Within-group relatedness and patterns of reproductive sharing and cooperation in the tropical chestnut-crested yuhina

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ARTICLE INFO

In cooperatively breeding animals, genetic relatedness among group members often determines the extent of reproductive sharing, cooperation and competition within a group. Studies of species for which cooperative behaviour is not entirely based on kinship are key for understanding the benefits favouring the evolution and maintenance of cooperative breeding among nonrelatives. In the cooperatively breeding chestnut-crested yuhina, *Yuhina everetti*, a songbird endemic to Borneo, we tested whether unrelated helpers are more likely to gain parentage than are related helpers consistent with the hypothesis that inbreeding risk constrains reproduction by related helpers. We also examined whether related or unrelated helpers provision broods more because of differences in their potential indirect or direct fitness benefits of helping. Kin structure of breeding groups (breeding pair and up to eight helpers of both sexes, median = 2 helpers, 96% of 57 pairs had helpers) based on genetic analysis was mixed; 48% of 76 breeder/helper dyads were first-order (26%) or second-order (22%) relatives of one or both members of the breeding pair, and 52% were nonrelatives. Only unrelated male and female helpers gained parentage, and helpers did not differ in their provisioning rate according to their relatedness to the broods. We documented quasi-parasitism or co-breeding by female helpers in 14% of 29 broods and extrapair paternity by male helpers in 21% of 47 broods. This rate of extrapair paternity is relatively high among the few tropical species examined but fit with predictions for mixed-kin groups where inbreeding is avoided. These findings support the emerging pattern for cooperative breeding in birds with mixed-kin groups, wherein unrelated helpers are more likely to gain parentage than are related helpers and helping effort is not necessarily predicted by kinship.

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Within-group relatedness is a strong driver of social and reproductive dynamics in cooperatively breeding animals (Hamilton, 1963; Keller & Reeve, 1994). In many cooperative societies, social groups form through delayed dispersal of juveniles that remain on their natal territory (Brown, 1987; Emlen, 1991; Koenig, Pitelka, Carmen, Mumme, & Stanback, 1992), resulting in high genetic relatedness of breeders and helpers (e.g. southern pied babbler, *Turdoides bicolor*: Nelson-Flower, Hockey, O'Ryan, & Ridley, 2012; red wolf, *Canis rufus*: Sparkman, Adams, Steury, Waits, & Murray, 2012; cave-dwelling cichlid, *Neolamprologus obscurus*: Tanaka et al., 2015). Related helpers might obtain indirect fitness benefits from helping the breeding pair to rear nondescendent kin (Green, Freckleton, & Hatchwell, 2016; Hamilton, 1964). However, a growing number of studies have found low mean levels of genetic relatedness within animal social groups, especially in tropical birds (Cockburn, 1998; Riehl, 2013), with the presence of both related and unrelated group members (e.g. dwarf mongoose, *Helogale parvula*: Creel & Rabenold, 1994; daffodil cichlid, *Neolamprologus pulcher*: Dierkes, Heg, Taborsky, Skubic, & Achmann, 2005; grey-throated babbler, *Stachyris nigriceps*: Kaiser, Martin, Oteyza, Armstad, & Fleischer, 2018). With no indirect benefits, why should unrelated individuals help and how much should they help?

Both related and unrelated helpers might obtain direct benefits, including the inheritance of mates or territories, acquisition of parenting skills and enhanced survival through improved foraging success and protection from predators (Cockburn, 1998, 2004; Griesser, Nystrand, & Ekman, 2006; Taborsky, Frommen, & Riehl,

2016). Helpers might also have access to extrapair mating opportunities and obtain direct fitness benefits from helping to raise their genetic offspring (Cockburn, 2004; Taborsky et al., 2016). However, reproduction by related helpers should be constrained by inbreeding avoidance (Riehl, 2017). In the absence of inbreeding risk, unrelated helpers might gain a share of reproduction (Brouwer, van de Pol, Atema, & Cockburn, 2011; Sanderson, Wang, Vitikainen, Cant, & Nichols, 2015). As a result, unrelated helpers might help more than related helpers if they gain parentage and have a direct reproductive interest in the brood (e.g. white-browed scrubwrens, *Sericornis frontalis*: Magrath & Whittingham, 1997). On the other hand, related helpers might help more than unrelated helpers because of indirect benefits (e.g. western bluebirds, *Sialia mexicana*: Dickinson, Akçay, Ferree, & Stern, 2016; long-tailed tits, *Aegithalos caudatus*: Hatchwell, 2016). Thus, within-group relatedness predicts different degrees of reproductive sharing and cooperation among relatives and nonrelatives because of differences in the potential indirect or direct fitness benefits of helping. Studies of species for which cooperative behaviour is not entirely based on kinship are key for understanding the benefits favouring the evolution and maintenance of cooperative breeding among nonrelatives (Koenig & Dickinson, 2016).

In this study, we examined how within-group relatedness influences patterns of reproductive sharing and cooperation in the cooperatively breeding chestnut-crested yuhina, *Yuhina everetti* — a songbird endemic to the island of Borneo in Southeast Asia (Collar & Robson, 2016). The social and genetic mating system of this species has not been described and the genetic relatedness among group members has not been quantified. We expected that chestnut-crested yuhina breeding groups contained some unrelated helpers because most second-year adults banded as nestlings were observed outside of their natal group (T. E. Martin, personal observations) and detection rates were high (Martin, Riordan, Repin, Mouton, & Blake, 2017). We first characterized the social structure of breeding groups by determining the size and composition of groups and the genetic relatedness of group members. Next, we determined the genetic mating system and the proportion of broods containing offspring produced by one or more male and/or female breeders within breeding groups. We then combined behavioural and genetic data and tested whether helpers are more likely to gain parentage if they are unrelated to the opposite-sex breeder consistent with the hypothesis that inbreeding risk constrains reproduction by related helpers. We also examined whether related or unrelated helpers provision broods more because of differences in potential fitness benefits from helping.

METHODS

Study Population

We studied an individually marked population of the chestnut-crested yuhina in seven study plots in montane forest (1450–1950 m) at Kinabalu Park, Sabah, Malaysian Borneo (6°N, 116°E) during 2009–2015 (Martin, Oteyza, Boyce, Lloyd, & Ton, 2015; Martin, Oteyza, Mitchell, Potticary, & Lloyd, 2015). The monomorphic, chestnut-crested yuhina is endemic to Borneo and a common resident in montane tropical forests (Collar & Robson, 2016), occurring from 550 m to 2600 m at Kinabalu Park (Sheldon, Moyle, & Kennard, 2001). Chestnut-crested yuhinas breed cooperatively and are highly social, but their natural history is poorly known. Individuals live year-round in fluid social groups of up to 20 or more conspecifics (Collar & Robson, 2016). The social groups are not territorial and travel long distances to forage, often interacting with other groups. During the breeding months, social groups can contain multiple, distinct breeding groups (i.e. breeding

pair and helpers), which regularly join these larger flocks to forage (T. E. Martin & J. C. Oteyza, personal observations). Membership in breeding groups is mostly stable within the breeding season (groups were not monitored during the nonbreeding season), but pair bonds and group members can change between years and sometimes between breeding attempts (Oteyza, 2016).

Breeding groups consist of a breeding pair that shares parental activities and helpers that assist in incubation, brooding and nestling provisioning, and occasionally, with nest building (i.e. more than two adults from a breeding group observed carrying nest material) (Martin, Oteyza, Boyce, et al., 2015; Martin, Oteyza, Mitchell, et al., 2015). However, because all adults can exhibit parental behaviours during each stage of the nesting cycle, visually distinguishing breeders from helpers at the nest can be unreliable. Usually, the putative breeding pair builds the nest, an open cup placed in a moss-covered recess along a bank (Collar & Robson, 2016). Females lay their eggs on consecutive days, with 87% laying three eggs and 12% laying two eggs (mean ± 1 SE; clutch size = 2.91 ± 0.02 eggs; $N = 571$ nests; Martin, 2015). Single-egg clutches were not observed. The rare appearance of multiple eggs laid on the same day in unusually large clutches (clutch size = 4–8 eggs, $N = 6$ of 571 nests), suggests co-breeding or conspecific brood parasitism might occur uncommonly in this species (T. E. Martin, J. C. Oteyza, & C. E. Armstad, personal observations). Helpers typically join the breeding pair before or during egg laying and remain with the breeding group until nestlings fledge. Members of the breeding group often visit the nest together to provision nestlings and are repeatedly observed associating with each other at the nest. Individuals from nearby social groups also have been infrequently observed visiting nests with nestlings or seen feeding fledglings produced from multiple breeding groups (T. E. Martin, J. C. Oteyza, & C. E. Armstad, personal observations).

During the primary breeding season (February–June), we captured and marked adults and nestlings, collected blood samples and monitored nesting attempts. We captured adults in mist nets and uniquely marked each adult with a combination of three coloured leg bands and a numbered metal band (Gey Band and Tag Company; Norristown, PA, U.S.A.), two bands on each leg, which allowed us to distinguish among individuals. Nestlings were handled at the nest and marked with a uniquely numbered metal band, typically 6–7 days posthatching once primary feathers had broken their sheaths. Small blood samples (20–70 µl) were collected from the brachial vein and stored in lysis buffer (White & Densmore, 1992) for sexing and parentage analyses. We found nests from parental behaviour and systematic searching. Nests were monitored every 1–3 days throughout all nest stages; once per day during egg laying and twice daily near hatch and fledge dates.

Breeding Group Size and Composition

We determined the size of breeding groups in which all group members were individually identifiable ($N = 57$ breeding groups). The members of the breeding group were identified through resighting surveys conducted during daily nest searching and monitoring activities on each study plot and verified from 6 h video recordings taken on multiple days during the incubation and nestling stages (Martin, 2015; Martin, Oteyza, Boyce, et al., 2015; Martin, Oteyza, Mitchell, et al., 2015). In addition, we performed behavioural observations of the breeding group at a third of the nests used in analyses to corroborate group size and composition obtained from video data. These efforts resulted in reasonable resight and detection rates of associated group members (Martin et al., 2017), such that breeding group membership was clear. The estimated size of a breeding group included all adults that provided parental care at a nest (i.e. breeding pair and helpers). These

estimates excluded individuals not consistently associated with members of the breeding group that were recorded visiting a nest with nestlings on a few occasions, but without feeding (i.e. individuals from social groups foraging nearby).

We only examined the composition of breeding groups for which all group members were both marked and sampled for genetic sexing ($N = 8$ breeding groups). We sexed individuals using methods following Griffiths, Double, Orr, and Dawson (1998). Genomic DNA was extracted from whole blood with the BioSprint 96 Tissue DNA Kit (Qiagen, Valencia, CA, U.S.A.). We amplified from 2 µl of genomic DNA extract with highly conserved primers, P2 and P8, that anneal to exonic regions flanking the introns of the CHD-W (carried by females) and CHD-Z genes (carried by females and males) in a 10 µl polymerase chain reaction (PCR). The PCR products were size-sorted on an Applied Biosystems (ABI, Foster City, CA, U.S.A.) 3130 Genetic Analyzer run with the GeneScan 500 base pair ROX internal size standard (ABI) and alleles scored using Genemapper v.4.1 (ABI). We validated sexing methods by assaying adults of known sex based on brood patch and cloacal protuberance development ($N = 12$ of each sex). We tested for sex bias among helpers in breeding groups and brood sex ratio bias using methods described in Neuhäuser (2004). Sample sizes for determining the size and composition of breeding groups differed because of missing genetic data from helpers (Supplementary Table S1).

Genetic Relatedness and Parentage

We genotyped 480 individuals, including 263 offspring from 104 broods and 215 candidate parents (60 adult females and 155 adult males; putative breeders and helpers) at eight polymorphic microsatellite loci isolated from the chestnut-crested yuhina (Kaiser, Danner, Bergner, & Fleischer, 2015). We followed previously described methods for PCR amplification (Kaiser et al., 2015) and analysed PCR products and scored alleles as described above for genetic sexing. We repeated PCRs at homozygous loci to verify genotypes and assessed potential sources of genotyping error using Micro-Checker v.2.2.3 (van Oosterhout, Hutchinson, Wills, & Shipley, 2004).

We estimated genetic relationships among breeding pairs, breeders and helpers, and helpers and offspring using ML-Relate (Kalinowski, Wagner, & Taper, 2006). We calculated the allele frequencies for the adult population and tested for heterozygote deficiency with the Hardy–Weinberg equilibrium test. We specified the frequency of null alleles detected at two loci (StEv122, StEv118) in analyses calculating maximum likelihood estimates of genetic relatedness (r; Goodnight & Queller, 1999) between pairs of individuals (Kalinowski & Taper, 2006). We calculated the log likelihood of four types of relationships (nonrelatives, half siblings, full siblings and parent/offspring) for each dyad. We used likelihood data to estimate the putative relationship with the highest likelihood and the relative likelihoods of alternative relationships and to identify relationships excluded by the genetic data (e.g. parent/offspring excluded if no alleles at a locus were shared between individuals). We then conducted specific hypothesis tests to evaluate cases where the likelihood of an alternative relationship was not much lower than the likelihood of the putative relationship. The primary hypothesis was that the dyad's putative relationship would have the highest likelihood estimate against the alternative hypothesis that the dyad's relationship would have a lower likelihood. We ran 10 000 simulations to calculate the uncertainty surrounding the estimated relationships at the 95% confidence level (Kalinowski et al., 2006). The alternative hypothesis was rejected when $P < 0.05$. We failed to reject the alternative hypothesis when $P > 0.05$, indicating that both the putative and alternative relationships were consistent with the genetic data.

We averaged pairwise genetic relatedness estimates for assigned first-order relatives (parent/offspring: $N = 104$; full siblings; $N = 41$) and second-order relatives (half siblings; $N = 14$) identified from parentage analyses (see below), breeders and helpers ($N = 76$), the breeding pair ($N = 20$), and helpers and offspring (i.e. mean helper–brood relatedness; $N = 47$). Sample sizes of female breeder/helper dyads and male breeder/helper dyads differed because of missing genetic data from female or male breeders in the same breeding groups (Supplementary Table S1). We conducted a Fisher's exact test to examine whether helpers related to the male or female breeder were more likely to be relatives of the same-sex breeder. We conducted all statistical analyses in R v.3.5.3 (R Core Team, 2019).

Parentage and Sibship Assignment

We conducted maternity (69 offspring from 29 broods) and paternity analyses (120 offspring from 49 broods) for offspring from breeding groups in which we obtained genetic data from both putative breeders (60 offspring from 26 broods), only the female breeder (9 offspring from 3 broods), or only the male breeder (60 offspring from 24 broods) using CERVUS v.3.0.7, which uses a maximum likelihood-based approach to infer parentage (Kalinowski, Taper, & Marshall, 2007; Marshall, Slate, Kruuk, & Pemberton, 1998). CERVUS calculates the natural logarithm of the likelihood ratio (LOD score), which provides the likelihood of parentage of each candidate parent relative to a random female or male in the population for each offspring. CERVUS uses simulations of the allele frequencies of adults in the population to calculate critical differences in LOD scores between the most likely female and male breeders and all other candidate parents to assign parentage at 95% confidence. We simulated maternity and paternity for 10 000 offspring to calculate critical LOD scores. Simulations consider the average number of candidate parents per offspring and the proportion of candidate parents sampled. Therefore, the number of candidate parents in simulations of parentage using all candidate parents sampled in the population for each breeding season included 20% more than the total number of females or males associated with sampled breeding groups to account for unsampled individuals in the population from both monitored and unmonitored breeding groups (Berg, 2005; Riehl, 2012). We conducted the maternity analysis first and then ran the paternity analysis including known mothers for offspring, when possible.

We assessed CERVUS assignments using likelihood scores and developed decision rules for assigning parentage in this species. For parentage analyses, we evaluated CERVUS assignments using pair LOD scores, which statistically distinguishes among nonexcluded candidate parents. For paternity analyses with known mothers, we evaluated CERVUS assignments of putative male breeders using trio LOD scores, which statistically distinguishes among nonexcluded candidate males while considering the genotypes of the known mother. Briefly, in the rare case when the highest-ranking female and male candidates had a high, positive likelihood score but mismatched the nestling at one or two loci, we investigated the possibility of null alleles or mistyping by repeating PCRs at mismatched loci and rescoring the alleles. We accepted the CERVUS assignment of the female and male candidates if they mismatched at ≤ 1 locus and had the highest positive pair or trio LOD score. A nestling was considered to result from an unsampled breeder if all candidate parents listed by CERVUS had negative pair or trio LOD scores and/or mismatched the nestling at ≥ 2 loci. Because distinguishing breeders from helpers in the field was not always reliable based on behavioural observations, we did not assess within-pair and extrapair paternity rates based on comparisons to

putative breeders. Instead, we report the proportion of broods containing offspring produced by one or more male and/or female breeders and identify whether breeders were from within or outside of the breeding group. The combined nonexclusion probability for the set of eight microsatellite loci was 0.002.

We used sibship reconstruction to identify full and half siblings for nests containing offspring but no parental genotypes (134 offspring from 52 broods). The presence of half siblings in a brood could indicate multiple female breeders (co-breeding or conspecific brood parasitism) or mating with multiple male breeders. We tested the primary hypothesis that offspring were full siblings against the alternative hypothesis that offspring were half siblings using likelihood ratio tests implemented in KINGROUP (Konovalov, Manning, & Henshaw, 2004). We ran 10 000 simulations to calculate the uncertainty surrounding the estimated relationships at the 95% confidence level. The critical log likelihood ratios for this hypothesis was 3.38 and the associated type II error rates at $P < 0.05$ was 0.57.

Breeder and Helper Provisioning Effort

We quantified provisioning effort by breeders and helpers (assigned based on behaviour) using video recordings. Nests were filmed starting within 30 min of sunrise for 6–8 h (Martin, 2015; Martin, Oteyza, Boyce, et al., 2015; Martin, Oteyza, Mitchell, et al., 2015). We mounted video cameras (Hi8 handycam camcorder, Sony Corporation; New York, NY, U.S.A.) on tripods 2–10 m from nests set to view the nest entrance (optical zoom of 30×) and concealed with vegetation and a camouflaged rain cover. Video cameras were left unattended to avoid behavioural disturbance. Parents resumed parental activities soon after camera placement. For each nest visit, we identified the individual attending the nest based on colour band combinations, recorded arrival and departure times, and noted when food was delivered (Martin, 2015; Martin, Auer, Bassar, Niklison, & Lloyd, 2007; Martin et al., 2011; Martin, Oteyza, Boyce, et al., 2015; Martin, Oteyza, Mitchell, et al., 2015). Most nests were filmed more than once during the nestling stage. We quantified provisioning rate as the number of feeding trips to a nest per nestling per hour for each observed member of a breeding group. To obtain a single measure of provisioning rate for each group member per nest, we averaged across all videos for a nest. We tested whether mean provisioning rate differed among breeders and helpers using a Kruskal–Wallis test.

Genetic Relatedness, Helper Provisioning Effort and Reproductive Sharing

We tested whether relatedness between helpers and the breeding pair (assigned based on behaviour) influenced the likelihood that helpers gained parentage. We built two generalized linear mixed models (GLMMs) with a binomial error distribution and logit link function using 'lme4' (Bates, Maechler, & Bolker, 2012) to examine relationships between male helpers and the male and female breeders separately. In each model, the binary response variable was parentage (whether a male helper gained parentage within the brood or not). We included the pairwise genetic relatedness of each male breeder/male helper dyad ($N = 49$ dyads) and female breeder/male helper dyads ($N = 30$ dyads) in the breeding group. Each model included group size as a fixed effect and group identity and individual identity as a random intercept. All fixed effects were standardized to have sample mean = 0 and sample variance = 1. Because of limited sample sizes of genotyped female helpers assisting broods with parentage assigned, we conducted Fisher's exact tests to examine whether gaining parentage in a brood depended on whether the female helper was related to

the female breeder ($N = 7$ dyads) or male breeder ($N = 7$ dyads). Lastly, we conducted Fisher's exact tests to examine whether mixed-parentage in a brood depended on the breeding group containing a male relative of the male breeder ($N = 49$ broods) or a female relative of the female breeder ($N = 29$ broods).

We tested whether mean relatedness to young in the brood influenced provisioning effort by helpers. We categorized helper–brood relatedness describing mean genetic relatedness between helpers and young in the brood (3 levels: first-order, second-order, unrelated). First, we conducted a Kruskal–Wallis test to compare provisioning rates (i.e. number of feeding trips per nestling/h) among the helper–brood relatedness categories. We then examined the distribution of data using 'fitdistrplus' (Delignette-Muller & Dutang, 2015) and constructed a linear mixed model (LMM) using lme4. The response variable was provisioning rate, which was square-root transformed to normalize its distribution and to reduce residual variance. We included fixed effects that were of interest and biologically relevant to avoid over-parameterization. As the primary predictor, we fitted helper–brood relatedness as a categorical factor. We also included age of young and number of helpers as potential confounding factors. All fixed effects were standardized to have sample mean = 0 and sample variance = 1. Breeding group identity and individual identity were fitted as random intercepts to account for repeated observations of the same breeding group or individual across years ($N = 47$ helper/brood dyads).

Ethical Note

This study was conducted under the auspices of the University of Montana Institutional Animal Care and Use Committee (IACUC protocol no. 059-10TMMCWRU) and the Smithsonian National Zoological Park (Approval letter no. 12-30). All federal and international permits were in hand when the research was conducted. We followed approved protocols to capture, handle, mark and sample chestnut-crested yuhinas. All work was performed under scientific permits from the Sabah Biodiversity Council. All procedures performed in this study were in accordance with the ABS/ASAB Guidelines for the ethical treatment of animals in behavioural research. Sampling and processing had no discernible negative impacts on individuals. Behavioural observations did not disrupt the normal activities of individuals.

RESULTS

Breeding Group Size and Composition

We recorded up to eight helpers at a nest, forming breeding groups that ranged in size from 2 to 10 individuals (mean ± SD = 4.2 ± 1.4; $N = 57$ breeding groups; see Appendix, Fig. A1a). Only four breeding pairs were observed without helpers. However, two of these four breeding pairs were not sufficiently monitored because of their remote location at the edge of the study area. Thus, 55 of 57 (96%) well-monitored breeding pairs had helpers. Breeding groups generally included a socially bonded pair and both male and female helpers. In the few groups in which all helpers were sampled for genetic sexing, the ratio of males to females was not significantly different from parity (mean ± SE = 0.61 ± 0.01; $N = 8$ breeding groups; $z = 0.96$, $P = 0.34$). Brood size ranged from one to five offspring (mean ± SD = 2.53 ± 0.73; $N = 109$ broods), although unusually large broods (>3 nestlings) were rare (Fig. A1b). Brood sex ratio (male:female) was not sex biased (mean ± SE = 0.49 ± 0.01; $N = 98$ broods; $z = -0.39$, $P = 0.69$).

Genetic Relatedness

The mean ± SE pairwise relatedness estimate identified from parentage assignment for first-order relatives was 0.56 ± 0.02 ($N = 145$ dyads), including mother/offspring, father/offspring, and full sibling relationship dyads, and 0.22 ± 0.02 ($N = 14$ dyads) for second-order relatives (half siblings) (Fig. 1). Breeding groups consisted of a mix of related and unrelated helpers (mean $r \pm SE = 0.20 \pm 0.03$; $N = 76$ female breeder/helper and male breeder/helper dyads). We documented some first-order and second-order relatives of one or both members of the breeding pair (see Appendix, Fig. A2). Of the female breeder/helper dyads, 5 of 26 (19%) were first-order relatives, 5 of 26 (19%) were second-order relatives, and 16 of 26 (62%) were nonrelatives (Fig. 2). Of the male breeder/helper dyads, 15 of 50 (30%) were first-order relatives, 12 of 50 (24%) were second-order relatives, and 23 of 50 (46%) were nonrelatives (Fig. 2). Helpers that were only related to either the female or the male breeder were not more likely to be relatives of the same-sex breeder than they were to be relatives of the opposite-sex breeder (Fisher's exact test: $P = 0.55$). Of the breeder/helper dyads, for which both the male and female breeder were compared to the helper, 3 of 20 (15%) were first-order relatives of the breeding pair, 13 of 20 (65%) were either first- or second-order relatives of the male or female breeder, and 4 of 20 (20%) helpers were unrelated to both breeders (mean $r \pm SE$; female breeder/helper dyads: 0.50 ± 0.00, male breeder/helper dyads: 0.42 ± 0.02). These individuals were most likely offspring of the breeding pair from previous broods that had not yet dispersed from their natal group (two male helpers, one female helper). The mean ± SE relatedness of helpers and young in broods was 0.20 ± 0.02 ($N = 47$ helper/brood dyads, $N = 26$ broods). Males and females of most breeding pairs were unrelated to each other (0.12 ± 0.04; $N = 20$ dyads; Fig. 1), although we did find a few cases of distantly related partners within breeding pairs.

Parentage and Sibship

For 29 broods with sampled social mothers, we assigned maternity to 69 (100%) genotyped offspring using CERVUS. We assigned 36 (52%) offspring to the social mother at the 95% confidence level and 10 (14%) offspring to the social mother at the 80% confidence level. For 17 (25%) offspring from seven broods, we assigned all offspring in each brood to a female designated as a helper in the breeding group (based on behavioural data and brood patch development) at the 95% confidence level (i.e. no mixed parentage in these broods). We assigned six (9%) offspring from four broods with mixed parentage to a second female in the breeding group at the 95% confidence level. All six offspring were produced by female helpers unrelated to the male breeder. In summary, 25 (86%) broods contained offspring produced by a single female breeder and four (14%) broods contained offspring produced by two female breeders sired by the same male breeder (i.e. possibly quasi-parasitism) (Fig. 3).

For 49 broods with sampled social fathers, we assigned paternity to 115 of 120 (96%) genotyped offspring from 47 broods using CERVUS. We assigned 76 (66%) offspring to the social father at the 95% confidence level and 13 (11%) offspring to the social father at the 80% confidence level. For 12 (10%) offspring from five broods, we assigned all offspring in each brood to a male designated as a helper in the breeding group at the 95% confidence level (i.e. no mixed parentage in these broods). We assigned 14 (12%) offspring from 10 broods with mixed parentage to a second male in the breeding group at the 95% confidence level. All 14 offspring were produced by male helpers unrelated to the female breeder. For the five (4%) unassigned offspring from two broods, all offspring in the brood mismatched the social father, but breeding groups contained at least one unsampled group member of unknown sex. Thus, we were unable to determine whether these offspring were sired by a male from within or outside of the breeding group to distinguish
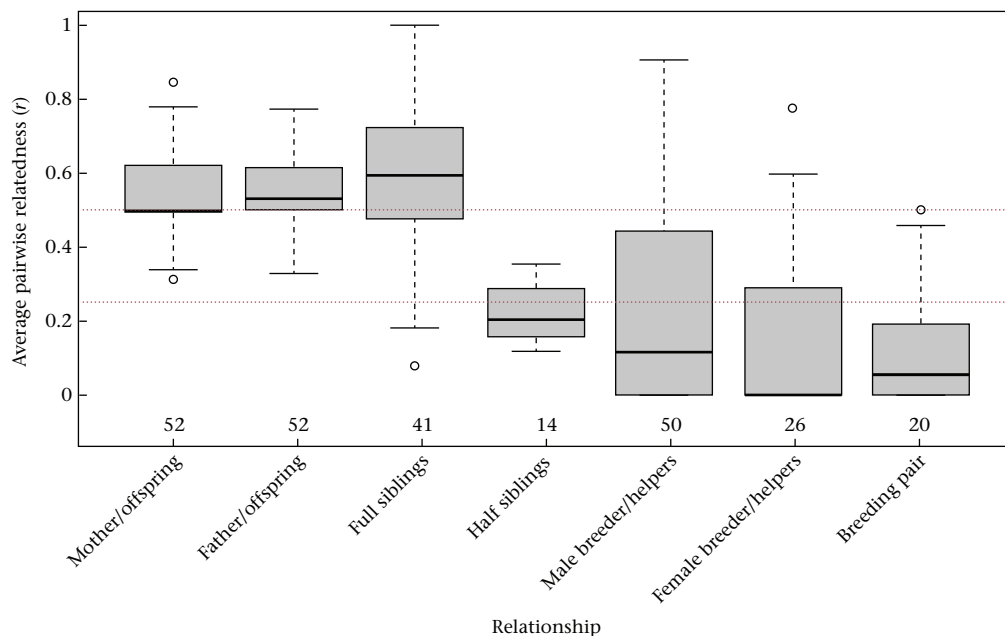
Figure 1. Mean pairwise relatedness (r) estimates of chestnut-crested yuhinas for first-order relative dyads (mother/offspring, father/offspring and full siblings) and second-order relative dyads (half siblings, female breeder/female helpers, male breeder/male helpers and breeding pairs). The box and midline represent the 25th, 50th and 75th quartiles; whiskers extend to the most extreme value within interquartile ranges beyond the 25th and 75th percentiles, and outliers beyond those values are shown as open circles. The horizontal red, dotted lines indicate the predicted coefficient of relatedness values for first-order ($r = 0.5$) and second-order dyads ($r = 0.25$). Sample sizes refer to the number of dyads in each relationship category and are given above the X axis.
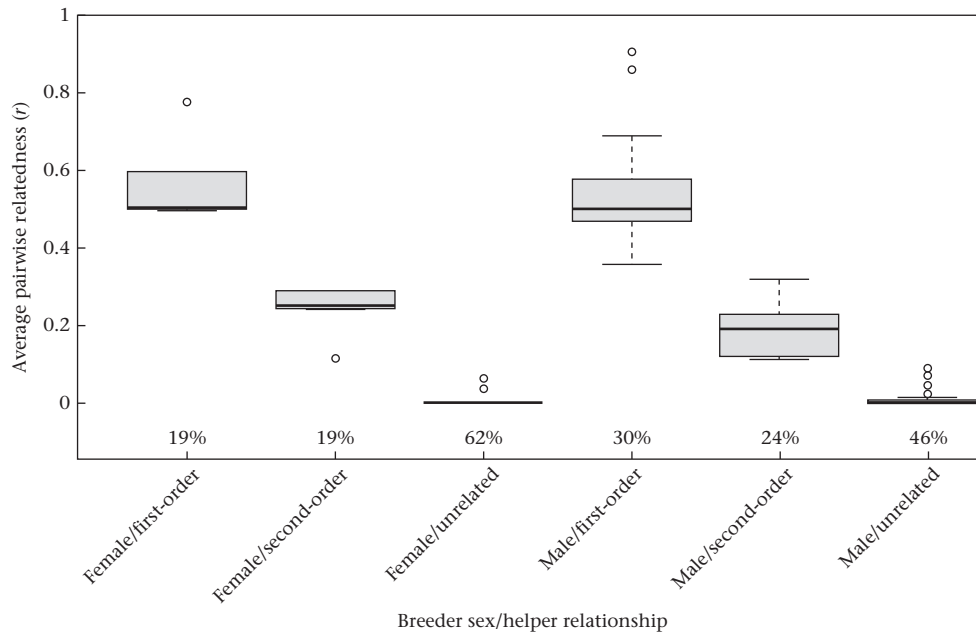
Figure 2. Mean pairwise relatedness (r) estimates of helpers that were unrelated, first-order relatives or second-order relatives of the female and male breeders in chestnut-crested yuhina breeding groups. The box and midline represent the 25th, 50th and 75th quartiles; whiskers extend to the most extreme value within interquartile ranges beyond the 25th and 75th percentiles, and outliers beyond those values are shown as open circles. The percentage of the breeder/helper dyads in each of the three relationship categories are given above the X axis (N = 26 female breeder/helper dyads, N = 50 male breeder/helper dyads).
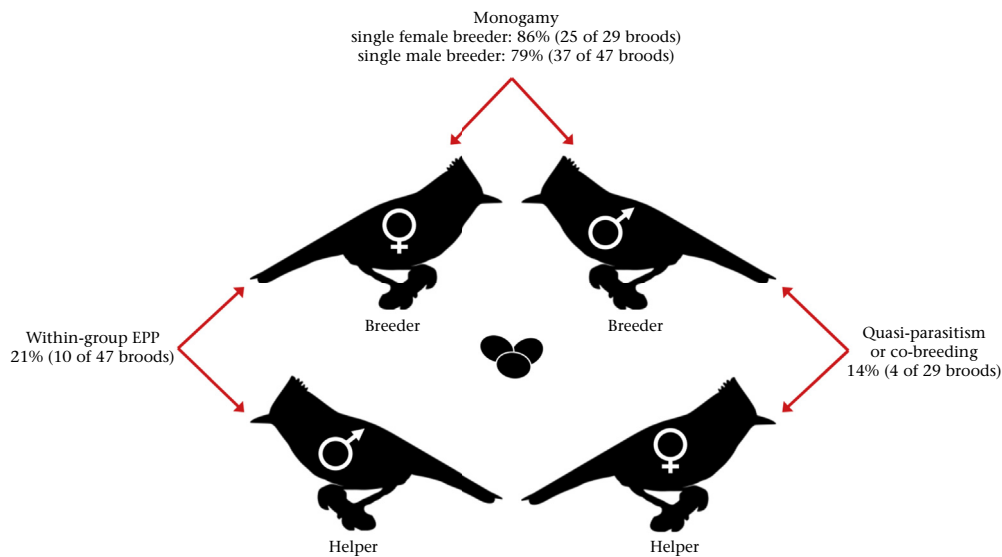
Figure 3. Percentage of chestnut-crested yuhina broods containing within-pair offspring produced by a single male and female breeder (monogamous breeding pair), at least one extrapair offspring produced by a male helper (within-group extrapair paternity (EPP)), and at least one offspring produced by a female helper (quasi-parasitism or co-breeding). Sample sizes for paternity analysis included broods in which the social father was genotyped (47 broods) and maternity analysis included broods in which the social mother was genotyped (29 broods). Chestnut-crested yuhina image produced by Madhvi X. Venkatramen.

between extrapair versus extragroup paternity or whether more than one male contributed paternity. In summary, 37 (79%) broods contained offspring sired by a single male breeder and 10 (21%) broods contained offspring sired by two male breeders produced by the same female breeder (Fig. 3). We therefore found evidence of extrapair paternity, but no evidence of extragroup paternity.

Of 134 genotyped nestlings from 52 broods with no parental genotypes (116 offspring dyads), we found that 109 (94%) offspring dyads were grouped as full siblings and seven (6%) offspring dyads were grouped as half siblings using KINGROUP. These results

reflected that 46 (79%) broods contained only full siblings and six (13%) broods contained both full and half siblings. This estimate is comparable to the CERVUS estimate of 18% of 76 broods with known parental genotypes containing mixed parentage.

Breeder and Helper Provisioning Effort

We videotaped nests where group members (breeders and helpers) were individually identifiable and sampled for genetic sexing during the nestling stage (mean ± SE: 5.58 ± 0.24 h, N = 83
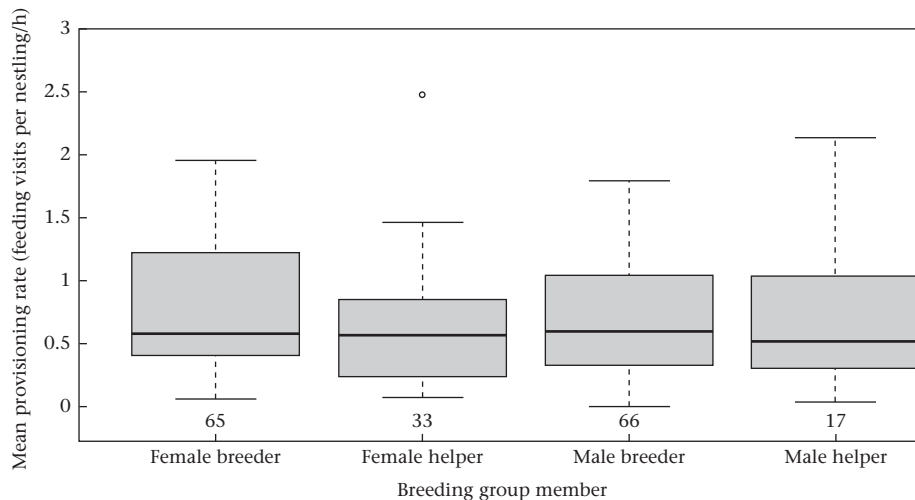
Figure 4. Mean provisioning rate by breeders and helpers (assigned based on behaviour) in the chestnut-crested yuhina. The box and midline represent the 25th, 50th and 75th quartiles; whiskers extend to the most extreme value within interquartile ranges beyond the 25th and 75th percentiles, and outliers beyond those values are shown as open circles. Sample sizes are given above the X axis.

videos, $N = 34$ nests). Mean provisioning rate did not differ between breeders and helpers (Kruskal–Wallis test: $H_3 = 0.98$, $N = 181$ group members, $P = 0.81$; Fig. 4).

Genetic Relatedness, Helper Provisioning Effort and Parentage

We found that relatedness influenced parentage, but not provisioning effort. The probability that a male helper sired offspring was not associated with the male helper's relatedness to the male breeder ($N = 49$ male breeder/male helper dyads; GLMM: $\beta \pm SE = -0.16 \pm 0.38$, $z = -0.42$, $P = 0.68$) or group size ($\beta \pm SE = -0.42 \pm 0.40$, $z = -1.04$, $P = 0.30$). However, the probability that a male helper sired offspring decreased with increasing relatedness to the female breeder ($N = 30$ female breeder/male helper dyads; GLMM: $\beta \pm SE = -12.71 \pm 5.17$, $z = -2.46$, $P = 0.014$). Female helpers gained parentage only when they were unrelated to the female breeder (Fisher's exact test: $N = 7$ female breeder/female helper dyads, $P = 0.029$), but relatedness to the male breeder did not predict parentage ($N = 7$ male breeder/female helper dyads, $P = 0.14$) although sample sizes for both analyses were limited. Mixed parentage in a brood was independent of whether the breeding group contained a first-order relative of the same-sex breeder (Fisher's exact test: male helper: $N = 49$ broods, $P = 0.23$; female helper: $N = 29$ broods, $P = 0.99$). In all broods with mixed parentage, same-sex breeders were unrelated, female co-breeders produced young sired by the same male breeder (i.e. possibly quasi-parasitism) and multiple males sired different young in a brood produced by the same female breeder.

Mean provisioning rate did not differ between helpers that were first-order relatives, second-order relatives or nonrelatives of the brood (Kruskal–Wallis test: $H_2 = 4.38$, $N = 47$ helper/brood dyads, $P = 0.11$). Helper provisioning rate was not associated with helpers' mean relatedness to the brood ($N = 47$ helper/brood dyads; LMM: helper–brood relatedness (second-order): $\beta \pm SE = 0.26 \pm 0.13$, $t = 2.01$, $P = 0.09$; helper–brood relatedness (unrelated): $\beta \pm SE = 0.07 \pm 0.13$, $t = 0.56$, $P = 0.60$) or number of helpers ($\beta \pm SE = 0.02 \pm 0.05$, $t = 0.30$, $P = 0.78$). Helper provisioning rate increased with the age of young ($\beta \pm SE = -0.20 \pm 0.03$, $t = 6.23$, $P < 0.001$).

DISCUSSION

Studies of cooperative breeding have focused largely on species with related helpers (Cockburn, 2004; Koenig & Dickinson, 2016; Russell, 2004; Solomon & French, 1997). We found that kin structure of breeding groups of the chestnut-crested yuhina was mixed, such that most groups contained both relatives (48% of 76 of breeder/helper dyads) and nonrelatives (52%) of one or both members of the breeding pair. Such mixed kin structure is more common than previously appreciated in birds, but tests of how related and unrelated group members differ in parentage and helping effort are limited (Riehl, 2013, 2017). We documented co-breeding or quasi-parasitism (i.e. when a female helper lays an egg fertilized by the male attending the parasitized nest) by female helpers in 14% of 29 broods and extrapair paternity by male helpers in 21% of 47 broods. Consistent with inbreeding avoidance, only male and female helpers that were unrelated to the breeding pair were successful at gaining parentage. We found that provisioning rate did not differ among helpers that were, on average, first-order relatives, second-order relatives or nonrelatives of the offspring they provisioned. These findings fit the emerging pattern of cooperative breeding in birds with mixed-kin groups, wherein unrelated helpers are more likely to gain parentage than are related helpers and helping effort is not necessarily predicted by kinship (Riehl, 2017).

Cooperatively breeding birds vary in how reproduction is shared among group members on a continuum from high reproductive skew (one breeding pair and several nonbreeding helpers that provide alloparental care to young) to low reproductive skew (several breeders that produce young in a shared nest and cooperate to raise young) (Magrath & Heinsohn, 2000; Vehrencamp, 2000). We show that the chestnut-crested yuhina is intermediate along this continuum, in which male helpers sire a minority of offspring and female helpers lay a minority of eggs in the nest of the breeding pair. Thus, in the chestnut-crested yuhina, most nestlings in a brood are produced by a socially monogamous pair, some unrelated males and females gain extrapair paternity and both males and females mate with multiple partners within, but not outside their breeding group (i.e. no extragroup parentage).

The closest babbler species for which the social and genetic mating system has been described in the Zosteropidae clade is the Taiwan yuhina, *Yuhina brunneiceps* (Cai et al., 2019). The Taiwan yuhina breeds cooperatively in groups composed of unrelated joint-nesters, wherein more than one breeding pair contributes eggs to a group nest (Shen, Yuan, & Liu, 2016). Unlike the Taiwan yuhina, no broods of the chestnut-crested yuhina contained young produced by more than one breeding pair.

Congruent with findings from other recent studies, we found that helpers unrelated to the breeding pair were more likely to produce offspring within the group than were related helpers. Broad patterns of reproductive sharing have only recently emerged that predict under what circumstances male and female helpers might gain parentage. A recent meta-analysis of 83 cooperatively breeding bird species showed that reproductive sharing is less common in kin-based groups than in mixed-kin groups and that unrelated helpers of both sexes are more likely to gain parentage than are related helpers (Riehl, 2017; see also Koenig, Shen, Krakauer, & Haydock, 2009). Moreover, genetic relatedness of the helper to the opposite-sex breeder, rather than relatedness of the helper to the same-sex breeder, was the primary driver of this pattern (Riehl, 2017). Thus, the proximate mechanism constraining reproduction by related helpers is inbreeding avoidance with the opposite-sex breeder rather than reproductive suppression by the same-sex breeder (Riehl, 2017).

Mixed parentage in broods from multiple females occurs rarely in birds (Koenig & Dickinson, 2016; Vehrencamp & Quinn, 2004). Mixed parentage could be the result of co-breeding, when more than one individual of one or both sexes contribute to the clutch of a single breeding group and share parental care (Brown, 1987; Vehrencamp, 2000), or conspecific brood parasitism, the latter of which is hypothesized to be an evolutionary precursor of joint laying by females (Vehrencamp, 2000). We were unable to differentiate between these possibilities in the chestnut-crested yuhina. Female co-breeding usually involves females that join groups as unrelated helpers that assist the female breeder over several breeding attempts (Vehrencamp, 2000). Some species tolerate eggs laid by co-breeders despite suffering reproductive costs from the addition of eggs by a secondary female (e.g. pukeko, *Porphyrio porphyrio melanotus*: Quinn, Haselmayer, Dey, & Jamieson, 2012; Taiwan yuhinas: Shen et al., 2016). Although reproductive conflict is evident in many of these species (e.g. egg burying in the guira cuckoo, *Guira guira*: Macedo, 2016), helping can lead to stable social relationships between familiar females (e.g. joint laying in the greater ani, *Crotophaga minor*: Riehl & Strong, 2018). Direct fitness benefits (e.g. parentage, cooperative nest defence) may favour the evolution of associations between unrelated females, which contribute to the long-term stability of social groups (Riehl & Strong, 2018).

Our knowledge of extrapair paternity patterns in cooperatively breeding species is largely biased towards kin-based groups (Koenig & Dickinson, 2016; Taborsky et al., 2016) and species found in the temperate zone and New World tropics (Macedo, Karubian, & Webster, 2008; Tori et al., 2008). In species that cooperate in kin groups, male helpers obtain inclusive fitness benefits (Green et al., 2016; Hamilton, 1964) and typically gain little to no extrapair paternity (e.g. 0–7%; Dickinson & Akre, 1998; Haig, Walters, & Plissner, 1994; Haydock, Parker, & Rabenold, 1996; Townsend, Clark, Mcgowan, & Lovette, 2009; but see exceptions: Double & Cockburn, 2000; Webster, Varian, & Karubian, 2008). Our finding that 21% of 47 chestnut-crested yuhina broods contained extrapair offspring — a higher rate than most cooperative breeders with kin groups — is similar to the rate of extrapair paternity (25% of offspring, 34% of broods) in our study of the grey-throated babbler in Borneo, which is also a cooperative breeder with mixed-kin

groups (Kaiser et al., 2018). These extrapair paternity rates are notably high for tropical species compared to the limited data from the New World tropics (Macedo et al., 2008; Tarwater, Brawn, & Maddox, 2013; Tori et al., 2008). This study underscores the need for parentage studies of species that cooperate with nonkin, especially from the Old World tropics, to examine the importance of extrapair paternity to the evolution of cooperative behaviour among nonrelatives (Riehl, 2013; Taborsky et al., 2016) and the life history traits that influence extrapair paternity patterns in the tropics (Macedo et al., 2008; Martin, 2004; Tori et al., 2008).

Helping through provisioning is one important benefit of cooperative breeding (Cockburn, 2004; Koenig & Dickinson, 2016). The relationship between helper–brood relatedness and helping effort have been mixed across studies of cooperatively breeding species. Positive associations between helper–brood relatedness and helping effort have been interpreted as support for kin selection and the ability to discriminate kin from nonkin (e.g. long-tailed tit, *A. caudatus*: Nam, Simeoni, Sharp, & Hatchwell, 2010; noisy miner, *Manorina melanocephala*: Barati, Andrew, Gorrell, Etezadifar, & McDonald, 2018). In contrast, helping effort was not predicted by relatedness in some species (e.g. Arabian babbler, *Turdoides squamiceps*: Wright, Parker, & Lundy, 1999; daffodil cichlid: Le Vin, Mable, Taborsky, Heg, & Arnold, 2011; banded mongoose: Vitikainen et al., 2017). More rarely observed, greater helping by unrelated individuals has been interpreted as support for 'pay to stay' (Gaston, 1978; Kokko et al., 2002) and a mechanism for control by dominant breeders over group membership (daffodil cichlid; Zöttl, Heg, Chervet, & Taborsky, 2013). In the chestnut-crested yuhina, we found no association between helper–brood relatedness and provisioning rate.

We also observed feeding of nestlings and fledglings by members of the larger social group (>20 or more conspecifics), which contains multiple, distinct breeding groups that sometimes nest in close proximity (<10 m, T. E. Martin & J. C. Oteyza, personal observations). Nonbreeding adults from the social group would visit nests with nest material and food (although often mistimed with the appropriate nest stage) and feed fledglings produced by other breeding groups. This form of helping has been observed in bird species that cooperate in extended family networks in 'kin neighbourhoods' that form through localized dispersal (Hatchwell, 2016; Wright & McDonald, 2016). Spatial genetic structuring can generate opportunities for repeated interactions with close relatives even after natal dispersal. For example, helping has been shown to be redirected towards neighbouring breeding pairs by related failed breeders (e.g. long-tailed tit: Russell & Hatchwell, 2001; Nam et al., 2010) and nonbreeding helpers (e.g. western bluebirds: Dickinson et al., 2016). Nonbreeding helpers can gain substantial indirect fitness benefits from indiscriminate helping in kin neighbourhoods (Leedale, Sharp, Simeoni, Robinson, & Hatchwell, 2018). However, the risk of caring for nonkin is predicted to increase selection for kin discrimination in species living in kin neighbourhoods (Cornwallis, West, & Griffin, 2009). Our preliminary data indicates that adult chestnut-crested yuhinas disperse <115 m from their natal area to join nearby breeding groups (T. E. Martin & J. C. Oteyza, personal observations). If neighbouring breeding groups of the chestnut-crested yuhina include relatives, helpers might provision multiple broods simultaneously and feed fledglings they encounter while foraging with their social group. Local kin structure from localized dispersal might also explain why we documented a few cases of inbreeding.

Conclusions

Behavioural and genetic data indicate that the chestnut-crested yuhina has a complex social structure with mixed-kin breeding

groups that produce some broods containing mixed parentage of multiple female or male breeders. We show that within-group relatedness predicts patterns of reproductive sharing but does not influence patterns of cooperation by relatives and nonrelatives. Specifically, both male and female helpers that are unrelated to the breeding pair are more likely to gain parentage than are related helpers. The proximate mechanism constraining reproduction by related male helpers appears to be inbreeding avoidance. It is less clear why female breeders tolerate co-breeding or quasi-parasitism by unrelated female helpers in this system. Helpers did not differ in their provisioning rate according to their relatedness to the broods. These findings are consistent with emerging patterns of reproductive sharing and cooperation found in cooperatively breeding species of birds with mixed-kin groups. Moreover, occasional indiscriminate helping by members of the larger social group suggest that the population may exhibit local kin structure from localized dispersal. Future work examining factors that generate differences in within-group relatedness will help us better understand variation in social and reproductive dynamics in cooperatively breeding animals.

Acknowledgments

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Supplementary Material

Supplementary material associated with this article is available, in the online version, at https://doi.org/10.1016/j.anbehav.2019.10.007.

Appendix
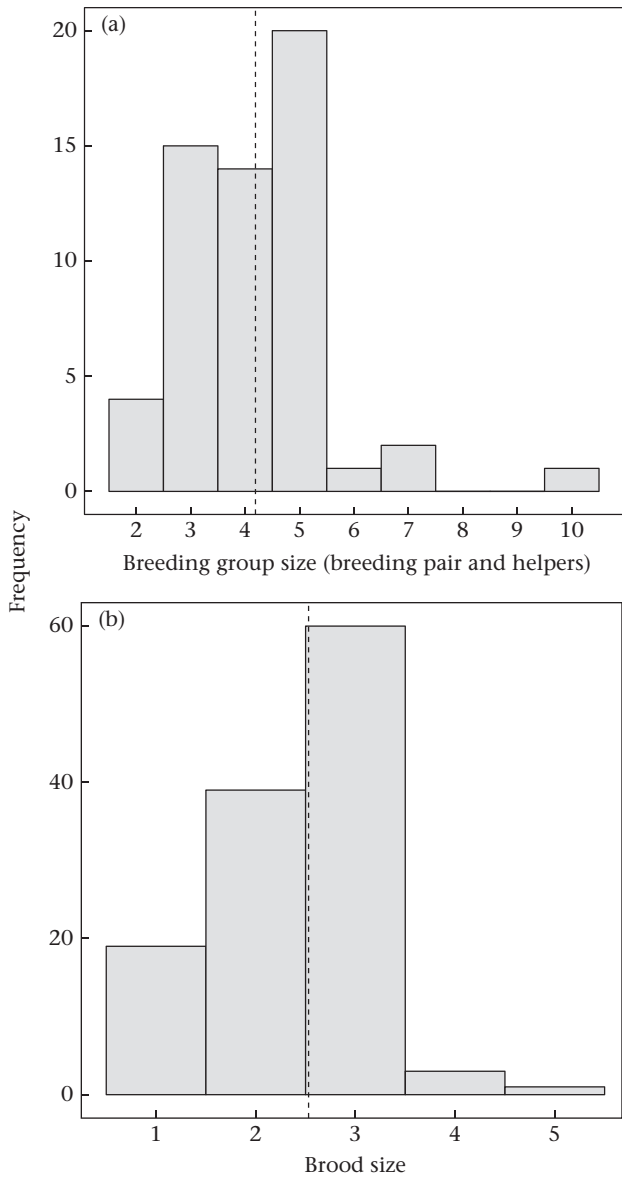
Figure A1. Frequency distribution of the size of (a) breeding groups (*N* = 57) and (b) broods (*N* = 109) of the chestnut-crested yuhina at Kinabalu Park, Sabah, Malaysian Borneo, 2009–2015. The dashed lines indicate mean values.
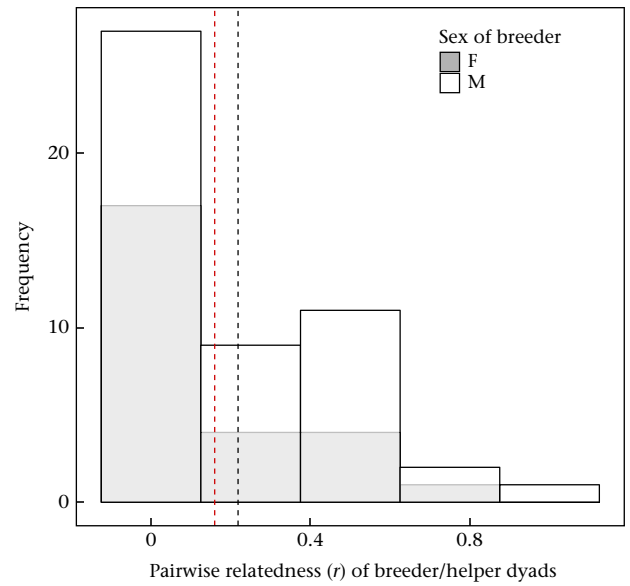
Figure A2. Frequency distribution of the pairwise relatedness (*r*) estimates of helpers that were related to the female breeder (grey bars) and/or the male breeder (white bars) in chestnut-crested yuhina breeding groups. We only included dyads with helpers that were significantly related to the female breeder, male breeder, or both. The mean pairwise genetic relatedness estimates are given for the male breeder (black dashed line) and female breeder (red dashed line).