1.a. Project title:
Identification of elephants in conflict with people using molecular techniques.

1.b. **FINAL REPORT**

1.c. Names of Investigators, Affiliation and contact information

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1.d. Project starting date: **February 1, 2014**

1.e. Anticipated completion date: **February 28, 2015.** The peak crop-raiding season in our study site is from October to January. We collected samples during the crop-raiding

season of 2014-15, and have tried to include the molecular analyses of some of those samples in our final report.

2. Conservation needs

A wide range of methods are used by governments, wildlife departments, NGOs and local communities to minimize crop damage by elephants, ranging from traditional methods such as noise making or chasing to construction of physical barriers, electric fencing and even occasional killing. Nevertheless, no single method has been entirely successful in preventing crop raiding. Moreover, animosity towards elephants among the local communities erodes goodwill towards the species and efforts for its conservation. Even in regions where elephant populations are on the rise due to successful conservation strategies, there is a need to minimize conflict to ensure sustainable co-existence with people into the future.

An assessment of the nature of conflict (whether by habitual or occasional raiders), accurate identification of habitual crop-raiders and information about their raiding frequency and ranging patterns can provide vital clues to devise effective mitigation measures and reduce conflict in the region.

3. Summary of goals and objectives

We aimed to identify crop-raiding elephants to gain a better understanding of the acute levels of conflict in the Kodagu district of Karnataka (southern India), using molecular techniques. Through our study, we planned to estimate the proportion of crop raiders versus non-raiders, the proportion of habitual raiders versus occasional raiders and the proportion of male versus female crop raiders in the region, across a habitat fragmentation gradient.

Changes in goals

In keeping with the recommendations of the grant review committee and the limited funds, we focused only on the crop raiding elephant samples. The analyzed samples included those collected between October to December, 2012 and 2013 from paddy field crop raids. No forest elephant samples were analyzed.

We observed a low amplification success in many of the 2012 and 2013 samples, which could be because the samples collected were from old dung piles, where the DNA was too degraded to amplify. Therefore, during our dung sample collection during the crop raiding season of Oct 2014- Jan 2015, we aimed to collect very fresh samples, with a focus on less than a day to upto 2 days old dung. Since the landscape is a mosaic of the two major crops, it is likely that the same elephants that raid paddy fields may also raid coffee plantations. Therefore, we included samples collected from raids in coffee plantations in addition to paddy fields during the 2014-15 season.

Since we had limited funds from IEF, we utilized a different source for funding the field work and sample collection for the 2014-15 crop raiding season. **The IEF grant was utilized exclusively for lab analyses of the collected samples.**

4. Actions taken

Objective 1. To identify the gender and individual identity of crop raiding elephants in the study area.
Most of our work so far has been towards this objective, which is our short term objective.

Sample collection: Members of our group had already collected samples from the Oct-Dec crop raiding seasons of 2012 and 2013 form paddy fields. We collected samples during the peak crop-raiding season, Oct 2014 to Jan 2015 from areas with high levels of conflict. Samples were collected from both paddy fields as well as coffee and other plantations. In this process, we were also able to interact with the local farmers, plantation owners, and forest department officials and build a network of informants who could provide details about crop raids quickly and efficiently. This enabled us to collected relatively fresh dung samples to ensure a better amplification success. We plan to continue to collect samples during April to June 2015, the season of jackfruit ripening (which also experiences high elephant-human conflict) using our continuation grant.

Molecular analyses to identify the gender of crop raiding elephants:

Our group had previously collected elephant fecal samples from paddy crop raid sites during the peak crop raiding seasons (October to December) of 2012 and 2013. We used these dung samples to extract DNA for PCR-based molecular sexing of the crop raiding animals. All extractions and PCRs were carried out in duplicates.

We standardized the dung DNA extraction protocol by trying out two different methods of extraction. First, we used the Qiagen stool DNA extraction kit and the manufacturer's protocol to extract DNA from 44 extractions collected from crop raid sites. We obtained about a 60-70% amplification success, that is, only about 60-70% of the DNA extracts amplified and gave results in the subsequent molecular sexing PCRs. Next, we explored another method for DNA extraction and found that the protocol described in Fernando et. al (2003) gave a better amplification success.

All extractions were repeated at least twice per dung sample, and PCRs were repeated at least twice per extraction to ensure consistency in the results. In some cases, the same samples were extracted using the two different protocols to compare results. We were thus able to analyze a total of 62 samples from the 2012 and 2013 collection.

We observed that the DNA extraction protocol described in Fernando et. al (2003) gave an almost 100% with fresh dung samples, but amplification success decreased with increase in the age of the dung at the time of sample collection. This is consistent with the observations of Vidya et. al, 2005 where they found an inverse correlation between dung sample age at the time of collection and amplification success, which is likely due to the degradation of DNA in the dung, which decreases the likelihood of successful amplification.

We also analyzed 22 samples collected during the 2014-15 crop raiding season, and preliminary results show a much higher amplification success (18 out of 22 samples). This further points to the possibility that the age of the dung pile at the time of collection

is crucial in determining the amplification success of the extracted DNA. We would like to confirm these findings further by analyzing more samples and looking for correlations between sample age and amplification success.

Molecular analyses to determine the individual identity of crop raiding elephants:

We procured and standardized the conditions for PCR amplification of eight microsatellite loci, which have already been used for genotyping a neighboring population (Shubhankar 2014). All the eight loci amplified consistently with a high quality DNA template obtained from a blood sample used as a control. However, we observed high variability in the amplification success of the eight loci, when tested with actual dung DNA samples from crop raid sites. This could be because of the poor quality of DNA obtained from dung samples (as compared to tissue or blood), compounded by the degradation of DNA due to the age of the dung piles at the time of sample collection. We therefore standardized four more microsatellite loci, to ensure that we had amplification from sufficient number of loci from each sample to enable individual identification.

We are in the process of standardizing the protocol for the analysis of genotyping data and individual identification of the crop raiding elephants.

Objective 2. To determine the proportion of opportunistic and habitual raiders in the population and examine whether crop-raiding patterns vary with habitat fragmentation.
This is an intermediate-term objective. Based on the results from objective 1, we would be able to identify unique genotypes of crop raiding elephants. Only after we build a database of unique genotypes from the region can we start to identify the opportunistic versus habitual crop raiding elephants.

Objective 3. To suggest effective conflict mitigation measures in the context of elephant population management in the Kodagu region.
We would like to make recommendations to the forest department for effective management of crop raiding elephant in the region upon completion of Objectives 1 and 2.

5. Activities that differ from the original proposed action

- Ms. Aditi Sridhar is assisting in this project instead of Ms. Evangeline Arulmalar as originally proposed.
- Based on the recommendations of the grant review committee, we did not collect or analyze any forest samples in the first year. Instead, we focused on analysis of the crop raid samples collected in 2012 and 2013, and did some fresh crop raid sample collection in 2014.
- With the limited funds available for lab analyses, we were able to complete the molecular sexing analyses for most of our samples, but were not able to complete the genotyping analyses.
- Moreover, since we observed low efficiency and higher variability in amplification success for the molecular sexing and microsatellite loci, we had to spend a lot of time on further standardization to ensure higher confidence in our results. As a result, we were not able to complete the genotyping analysis of all the samples.

- Since the genotyping analysis is still in progress, we have also not been able to identify any habitual or opportunistic crop raiders. However, we plan to continue our project, and hope to add some more definitive results to our analyses.

6. Conservation outcomes

Our results would provide valuable information to devise measures for reducing crop raids that could be specifically customized to a few habitual raiders that may be causing most of the damage versus raiding that is found to be largely opportunistic. These observations would also have wide-ranging lessons for the study of elephant-human conflict, and subsequently, for informing policy decisions across the entire habitat of the Asian and African elephant.

Major findings and accomplishments to date

- We standardized the DNA extraction protocol from dung samples to obtain good amplification success using the protocol from Fernando et.al. (2003) using the samples collected in 2012 and 2013.
- We adapted the molecular sexing protocol from Ahlering et. al. (2011) for Asian elephants.
- Of the 62 samples analyzed from the 2012 and 2013 samples, we were able to successfully and consistently determine the sex of 33 samples, of which 30 were males and 3 were females.
- We observed that amplification success decreases with increase in the dung sample age. This has implications for our sample collection strategy in the future, with a strong need to minimize the delay between a raid, and sample collection.
- We standardized the PCR conditions for 12 microsatellite loci. These loci are being used for the genotype analysis of the samples to determine individual identity.
- We collected an additional ~100 dung samples from crop raid sites in paddy fields and plantations during October 2014 – January 2015.
- Extraction of DNA and molecular sexing analyses from the 2014-15 samples is in progress.

7. Problems discovered or occurring during this grant period

One of the problems we faced was the age of the dung sample at the time of collection. Kodagu is spread out over a large area (4000 sq km), and obtaining information about crop raids quickly is not always possible. As a result, many of the samples we encounter in crop raid sites are already a few days old and unlikely to provide good amplification success. We have decided to focus on building a better network through which we can get information faster, and be able to reach the crop raid sites well in time to collect fresh samples.

8. Was your project successful? We obtained some very promising results from our preliminary work in the year 2014. While we have not been able to achieve all our goals

yet, we have been able to iron out several difficulties in the methods used for analyzing samples and hope that in the coming year, we will be able to produce more data using the standardized techniques. We have also built connections within the local communities that will enable us to collect more fresh samples in the future.

Short term goals: To identify the gender and individual identity of crop raiding elephants from dung samples collected from **paddy fields and coffee plantations** in the Kodagu region.

Long term goals: To identify habitual crop-raiders and determine the proportion of habitual raiders in the population, the proportion of habitual raiders versus occasional raiders and the proportion of male versus female crop raiders in the region. We plan use this information to devise measures for reducing crop raids that could be specifically targeted at deterring the identified habitual crop raiders in the region.

9. Next steps

We are currently in the process of carrying out genotyping analyses of the samples collected in 2012 and 2013, as well as the molecular sexing and genotyping of samples collected in 2014-15. The next step would be to identify the **unique** genotypes from our samples across different regions, seasons and years. We would then try to determine whether a single or a few unique genotypes appear multiple times in our samples. This would indicate them to be individuals that raid crops repeatedly, and help us identify the number of habitual crop raiders in the region.

10. Summary of progress

We plan to estimate the proportion of crop raiders versus non-raiders, the proportion of habitual raiders versus occasional raiders and the proportion of male versus female crop raiders in the Kodagu region, of Karnataka in southern India. We have collected fecal samples from different crop raid sites in Kodagu. The fecal samples would be used for DNA extraction and analyzed to identify gender as well as for variability in alleles at eight to twelve different microsatellite loci, which would be sufficient to assign a unique genetic signature to each individual elephant.

We standardized the protocol for extraction of DNA from dung samples and carried out molecular sexing of 62 dung samples collected in 2012 and 2013. We were able to obtain a confirmed result for 33 samples, of which 30 were males and 3 females. Since many of the samples collected from crop raid sites are a few to several days old, the DNA may have been fragmented and degraded. More efficient extraction methods can increase the amplification success to a certain extent, but some samples may be degraded to the point that they no longer have any amplifiable DNA.

We are currently carrying out the genotyping of the same 62 samples, to determine the individual identity of the samples. We have also collected about 100 samples during the crop raiding season of October 2014 – Jan 2015 for which we are in the process of carrying out extractions and molecular sexing analyses. Since these samples were collected with extra precautions to ensure that we reached the crop raid sites within 2-3

days of the raid, we have been able to get a much better amplification success with these samples. We plan to continue our work in 2015 and into early 2016 with our continuation grant from IEF.

This analysis would enable us to identify the proportion of crop raiding individuals in the Kodagu elephant population. We would also identify individuals that appear at multiple crop raid sites, if any, and the number and proportion of such habitual raiders compared to opportunistic raiders. The results from our study would provide valuable information to devise effective measures specifically targeted at habitual or opportunistic raiders as a potential management intervention for reducing crop raids in the region. Landscape or region-specific efforts would be more effective in minimizing local conflict and would therefore ensure sustainable co-existence of Asian elephants with humans in the future. Lessons from this project could be applied to other regions both in India as well as other countries that harbor Asian or African elephants in the wild.

11. Organizations associated with this project and their roles in the project.

The Center for Ecological Sciences, Indian Institute of Science, Bangalore, India is the parent institution to which members of this project are affiliated. All the genetic analyses have been carried out in the molecular biology laboratory at the Center for Ecological Sciences (CES). Office workspace as well as existing infrastructure support for major laboratory equipment has also been provided by CES.

The Asian Nature Conservation Foundation (ANCF) has been actively involved in the conservation of the Asian Elephant and promotes field research projects and on the ground conservation initiatives pertaining to the Asian elephant and its habitat. The organization has provided provide logistic support for sample collection and fieldwork, and assistance in managing the project funds.

12. **Financial report**

Amount sanctioned in Jan 2014 : $6000

Budget item	**Amount utilized during Feb 2014 to Feb 2015**
Consumables for DNA extraction x 100 samples @ approx. $12/sample	$1,256
Consumables for molecular sexing x 100 samples @ approx. $7/sample	$722
Synthesis of fluorescent labeled primers for amplification of microsatellite loci	$880
Consumables for PCR amplification of microsatellite loci x 30 samples @ approx. $30/sample	$994
Genotyping services x 30 samples @ approx. $30/sample	$1,107
Miscellaneous laboratory consumables (agarose, buffers, reagents, and plasticware)	$798
Total	**$5,757**

Notes:

1. Expenses were incurred in INR, and converted to an approximate value in dollars at an exchange rate of 1 USD = 60 INR.
2. In the initial standardization process, the cost was higher than estimated for the extraction, molecular sexing, PCR amplification of microsatellite loci, and genotyping services because we did more repeats.
3. The expense on synthesis of fluorescent labeled primers for analysis of microsatellite loci is a one time cost.
4. The remaining balance of $243 will be used to continue the work into the coming year, along with the continuation grant received on 2015.

13. Details of images

1. The mosaic landscape of Kodagu with paddy fields, coffee plantations and forests in a hilly terrain. Photo credit: Ishani Sinha
2. A fruiting coffee tree damaged by elephant. Photo credit: Ishani Sinha
3. Dung sample collection from a roadside near paddy fields. Photo credit: Ishani Sinha
4. Elephant crossing a road while a bus passes by Photo credit: Ishani Sinha
5. Agarose gel picture of a typical molecular sexing analysis. Photo credit: Aditi Sridhar