

SEXUAL BEHAVIORS OF INDOCHINESE LONG-TAILED MACAQUE *Macaca fascicularis* IN COMPARISON WITH SUNDAIC LONG-TAILED MACAQUE



A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Zoology

Department of Biology

FACULTY OF SCIENCE

Chulalongkorn University

Academic Year 2020

Copyright of Chulalongkorn University

พฤติกรรมทางเพศของลิงหางยาว *Macaca fascicularis* อินโดจีน เปรียบเทียบกับลิงหางยาวซุน

ดา



วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชาสัตววิทยา ภาควิชาชีววิทยา

คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2563

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย



ปรมาศ คุ่มภัย : พฤติกรรมทางเพศของลิงหางยาว *Macaca fascicularis* อินโดจีน เปรียบเทียบกับลิงหางยาวซุนดา . ( SEXUAL BEHAVIORS OF INDOCHINESE LONG-TAILED MACAQUE *Macaca fascicularis* IN COMPARISON WITH SUNDAIC LONG-TAILED MACAQUE) อ.ที่ปรึกษาหลัก : ศ. ดร.สุจินดา มาลัยวิจิตรนนท์

จากที่มีรายงานเกี่ยวกับการผสมข้ามสายพันธุ์ในอดีตระหว่างลิงวอก (*Macaca mulatta*; *Mm*) และลิงหางยาว (*M. fascicularis*; *Mf*) ที่เกิดจาก *Mm* เพศผู้รูกำลังไปทางใต้และผสมกับ *Mf* จึงทำให้ *Mf* ที่อาศัยอยู่ในเขตภูมิศาสตร์อินโดจีนมีพันธุกรรมของออดีโคมของ *Mm* มาปะปนสูงกว่า *Mf* ที่อาศัยอยู่ในเขตภูมิศาสตร์ซุนดา และจากที่มีรายงานว่า *Mf* สามารถสืบพันธุ์ได้ทั้งปี ในขณะที่ *Mm* มีฤดูกาลในการสืบพันธุ์ จึงเป็นที่น่าสนใจว่าพฤติกรรมทางเพศระหว่าง *Mf* อินโดจีนและ *Mf* ซุนดามีความแตกต่างกันหรือไม่ จึงได้คัดเลือกประชากร *Mf* จากวัดหาดมูลกระบือ อ. พิจิตร (WHM; 16° 51'N) และจากเขาน้อยเขาตั้งกวน จ.สงขลา (KN/KTK; 7° 12'N) ที่มีระดับพันธุกรรมของ *Mm* มาปะปนร้อยละ 50 และร้อยละ 15 ตามลำดับ มาเป็นตัวแทน *Mf* อินโดจีน และ *Mf* ซุนดา ในการศึกษาค้นคว้า การคัดเลือก *Mf* เพศเมีย จำนวน 10 ตัว จากแต่ละประชากร ติดตาม สังเกต และเก็บข้อมูลความถี่ของพฤติกรรมทางเพศ 3 แบบ (proceptivity, attractivity และ receptivity) จำนวนลูกที่เกิดใหม่ การเปลี่ยนแปลงของผิวหนังรอบอวัยวะเพศ (มีค่าตั้งแต่ 0-4) และวัดระดับฮอร์โมนในน้ำลายที่เก็บด้วยวิธีเยื่อเชือก เป็นเวลานาน 12 เดือน (7วัน/เดือน/ประชากร) ตั้งแต่เดือนมกราคม – ธันวาคม 2561 พบว่าลิงทั้งสองประชากรแสดงพฤติกรรมทางเพศทั้ง 3 แบบ ตลอดทั้งปี แต่ WHM มีช่วงการแสดงออกสูงสุด (พฤศจิกายน – มีนาคม) เร็วกว่าของ KN/KTK (ธันวาคม – เมษายน) 1 เดือน ในการให้กำเนิดลูก ถึงแม้ว่ามีลูกเกิดใหม่ตลอดทั้งปี แต่ก็มีช่วงที่มีการให้กำเนิดลูกสูงสุดอย่างสอดคล้องกับระยะเวลาตั้งท้องนาน 24 สัปดาห์ (หลังจากการผสมพันธุ์) โดย WHM มีช่วงการเกิดสูงสุดในเดือนเมษายน-กรกฎาคม และ KN/KTK สูงสุดในช่วงมิถุนายน-กันยายน จากระบบการแยกการมีฤดูกาลในการสืบพันธุ์ในไพรเมทของ van Schaik และคณะ (1999) พบว่า *Mf* ทั้งสองประชากรมีการสืบพันธุ์แบบ moderately seasonal breeder โดยคำนวณจากการนับจำนวนลูกที่เกิดสูงสุดในรอบ 3 เดือนเมื่อเทียบกับจำนวนลูกที่ถึงเกิดทั้งปี ที่มีค่าอยู่ระหว่างร้อยละ 33-67 (WHM มีค่าเท่ากับร้อยละ 56 (จำนวนลูกถึง 5 ตัว จากลูกถึงทั้งหมด 9 ตัวในรอบปี) และ KN/KTK มีค่าเท่ากับร้อยละ 52 (จำนวนลูกถึง 15 ตัว จากลูกถึงทั้งหมด 29 ตัวในรอบปี)) การรวมและแดงของผิวหนังรอบอวัยวะเพศมีค่าคงที่ตลอดทั้งปีในทั้งสองประชากรจึงทำให้ไม่มีสหสัมพันธ์กับค่าพฤติกรรมทางเพศ แต่อย่างไรก็ตามพบว่าค่าเฉลี่ยของการแดงที่ผิวหนังรอบอวัยวะเพศ (ซึ่งเป็นลักษณะของ *Mm*) ใน WHM ( $2.70 \pm 0.82$ ) มีค่าสูงกว่าของ KN/KTK ( $2.00 \pm 0.0$ ) ในขณะที่ค่าเฉลี่ยการรวมที่โคนหาง (ซึ่งเป็นลักษณะของ *Mf*) ใน WHM ( $0.70 \pm 0.48$ ) มีค่าต่ำกว่าของ KN/KTK ( $1.50 \pm 0.85$ ) ด้วยการวัดระดับฮอร์โมนเอสตราไดโอดในน้ำลายไม่ประสบความสำเร็จ จึงมีการประเมินถึงปัจจัยอื่น ๆ ที่อาจส่งผลกระทบต่อ การตรวจวัด เช่น ระดับฮอร์โมนเอสตราไดโอดที่ต่ำเกินไปในน้ำลาย ผลการทดลองที่ได้ชี้ให้เห็นว่าการเข้ามาปะปนของพันธุกรรมของออดีโคมของ *Mm* ไม่มีผลต่อพฤติกรรมทางเพศของ *Mf* แต่มีผลต่อความสามารถในการให้ลูกและการเปลี่ยนแปลงของผิวหนังรอบอวัยวะเพศ ดังนั้นจึงทำให้ไม่มีความแตกต่างของฤดูกาลในการสืบพันธุ์ระหว่างประชากรลิงหางยาวอินโดจีน WHM และลิงหางยาวซุนดา KN/KTK ในขณะที่ความสามารถในการให้ลูกและการเปลี่ยนแปลงของผิวหนังรอบอวัยวะเพศของลิงหางยาวอินโดจีน WHM มีแนวโน้มไปในทิศทางของ *Mm* มากกว่าลิงหางยาวซุนดา KN/KTK

สาขาวิชา                   สัตววิทยา  
ปีการศึกษา               2563

ลายมือชื่อ นิสิต .....  
ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

## 5972004623 : MAJOR ZOOLOGY

KEYWORD: reproductive patterns, seasonal breeder, sexual behaviors, sex skin, fecundity

Prangmas Kumpai : SEXUAL BEHAVIORS OF INDOCHINESE LONG-TAILED MACAQUE *Macaca fascicularis* IN COMPARISON WITH SUNDAIC LONG-TAILED MACAQUE. Advisor: Prof. SUCHINDA MALAIVIJITNOND, Ph.D.

Regarding to the previous studies reported that the past hybridization event between rhesus (*Macaca mulatta*; *Mm*) and long-tailed (*M. fascicularis*; *Mf*) macaques was occurred by the southward introgression of male *Mm* into *Mf* population, thus the *Mf* originating from Indochinese region carried higher level of genetic admixture of *Mm* ancestry than the *Mf* living in Sundaic region. Since it was reported that *Mf* are non-seasonal breeder while *Mm* are seasonal breeder, thus, it is interesting to compare the sexual behaviors between Indochinese and Sundaic *Mf. Mf* populations at Wat Haad Moon Kra Beau, Pichit province (WHM; 16° 51'N) and Khao Noi/Khao Tangkuan, Songkhla province (KN/KTK; 7° 12'N) who carried 50% and 15% of *Mm* genetics were selected as representatives of Indochinese and Sundaic *Mf. Mf*. Ten adult females from each population were selected as focal animals and followed for 12 months (7 days/month/population) from January-December, 2018. Frequency of three sexual behaviors (proceptivity, attractivity and receptivity), number of newborn, changes of sex skin (scoring from 0 – 4) and salivary hormonal analysis collected by rope bait method were determined and recorded. Both populations showed three sexual behaviors throughout the year, but the peak period of the WHM (November-March) was one-month earlier than the KN/KTK (December-April). In association with a 24-week gestation period of *Mf*, though births were observed all year round, the birth peaks occurred in April-July for WHM and June-September for KN/KTK. Following the reproductive seasonality classification system of van Schaik and colleagues (1999), both populations were classified as moderately seasonal breeder that the birth count with a three-month period was 33-67% (56%; 5 out of 9 birth count for WHM, and 52%; 15 out of 29 birth count for KN/KTK). Sex skin swelling and reddening were consistent in both populations and thus no correlation with sexual behaviors. However, average score of sex skin reddening (*Mm* characters) in WHM (2.70±0.82) was higher than the KN/KTK (2.00±0.0), while the average score of swelling at the base of the tail (*Mf* characters) in WHM (0.70±0.48) was lower than the KN/KTK (1.50±0.85). Since the measurement of salivary estradiol levels was not succeeded, several interfering factors were predicted such as low estradiol levels in saliva. From these results, it indicates that autosomal genetic admixture of *Mm* has no effect on sexual behaviors of *Mf*, but it can intervene the fecundity and changes of sex skin. Thus, the reproductive seasonality was no differences between WHM Indochinese and KN/KTK Sundaic *Mf*, while the fecundity and sex skin reddening of WHM females were more prone to *Mm*'s pattern than the KN/KTK females.

Field of Study: Zoology

Student's Signature .....

Academic Year: 2020

Advisor's Signature .....

## ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to my thesis advisor, Professor Dr. Suchinda Malaivijitnond, for her invaluable advice, support, and encouragement throughout my master journey. She is not only an advisor in the study or the research, but she is also an advisor in the living life. Without her, I would have never been able to come this far. She is one of the most important persons in my life.

I would also extend my deepest gratitude to Professor Dr. Yuzuru Hamada for his advice about sex skin changes in monkeys, Dr. Florian Trebouet for his guidance on behavioral data collection and interpretation, Dr. Kazunari Matsudaira and Dr. Aru Toyoda for saliva collection's guidance, Assistant Professor Dr. Sukanya Jaroenporn for her teaching and guidance on hormonal analysis and kind supports. I also thank all staff at the National Primate Research Center of Thailand-Chulalongkorn University for their support and help during working there. I am very overwhelmed to monks and people around Wat Haad Moon Kra Beau, Pichit, and also Ms. Kwan, Mr. Watcharin, Ms. Jai, and uncle Sayun for their love and care while I was doing my fieldwork at Khao Noi/Khao Tangkuan, Songkhla.

I gratefully acknowledge the funding of the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund), for financial supports which allowed me to undertake this research, and Thailand Research Fund (grant number DBG6080008). Finally, I am very thankful to my family for all of their love and understanding, to all members of the Primate Research Unit, Department of Biology, Faculty of Science, Chulalongkorn University, for their help, friendship, and encouragement. My special thank also goes to my boyfriend for being my rainbow after the storm.

Prangmas Kumpai

## TABLE OF CONTENTS

	Page
ABSTRACT (THAI).....	iii
ABSTRACT (ENGLISH) .....	iv
ACKNOWLEDGEMENTS.....	v
TABLE OF CONTENTS.....	vi
LIST OF TABLES .....	ix
LIST OF FIGURES .....	x
LIST OF ABBREVIATIONS.....	xiv
CHAPTER I GENERAL INTRODUCTION .....	1
CHAPTER II LITERATURE REVIEW .....	5
1. Distributions of rhesus and long-tailed macaques .....	6
2. Hybridization between rhesus and long-tailed macaques .....	8
3. Morphological characters .....	10
4. Demography and reproduction .....	11
4.1 Demography and mating system .....	11
4.2 Reproductive seasonality.....	13
4.3 Sexual behaviors .....	14
4.4. Sex skin: a visual cue of peri-ovulatory period for males .....	16
5. Environment factors and reproduction .....	18
CHAPTER III MATERIALS AND METHODS.....	20
1. Animals and study sites .....	20
2. Population demography and selected subjects .....	22

3. Behavioral observation and data collection .....	26
3.1 Behavioral observation .....	26
3.2 Behavioral data collection .....	27
4. Birth observation .....	31
5. Assessment of sex skin swelling and reddening and data collection .....	32
6. Saliva collection and salivary hormonal analysis .....	34
6.1 Rope bait method for saliva collection .....	34
6.2 Saliva collection .....	34
6.3 Hormone assays .....	35
7. Physical data collection .....	37
8. Data presentation and statistical analyses .....	37
CHAPTER IV RESULTS .....	38
1. Sexual behaviors .....	38
2. Birth observation .....	44
3. Sex skin swelling and reddening .....	45
4. Saliva collection and salivary estradiol analysis .....	49
5. Physical data .....	52
CHAPTER V GENERAL DISCUSSION AND CONCLUSION .....	56
1. Sexual behaviors and birth .....	57
2. Sex skin swelling and reddening .....	60
3. Saliva collection and salivary estradiol assay .....	62
4. Hybridization between rhesus and long-tailed macaques and genetic analyses ...	63
5. Conclusion .....	64



REFERENCES .....	70
APPENDICES.....	79
VITA.....	90



จุฬาลงกรณ์มหาวิทยาลัย  
**CHULALONGKORN UNIVERSITY**

## LIST OF TABLES

	Page
Table 3.1 Ten selected female long-tailed macaques from WHM and KN/KTK population for the study. The names are listed in alphabetical order and the rank is given in the parenthesis. ....	23
Table 3.2 Ethogram of three sexual behaviors (proceptivity, attractivity and receptivity) .....	28
Table 4.3 Ranged (mean $\pm$ SE) monthly temperature ( $^{\circ}$ C), rainfall (mm), relative humidity (%) and photoperiod (hour) at WHM and KN/KTK from January to December 2018.....	53



## LIST OF FIGURES

	Page
Figure 2.1 A distribution map of rhesus macaques ( <i>Macaca mulatta</i> ) shaded in green color, long-tailed macaques ( <i>M. fascicularis</i> ) shaded in purple color, and the hybrids between these two species shaded in blue color .....	8
Figure 2.2 Genetic admixture panel of the rhesus ancestry (red color) in long-tailed macaques (green color) determined by autosomal SNPs markers and structure analysis. Nos. 8 to 11 on the upper panel indicate long-tailed populations from the north to the south of Thailand. ....	10
Figure 2.3 Newborn and their long-tailed macaque mothers. Black pelage color with streak at the crest and pink skin are identifiable morphological characters of the newborn. ....	12
Figure 2.4 Attractivity behaviour displaying by long-tailed macaque male, mounting behavior. This is a complete mounting because male uses double feet to clasp the hind limbs and uses his hands to grasp the female's hips. ....	15
Figure 2.5 Receptivity behavior indicating by a copulatory sperm plug at the vagina of the female. ....	16
Figure 2.6 Representative photos of patterns of sex skin reddening of rhesus macaques (left) and sex skin reddening and swelling at the base of the tail of long-tailed macaques (right).....	17
Figure 3.1 Home range of the Wat Haad Moon Kra Beau (WHM) population at Pichit province, Thailand (framed by red line). It covers with fragmented forest and temple ground.....	21
Figure 3.2 Home range of the Khao Noi (right hill) and Khao Tangkuan (left hill) population at Songkhla province, Thailand (framed by red line). It covers with a forestry recreational park.....	21

Figure 3.3 Ten selected female long-tailed macaques from WHM population. ....	24
Figure 3.4 Ten selected female long-tailed macaques from KN/KTK population. ....	25
Figure 3.5 Proceptivity behaviors recorded in this study; (A) female solicitation, (B) affiliative behavior, (C) contact behavior, and (D) female following adult male. .	29
Figure 3.6 Attractivity behaviors recorded in this study; (A) male solicitation, (B) male genital inspection, (C) male grooming, (D) masturbation, (E) male following adult female, and (F) mating. ....	30
Figure 3.7 Receptivity or copulation behavior. Male mounts the female with intromission and pelvic thrust resulting in ejaculation. Sperm plug can usually be seen at the vagina of the female. ....	31
Figure 3.8 Sex skin swelling at the base of the tail, from Level 1 to 4. ....	33
Figure 3.9 Sex skin reddening from Level 1 to 4. ....	33
Figure 3.10 Rope bait wrapped with corn seed (A). Focal monkey picked up and chewed the rope bait (B). ....	36
Figure 3.11 The procedure of validation of salivary estradiol assay in comparison with that of plasma sample. ....	36
Figure 4.1 Proceptivity, attractivity, and receptivity behaviors of 10 female long-tailed macaques in WHM population. Nos 1, 6, 8 and 10 were high ranking females..	40
Figure 4.2 Proceptivity, attractivity, and receptivity behaviors of 10 female long-tailed macaques in KN/KTK population. Nos 3, 4, 9 and 10 were high ranking females. ....	41
Figure 4.3 Average frequency of sexual behaviors (proceptivity, attractivity, and receptivity) of female monkeys in WHM population .....	42
Figure 4.4 Average frequency of sexual behaviors (proceptivity, attractivity, and receptivity) of female monkeys in KN/KTK population .....	42

Figure 4.5 Average frequency of sexual behaviors (proceptivity, attractivity, and receptivity) between surge period (November-March) and non-surge period (April-October) of WHM population. * and ** represents $p < 0.05$ and $p < 0.01$ , respectively.....	43
Figure 4.6 Average frequency of sexual behaviors (proceptivity, attractivity, and receptivity) between surge period (December-April) and non-surge period (May-November) of KN/KTK population. * and ** represents $p < 0.05$ and $p < 0.01$ , respectively.....	43
Figure 4.7 Percentage of births of the WHM and KN/KTK population .....	44
Figure 4.8 Averaged score of sex skin swelling of all females in the WHM and KN/KTK population. ....	47
Figure 4.9 Averaged score of sex skin reddening of all females in the WHM and KN/KTK population. ....	47
Figure 4.10 Representative photos of patterns of sex skin reddening (score 3) of WHM female (A), and sex skin reddening (score 1) and swelling at the base of the tail (score 3) of KN/KTK female (B). Arrow indicates sperm plug. ....	48
Figure 4.11 Average score of sex skin swelling and reddening of the WHM and KN/KTK population. * and ** represents $p < 0.05$ and $0.01$ , comparing between two populations. ....	48
Figure 4.12 Average monthly temperature ( $^{\circ}\text{C}$ ), rainfall (mm), relative humidity (%) and photoperiod (hour) at WHM and KN/KTK from January to December 2018. Gray box indicates the period of the higher rainfall in the KN/KTK than the WHM population.....	54
Figure 4.13 Monthly rainfall (mm) and number of newborns from January to December 2018 of the WHM and KN/KTK population.....	55
Figure 5.1 Scheme of gene flow and genetic admixture between rhesus and long-tailed macaques. ....	67

Figure 5.2 In association with the autosomal genetic admixture event between rhesus and long-tailed macaques and the results of that there were no differences in reproductive seasonality between WHM and KN/KTK macaques, while the fecundity and sex skin reddening of WHM females were more prone to rhesus macaques' pattern than the KN/KTK females, it can propose that the reproductive seasonality is controlled by the X-linked gene and the latter two parameters are controlled by the autosomal chromosomes. .... 68



## LIST OF ABBREVIATIONS

A	Attractivity
A <sup>ln</sup>	Autosome of long-tailed macaque
A <sup>rh</sup>	Autosome of rhesus macaque
CV	Coefficient of variation
ddRAD	Double-digest restriction site-associated DNA sequencing
DNA	Deoxyribonucleic acid
ha	Hectare
KKZ	Khao Khieow Open Zoo
KN/KTK	Khao Noi/Khao Tangkuan
mtDNA	Mitochondrial deoxyribonucleic acid
P	Proceptivity
PBS	Phosphate buffer saline
R	Receptivity
RTL	Relative tail length
SNP	Single nucleotide polymorphism
WHM	Wat Haad Moon Kra Beau
X <sup>ln</sup>	X chromosome of long-tailed macaque
X <sup>rh</sup>	X chromosome of rhesus macaque
Y <sup>ln</sup>	Y chromosome of long-tailed macaque
Y <sup>rh</sup>	Y chromosome of rhesus macaque

## CHAPTER I

### GENERAL INTRODUCTION

Rhesus (*Macaca mulatta*) and long-tailed (*M. fascicularis*) macaques are the two most species commonly used for biomedical research (Bonhomme et al., 2009; Osuna et al., 2017) and have the widest distribution range among non-human primates. The distribution of rhesus macaques is at 15 – 36°N including Afghanistan, China, Myanmar, Thailand, Laos and Vietnam (Fooden, 2000, 2006; Malaivijitnond and Varavudhi, 2002), while long-tailed macaques live at 20°N – 10°S including Bangladesh, Myanmar, Laos, Cambodia, Vietnam, Thailand, Malaysia, Singapore, Indonesia, the Philippines and Timor (Fooden, 1995, 2006; Malaivijitnond and Hamada, 2008). In Thailand, long-tailed macaques are the most encountered species among 17 existing species of non-human primates (Grove, 2001; Malaivijitnond and Hamada, 2008). They distribute from the lower northern (Pichit and Mukdahan province) to the southern part (Yala and Satun province) of Thailand, at approximately 16°30' - 6°30'N. Isthmus of Kra, Ranong province (ca. 10°N) is a proposed biogeographical barrier between two forms of long-tailed macaques; Indochinese and Sundaic forms.

Based on their distribution range, long-tailed and rhesus macaques lived overlapping at 15 - 21°N in Southeast Asian region and it was proposed as a hybrid zone between the two species revealed by morphological characteristics (Hamada et al., 2016), ABO blood group (Kanthaswamy et al., 2017; Malaivijitnond et al., 2008) and various genetic markers including mitochondrial (mt) DNA, Y-chromosome, nuclear DNA, and microsatellite DNA (Tosi et al., 2002; Street et al., 2007; Kanthaswamy et al., 2008; Bonhomme et al., 2009; Stevison and Kohn, 2009; Osada et al., 2010; Bunlungsup



et al., 2017a). Previously, the hybridization between the two species was proposed as a unidirectional introgression of male rhesus macaques into long-tailed macaque populations (Tosi et al., 2002; Street et al., 2007), but recently it was confirmed that the hybridization occurred two directions (Kanthaswamy et al., 2008; Bonhomme et al., 2009; Stevison and Kohn, 2009; Osada et al., 2010; Bunlungsup et al., 2017a). Using autosomal SNP markers, Bunlungsup et al. (2017a) reported that the introgression of male rhesus macaques into long-tailed macaque populations was beyond the Isthmus of Kra and the level of genetics admixture of rhesus ancestry declined gradually from northern to the southern part of Thailand. For example, Indochinese long-tailed macaques at Wat Haad Moon Kra Beau (WHM), Pichit province (16°30'N) carried 50% of rhesus ancestry and Sundaic long-tailed macaques at Khao Noi Khao Tangkuan (KN/KTK), Songkhla province (7°12'N) carried 15% of rhesus ancestry (Bunlungsup et al., 2017b).

The species specific morphological characteristics that have been used to identify rhesus and long-tailed macaques are the pelage color and the relative tail length (RTL; a proportion between tail length and crown rump length). Rhesus macaques have shorter RTL (<70%) whereas long-tailed macaques have longer RTL (>90%) (Fooden, 1995; Hamada et al., 2006). Rhesus macaques have a bipartite pattern of the pelage color (body contrast between back and thigh) that the lower part of their body is more reddish-yellowish than the upper part, while it is absent in long-tailed macaques. Thus, the hybrid individuals have the intermediate values between the two species; for example, 69.6-95.6 %RTL and contrast of pelage color ( $b^*$  values) as 1.33-2.01 (Jadejaroen et al., 2015). Regarding the higher level of genetic admixture of rhesus ancestry, the morphological characteristics of Indochinese long-tailed macaques were similar to those of rhesus macaques, such as shorter RTL and higher body contrast,

than the Sundaic long-tailed macaques (Hamada et al., 2008).

Rhesus and long-tailed macaques live in multi-male multi-female group in which adult males mate with several females and *vice versa*. The males migrate out of the group when they reach maturity age (or male dispersal) and females stay permanently in the group (or female philopatry). Thus, changes of sex skin which indicate the fertile phase in females and increase attractiveness to male conspecifics are very important (Nunn, 1999). Rhesus macaques had large areas of sex skin reddening around hindquarter including anogenital area, legs and thighs without swelling (Dixon, 1998; Dubuc et al., 2009), while long-tailed macaques show narrow area of sex skin reddening from subcaudal to inguinal regions, but a sex skin swelling at the base of tail is conspicuous (Engelhardt et al., 2005).

In view of the reproductive pattern, rhesus macaques showed strong reproductive and birth seasonality (or strictly seasonal breeder) (Vandenbergh and Vessey, 1968). They showed a mating peak mostly during the fall and early winter, for instance, in November – April for Indochinese rhesus macaques (Gordon, 1981), October – February for Chinese rhesus macaques (Du et al., 2010) and October – December for Indian rhesus macaques (Lindburg, 1971), and a birth peak in May - June (Fooden, 1995). For long-tailed macaques, they are non-seasonal breeder (Hobbs et al., 1987; Tardif et al., 2012) that can mate throughout the year, although the mating peak was detected in January – July for northern Sumatra long-tailed macaques (van Schaik and Noordwijk, 1985) and birth peak in July – September (Fooden, 1995). So far, only one study reported sexual behaviors of the hybrids between long-tailed and rhesus macaques in eastern Thailand, however, the hybridization was occurred by the human-made introduction of both sexes of few individuals of rhesus macaques into long-tailed populations (Jadejaroen, 2016). The breeding season in September – January was

observed in this population. Recently, Ito and his colleagues (2020) reported that the genes related to reproduction were selected during a natural hybridization between these two species.

Thus, the basic knowledge on reproductive patterns, fecundity and sex skin changes of the wild long-tailed macaques in Thailand that carried different levels of genetic admixture of rhesus ancestry are important in view of conservation and species identification as well as animal model for biomedical research. Thus, one population each of Indochinese (WHM population) and Sundaic (KN/KTK population) long-tailed macaques which carried 50% and 15% of rhesus ancestry was selected for this study.

### Objectives

1. To study sexual behaviors of Indochinese and Sundaic long-tailed macaques.
2. To compare sexual behaviors between Indochinese and Sundaic long-tailed macaques in association with the distribution and the levels of genetic admixture of rhesus ancestry.

## CHAPTER II

### LITERATURE REVIEW

Animal studies are essential to basic biological and medical knowledge, especially the non-human primate study because they are the closest living organisms to humans. They have many aspects that are unique and similar to that of humans such as genetic and morphological characters and behaviors. For morphological structures, they have large brains comparing to their body sizes, have eye sockets facing forward which can produce 3D-visible, and are advance in evolution. Thus, the knowledge gained from non-human primates can be applied to the humans. Charles Southwick is the first primatologist who began the primate survey in 1959 and many more were following him afterwards. Among 505 species of non-human primates exist across the world, rhesus macaques and long-tailed macaques are the two species commonly used as animal models for biomedical research. One of the reasons is that they distribute in the widest ranges among any other non-human primate species. Although the vast knowledge on physiological characters and biomedical results in these two species have been reported, the information on their reproductive patterns, especially in the wild, is very scarce.

As mentioned in Chapter I that these two species hybridized at the areas where they lived close to each other (or sympatric) at 15 - 21°N, thus the basic knowledge on morphological and genetic characters, and reproduction in the pure breed are important for species identification. Thus, this chapter summarizes the basic knowledge about those characters and also distributions in rhesus and long-tailed macaques which can be essential for understanding the existing of the species and the hybrids.

## 1. Distributions of rhesus and long-tailed macaques

Rhesus macaques have a broader geographical distribution among all non-human primates (Southwick et al. 1996), encompassing 11 countries throughout south and southeast Asia. The distribution of rhesus macaques is around 15 - 36°N, covering Afghanistan, Pakistan, India, Nepal, Myanmar, Thailand, Laos, Vietnam, and China (Fooden, 2000, 2006; Malaivijitnond and Varavudhi, 2002) (Figure 2.1). Rhesus macaques can be classified into three main groups including China (eastern group), India (western group) and Indochina (southern group) (Fooden, 2000; Hamada et al., 2006), and most of them that have been used in biomedical research came from India (Richard et al. 1989). The southern distribution of rhesus macaques was climatologically limited by interspecific competition with long-tailed and bonnet macaques (*M. radiata*) (Trewartha, 1978; Fooden, 1982; 1997).

In Thailand, rhesus macaques are rare to find and mostly live in the northern or northeastern part (Lekagul and McNeely, 1988; Malaivijitnond et al., 2007). Since 1998, no report on any wild populations in Thailand was found, until in 2002 the wild troop was discovered at Wat Tham Pa Mak Ho, Loei province (Malaivijitnond and Varavudhi, 2002). Hamada et al. (2006) measured body size, RTL and pelage color of this population and proposed the past hybridization with long-tailed macaques. Recently, the new location of rhesus macaques at Wat Phrabuddhabat Pa Reau in Chiangrai province was reported (Kyes et al., 2018).

Long-tailed macaques have the widest geographical range, only next to rhesus macaques (Wheatley, 1978), at 20°N - 10°S in southeast Asia, encompassing Bangladesh, Myanmar, Laos, Cambodia, Vietnam, Thailand, Malaysia, Singapore, Indonesia, the Philippines, and Timor (Fooden, 1995, 2006; Malaivijitnond and Hamada, 2008). Long-tailed macaques are the most frequently encountered non-human primate

species in Thailand which can be found throughout the country from the lower northern (Pichit and Mukdahan province) to the southern part (Yala and Satun province), at approximately 16°30'- 6°30'N. Because they distributed across the zoogeographical barrier, namely Isthmus of Kra (ca. 10°N), they are divided into two groups of Indochinese and Sundaic long-tailed macaques.

Regarding the distribution range of rhesus and long-tailed macaques mentioned above, the areas at 15 - 21°N covering Myanmar, Laos, Vietnam, and Thailand were overlapped and proposed as a hybrid zone between the two species. A recent hybrid population of these two species has been reported in Khao Khieow Open Zoo (KKZ; 13°21' N, 101°06'E) in the eastern part of Thailand (Malaivijitnond et al., 2011) which is situated further south from the proposed hybrid zone. They are human-made hybrid population of which rhesus macaques were released into the long-tailed population approximately 20 years ago (Malaivijitnond et al., 2011; Jadejaroen et al., 2015; Jadejaroen, 2016).

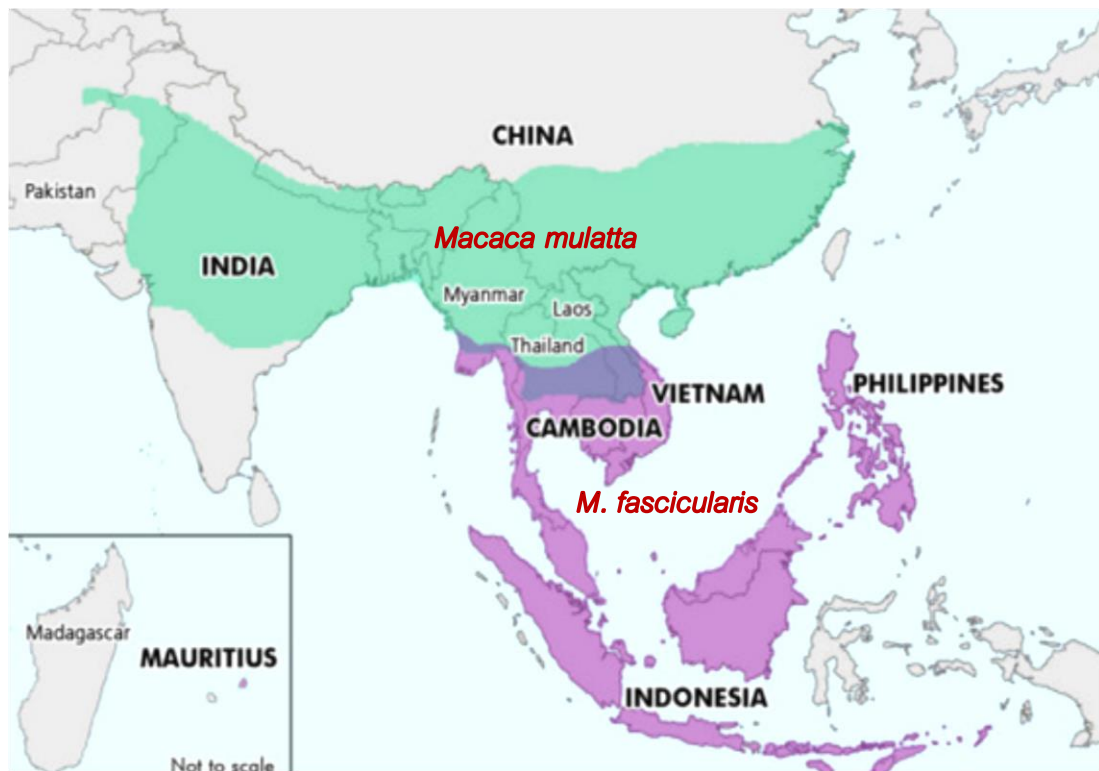


Figure 2.1 A distribution map of rhesus macaques (*Macaca mulatta*) shaded in green color, long-tailed macaques (*M. fascicularis*) shaded in purple color, and the hybrids between these two species shaded in blue color (Source: Street et al, 2007).

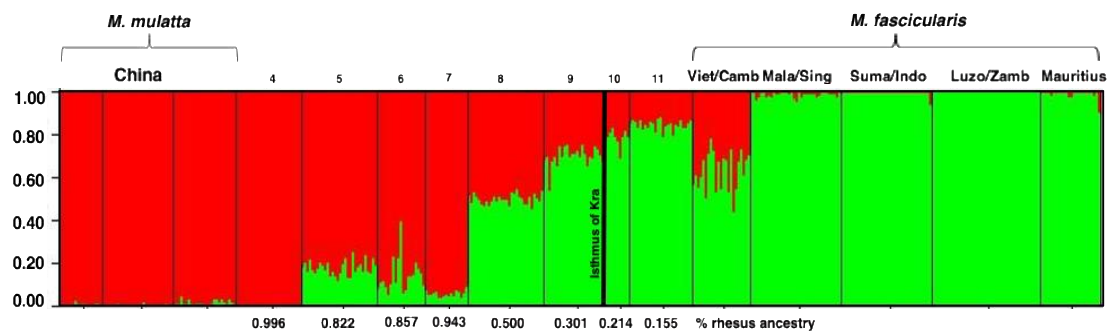
จุฬาลงกรณ์มหาวิทยาลัย  
CHULALONGKORN UNIVERSITY

## 2. Hybridization between rhesus and long-tailed macaques

Hybridization between rhesus and long-tailed macaques was first discovered in Thailand and reported in 1964 (Fooden, 1964) based on morphological observation. Afterward, the hybridization between the two species was confirmed by morphological measurements (Hamada et al., 2016), ABO blood group (Kanthaswamy et al., 2017; Malaivijitnond et al., 2008) and various genetic markers including mtDNA, Y-chromosome gene, nuclear DNA, microsatellite DNA and single nucleotide

polymorphisms (SNPs) (Tosi et al., 2002; Street et al., 2007; Kanthaswamy et al., 2008; Bonhomme et al., 2009; Stevison and Kohn, 2009; Osada et al., 2010; Bunlungsup et al., 2017a; 2017b). Using uniparental markers such as Y-chromosome gene or mtDNA (Tosi et al., 2002; Bunlungsup et al., 2017a), the hybridization between rhesus and long-tailed macaques was detected southerly to the proposed hybrid zone and terminated at the Isthmus of Kra (10°20'N). The hybridization hypothesis based on those genetic markers was by the introgression of male rhesus macaques into the populations of long-tailed macaques. However, the recent results using the more sensitive markers such as genome-wide or autosomal SNPs, the hybridization was found far beyond the Isthmus of Kra to the southernmost Thailand, and the genetic admixture of rhesus ancestry into long-tailed populations was diluted when it was far from the proposed hybrid zone (Osada et al., 2010; Bunlungsup et al., 2017b) (Figure 2.2).





**Figure 2.2** Genetic admixture panel of the rhesus ancestry (red color) in long-tailed macaques (green color) determined by autosomal SNPs markers and structure analysis. Nos. 8 to 11 on the upper panel indicate long-tailed populations from the north to the south of Thailand. (Source: Bunlungsup et al., 2017b).

### 3. Morphological characters

Rhesus and long-tailed macaques had different morphological characters on pelage color, RTL, and cheek hair patterns. Generally, species-specific characters of rhesus macaques are bipartite pattern of the pelage color that the lower part of their body is more reddish-yellowish than the upper part which varies from yellowish gray to golden brown to burnt orange, their RTL is <70%, the crown hairs are smoothly directed posteriorly, and the cheek hair usually forms a small crest or whorl near the angle of the jaw (Fooden, 2000; Malaivijitnond et al., 2005; Jadejaroen et al., 2015; Hamada et al., 2016). Their body mass is about 6.50 – 9.82 kg for females and 7.29 - 13.95 kg for males (Hamada et al., 2016). Long-tailed macaques have evenly pelage color of buffy to yellowish gray to golden brown to reddish brown to blackish throughout their bodies (Fooden, 1990). Most of them have irregular tuft or crest at the vertex of the crest. Hairs on the cheek form a variably prominent lateral facial crest that usually extend from near

the angle of the jaw to the crown (Fooden, 1990). Long-tailed macaques, by their names, have longer tail than the rhesus macaques ( $RTL > 90\%$ ) (Fooden, 1995; Hamada et al., 2006), however, they are lighter than rhesus macaques and their body mass is about 3.61 – 6.56 kg for females and 5.30 – 10.49 kg for males (Hamada et al., 2016). Hamada et al. (2008) reported that the northern long-tailed macaques living close to the hybrid zone had the lighter pelage color and shorter RTL than the southern ones.

#### 4. Demography and reproduction

##### 4.1 Demography and mating system

Rhesus and long-tailed macaques live in multi-male and multi-female societies with promiscuous mating. The estimated population size was around 40-400 individuals, and the male to female ratio was 1:7 to 1:10 for long-tailed macaques (Valerio et al., 1969; Fooden, 2000) and 1:1.2 to 1:5.7 for rhesus macaques (Beisner et al., 2012). Female macaques remain in their natal group throughout their life (so-called female philopatry) whereas male macaques migrate out of the group when they reach the maturity age (so-called male dispersal) (Dixon, 1998). Female macaques become sexual maturity at 4 years. Estrous cycle was approximately 26-29 days for rhesus macaques and 26-38 days for long-tailed macaques (Kumar et al. 2011). Gestation length was approximately 5.5 months for both species (146 – 180 days for rhesus macaques and 153 – 179 days for long-tailed macaques; Wolfensohn and Honess, 2005). Normally, a mother gives birth at night time, black hair and pink skin are the morphological characters of newborns (Figure 2.3). The newborn babies are cuddled at the belly of their mothers during the first three months while they are carried, nursed and protected. Infants begin weaning at 4 months of age and complete the nutritional

weaning at 12-14 months (the nutritional weaning was as early as 8 months of age for captive macaques; Fooden, 2000; Semple et al. 2009). The juveniles stay close to their mothers until the next mating season (inter-birth interval is about 1 year). The daughter of the high-ranking female is more likely to achieve the high rank when she grows up, and the son tends to migrate from their natal group later than that of the low-ranking male (Chapais, 1983).



**Figure 2.3** Newborn and their long-tailed macaque mothers. Black pelage color with streak at the crest and pink skin are identifiable morphological characters of the newborn.

## 4.2 Reproductive seasonality

Based on the reproductive information, rhesus and long-tailed macaques have different breeding patterns. Rhesus macaques have strong reproductive and birth seasonality (or strictly seasonal breeder) (Vandenbergh and Vessey, 1968), while long-tailed macaques are both moderately seasonal breeder (*M. fascicularis fascicularis*; Janson and Versolin, 2005; Trebouet et al., 2020, and *M. fascicularis aurea*; San and Hamada, 2009) and non-seasonal breeder (*M. fascicularis umbrosa*; Hobbs et al., 1987; Tardif et al., 2012; Pal et al., 2018). Duration of mating season in rhesus macaques is approximately 3-6 months (Lancaster and Lee 1965; Southwick et al. 1965; Lindburg, 1971). Mating peak period varies in timing and length between locations but most of them are during the fall and early winter season. Indochinese rhesus macaques showed the mating peak in November – April (Gordon, 1981), while Chinese rhesus macaques had the mating season in October – February (at Chongqing, China; Du et al., 2010) or in September – November (at Taihang, China; Wenyuan et al, 1991). Indian rhesus macaques showed the mating season in October – December (north India; Lindburg, 1971) or November – January (north India; Dixon, 1998) with birth peak in May - June (Fooden, 1995). Generally, the birth season in the natural habitat was around spring. Long-tailed macaques, on the other hand, can mate throughout the year, but the mating peak varies across their locations (Hobbs et al., 1987; Tardif et al., 2012). The mating peak was detected in January – July for northern Sumatra long-tailed macaques (van Schaik and Noordwijk, 1985), and birth dispersed in at least 10 months of the year (Wheatley, 1978) with birth peak in July – September (for north peninsula long-tailed macaques) or May - July (for south peninsula long-tailed macaques; Fooden, 1995).

### 4.3 Sexual behaviors

Sexual behaviors of rhesus and long-tailed macaques are similar because both of them belong to the *fascicularis species* group (Fooden, 2006). They are multiple brief intromissions culminating in ejaculation at the end of mount (Dixson, 1998), although some previous report showed that long-tailed macaques are either multi-mounting or single-mounting. Two species have similar sexual pattern such as duration of mounting, duration of sequence, number of mounts, and number of thrusts (Shively et al., 1982)

Sexual behaviors in estrous female macaques can be categorized into three main groups, including proceptivity, attractivity and receptivity (Beach, 1976). **Proceptivity** is the behavior that the female approaches to male or that the female has a purpose to initiate and sustain sexual interactions with male (Beach, 1976), for example, distance-reducing display, lip smacking with eye contact, mounting by female, and sexual presentation postures which could be seen in many catarrhine species (Young, 1941; Beach, 1976; Dixson, 1998). A peak of proceptivity occurs during the peri-ovulatory period of the menstrual cycle (Dixson, 1998). **Attractivity** is the behavior that the male approaches to female or male's precopulatory behaviors, for example, male investigating the anogenital area of female (sniffing, licking, visual inspection), jaw thrust, following female, body postures and mating without ejaculation (Figure 2.4) (Hrdy and Whitten, 1987; Dixson, 1998). This behavior is influenced by female sexual attractiveness or reflects successes of the female. Female macaques practically show the physiological or chemical signals (or pheromones) to attract the males. **Receptivity** is the behavior indicating a successful copulation or the male's attainment of intromission with intravaginal ejaculation (Beach, 1976). Normally, the ejaculation is evidenced by sperm plug that the semen coagulates or is dense copulatory at the vagina of the female (Figure 2.5). The sperm plug is a reproductive advantage seen in multimale-multifemale

mating primates which increases the sperm competition (Dixson and Anderson, 2002).

Previous studies reported that the hierarchy of female macaques affected the successful rate of mating from males. High ranking females gained higher possibility of successful copulation from males, especially from high dominant ranking male, than the low ranking females, while both rhesus and long-tailed macaque males gain benefit from choosing dominant female (Smith and Smith, 1988; van Noordwijk and van Schaik 1999). Thus, the ranking of female may affect the display and frequency of sexual behaviors.



**Figure 2.4** Attractivity behaviour displaying by long-tailed macaque male, mounting behavior. This is a complete mounting because male uses double feet to clasp the hind limbs and uses his hands to grasp the female's hips.



Figure 2.5 Receptivity behavior indicating by a copulatory sperm plug at the vagina of the female.

#### 4.4. Sex skin: a visual cue of peri-ovulatory period for males

Rhesus and long-tailed macaques live in a multi-male multi-female social group in which adult males mate with multiple females and *vice versa*. To increase a possibility of successful copulation, the females use both olfactory (pheromones) and visual (changes of sex skin) cues to convey the message of the fertile phase and to attract the male conspecifics (Nunn, 1999). Changes of sex skin during peri-ovulatory period are different between rhesus and long-tailed macaques. Rhesus macaques have large areas of sex skin reddening around hindquarter including anogenital area, legs and thighs without swelling (Dixon, 1998; Dubuc et al., 2009) (Figure 2.6), while long-tailed macaques show the narrow area of sex skin reddening from subcaudal to inguinal regions and the conspicuous swelling at the base of the tail (Engelhardt et al., 2005) (Figure 2.6).



Exaggerated sex skin swelling in long-tailed macaques and large reddening area in rhesus macaques reflected high estrogen levels and ovulation time. These changes can transmit the visual signals to male conspecifics. In rhesus macaques, there are a plexus of large, thin-walled blood vessel below the epidermis around sex skin (Collings, 2005), thus, sex skin reddening covers a large area (Dixon, 1998). Sex skin reddening in rhesus macaques indicates the start of the mating season (Fooden, 2000), and sex skin swelling at the base of the tail in non-seasonal breeders as long-tailed macaques indicates the ovulation time (Fooden, 1995; Engelhart et al., 2015). It is possible that the genetic admixture of rhesus ancestry into long-tailed macaques could affect the pattern of changes of sex skin of female long-tailed macaques in Thailand (Bunlungsup et al., 2017).



**Figure 2.6** Representative photos of patterns of sex skin reddening of rhesus macaques (left) and sex skin reddening and swelling at the base of the tail of long-tailed macaques (right)



## 5. Environment factors and reproduction

Long-tailed macaques live in several types of habitats, but most commonly found at the mangrove forest and swamp forest (Fooden, 1995). They inhabit the non-broadleaf evergreen forest (Fooden, 1982) and can adapt to live with other species (Gumert et al., 2011). Long-tailed macaques are arboreal or semiterrestrial primates that prefer the low elevations of the habitats (Henderson, 2017). They use their long tail as balancers during long leaps (Rodman 1991). Their feeding habit is a frugivore and omnivore (Wilson and Wilson, 1977); 86% for fruits and 14% for grass, leaves and some insects (Wheatley, 1976; Rijksen, 1978). Rhesus macaques are semi-terrestrial primates (Vanhoof et al., 2020), however, the ones who live in forest tend to be somewhat more arboreal than the non-forest ones. Rhesus macaques can feed on a variety of foods (Majumder et al., 2012) such as fruits, seeds, flowers and leaves (Fooden, 2000) and occasionally eat termites, grasshoppers, ants and beetles (Lindburg, 1971).

With regard to their seasonal reproductive pattern, the behaviors of rhesus macaques varied between seasons. They spent the greatest proportion of their diurnal time for feeding and resting in spring (33.3% and 22.9%), moving in summer (20.0%), and social behaviors (e.g. grooming, sexual interaction, and playing) in winter (35.9%) (Khatiwada et al., 2020). This indicates that environmental factors could influence the behaviors including reproductive behaviors, especially in temperate non-human primate species such as rhesus and Japanese macaques (*M. fuscata*). Kappeler and colleagues (2003) explained that the primate species that had slow reproduction and long gestation period exhibited longer periods of infant dependency which associated with the availability of foods. This is so-called an income – capital continuum theory which is the model to predict or illustrate the diversity of primate reproductive response to seasonality (Brockman and van Schaik, 2005). For instant, female long-tailed

macaques showed a peak period of pregnancy and births in a peak period of food abundance which is a character of capital breeder (van Schaik and van Noordwijk, 2005). Thus, the same species of macaques that live in different environmental factors such as rainfall, photoperiod, temperature and humidity may show the inconsistency of the reproductive patterns across populations.



## CHAPTER III

### MATERIALS AND METHODS

#### 1. Animals and study sites

Two (WHM and KN/KTK) populations of long-tailed macaques which carried different levels of genetic admixture of rhesus ancestry analyzed by autosomal SNP markers (Bunlungsup et al., 2017) were selected for this study. WHM population lived at Wat Haad Moon Kra Beau, Pichit province, Thailand (16°30'21"N, 100°16'55"E), and was counted as Indochinese long-tailed macaques. KN/KTK population lived at Khao Noi Khao Tangkuan, Songkhla province (7°12'33.2"N, 100°35'49.8"E) and was counted as Sundaic long-tailed macaques. WHM carried 50% of the rhesus ancestry, while KN/KTK carried 15% of the rhesus ancestry.

WHM was a temple near Nan river which was surrounded by a fragmented forest. Various kinds of trees were found in the forest such as *Ficus* sp. of which macaques could access to their leaves and fruits throughout the year, and *Azelia* sp. that the flowers were blooming during February to March (Figure 3.1). KN/KTK was two small hills of KN and KTK that located near the sea. Its elevation was approximately 15 meters above sea level. The hills were covered with a tropical rain forest including *Callerya* sp. and *Garcinia* sp. trees and recreational park (Figure 3.2). Home range of the WHM population was 9 ha, and that of the KN/KTK population was approximately 33 ha but the foraging area was only 8 ha (see Figure 3.2)



Figure 3.1 Home range of the Wat Haad Moon Kra Beau (WHM) population at Pichit province, Thailand (framed by red line). It covers with fragmented forest and temple ground. (Map from Google Earth, accessed December 2019).



Figure 3.2 Home range of the Khao Noi (right hill) and Khao Tangkuan (left hill) population at Songkhla province, Thailand (framed by red line). It covers with a forestry recreational park. (Map from Google Earth, accessed December 2019).

## 2. Population demography and selected subjects

WHM population consisted of 52 macaques including 26 adult females and 7 adult males, and the KN/KTK population consisted of 125 macaques including 55 adult females and 16 adult males. Note that more than 4 groups of macaques were found at KN/KTK, but only the targeted group was counted and studied. Both WHM and KN/KTK populations were habituated with human appearance. Ten females in each population were selected. The major criterion of group and animal subject selection for this study was that they were often encountered and they must carry some unique morphological characters, e.g., scar, distinct color of nipples, or some unique behaviors which could be easily identified and recognized. The selected macaques were divided into two groups, high and low rank, according to their social organization. The social rank was identified by observing their behaviors such as agonistic behaviors (supplant, threat, chase, attack) and submissive behavior (grimace). All selected 10 female monkeys were named and the name list was arranged in alphabetical order (see Table 3.1 and Figure 3.3 and 3.4). The female monkeys were selected as representatives of the populations for sexual behavior observation based on the knowledge that female lives permanently in the group while male migrates out of the group when he reaches maturity age. For the long-term observation, the female subjects should suit best to the study. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Faculty of Science, Chulalongkorn University, Thailand (Protocol Review no. 1723017).

**Table 3.1** Ten selected female long-tailed macaques from WHM and KN/KTK population for the study. The names are listed in alphabetical order and the rank is given in the parenthesis.

Number	WHM	KN/KTK
1	Gorilla (high rank)	Bob (low rank)
2	Hang (low rank)	Ceasar (low rank)
3	Jaime (low rank)	Dragon (high rank)
4	Leam (low rank)	Fu (high rank)
5	Mhob (low rank)	Jam (low rank)
6	Red (high rank)	Lord (low rank)
7	Rhino (low rank)	Milk (low rank)
8	Tarly (high rank)	Pang (low rank)
9	The hound (low rank)	Raven (high rank)
10	Yib (high rank)	Tumkhaw (high rank)





Figure 3.3 Ten selected female long-tailed macaques from WHM population.



Figure 3.4 Ten selected female long-tailed macaques from KN/KTK population.



### 3. Behavioral observation and data collection

#### 3.1 Behavioral observation

Behavioral observation was divided into two phases. Phase I was for group and animal subject selection which was done in October – December 2017. In this session, the animals were also habituated to the appearance of the observer. Phase II was the main data collection period which was in January to December 2018. Note that no food provision from the observer and at least 3 m of the distance between the observer and monkeys were performed during behavioral observation (Altmann, 1974). This aimed to avoid the disturbance of the daily activities of the animal subject.

Sexual behaviors were collected using focal animal sampling method (Altmann, 1974). Behavioral data collection was initiated when one of the selected female monkeys in the name list was first encountered. After the observation was completed, the subsequent female was alphabetically selected and followed (see Table 3.1). The procedure was done until the last animal in the list was observed and another round of the observation was begun again. However, if the selected subsequent monkey could not be found within 10 minutes, it would be shifted to the next order of the monkey. Each monkey was followed for 1 hour or until she could not be followed further such as she climbed to the very steep area or moved to restricted areas. However, during the focal follow, if another female monkey in the list appeared within the sight distance, the observer simultaneously recorded the behaviors of two animals at the time, but no more than two females were conducted for a simultaneous observation. The period of observations was from the sunlight to the sunset (approximately from 0600 am – 0600 pm). Focal female monkeys were followed for 7 days in each month for 12 consecutive

months, from January to December 2018. The first week of the month was spent for the KN/KTK population and the third week of the month was for the WHM population.

### 3.2 Behavioral data collection

The sexual behaviors included in this study were followed that of Jadejaroen (2016) with a slight modification. Sexual behaviors were categorized into three groups; proceptivity, attractivity and receptivity. Proceptivity was the behaviors acted by the females including female solicitation, affiliative behavior, contact behavior, and female following adult male (Figure 3.5). Attractivity was the behaviors acted by the males consisting of male solicitation, male genital inspection, male grooming, masturbation, male following adult female, and mating (Figure 3.6). Receptivity was the behavior cooperated by both sexes which indicated a successful copulation. Frequency of these three sexual behaviors were recorded. Ethogram of the three behaviors was described in Table 3.2 and the representative pictures were shown in Figure 3.5, 3.6 and 3.7.

Table 3.2 Ethogram of three sexual behaviors (proceptivity, attractivity and receptivity)

Behaviors		Definition
Proceptivity (P)	Female solicitation	Female invites male by presenting her anogenital region towards the male (or sexual context)
	Affiliative behavior	Female stays beside the male without contacting his body (if it occurs during feeding, it is not recorded).
	Contact behavior	Female touches, but does not groom, any part of the male's body including mounting by the female.
	Female following adult male	Female keeps following the male within 3-meter distance.
Attractivity (A)	Male solicitation	Male presents his erected penis to the female.
	Male genital inspection	Male visually inspects, touches or sniffs of female genitalia.
	Male grooming	Male cleans the female's fur (if the focal animal pauses acting this behavior longer than 5 seconds and starts again, the observation is counted as a new round of the behavior).
	Masturbation	Male tugs and rubs his penis until it becomes erected with or without ejaculation. The tip is usually touched with his fingers or any objects and is often sniffed and/or licked.
	Male following adult female	Male keeps following the female within 3 meters distance.
	Mating	Male mounts the female with or without intromission and pelvic thrust and without ejaculation.
Receptivity (R)	Copulation	Male mounts the female with intromission and pelvic thrust resulting in ejaculation. The ejaculation is evidenced by sperm plug which often involves an intermission during intromission for approximately 3-5 second before the male pulls his penis off the vagina of the female and the female usually vocalizes. If the observer could not see any evidences mentioned above, it is recorded as mating behavior.

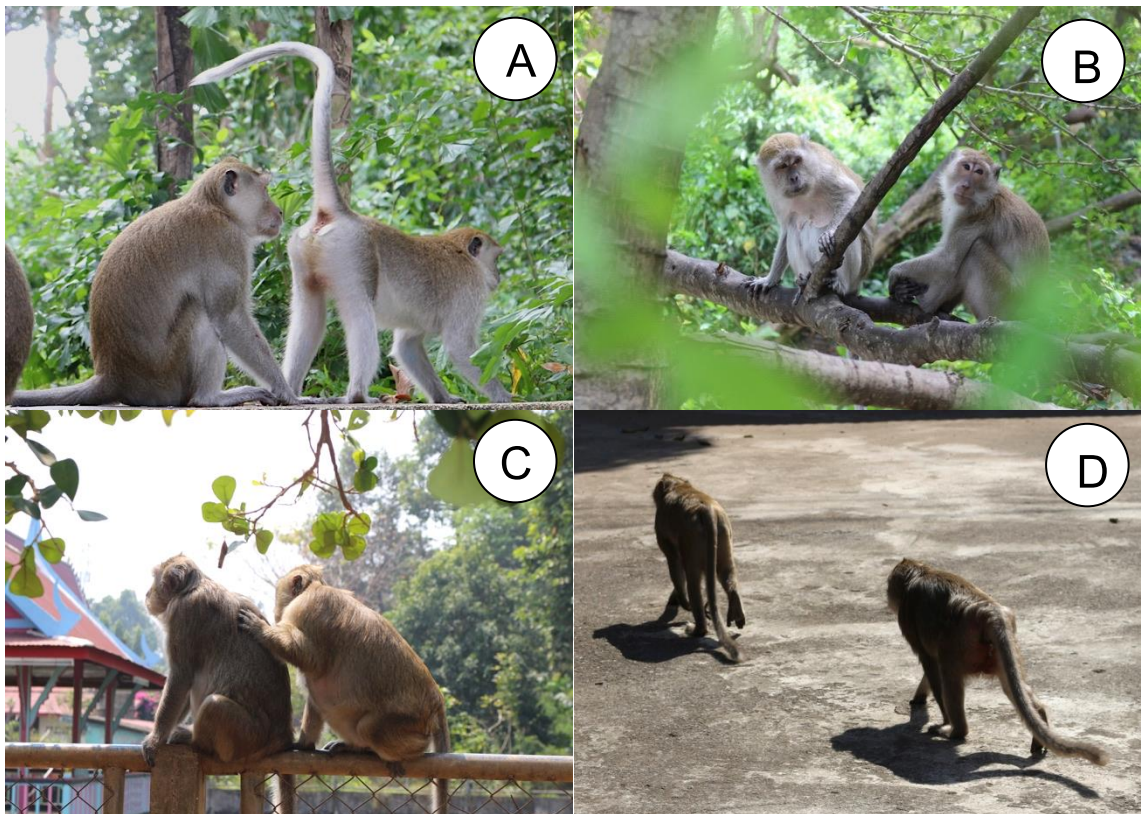


Figure 3.5 Proceptivity behaviors recorded in this study; (A) female solicitation, (B) affiliative behavior, (C) contact behavior, and (D) female following adult male.



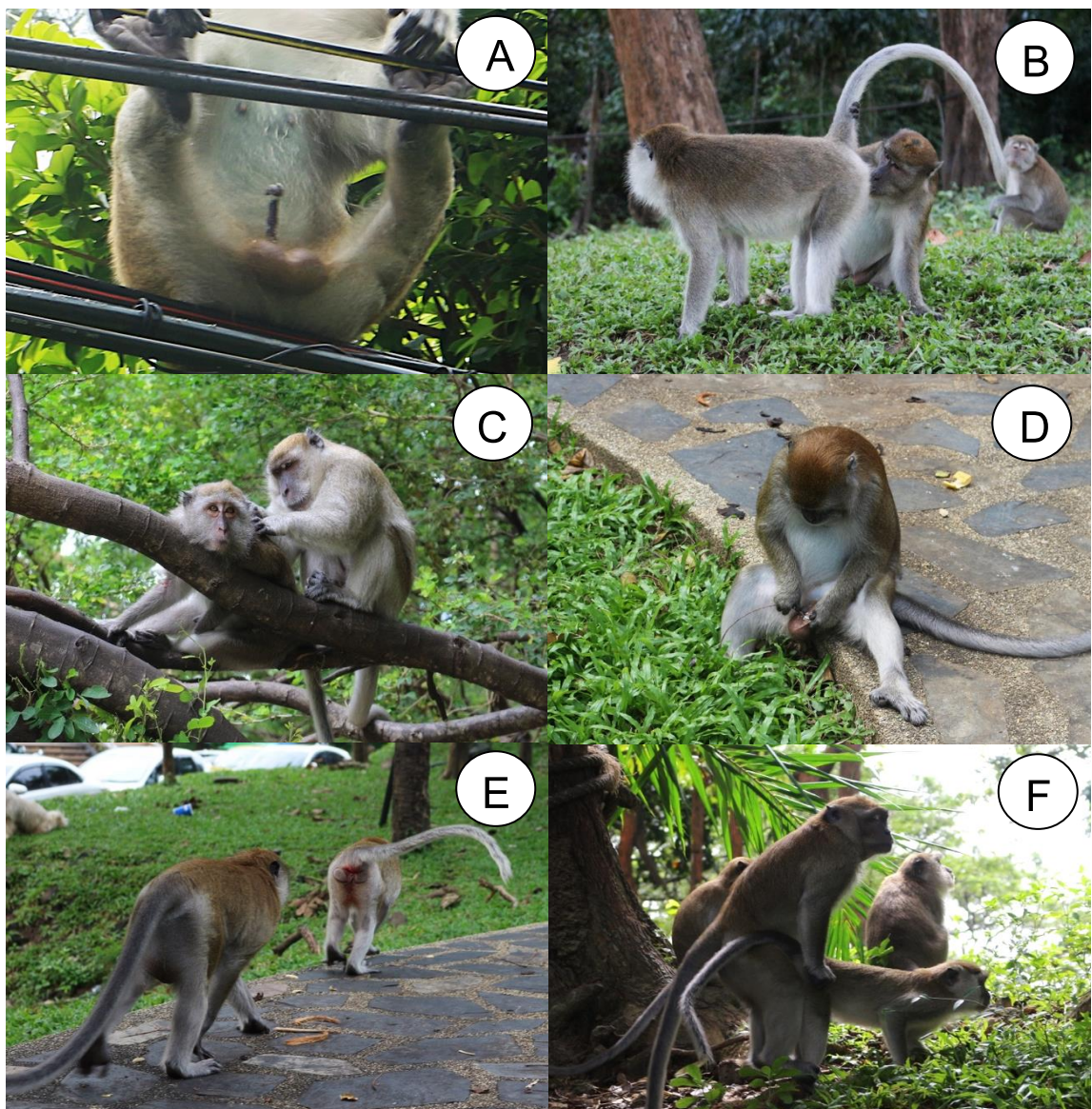


Figure 3.6 Attractivity behaviors recorded in this study; (A) male solicitation, (B) male genital inspection, (C) male grooming, (D) masturbation, (E) male following adult female, and (F) mating.



Figure 3.7 Receptivity or copulation behavior. Male mounts the female with intromission and pelvic thrust resulting in ejaculation. Sperm plug can usually be seen at the vagina of the female.

#### 4. Birth observation

The numbers of newborn were counted in every month for 12 months (January – December 2018) from all females in each population, and the pictures of both newborn and his/her mother was taken for a record to prevent the newborn recounting. Identification of the newborn was relied on the pink facial skin and blackish pelage color. Pelage color is generally similar among adult, subadult, and juvenile males and females (Fooden, 1995; Hamada et al., 1988; Harrison et al., 2006). The pelage color of the infant is changed to be that of the older ages at 2 to 3 months of age, and the progression to adult pelage is complete by 1-year-old (Fooden, 1995; Rowe, 1996).

## 5. Assessment of sex skin swelling and reddening and data collection

Focal female monkeys were followed and photographed using a digital camera (Canon EOS 700D, lens EF-S18-55mm, Japan) in every month for 12 months (January – December 2018) during sexual behavior observation. The photographs were taken in the distance of at least three meters from the monkeys in an avoidance to the disturbance of their daily activities. To lessen the effect of the different reflection of the sunlight, animals should not be under the shade during photographed. As it was reported that long-tailed macaques exhibited a sex skin reddening at the narrow area of subcaudal to inguinal regions, while sex skin swelling is conspicuous at the base of the tail (Engelhardt et al., 2005), and rhesus macaques had large areas of sex skin reddening around hindquarter including anogenital area, legs and thighs without swelling (Dixon, 1998; Dubuc et al., 2009), the scoring system of sex skin swelling and reddening in this study was integrated between two macaque species. Scoring of sex skin swelling at the base of the tail was followed Engelhart et al. (2015) with slight modification; scoring ranging from 0 to 4 as follows; 0 : no swelling at the base of the tail, 1: slight swelling at the base of the tail, 2: protuberant swelling at the base of the tail, 3: protuberant swelling and small lateral lumps at the base of the tail can be detected, and 4: protuberant swelling and large lateral lumps at the base of the tail can be detected (see Figure 3.8). Scoring of sex skin reddening was modified from Jadejaroen et al. (2015). The scoring of sex skin reddening ranged from 0 to 4 as follows; 0: no reddening is detected; 1: reddening area is between ischial callosity and/or at the base of the tail; 2: reddening area of (1) plus the inguinal part; 3: reddening area of (2) plus the hindquarter area (or outside the ischial callosity); and 4: reddening area of (3) plus the thigh (see Figure 3.9).



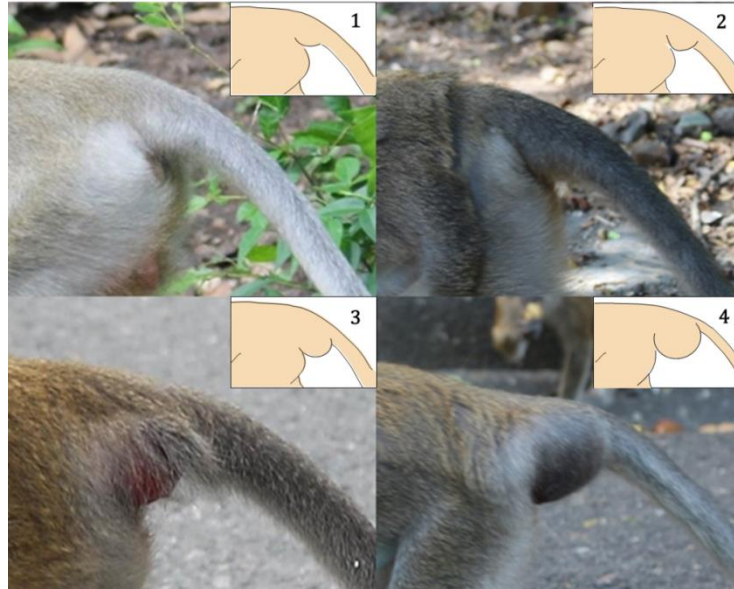


Figure 3.8 Sex skin swelling at the base of the tail, from Level 1 to 4

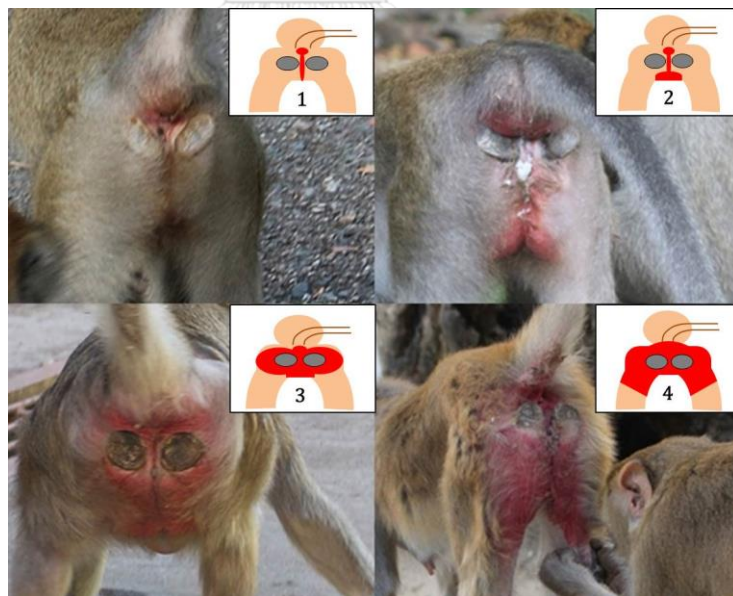


Figure 3.9 Sex skin reddening from Level 1 to 4



## 6. Saliva collection and salivary hormonal analysis

### 6.1 Rope bait method for saliva collection

According to the previous plan, the sex steroid hormone (estradiol) levels were proposed to analyze from the fecal samples and to support the sexual behavioral data, but collection of the fecal samples from each monkey individual in the natural habitat during observation period of January – December 2018 were implausible. This is because researcher must patiently follow the targeted monkeys until they defecate which is time-consuming (Toyoda et al., 2020), thus determination of estradiol levels in saliva was considered for this study. Saliva was collected using non-invasive rope bait method which was slightly modified from that of Toyoda et al. (2020). The 2-mm diameter polyester ropes (Takagi Corporation, Kagawa, Japan) were cut into 6 cm long. When used as bait, the ropes were soaked in 20% sugary water (70 g of cane sugar dissolved in 350 ml of distilled water) for 30 min and dried at 120°C for 2 hours.

### 6.2 Saliva collection

To stimulate a monkey's attraction, the rope was wrapped with corn seed (Figure 3.10A) and dipped into honey (Thai Royal Chitralada Honey, Thailand) at the tip (approximately 2-cm long) of the rope. Ropes were scattered on the open ground when the focal monkeys appeared. After a targeted monkey picked up the rope, chewed (longer than 10 sec) (see Figure 3.10B) and discarded, the rope was quickly collected and stored in 8 ml of 95% ethanol of 5-ml tube size. The tube containing rope was kept in -20°C when the researcher returned back to the laboratory until analyzed. Saliva samples were collected every month from January to December, 2018 while the researcher followed the animals.

### 6.3 Hormone assays

Since the commercial kits for estradiol assay available in the market was for plasma or serum samples, not for saliva, the assay needs to be validated. Thus, the blood plasma and rope-bait-saliva were collected from the same individuals of six female long-tailed macaques at the National Primate Research Center of Thailand during the time of semiannual health check of the animals. For the preparation of the saliva samples, frozen samples (ropes and 95% ethanol) were thawed at room temperature for 1 hour, mixed by rotating shaker (40R/min) for 30 min and centrifuged at 3000 x g at 4°C for 20 min. The mucus, corn, soil and small leaves were precipitated at the bottom of the tube. The 5 ml of supernatant was pipetted into the new 12-ml tube, evaporated at 60°C in bioshaker (180R/min) until the volume was decreased to 1 ml, and then 2 ml of ether were added and vortexed for 2 min. After that, the tube containing 3 ml of sample (saliva mixed in ether) was submersed into 95% ethanol solution with the chip of dry ice until the saliva sample was frozen and separated from ether. Ether solution was poured into a new 12-ml tube, evaporated in water bath shaker at 60°C until completely dry, and added 250 µl of PBS solution (pH 7.2) (Figure 3.11). Plasma and extracted saliva samples (50 microliters each) from the same monkey was assayed for estradiol levels using Monkey Estradiol (E2) ELISA Kit (no. CSB-E16426Mk, Cusabio, Hubei, China). Detection range was 40-1000 pg/ml and intra and inter-assay coefficient of variation (%CV) were <15%.

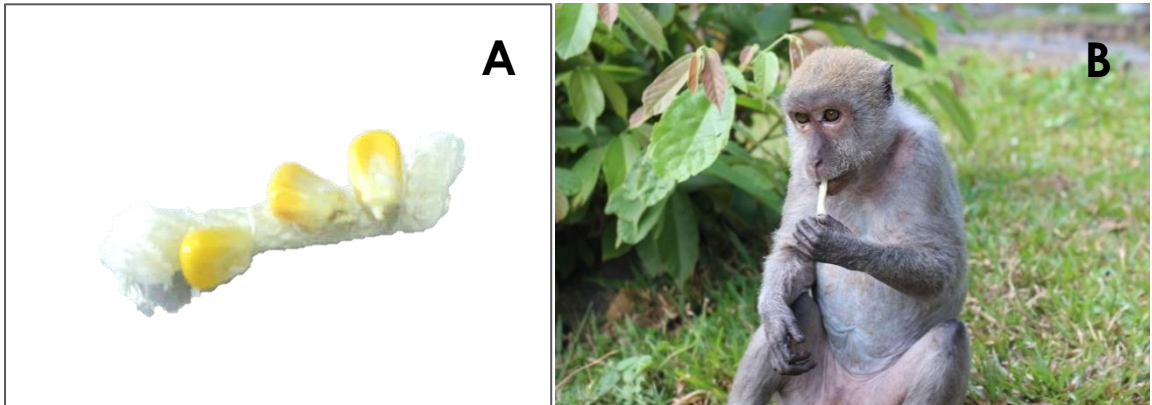


Figure 3.10 Rope bait wrapped with corn seed (A). Focal monkey picked up and chewed the rope bait (B).

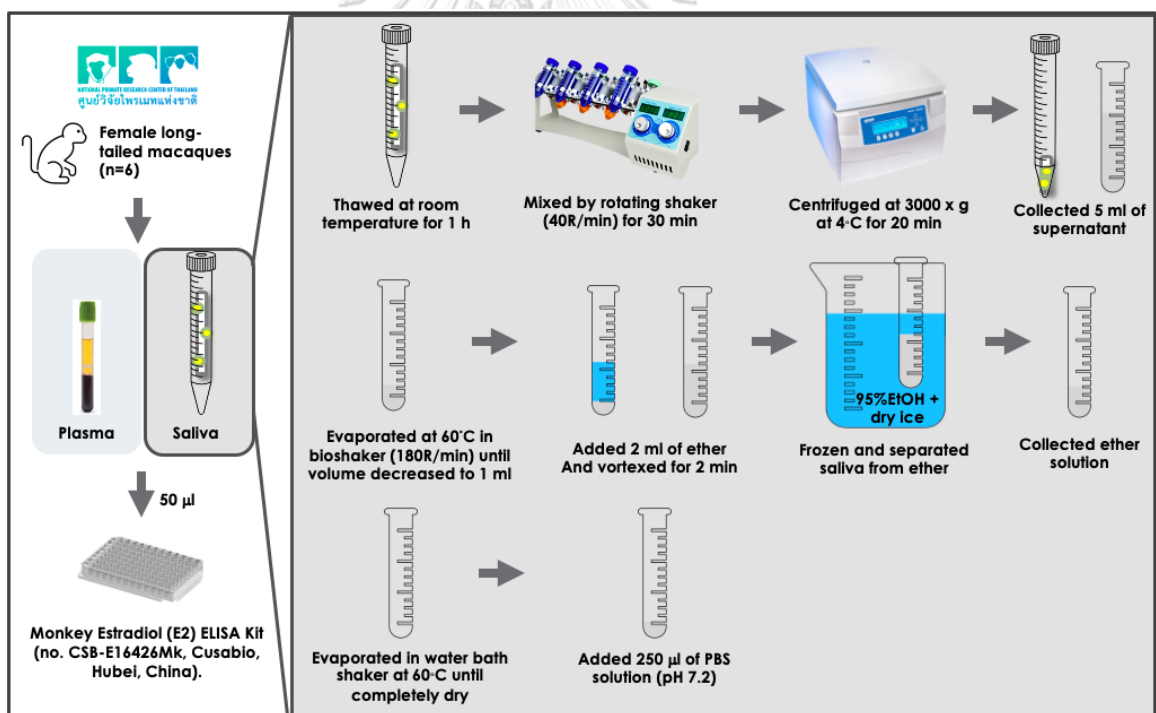


Figure 3.11 The procedure of validation of salivary estradiol assay in comparison with that of plasma sample.

## 7. Physical data collection

Average monthly values of temperature, rainfall, relative humidity and photoperiod were retrieved from Thai Meteorological Department. The distance from WHM to Pichit Agromet Meteorological Station was approximately 31 kilometers, and from KN/KTK to Songkhla Meteorological Station and Khohong Agromet Meteorological Station (for photoperiod data) were 3.6 and 36 kilometers, respectively.

## 8. Data presentation and statistical analyses

For individual data presentation, frequency of the proceptivity, attractivity and receptivity behaviors of each female in each month was calculated as frequency per hour of observation time, and 12 months of data collection was shown. For population data presentation, frequency of the sexual behaviors, sex skin scores and physical data in each month were averaged values from 10 animals in each population and presented as mean  $\pm$  SE for 12 months. Mann-Whitney U test was used to determine the significant difference between two populations of three sexual behaviors, sex skin score, and physical data. Correlation between three sexual behaviors, between sexual behaviors and sex skin scores or physical data were analyzed by Spearman correlation test. Statistic tests were conducted with SPSS software program version 22 for Windows and a significant level was set at  $p < 0.05$ .

## CHAPTER IV

### RESULTS

#### 1. Sexual behaviors

From the behavioral observation of totally 2,016 hours, from January to December 2018, 20 focal female macaques at WHM and KN/KTK showed sexual behaviors of proceptivity (female solicitation, affiliative behavior, contact behavior, and female following adult male), attractivity (male solicitation, male genital inspection, male grooming, masturbation, male following adult female, and mating) and receptivity in the similar pattern, that is, the behaviors were observed throughout the year (the individual data of each P, A, and R behavior were shown in the Appendix). Female macaques at WHM showed the scattering of three sexual behaviors (P, A, and R) throughout the year and the patterns were similar among 10 animals (Figure 4.1). As a matter of fact, the correlation between the average frequencies of P, A and R behaviors of all females were significant (r-value: between P and A = 0.872, between P and R = 0.834, and between A and R = 0.799;  $p < 0.01$ ). The frequencies of sexual behaviors were high at the end and the early of the year (November and March; surge period), which were highest in January, and low in the remaining months of the year (April – October; non-surge period) (Figure 4.3). If the data within surge period and within non-surge period of P, A, and R behaviors were pooled and compared, the statistically significant differences were detected (P:  $p = 0.018$ ; A:  $p = 0.003$ ; R:  $p = 0.030$ ) (Figure 4.5) .

For KN/KTK population, the 10 female macaques also had similar patterns of three sexual behaviors throughout the year as seen in the WHM population (except the female No. 8 that was lost from the observation since May) (Figure 4.2). The correlation

between average frequencies of P, A and R behaviors of all females were significant (r-value: between P and A = 0.895, between P and R = 0.918, between A and R = 0.826;  $p < 0.01$ ). The frequencies of sexual behaviors were high at the end and the early of the year (December and April; surge period), which were highest in March, and low in the remaining months of the year (May – November; non-surge period) (Figure 4.4). If the data within surge period and within non-surge period of P, A, and R behaviors were pooled and compared, the statistically significant differences were detected (P:  $p = 0.003$ ; A:  $p = 0.003$ ; R:  $p = 0.018$ ) (Figure 4.6).

Although averaged frequency of P, A, and R between individuals of the WHM and KN/KTK population were not statistically significant differences, if the months with the highest three sexual behaviors were analyzed, the significant differences can be detected in high ranking females. In WHM population, the high ranking female Nos.6 and 10 showed higher frequencies of the three sexual behaviors than other females which were peaked in January. In KN/KTK population, high ranking female Nos. 3 and 10 showed the peaks of behaviors in January and March.

Some males at WHM population ate sperm that attached at the tip of his penis. One male at KN/KTK population used his finger and branch for masturbating and sometimes scraped his penis by hand until his penis becomes erected in front of the females. For copulation (receptivity), only some females called (cue of female reproductive status in macaques) during copulation. Besides, female reaching back during copulation has appeared only in some females in both populations.

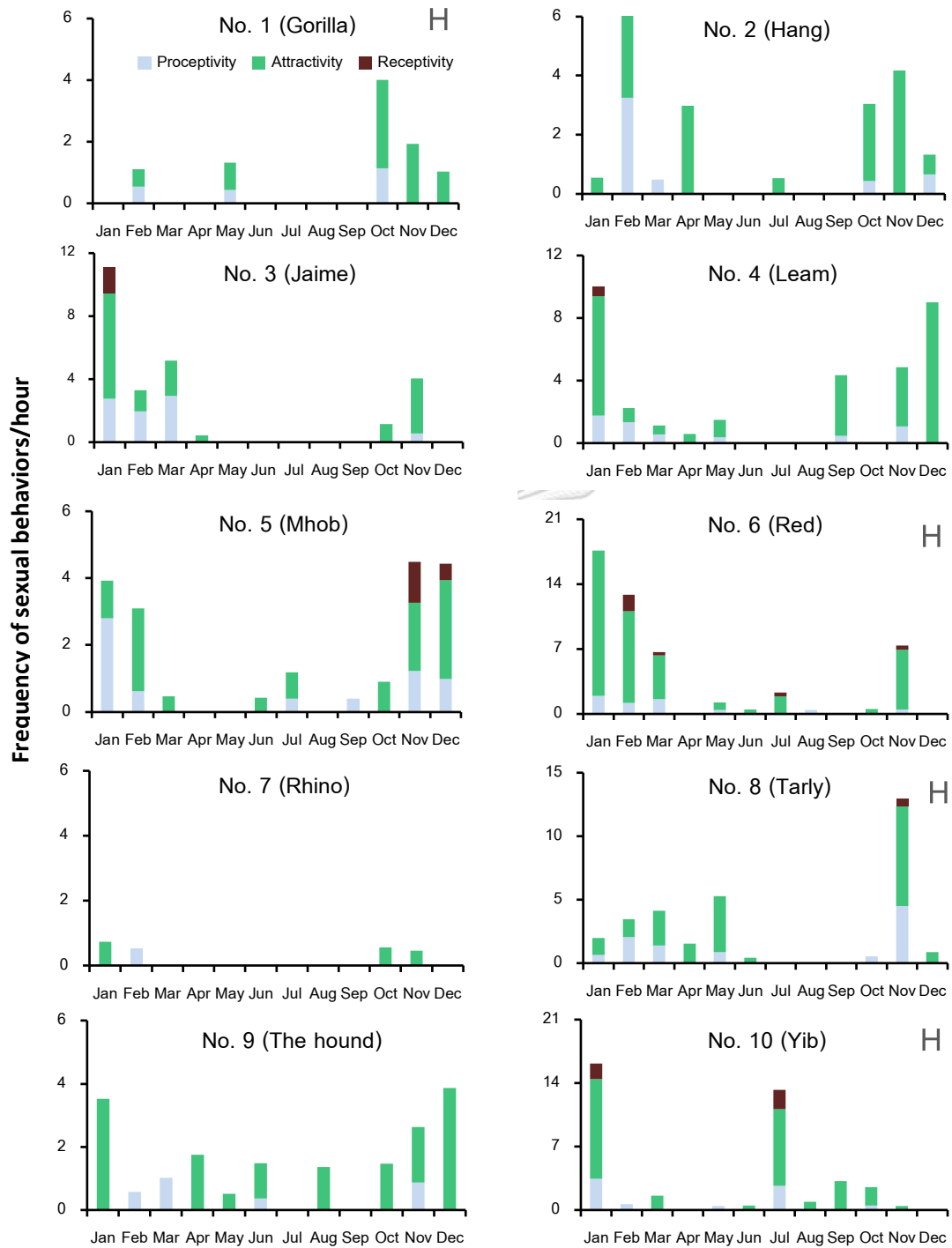


Figure 4.1 Proceptivity, attractivity, and receptivity behaviors of 10 female long-tailed macaques in WHM population. Nos 1, 6, 8 and 10 were high ranking females.

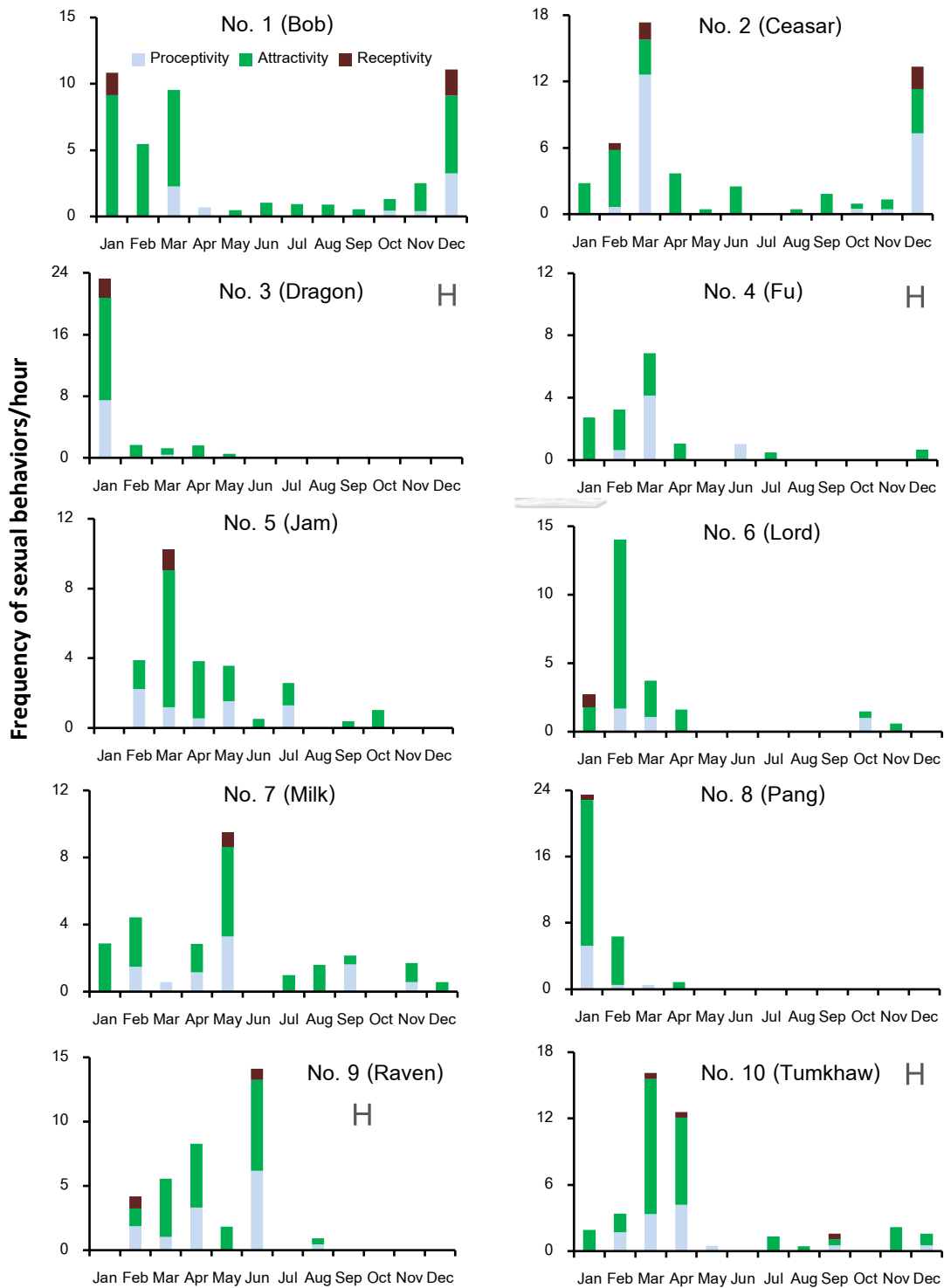


Figure 4.2 Proceptivity, attractivity, and receptivity behaviors of 10 female long-tailed macaques in KN/KTK population. Nos 3, 4, 9 and 10 were high ranking females.



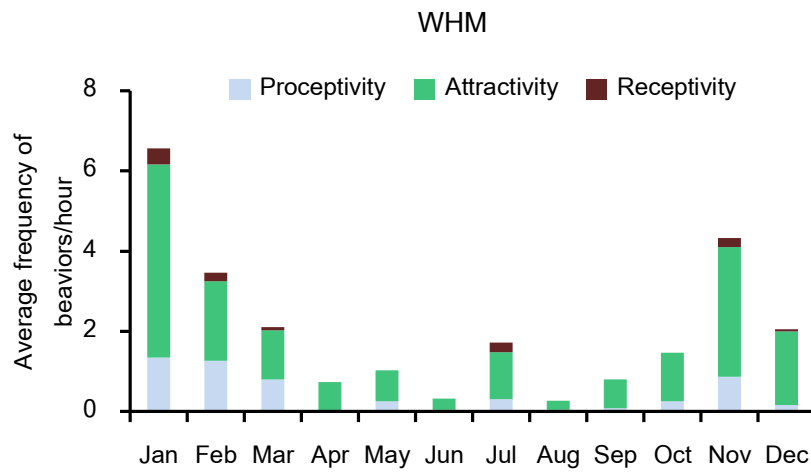


Figure 4.3 Average frequency of sexual behaviors (proceptivity, attractivity, and receptivity) of female monkeys in WHM population

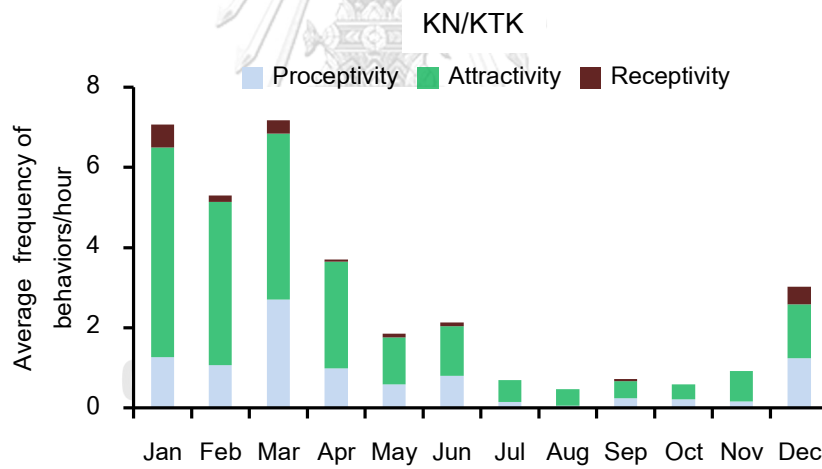


Figure 4.4 Average frequency of sexual behaviors (proceptivity, attractivity, and receptivity) of female monkeys in KN/KTK population

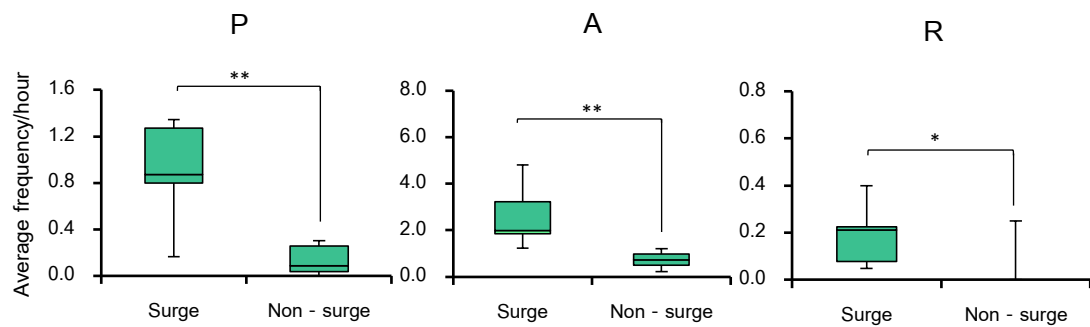


Figure 4.5 Average frequency of sexual behaviors (proceptivity, attractivity, and receptivity) between surge period (November-March) and non-surge period (April-October) of WHM population. \* and \*\* represents  $p < 0.05$  and  $p < 0.01$ , respectively.

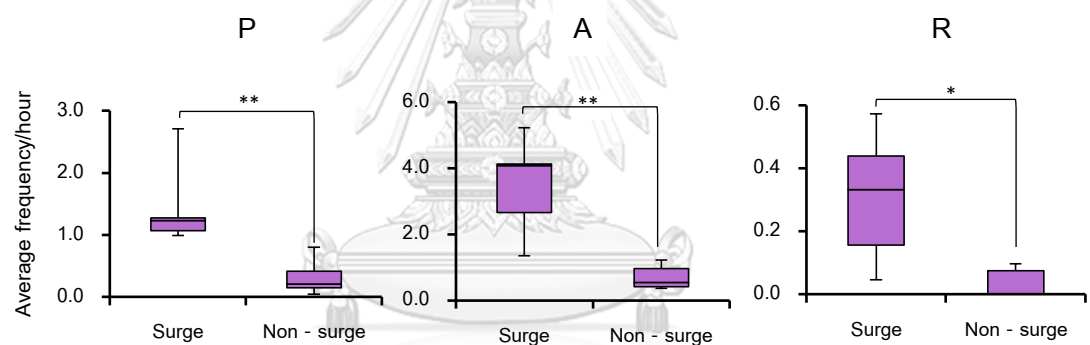


Figure 4.6 Average frequency of sexual behaviors (proceptivity, attractivity, and receptivity) between surge period (December-April) and non-surge period (May-November) of KN/KTK population. \* and \*\* represents  $p < 0.05$  and  $p < 0.01$ , respectively.

## 2. Birth observation

For WHM population, birth was not observed every month within the observation period (only from April to December). One female gave births of twin in April which is the month with the highest birth in this population (44.4%) (Figure 4.7). Based on the reproductive seasonality classification system of van Schaik and colleagues (1999), the percentage of births concentrated in a three-month period were calculated. In three-month period between April-June, 56% of births (5 births out of 9) were observed in the WHM population. Contrarily, in the KN/KTK population, births occurred every month throughout the year, except in March and April. Birth surge in a three-month period in this population was between June-August (15 out of 29 births or 52%) and highest in July (20.69%) (Figure 4.7). This indicates that the WHM population had a narrower birth peak than the KN/KTK population.

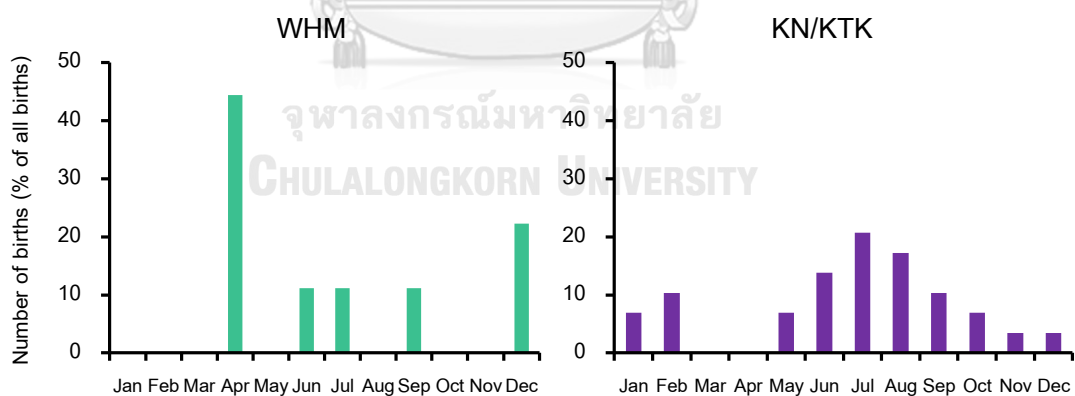


Figure 4.7 Percentage of births of the WHM and KN/KTK population

### 3. Sex skin swelling and reddening

Sex skin swelling score of each female in WHM and KN/KTK population was fluctuated throughout the year and the surge was not clear, thus the data of all females in each population were pooled (Figure 4.8). After the data were pooled, it was found that the average score of sex skin swelling of WHM population was lower than 1 in every month from January-December (Figure 4.8), and the KN/KTK was higher than 1 in every month, except October that it was 0.78 (Figure 4.8). Similar to that of sex skin swelling, each female macaque in both WHM and KN/KTK population was not detected any significant changes of sex skin reddening score from January-December, however, the value of the reddening score of female macaques in the WHM population was mostly at score 3 (Figure 4.9 and 4.10A; 60%, 20% and 10% for score 3, 2, and 1/4), while the females in KN/KTK population were mostly at score 2 (Figure 4.9 and 4.10B).

Neither the averaged sex skin swelling score nor the averaged sex skin reddening score showed a statistically significant difference between surge and non-surge period in both populations (data not shown), the 12-month data of all 10 monkeys in each population were pooled and compared between two populations. The significant differences of sex skin swelling and reddening between populations were detected. The average score of sex skin swelling in the WHM population ( $0.70 \pm 0.48$ ) was significantly lower than the KN/KTK population ( $1.50 \pm 0.85$ ;  $p < 0.05$ ) (Figure 4.11). Contrarily, the average score of sex skin reddening of the WHM population ( $2.70 \pm 0.82$ ) was significantly higher than the KN/KTK population ( $2.00 \pm 0.00$ ;  $p < 0.01$ ) (Figure 4.11). By combining the data of sex skin swelling and reddening together, it indicates that the WHM females who carried 50% of genetic admixture of rhesus ancestry had a smaller sex skin swelling at the base of the tail but larger sex skin reddening area than the KN/KTK females who gained only 15% of genetic admixture of rhesus ancestry.

As the values of sex skin swelling and reddening were consistent throughout the year in both populations, no significant correlation between averaged frequencies of P, A and R behaviors and averaged scores of sex skin swelling (p-values between P, A and R behaviors and sex skin swelling score were 0.481, 0.947 and 0.474 for WHM populations and 0.687, 0.820 and 0.766 for KN/KTK population) and reddening were detected (p-value between P, A and R behaviors and sex skin reddening could not be calculated).



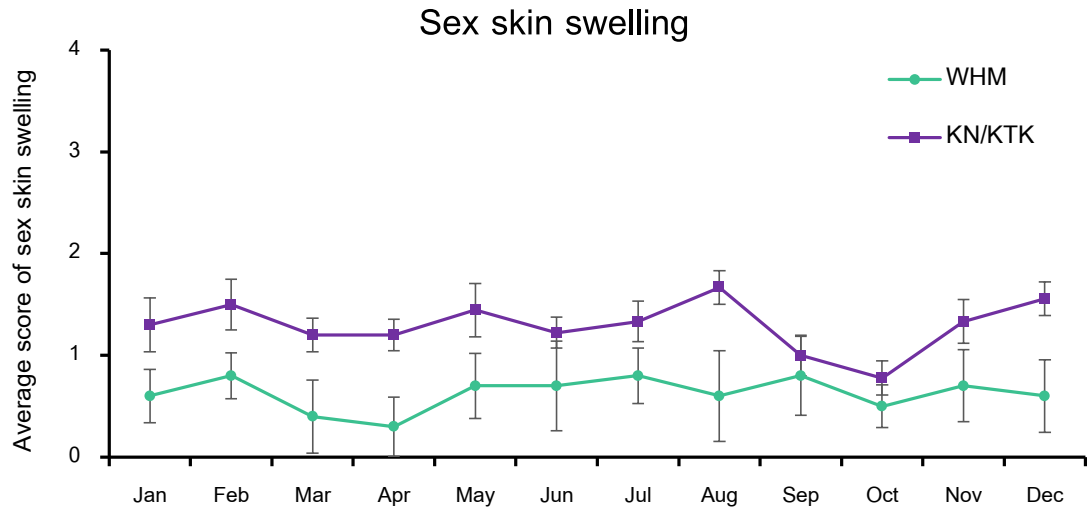


Figure 4.8 Averaged score of sex skin swelling of all females in the WHM and KN/KTK population.

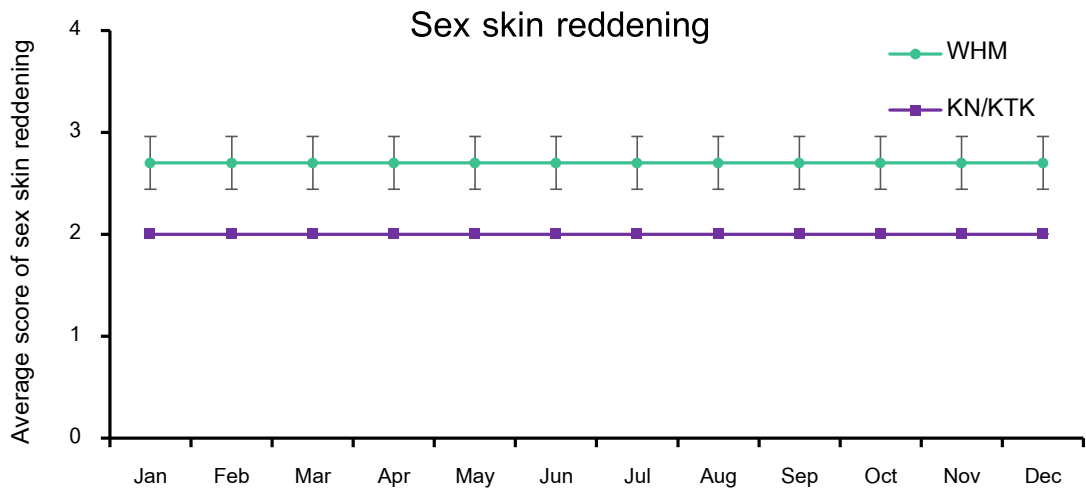


Figure 4.9 Averaged score of sex skin reddening of all females in the WHM and KN/KTK population.

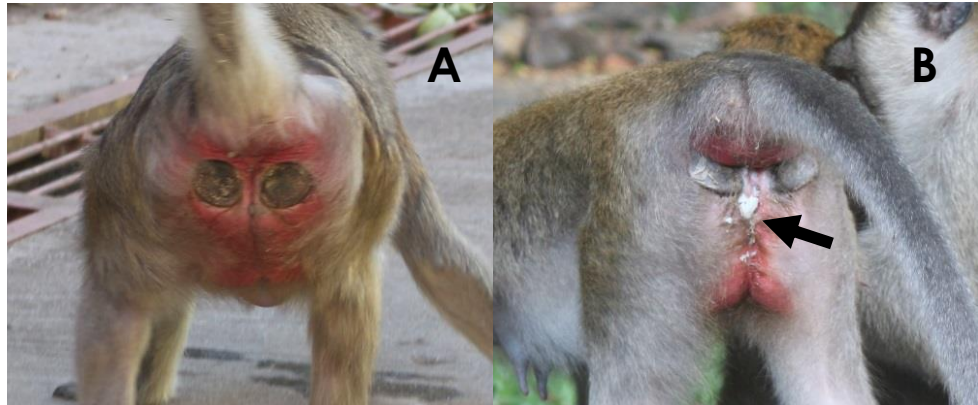


Figure 4.10 Representative photos of patterns of sex skin reddening (score 3) of WHM female (A), and sex skin reddening (score 1) and swelling at the base of the tail (score 3) of KN/KTK female (B). Arrow indicates sperm plug.

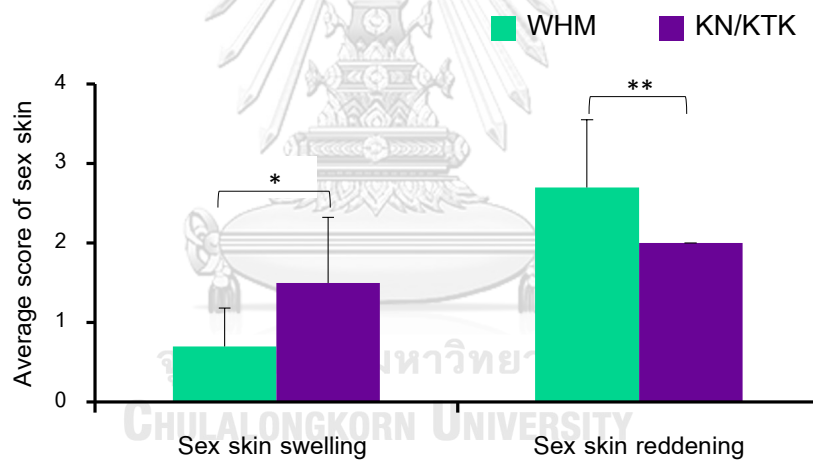


Figure 4.11 Average score of sex skin swelling and reddening of the WHM and KN/KTK population. \* and \*\* represents  $p < 0.05$  and  $0.01$ , comparing between two populations.

#### 4. Saliva collection and salivary estradiol analysis

Although as many as 176 salivary samples were collected from 20 monkeys (96 samples for WHM population and 80 samples for KN/KTK population) during the study period, but not all monkeys could be collected the saliva samples in all 12 consecutive months. For example, Monkey Nos. 1 and No. 3 in the WHM population and No. 2 in the KN/KTK population could be collected the saliva only in 11 months. Female No.2 in the WHM population and Nos.4 and 8 in the KN/KTK population did not pick up the rope bait during 12-month period. Two females (Nos.9 and 10) in the KN/KTK population picked up the rope bait, ate the corn, but did not chew the rope, and female No.10 did not pick up the rope bait from May onwards (Table 4.1; Table 4.2).

Following the salivary estradiol assay validation, the result showed that the levels of estradiol in extracted saliva and plasma were not correlated, and thus the measurement of salivary estradiol levels could not be continued. There were several factors that might interfere with the reaction such as honey and corn which might affect an optical density (OD) reading and estradiol levels in saliva was too low under the detection limit of the kit (0.3 pg/ml; Lubinsky, 2013).



**Table 4.1** Number of salivary samples collected from each WHM female. \*monkeys picked up the rope bait, ate corn, but not chewed the rope. # monkey did not pick up the rope bait.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
1. Gorilla	3	3	0*	2	1	1	3	2	1	1	1	1	19
2. Hang	0#	0#	0#	0#	0#	0#	0#	0#	0#	0#	0#	0#	0
3. Jaime	2	2	2	3	2	3	2	2	1	0*	2	1	22
4. Leam	1	0*	0*	1	1	0*	0*	0*	0*	0*	0*	0*	3
5. Mhob	3	0#	0*	0*	2	0#	0*	0*	0*	0*	0*	0*	5
6. Red	3	2	2	1	1	0*	1	0*	0#	0#	0#	1	11
7. Rhino	0*	0*	0*	0*	0*	0*	0*	0*	1	0*	1	0*	2
8. Tarly	2	3	1	0*	0*	0*	1	2	0*	1	2	1	13
9. TH	2	2	3	0*	0*	1	0#	0#	0#	0*	0*	0*	8
10. Yib	2	0#	0*	3	3	2	0*	1	1	0*	0*	1	13
<b>Total</b>	<b>18</b>	<b>12</b>	<b>8</b>	<b>10</b>	<b>10</b>	<b>7</b>	<b>7</b>	<b>7</b>	<b>4</b>	<b>2</b>	<b>5</b>	<b>5</b>	<b>96</b>

Table 4.2 Number of salivary samples collected from each KN/KTK female. \*monkeys picked up the rope bait, ate corn, but not chewed the rope. # monkey did not pick up the rope bait. Female No.8 was lost from the observation since May.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
1. Bob	5	3	0*	2	2	0*	2	0*	2	2	2	1	21
2. Caesar	2	2	3	1	1	2	0*	1	1	3	2	1	19
3. Dragon	0*	2	4	1	2	0*	1	1	2	1	1	1	16
4. Fu	0 <sup>#</sup>	0 <sup>#</sup>	0 <sup>#</sup>	0 <sup>#</sup>	0 <sup>#</sup>	0 <sup>#</sup>	0 <sup>#</sup>	0 <sup>#</sup>	0 <sup>#</sup>	0 <sup>#</sup>	0 <sup>#</sup>	0 <sup>#</sup>	0
5. Jam	1	1	2	1	0 <sup>#</sup>	0*	1	1	1	1	0*	0*	9
6. Lord	1	3	2	0*	1	1	2	0*	1	0*	2	1	14
7. Milk	0*	0*	1	0*	0*	0*	0*	0*	0*	0*	0*	0*	1
8. Pang	0 <sup>#</sup>	0 <sup>#</sup>	0 <sup>#</sup>	0 <sup>#</sup>	-	-	-	-	-	-	-	-	0
9. Raven	0*	0*	0*	0*	0*	0*	0*	0*	0*	0*	0*	0*	0
10. TK	0*	0*	0*	0*	0 <sup>#</sup>	0 <sup>#</sup>	0 <sup>#</sup>	0 <sup>#</sup>	0 <sup>#</sup>	0 <sup>#</sup>	0 <sup>#</sup>	0 <sup>#</sup>	0
Total	9	11	12	5	6	3	6	3	7	7	7	4	80

## 5. Physical data

The physical data of temperature, rainfall, humidity and photoperiod were accessed from the Meteorological Station during January – December 2018. The values were fluctuated throughout the year and not significantly different between two populations. However, rain fall in the KN/KTK was higher than the WHM at the end of the year (October – December) (Figure 4.12). Monthly rainfall ranged from 0 to 230.2 mm (mean±SE = 87.9±83.4 mm; totally 1,054.4 mm) for WHM and from 1.40 to 456.6 mm (mean±SE = 165.8±139.5 mm; totally 1,989.7 mm) for KN/KTK. Monthly photoperiod ranged from 3.69 to 8.47 hour (mean±SE = 7.03±1.76 hour) for WHM and from 4.0 to 9.4 hour (mean±SE = 5.90±1.75 hour) for KN/KTK. Monthly temperature ranged from 26.1 to 29.5°C (mean±SE = 28.1±1.1°C) and from 26.8 to 29.2°C (mean±SE = 28.0±0.7°C) for WHM and KN/KTK, respectively. Monthly relative humidity ranged from 72 to 83% (mean±SE = 77±3%) for WHM and from 74 to 85% (mean±SE = 80.3±3%) for KN/KTK (Table 4.3). Significant correlations of physical data and sexual behaviors were detected only for the WHM population as follows; rainfall and P behavior ( $r = -0.690$ ,  $p < 0.05$ ), rainfall and A behavior ( $r = -0.867$ ,  $p < 0.01$ ), temperature and A behavior ( $r = -0.595$ ,  $p < 0.05$ ) and temperature and R behavior ( $r = -0.737$ ,  $p < 0.01$ ). The association between the number of newborns and the rainfall was also observed that the number of newborns tended to be high before the peak period of the rainfall in both WHM and KN/KTK population (Figure 4.13).

**Table 4.3** Ranged (mean $\pm$ SE) monthly temperature ( $^{\circ}$ C), rainfall (mm), relative humidity (%) and photoperiod (hour) at WHM and KN/KTK from January to December 2018 (Source: Thai Meteorological Department)

	WHM	KN/KTK
Temperature	26.1 - 29.5 $^{\circ}$ C (28.1 $\pm$ 1.1 $^{\circ}$ C)	26.8 - 29.2 $^{\circ}$ C (28.0 $\pm$ 0.7 $^{\circ}$ C)
Rainfall	0 - 230.2 mm (87.9 $\pm$ 83.4 mm)	1.40 - 456.6 mm (165.8 $\pm$ 139.5 mm)
Relative humidity	72 - 83% (77 $\pm$ 3%)	74 - 85% (80.3 $\pm$ 3%)
Photoperiod	3.69 - 8.47 hour (7.03 $\pm$ 1.76)	4.0 - 9.4 hour (5.90 $\pm$ 1.75 hour)

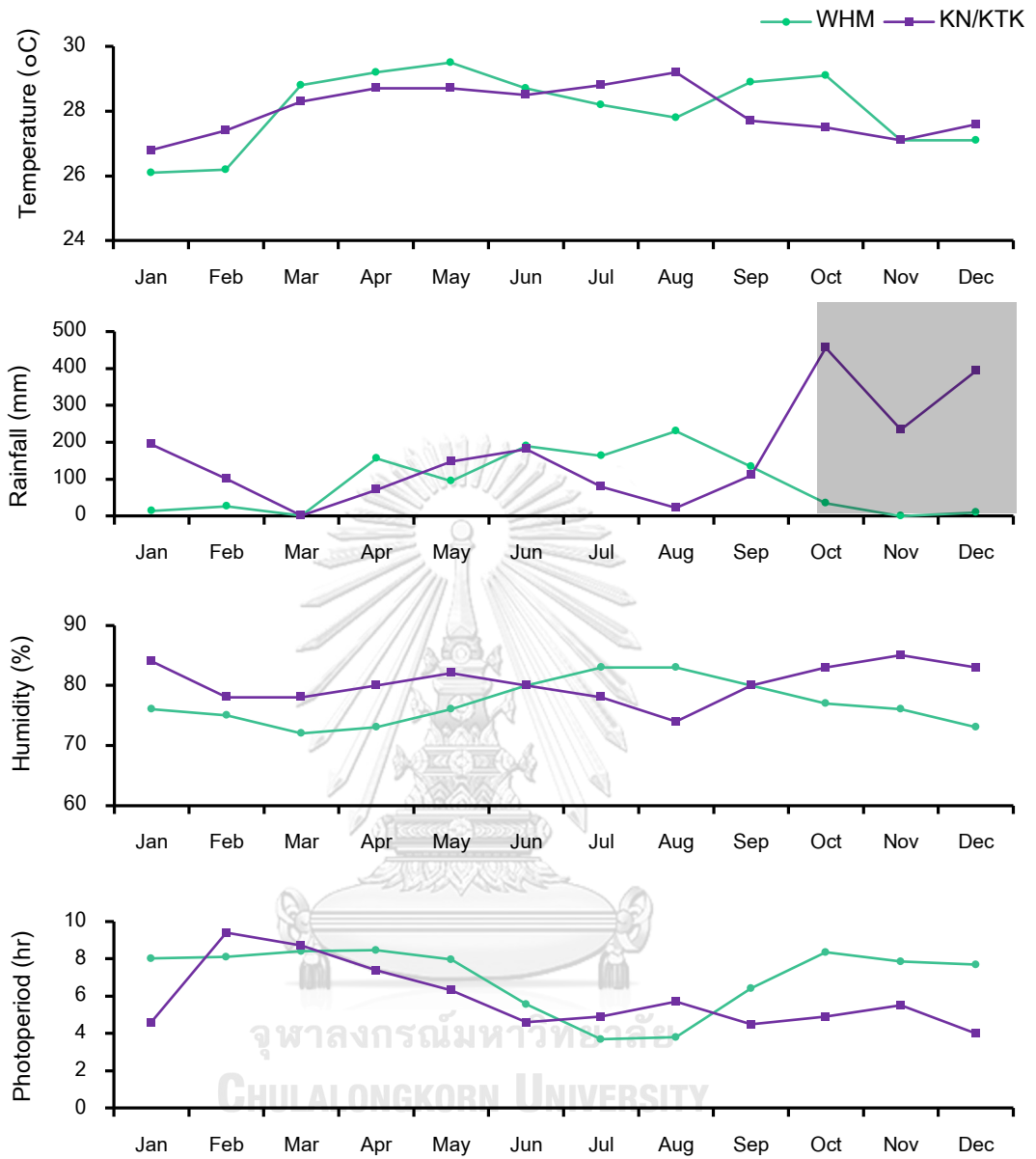


Figure 4.12 Average monthly temperature (°C), rainfall (mm), relative humidity (%) and photoperiod (hour) at WHM and KN/KTK from January to December 2018. Gray box indicates the period of the higher rainfall in the KN/KTK than the WHM population (Source: Thai Meteorological Department).

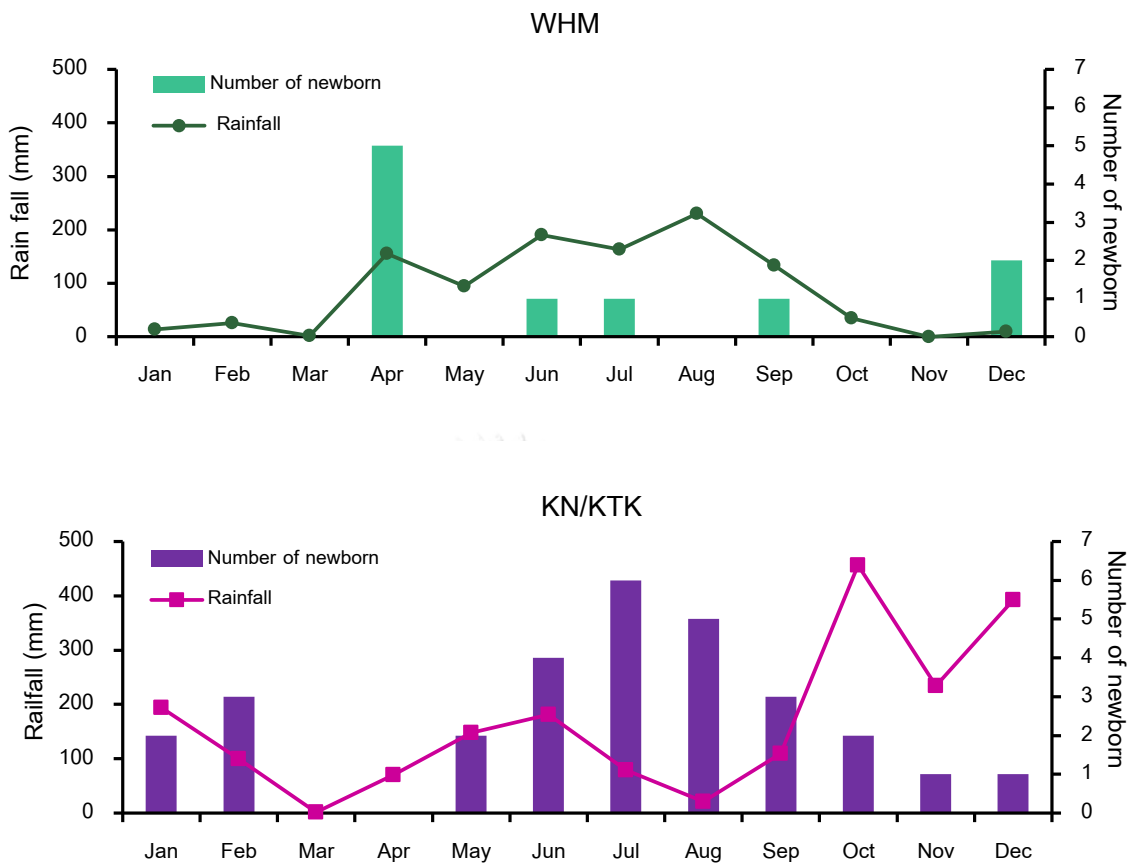


Figure 4.13 Monthly rainfall (mm) and number of newborns from January to December

2018 of the WHM and KN/KTK population

## CHAPTER V

### GENERAL DISCUSSION AND CONCLUSION

Long-tailed macaques are the most encountered species among 17 non-human primates in Thailand (Grove, 2001; Malaivijitnond and Hamada, 2008; Malaivijitnond et al., 2012). They live closely with rhesus macaques at 15 - 21°N, which was proposed as a hybrid zone, and a hybridization between the two species was confirmed by morphological characteristics (Hamada et al., 2016), ABO blood group (Kanthaswamy et al., 2017; Malaivijitnond et al., 2008) and various genetic markers including mtDNA, Y-chromosome, nuclear DNA, microsatellite DNA and SNPs (Tosi et al., 2002; Street et al., 2007; Kanthaswamy et al., 2008; Bonhomme et al., 2009; Stevison and Kohn, 2009; Osada et al., 2010; Bunlungsup et al., 2017a; 2017b), however, no reports on reproductive pattern in hybrid populations were found. Reproduction is essential for the existing of the species, thus understanding the influence of hybridization on sexual behaviors, fecundity (or birth) and physiological signs of ovulation timing (or sex skin swelling and reddening) in hybrid macaques is important. Because rhesus and long-tailed macaques are the two most species commonly used for biomedical research, thus, knowledge on the reproduction and fecundity of the species that carry different levels of genetic admixture of another species will help to improve the breeding program of the primate centers.

Regarding the recent study of Bunlungsup et al. (2017b), long-tailed macaques originating at WHM (16°51'N; Indochinese region) and at KN/KTK (7°12'N; Sundaic region) carried different levels (50% and 15%, respectively) of genetic admixture of rhesus ancestry, analyzed by autosomal SNPs, thus these two populations should be

good representatives of the Indochinese and Sundaic long-tailed macaque populations to study sexual behaviors and fecundity.

### 1. Sexual behaviors and birth

In this study, mounting by the females (P behavior) and masturbation by males (A behavior) were rarely seen; it was observed a few times in January (a highest month of sexual behavior performed) in the WHM population. This was in line with the previous report in Japanese macaques (*M. fuscata*) that the masturbation was high in mating season and mostly occurred in low-ranking males (Ruth and Soltis, 2004). In consistent with the previous study in long-tailed macaques species that copulation calls occurred equally in copulations with and without ejaculation (Nikitopoulos et al., 2004). Although the sperm plug was the important clue to confirm successful copulation, it was difficult to see in free-ranging macaques. Thus, sperm plug was not always visible to the observer during focal animal following, and some female macaques ran away immediately from their partners after the complete copulation. Some other factors affected the sperm plug visibility were depth of reproductive tract (Danzy et al., 2009), the volume of seminal fluid which was usually high amount in mating season in male rhesus macaques (Zamboni et al., 1974; Settlage and Hendrickx., 1974), and some females took the sperm plug on their vagina or ate. The sexual behavior that was observed often was female solicitation, male genital inspection, and then mating. Noting that long-tailed macaques in this study were aggressive during the peak period of sexual behaviors which led to the difficulty on behavioral observation.

Although both WHM and KN/KTK populations showed three sexual behaviors of proceptivity, attractivity and receptivity throughout the year, the different peak periods



were detected between populations; the peak period of the WHM population (November – March) was approximately one-month earlier than the KN/KTK population (December – April). Following the period of receptive behavior (or copulation), the birth peaks that occurred in April - July (for WHM population) and June - September (for KN/KTK population) were associated with a 24-week gestation period in long-tailed macaques (Fooden, 1995). The birth peaks of Thai long-tailed macaques aligned with that of long-tailed macaques housed in the primate breeding facility located near Kula Lumpur, Malaysia where births occurred throughout the year and peaked during May – August (Kavanagh and Laursen, 1982). Although the birth patterns were in line with that of sexual behaviors which were scattering throughout the year in both populations, the fecundity rate was different between two populations. The KN/KTK population that lived in Sundaic region had a higher fecundity rate than the WHM population who lived in Indochinese region (29 newborns counted for KN/KTK and 9 newborns for WHM during January – December 2018). van Schaik and colleagues (1999) classified the reproductive seasonality in primates into three groups based on birth count with a three-month period, that is, >67% of births is a strictly seasonal breeder, 33-67% is a moderately seasonal breeder, and <33% is non-seasonal breeder. The difference between moderately and strictly seasonal breeders is that when the moderately seasonal breeders are housed in captivity at temperate latitudes they lose their seasonality, but the strictly seasonal breeders keep retaining their high seasonality despite abundance of foods. Following the classification of van Schaik and colleagues (1999), both WHM (56% of births between April-June) and KN/KTK (52% of births between June-August) long-tailed macaques were fallen into the classification of moderately seasonal breeders. This finding is discorded with many previous studies concluding that long-tailed macaques are non-seasonal breeder (Hobbs et al., 1987; Tardif et al., 2012). Recently, Trebouet et

al. (2020) analyzed the effects of environment (ecological hypothesis; (Janson and Verdolin 2005)) and genetics (phylogenetic inertia hypothesis; (Hansen and Orzack 2005)) on reproductive seasonality in 13 out of 23 existing species of macaques (Groves 2001; Roos and Zinner 2015; Fan et al. 2017). They suggested that a species latitudinal location with different environment had strong influenced on reproductive seasonality. Their analysis also indicated that the reproductive seasonality in macaques did not entirely follow the phylogenetic inertia hypothesis, because some macaques within the same genetic clade based on mitochondrial genome analysis (Evans et al. 2020) showed large variation of birth seasonality from non-seasonal to moderately seasonal breeding (25% < births count < 56%). This agrees with the previous studies on the reproduction of Old World Monkeys (Tardif et al., 2011; 2012) denoting that the degree of seasonality in Old World Monkeys is highly variable and not related to phylogeny. Thus, it is not surprise here that WHM and KN/KTK macaques who carried different levels of genetic admixture of rhesus ancestry as large as 35% showed a similar pattern of moderately seasonal breeding.

The slightly shifted peak of sexual behaviors and births between the two populations may cause by the different environmental factors. Although the latitudinal locations between these two populations were only 9 degrees apart, they belong to different zoogeographical regions; Indochina and Sunda. In many primate species, environmental factors, such as climate conditions and food availability (van Schaik and van Noordwijk, 1985) are associated with reproductive seasonality and birth peak. The result in this study is in line with the previous report denoting that long-tailed macaques in Peninsular Malaysia which located near a species' southernmost geographic range may give birth any time of years depending on vegetation phenology. Macaques inhabiting different locations that had different timings of food abundance presented

different timings of birth peak (Kavanagh and Laursen, 1982). A birth window can be wider according to the optimal timing of resource availability (Wilson and Mittermeier, 2009; Stein and Hayssen, 2013). Ridley (1986) also hypothesized that primate species who lived in multimale-multifemale group with a higher proportion of operational sex ratio showed the short birth season, however the sex ratio in WHM (1 male : 3.7 females) and KN/KTK (1 male : 3.4 females) populations were low which should not affect the birth season.

## 2. Sex skin swelling and reddening

Although rhesus and long-tailed macaques lived in multimale-multifemale group, females of these two species transmitted different visual signals of periovulatory period to the males. In rhesus macaques, there are a plexus of large, thin-walled blood vessel below the epidermis around sex skin (Collings, 2005), thus, sex skin reddening covered a large area (Dixon, 1998). Sex skin reddening in rhesus macaques indicated the start of the mating season (Fooden, 2000). In long-tailed macaques, the sex skin was swollen at the base of the tail with a narrow area of reddening which indicated the ovulation time (Fooden, 1995; Engelhart et al., 2015). In this study, the WHM female long-tailed macaques who carried 50% of genetic admixture of rhesus ancestry had a smaller sex skin swelling at the base of the tail, but larger sex skin reddening area, than the KN/KTK females who gained only 15% of genetic admixture of rhesus ancestry. Changes of sex skin in the WHM long-tailed macaques were somewhat intervened by that of rhesus macaques' pattern, while that of the KN/KTK population was inclined to the long-tailed macaques. This finding confirms the previous study reporting that Indochinese long-tailed macaques less developed the swelling at the base of the tail, only Level 1 or 2,

comparing to the Sundaic ones which were mostly at Level 4 (Malaivijitnond et al., 2007a). In regard to the scenario of hybridization between long-tailed and rhesus macaques (Tosi et al., 2002; Street et al., 2007; Kanthaswamy et al., 2008; Bonhomme et al., 2009; Stevison and Kohn, 2009; Osada et al., 2010; Bunlungsup et al., 2017a; 2017b) was that the male rhesus macaques introgressed into and mated with females of long-tailed populations (Tosi et al., 2002; Bunlungsup et al., 2017a). These results indicate that the autosomal SNPs genetic admixture of rhesus ancestry had an effect on physiological signs of ovulation timing (sex skin swelling and reddening) of female long-tailed macaques.

Although the peak periods of sexual behaviors of the WHM population (November – March) and the KN/KTK population (December – April) were detected, the scores of sex skin swelling and reddening area were consistent throughout the year in both populations. Thus, no correlation between behaviors and changes of sex skin was detected. This finding confirms previous study reporting that sex skin swelling was an unreliable indicator for the fertile phase in long-tailed macaques, but sexual behavior reliably indicated the timing of fertile phase (Engelhardt et al., 2005), thus the period of birth peaks of long-tailed macaques in this study was still followed the peak periods of sexual behaviors. Another possibility is that female long-tailed macaques might use sex pheromones, potential olfactory clues, to signal the fertile phase to male long-tailed macaques (Goldfoot et al., 1978; Michael and Keverne, 1968, 1970; Michael et al., 1971).

### 3. Saliva collection and salivary estradiol assay

Although the method developed in this study was not successful for salivary estradiol assay, it could give some clues for future studies as follows;

1. As mentioned earlier that the method of saliva collection was modified from that of Toyoda and his colleagues (2020) which was conducted in stump-tailed macaques (*M. arctoides*), because stump-tailed macaques were heavier, the size of the rope bait used for long-tailed macaques should be smaller than that for stump-tailed macaques; 6 mm in diameter and 10 cm long for stump-tailed macaques and 2 mm in diameter and 6 cm long for long-tailed macaques.
2. The dried sugary rope bait was hard and difficult to be chewed, it should be crushed before being scattered on the ground.
3. To bait the rope to the monkeys, it is important to notice the rank of the monkeys before experimenting. Usually, the high-ranking female picked up the rope before the low-ranking female and sometimes the discarded rope was re-picked and chewed by low-ranking female.
4. Infant sometimes took the chewed rope from the mouth of his/her mother.
5. During a peak period of sexual behaviors, animals were very aggressive, and difficult to collect the chewed rope.
6. The researcher should train the targeted monkeys to learn about the rope bait (with corn and honey), after they learned to recognize the bait, the corn and honey can be omitted afterwards.
7. To collect the saliva, it should be performed in the morning, this is to eliminate the interference of feeding.
8. The duration of rope-chewing should be recorded.

9. This rope bait method may not be suitable for non-habituated monkeys.
10. After five months of the rope bait method was initiated, the number of salivary samples collected was decreased, researcher should create the new bait then.
11. Because in the present study, the chewed rope was directly immersed in 95% ethanol, without measuring the volume of the saliva, thus it is recommended to separate saliva from the rope, measure the volume and record before hormonal analysis in the laboratory.

#### 4. Hybridization between rhesus and long-tailed macaques and genetic analyses

Recently, Ito et al. (2020) used a very sensitive marker, a double-digest restriction site-associated DNA sequencing (or ddRAD), to draw the scenario of the hybridization between rhesus and long-tailed macaques. They reported that some rhesus macaque-biased alleles (especially on autosomal chromosomes) widely introgressed into long-tailed macaque populations, while some alleles (especially on X chromosomes) infected and concentrated around the hybrid zone of 15 – 21°N. In agreement with the previous report using autosomal SNP makers (Bunlungsup et al., 2017b) indicated that an introgression of the rhesus ancestry was far beyond the proposed hybrid zone and the rhesus gene flow to long-tail macaques (from north to south) declined gradually in proportion to the distance. All in all, it can signify that the autosomal genetic admixture of rhesus ancestry into long-tailed populations does not affect the reproductive seasonality (or sexual behaviors) of long-tailed macaques, but it can interfere their fecundity and pattern of changes of sex skin. Thus, the WHM female long-tailed macaques that carried 50% of rhesus genetic admixture showed similar patterns of sexual behaviors with that of the KN/KTK female long-tailed macaques who

carried only 15% of rhesus genetic, while the fecundity and sex skin reddening pattern of WHM females were more prone to that of rhesus macaques than the KN/KTK females.

## 5. Conclusion

In conclusion and in reference to various genetic marker analyses (Tosi et al., 2002; Street et al., 2007; Kanthaswamy et al., 2008; Bonhomme et al., 2009; Stevison and Kohn, 2009; Osada et al., 2010; Bunlungsup et al., 2017a; 2017b; Ito et al., 2020), the scenario of the hybridization between rhesus and long-tailed macaques in association with sexual behaviors (reproductive seasonality), births (fecundity) and changes of sex skin (physiological sign of ovulation) conducted in this study can be drawn as follows (see Figure 5.1 and 5.2);

1. Hybridization between the two species was mainly led by the male dispersal of rhesus macaques introgressed into long-tailed macaque populations (the direction is from north to south).
2. Male rhesus macaques who carried rhesus sex chromosomes ( $A^{rh}A^{rh}-X^{rh}Y^{rh}$ ) hybridized with female long-tailed macaques who carried long-tailed sex chromosomes ( $A^{ln}A^{ln}-X^{ln}X^{ln}$ ) and the two types of the hybrid offspring could plausibly be produced;  $A^{ln}A^{rh}-X^{ln}X^{rh}$  female and  $A^{ln}A^{rh}-X^{ln}Y^{rh}$  male.
3. Regarding the meiotic inactivation or heterochromatinization hypothesis (Jablonka and Lamb, 1990), the different region of the X chromosome between rhesus and long-tailed macaques that lacks of conformational or structural homology can cause the failure on the meiotic pairing. Thus, the fertility of  $A^{ln}A^{rh}-X^{ln}X^{rh}$  hybrid female was reduced and she could not pass the  $X^{rh}$  chromosome along another generation of long-tailed macaques ( $X^{rh}$  is

thus concentrated at the hybrid zone; Ito et al., 2020), while the  $A^{ln}A^{rh}-X^{ln}Y^{rh}$  male could propagate his genes to the next generations of long-tailed macaques ( $Y^{rh}$  is thus passed the hybrid zone and terminated at the Isthmus of Kra, a zoogeographical barrier between Indochina and Sunda; Tosi et al., 2002; Street et al., 2007; Bulungsup et al., 2017a).

4. Since in multi-male and multi-female group of macaques, males left their natal group before sexual maturity (or male dispersal) while females lived permanently in the group (or female philopatry) and the hybridization between these two species was led by the introgression of male rhesus macaques (as stated in No.1), the rhesus gene flow was occurred from the north to the south of long-tailed population. Thus, the level of autosomal genetic admixture of rhesus ancestry ( $A^{rh}$ ) in long-tailed populations ( $A^{ln}$ ) was diluted in the distant that was far beyond the proposed hybrid zone (15 - 21°N) (Bunlungsup et al., 2017b). As a result of this propagation of the male rhesus macaques' genetics, the WHM long-tailed population ( $A^{rh} = 50\%$ ;  $A^{ln} = 50\%$ ) carried higher genetic admixture of rhesus ancestry than the KN/KTK long-tailed population ( $A^{rh} = 15\%$ ;  $A^{ln} = 85\%$ ).
5. From the above mentioned Nos.3 and 4 combining with the results in this study that there were no differences in reproductive seasonality between WHM and KN/KTK macaques, while the fecundity and sex skin reddening of WHM females were more prone to rhesus macaques' pattern than the KN/KTK females, it can propose that the reproductive seasonality is controlled by the X-linked gene and the latter two parameters are controlled by the autosomal chromosomes.



6. This study indicates that reproduction and its mechanism (including sexual behaviors, births, and changes of sex skin in this study) are complicated. It is not controlled by a single gene or a single type of gene and the effect of environment needs to be included. Thus, the interpretation of the effect of the different levels of autosomal genetic admixture of rhesus ancestry into WHM and KN/KTK long-tailed macaque populations is not straightforward as it was previously thought.



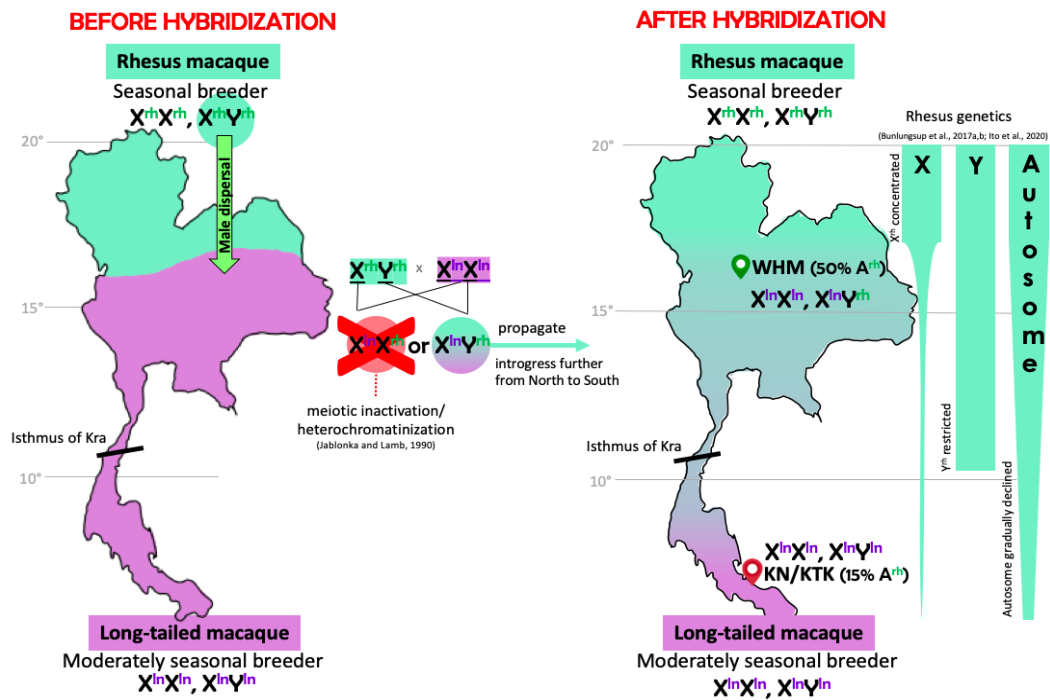


Figure 5.1 Scheme of gene flow and genetic admixture between rhesus and long-tailed macaques. Before the hybridization event (left panel), rhesus and long-tailed macaques are separated and live closely at the 15-21°N. After the hybridization event (right panel), male rhesus macaques introgressed into long-tailed population and hybridized with female long-tailed macaques. The level of autosomal genetic admixture of rhesus ancestry in long-tailed populations was diluted in the distant that is far beyond the proposed hybrid zone, thus the WHM population carries higher level of genetic admixture of rhesus ancestry ( $A^{rh} = 50\%$ ) than the KN/KTK population ( $A^{rh} = 15\%$ ). X-linked genetic of rhesus macaques is infected and concentrated at the hybrid zone, while the Y-linked genetic of rhesus macaques is terminated at the Isthmus of Kra. A, X, Y, rh and ln = autosome, X chromosome, Y chromosome, rhesus and long-tailed macaques, respectively.

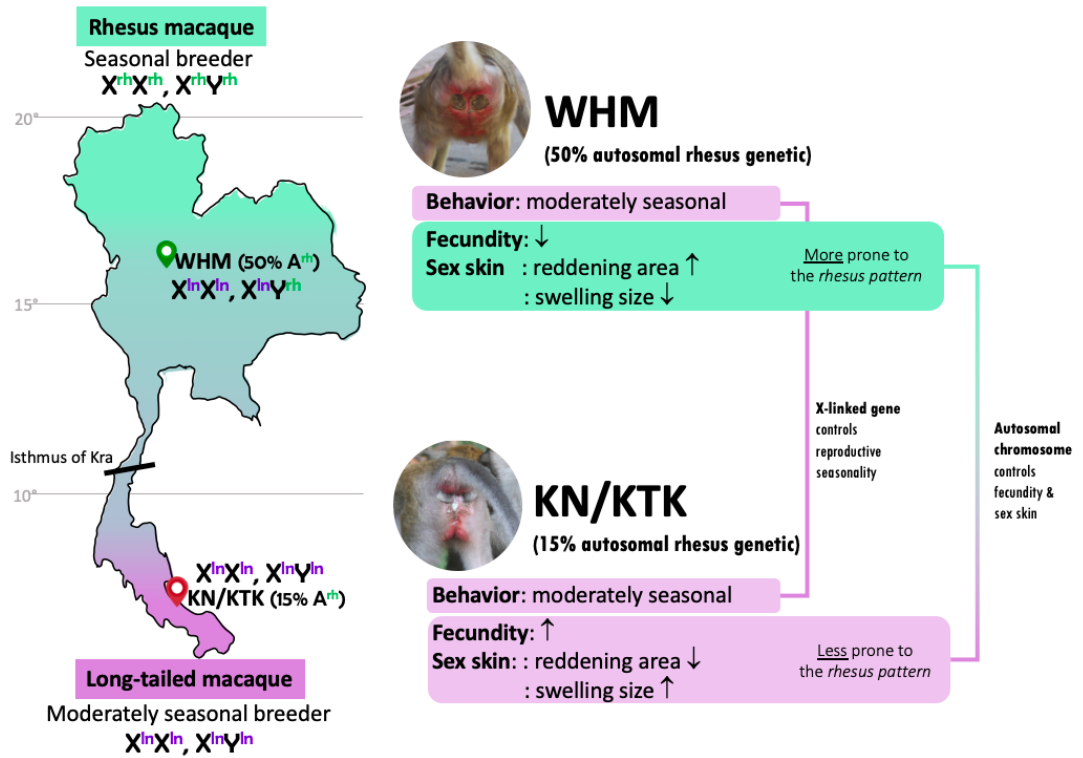


Figure 5.2 In association with the autosomal genetic admixture event between rhesus and long-tailed macaques and the results of that there were no differences in reproductive seasonality between WHM and KN/KTK macaques, while the fecundity and sex skin reddening of WHM females were more prone to rhesus macaques' pattern than the KN/KTK females, it can propose that the reproductive seasonality is controlled by the X-linked gene and the latter two parameters are controlled by the autosomal chromosomes.

## Recommendations

1. The future study on hybrid macaques that combines various factors such as genetic, behavior, morphology, environmental factors and hormonal analysis might give a better clue.
2. Because this study was conducted at the population level, and each macaque individual may have different level of genetic admixture and behavioral background, thus the comparison at the individual level is recommended. Besides the comparison between two populations that live separately in a far distance is very difficult for a good data collection from both sites.



## REFERENCES

- Altmann, J. (1974). Observational study of behavior: sampling methods. *Behaviour*, 49(3-4), 227-266.
- Beach, F. A. (1976). Sexual attractivity, proceptivity, and receptivity in female mammals. *Hormones and behavior*, 7(1), 105-138.
- Beisner, B. A., Jackson, M. E., Cameron, A., & McCowan, B. (2012). Sex ratio, conflict dynamics, and wounding in rhesus macaques (*Macaca mulatta*). *Applied Animal Behaviour Science*, 137(3-4), 137-147.
- Bonhomme, M., Cuartero, S., Blancher, A., & Crouau-Roy, B. (2009). Assessing natural introgression in 2 biomedical model species, the rhesus macaque (*Macaca mulatta*) and the long-tailed macaque (*Macaca fascicularis*). *The Journal of heredity*, 100, 158-169.
- Brockman, D., & van Schaik, C. (2005). Seasonality and reproductive function. In *Seasonality in primates* (pp. 269-298). Cambridge: Cambridge University Press.
- Bunlungsup, S., Imai, H., Hamada, Y., Matsudaira, K., & Malaivijitnond, S. (2017a). Mitochondrial DNA and two Y-chromosome genes of common long-tailed macaques (*Macaca fascicularis fascicularis*) throughout Thailand and vicinity. *American Journal of Primatology*, 79.
- Bunlungsup, S., Oldt, R., Smith, D., Houghton, P., Hamada, Y., & Malaivijitnond, S. (2017b). Genetic analysis of samples from wild populations opens new perspectives on hybridization between long-tailed (*Macaca fascicularis*) and rhesus macaques (*Macaca mulatta*). *American Journal of Primatology*, 79.
- Chapais, B. (1983). Reproductive activity in relation to male dominance and likelihood of ovulation in rhesus monkeys. *Behavioral Ecology and Sociobiology*, 12, 215-228.
- Collings, M. (2005). A study of the cutaneous reddening and swelling about the genitalia of the monkey, macacus rhesus. *The Anatomical Record*, 33, 271-287.
- Danzy, C., Gutierrez, V., Pampush, J., & Campbell, B. (2009). Factors affecting the distribution of copulatory plugs in rhesus monkeys (*Macaca mulatta*) on Cayo

- santiago. *Folia Primatologica; International Journal of Primatology*, 80, 264-274.
- Dixson, A. (1998). Comparative studies of the prosimians, monkeys, apes, and human beings. In *Primate Sexuality* (pp. 315-353). Oxford: Oxford University Press.
- Du, Y., Fan, T.-Y., Tan, Y., Xiong, Z., & Wang, Z. (2010). Seasonal changes in the reproductive physiology of female rhesus macaques (*Macaca mulatta*). *Journal of the American Association for Laboratory Animal Science : JAALAS*, 49(3), 289-293.
- Dubuc, C., Brent, L., Accamando, A., Gerald, M., MacLarnon, A., Semple, S., . . . Engelhardt, A. (2009). Sexual skin color contains information about the timing of the fertile phase in free-ranging *Macaca mulatta*. *International Journal of Primatology*, 30, 777-789.
- Engelhardt, A., Hodges, K., Niemitz, C., & Heistermann, M. (2005). Female sexual behavior, but not sex skin swelling, reliably indicates the timing of the fertile phase in wild long-tailed macaques (*Macaca fascicularis*). *Hormones and Behavior*, 47, 195-204.
- Evans, B., Gansauge, M.-T., Tocheri, M., Schillaci, M., Sutikna, T., Jatmiko, . . . Meyer, M. (2020). Mitogenomics of macaques (*Macaca*) across Wallace's Line in the context of modern human dispersals. *Journal of Human Evolution*, 146, 102.
- Fan, P.-F., Zhang, Z., Zhao, C., Li, C., Liu, W., Liu, Z., & Li, M. (2017). Phylogenetic position of the white-cheeked macaque (*Macaca leucogenys*), a newly described primate from Southeastern Tibet. *Molecular Phylogenetics and Evolution*, 107, 80-89.
- Fooden, J. (1964). Rhesus and crab-eating macaques: intergradation in Thailand. *Science* 143, 363-364.
- Fooden, J. (1982). Ecogeographic segregation of macaque species. *Primates*, 23, 574-579.
- Fooden, J. (1990). The bear macaque, *Macaca arctoides*: a systematic review. *Journal of Human Evolution*, 19, 607-686.
- Fooden, J. (1995). Systematic review of southeast Asian long tail macaques, *Macaca fascicularis* (Raffles, 1821). *Fieldiana Zoology*, 8, 1-206.

- Fooden, J. (1997). Tail length variation in *Macaca fascicularis* and *M. mulatta*. *Primates*, 38(3), 221-231.
- Fooden, J. (2000). Systematic review of the rhesus macaque, *Macaca mulatta* (Zimmermann, 1780). *Fieldiana Zoology*, 96, 1-180.
- Fooden, J. (2006). Comparative review of fascicularis-group species of macaques (primates: *Macaca*). *Fieldiana Zoology*, 107, 1-43.
- Gordon, T. (1981). Reproductive behavior in the rhesus monkey: Social and endocrine variables. *Integrative and Comparative Biology* 21, 185-195.
- Groves, C. P. (2001). Primate taxonomy. In *Primate taxonomy* (pp. 350). Washington, DC: Smithsonian Institution Press.
- Gumert, M. (2011). Long-tailed macaque populations, ethnoploresy, and their occurrence in human environments. In *The common monkey of Southeast Asia* (pp. 3-44). Cambridge: Cambridge University Press.
- Hamada, Y., San, A. M., & Malaivijitnond, S. (2016). Assessment of the hybridization between rhesus (*Macaca mulatta*) and long-tailed macaques (*M. fascicularis*) based on morphological characters. *American Journal of Physical Anthropology*, 159(2), 189-198.
- Hamada, Y., Urasopon, N., Hadi, I., & Malaivijitnond, S. (2006). Body size and proportions and pelage color of free-ranging *Macaca mulatta* from a zone of hybridization in northeastern Thailand. *International Journal of Primatology*, 27, 497-513.
- Hamada, Y., Watanabe, T., Takenaka, O., Suryobroto, B., & Kawamoto, Y. (1988). Morphological studies on the sulawesi macaques I: Phyletic analysis of body color. *Primates*, 29, 65-80.
- Hansen, T., & Orzack, S. (2005). Assessing current adaptation and phylogenetic inertia as explanations of trait evolution: The need for controlled comparisons. *Evolution; International Journal of Organic Evolution*, 59, 2063-2072.
- Harrison, T., Krigbaum, J., Manser, J., Lehman, S., & Fleagle, J. (2006). A paleontological and zooarchaeological perspective. In *Primate biogeography and ecology on the sunda shelf islands* (pp. 331-372).

- Hobbs, K. R., Welshman, M. D., Nazareno, J. B., & Resuello, R. G. (1987). Conditioning and breeding facilities for the cynomolgus monkey (*Macaca fascicularis*) in the Philippines: a progress report on the SICONBREC project. *Laboratory Animals*, 21(2), 131-137.
- Hrdy, S., & Whitten, P. (1987). Patterning of sexual activity. In *Primate societies* (pp. 370-384). Chicago: University of Chicago Press.
- Ito, T., Kanthaswamy, S., Bunlungsup, S., Oldt, R., Houghton, P., Hamada, Y., & Malaivijitnond, S. (2020). Secondary contact and genomic admixture between rhesus and long-tailed macaques in the Indochina Peninsula. *Journal of Evolutionary Biology*, 33, 1164-1179.
- Jablonka, E., & Lamb, M. (1990). The evolution of heteromorphic sex chromosomes. *Biological reviews of the Cambridge Philosophical Society*, 65, 249-276.
- Jadejaroen, J. (2016). *Morphological characters, genetics and behaviors of hybrids between rhesus (Macaca mulatta) and long-tailed macaque (M. fascicularis) at Khao Khieow Open Zoo*. (Doctoral dissertation). Chulalongkorn University,
- Jadejaroen, J., Hamada, Y., Kawamoto, Y., & Malaivijitnond, S. (2015). Use of photogrammetry as a means to assess hybrids of rhesus (*Macaca mulatta*) and long-tailed (*M. fascicularis*) macaques. *Primates*, 56.
- Janson, C., & Verdolin, J. (2005). Seasonality of primate births in relation to climate. In *Seasonality in primates: Studies of living and extinct human and non-human primates* (pp. 307-350). Cambridge: Cambridge University Press.
- Kanthaswamy, S., Ng, J., Oldt, R. F., Valdivia, L., Houghton, P., & Smith, D. (2017). ABO blood group phenotype frequency estimation using molecular phenotyping in rhesus and cynomolgus macaques. *HLA*, 90.
- Kanthaswamy, S., Satkoski Trask, J., George, D., Kou, A., Erickson, B., & Smith, D. (2008). Interspecies hybridization and the stratification of nuclear genetic variation of rhesus. *International Journal of Primatology*, 29, 1295-1311.
- Kappeler, P., & Pereira, M. (2003). Primate life histories and socioecology. In *Primate life histories and socioecology* (pp. 1-20). Chicago: The University of Chicago Press.



- Kavanagh, M., & Laursen, E. (1984). Breeding seasonality among long-tailed macaques, *Macaca fascicularis*, in peninsular Malaysia. *International Journal of Primatology*, 5, 17-29.
- Kumar, R., Radhakrishna, S., & Sinha, A. (2011). Of least concern? range extension by rhesus macaques (*Macaca mulatta*) threatens long-term survival of bonnet macaques (*M. radiata*) in Peninsular India. *International Journal of Primatology*, 32(4), 945-959.
- Kyes, P., Thamsenanupap, P., Tanee, T., Intralawan, A., & Kyes, R. (2018). Previously unreported population of rhesus macaques *Macaca mulatta* in Chiang Rai province, Thailand: preliminary observations. *Asian Primates Journal : a journal of the Southeast Asia, South Asia and China of the IUCN/SSC Primate Specialist Group*, 7, 6-13.
- Lekagul, B., & McNeely, J. A. (1988). *The mammals of Thailand* (Vol. 60). Bangkok: Darnsutha Press.
- Lindburg, D. G. (1971). The rhesus monkey in north India: An ecological and behavioral study. In L. A. Rosenblum (Ed.), *Primate behaviour: Developments in the field and laboratory research* (Vol. 2, pp. 83-104). New York: Academic Press.
- Lubinsky, M. (2013). *Estradiol extraction and quantification in macaques: development and evaluation of sampling methods*. (Master of science). York University,
- Majumder, J., Lodh, R., & Agarwala, B. (2012). Fish feeding adaptation by rhesus macaque *Macaca mulatta* (Cercopithecidae) in the Sundarban mangrove swamps, India. *Journal of Threatened Taxa*, 4, 2539-2540.
- Malaivijitnond, S., Arsaithamkul, V., Tanaka, H., Pomchote, P., Jaroenporn, S., Suryobroto, B., & Hamada, Y. (2012). Boundary zone between northern and southern pig-tailed macaques and their morphological differences. *Primates; journal of primatology*, 53, 377-389.
- Malaivijitnond, S., & Hamada, Y. (2008). Current situation and status of long-tailed macaques (*Macaca fascicularis*) in Thailand. *The Natural History Journal of Chulalongkorn University*, 8, 185-204.

- Malaivijitnond, S., Hamada, Y., Suryobroto, B., & Takenaka, O. (2007). Female long-tailed macaques with scrotum-like structure. *American Journal of Primatology*, 69, 721-735.
- Malaivijitnond, S., Hamada, Y., Varavudhi, P., & Takenaka, O. (2005). The current distribution and status of macaques in Thailand. *The Natural History Journal of Chulalongkorn University*, 1, 35-45.
- Malaivijitnond, S., Sae-Low, W., & Hamada, Y. (2008). The human-ABO blood groups of free-ranging long-tailed macaques (*Macaca fascicularis*) and parapatric rhesus macaques (*M. mulatta*) in Thailand. *Journal of Medical Primatology*, 37, 31-37.
- Malaivijitnond, S., & Varavudhi, P. (2002). The last possible troop of semi-wild rhesus macaque (*Macaca mulatta*) in Thailand. *The Natural History Journal of Chulalongkorn University*, 2, 59-61.
- Malaivijitnond, S., Vazquez, Y., & Hamada, Y. (2011). Human impact on long-tailed macaques in Thailand. In A. Fuentes, L. Jones-Engel, & M. D. Gumert (Eds.), *Monkeys on the edge: Ecology and management of long-tailed macaques and their interface with humans* (pp. 118-158). Cambridge: Cambridge University Press.
- Michael, R., & Keverne, E. (1968). Pheromones in the communication of sexual status in primates. *Nature*, 218, 746-749.
- Michael, R., & Keverne, E. (1970). Primate sex pheromones of vaginal origin. *Nature*, 225, 84-85.
- Michael, R., Keverne, E., & Bonsall, R. (1971). Pheromones: Isolation of male sex attractants from a female primate. *Science* 172, 964-966.
- Nunn, C. (1999). The evolution of exaggerated sexual swellings in primates and the graded-signal hypothesis. *Animal Behaviour*, 58, 229-246.
- Osada, N., Uno, Y., Mineta, K., Kameoka, Y., Takahashi, I., & Terao, K. (2010). Ancient genome-wide admixture extends beyond the current hybrid zone between *Macaca fascicularis* and *M. mulatta*. *Molecular Ecology*, 19, 2884-2895.
- Osuna, C., & Whitney, J. (2017). Nonhuman primate models of zika virus infection,

- immunity, and therapeutic development. *The Journal of Infectious Diseases*, 216, 928-934.
- Pal, A., Kumara, H., Velankar, A., Mishra, P., & Singh, M. (2018). Demography and birth seasonality in the nicobar long-tailed macaque (*Macaca fascicularis Umbrosus*). *Current Science*, 114, 1732.
- Richard, A. F., Goldstein, S. J., & Dewar, R. E. (1989). Weed macaques: The evolutionary implications of macaque feeding ecology. *International Journal of Primatology*, 10(6), 569.
- Ridley, M. (1986). The number of males in a primate troop. *Animal Behaviour*, 34(6), 1848-1858.
- Rijksen, H. D. (1978). *A field study on Sumatran orang utans (Pongo pygmaeus abelii Lesson 1827); ecology, behaviour and conservation*. (Doctoral dissertation). Wageningen University,
- Rodman, P. (1991). Structural differentiation of microhabitats of sympatric *Macaca fascicularis* and *M. nemestrina* in East Kalimantan, Indonesia. *International Journal of Primatology*, 12, 357-375.
- Roos, C., & Zinner, D. (2015). Diversity and evolutionary history of macaques with special focus on *Macaca mulatta* and *Macaca fascicularis*. In B. Joerg, K. Sven, S. Emanuel, F. Gerhard, & Weinbauer (Eds.), *The nonhuman primate in nonclinical drug development and safety assessment* (pp. 3-16). San Diego: Academic Press.
- Rowe, N. (1996). The pictorial guide to the living primates. In *The pictorial guide to the living primates* (pp. 263). New york: Pogonias Press.
- San, A., & Hamada, Y. (2009). Reproductive seasonality of Myanmar long-tailed macaque (*Macaca fascicularis aurea*). *The Natural History Journal of Chulalongkorn University*, 9, 223-234.
- Semple, S., Gerald, M. S., & Suggs, D. N. (2009). Bystanders affect the outcome of mother–infant interactions in rhesus macaques. *Proceedings of the Royal Society B: Biological Sciences*, 276(1665), 2257-2262.
- Shively, C., Clarke, A., King, N., Schapiro, S., & Mitchell, G. (1982). Patterns of sexual

- behavior in male macaques. *American Journal of Primatology*, 2, 373-384.
- Smith, D., & Smith, S. (1988). Parental rank and reproductive success of natal rhesus males. *Animal Behaviour*, 36, 554-562.
- Southwick, C., & Siddiqi, M. (1966). Population changes of rhesus monkeys (*Macaca mulatta*) in India, 1959 to 1965. *Primates*, 7, 303-314.
- Southwick, C. H., Yongzu, Z., Haisheng, J., Zhenhe, L., & Wenyuan, Q. (1996). Population ecology of rhesus macaques in tropical and temperate habitats in China. *Primates*, 2, 95.
- Stein, A., & Hayssen, V. (2013). *Panthera pardus* (Carnivora: Felidae). *Mammalian Species*, 900, 30-48.
- Stevison, L., & Kohn, M. (2009). Divergence population genetic analysis of hybridization between rhesus and cynomolgus macaques. *Molecular Ecology*, 18, 2457-2475.
- Street, S. L., Kyes, R. C., Grant, R., & Ferguson, B. (2007). Single nucleotide polymorphisms (SNPs) are highly conserved in rhesus (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) macaques. *BMC Genomics*, 8(1), 480.
- Tardif, S., Carville, A., Elmore, D., Williams, L., & Rice, K. (2012). Reproduction and breeding of nonhuman primates. In *Nonhuman primates in biomedical research* (pp. 197-249). San Diego: Academic Press.
- Tardif, S., Rutherford, J., & Saltzman, W. (2011). Hormones and reproductive cycles in primates. In *hormones and reproduction of vertebrates* (Vol. 5, pp. 291-327). San Diego: Academic Press.
- Tosi, A., Morales, J., & Melnick, D. (2002). Y-chromosome and mitochondrial markers in *Macaca fascicularis* indicate introgression with indochinese *M. mulatta* and a biogeographic barrier in the Isthmus of Kra. *International Journal of Primatology*, 23, 161-178.
- Toyoda, A., Matsudaira, K., Maruhashi, T., Malaivijitnond, S., & Kawamoto, Y. (2020). Highly versatile, non-invasive method for collecting buccal DNA from free-ranging non-human primates. *BioRxiv*, 1, 50.
- Trébouet, F. (2019). *Male reproductive strategies in wild northern pig-tailed macaques*

- (*Macaca leonina*): testing the priority-of-access model. (Doctoral dissertation). Southern Illinois University
- van Noordwijk, M., & van Schaik, C. (1999). The effects of dominance rank and group size on female lifetime reproductive success in wild long-tailed Macaques, *Macaca fascicularis*. *Primates*, 40, 105-130.
- van Schaik, C., & van Noordwijk, M. (1985). Interannual variability in fruit abundance and the reproductive seasonality in Sumatran long-tailed macaques (*Macaca fascicularis*). *Journal of Zoology*, 206, 533-549.
- van Schaik, C., van Noordwijk, M., & Nunn, C. (1999). Sex and social evolution in primates. In P.C.Lee (Ed.), *Comparative primate socio-ecology* (pp. 204-240). Cambridge: Cambridge University Press.
- Vandenbergh, J., & Vessey, S. (1968). Seasonal breeding of free-ranging monkeys and related ecological factors. *Journal of Reproduction and Fertility*, 15, 71-79.
- Vanhoof, M., van Leeuwen, T., Galletta, L., & Vereecke, E. (2020). The forearm and hand musculature of semi-terrestrial rhesus macaques (*Macaca mulatta*) and arboreal gibbons (fam. Hylobatidae). Part II. Quantitative analysis. *Journal of Anatomy*, 0, 1-17.
- Wenyuan, Q., Yongzu, Z., Manry, D., & Southwick, C. H. (1993). Rhesus monkeys (*Macaca mulatta*) in the Taihang mountains, Jiyuan county, Henan, China. *International Journal of Primatology*, 14(4), 607-621.
- Wheatley, B. (1978). *The behavior and ecology of the crab-eating macaque (Macaca fascicularis) in the Kutai Nature Reserve, East Kalimantan, Indonesia*. (Doctoral dissertation). University of California, Davis,
- Wolfensohn, S., & Honess, P. (2005). Handbook of primates husbandry and welfare. *Primates*, 47(2), 129-149.

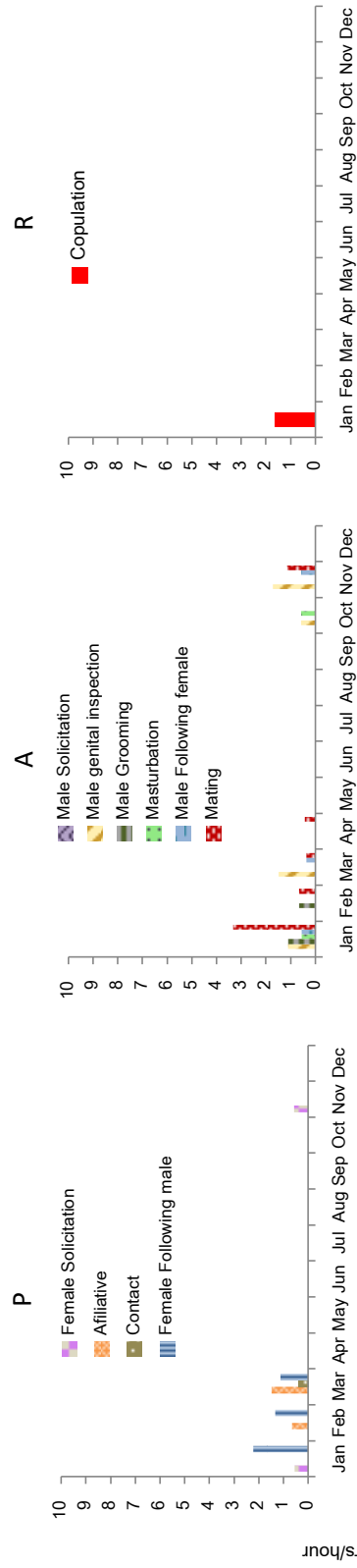


APPENDICES

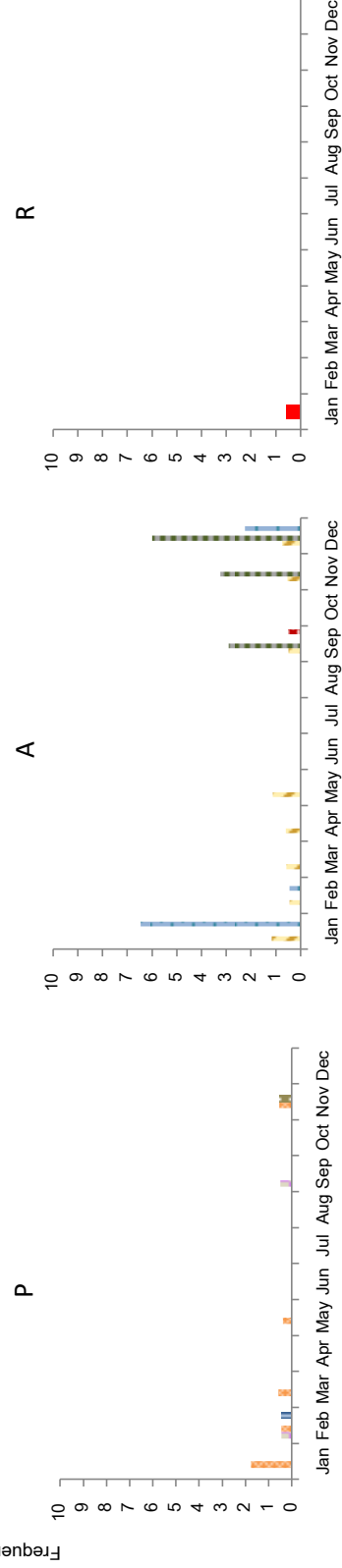
จุฬาลงกรณ์มหาวิทยาลัย  
**CHULALONGKORN UNIVERSITY**



No. 3 (Jaime)



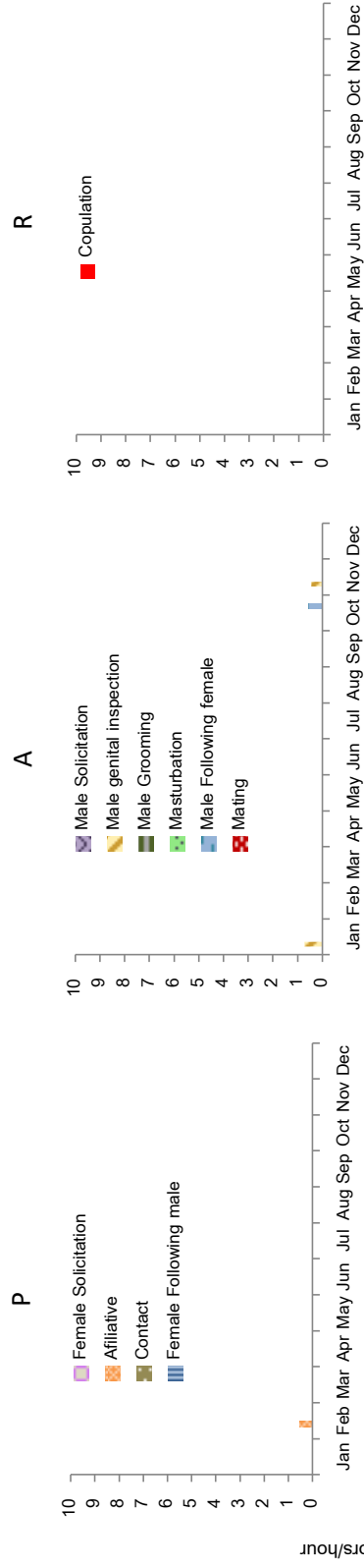
No. 4 (Leam)



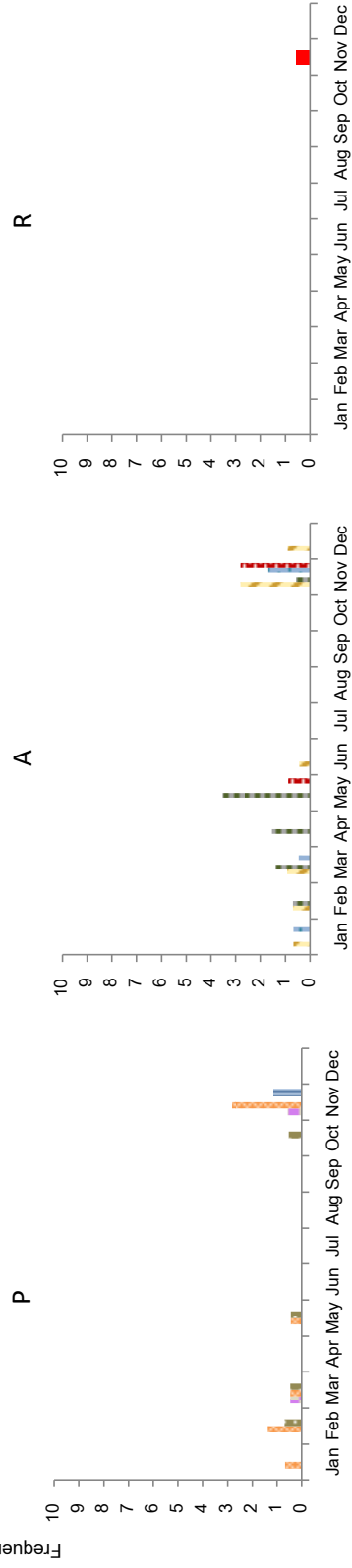




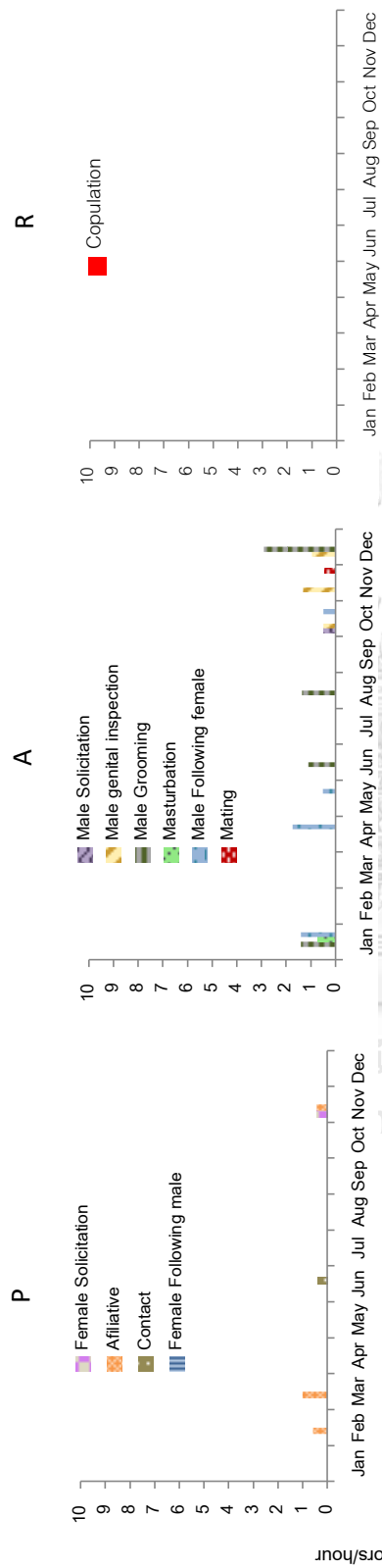
No. 7 (Rhino)



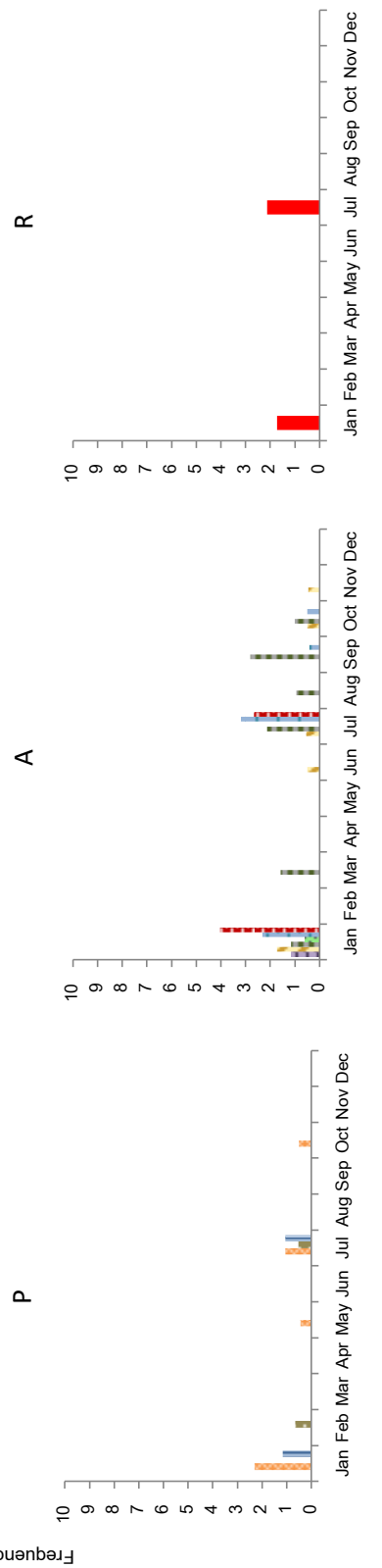
No. 8 (Tary)



No. 9 (The hound)



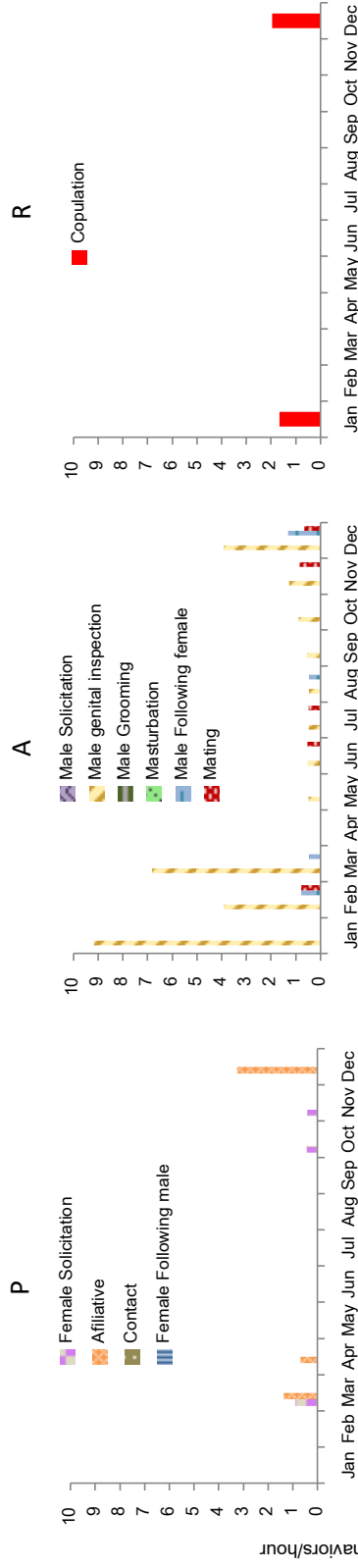
No. 10 (Yib)



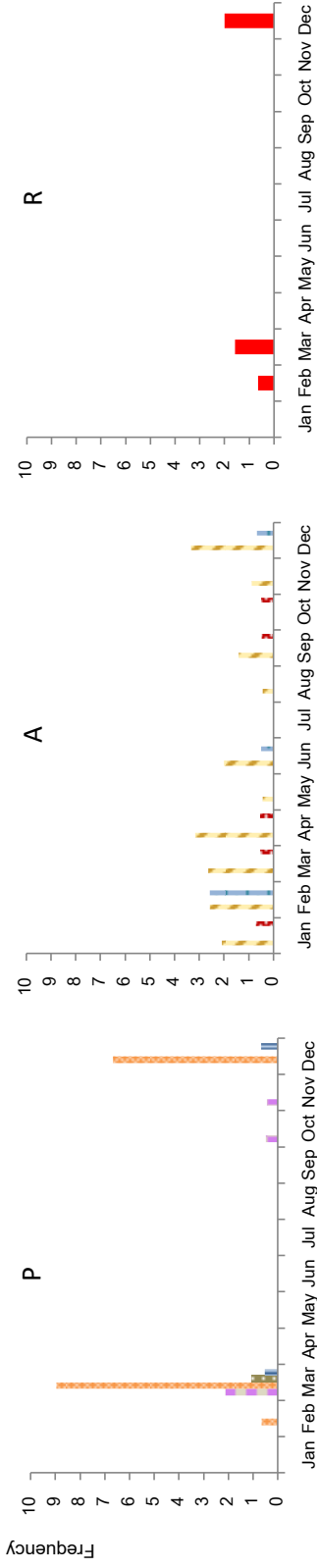
APPENDIX B; Individual data of each proceptivity (P), attractivity (A), and receptivity (R) behaviors of 10 female long-tailed macaques in

KN/KTK population.

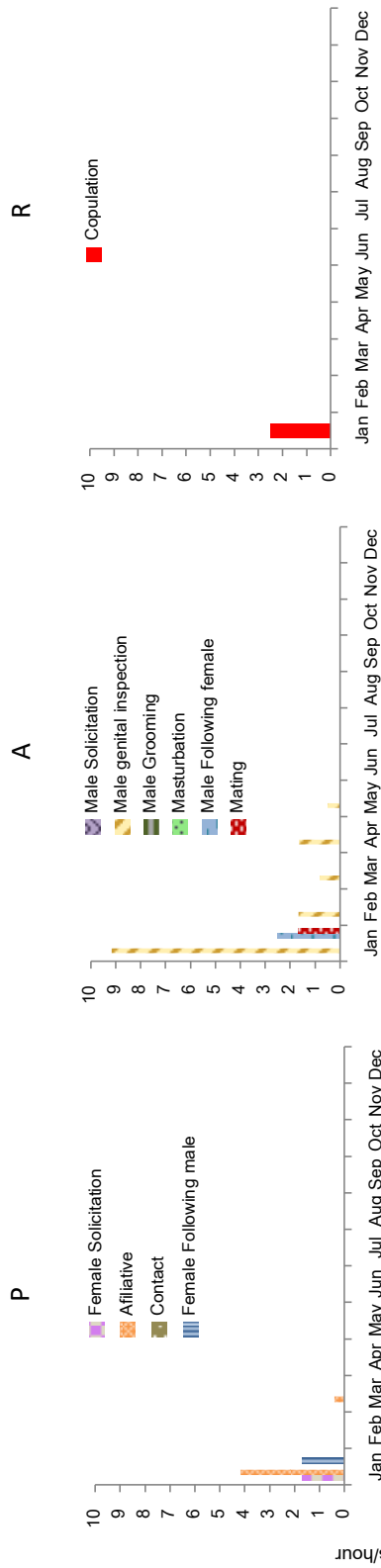
No. 1 (Bob)



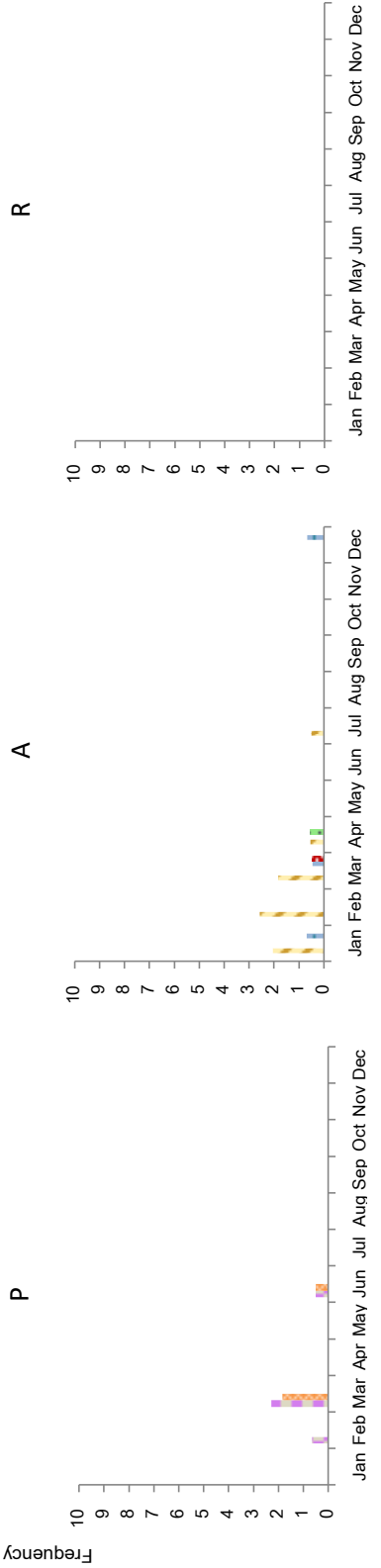
No. 2 (Cesar)



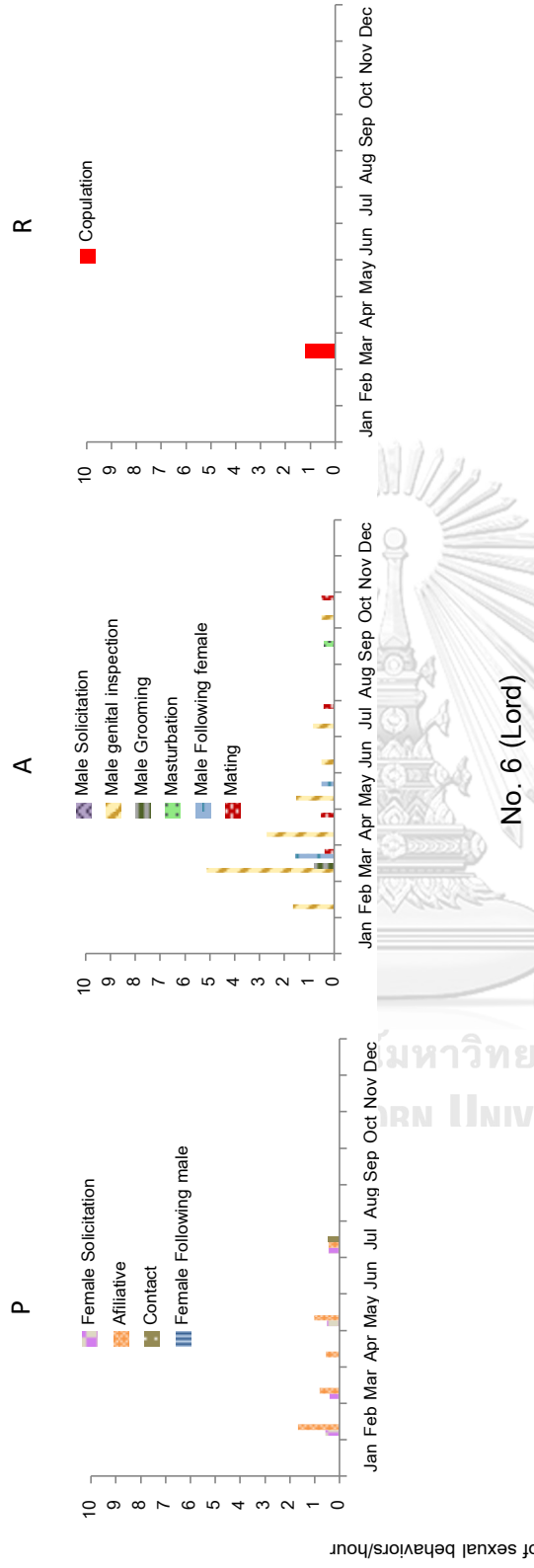
No. 3 (Dragon)



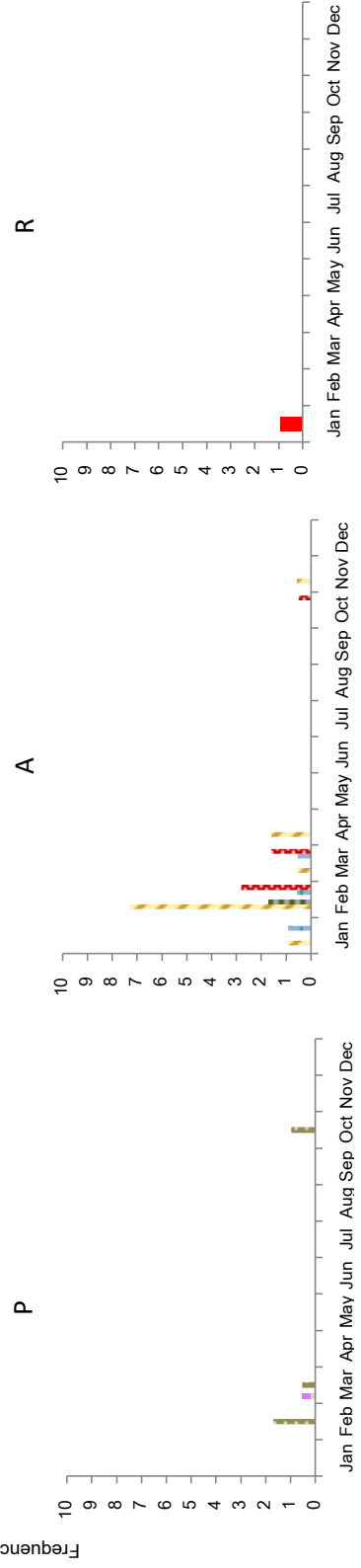
No. 4 (Fu)



No. 5 (Jam)

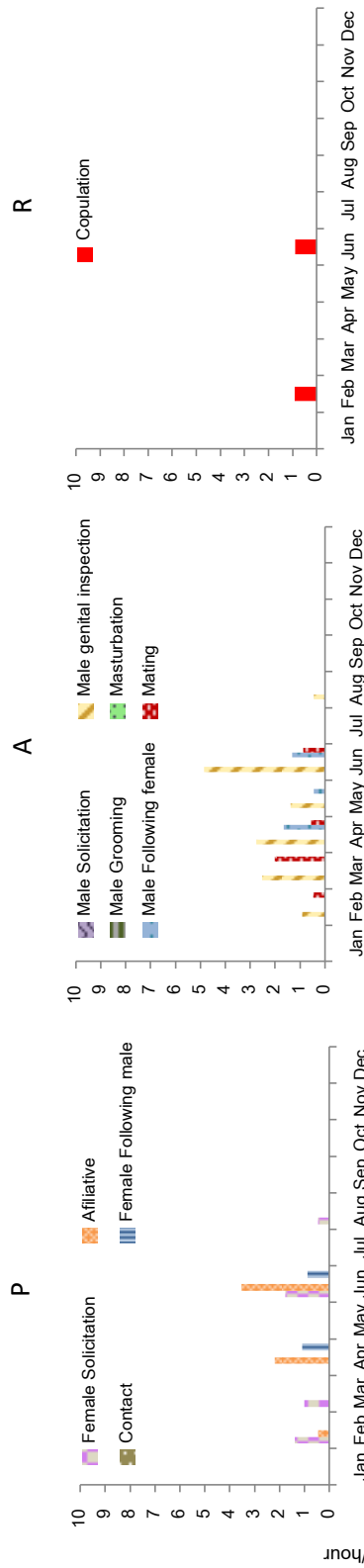


No. 6 (Lord)

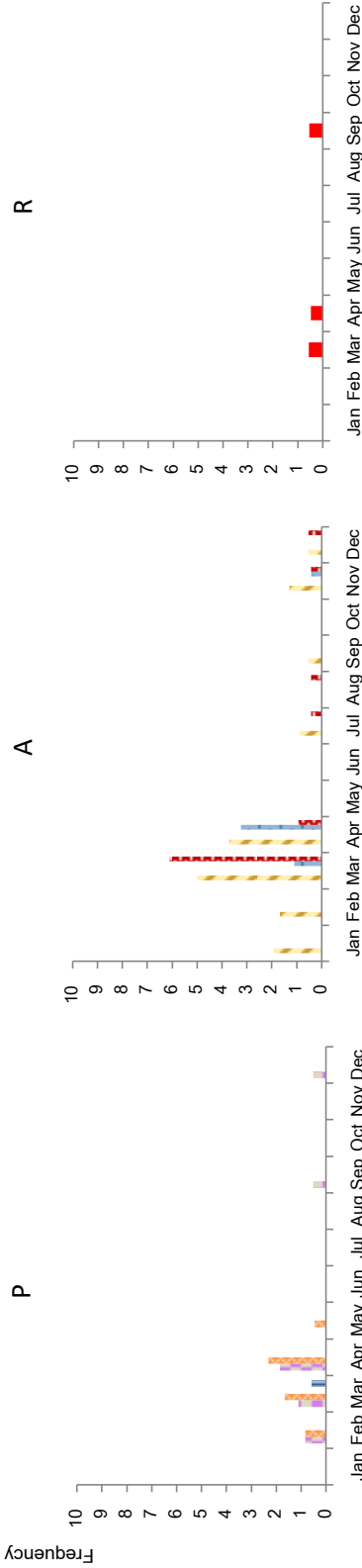




No. 9 (Raven)



No. 10 (Tumkhaw)





## VITA

**NAME** Prangmas Kumpai

**DATE OF BIRTH** 25 July 1991

**PLACE OF BIRTH** Ayutthaya province, Thailand

**INSTITUTIONS ATTENDED** Master of Science Program in Zoology at Department of Biology, Faculty of Science, Chulalongkorn University

**HOME ADDRESS** 131/20 M.2 Bankoh, Phra Nakhon Si Ayutthaya, Phra Nakhon Si Ayutthaya 10900

**PUBLICATION** She published a part of her research work as a research article in the Proceedings of the 10th National Graduate Study Conference, Silpakorn University, Thailand.

**AWARD RECEIVED** She was received a research grant from the 90th Anniversary of Chulalongkorn University. During her Master journey, she had participated in several conferences both national and international levels, and presented a part of her thesis there. In 2017, she had a poster presentation at the 7th International Symposium on Asian Vertebrate Species Diversity (AVIS), University of Yangon, Myanmar which was hosted by Kyoto University, Japan and University of Yangon, Myanmar. In 2018, she had an oral presentation at the International Conference on Animal and Plant Ecology and Management (APEM), University Malaysia Terengganu, Terengganu, Malaysia. In the same year of 2018, she had an oral presentation entitled, "Comparison of sexual behaviors of northern and southern Thai long-tailed macaques which carry different levels of genetic admixture of rhesus macaques" at the 8th International Symposium on Asian Vertebrate Species Diversity (AVIS), National University of Laos, Laos, and received the Best Young Researcher Presentation Award. In 2019, she had a poster presentation at the International Conference on Biodiversity, Centara Grand & Bangkok Convention Centre at Central World, Thailand. Lastly, in 2020, she had presented and published part of her work entitled "Comparing the physiological signs of ovulation timing between northern and southern Thai long-tailed macaques" in the Proceedings of the 10th National Graduate Study Conference, Silpakorn University, Thailand.