INTRODUCTION

Among 505 species of non-human primates that exist across the world, rhesus (Macaca mulatta) and cynomolgus macaques (M. fascicularis) are the two species most commonly used as animal models for biomedical research.1,2 One of the reasons is that they have one of the widest distribution ranges among non-human primate species. The distribution of rhesus macaques is from 15° N to 36° N, including Afghanistan, Pakistan, India, China, Myanmar, Thailand, Laos, and Vietnam,3 while cynomolgus macaques range from 20°N to 10°S, including Myanmar, Laos, Cambodia, Vietnam, Thailand, Malaysia, Singapore, Indonesia, the Philippines, Timor, and the Nicobar Islands.4–6 Based on their distribution range, rhesus and cynomolgus macaques overlap at 15–20° N in the Southeast Asian region, which was proposed as a hybrid zone between the two species, as revealed by morphological characters,7 ABO blood group,8,9 and various genetic markers.1,10–16

Based on mitochondrial (mt)DNA and Y-chromosome markers, an interspecific hybridization scenario was proposed illustrating a southwardly unidirectional introgression of male rhesus macaques...
into cynomolgus macaque populations.\textsuperscript{11,14,16} According to this scenario, the mtDNA of female rhesus macaques did not introgress into the hybrid zone (at 15°–20°N), but the Y chromosomes of their male counterparts reached far beyond the hybrid zone and terminated at the Isthmus of Kra (ca. 10°N), a proposed zoogeographical barrier between the Indochina and Sundaic regions.\textsuperscript{14,16}

Using nuclear and microsatellite markers, it was recently confirmed that the hybridization occurred in two directions,\textsuperscript{1,10,12,13,15} and the introgression of male rhesus macaques into cynomolgus macaque populations was beyond the Isthmus of Kra with the level of genetic admixture of rhesus ancestry declining gradually from the north to the south.\textsuperscript{10} In Thailand, cynomolgus macaques are distributed from the lower North (16°30′ N) to the Southernmost part (6°30′ N) across the Isthmus of Kra. Due to the different levels of autosomal genetic admixture of rhesus macaque ancestry into cynomolgus macaque populations, the Indochinese and Sundaic Thai cynomolgus macaques were, therefore, genetically clustered into different clades,\textsuperscript{11,14,16} and their morphological characters were separated into two forms.\textsuperscript{7}

The species-specific morphological characters used to identify rhesus and cynomolgus macaques are the pelage color and the relative tail length (RTL; a proportion between the tail length and crown-rump length). Rhesus macaques have a shorter RTL (<70%), whereas cynomolgus macaques have a longer RTL (>90%).\textsuperscript{4,17} Rhesus macaques have a bipartite pattern of pelage color (body contrast between back and thigh). The lower part of their body is more reddish-yellowish than the upper part, which is absent in cynomolgus macaques.\textsuperscript{3,4,7} The hybrid individuals have intermediate values between the two species; for example, a 69.6%–95.6% RTL and 1.33–2.01 contrast of yellow pelage color.\textsuperscript{18} Regarding their higher level of autosomal genetic admixture of rhesus ancestry, the morphological characters of Indochinese cynomolgus macaques were similar to those of rhesus macaques, such as a shorter RTL and higher body contrast, than the Sundaic cynomolgus macaques.\textsuperscript{7,19}

Rhesus and cynomolgus macaques live in multimale-multifemale groups in which adult males mate with several females and vice versa. Thus, changes in the sex skin, which indicates the fertile phase in females and increases their attractiveness to male conspecifics, are reported to be important for reproductive success.\textsuperscript{20} Rhesus macaques have large areas of sex skin reddening around the hindquarter, including the anogenital area, legs, and thighs without swelling.\textsuperscript{21,22} while cynomolgus macaques show a narrow area of sex skin reddening from the subcaudal to inguinal regions, but the sex skin swelling at the base of the tail is conspicuous.\textsuperscript{22}

With respect to the reproductive pattern, rhesus macaques show a strong reproductive and birth seasonality (strictly seasonal breeder).\textsuperscript{24} Their mating peak occurs mostly during the fall and early winter, for instance, in November–April for Indochinese rhesus macaques,\textsuperscript{25} October–February for Chinese rhesus macaques,\textsuperscript{26} and October–December for Indian rhesus macaques,\textsuperscript{27} and their birth peak occurs in May–June.\textsuperscript{4} Whereas cynomolgus macaques are non-seasonal breeders,\textsuperscript{28,29} that is, they mate throughout the year, although the mating peak has been detected in January–July for Northern Sumatra cynomolgus macaques\textsuperscript{30} with a birth peak in July–September.\textsuperscript{4} Currently, the reproductive seasonality of the hybrids between cynomolgus and rhesus macaques is unknown. However, X-linked gene(s) related to reproduction were reported to have been selected during the natural hybridization between these two species.\textsuperscript{31}

It is important to understand 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 for species identification and captive breeding as well as its use as an animal model for biomedical research, such as reproductive health and behavior. Although a vast knowledge on the genetic and morphological characters in these hybrid macaques has been reported,\textsuperscript{1,7–16} information on their reproductive patterns, especially in the wild, is very scarce. Thus, this study aimed to assess the effect of the different levels of autosomal genetic admixture of rhesus ancestry into wild Indochinese and Sundaic cynomolgus macaque on reproduction and the scenario of the underlying mechanism of genetics on reproduction.

## METHODS

### 2.1 Ethical note

The permit for research in Thailand was approved by the National Research Council of Thailand and the Department of National Parks, Wildlife, and Plant Conservation of Thailand (DNP). The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Faculty of Science, Chulalongkorn University (Protocol Review no. 1723017). The research adhered to the American Society of Primatologists (ASP) Principles for the Ethical Treatment of Non-Human Primates.

### 2.2 Animals and study sites

One population each of Indochinese and Sundaic cynomolgus macaques was selected for the study. The Wat Haad Moon Kra Beau (WHM) population (16°30′ N, 100°16′ E), which carries a 50% genetic admixture of rhesus ancestry as analyzed by autosomal SNP markers\textsuperscript{10} and belongs to the Indochinese clade based on phylogenetic analysis of the mtDNA sequences of rhesus and cynomolgus macaques,\textsuperscript{11} was selected as representative of Indochinese cynomolgus macaques. The Khao Noi/Khao Tangkuan (KN/KTK) population (7°12′ N, 100°35′ E), which carries a 15% rhesus ancestry\textsuperscript{10} and is grouped with Sundaic cynomolgus macaques from mtDNA analysis,\textsuperscript{11} was selected as a Sundaic cynomolgus macaque representative. The linear distance between these two populations is about 1300 km.

The WHM is located at a temple near the Nan River and is surrounded by a fragmented dry dipterocarp forest. Various tree species are found in the forest, such as Ficus sp., whose leaves and fruits
are accessible to macaques throughout the year, and *Afzelia* sp., whose flowers are accessible to macaques from February to March. The KN/KTK population is centered between the two small hills of KN and KTK and is located near the coast, approximately 15 m above sea level. The hills are covered with a tropical rainforest, including *Calleya* sp. and *Garcinia* sp. trees, and a recreational park. The home range of the WHM population is nine hectares (ha), and that of the KN/KTK population is approximately 33 ha, but the foraging area is only eight ha. Since tourists or pilgrims visited these locations often, the animals were provided food mainly by humans.

### 2.3 Population demography and selected subjects

The WHM population comprised 52 macaques, including 26 adult females and seven adult males, while the KN/KTK population consisted of 125 macaques, including 55 adult females and 16 adult males. Both the WHM and KN/KTK populations were habituated to human presence. Based on the knowledge that females live permanently in the group while males migrate out of the group upon reaching maturity, ten adult female monkeys in each population were selected as representatives of the population for long-term observation of sexual behaviors. The major criteria for animal selection in this study were that they were encountered often, and they must carry some unique morphological characters, for example, scars, a distinct color of nipples, or some unique behaviors that would allow their easy identification. To account for the effect of social ranking on sexual behaviors, both high (four animals in each population) and low (six animals in each population) ranked females were recruited for the study. Social rank was identified by observing their agonistic behaviors (supplant, threat, chase, or attack) and submissive behavior (grimace). All of the ten focal female monkeys were named based on their unique morphological characters; for example, the name “Gorilla” was given to a big, high-ranked adult female, and the name list was arranged alphabetically for data collection purposes.

### 2.4 Behavioral observation and data collection

Behavioral observations were performed in two phases. Phase I was for animal subject identification and selection, which was performed from October to December 2017. In this session, the animals were also habituated to the presence of the observer. Phase II was the main data collection period and was from January to December 2018. Note that no food was provisioned by the observer, and to avoid disturbing their daily activities, at least a 3 m distance between the observer and monkeys was maintained during all behavioral observation.

The frequency of sexual behaviors was recorded using a focal animal sampling method. Behavioral data collection was initiated when one of the focal female monkeys in the name list was encountered by the observer (PK). After the observation was completed, the next female on the alphabetical list was selected and monitored. The procedure was repeated until the last animal on the list was observed prior to starting another round of observation. However, if the selected monkey could not be found within 10 min, the observation was shifted to the next monkey on the list. Each monkey was observed for one hour or until she could not be followed further, such as when she climbed to a very steep area or moved to restricted areas. During the focal follow, if another female monkey on the list appeared within sighting distance, the observer simultaneously recorded the behaviors of the two animals. However, no more than two females were simultaneously observed. Observations were made from sunlight to sunset (from approximately 0600 to 1800 h). Focal females were observed for seven days each month for 12 consecutive months, from January to December 2018. The first week of the month was spent with the KN/KTK population, and the third week was spent with the WHM population.

The sexual behaviors included in this study were categorized into three groups: proceptivity (P), attractivity (A), and receptivity (R). Proceptivity is a female behavioral activity, including solicitation, affiliative behavior, contact behavior, and following adult males. Attractivity has reflected the male behavioral activities, including solicitation, male genital inspection, male grooming, masturbation, male following adult female, and mating. Receptivity is the behavior exhibited by both sexes, which is indicated by successful copulation. An ethogram of the three behaviors is described in Table 1.

### 2.5 Birth observation

The number of newborns from all females in each population was counted every month for 12 months. Photographs of newborns and their dam were also taken to prevent overestimating the number of newborns. Identification of the newborn relied on their pink facial skin and blackish pelage color. Pelage color is generally similar among adult, subadult, and juvenile males and females. The pelage color of cynomolgus infant changes to that of the older ages at 2–3 months of age, and the progression to adult pelage is complete by one year of age.

### 2.6 Assessment of sex skin swelling and reddening

Focal female monkeys were followed and photographed during the sexual behavior observations using a digital camera (Canon EOS 700D, lens EF-S18-55 mm, Japan) every month for 12 months. As mentioned above, cynomolgus macaques subjected to this study carry different levels of genetic admixture of rhesus macaques, the scoring system of sex skin swelling and reddening was integrated between the two macaque species. Scoring of sex skin swelling at the base of the tail followed Engelhardt et al. with slight modification and ranged from 0 to 4, as follows: 0: no swelling at the base of the tail, 1: slight swelling, 2: protuberant...
swelling, 3: protuberant swelling and slightly detectable lateral lumps, and 4: protuberant swelling and greatly detectable lateral lumps (Figure 1). Scoring of sex skin reddening was modified from that previously reported\(^{18} \) and ranged from 0 to 4, as follows, 0: no reddening is detected; 1: reddening area between the 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 (Figure 2).

### 2.7 Meteorological data collection

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

### 2.8 Statistical analysis

The frequency of the sexual behaviors of the ten animals in each population for each month was averaged and presented as mean ± one standard error (SE). The Mann–Whitney U test was used to determine the significance of any differences in these three sexual behaviors and sex skin scoring between the two ranks in each population and between two populations. Correlations between the three sexual behaviors and between the sexual behaviors and meteorological data were analyzed using the Spearman correlation test. The SPSS software program version 22 for Windows was used for all statistical analyses, and significance was defined as \( p < .05 \).

### 3 RESULTS

#### 3.1 Sexual behaviors

Behaviors of the 20 focal female macaques at WHM and KN/KTK were observed for 2016 h. The averaged frequency estimates of P, A, and R between the high and low-rank females within the WHM and KN/KTK populations were not significantly different. Therefore, the data were analyzed without concern of the social ranking. Female macaques at WHM showed a scattering of the three sexual behaviors (P, A, and R) throughout the year. The patterns were similar among the ten animals studied (Figure S1). The correlation between the average frequencies of P, A, and R behaviors of all females was significant (Spearman rank correlation: \( r_s \)-value between P and A = .872, between P and R = .834, and between A and R = .799; \( N = 10 \) females, \( p < .01 \)). The average frequencies of sexual behaviors were high at the end of and the start of the year (November–March; surge period), highest in January, and low in the remaining months of the year (April–October; non-surge period; Figure 3). If the data within the surge and non-surge periods of P, A, and R behaviors were

### TABLE 1 Ethogram of proceptivity, attractivity, and receptivity

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Proceptivity (P)</td>
<td>Female solicitation Female invites male by presenting her anogenital region towards the male (or sexual context).</td>
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<tr>
<td></td>
<td>Affiliative behavior Female stays beside the male without contacting his body (if it occurs during feeding, it is not recorded).</td>
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<tr>
<td></td>
<td>Contact behavior Female touches, but does not groom, any part of the male's body, including mounting by the female.</td>
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<tr>
<td></td>
<td>Female following adult male Female keeps following the male within a 3-m distance.</td>
</tr>
<tr>
<td>Attractivity (A)</td>
<td>Male solicitation Male presents his erect penis to the female.</td>
</tr>
<tr>
<td></td>
<td>Male genital inspection Male visually inspects, touches, or sniffs the female genitalia.</td>
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<tr>
<td></td>
<td>Male grooming Male cleans the female's fur (if the focal animal pauses acting this behavior longer than 5 s and starts again, the observation is counted as a new round of the behavior).</td>
</tr>
<tr>
<td></td>
<td>Masturbation Male, who follows the focal female(s) within &lt;3 m, tugs and rubs his penis until it becomes erect with or without ejaculation. The tip is usually touched with his fingers or any objects and is often sniffed and/or licked.</td>
</tr>
<tr>
<td></td>
<td>Male following adult female Male keeps following the female within a 3-m distance.</td>
</tr>
<tr>
<td></td>
<td>Mating Male mounts the female with or without intromission and pelvic thrust and without ejaculation.</td>
</tr>
<tr>
<td>Receptivity (R)</td>
<td>Copulation Male mounts the female with intromission and pelvic thrust resulting in ejaculation. The ejaculation is evidenced by a sperm plug, which often involves an intermission of approximately 3–5 s before the male pulls his penis out of the vagina and the female usually vocalizes. If the observer could not see this event, it is recorded as mating, not copulation.</td>
</tr>
</tbody>
</table>
pooled and compared, statistically significant differences were detected (Mann–Whitney U test: P: \( p = .018 \); A: \( p = .003 \); R: \( p = .030 \)) (Figure 4).

For the KN/KTK population, the ten female macaques showed patterns for the three sexual behaviors throughout the year similar to those seen in the WHM population (except for female No. 8, who was lost from observation since May) (Figure S2). Correlation between the average frequencies of P, A, and R behaviors of all females was significant (Spearman rank correlation: \( r_s \)-value: between P and A = .895, between P and R = .918, between A and R = .826; \( N = 10 \) females, \( p < .01 \)). The average frequencies of sexual behaviors were high at the end of and the start of the year (December–April; surge period), highest in March, and low in the remaining months of the year (May–November; non-surge period; Figure 3). If the data within the surge and non-surge periods of P, A, and R behaviors were pooled and compared, significant differences were detected (Mann–Whitney U test: P: \( p = .003 \); A: \( p = .003 \); R: \( p = .018 \); Figure 5).
3.2 | Birth observations

For the WHM population, evidence of recent birth was sporadically observed from April to December. One female gave birth to twins in April, the month with the highest birth rate in this population (4/9 births; 44.4%; Figure 6). In contrast, in the KN/KTK population, births occurred every month throughout the year except in March and April. The percentage of births during the three-month period was computed based on a reproductive seasonality classification system. During the three months of April–June, 56% of births (5/9) were observed in the WHM population. The birth surge in the three months in the KN/KTK population occurred between June and August (15/29 births; 52%) and was highest in July (6/29 births; 20.69%; Figure 6).

3.3 | Sex skin swelling and reddening

The sex skin swelling score (Figure 1) for each female in the WHM and KN/KTK populations was consistent throughout the year, and a dramatic change was not apparent (data not shown). Thus, each month’s data of all females in a population were pooled. The average score of sex skin swelling in the WHM population was <1 from January to December, while it was >1 in every month in the KN/KTK population, except for October when it was 0.78. When the average scores of 12 months in each population were pooled, the average score of sex skin swelling in the WHM population (X ± SE = 0.70 ± 0.48, N = 12 months, p < .05) was significantly lower than that in the KN/KTK population (X ± SE = 1.50 ± 0.85, N = 12 months; Figure 7).

The sex reddening score (Figure 2) for each female macaque in the WHM and KN/KTK populations was consistent throughout the year (data not shown). However, the value of the reddening score in female macaques in the WHM population was mostly scored at 3 (60%), while the females in KN/KTK population were all scored at 2 (100%). The average score of 12 months of sex skin reddening in the WHM population (X ± SE = 2.70 ± 0.82, N = 12 months, p < .01) was significantly higher than in the KN/KTK population (X ± SE = 2.00 ± 0.00, N = 12 months; Figure 7).

By combining the data of sex skin swelling and reddening together, the WHM female cynomolagus macaques had a reduced sex skin swelling at the base of the tail but a much greater sex skin reddening area than the KN/KTK females. No significant correlation between the averaged frequencies of P, A, and R behaviors and averaged scores of sex skin swelling and reddening were detected.

3.4 | Meteorological data

The values of temperature, rainfall, humidity, and photoperiod fluctuated throughout the year and were not significantly different between the two populations (data not shown). The average 12-month rainfall, photoperiod, temperature, and humidity data are presented in Table 2. Although the average rainfall at KN/KTK was higher than at WHM, the range of average rainfall at KN/KTK was wider than that at WHM, and no significant difference in the values between the two populations was detected. Significant correlations between the meteorological data and sexual behaviors were detected only in the WHM population as follows: rainfall and P behavior (Spearman rank correlation: rs = −.690, N = 12 months, p < .05), rainfall and A behavior (Spearman rank correlation: rs = −0.867, N = 12 months, p < .01), temperature and A behavior (rs = −0.595, N = 12 months, p < .05), and temperature and R behavior (Spearman rank correlation: rs = −0.737, N = 12 months, p < .01). An association between the number of newborns and the rainfall was also observed in both WHM and KN/KTK populations; for example, the number of newborns tended to be high (14/29 newborns in July–September) before the peak period of the rainfall in October–December for KN/KTK population.

4 | DISCUSSION

4.1 | Sexual behaviors

Although both the WHM and KN/KTK populations showed the three groups of sexual behaviors, P, A, and R, throughout the year, different peak periods were detected between the two populations. The peak period of the WHM population (November–March) was one month earlier than that of the KN/KTK population (December–April). Following the period of receptive behavior (or copulation), the birth peaks that occurred in April–July for the WHM population and June–September for the KN/KTK population were associated with the 24-week gestation period of cynomolgus macaques. The birth peaks of Thai cynomolgus macaques in the present study aligned with that of cynomolgus macaques housed in a primate breeding facility near Kuala Lumpur, Malaysia, where births occurred throughout the year and peaked between May and August.
Births

Although the birth patterns were consistent with the sexual behaviors exhibited in both populations throughout the year, the fecundity differed between the two populations. Between January and December 2018, the number of adult females in the KN/KTK population (55) was two times higher than that in the WHM population (26). However, the KN/KTK population had a threefold higher fecundity (29 newborns) than the WHM population (nine newborns).

Following the classification of van Schaik et al., the reproductive seasonality in primates was divided into three categories based on the number of births during the three consecutive months, where primates that have >67% births are considered a strictly seasonal breeder. In contrast, those with 33–67% are moderately seasonal breeders, and those with <33% are non-seasonal breeders. The difference between moderately and strictly seasonal breeders is that when the former is housed in captivity at temperate latitudes, they lose their reproductive seasonality when food is available throughout the entire year. However, strictly seasonal breeders retain their high reproductive seasonality. Following this classification, both the WHM (56% of births between April and June) and KN/KTK (52% of births between June and August) cynomolgus macaque...
populations fall into the classification of moderate seasonal breeders. This finding is not in line with previous studies that concluded that cynomolgus macaques are non-seasonal breeders.\textsuperscript{28,29}

Although the WHM and KN/KTK macaques carried as much as 35% difference of genetic admixture of rhesus ancestry, they showed a similar pattern of moderately seasonal breeding. Recently, the effects of the environment (ecological hypothesis),\textsuperscript{37} and genetics (phylogenetic inertia hypothesis);\textsuperscript{38} on the reproductive seasonality in 13 out of the 23 existing species of macaques\textsuperscript{39–41} were analyzed.\textsuperscript{42} They suggested that the species’ latitudinal locations with different environments strongly influenced reproductive seasonality. Their analysis also indicated that the reproductive seasonality in macaques was not entirely consistent with the phylogenetic inertia hypothesis because some macaques within the same genetic clade, based on mitochondrial genome analysis,\textsuperscript{43} showed a large variation in birth seasonality, from non-seasonal (25% birth count) to moderately seasonal breeding (56% birth count). This agrees with previous studies on the reproduction of Old World Monkeys,\textsuperscript{29,44} denoting that the degree of reproductive seasonality in Old World Monkeys is highly variable and not related to genetics.

The slightly shifted peak of sexual behaviors and births between the two populations may be caused by different environmental conditions such as rainfall. Although the latitudinal locations between the WHM and KN/KTK populations were only 9° apart, they belong to different zoogeographical regions, Indochina and Sunda.\textsuperscript{45} In many primate species, environmental factors, such as climate conditions and food availability,\textsuperscript{30} are associated with reproductive seasonality and birth peaks. Macaques inhabiting different locations with different timings of food abundance present different birth peak timings.\textsuperscript{36} However, foods for the WHM and KTK populations were available throughout the year, by provisioning and from natural habitats, which should not be a factor in differences of sexual behaviors and births between the two populations. One limitation of this study is that it involves only one group per population and reflects only 1 year of data collection; therefore, it is possible that the fecundity reported here might not be representative of an annual average estimate.

<table>
<thead>
<tr>
<th>Physical factor</th>
<th>WHM (Range, X ± SE)</th>
<th>KN/KTK (Range, X ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>26.1–29.5 (28.1 ± 1.1)</td>
<td>26.8–29.2 (28.0 ± 0.7)</td>
</tr>
<tr>
<td>Rainfall (mm)</td>
<td>0–230.2 (87.9 ± 83.4)</td>
<td>1.40–456.6 (165.8 ± 139.5)</td>
</tr>
<tr>
<td>Relative humidity (%)</td>
<td>72–83 (77 ± 3)</td>
<td>74–85 (80.3 ± 3)</td>
</tr>
<tr>
<td>Photoperiod (h)</td>
<td>3.69–8.47 (7.03 ± 1.76)</td>
<td>4.00–9.40 (5.90 ± 1.75)</td>
</tr>
</tbody>
</table>

Although peak periods in sexual behaviors were detected in the WHM (November–March) and KN/KTK (December–April) populations, the sex skin swelling and reddening scores were consistent throughout the year in both populations. Thus, no correlation between sexual behaviors and changes in the sex skin was detected. This finding confirms a previous study that sex skin swelling was an unreliable indicator for the fertile phase in cynomolgus macaques, but sexual behavior reliability indicated the timing of the fertile phase.\textsuperscript{23} Thus, the period of birth peaks in the cynomolgus macaques in this study still followed the peak periods of sexual behaviors.

### 4.4 Hybridization between rhesus and cynomolgus macaques and recent genetic analyses

The sensitive double-digest restriction site-associated DNA sequencing (or ddRAD) was recently used to elucidate the hybridization scenario between rhesus and cynomolgus macaques.\textsuperscript{31} They reported that some rhesus macaque-biased alleles (especially on autosomal chromosomes) were widely introgressed into cynomolgus macaque populations. In contrast, some alleles (especially on X chromosomes) were concentrated around the hybrid zone of 15–20°N. In agreement with the previous report using autosomal SNP markers,\textsuperscript{10} their study indicated that introgression of the rhesus ancestry went far beyond the proposed hybrid zone. The rhesus gene flow to
cynomolgus macaques (from north to south) declined gradually and was proportionate to distance.

Overall, based on the autosomal SNP markers used in the previous study, we can signify that the genetic admixture of rhesus ancestry in cynomolgus populations does not affect the reproductive seasonality (or sexual behaviors) of cynomolgus macaques. However, the genetic admixture can interfere with the pattern of changes of sex skin. Thus, the WHM female cynomolgus macaques (50% rhesus autosomal SNP genetic admixture) showed similar patterns of sexual behaviors with those of the KN/KTK female cynomolgus macaques (15% rhesus genetic admixture). In contrast, the WHM females were more predisposed than the KN/KTK females to exhibit the rhesus sex skin reddening pattern.
5 | CONCLUSIONS

With reference to various previous genetic marker-based analyses,1,10-16,31 the scenario of the hybridization between rhesus and cynomolgus macaques concerning sexual behaviors (reproductive seasonality, births (fecundity), and changes in sex skin (a physiological sign of ovulation) examined in this study can be drawn in sequence as follows (Figures 8 and 9).

1. Hybridization between the two species was mainly led by male dispersal of rhesus macaques that introgressed into cynomolgus macaque populations from north to south.

2. Male rhesus macaques who carried rhesus sex chromosomes (AcyAcy- XcyXcy) hybridized with female cynomolgus macaques who carried cynomolgus sex chromosomes (AcyAcy- XcyXrh), and so two types of the hybrid offspring could plausibly be produced: AcyAcy- XcyXrh females and AcyAcy- XcyYrh males.

3. Regarding the meiotic inactivation or heterochromatization hypothesis,57 the different regions of the X chromosome between rhesus and cynomolgus macaques that lack conformational or structural homology can cause the failure of meiotic pairing. Thus, the fertility of AcyAcy- XcyXrh hybrid females was reduced and could not pass the Xth chromosome to another generation of cynomolgus macaques (Xrh is, thus, concentrated at the hybrid zone;31 while the AcyAcy- XcyYrh male could propagate his genes to the next generations of cynomolgus macaques (Yrh is, thus, passed beyond the hybrid zone and terminated at the Isthmus of Kra).11,14,16

4. Since macaque males in multimale–multifemale groups leave their natal group around sexual maturity (male dispersal) and eventually join another group while females live permanently in the group (female philopatry), the introgression of male rhesus macaques led to the hybridization between these two species (as stated in No.1). Therefore, the rhesus gene flow occurred from the north to the south of the cynomolgus populations. Thus, the level of autosomal genetic admixture of rhesus ancestry (Athr) in cynomolgus populations (Acy) was diluted over a distance that was far beyond the proposed hybrid zone (15–20° N). As a result of this propagation of the male rhesus macaques’ genetics, the northern WHM cynomolgus population (Athr = 50%; Athy = 50%) carried a higher level of autosomal genetic admixture of rhesus ancestry than the southern KN/KTK cynomolgus population (Athr = 15%; Athy = 85%).10

5. From points 3 and 4 above, combined with the results of this study, indicate that there were no differences in reproductive seasonality between Indochinese WHM and Sundai KN/KTK cynomolgus macaques. Compared to the KN/KTK females, the WHM females were more prone to exhibit rhesus macaque sex skin reddening pattern. Therefore, it can be proposed that the macaque X-linked gene(s) controls the reproductive seasonality, while genes on the macaque autosomal chromosomes govern changes in sex skin.

6. This study indicated that reproduction and its mechanism (including sexual behaviors, births, and changes of sex skin) are complicated. They are not controlled by a single gene or a single type of gene, and the effect of the 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 cynomolgus macaque populations on reproduction is not as straightforward as previously thought.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher’s website.

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