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Disassociation of social and sexual partner relationships in a gibbon population with stable one-male two-female groups

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Abstract

Adult males living in a one-male multi-female social group are expected to try to monopolize copulations with resident females to increase reproductive fitness. Gibbons have traditionally been described as living in monogamous groups, with the sole resident adult male assumed to sire all of the group's offspring. Here, we used microsatellite analyses and behavioral observations to examine rates of extra-group paternity (EGP) over 16 years in a population of crested gibbons (*Nomascus concolor*) that form stable and long-term one-male two-female social units. Forty percent of offspring ($N = 14$) were sired by extra-group males. To understand this high level of EGP, we tested whether inbreeding avoidance was related to EGP. Females who engaged in EGP did not show larger pairwise relatedness with their resident male compared to females who did not engage in EGP. Nevertheless, the standardized heterozygosity of EGP offspring was significantly higher than for offspring sired by the group's resident male. These results provide partial support for the inbreeding avoidance hypothesis. It appears that resident male crested gibbons are unable to monopolize resident females' matings. Our results indicate that long-term social partners are often distinct from sexual partners in this population. Clearly, the breeding system of crested gibbons is more flexible than previously thought, indicating a need for integrating long-term behavioral data and genetic research to re-evaluate gibbon social and sexual relationships derived from concepts of monogamy and pair-bonding.

KEYWORDS

breeding system, extra-group paternity, heterozygosity, *Nomascus concolor*, reproductive strategy

1 | INTRODUCTION

In polygynous groups containing a single adult male and multiple females, the resident male is expected to invest considerable time and energy in monopolizing resident females' matings, so as to increase his reproductive success. Theoretically, males should be able to monopolize copulations when the number of resident females is small and females come into estrus at different times (Clutton-Brock, 1989; Trivers, 1972). However, this is not always the case (Isvaran & Clutton-Brock, 2007). A recent study of golden snub-nosed monkeys (*Rhinopithecus roxellana*), a species in which the breeding unit has traditionally been described as a uni-male multi-female or harem unit (average male to female adult sex ratio 1:5.7) (Guo et al., 2010), found that even though the resident male was intolerant of extra-group males attempting to mate with harem females, 56.3% of group infants were sired by extra-group males (Qi et al., 2020).

Across several mammalian taxa, resident females actively seek extra-group copulations (EGCs) (Cant et al., 2002; Fang et al., 2018). The inbreeding avoidance hypothesis has been proposed to account for EGCs. It suggests that adult females will preferentially mate with males that are more genetically dissimilar with themselves than with their close social mates (Varian-Ramos & Webster, 2012). Furthermore, it posits that females engage in EGCs to increase offspring heterozygosity and thereby produce higher quality offspring (Ferretti et al., 2011; Foerster et al., 2003). For example, a study of mole-rats (*Cryptomys hottentotus*) found that females were less closely related to EGC males than resident mates and that the heterozygosity of offspring sired by EGC males was increased through extra-group matings (Bishop et al., 2007). In a study of meerkats (*Suricata suricatta*), Leclaire et al. (2013) found that females engaged in EGCs to increase the heterozygosity of offspring, but the EGC males were not more distantly related to females than social mates.

Gibbons (Family Hylobatidae) are small, territorial apes exhibiting low levels of sexual dimorphism in body mass (Bartlett & Light, 2017). Most gibbons live in monogamous family groups consisting of a single adult male, a single adult female, and their putative offspring. They form a long-term pair bond that ends when one of the pair dies or is replaced by a same-sex individual (Mitani, 1987). In some cases, the pair bond can be dissolved in the absence of an invader when an adult abandons its mate permanently or temporarily (Palombit, 1994a). Given the close spatial and social coordination of gibbon groups, it is often assumed that all of the group's offspring are sired by the resident male. In support of this view, a study of Müller's gibbon (*Hylobates muelleri*) (*N* = 4) at the Bukit Soeharto Education Forest of Mulawarman University genetically confirmed that resident adult males monopolized paternity (Oka & Takenaka, 2001). And, although EGCs have been observed in several gibbon species (Palombit, 1994b; Reichard, 1995), genetic testing confirmed extragroup paternity (EGP) accounted for less than 10% of births in a population of white-handed gibbons (*Hylobates lar*) (*N* = 41) at Khao Yai National Park (Barelli et al., 2013). Similarly, 10% of births in a population of golden-cheeked gibbons (*Nomascus gabriellae*) (*N* = 10) in Cat Tien National Park were confirmed to be the results of EGP (Kenyon et al., 2011).

In contrast to tropical gibbons, the western black crested gibbon (*N. concolor*) inhabits temperate forests and lives in stable polygynous groups consisting of one resident adult male, two breeding females, and their offspring (Hu et al., 2018). In response to seasonal fruit shortages, crested gibbons consume a leaf-heavy diet (Fan et al., 2009). According to a recent model of the evolution of gibbon social and mating systems, habitat heterogeneity in these temperate forests and the ability to increase their intake of leaves reduce female intrasexual feeding competition and provide an increased opportunity for social polygyny (Guan et al., 2018).

Here, we combine 16 years of continuous field observations and noninvasive genetic analysis to assess the social structure and breeding system of a one-male two-female population of western black crested gibbons (*N. concolor*) inhabiting Mt. Wuliang, Central Yunnan, China. Given that females in our three gibbon groups gave birth every 3–4 years, and each group's two resident adult females rarely were fertile at the same time and generally bred in different years (Hu et al., 2018), we expected that the resident male would monopolize the matings of both females and sire all the group's offspring. However, EGCs have been reported in this population (Huang et al., 2013), and therefore, we examined whether inbreeding avoidance was related to EGP and tested two related predictions: (1) females who engaged in EGP was more closely related to their resident male than females who did not engage in EGP, and (2) the heterozygosity of offspring sired by extra-group males was higher than offspring sired by social mates.

2 | METHODS

2.1 | Study species, site, and groups

Our study was carried out at Dazhaizi (24°21′N, 100°42′E), a long-term field station established in 2003, on the western slope of the Wuliang National Nature Reserve, central Yunnan, China. The site is characterized by temperate primary semi-humid evergreen broad-leaved forest and mid-montane humid evergreen broad-leaved forest at an elevation of between 2100 and 2700 m, and rhododendron dwarf forest above 2700 m (Tian et al., 2007). Beginning in 2003, we monitored the composition and identified all individuals in three groups of western black crested gibbons (G2, G3, and G4) each month over 16 years. During this period, four adult male replacements and two adult female replacements were recorded. Replaced adults were never observed to re-enter an established group, and their fates remained unknown. Across our 16-year study period, there were eight females and seven males resident in our study groups. Group composition and replacements of resident adults can be found in Hu et al. (2018).

2.2 | Fecal sample collection and DNA extraction

Between 2010 and 2018, we collected fresh fecal samples from 76% (29/38) of the individuals in our study population from 2003 to 2018.

We collected feces from all but one breeding female ($N = 7$), all but one resident male ($N = 6$), and 16 offspring. We stored all fecal samples in 99.9% ethanol immediately after collection and each sample was desiccated using silica after more than 24 h of storage in 99.9% ethanol (Nsubuga et al., 2004). All samples were then stored at −20°C in the laboratory for long-term preservation. The total genomic DNA of each sample was extracted using a QIAamp Fast DNA Stool Mini kit (Qiagen, GmbH) following the protocol provided by the manufacturer. DNA quality was quantified using a NanoDrop ND-1000 spectrometer (Thermo Fisher Scientific). We extracted 2–3 fecal samples from each individual.

2.3 | Mitochondrial DNA sequencing and STR genotyping

We used two pairs of primers to amplify 1025 base pairs of the first hypervariable region of the mitochondrial (mtDNA) D-loop gene of each individual (Hu et al., 2018). This was done to confirm mother-offspring relationships and to minimize errors in sample collection (Barelli et al., 2013). Polymerase chain reaction (PCR) amplifications were performed in a 20 µl reaction volume which contained 1–2 µl template DNA (50–100 ng), 2 µl 10×buffer,1.6 µl dNTP (2.5 mM each), 1 µl of each primer (10 µM), and 0.2 µl BSA (10 mg/ml) and 0.1 µl (5U/µl) TaKaRa Ex Taq. We used touch-down PCR to improve the specificity of primer annealing and DNA production. The first step in PCR cycling conditions was an initial denaturing at 94°C for 5 min, then 10 cycles of denaturation at 94°C for 40 s, followed by annealing at 55°C for 40 s (decreased 0.5°C per cycle), and extension at 72°C for 40 s; then 25 cycles of denaturation at 94°C for 40 s, followed by annealing at 50°C for 40 s and extension at 72°C for 40 s; finally, each sample was extended at 72°C for 10 min. All PCR products were sent to Sangon Biotech Company in Shanghai, China, and sequenced on an ABI 3730xl.

For each individual, we genotyped 12 microsatellites loci: NC15, NC20, NC23, NC32, NC34, NC35, NC36, NC37, NC17, NC28, NC38, and NC22, which are polymorphic in western black crested gibbons (Hu et al., 2014). The PCR amplifications were performed in a reaction volume of 10 µL, containing 5 µL 2×PCR mix (QIAGEN Multiplex Kit), 0.1 µM of each primer (3 to 4 pairs of primers mixed), and 2.5 µL of template DNA. Multiplex PCR conditions were as follows:95℃ for 15 min, followed by 30 cycles (85℃ for 30 s, 57℃ for 3 min, 72℃ for 60 s) and 60℃ for 30 min. A second multiplex PCR was performed with the same reaction system and PCR conditions as above, but with fluorescently labeled forward primers (FAM, HEX) and 1:10 dilutions of the first PCR product in place of template DNA. To avoid contamination, a negative control was processed along with each set of PCRs. To minimize the possibility of allelic dropout and genotyping errors when amplifying microsatellite loci with fecal samples, we confirmed genotypes from two duplicate samples and a multiple-tube replication procedure (Taberlet et al., 1999). The PCR products were electrophoresed on an ABI 3730xl genetic analyzer (Applied Biosystems) at Sangon Biotech Company in Shanghai, China.

2.4 | Genetic analyses

All mtDNA sequences were assembled using the DNAstar Lasergene Seqman Pro Version 7.1.0. Then we aligned and compared the assembled sequences using MEGA X (Kumar et al., 2018). All mtDNA sequences of 29 individuals were uploaded to GenBank (MZ356168-MZ356196). The genotyping analysis for each microsatellite locus was determined against an internal size standard GeneScan™ 500 LIZ (Applied Biosystems) with GENEMAKER v. 1.91. Scoring errors were tested with MICRO-CHECKER v. 2.2.3 (Van Oosterhout et al., 2004). We used CERVUS 3.0 (Kalinowski et al., 2007) to calculate the number of different alleles, polymorphic information content (*PIC*), observed heterozygosity (H_O), expected heterozygosity (H_E), and null allele frequency of the12 loci. Hardy–Weinberg equilibrium and pairwise linkage disequilibrium were tested using ARLEQUIN v. 3.5 (Excoffier & Lischer, 2010). The *p* value of pairwise linkage disequilibrium was adjusted using the sequential Bonferroni test (Rice, 1989). Four pairs of loci showed pairwise linkage disequilibrium which meant inheritance by descent was not independent. This could affect calculation of the relatedness coefficient and individually standardized heterozygosity, thus we excluded three loci. NC37 showed linkage disequilibrium to two other loci and was excluded and NC15 and NC36 were each linked to another locus and were excluded from further pairwise relatedness analyses and from individually standardized heterozygosity analyses. After excluding these loci, the remaining loci did not show pairwise linkage disequilibrium.

2.5 | Paternity analyses

We used all sampled individuals ($N = 29$) to estimate the reference allele frequency using CERVUS. If we knew the genotype of the offspring and its mother, the probability of excluding a candidate parent from parentage was 99.5% based on the genotype data. We conducted paternity analyses for 16 mother-infant pairs identified via field observations. For 15 offspring, we were able to unambiguously identify the resident male in their natal group at the time of conception (Table 1). We performed maternity analysis via CERVUS based on individual STR genotypes to test for inconsistencies. We assessed the likelihood of paternity using LOD scores with CERVUS. For the paternity simulation in CERVUS, we used 70% as the proportion of candidate fathers sampled, considering a 1% genotyping error and 10,000 simulation cycles. The confidence levels were set at 80% for relaxed levels of confidence and 95% for strict levels of confidence. We used the strict exclusion method assignment for individuals with positive and high LOD scores and allowed one mismatch per parent-offspring pair for possible mutations (Guo et al., 2010; Pemberton et al., 1992). EGP was determined when the sire was confirmed not to be the resident

TABLE 1 Results of paternity analysis for a population of *Nomascus concolor* at Dazhaizi Mt. Wuliang, China

Group	Mother	Offspring	Resident male when offspring was conceived	Identified father	Confidence	Paternity
G2	G2AFR	G2AFR2009	G3FB1998	G3FB1998	95%	IGP
		G2AFR2012	G3FB1998	Unknown	N/A	EGP
		G2AFR2015	G3FB1998	G3M-Floater	80%	EGP
	G2AFI	G2AFI2008	Unknown	G3FB1998	95%	N/A
		G2AFI2012	G3FB1998	G3FB1998	95%	IGP
	G2AFR2006	G2AFG2015	G3FB1998	G3FB1998	80%	IGP
G3	G3FB	G3FB2004	AM3	Unknown	N/A	EGP
	G3FY	G3FY2005	AM3	AM3	95%	IGP
		G3FY2013	G3FB2001	AM3/G3FB2001	80%	UP
		G3FY2016	G3M	G3M	95%	IGP
G4	G4FB	G4FB2006	OAM4	OAM4	95%	IGP
		G4FB2013	OAM4	OAM4	95%	IGP
		G4FB2016	RAM4	G3FB2001-Floater	95%	EGP
	G4FY	G4FY2007	OAM4	Unknown	N/A	EGP
		G4FY2012	OAM4	Unknown	N/A	EGP
		G4FY2015	RAM4	RAM4	95%	IGP

Note: UP, paternity could not be assigned as there were two individuals who could not unambiguously be excluded as the possible father.
Abbreviations: EGP, extragroup paternity; IGP, intragroup paternity.

male in the group in which the infant was conceived (7 months before birth).

2.6 | Hypotheses testing

To analyze whether females avoided inbreeding, we first compared pairwise relatedness between the resident male and females who did not engage in EGP versus females who did. From 2003 to 2018, we observed four male replacements. The resident adult females may show different reproductive strategies in response to the resident male status and floater male behavior. So, we considered each male–female dyads pairwise relatedness as an independent case. We estimated relatedness using Wang's unbiased estimators (Wang, 2002, 2017), which is suitable for analyzing small sample sizes using COANCESTRY v.1.0.0.9 (Wang, 2011). We ran a Wilcoxon test to compare the pairwise relatedness between a non-EGP female and the resident male versus an EGP female and the resident male in R 4.0.3.

Second, we used GENHET v. 3.1 (Coulon, 2010) in R 4.0.3 to determine the standardized heterozygosity of each individual. We compared the standardized heterozygosity between intra-group paternity (IGP) and EGP offspring in R 4.0.3 with a Wilcoxon test.

The Wilcoxon effect size *r* for each Wilcoxon test was estimated in R 4.0.3 by using "wilcox_effsize" (Tomczak & Tomczak, 2014).

2.7 | Ethical note

This project strictly complied with the ethical specifications of the Chinese Animal Welfare Act (20090606) and the American Society of Primatologists (ASP) Principles for the Ethical Treatment of Non-Human Primates.

3 | RESULTS

3.1 | Social structure

Each of the three groups consisted of one resident adult male and two breeding females since we began behavioral observations in 2003. Over the next 16 years, there were four male replacements and two female replacements. Three of the resident males were replaced by floaters or males that were solitary. One male replacement occurred when a natal male remained in the group and assumed the breeding position. The resident male he replaced was not his father. Both female replacements involved members born into the group (Figure 1). Although each group experienced the replacement of one or more members between 2003 and 2018, all three groups maintained a one adult male and two adult females residence patterns during an average of 98.4% of observation months (G2: 190/192 months; G3: 191/192 months, G4: 186/192 months). During the process of resident adult displacement, our groups

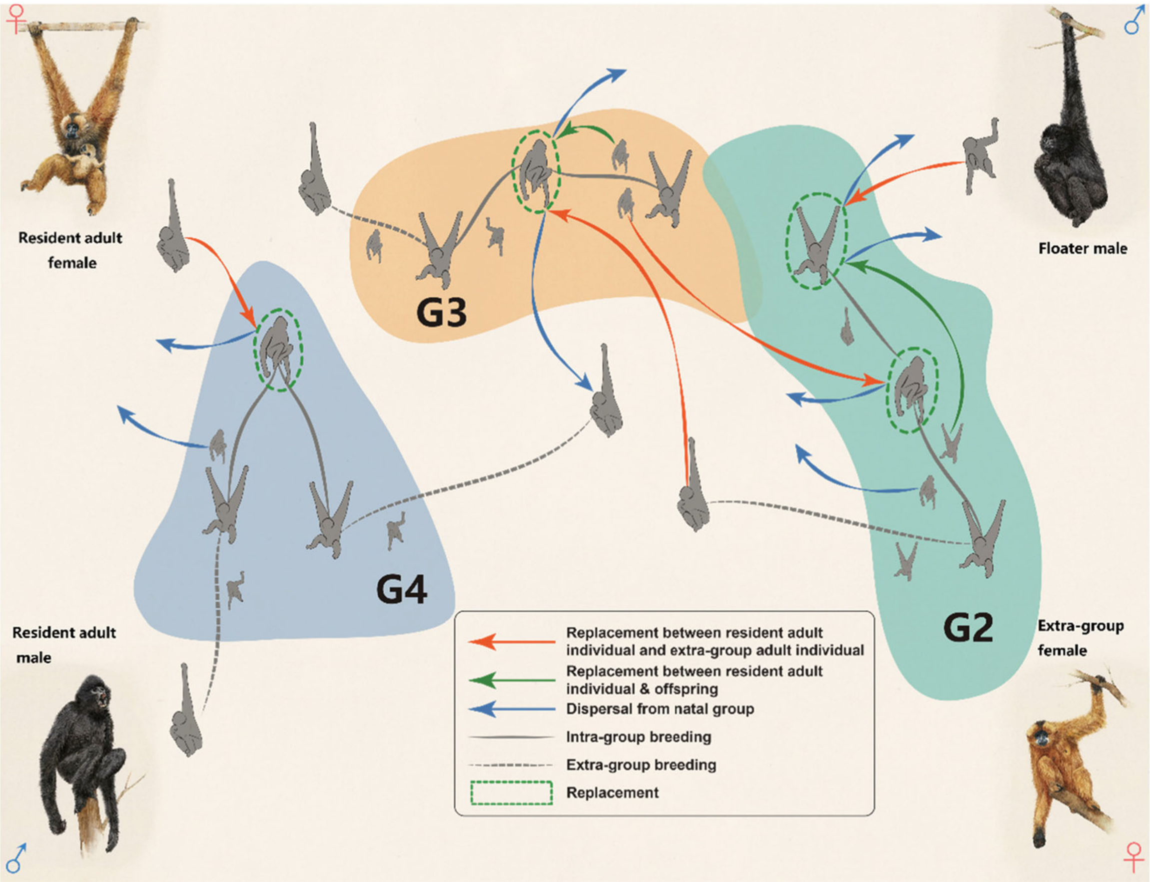

FIGURE 1 Residence relationships among individuals in three one-male two-female gibbon (*Nomascus concolor*) groups at Dazhaizi, China. Different shapes in the central area correspond to differences in the breeding status of males and females depicted in the drawings in the four corners. Females mated and reproduced with both their resident adult males and floater males. We observed incidences of both resident adult males and resident adult females replaced by maturing offspring in their group, as well as by and floater males and extra-group females. Some offspring that reached sexual maturity remained in their natal group while others dispersed. Not all relationships discussed in the main text are shown in this figure.

contained either two adult males or three adult females, with this transition period lasting for approximately 2 weeks to 6 months.

3.2 | Paternity assignments and determination of EGP

We obtained reliable microsatellite genotypes for 29 individuals (Table S1). The number of different alleles per locus ranged from 2 to 5. H_O ranged from 0.310 to 0.828, and H_E from 0.390 to 0.792. All loci were in Hardy–Weinberg equilibrium except for NC20 (Table S2).

We confirmed paternity for nine (56.25%) offspring at a confidence level of 95%. When using an 80% confidence level, the paternity of two (12.5%) additional offspring could be determined. One offspring (6.25%) had two potential sires at the 80% level (Table 1). By comparing the genotype of the assigned biological father with the genotype of the resident male in the group when the infant was conceived, we identified eight cases of IGP and six cases of EGP (Table 1). All but one of the 14 offspring survived to the end of the study or dispersed. The infant who did not survive was sired through an EGP and died as a result of falling from a tree.

None of the resident males sired offspring in neighboring groups. Two floater males, however, were each found to have sired one offspring in this population. We could not identify paternity for the remaining four offspring to any of the known males in our database, suggesting the existence of undetected floater males in the population. Among the seven adult females that bred, four were found to engage in EGP (Table 1).

3.3 | Inbreeding avoidance hypothesis

The pairwise relatedness between females who did not engage in EGP and resident male was higher than that between females who engaged in EGP and resident male, but this was not statistically different, possibly because of the small sample size (Figure 2a, Table S3, Wilcoxon test: $N_1 = 6$, $N_2 = 4$, $W = 17$, $p = 0.880$, $r = 0.337$). Thus, the first prediction of the inbreeding avoidance hypothesis was

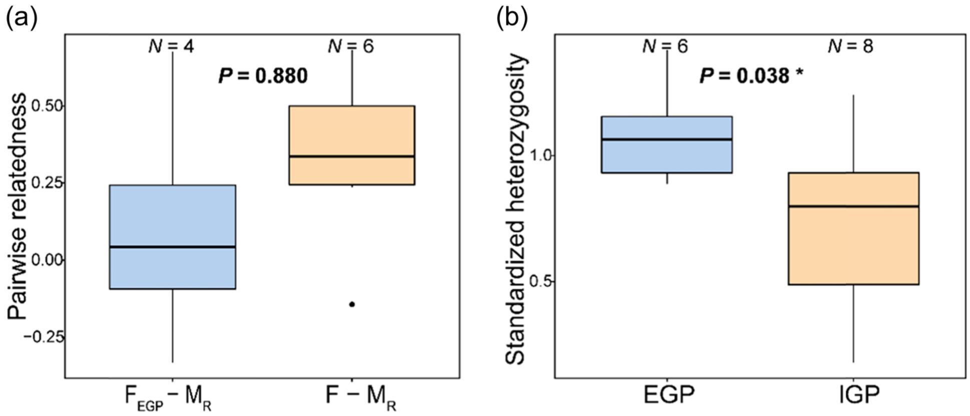

FIGURE 2 (a) Comparison of the pairwise relatedness between EGP females and resident males (F_{EGP}-M_R) and relatedness between other females and resident males (F-M_R); (b) Comparison of the standardized heterozygosity between EGP and IGP offspring. EGP, extra-group paternity; F, females who did not engage in extra-group paternity; F_{EGP}, females who engaged in extra-group paternity; IGP, intra-group paternity; M_R, resident male

not supported. Moreover, we have one case of inbreeding in our population. In G3, we found that the younger breeding female, G3FY, was sired by male AM3. G3FY and AM3 produced offspring together, G3FY2005. In G4, the younger breeding female, G4FY, was closely related to the resident male of her group, OAM4 (*pairwise relatedness* = 0.677). G4FY produced two offspring when OAM4 was the resident male, however, both of her offspring were sired by different extra-group males. After OAM4 was replaced by RAM4, G4FY reproduced with the new resident male, suggesting G4FY avoided breeding with OAM4. Moreover, the standardized heterozygosity of the six EGP offspring was significantly higher than for the eight IGP offspring (Wilcoxon test: $N_1 = 6$, $N_2 = 8$; $W = 38$, $p = 0.038$, $r = 0.491$; Figure 2b, Table S4), supporting a pattern of inbreeding avoidance.

4 | DISCUSSION

4.1 | Stable one-male two-female gibbon groups

Based on 16 years of field observations of three crested gibbon groups, we found that a one-male two-female group was the dominant social or residence pattern in this population. Although each group experienced the replacement of one or more breeding members, no group deviated from a one adult male and two adult females social grouping for more than 6 months, and no group was characterized by a single male-female pair.

4.2 | Male reproductive strategy

In this population, the two breeding females in each group generally reproduced during different years (Hu et al., 2018). Thus, we expected that the lone resident male could monopolize breeding success in his group and EGP would be minimal. However, contrary to our prediction, 40% of the infants born were sired by males other than the resident. This is considerably higher than that reported in other gibbon populations, for which EGP ranges from 0% to 10% (Barelli et al., 2013; Kenyon et al., 2011; Oka & Takenaka, 2001).

Resident male crested gibbons face several challenges in attempting to monopolize mating with resident females. First, there is no clear evidence of sexual swellings in *Nomascus* gibbons (Bolechova et al., 2019). Sexual swellings occur in two gibbon genera, *Hylobates* (Cheyne & Chivers, 2006) and *Symphalangus* (Chivers, 1974), and in these taxa copulations between the resident male and the group's lone female were most common when females were swollen (Barelli et al., 2008; Cheyne & Chivers, 2006). However, in *N.* gibbons, males often copulate with pregnant females, suggesting that they either are unable to accurately detect ovulation (*N. nasutus*, Fan et al., 2016; *N. concolor*, unpublished data) or that copulations represent a form of affiliative behavior reinforcing a social bond between the resident male and group females (Fan et al., 2016).

Second, when foraging, females can be >30 m from the adult male for more than 1 h (Fan & Jiang, 2010) and since copulations are silent and typically last only a few seconds (Fan et al., 2016; Huang et al., 2013), it may be difficult for resident males to defend promiscuous females.

Third, it is difficult for resident males to detect floater males in the dense forest that characterizes gibbon habitats because floater males move cryptically and rarely vocalize. During our study, nine males and three females reached sexual maturity and dispersed from their natal groups (Hu et al., 2018). This, along with a 1:2 adult male to adult female sex ratio in established groups, suggests that either the mortality of males is higher than that of females or that male crested gibbons spend several years as floaters waiting to enter an established group. A high density of floater males and their silent nature appear to make it difficult for resident males to guard or defend females.

Based on paternity data, no resident male sired offspring in a neighboring group. However, we did observe a resident male in one group copulating with a female residing in a neighboring group during an intergroup conflict (Huang et al., 2013). Thus, it is likely that some resident males can increase their breeding success by occasionally mating outside their group. Similarly, the ranges of floater males may overlap with the ranges of several gibbon groups, and floaters may spend considerable time following established groups in an attempt to increase their reproductive opportunities. In general, we believe that the primary strategy for resident males is to defend their territory and the group's two breeding females. This appears to be a generally successful strategy in this population, given that except for adult male G3FB2001, who was one of two potential fathers of offspring G3FY2013, all other resident males sired at least one offspring during their tenure.

We found that two floater males each sired one offspring. Paternity could not be assigned to a known male in our database for four additional offspring, suggesting that there were additional undetected floater males in the population. Offspring sired by floater males also have been reported in a population of golden-cheeked gibbons (*N. gabriellae*) (Kenyon et al., 2011). Little is known about the length of time a male typically remains a floater, the percentage of floater males that eventually become resident males, or the reproductive output of floater males compared to resident males.

Gibbons are characterized by a slow life history, an extended juvenile period, a late age (10 years old) at first reproduction and a long inter-birth interval (3–4 years), and females produce relatively few offspring during their lifetime (Fan et al., 2021). This, coupled with the fact that gibbon groups contain only two adult females, means that even after 16 years of observations our sample size of reproductive output is small. However, our results show that both a floater male reproductive strategy and a resident male reproductive strategy can result in successfully siring offspring, and the pool of potential sires for female western black crested gibbons is larger than the number of resident males in her group. Thus, our study has clearly demonstrated that in *Nomascus* gibbon social and breeding systems, residence and breeding are often decoupled.

4.3 | Female reproductive strategy

An earlier study by Hu et al. (2018) showed the two breeding females in each of our three groups were mother–daughter pairs, suggesting the possibility of a high degree of female philopatry. These females formed coalitions that defended their territory, evicted floater females (Fan et al., 2006), and shared the meat of a flying squirrel (Fan & Jiang, 2009). By remaining in their natal group with their mother, a young adult female may benefit from the joint defense of food resources within their territory, as well as reducing risks associated with dispersal. However, adult females also actively seek EGCs, which may serve to increase opportunities for mate choice by allowing them to breed with dissimilar males or to increase offspring heterozygosity.

Our results offer partial support for the inbreeding avoidance hypothesis. Four of seven breeding females in our western black crested gibbon population successfully produced offspring with an extragroup male. We confirmed two cases of inbreeding avoidance in G4, in which a young adult female was resident in the same group with a close male relative, and her offspring was sired via EGC. However, we also identified one case of father–daughter incest that resulted in the birth of an offspring in G3. Inbreeding avoidance appears to be influenced by whether the breeding pair had spent (part of) their developmental period together (de Boer et al., 2021). We note, however, that we do not know whether the female's father was the resident male or a floater male when she was born in G3, because the female was born prior the start of the study. It is possible that this female was sired by a floater male who later became the resident male of her natal group. If this is the case, this female would not be expected to avoid breeding with her father because they did not live together when the female was young. But this speculation needs further investigation in the future.

Overall, our results indicate that the standardized heterozygosity of EGP offspring was higher than that of IGP offspring. A similar result was found in a study of meerkats (Leclaire et al., 2013). However, the degree to which offspring heterozygosity is related to offspring fitness requires further research (Leclaire et al., 2013). In this regard, it is important to distinguish between a social or residence unit, which represents the set of individuals who regularly travel, feed, forage, rest, and socially interact, and the breeding system, which represents the set of individuals who contribute genes to the next generation (Garber et al., 2016).

4.4 | Flexibility in the social and mating system of gibbon

Traditionally, gibbons were reported to live in small family groups consisting of an adult pair and their putative offspring. However, as more species have been studied and for longer periods of time, additional social and mating system have been described. For example, multimale groups have been observed in white-handed gibbons, *Hylobates lar* (Barelli et al., 2007, 2008) and siamangs, *Symphalangus syndactylus* (Lappan et al., 2017). Stable one-male two-female groups are commonly reported in *N. nasutus*, *N. hainanus*, and *N. concolor* (Guan et al., 2018). Temporary one-male and two-female groups also have been documented in white-handed gibbons (Reichard et al., 2012). Overall, approximately 10% of gibbon groups have more than two adult individuals (Fuentes, 2000). Thus, the social and mating system of the Hylobatidae is more flexible than previously thought. Unfortunately, this flexibility has not been fully acknowledged in the literature that has explored the evolution of primate social systems (Opie et al., 2012; Shultz et al., 2011), but sees Kappeler and Pozzi (2019).

5 | CONCLUSIONS

Genetic analyses revealed an unexpectedly high level of EGP in a population of crested gibbons that form single adult male two adult females social units. Although groups contain only two resident females and each generally breeds in a different year, resident males are unable to monopolize female' mating and paternity. Floater males reproduced with females living in established groups, presumably through a process of female mate choice and sneaky copulations. Female engagement in EGP resulted in an increase in offspring heterozygosity, providing partial support for the inbreeding avoidance hypothesis. Our study reveals that the gibbon breeding system is more flexible than previously thought and underscores the important distinction between a species' social system and its breeding system. Although it has traditionally been assumed that the gibbon social system and breeding system are composed of the same set of individuals, this is clearly not the case for western black crested gibbons.

AUTHOR CONTRIBUTIONS

Xia Huang: Formal analysis (lead); investigation (lead); visualization (lead); writing—original draft (lead). **Nai-qing Hu**: Data curation (supporting); formal analysis (equal); methodology (supporting). **Kai He**: Methodology (supporting); writing—review & editing (supporting). **Zhen-hua Guan**: Formal analysis (supporting); methodology (equal). **Colin A. Chapman**: writing—review & editing (equal). **Paul A. Garber:** writing—review & editing (equal). **Xue-long Jiang:** Conceptualization (lead); funding acquisition (lead). **Peng-Fei Fan**: Conceptualization (lead); funding acquisition (lead); methodology (lead); project administration (lead); supervision (lead); writing—review & editing (lead).

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The raw data used and analyzed during the present study are available in supporting files.

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SUPPORTING INFORMATION

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