

Studies on male mating strategy, reproductive success, and copulation related behaviors of stump-tailed macaques in Khao Krapuk Khao Taomor Non-Hunting Area, Thailand

Aru Toyoda

Summary

Introduction

Macaca species have been categorized into four species groups based on morphological characteristics of the male reproductive organs. Since the stump-tailed macaque (*Macaca arctoides*) has a unique glans penis morphology, it has been classified as the sole representative of the *arctoides* group. Not only morphological characteristic but the sexual behaviors are also reported as unique traits. Although there have been some previous studies on sexual behavior in this species, the animals studied were captive-born or reared in laboratory conditions. Thus, 18 months of fieldwork on a population of wild stump-tailed macaques was conducted to elucidate why certain unique reproductive characteristics have evolved in this species.

Methods

Study site: The present study was conducted at the Khao Krapuk Khao Taomor Non-Hunting Area, Phetchaburi Province, Thailand, which harbors five free-ranging groups with in total approximately 391 individuals of stump-tailed macaques.

Development of non-invasive DNA collection method: A method using a rope swab to non-invasively collect buccal cells from the macaques was designed for this study. The amount of host DNA collected from both buccal cells and intestinal slough cells was quantified by qRT-PCR and compared. Allelic dropout rates, false allele rates, and genotype failure rate were also compared respectively.

Behavioral observation: Behavioral observation was carried out between September 25th, 2015 to June 15th, 2017. In total, 289 days of observation were conducted. All copulations observed during the observation period were recorded.

Paternity determination: Ten polymorphic loci which exhibited sufficient variation and successful amplification, were selected for paternity determination. In total, 248 individual's samples collected using rope swab method were genotyped and used for analysis.

Facial expression: The facial expressions of stump-tailed macaques that occur in the context of copulation were also analyzed in comparison with the facial expressions of other macaques. Teeth chattering, a unique facial expression of this species which occurred in the context of copulation was analyzed from video clips by using the ELAN software program.

Results

Availability of rope swab method: Analysis of the 82 DNA samples (41 buccal and 41 intestinal DNA samples) revealed that the buccal samples yielded significantly more DNA than did the fecal samples. the allelic dropout rate of the 10 microsatellite loci was significantly lower for the buccal than for the fecal DNA samples. Similarly, the genotyping failure rate was significantly lower for buccal DNA

samples than for fecal DNA samples, although the rate was variable among the loci examined.

Male mating strategy and reproductive success: Results showed two different male mating strategies: 1) copulations were monopolized solely by the alpha male; or 2) copulations were shared by the alpha male and his coalition partner(s). In both strategies, the alpha male or the alpha male plus his coalition males occupied more than 80% of the observed ejaculatory copulations. However, paternity determination by microsatellite analysis showed that copulatory success did not directly reflect reproductive success.

Dizygotic twin: Paternity testing confirmed that these dizygotic twins were produced by a single female and sired by different males.

Facial expression analysis: In total, 46 video recordings of TCs from 23 subjects (1–7 video recordings per subject) were obtained. When measuring the open-to-open mouth duration of vertical mouth actions, the mean and standard deviation of open-to-open time was 181.04635.4 ms in TCs (i.e., the oscillation rate for TCs was 5.7461.19 Hz).

Discussion

The rope swab method was advantageous in: 1) obtaining high-quantity and in particular higher quality DNA than that contained in fecal samples; 2) obtaining good DNA samples consistently regardless of the collector's level of experience; and, 3) obtaining accurate genotyping results with the minimum costs in time and expense.

This study revealed that there were two mating strategies for the males to monopolize copulations. These strategies played an important role mainly for alpha male, and which strategy the alpha male chose depended on the group. The group size, number of males, or kinship structure among males in group were considered to be factors affecting the choice of strategy.

The DNA analysis on a case of twins indicated that it is highly possible that the twins had multiple paternity. In other words, the female copulated with multiple males during the fertile period, but these two males could successfully produce infant due to sperm competition, although it is very unusual that two ova are ovulated simultaneously.

The analysis of facial expression showed that teeth chattering of stump-tailed macaques shares the action rhythms with lip-smacking of long-tailed macaques, but not for contexts. Of particular importance is the oscillatory rhythm consistency despite differences in species, action forms, and social contexts. Particularly, such as stamp-tailed macaques which have diverse sexual interactions might use such rhythmic facial signals in copulations with the different ways of the other macaques.

Conclusion

The four studies summarized in this thesis clarified aspects of the reproductive strategies of stump-tailed macaques which previously were not elucidated. Although this research remains at a basic stage, and the full scope of reproductive strategies of this species is not yet understood due to deficiencies in data, long-term continuous observation at this study site and other sites in the future will elucidate

species-specific characteristics of this species as well as our knowledge of the evolution of the genus *Macaca*.