21st Century Tiger Final Report

Project title: Field based forensics for protection of tigers and their prey.

Project dates: January 2017 - July 2017

Grantee: Olutolani Smith, Panthera Tiger Program

Executive Summary:
Several countries in South and Southeast Asia have been recognised as trafficking hotspots for tiger skins and body parts, with Thailand, Vietnam, and Bangladesh reported to have the highest numbers of tigers per seizure (Stonor 2013). The Bangladesh Sundarbans is subject to high rates of poaching (Aziz 2017) with species such as tigers and spotted deer (chital) often the primary targets. Up to 10 tiger and 81 chital skins were seized over a 14 month period in 2014-2015 (Dhaka Tribune 2015), and it is thought that much of the trade is driven by both local and international demand for personal use, community protection, or commercial gain (Saif 2016). Pelts from tigers and spotted deer are easily identified by their distinctive coat pattern, but body parts such as bone and meat are more difficult to assign to a particular species.

The main objective of this project was to develop and deploy a portable genetic test that could be used by law enforcement personnel *in situ*. Thus, the tests were designed to be straightforward to interpret and quick to run. Following Phase I of the project, we now have species identification tests for tiger and chital that can be run on small battery-operated devices. Care must be taken to handle the samples and reagents appropriately, but results can be obtained within 30 – 90 minutes.

Project development in ongoing with plans to: (i) design DNA tests for other species, e.g. leopard, sambar, (ii) organise field trials in key tiger sites, and (iii) assess the effectiveness of other portable technologies for field use.

Project Achievements:

1. Development of a portable LAMP test for tigers and spotted deer.

21st Century Tiger funds have been used to develop and validate a LAMP-based species identification test for tigers and spotted deer.

LAMP primers were designed using mitogenome sequences from multiple species. Blood, tissue (skin or muscle) and buccal swabs were collected from a variety of individuals and DNA was extracted using commercial kits. Archived faecal DNA taken from captive tigers between 2008 – 2015 was also tested. LAMP reactions were conducted with a portable (battery-operated) isothermal amplification device that measures fluorescence.

The final primer sets amplified a 301 bp NADH4 fragment for tigers and a 292 bp Cytochrome b fragment for chital. Peak amplification was achieved after 40 minutes at 67 °C for the tiger assay and after 28 minutes at 66 °C for the chital assay (Figs. 1 and 2). DNA could be amplified from all the samples tested, including the faecal samples, though amplification times were extended by 5 - 10 minutes.

The tiger and chital LAMP assays have been designed to be portable so that reactions can be monitored in real time and crude DNA extracts can be used following a simple heating step. Most field applications can therefore be performed with a portable thermocycler and a heat block. The LAMP assays have also been shown to work with low quality samples such as faeces. Further work with DNA extracted from tanned hides shows that the LAMP reaction can be inhibited, and therefore false negatives are possible. We have therefore been developing a second test which can be used to confirm the result of the LAMP test.

1b. Development of a portable real-time species ID test for tigers.

Two primers and a fluorescent probe were designed using the Cytochrome b sequence for tigers. DNA was extracted from the carnivore samples listed above to test for cross-reactivity between the tiger probe and non-target species. Reactions were performed on a portable (battery-operated) thermocycler, with positive amplification shown by an increase in fluorescence. Positive amplification was seen only with DNA from tigers (Sumatran and Amur samples), including faecal DNA, after 20 - 28 cycles.

1c. Development of a portable real-time species ID test for chital.

Primers targeting alleles specific to spotted deer were designed using Cytochrome b sequences from all non-target deer species. Reactions were performed on the same portable thermocycler as the tiger probe study using DNA extracted from the deer species described above. Positive amplification was seen only with the chital samples, though there was some evidence of inhibition using DNA from tanned hides. Work to overcome this inhibition is still ongoing.

In conclusion, we have been able to design a method for identifying samples containing DNA from tigers and chital that can be run on portable amplification/thermocycling devices. The LAMP tests are intended to be used as a first line screening tool to identify those samples that require further investigation. The real-time tests can then be used as an additional means of verification to confirm that the samples contain DNA from chital or tigers.

2. Training of local law enforcement personnel.

Panthera has been working with organisations such as the USAID BAGH Project in Bangladesh to train forest department and police officials in criminal investigation procedures, such as surveillance and intelligence-gathering. As part of this training, the chital LAMP test was introduced to representatives from the Bangladesh government and police, and to the head of the CID forensics lab in Dhaka in August 2016 and April 2017.

Panthera has ongoing collaborations with NGOs in Malaysia, Thailand and Indonesia, that represent other suitable sites for the test kits. We are also in discussion with other anti-wildlife trafficking organisations that could use the tests as part of their investigative work.

3. Dissemination of testing protocols to project partners and publishing findings in peer-reviewed journals.

We plan to share full details of the LAMP and real-time PCR protocols with the Bangladesh Police CID forensics lab in Dhaka. The CID lab have access to the necessary equipment to perform LAMP and real-time PCR tests on-site without having to rely on an external lab. In addition, Dr. Olutolani Smith is co-supervising a Master's student in the UCL's Crime & Forensic Science Dept. who is investigating DNA sampling and recovery rates from wildlife snares under different climate conditions. There is also an ongoing collaboration with the University of Leicester to develop other field-based sequencing technologies.

Details of the chital LAMP test have been published in Conservation Genetics Resources, and a second paper outlining the complete tiger tool kit will be submitted for publication by December 2017. Results of the snare sampling work are also due to be included in a supplement of Forensic Science International: Genetics.

Figure 1. Fluorescence curve showing the specificity of the Cytochrome b chital LAMP test. Only the chital sample generated an increase in fluorescence. Peak fluorescence is achieved in ~28 minutes.

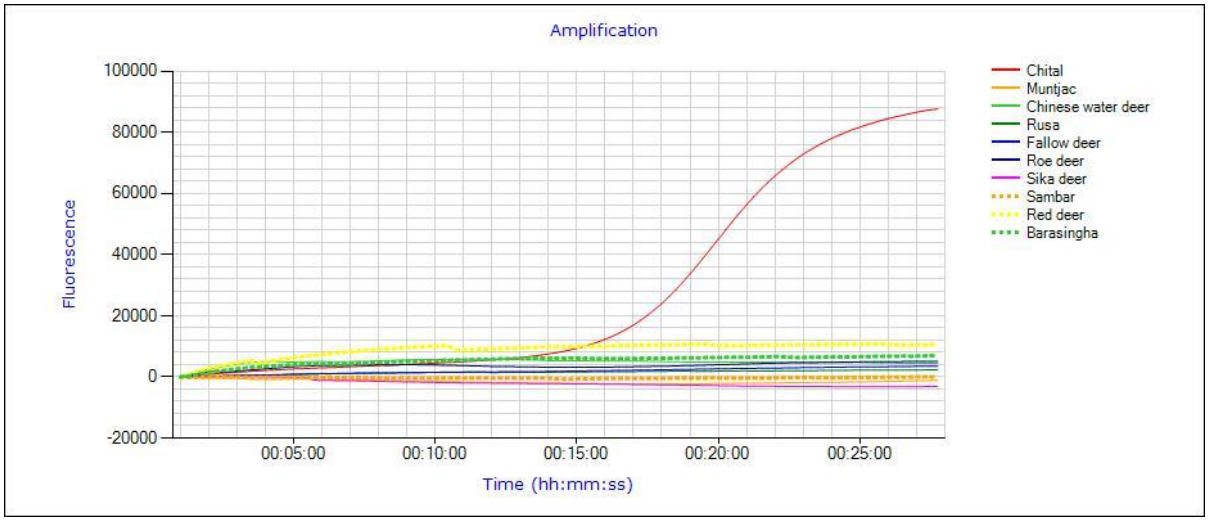
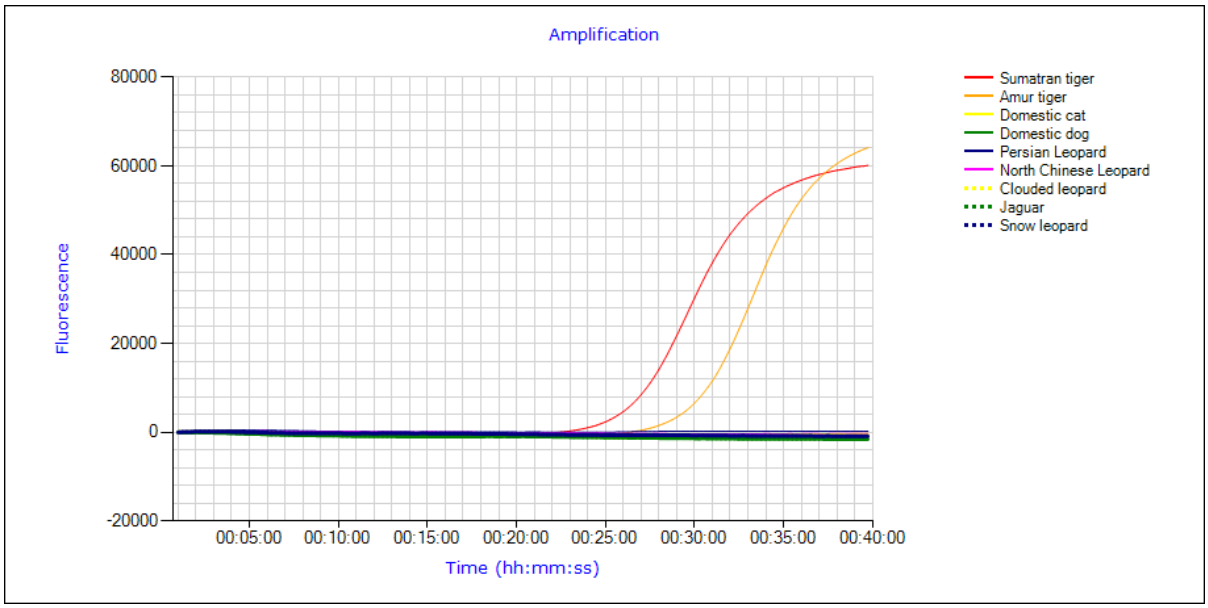
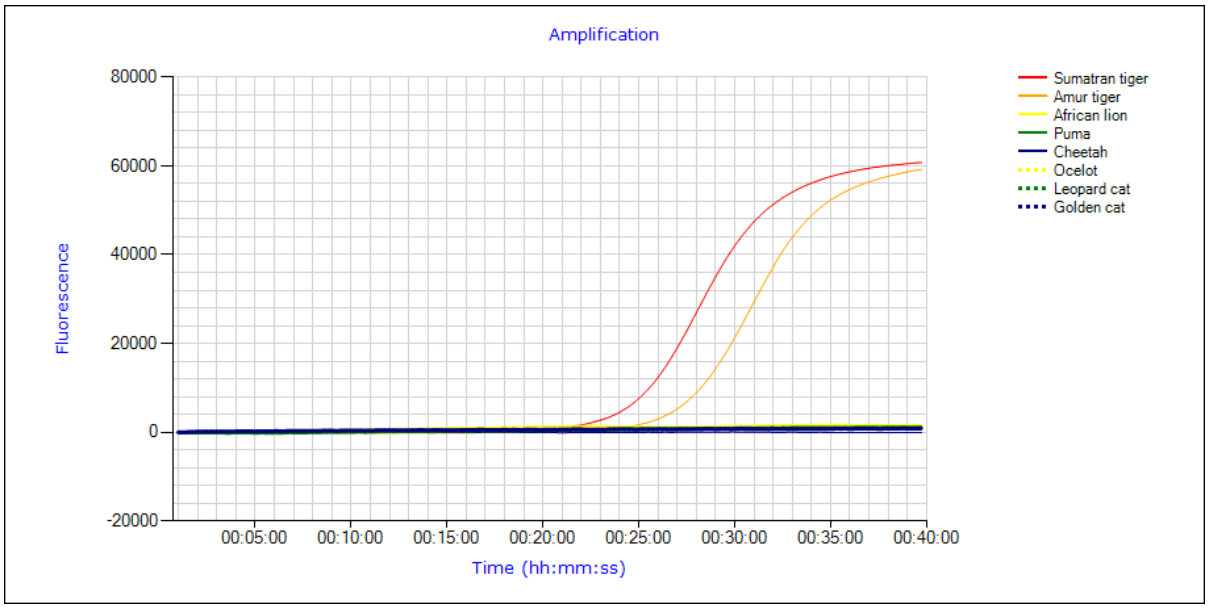


Figure 2. Fluorescence curve showing the specificity of the NADH4 tiger LAMP primers. Only the Amur and Sumatran tiger samples generated an increase in fluorescence. Peak fluorescence is achieved in ~40 minutes.




Amplification
Fluorescence
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Time (hh:mm:ss)
Sumatran tiger
Amur tiger
African lion
Puma
Cheetah
Ocelot
Leopard cat
Golden cat