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INFLUENCE OF ASIAN ELEPHANT DUNG DECAY ON DNA RECOVERY

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Abstract: Evaluation on Asian elephant dung decay rate affecting the yield of DNA was carried out in National Elephant Conservation Center (NECC) Kuala Gandah, Pahang, Peninsular Malaysia. The attempt is intended to optimise the current field DNA sampling method. Two distinguished environmental sites of dung piles were sampled; direct sunlight exposures and under shades of voluminous tree canopies. Dung piles of Sanum, a cow; and Rajah**,** a bull were being tested. Results showed that the environmental factors of sample sources have not affected the purity of DNA. Nonetheless, the DNA quantity varies between both study sites samples. The average of DNA concentration amongst the shady area samples was 7.6 ng/μL, whereby in sunny area the concentration was lower at 6.0 ng/μL. It is showed that better quantity of DNA are related to dung decay rates where faecal samples with direct sunlight will degrade faster compared to shady areas covered by canopies. This outcome contributes a supporting data for non-invasive field DNA sampling on Asian elephants.

Keywords: Asian elephant, Sanum, Rajah, dung piles, non-invasive, National Elephant Conservation Center Kuala Gandah.

Introduction

Conducting a research on wildlife species in our tropical rainforests had always been a great challenge especially on investigations involving molecular examinations. Such an exercise requires good preserved sample collections. In conservation genetic studies, non-invasive sampling method is the best choice to collect precious wildlife samples without handling or observing animals (Aifat *et al*., 2016; Abdul-Latiff *et al*., 2017). Previous researches on Malaysian wildlife genetics had succeed in the use of faecal samples in their systematic, population genetics, phylogeography and molecular ecology studies (Hedges, 2012; Abdul-Latiff *et al*., 2014a; Abdul-Latiff *et al*., 2014b; Rosli *et al*., 2014; Md-Zain *et al*., 2018; Abdul-Latiff *et al*., 2019). Similarly, dung piles of wild Asian elephants have strong potential to reveal their genetic information (Vidya *et al*., 2005; Sripiboon, 2013), where fresh dung would provide good DNA quality (Hedges, 2012). The outermost layer of a feces contains the epithelial cells being the last contact with the intestinal mucosa (Perry *et al*., 2010) and it is also the first layer to dry after defecation. Therefore, samples collected from this layer result in the highest probability of obtaining DNA for isolation (Fernando *et al*., 2003; Okello *et al*., 2008).

Environmental factors influenced the excreta decay rate and thus affecting the freshness of faecal. The elephant dung decay rates is a non-linear, it is affected by various environmental parameters such as rainfall, exposure to sunlight, temperature; and biological factors such as the elephant diet and the action of decomposers (Vanleeuwe & Probert, 2014). According to Dawson (1992), dung decay rate is

in contribution of the different climate, different forest condition such as a closed or open canopy and the composition of the particular excreta (Raymond *et al*., 2010).

It is important to evaluate faecal samples from different decay categories in seek of optimising the best high DNA quantity samples for molecular related studies. Therefore, this paper tested different Asian elephant dung decay rate which correlated with the degradation of DNA. These examinations add supporting database and guide during field sampling to the literatures.

Materials and Methods

The study site was the National Elephant Conservation Centre (NECC) in Kuala Gandah (N 3° 35'20.684, E 102° 8'45.394), in the state of Pahang. Two captive elephants, Rajah, a sub-adult bull and Sanum, an adult cow were being selected for this experiment (Figure 1).

The elephants exercise yard was chosen as the study site, where it settles a huge field area with trees and open grassy areas (Figure 2). The area with many tree canopies has cool and moist environment, while the grassy land area was exposed to the direct sunlight with hot and dry environment. These areas were tagged as a) Shady and b) Sunny, being two different environments as variables in this experiment. The sampling was done during the elephant's daily routine of 2 hours morning exercise in the yard. Dung bolus of Rajah and Sanum in both environmental sites were being marked. The outer layer of their excretion piles was swabbed or scabbed, and were preserved into absolute ethanol containers. Asian elephant dung piles were then classified further into five categories based on the 'S system' as described by Hedges and Lawson (2006) (Table 1). Continuous collections of samples from the outer layers were made for five (5) days or until class S3 revealed (Figure 3). Faecal samplings were discontinued at class S3 as DNA sampling only can be performed on intact boli (Gray *et al*., 2014). Throughout this study, no quantifiable environmental parameters like temperature or total rainfall were taken into account. However, general observations on daily weather were recorded as shown in Table 2.

Figure 1: Two captive elephants as studied subjects: Rajah (A) and Sanum (B)

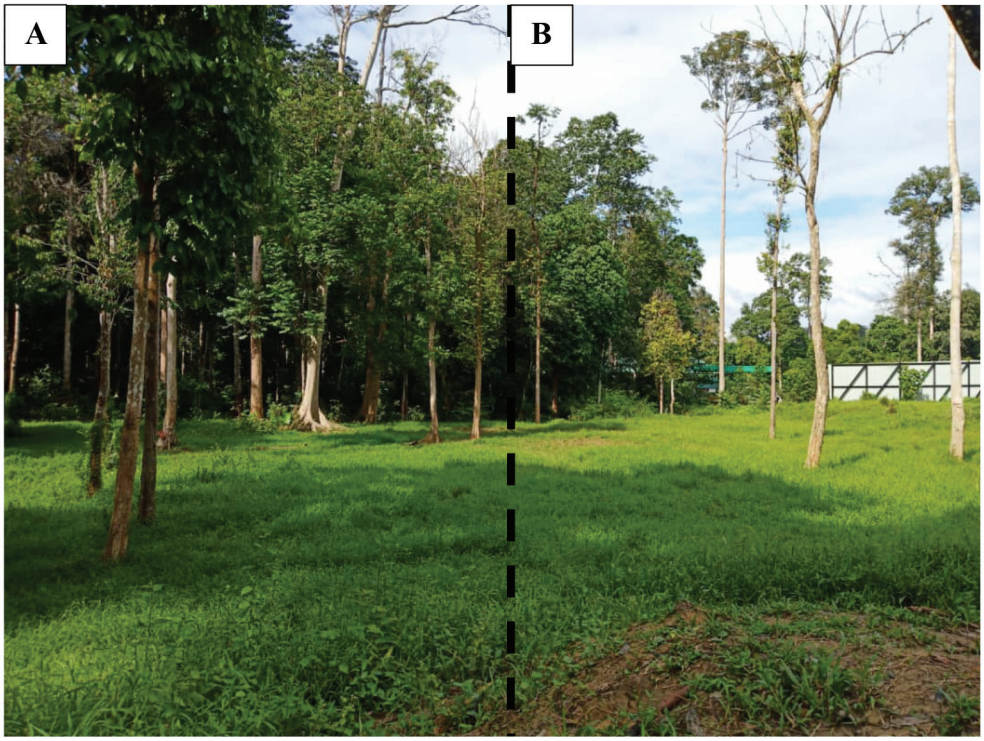

Figure 2: Elephant exercise yard with shady (A) and sunny (B) environment

Table 1: Asian elephant dung pile categories

Categories	Description
S1	Very fresh and intact bolus. Looks moist with the odour
S2	Fresh and intact bolus. Looks dry with no odour
S3	Some disintegrated bolus with some recognisable boli
S4	Completely disintegrated bolus
S5	Completely decayed

Figure 3: Dung piles from class S1 (A), S2 (B) and S3 (C)

Table 2: General observation on daily weather conditions

Weather conditions/ Day	1	2	3	4	5
Morning (7 am to 12 noon)	Sunny	Sunny	Sunny	Cloudy	Sunny
Afternoon (12 noon to 3 pm)	Sunny	Cloudy	Cloudy & windy	Partly cloudy	Sunny
Evening (3 pm to 7 pm)	Sunny	Cloudy	Raining	Partly cloudy	Cloudy
Night (7pm onwards)	No rain	Cloudy	Raining	No rain	No rain

A complete laboratory analyses were carried out in the National Wildlife Forensics Laboratory, Ex-situ Conservation Division DWNP. DNA extractions from the collected samples were carried out by using Qiagen's QIAamp DNA Stool Mini Kit. Quantification and purity of nucleic acids were conducted using NanoDrop 2000 Spectrophotometer. Measurements were taken out by adding 1 µL of sample onto the pedestal. Gel electrophoresis by using 1 kb ladder (Promega) was being run on all samples collected from Rajah (Figure 4).

Results and Discussions

The DNA purity was based on 260/280 nm ratio with accepted range of 1.8 to 2.0. Samples with 260/280 ratio with more than 2.0 indicates RNA contamination (William *et al*., 2012). All 20 samples from Rajah and Sanum had an average of 1.89 of 260/280 ratio which proved that pure nucleic acids from all the collected faecal samples had been successfully retrieved. Samples collected from both study sites had similar DNA quality, wherein the average ratio reading for all the samples were 1.9 and hence it showed that the dung decay rate does not affect the purity. However, this similarity pattern did not occurred in the DNA quantification analysis.

The values of DNA concentration of all the 20 samples were summarized in Table 3, while figures 5 and 6 displayed the comparisons pattern of DNA yield from both environments sites with the different days from the two tested elephants. The samples collected from the shady area showed thick DNA bands compared to samples taken from the sunny area. In overall, the DNA concentrations on both sites were slightly different. The average of DNA quantification among samples collected in shady area was at 7.6 ng/µL, while in the sunny area the concentration was lower at 6.0 ng/µL. Samples collected from the cool and moist environment recorded a higher concentration of nucleic acid compared to the samples taken from the dry and hot environment. It shows that these two natural environments gave different effect on the DNA degradation readings. The results also showed that the DNA samples from 1st and 2nd day have a good yield compared to the other continuing days, which indicates that dung decay rate had also played an important parameter in non-invasive sampling such as the faecal.

The examinations had obtained a technically supporting data on dung decay rate which produced an effect to the DNA pattern yield. The fresh samples (1st day) or reasonably fresh (2nd day) samples were the best faecal test-samples for molecular related works compared to the later than 2 days old samples. On the third day and onwards, the boli will start to disintegrate these were the points to focus on during such field sampling. These outcomes are in support to the findings of Perry *et al*., (2010) where DNA starts to degrade in older dung piles. The overall outcome from this studies showed that the quality of DNA from the fresh elephant faecal samples are equally as good as the elephant blood samples (Elliza *et al*., 2015).

Conclusions

The outcome of this study had set an aid on field work planning. By focusing on day 1 or 2 dung piles samples will minimise the possibility of collecting samples with low DNA, thus less wastage of reagents and other consumables. The success of these validations had also proven

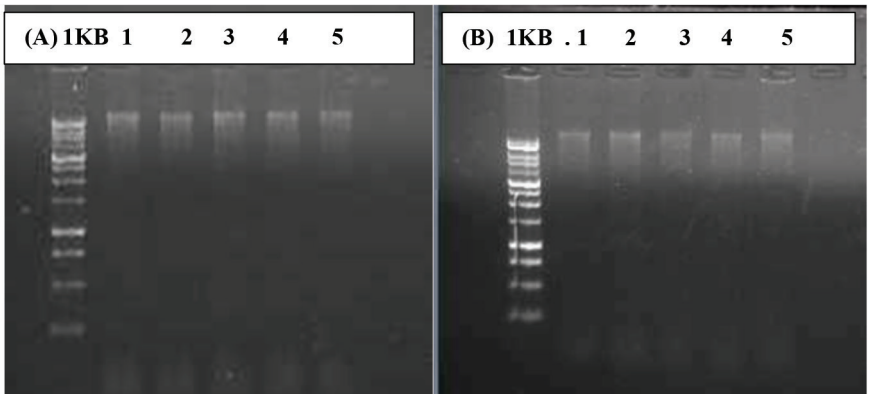

Figure 4: DNA extraction from Shady Rajah (A) and Sunny Rajah (B)

Table 3: The summary of DNA Concentration of faecal samples collected from both sites

Sites	Elephants	N	Mean±SD	Min-Max
Sunny	Rajah	5	6.12 ± 7.90	0.31 -17.62
	Sanum	5	6.25 ± 7.81	0.73 - 19.10
Shady	Rajah	5	7.14 ±7.54	0.74 - 18.92
	Sanum	5	6.39 ± 6.67	1.00 - 16.24

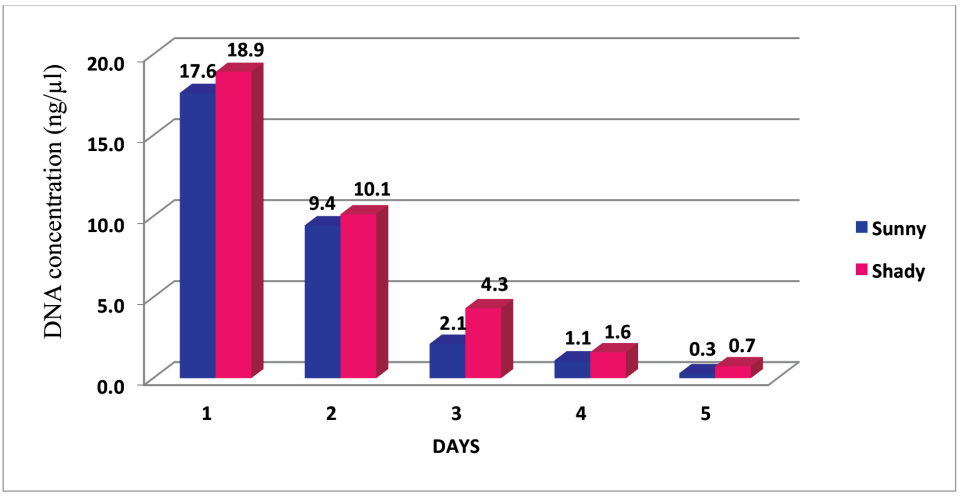

Figure 5: Comparisons on Rajah's DNA concentration based on sites and days

that good non-invasive samples will benefit all type molecular genetics-based researches. As a continuation, DWNP had proposed to carry out a major DNA sampling programme of Asian elephant in Taman Negara National Parks (TNNP), Peninsular Malaysia. The sampling programme design will incorporate investigations to understand the pattern of genotype, haplotype and sex distribution among the Asian elephant individuals in TNNP by using non-invasive method. The proposed study is in cognisant with the action plan efforts in NECAP. The anticipated outcome from this genetic assessment of Asian elephant in TNNP will

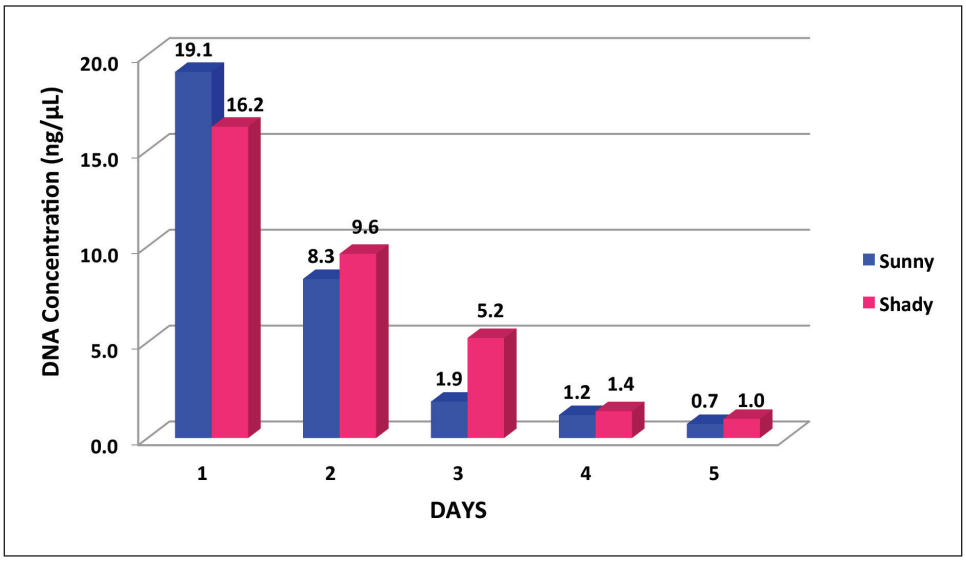

Figure 6: Comparisons on Sanum's DNA concentration based on sites and days

generate key data for evidence-based elephant conservation endeavours in the Peninsular Malaysia.

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