ZOOLOGICAL RESEARCH

Determining the level of extra-pair paternity in yellow-bellied prinias, a socially monogamous passerine

DEAR EDITOR,

Previous work based on molecular evidence has shown that most socially monogamous birds follow a genetic polyandrous mating system. However, our knowledge about avian mating systems is heavily biased toward the north temperate zone, with data on tropical birds remaining relatively scarce. This uneven distribution of both phylogenetic and spatial sampling has hampered our understanding and interpretation of results. In this study, we investigated the frequency of extra-pair paternity (EPP) in a tropical population of yellow-bellied prinias (*Prinia flaviventris*) in Guangxi, southern China. A total of 129 individuals belonging to 24 nests were sampled, among which 12 out of 83 chicks (14.46%) in seven nests were found to be EPP offspring. In nests in which all nestlings were sampled, only five out of 56 chicks were EPP offspring, accounting for an unbiased EPP rate of 8.93%. This rate is below the average rate of EPP in the family Sylviidae. The possible causes of EPP in prinias and the occurrence of EPP in birds with high resource investment and intensive parental care are discussed. This study highlights the value of genome-wide markers in determining relatedness in a wild bird species without a reference genome.

More than 90% of all bird species are socially monogamous (Lack, 1968). In socially monogamous species, one expects breeding pairs to have low mating success variance due to limited sexual selection forces on males and females, and thus both sexes would play more cooperative roles in parental care (Remeš et al., 2015). However, even for sexually monomorphic species, both males and females can copulate with non-social partners through extra-pair mating, although it is usually difficult to discover such a reproductive strategy in socially monogamous birds without determining parentage using DNA samples. Based on meta-analysis, Brouwer & Griffith (2019) found that nearly 76% of socially monogamous passerine species (255 species) exhibit rates of extra-pair paternity (EPP thereafter). However, despite numerous studies collecting data on EPP rates in many species (a total of 342), this represents less than 4% of bird species and 44% of bird families (Brouwer & Griffith, 2019). This bias in sampling (i.e., uneven distribution of sampling both phylogenetically and geographically) has hampered our understanding and interpretation of results. Thus, to obtain comprehensive insights into variation in overall rates of EPP, more studies across representative bird species from various regions are needed, especially in Asia, where few studies have been reported on this topic (Figure 6 in Brouwer & Griffith, 2019).

Several factors have been proposed to explain variation in EPP (reviewed in Brouwer & Griffith, 2019; Griffith et al., 2002; Westneat & Stewart, 2003), which can be grouped into adaptive and non-adaptive hypotheses, each including the role of ecology, life history, and/or genetic diversity. Among them, the constrained female hypothesis proposed by Gowaty (1996) is considered a possible explanation for variation in EPP. This hypothesis states that females are constrained in regard to EPP because of limited energy. For example, females are likely constrained by the requirement of resource investment for egg production (Cordero et al., 1999) and by greater investment in parental care (i.e., a trade-off between female parental investment and certainty of EPP, Trivers, 1972). Therefore, a higher level of EPP would be expected under increasing food abundance (Hoi-Leitner et al., 1999).

The above hypothesis might be explicable for small passerines with certain life-history traits, e.g., short life span and intensive reproductive investment in regard to number of offspring reproduced and parental care (Sæther, 1988). In addition, male parental care is negatively related to paternity, i.e., a low level of EPP will occur when the rates of male parental care are high (Møller & Cuervo, 2000). Based on

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these assumptions, short-lived species with intensive parental care should exhibit a low rate of EPP. However, recent reviews suggest that a single factor is unlikely to explain all patterns of EPP (Griffith et al., 2002), especially for poorly studied species in tropical zones (Macedo et al., 2008).

The yellow-bellied prinia (*Prinia flaviventris*) is a small passerine bird with a monogamous social mating system (Ding et al., 2007, 2016, 2017a). This species demonstrates subtle sexual dimorphism (color difference in upper back), unique seasonal changes in tail length (with shorter tails in the breeding season than in the non-breeding season; Ding et al., 2007), and wide distribution in southern China. Previous work has shown that yellow-bellied prinias display high parental investment during the breeding season and may suffer energy deficits (Ding et al., 2016, 2017b). This species has an average clutch-size of 4.34±0.84 (*n*=18), and exhibits elaborate biparental care, with both parents participating in all breeding activities such as nest building, egg incubation, and nestling feeding (Ding et al., 2016, 2017b). Specifically, yellow-bellied prinias show enormous overlap between spring molting and breeding activities (both are energy-consumptive behaviors) as well as higher feeding frequencies (compared to sister species with similar body size belonging to the same family) and relatively higher annual productivity than the nine passerine bird species with similar body mass (Ding et al., 2016, 2017b).

In this study, we determined the level of EPP in yellow-bellied prinias using parental analysis based on genetic variants of genome-wide polymorphic markers, i.e., single-nucleotide polymorphisms (SNPs). Conventionally, genetic-based parental analysis for birds employs polymorphic microsatellite markers (Burke & Bruford, 1987; Gibbs et al., 1990). Given the rapid developments of high-throughput whole-genome sequencing, genome-level SNPs obtained from cost-effective genotype approaches should provide a more powerful and time-effective solution (Flanagan & Jones, 2019; Hauser et al., 2011; Manichaikul et al., 2010; Weinman et al., 2014). Based on known life-history traits of the studied species, we predicted that yellow-bellied prinias would likely display a low level of EPP because they are more energetically limited.

Data collection from a breeding population of yellow-bellied prinias was carried out in Nonggang National Nature Reserve (N23°39′, E107°04′), Guangxi, southern China, from May to July in the 2013 breeding season (see Ding et al., 2017a; Yang et al., 2014 for details). Birds were banded and blood samples were collected in the field. Blood samples were obtained via brachial venipuncture of the left wing of adults and at least one chick from each clutch following standard protocols. These samples were stored in 96% ethanol and kept in a cool condition in the field before transfer to a –40 °C freezer. All experiments conducted in this study complied with the current laws of China, where they were performed. All experimental procedures were in accordance with the Animal Research Ethics Committee of Hainan Provincial Education Centre for Ecology and Environment, Hainan Normal University (Permit No. HNECEE-2012-002).

In total, 129 individuals from 24 nests were collected. Among these, one nest (Nest ID: C) lacked data on the female parent, one nest (Nest ID: O) lacked data on the male parent, and 11 nests had incomplete sampling of nestlings (range 1–4 missing individuals). In total, our dataset consisted of 46 social parent birds and 83 nestlings for subsequent sequence analysis (Supplementary Table S1). Complete broods (*n*=56 nestlings) were sampled in 13 nests, including one nest with a missing sample from the social mother.

DNA extraction for all samples was performed with a DNEasy Blood and Tissue Kit (Qiagen, Germany) following the manufacturer's protocols for blood. We then quantified DNA concentration and quantity using three methods: i.e., (1) bands based on 1% agarose gel electrophoresis; (2) Nanodrop (Wilmington, USA) test for DNA purity (OD260/OD280); and (3) Qubit® DNA Assay Kit with a Qubit® 2.0 Fluorometer (Life Technologies, USA) to measure DNA concentration.

Genotyping-by-Sequencing (GBS) library preparation and sequencing were performed using the protocols in Elshire et al. (2011) at NovoGene Bioinformatics Technology Co., Ltd., China (www.novogene.cn). In brief, individual genomic DNA was digested with two restriction enzymes, i.e., MseI and HaeIII. The digested DNA was then ligated using one of 96 uniquely barcoded sequencing adaptor pairs (Elshire et al., 2011; Morris et al., 2011). Library amplicons between 250 and 600 bp were extracted from agarose gel and the quality of the libraries was quantified using a Qubit High Sensitivity Assay Kit (Novogene, China). Finally, the libraries were sequenced using the Illumina HiSeq2500-PE125 platform (Illumina, USA), which generates 150 bp paired-end reads. Data with sequenced reads were deposited in Dryad: https://doi.org/10.5061/dryad.73n5tb2v7 (Ding et al., 2020). The raw data obtained by high-throughput sequencing were filtered to remove adapters, missing data, and low-quality reads. After these steps, we obtained 61.27 Gb of clean data, with an average of 474.96 Mb of data (336.11–713.09 Mb) for each individual. We used the standard STACK (v2) program (Catchen et al., 2011, 2013) to align the sequences into matching stacks using the command "Ustacks" with the parameters "-t gzfastq -i -m 3 -M 3 -p 15 -d -r –f –o". We then built the catalog from this set of samples and created a set of consensus loci, merging alleles together using the command "Cstacks" with the parameters "b 1 –o –s –n 2 –p 15". In the case of a genetic cross, a catalog was constructed from the parents of the cross to create a set of all possible alleles expected in the progeny of the cross. We searched sets of stacks against a catalog produced by "Cstacks" using the command "Sstacks" with the parameters "-b 1 –c –p 15". In the case of a genetic map, stacks from progeny were matched against the catalog to determine which progeny contained which parental alleles. We finally corrected genotypes using the command "Sstacks" with the parameters "-b 1 –P -r 1 -c -s -t CP". After these steps, to exclude SNP calling errors, only high-quality SNPs (coverage depth ≥5 and l, rate of missing

data ≤0.5) were selected for further analyses. Genotype data were cleaned using PLINK v1.07 (Purcell et al., 2007).

Because of incomplete sampling of parents and nestlings in some nests, we were not able to apply the conventional parentage program, CERVUS (Kalinowski et al., 2007; Marshall et al., 1998), which assigns parents to offspring based on the possibility that the genotypes of parents and offspring match. Instead, we used two methods with different principles to infer relatedness within and between nests, and thus identify potential EPP nestlings.

First, to visualize the genetic relationships among individuals within nests, we reconstructed a neighbor-joining tree using the program "*fneighbor*" in PHYLIP v3.5 (Felsenstein, 1993). This method is based on the uncorrected genetic *P*-distance (Nei & Kumar, 2000) between each pair of individuals (dyad) using individual SNP genotypes. The resulting phylogenetic tree was visualized in MEGA v6 (Tamura et al., 2013). Because genetic relationships between nestlings could be either full-siblings (no EPP offspring) or half-siblings (EPP offspring), we expected individuals from the same nest to have shorter genetic distances than individuals among nests, and that nestlings would be closely related to their mother. If nestlings in a nest were not closely related to their social father, they were considered EPP offspring sired by another male. Because the sampling of all possible males in tropical habitats is difficult, it is highly unlikely we would be able to find the genetic father of EPP offspring. Overall, this method offers rapid and qualitative detection of EPP offspring when sampling is not perfect but is not a quantitative means to offer relatedness estimates.

Second, we estimated pairwise relatedness using a population genetic method, in which identical-by-descent (IBD) statistics between dyads are determined based on the estimation of allele frequencies at each SNP under Hardy-Weinberg Equilibrium (HWE) assumptions. Because population substructures and high levels of inbreeding deviate from the HWE and thus affect parental analysis (Huang et al., 2018), we ascertained several population genetic estimates, such as observed and expected heterozygosity of genotypes, inbreeding coefficients (F_{IS}), and population substructures for the study population using POLYRELATEDNESS v1.4 (Huang et al., 2016). We estimated the relatedness of all samples in all nests using KING v2.1.3 (Manichaikul et al., 2010). This method estimates the kinship coefficient using genome-wide SNPs with no missing sites. The analytical framework of KING accurately estimates genetic distance between a pair of individuals as a function of their allele frequencies and kinship coefficients with or without population substructure. According to the method description and program recommendations (Manichaikul et al., 2010), a kinship coefficient threshold between 0.177 and 0.354 for a pair of individuals (a dyad) indicates a full-sibling or parent-offspring relationship (first-degree relative). Half-siblings (second-degree relative) have kinship coefficients between 0.088 and 0.177 and third-degree relatives have kinship coefficients between 0.022 and 0.088. Unrelated individuals have a negative kinship coefficient value or a value that never exceeds 0.022 (Manichaikul et al., 2010). These threshold values are based on simulations using a high density of SNPs. For smaller datasets, missing data, heterozygosity rates, and rare alleles may influence the estimation of kinship coefficients. Thus, we further checked the values between individuals within a nest and plotted kinship coefficients using the R program (R Core Team, 2018). We considered an individual to be an EPP offspring if it was not closely related to one of its parents (usually the male) or did not show a full-sibling relationship (half-sibling or unrelated) with at least one other nestling within the same nest.

We successfully sequenced 129 individuals using the GBS approach based on the Illumina short-read sequencing platform. In total, 12 nests had complete data, with the remaining nests lacking information on some nestlings and/or parent birds (Supplementary Table S1). Because there is currently no genome available for closely related species of prinias, we generated genetic maps using the STACK pipeline and acquired a total of 532 411 SNPs. For each individual, we obtained an average of 2 738.85±1 676.07 (mean±*SD*, same below) SNPs, with an average heterozygosity of 1 115±792.33 (heterozygosity rate of 39.33%±6.23%) (Figure 1A). After all filtering and correction steps, 210 388 SNPs were retained for further analysis.

The neighbor-joining tree based on uncorrected genetic *P*-distances using individual SNP genotypes of the yellow-bellied prinia showed that individuals within the same nest formed an inclusive cluster. We identified 24 clusters, representing the 24 sampled nests. In all cases, nestlings were non-exclusively clustered together with their female parent (Figure 1C). Most male parents were also clustered with nestlings of the same nests, except for two males (S3 and TT1), suggesting that they were not the genetic father of their specific nestlings. In the nest CC, two nestlings were clustered with the male (CC7), whereas the other three offspring were clustered with the female (CC6). Therefore, these three nests likely contained EPP offspring.

Using the program POLYRELATEDNESS, we determined certain population genetics of our study population. The observed and expected heterozygosities of genotypes were 0.1012 and 0.1277, respectively. The estimated inbreeding coefficient (F_{IS}) was 0.166, suggesting a low level of inbreeding, which may have a neglected effect on parental analysis (Huang et al., 2018). In addition, we did not detect any population substructure signatures (global F_{st}=0), meaning that the overall samples could be treated as a single population.

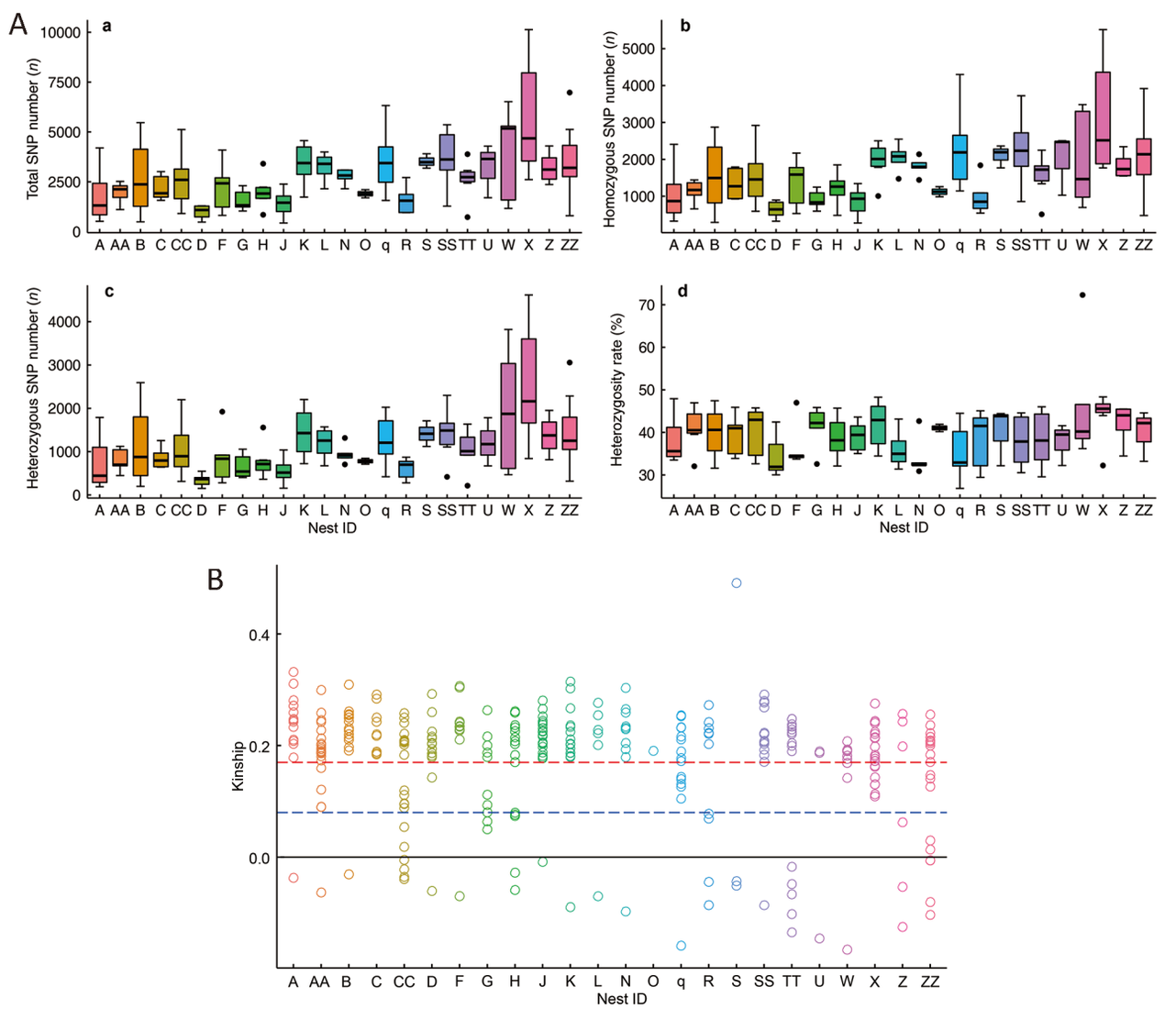
We obtained a matrix of pairwise kinship coefficients based on 642 non-missing SNPs with a minimum allele frequency ≥ 0.05 using KING. As expected, individuals among nests had negative values, indicating unrelated relationships (data not shown). We obtained pairwise kinship coefficients between dyads within each nest (Supplementary Table S2) and plotted these values based on thresholds of different relative degree (Figure 1B). The average kinship coefficient of this population

was 0.1747±0.09. We found parents of each nest were usually unrelated individuals, except in nests X and G, where parents had a positive kinship value, indicating the cause of inbreeding in this population. In three nests (CC, TT, and S), we found evidence of unrelated relationships between males and nestlings (*n*=3, 4, 1, respectively). We also detected other candidate EPP individuals (Table 1), which showed consistent patterns of kinship coefficients, as described above. In a few cases, although we detected a lower degree of relatedness within a nest, e.g., AA, D, G, and q, we were able to determine the occurrence of EPP for certain individuals. We assumed that these low kinship coefficients may be an artefact of SNP data due to the low rate of heterozygosity.

Based on the above results, 12 out of 83 chicks (14.46%, 95% CI=6.89%–22.02%, Rohlf & Sokal, 1981) from seven nests were found to be the offspring of extra-pair copulations. If we only consider fully sampled nests (13), five out of 56 chicks from three nests were EPP offspring, accounting for an unbiased EPP rate of 8.93% (95% CI=1.46%–16.40%) and a rate per nest of 23.07% (95% CI=5.04%–53.79%). Notably, we did not find EPP offspring in the two nests (G and X) with inbred parents.

In this study, we found EPP rates ranging from 8.93% to 14.46%. In addition, the sampled prinia population was characterized by a relatively low level of genetic diversity and low level of inbreeding. Overall, using two methods with different principles, we showed that the likelihood of EEP was fairly low in the yellow-bellied prinia population.

Bird species within a family tend to exhibit similar rates of EPP (Brouwer & Griffith, 2019). In Sylviidae birds, EPP rates are about 10% (Brouwer & Griffith, 2019), which is slightly higher than that observed for the yellow-bellied prinias (8.93%, unbiased EPP rate). Nevertheless, family-level variation in EPP is still striking, even when considering monogamous passerine bird species with biparental care (Brouwer & Griffith, 2019). Our observations, in contrast to those reported by



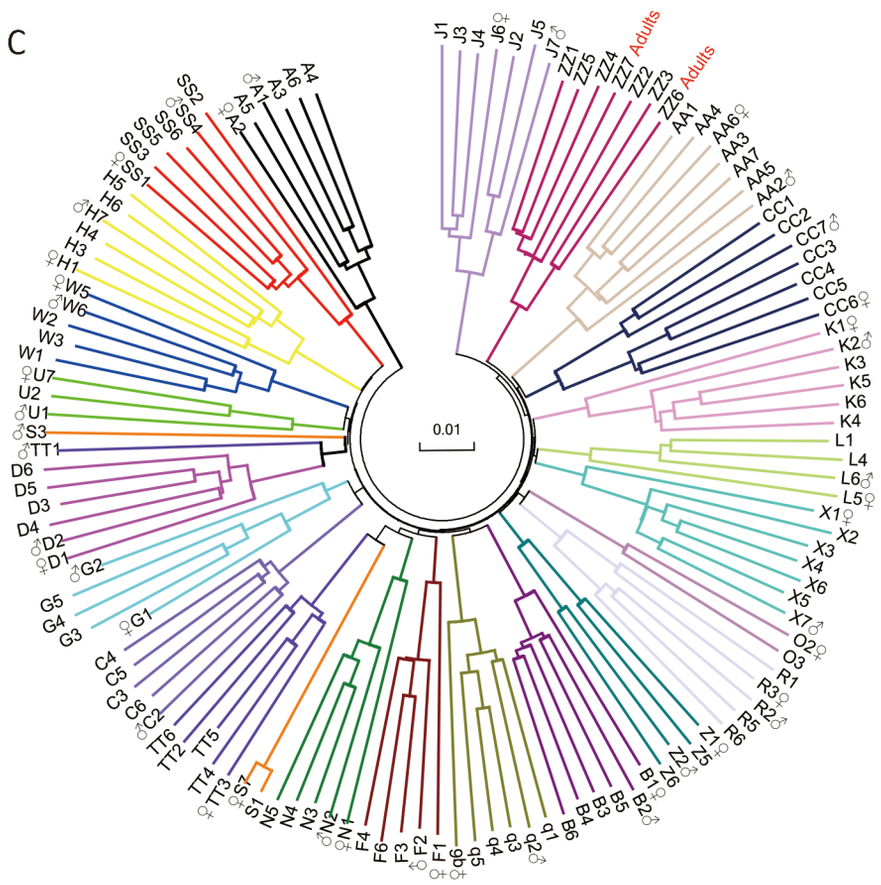


Figure 1 Summary of SNPs and genetic relationships among yellow-bellied prinia individuals

A: Summary information of SNP number and heterozygosity of all sequenced samples of yellow-bellied prinias (a refers to total SNP number, b refers to homozygous SNPs, c refers to heterozygous SNPs, and d refers to heterozygosity rate). B: Kinship coefficients of individuals within each nest. Dashed red line represents threshold value (0.177) of first-degree relatives and dashed blue line represents threshold value (0.088) of second-degree relatives. Black line indicates zero value of coefficient. C: Neighbor-joining tree based on genetic *P*-distances between any two individuals using individual SNP genotypes of yellow-bellied prinia (different colors refer to different nests, codes at tips of branches show individual IDs, information about male and female parents (we did not identify the sex of parents in one nest (Nest ID: ZZ), see details in Supplementary Table S1). Red box indicates low kinship coefficients with 'offspring' of specific nests.

meta-analysis, may be due to differences in phylogeny and geographical variation. For example, previous research has reported that EPP rates are quite different across continents, with average EPP levels of 11% in Europe, 20% in Africa, 23% in Australasia, 17% in South America, and 20% in North America (Brouwer & Griffith, 2019). In addition, variation in EPP may be related to different study methodologies and/or estimate quality. As few studies have reported on EPP rates in China, whether the low level of EPP presented in this study is a general or unique phenomenon needs further investigation.

Nevertheless, we suggest that the low EPP rate in yellow-bellied prinias may be related to energy constraints, as both parents exhibit high energy-consuming behavior during the breeding season (Ding et al., 2007, 2016, 2017b). Indeed, a negative relationship between female and/or male parental investment and certainty of EPP has been reported in other studies (e.g., Bonier et al., 2014; Dixon et al., 1994; Perlut et al., 2012; Petrie & Kempenaers, 1998). During breeding, both parents engage in nest-building, which is regarded as an energetically and temporally expensive activity (Mainwaring & Hartley, 2013). Furthermore, partner and nestling feeding are frequently observed for both parents (Ding et al., 2016). Tail molting, which is another energy-demanding behavior (Hoye & Buttemer, 2011), also occurs during the breeding period. Taken together, these energetically costly activities in breeding yellow-bellied prinias may prevent extra-pair copulations, leading to a low level of EPP. Indeed, it is important to note that the low level of inbreeding in yellow-bellied prinias may indicate that females engage in extra-pair copulation to reduce inbreeding depression. However, this needs further confirmation. In addition, the relatively low population genetic diversity may also contribute to the low level of EPP as females obtain little scope for good genes (different males with similarly low genetic variability would be

Table 1 Summary of degree of kinship coefficients and number of EPP offspring in each nest of yellow-bellied prinia

Nest ID.	No. of nestlings and parents (*n*)	No. of relationships (*n*)	1st degree relative	2nd degree relative	3rd degree relative	Unrelated	EPP	Full brood
A	6	15	14	0	0	1	0	No
AA	7	21	16	4	0	1	0	Yes
B	6	15	14	0	0	1	0	Yes
C	5	10	10	0	0	0	0	No
CC	7	21	10	5	2	4	3	Yes
D	6	15	13	1	0	1	0	Yes
F	5	10	9	0	0	1	0	No
G	5	10	5	3	2	0	0	Yes
H	6	15	10	0	0	5	1	Yes
J	7	21	20	0	0	1	0	Yes
K	6	15	14	0	0	1	0	Yes
L	4	6	5	0	0	1	0	No
N	5	10	9	0	0	1	0	Yes
O	2	1	1	0	0	0	0	No
q	6	15	9	5	0	1	0	No
R	5	10	6	0	2	2	1	No
S	3	3	1	0	0	2	1	No
SS	6	15	14	0	0	1	0	Yes
TT	6	15	10	0	0	5	4	No
U	3	3	1	1	0	1	0	No
W	5	10	8	1	0	1	0	No
X	7	21	14	7	0	0	0	Yes
Z	4	6	3	0	1	2	1	No
ZZ	7	21	12	3	2	4	1	Yes
Total	129	304	228	29	9	38	12	12/12

expected to engage in EPP rarely, Møller & Cuervo, 2003; Petrie et al., 1998). Finally, evidence shows that male parental care is negatively related to the frequency of EPP (Møller & Cuervo, 2000), and the high rate of male parental care, as seen in yellow-bellied prinias, may prevent male extra-pair copulations because they not only participate in nest building and egg incubation, but also in nestling feeding.

In conclusion, the present study showed that EPP rates are low in yellow-bellied prinias, which may be related to energy constraints (i.e., female constraint hypothesis). However, caution is needed when interpreting the results due to several study limitations, namely the lack of experimental work, consideration of a single population, and interpretation using a single hypothesis (Brouwer et al., 2017; Brouwer & Griffith, 2019). In addition, the incomplete broods included in analysis may have affected the rates of EPP (Sardell et al., 2010). That is, incomplete sampling of offspring and males could underestimate or overestimate the frequency of EPP and restrict the use of genotype-matching approaches like CERVUS. Nevertheless, we applied an analytical framework using both phylogenetic and population genetic approaches, which were based on thousands of genome-wide SNP makers. Therefore, the results of this study are more powerful than results using tens of microsatellites (Weinman et al., 2014). Taken together, the merits and limitations of this study highlight the necessity for collecting more data at multiple temporal and spatial scales on this species and other prinia birds to gain insight into the underlying mechanisms of low-level EPP.

SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

Fieldwork, including capturing and sampling birds, was carried out under permission from the Nonggang National Nature Reserve, Guangxi, China.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

We declare that the authors have no competing interests.

AUTHORS' CONTRIBUTIONS

H.H. and W.L. conceived and designed the study. L.W. sampled and collected data in the field. Z.D., C.Z., W.Z., Q.Y., G.R., and E.L. performed laboratory work and data analyses. Z.D. wrote the draft manuscript. H.H. and W.L. improved the manuscript. All authors read and approved the final version of the manuscript.

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REFERENCES

Bonier F, Eikenaar C, Martin PR, Moore IT. 2014. Extrapair paternity rates vary with latitude and elevation in Emberizid sparrows. *The American Naturalist*, **183**(1): 54−61.

Brouwer L, Van De Pol M, Aranzamendi NH, Bain G, Baldassarre DT, Brooker LC, , et al. 2017. Multiple hypotheses explain variation in extra‐pair paternity at different levels in a single bird family. *Molecular Ecology*, **26**(23): 6717−6729.

Brouwer L, Griffith SC. 2019. Extra‐pair paternity in birds. *Molecular Ecology*, **28**(22): 4864−4882.

Burke T, Bruford MW. 1987. DNA fingerprinting in birds. *Nature*, **327**(6118): 149−152.

Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA. 2013. Stacks: an analysis tool set for population genomics. *Molecular Ecology*, **22**(11): 3124−3140.

Catchen JM, Amores A, Hohenlohe P, Cresko W, Postlethwait JH. 2011. *Stacks*: building and genotyping loci *De novo* from short-read sequences. *G3*, **1**(3): 171−182.

Cordero PJ, Wetton JH, Parkin DT. 1999. Extra-pair paternity and male badge size in the house sparrow. *Journal of Avian Biology*, **30**(1): 97−102.

Ding ZF, Zhang WS, Yuan QM, Wang LW, Ren G, Li E, et al. 2020. Determining level of extra-pair paternity in the yellow-bellied prinia, a socially monogamous passerine. Dryad, dataset: https://doi.org/10.5061/dryad.73n5tb2v7.

Ding ZF, Ji F, Huang QL, Wang LW, Jiang AW, Zhang CL, et al. 2017a. Brood sex ratio in the yellow-bellied prinia (*Prinia flaviventris*). *Avian Research*, **8**: 15.

Ding ZF, Liang JC, Pan XY, Hu HJ. 2016. Feeding behavior and nestling growth of yellow-bellied prinia (*Prinia flaviventris*). *Chinese Journal of Zoology*, **51**(6): 969−976. (in Chinese)

Ding ZF, Liang JC, Zhou ZX, Feng YJ, Hu HJ. 2017b. Comparisons of breeding parameters of two prinia species. *Chinese Journal of Zoology*, **52**(3): 417−422. (in Chinese)

Ding ZF, Tang SX, Zhang JX, Chen YZ, Hu HJ. 2007. Autumn moulting of the adults of yellow-bellied prinia, *Prinia flaviventris*. *Chinese Journal of Zoology*, **42**(6): 28−33. (in Chinese)

Dixon A, Ross D, O'Malley SLC, Burke T. 1994. Paternal investment inversely related to degree of extra-pair paternity in the reed bunting. *Nature*, **371**(6499): 698−700.

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, et al. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One*, **6**(5): e19379.

Felsenstein J. 1993. PHYLIP (Phylogeny Inference Package), version 3.5 c.

Flanagan SP, Jones AG. 2019. The future of parentage analysis: from microsatellites to SNPs and beyond. *Molecular Ecology*, **28**(3): 544−567.

Gibbs HL, Weatherhead PJ, Boag PT, White BN, Tabak LM, Hoysak DJ. 1990. Realized reproductive success of polygynous red-winged blackbirds revealed by DNA markers. *Science*, **250**(4986): 1394−1397.

Gowaty PA. 1996. Battles of the sexes and origins of mono-gamy. *In*: Black JM. Partnerships in Birds: The Study of Monogamy. Oxford: Oxford University Press, 21–52.

Griffith SC, Owens IPF, Thuman KA. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology*, **11**(11): 2195−2212.

Hauser L, Baird M, Hilborn R, Seeb LW, Seeb JE. 2011. An empirical comparison of SNPs and microsatellites for parentage and kinship assignment in a wild sockeye salmon (*Oncorhynchus nerka*) population. *Molecular Ecology Resources*, **11**(S1): 150−161.

Hoi-Leitner M, Hoi H, Romero-Pujante M, Valera F. 1999. Female extra-pair behaviour and environmental quality in the Serin (*Serinus serinus*): a test of the 'constrained female hypothesis'. *Proceedings of the Royal Society B: Biological Sciences*, **266**(1423): 1021−1026.

Hoye BJ, Buttemer WA. 2011. Inexplicable inefficiency of avian molt? Insights from an opportunistically breeding arid-zone species, *Lichenostomus penicillatus*. *PLoS One*, **6**(2): e16230.

Huang K, Ritland K, Dunn DW, Qi X, Guo S, Li B. 2016. Estimating relatedness in the presence of null alleles. *Genetics*, **202**(1): 247−260.

Huang K, Mi R, Dunn DW, Wang T, Li B. 2018. Performing parentage analysis in the presence of inbreeding and null alleles. *Genetics*, **210**(4): 1467−1481.

Kalinowski ST, Taper ML, Marshall TC. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, **16**(5): 1099−1106.

Lack D. 1968. Ecological Adaptations for Breeding in Birds. Methuen & Co., London.

Macedo RH, Karubian J, Webster MS. 2008. Extrapair paternity and sexual selection in socially monogamous birds: are tropical birds different?. *The*

Auk, **125**(4): 769−777.

Mainwaring MC, Hartley IR. 2013. The energetic costs of nest building in birds. *Avian Biology Research*, **6**(1): 12−17.

Manichaikul A, Mychaleckyj JC, Rich SS, Daly K, Sale M, Chen WM. 2010. Robust relationship inference in genome-wide association studies. *Bioinformatics*, **26**(22): 2867−2873.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM. 1998. Statistical confidence for likelihood‐based paternity inference in natural populations. *Molecular Ecology*, **7**(5): 639−655.

Møller AP, Cuervo JJ. 2000. The evolution of paternity and paternal care in birds. *Behavioral Ecology*, **11**(5): 472−485.

Møller AP, Cuervo JJ. 2003. Sexual selection, germline mutation rate and sperm competition. *BMC Evolutionary Biology*, **3**: 6.

Morris GP, Grabowski PP, Borevitz JO. 2011. Genomic diversity in switchgrass (*Panicum virgatum*): from the continental scale to a dune landscape. *Molecular Ecology*, **20**(23): 4938−4952.

Nei M, Kumar S. 2000. Molecular Evolution and Phylogenetics. Oxford: Oxford University Press.

Perlut NG, Kelly LM, Zalik NJ, Strong AM. 2012. Male savannah sparrows provide less parental care with increasing paternity loss. *Northeastern Naturalist*, **19**(2): 335−344.

Petrie M, Doums C, Møller AP. 1998. The degree of extra-pair paternity increases with genetic variability. *Proceedings of the National Academy of Sciences of the United States of America*, **95**(16): 9390−9395.

Petrie M, Kempenaers B. 1998. Extra-pair paternity in birds: explaining variation between species and populations. *Trends in Ecology & Evolution*, **13**(2): 52−58.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, et al. 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. *The American Journal of Human Genetics*, **81**(3): 559−575.

R Core Team. 2018. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Remeš V, Freckleton RP, Tökölyi J, Liker A, Székely T. 2015. The evolution of parental cooperation in birds. *Proceedings of the National Academy of Sciences of the United States of America*, **112**(44): 13603−13608.

Rohlf FJ, Sokal RR. 1981. Statistical Tables. 2nd ed. San Francisco, CA: W.H. Freeman.

Sæther BE. 1988. Pattern of covariation between life-history traits of European birds. *Nature*, **331**(6157): 616−617.

Sardell RJ, Keller LF, Arcese P, Bucher T, Reid JM. 2010. Comprehensive paternity assignment: genotype, spatial location and social status in song sparrows, *Melospiza melodia*. *Molecular Ecology*, **19**(19): 4352−4364.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, **30**(12): 2725−2729.

Trivers RL. 1972. Parental investment and sexual selection. *In*: Campbell B. Sexual Selection and the Descent of Man. Chicago: Aldine Press, 136–179.

Weinman LR, Solomon JW, Rubenstein DR. 2014. A comparison of SNP and microsatellite markers for analysis of parentage and kinship in a cooperatively breeding bird. *Molecular Ecology Resources*, **15**(3): 502−511.

Westneat DF, Stewart IRK. 2003. Extra-pair paternity in birds: causes, correlates, and conflict. *Annual Review of Ecology, Evolution, and Systematics*, **34**: 365−396.

Yang CC, Wang LW, Cheng SJ, Hsu YC, Liang W, Møller AP. 2014. Nest defenses and egg recognition of yellow-bellied prinia against cuckoo parasitism. *Naturwissenschaften*, **101**(9): 727−734.