous. Perhaps more important though, is the increasing requirement for wildlife population genetic researchers to make their genotype data freely available at point of publication. Large data repositories such as DRYAD (datadryad.org) offer applied scientists, including forensic practitioners, the opportunity to access large data sets generated by the research community. Although issues of data quality may still exist, this mechanism allows the location of species-specific sample collections to be rapidly identified amongst the world's academic research laboratories.

6. Enforcement application

Interpreting a geographic assignment result in an enforcement context is a two-stage process: first, samples are assigned to their most likely genetic population; second, using this information and additional biological or circumstantial evidence, the geographic origin of the evidence sample is inferred. It is important that forensic geneticists are aware of the risks of over-interpreting the genetic assignment result to directly conclude on the geographic origin of the evidence. For example, animals known to use the same breeding grounds generation after generation but which migrate during their life history will display a population genetic signal of the breeding ground, but it would not be appropriate to conclude that a poached animal was killed in that area based on its genetic assignment alone. When presenting such evidence, it would therefore be more informative to also consider known migration routes and lifetime dispersal patterns to evaluate the potential geographic range of the individual. This may, or may not, be something on which the forensic geneticist is competent to comment, but as a second stage analysis it encompasses independent estimations of accuracy, precision and error.

Geographic origin identification in marine fish offers another example of assignment interpretation that is worth consideration. Many species of fish display high spawning site fidelity, leading to associations between genetic markers and geography that can be used to infer the origin of commercially caught fish (see Box 2). However, it is inevitable that the occasional fish will disperse more widely, carrying with it the genetic signal of its natal region. The Marine Stewardship Council uses DNA profiling to verify the geographic origin of fish from certified sustainable fisheries. This leads to crucial questions such as, how should the discovery of a single fish displaying a genotype of a non-sustainable fishery be interpreted, and how many fish need to be discovered simultaneously to conclude that a fisherman has been straying from the certified fishing grounds. Ultimately these questions do not affect the population genetic assignment, but are crucial to the investigative question and therefore impact on the utility of any forensic genetic test employed. Without a way to evaluate the likelihood of such alternative scenarios, there may be little point in developing and deploying a geographic assignment test in the first place.

7. Validation

Validation is a fundamental requirement for the transfer of methods from research to forensic science. The process of designing and implementing validation studies for forensic genetic tests is well-established [54] and there has been considerable discussion surrounding the lengths to which wildlife forensic test validation must go to demonstrate fitness for use [7,16]. To fully validate a genetic geographic origin assignment method it would be necessary to validate the individual DNA markers in the assignment panel, the genotyping system employed to analyse them, the authenticity of the reference data and the data analysis method used for assignment. This is in addition to the validation of the DNA extraction process for the specific evidential sample type and a formal assessment of the standard operating procedures controlling the method. Where feasible, this process should be followed prior to the implementation of any test used in forensic investigation [10,13]. However, as discussed above, geographic assignment techniques are specific to both a single species and a defined investigative question, placing a huge validation burden on the development of assignment methods for wildlife law enforcement. This limitation, combined with the fact that geographic origin identification is often not considered prima facie evidence, raises the question of whether in certain circumstances, the evidential standard needs to be considered and potentially relaxed in certain areas.

8. Evidentiary use and value of geographical assignment

The original aim of much of the research underpinning geographical assignment tests applied to the illegal wildlife trade was to aid in an investigation by allowing authorities the means to determine the scale and location of any illegal activity and possibly inform decisions concerning the deployment of resources to a particular area. The aim may never have been to use the test in a court of law.

This draws open a familiar issue in forensic science of whether a method can be for intelligence purposes only, rather than having to meet the criteria for evidence presented at a criminal court. Geographic assignment for wildlife forensic science is in this case similar to ancestry or phenotypic testing in human identification. In both instances there is a body of knowledge that underpins the science, such as publications in the peer-reviewed scientific literature and broad acceptance of the science.

A forensic method designed only for intelligence use has a history of being presented ultimately, and often prematurely, in a court. If the test results in findings that are very valuable to the case then there is understandable eagerness by the investigating authorities to make known what has been achieved and present this to the court. In most jurisdictions if a test is performed during an investigation it must be disclosed to the defence and depending on the jurisdiction, if the judge accepts the test then it will be presented as part of the case. Unless the science presented has met the same standards as any other validated area of forensic science, its reliability may be open to challenge. On this basis it would be necessary that tests used in wildlife geographical assignment meet the requirements of any forensic test regardless of whether or not it was meant for intelligence purposes only.

This line of argument maintains evidential standards, enhances judicial confidence in forensic analysis and ultimately reduces the risk of miscarriages of justice. However when applied to wildlife geographic origin analysis, the simple truth is that the cost of full validation would often be prohibitive and therefore the vast majority of the geographic assignment results would not be available to investigators. So, while retaining the standards and assurances of a test described as 'forensic', should a lesser status, such as 'intelligence' be assigned to the application of peer-reviewed research that contributes to wildlife crime investigations but is not considered fundamental to proving the case? It is an unsatisfactory situation if no DNA-based testing is undertaken in alleged cases of serious wildlife crime where there is every indication that organised criminal networks are active. It would also be unsatisfactory to undertake testing that is not sufficiently developed to withstand challenge.

The use of novel scientific processes in courts has been debated globally and most countries have established criteria for accepting evidence. For example, in Australia a 'credible evidence' ruling is made by the judge at the start of a trial, relating to a landmark ruling in 1984 (R v Bonyathon), stating that a method should be accepted based on '*whether it forms part of a body of knowledge or experience which is, indeed, sufficiently organised or recognised to be accepted as a reliable body of knowledge or experience*'. The judge is the ultimate gatekeeper of whether a novel geographical assignment method meets this credible threshold. The wildlife DNA forensic community should seek to develop both consensus amongst its members and provide guidance to the judiciary if the potential for geographic origin testing is to be fully realised.

9. DNA in combination with other methods

As discussed here, the spatial resolution of DNA based methods is often limited and the use of DNA markers is compromised when individuals are moved beyond the borders of their natural population genetic range. In such situations, the use of other information that reflects the life history of the individual, rather than its genetic ancestry, may enhance our ability to determine the provenance of a biological sample. Chemical isotopes can be used to provide a signature of environmental origin, based on variation in naturally occurring isotopic ratios amongst regions. As with population genetic assignment, isotope analysis requires baseline reference data against which to assign unknown samples. By sampling across continents, 'isoscapes' are produced such as across North America [55] which have been used in wildlife forensic science [56]. Stable isotopes have the added advantage of determining very recent past movements so that for example, a member of a species that has migrated from one normal breeding area to another may show genetic ancestry to the former region but recent geographic association based on isotopes. In relation to the movement of humans, readers will be familiar with the very recent colonisation of the Americas and Australasia by persons of European genetic decent who will have isotope profiles in hair and bones associated with their new surroundings [57,58]. The same is true for wildlife species.

10. Discussion, summary and future direction

Poaching, illegal fishing and illegal logging are prime examples of why there is an increasing need for methods of geographic and population assignment. The potential excessive financial gain from decimating iconic species and habitats is in stark contrast to the low success rate in enforcing any legislation, even if perpetrators of such acts are ever apprehended. Developments in wildlife forensics offer some hope that methods will be available and applied as needed. This review has attempted to describe the real opportunities and problems in transferring these research type applications to the criminal justice system.

The science associated with genetic geographic origin assignment is complex requiring a greater number of analytical stages and more interpretation than other areas of DNA analysis in forensic science. Species testing using a mitochondrial locus and individual assignment using STRs are well developed and in many cases routine, but geographic and population assignment questions often require specific species $\times$ locality test development, as well as a comprehensive understanding of both population genetics and species life history. This type of forensic science is therefore much more resource intensive and will most likely continue to require close collaboration between forensic practitioners, universities and museums.

The present situation in forensic genetic origin assignment in wildlife is in some ways a stark example of the situation faced by the wildlife forensic science community as a whole. A reliance on academic researchers to develop new methods, combined with limited access to validated reference data and a lack of engagement from the established human forensic community, means that wildlife forensic scientists often find themselves in something of a quandary. We often walk a tightrope between rigid validation and loose research application. On the one side, validation and application requirements derived from human forensics that cannot be followed without appropriate resourcing; the result being that no forensic test is available to assist with law enforcement. And on the other side, the risks posed by over-zealous, sometimes frustrated conservation research scientists who fail to implement basic forensic controls and present ultimately flawed evidence that damages the long-term reputation of the wildlife forensic community. Clearly the solution lies in combining the strengths of all parties, sharing and incorporating best practise and collaborating closely to ensure that the impact of

cutting edge applications such as geographic origin assignment are maximised for wildlife law enforcement.

The future of the field will undoubtedly see steady progress in our technical abilities for determining geographic origin in more species, with greater accuracy. SNP DNA markers are likely to overtake other marker types due to their enhanced discovery through genomic research, their ease of laboratory transfer and potential assignment power. Obtaining new reference materials will remain a limitation, but much more could be achieved by fully exploiting existing collections. The greatest challenge by far lies in supporting the application of these technologies, creating appropriate wildlife forensic capacity, operating to agreed standards, where it is most needed.

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