SADC Regional Programme for Rhino Conservation

SUMMARY REPORT ON PROGRESS WITH RHINO HORN FINGERPRINTING AND THE WAY FORWARDS

Task 6.2-1.3
Rhino Horn Fingerprinting Validation

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Richard Emslie, Rajan Amin and Max Bramer

What is horn fingerprinting ?

Rhino horn fingerprinting seeks to determine the source and species of rhino horn recovered in police busts based on the chemical profile (fingerprint) of the horn. Horn fingerprinting is based on the premise that the chemical composition of rhino horns will to some extent be influenced by the chemistry of the food plants rhinos eat, with the latter in turn being influenced by underlying geology, soil chemistry and weather. Thus it could be expected that rhino horn chemistry should vary from one area to another.

The white rhino is a grazer (eating tropical grasses) and the black rhino is a browser (eating succulent plants and trees and herbs). As these different plant types have different photosynthetic pathways, horn chemistry should also vary between species.

Wildlife Investigators and Specialist Police Units dealing with wildlife crimes, as well as those study illegal trade routes, have indicated it would be very useful to have a forensic technique, which could both identify the species and source location of rhino horn recovered in busts.

In the early 1990s, several studies determined that element and isotope concentrations and their ratios found in rhino horns varied between species and park origin (Lee-Thorp et. al. 1992, Hall-Martin et.al. 1993, Hart et.al. 1994). The potential for horn fingerprinting indicated by these early pilot studies resulted in the AfRSG assembling an extensive rhino horn chemistry database and initiating a long-term project to further develop statistical models for the identification of the species and source of rhino horn. The development of a tool for identifying the source of an African rhino horn from its fingerprint was rated by the AfRSG as a *Continentally Important* project. The African Rhino Action Plan also calls for horn fingerprinting to be developed (Emslie & Brooks 1999).

It is however relatively easy to demonstrate that *on average* men are taller than women. Due to overlaps in the distributions of heights of men and women, it is much harder to accurately predict the sex of someone just by knowing they are 1.68 m tall. In the same way it is a greater challenge to build horn fingerprinting models that can reliably source a single sample of rhino horn based on its chemistry, as opposed to simply determining that *on average* horn samples from Area A have higher levels of element X than sample from Areas B and C.

The IUCN SSC African Rhino Specialist Group initiated a rhino horn-fingerprinting project with the aim of getting more samples from a greater range of parks. The AfRSG also sought to analyse the samples using a range of different lab techniques in contrast to the original pilot studies which each chemically analysed rhino horn using one lab technique at a time. The reason for this is that the more chemical descriptors we have for rhino horn, the more likely one will be able to source horn. Similarly the more different descriptions one has of a suspect, the more one can narrow down one's search for that subject.

This work has been strongly supported by SADC's Rhino and Elephant Security Group.

State of horn fingerprinting work prior to SADC RPRC

The AfRSG successfully obtained horn samples from South Africa, Namibia, Zimbabwe, Kenya and Swaziland. These samples came from 27 different black and 22 different white rhino populations covering about two-thirds of Africa's rhinos at the time. While there were gaps in the coverage (especially for Zimbabwe and Kenya) the AfRSG project has made significant progress in establishing a continental horn database. The initial sample sizes for most Parks were however small at 4-6 samples/park.

The horn samples were cut up into smaller samples and analysed in three different laboratories, each using a different technique:

- carbon and nitrogen analysis using mass spectrometry,
- common and trace element analysis using inductively-coupled-plasma optical-emission-spectroscopy (ICP-OES), and
- heavier isotope analysis using laser-ablation inductively-coupled-plasma mass spectrometry (LA-ICP-MS).

The carbon and nitrogen analyses were undertaken at the University of Cape Town (UCT). The other analyses were carried out by Anglo American Research Laboratories (AARL) in Johannesburg.

In the first analysis at UCT, four variables were measured: the percentage of carbon and nitrogen, together with carbon and nitrogen isotope ratios.

The second analysis (ICP-OES) at AARL quantified the abundance of four common elements (aluminium, iron, calcium and magnesium) and 16 trace elements.

The third chemical analysis at AARL measured the relative abundance of 132 isotopes of 58 elements using LA-ICP-MS. Some of the isotopes are not required by rhino for normal metabolic functions, and others are very rare, with the result that a number of elements and isotopes occurred in such low quantities in some horn samples that they were beyond the detection capabilities of the machines, and could not be measured. Summing the isotope values for elements with more than one isotope gave an additional 12 potential variables. In addition, some of the more common elements and isotopes were used to calculate additional potentially useful isotope/element ratios, e.g. Sr88Rb85 (strontium88/rubidium85).

Principle components analysis was also used to generate additional composite variables[1].

Prior to analysis data were examined for approximate normality, and where necessary the data were subjected to a Log + 1 transformation.

The AfRSG then used classical Discriminant Function Analyses (DFA) to build statistical models to identify both the species of rhino and source of horn samples based on their chemistry. The initial phase of the AfRSG study is described in detail in Emslie et.al. (2001).

While many of the DFA models built by the AfRSG successfully classified all the samples (100% post-hoc classification success), the AfRSG was aware of the danger of model over-fitting given the combination of small sample sizes per park and the large number of different chemistry variables per sample (Emslie et al. 2001). With only five horn samples for most parks and the very large number of chemistry variables, there was a high chance that most or all of a Park's horn samples could by random chance have high or low values for one or two variables. The problem is that DFA will detect this spurious chance "pattern" in the data, wrongly interpreting this as a real pattern. The DFA then models this "noise", and this leads to model over-fitting. While this helps the DFA model more accurately predict the source of samples used to build it, over-fitting reduces a model's ability to successfully predict new samples that were not used in building the models. The problem of model over-fitting can be illustrated graphically.

[1] Principle components were also used to reduce data dimensionality.

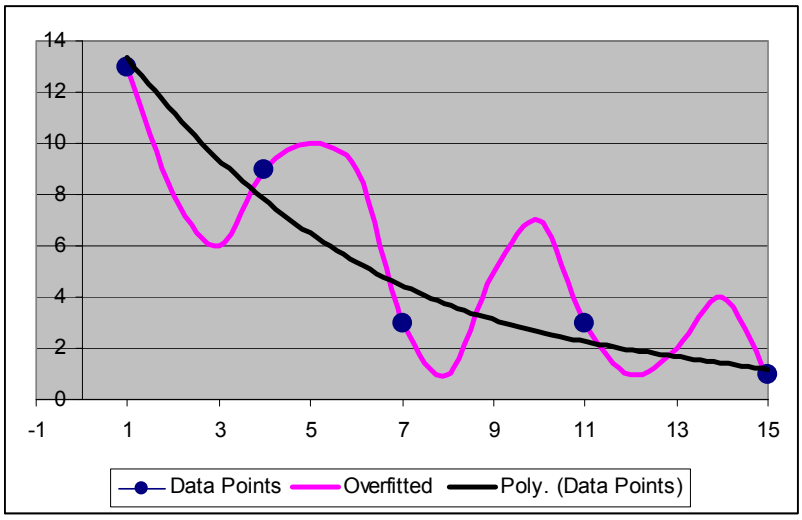

Figure 1: Illustration of the problem of model over-fitting. The crimson over-fitted model passes through each data point, and would accurately predict the Y values for all datapoints given their X values. However estimates of Y for other values of X derived using the crimson line will be poor. The best-fit (black line) polynomial model is clearly a better representation of the actual pattern in the data, and hence better at predicting. For example, when X = 10, the polynomial line predicts a Y value of just under 3 compared to the 7 predicted by the over-fitted line.

As Figure 1 illustrates, over-fitted models are very good at classifying the samples used to build them, but poor at correctly classifying new samples. To be of real practical use horn fingerprinting has to do the latter.

The real test of how good classification models are is how good they are at successfully classifying new samples that have not been used to build the models. Statisticians therefore seek to validate the derived DFA models using techniques such as k-fold cross-validation or 'jack-knifing'. In the case of k-fold cross-validation, the original data is first divided into k approximately equal parts (generally 5 or 10). One of the k parts of the data is excluded from the dataset, and the remaining data are used to build a model, which is then used to predict the withheld datapoints (that were not used in building the model). This process is repeated for each of the k parts. The results of these k models are then combined to give an estimate of the overall accuracy of the modelling process on genuinely unseen data. Jack-knifing is the extreme case of k-fold cross-validation, where k is equal to the total number of examples available in the data. In other words, with jack-knifing a single sample is left out, a model is then built with the rest of the data and used to predict the source of the single withheld sample. The process is repeated for the next sample and so on until this has been done for all samples. The overall classification success rate is then used to estimate the ability of a model to generalize (i.e. predict the species and source of new samples accurately). With jack-knifing as much as possible of the data are used in building the models and so this form of validation is mainly used where sample sizes are limited. Unfortunately facilities for automated k-fold or jack-knifing model validation were not available in the Statistica 5 package used by the AfRSG to do the initial DFA analyses. Given the small sample sizes per park, and based on manual trial analyses, Emslie et.al. (2001) concluded that 'jack-knife validation should be used to validate horn fingerprinting models in future'. In the absence of such validation the AfRSG concluded that 'while these results are very encouraging…readers still need to be cautious and treat these results as preliminary'.

The AfRSG analyses confirmed the earlier discovery by Lee-Thorp and co-workers (1992) that the $\delta^{13}C$ (carbon isotope ratio expressed using delta notation) variable was particularly good for discriminating amongst species. Analysis by the AfRSG found that variable $\delta^{15}N$ (nitrogen isotope ratio expressed using delta notation) was also useful for distinguishing amongst species. Emslie et. al. (2001) also discovered that these two variables were related to rainfall in different areas. However, it was found that species identification was not as straightforward as initially expected. Using only $\delta^{13}C$ (carbon isotope ratio), some black rhino samples from the very arid Kunene area of Namibia were misclassified (Emslie et al. 2001).

Initial work supported by SADC RPRC – Validation Phase 1

While the results of these initial analyses by the AfRSG were very promising; the problems associated with small sample sizes, high data dimensionality and the need for validation of models were recognised by the AfRSG. It was also possible that these issues would be better addressed using techniques other than DFA.

With SADC RPRC funding, the next phase of the horn-fingerprinting project sought to undertake the necessary validation of the DFA models produced, as well as to evaluate the success of alternative source and species determination models.

Two techniques of intelligent data analysis – Artificial Neural Networks and Automatic Induction of Classification Trees were used to build models, in addition to the classical DFA methods used by the AfRSG. A subset of 52 variables was used in these analyses. Jack-knifing was used to validate the success of the models (estimate their ability to successfully classify new samples not used to build the models). Artificial intelligence methods were tested as they often perform better than traditional statistical methods.

It will not be possible to get horn samples for every park, which currently has, or in the past, had rhino. Therefore, to be practically usable, any horn fingerprinting technique also needs to be able to identify samples that are likely to come from areas not yet covered by the AfRSG's horn sample database. The utility of using artificial intelligence techniques in doing this (novelty detection) was also investigated as part of this phase.

Results of Initial validation work in Layman's terms

The detailed results of the Validation Phase 1 work were written up in detail and published in a peer-reviewed paper (Amin et. al. 2003), which is attached as an appendix. This paper was presented at an International conference in Cambridge on Artificial Intelligence, and won the prize for the best paper in the conference.

In layman's terms the conclusions to emerge from this work can be summarized as follows:

- The jackknife validation confirmed the AfRSG were right to be cautious when interpreting the results of their earlier DFA classification models. These models clearly were over-fitted.
- In general the classification models derived using artificial intelligence techniques outperformed (were better at classifying new samples) those built using classical DFA techniques.
- Species identification:
    - The best model (tested by jack-knifing) was produced using a Neural Network[2]. This model correctly predicted the species for all 178 white rhino samples, and all but 2 of the 178 black rhino samples. The overall predictive accuracy was 99.44%. The output of the Neural Network gives probabilities that each sample is of a white or black rhino, and these can be used to

[2] More specifically a Multi-Layered Perceptron (MLP) Neural Network trained with the four Carbon and Nitrogen variables and using the Bayesian regularized Levenberg–Marquardt optimisation technique

calculate error bars. Both the misclassified samples had slightly higher error-bars than those for the rest of the data samples allowing one to flag these samples as species unknown.

- Although marginally inferior to the MLP Neural Network (giving one further misclassification) a Top Down Induction Decision Tree (TDIDT) came up with a simple rule for identifying the species - If the delta 13 carbon isotope ratio ($\delta^{13}C$) is less than -13.621304, then the horn is from a black rhino and if greater or equal to this number a white rhino.
- It was noticed that all the methods used, including DFA, misclassified the same black rhino samples and that these both originated from the Kunene area in North West Namibia. This was in keeping with the earlier work by the AfRSG, which indicated that possible confusion between the species would be in very arid areas (Emslie et al. 2001). Analysis of additional Kunene black rhino horn samples would assist in further refining species identification.

- Country of origin determination:
  - Earlier AfRSG analysis showed that decomposing the problem of classification into parks, areas, etc. by species gave better results than treating both species together in one model. This was borne out by further experiments with intelligent data analysis techniques (Amin et al. 2003).
  - Using artificial intelligence methods it was possible to build country determination models for black and white rhino with validated successes of 96.56% and 97.36% respectively (Amin et al. 2003).
    - A Probabilistic Neural Network classification model predicting the country of origin of white rhino samples was cross-validated by jack-knifing and had an overall predictive accuracy of 97.36%. This compared well with the use of DFA with the same 52 variables, which gave a classification accuracy of 94.7%. Although a TDIDT also gave 'only' 94% accuracy, only six variables and seven rules were needed to gain that level of accuracy.
    - A multi-layered feed-forward Neural Network was used to classify the country of origin of the black rhino samples.[3] The overall predictive accuracy was 96.56%, which again improved upon the result using classical DFA (95.9%). The same problem was tackled using TDIDT in a much simpler fashion achieving a predictive accuracy of 95.82%.
    - Country borders are somewhat arbitrary and it would make more sense in future to instead classify samples into broad regions with similar geomorphology and climate. Prediction accuracy should then increase further.

- Finer scale source determination:
  - Seeking to distinguish amongst different parks within a region within a country is a more rigorous test of data analysis techniques than distinguishing amongst parks that are widely separated geographically. As a severe test of fingeprinting's ability to source samples at a finer spatial scale, analyses tried to build and validate models designed to distinguish between horns from six different parks in the northern part of KwaZulu-Natal Province in South Africa.
    - Once again Intelligent Data Analysis methods gave the best results with a predictive accuracy of 64.9% for white rhino and 72.4% for black Rhino. The use of Neural Networks represents a considerable improvement on the DFA which only had a 40.5% predictive accuracy.

[3] The model was trained by Bayesian regularized Levenberg-Marquardt optimisation, and tested by 10-fold cross validation.

        - The very unbalanced distribution of classes and the small number of samples for some classes (parks) are likely to prove problematic for any method of analysis. However, although the predictive accuracy at the finer park level was not sufficiently high to be of real practical use, the results were very encouraging, as predictive accuracy should increase substantially in future as sample sizes per park increase.

- Novelty detection – Are samples from areas not yet covered by the database?:
    - One requirement for turning horn fingerprinting into a practical routine forensic test is that it is necessary to be able to detect whether some samples are likely to have come from areas not yet covered by the continental rhino horn chemistry database. An artificial intelligence technique was found to do this[4].

Application of lessons learned in Validation Phase 1

The technique developed to identify the species of horn based on Carbon isotope ratios was used to assist the Canadian CITES permit authority deal with an application for the import of two cut horns of unknown species being imported from South Africa.

These results could have wider applicability. The AfRSG was contacted by the President of the CITES Monitoring Centre (RECC) in Poland who was very interested in the techniques. Should this method be made to work for rhinos, then it is likely it could be used to develop methods to source products of other endangered species such as elephant ivory.

The Australian Antarctic Division's website also reviewed the Knowledge-based Systems paper by Amin et al. (2003), noting that the analytical horn fingerprinting work described in this paper had direct relevance to their Human Impacts Program which is using chemical fingerprinting techniques to assist in the management of petroleum product spills in the Antarctic.

Need for final experimental phase

While the horn fingerprinting work to date can reliably identify the species of horn, and the results of attempts to determine the source of horn were encouraging, samples sizes were clearly too small/park to be able to reliably determine the source of horn at a park level. A final experimental phase was needed where much bigger sample sizes would be analysed from a subset of parks (some close to each other and some further away) in order to provide data to be able to determine the spatial level to which horn samples can be reliably sourced, and the minimum sample sizes/park required to achieve this at different spatial scales.

The results of such experimental work can then be used to inform a decision (in consultation with representatives on the SADC Rhino and Elephant Security Group) as to whether to either proceed to full implementation of the method or abandon attempts to develop fingerprinting as a workable practical forensic tool. Such an experiment would also determine how many samples should ideally be sourced for each area in the horn database.

Clear direction will need to given in the final report on exactly how the technique would work in practice. Custom made software would need to be provided to undertake the species and source determinations in future. However before this should be done one first has to decide on whether fingerprinting will be able to reliably source samples at a practically useful spatial scale and how many samples per park are required to do this. A final experimental phase was planned to do this and the necessary additional samples collected from three countries and four conservation agencies.

[4] A Kohonen Self Organising Map was successfully trained as a novelty filter

A few additional samples from Kunene will also be analysed in an effort to improve upon the 99.6% validated success rate in determining the species from a sample of rhino horn.

The initial Laser Ablation Inductively-Coupled-Plasma Mass Spectrometry (LA-ICP-MS) at AARL generated horn chemistry data that were very bulky and prone to aberrant data spikes. This meant that extensive and very time consuming data preparation and checking was needed prior to analysis. Such data are not suited to routine use. If fingerprinting is going to become a routine technique, chemistry data needs to be available in a much simpler sample (row) by variable (column) spreadsheet table format, which could easily be importing into a standard horn fingerprinting analysis package. Another disadvantage of the original LA-ICP-MS data was that it was in the form of counts rather than in parts per million calibrated against known standards. The original LA-ICP-MS machine also got contaminated. The machine was also ageing and measurement variability was too high. There was therefore a need to use a different mass spectrometer to do the horn analyses.

Fortunately AARL obtained a Finegan Mat-Element Magnetic-Sector High-Resolution Inductively-Coupled-Plasma Mass Spectrometer (F-ME-MS-HR-ICP-MS) . This machine had a number of advantages over the LA-ICP-MS and offered the opportunity of getting higher resolution data and measuring the abundance of more elements. The use of a F-ME-MS-HR-ICP-MS would also mean that it no longer would be necessary to also undertake ICP-OES analyses as well reducing the number of lab analyses required from three used by the AfRSG initially to two. This also would significantly reduce the cost per sample. In addition the F-ME-MS-HR-ICP-MS is able to determine the abundance of some potentially very useful common elements (for example sodium), which previously could not be measured using the other methods.

Final experimental phase work sample collection and pre-preparation

Additional rhino horn samples (around 30 per area) were obtained for the final experimental phase of this project from South Africa (Great Fish River, iMfolozi, uMkhuze, Tembe, Ithala, Ndumo; Namibia (Etosha and some samples from other areas including Kunene) and Kenya (Nairobi). Thus larger sample sizes were obtained for some widely spaced populations (Great Fish River vs. Zululand vs. Nairobi vs. Etosha), as well as for populations found close together in a region within a country (iMfolozi vs. uMkhuze vs. Ithala vs. Tembe vs. Ndumo in KwaZulu-Natal Province in South Africa). This study area selection was chosen for the final experimental phase of the project to 1) enable a determination of just what level of spatial source determination could be reliably achieved using horn fingerprinting and 2) determine the number of samples per area required to do this.

All the additional horn samples were pre-prepared (cut up into lab sized pieces and numbered), entered into a sample database and delivered to the Labs at AARL and UCT.

Laboratory analyses of final experimental phase samples

Lighter isotope analyses at UCT

The abundance of carbon and nitrogen, and $\delta^{13}C$ (carbon) and $\delta^{15}N$ (nitrogen) isotope ratios were determined for all the new samples at the University of Cape Town's Department of Archaeology. The results were timeously sent to the AfRSG.

Element analyses at AARL

Unfortunately the corresponding multi-element analyses at AARL were not completed by the end of the SADC RPRC Phase 1 due to firstly a breakdown of the F-ME-MS-HR-ICP-MS at

AARL; and then to unexpected problems in developing a suitable method for pre-preparing the horn samples prior to analysis in the Mass Spectrometer.

Traditional methods to dissolve the samples in an acid Aqua Regia solution were unsatisfactory leaving a glob of orangeish material in the solution. Thus not all the horn went into solution. Test runs using the solution obtained, showed an inability to adequately record the abundance of many of the key rarer elements.

In subsequent trials, ashing the samples prior to analysis proved much more successful, although AARL staff indicated their concerns that this method would reduce the ability to measure some of the more volatalizable elements, (which may potentially be useful as source indicator variables).

Recently AARL became aware of a potentially better method to produce analysis solutions using a method successfully developed by Wits University to prepare samples of keratin (human hair) prior to Mass Spec analysis. A trial of this method, which uses a pressurised microwave to produce a horn solution, has been arranged.

Implications of delay in completing lab analyses at AARL

Due to the long delays experienced in getting final lab results back from AARL and the fact that additional time would be needed to statistically analyse the lab data using the artificial intelligence techniques developed and written up as part of an earlier phase of this project, the final experimental phase of this project could not be completed by the end of the SADC RPRC phase 1.

However, the fact that all the horn chemistry data (AARL and UCT) are required before undertaking the statistical analyses cannot be got around. At least the development of the new analytical approach at AARL, while being frustratingly slow, should in the end result in better measurements of element abundance (increasing the potential power of the technique to determine the source of horn), produce raw chemistry data in a form much more suitable to plug in to any eventual source determination software, as well as significantly reducing the cost/sample compared to the earlier analyses.

When the samples have been analysed at AARL and the horn chemistry data are analysed using the neural network techniques developed in an earlier (SADC RPRC funded) phase of this final experimental phase of the project will determine the level of spatial resolution possible and the sample sizes required to do this. The AfRSG will then take these results to the wildlife investigators, police and SADC RESG and based on feedback will take a decision to proceed to either full implementation or scrap the project.

Steps taken to ensure that the final experimental phase is completed as planned despite the end of Phase 1 of the SADC RPRC .

Given 1) the continued support for this work from wildlife investigators, specialised police units, the conservation agencies which have provided the samples and SADC's Rhino and Elephant Security Group; and 2) as effort and expense has been expended in sourcing, pre-processing the samples, sending them to the labs, analysing their carbon and nitrogen composition and in developing methods to pre-process the samples prior to Mass Spectrometer analysis at AARL, it is important that the experimental phase of this project is still completed and a full report on the results produced.

A funding proposal for the balance of funding required to complete the final experimental phase including doing all the necessary statistical analysis work was submitted to and has been funded by the European Association of Zoos and Aquaria's (EAZA) Rhino Campaign 2005-06. Thus the work in collecting and preparing additional horn samples, and analysing the carbon and nitrogen content of the final experimental phase samples will not have been wasted.

Discussion – Is this technique too high tech to be practical? How would such a technique be used in practice?

Some concerns have been expressed that horn fingerprinting may be too high tech and others have wondered how the technique could be used in practice.

As explained above, whether or not the technique proceeds to full implementation will depend upon the results of the final experimental phase. Assuming that the final experimental phase is successful and it is possible to reliably source samples down to a practically useful level additional samples will need to be collected for as many parks as possible to build up the AfRSG horn sample database. Once these samples have been analysed appropriate Neural Network models will be built and validated using the methods outlined in Amin et al. (2003). The results of these analyses would then be used to build specific species and source prediction fingerprinting software.

The steps in the process for routinely processing a sample would be the following.

1. Samples for analysis will need to be cut into small 1-2 cm^2 pieces and sent to both UCT and AARL labs for chemical analysis. The necessary CITES permits should be obtained and the AfRSG informed of the need to analyse the samples. The AfRSG would seek to raise sufficient funding to be able to pay for these sample analyses.
2. The raw chemistry data will then need to be compiled in a simple sample (row) by chemistry variable (column) spreadsheet format.
3. The standardized chemistry data file would then be imported into specific Horn Fingerprinting software that will need to be developed. Fortunately, it is relatively straightforward to take the results of Neural Network modeling and code it to produce predictive software. Some Neural Network software packages have options, which allow users to automatically generate such code automatically.
4. The Horn Fingerprinting software then will first seek to identify the species of rhino together with the estimated probability of being correct.
5. The software will then determine the likelihood that some samples are likely to have come from areas not yet covered by the continental rhino horn chemistry database [5].
6. If the sample is likely to come from a region/park covered by the database, then the software will determine the geographical region the sample is likely to have come from, and the estimated probability chance of being correct.
7. Finally the software will go on to estimate the Park (and possibly Area within a big park) that the sample is likely to have come from with associated probabilities of being correct.

Example output could be as follows …

```
Sample Number xxxx

Species – White rhino     (99.99% probability of being correct)

Novelty detection – Analysis indicates this sample comes from,
regions/areas covered by the AfRSG database,

Region of Origin – It is predicted that this sample comes from the
Northern KwaZulu-Natal Region with a probability of being correct of
99.82%)

Area of Origin – It is predicted that this sample comes from Ithala
Game Reserve (96.7% chance of being correct) with a 2.8% chance of
coming from uMkuze Game Reserve
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[5] Using a Kohonen Self Organising Map as a novelty filter

As horn continually grows from the base whilst being worn away at the tip, by sampling at different places up the horn it will be possible to determine if the sample has come from a rhino that was translocated, and if so to determine the donor and recipient areas.

The change to using F-ME-MS-HR-ICP-MS will mean that only two instead of three lab analyses will be required, simplifying the chemical analyses and significantly cutting the cost of analysing samples. In addition the data produced by both labs is calibrated against known standards and can easily be represented in a simple sample (rows) by chemistry variable (columns) spreadsheet data table format, which could easily imported by horn fingerprinting software. This will facilitate subsequent analysis.

Sustainability

Funding permitting, the AfRSG will continue to coordinate this project.

Should the final experimental phase of the work prove successful, and full backing for the continuation to full scale implementation is given by the SADC RESG, then there is a good chance that the cost of further work will be picked up and funded either by individual zoos involved with the EAZA rhino campaign, or from other funding agencies such as US Fish & Wildlife's Rhino and Tiger Conservation Fund or perhaps WWF.

Acknowledgements

The AfRSG Chairman, Dr Martin Brooks, is acknowledged for his role in initiating the AfRSG horn fingerprinting project, and his initial work in raising the necessary funding and obtaining many of the original samples.

The work of the initial pioneers in this field provided the base on which subsequent work has been built. JuliaLee-Thorp (University of Cape Town), Roger Hart (University of the Witwatersrand) and Anna Jolles (University of Princeton) are thanked for their advice during the initial project planning stage.

The AfRSG thanks all those in the field who provided it with samples. Julia Lee-Thorp also kindly made additional samples available to the AfRSG that were left over from her initial horn fingerprinting work. Julia also is thanked for facilitating the carbon and nitrogen analyses at the University of Cape Town. Richard Holdsworth and Nannete Schutte of Anglo American Research Laboratories (AARL) are thanked for expediting the analysis of the initial samples at AARL and for their useful comments.

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SADC RPRC are also thanked for budgeting to sponsor the F-ME-MS-HR-ICP-MS chemical analyses of the final experimental phase horn samples, and the statistical analyses of these data by Dr Amin. Unfortunately due to the delays in completing the F-ME-MS-HR-ICP-MS analyses at AARL, it was not possible to undertake this work as part of the SADC RPRC. Fortunately EAZA has chosen to fund this work as part of their 2005-06 rhino conservation campaign, and so the final experimental phase of this work will still be completed.

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