Sex Ratio Significance and Implications in the Cooperative Breeding Chestnut Crowned Babbler

Kelly Howell

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SEX RATIO SIGNIFICANCE AND IMPLICATIONS IN THE COOPERATIVE 
BREEDING CHESTNUT CROWNED BABBLER 

By 

Kelly Howell 

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Of the Requirements for the 
University Honors Program 

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The members of the Honors Thesis Committee appointed to examine the thesis of Kelly Howell find it satisfactory and recommend that it be accepted.

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ABSTRACT

Sex Ratio Significance and Implications in the Cooperative Breeding Chestnut Crowned Babbler

Kelly Howell

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The most simplified explanation to describe sex ratios comes from Fisher’s Principle, which assumes equal representation of both sexes following Mendelian segregation. However, violations to this principle create a biased sex ratio of more males or females. This bias has yet to be completely understood, as there have been conflicting study results. Hence, there is an importance of additional study, especially among cooperative avian species, where sex ratio variation may have greater consequences due to sex-specific dispersal and helping. One such cooperative breeder is the chestnut crowned babbler (*Pomatostomus ruficeps*), endemic to the Australian outback. In chestnut crowned babblers, females are generally dispersive and males philopatric. In this study blood was collected from chicks in the nest to molecularly determine the sex of each chick and examined for any sex ratio changes. This study found no significant variation in sex-ratios among chestnut crowned babblers at the population or individual levels; as well as no sex-ratio bias under six different variables – hatch date, brood size, breeding unit, number of helpers, year of birth, and number of attempts. These results are of great importance in cooperative breeding, as the ultimate goal for helpers is to pass on genetic information to continue the success of the species, even if it is not through their direct
offspring. Therefore, with no significant evidence showing sex ratio bias, future studies should focus on the significance of maternal age (costs versus benefits of the sex), social status, and hatch rank.

KEYWORDS: Sex Ratio, Chestnut Crowned Babbler, Cooperative Breeder, Philopatric
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DEDICATION

I would like to dedicate this Honors Thesis to my parents, Lisa and Andrew Howell, who have offered nothing but unconditional love and support my entire life. They instilled hard work and dedication in my brother, Michael and me that allowed this thesis to be possible.
CHAPTER ONE

Introduction

Sex ratios are most simply explained by Fisher’s Principle, which assumes that half of all an individual’s genes come from each contributing parent, regardless of its rarity, including those alleles determining sex; thus, sex ratios of populations are expected to be 50% female and 50% male (Carvalho et al 1997). Yet, evidence suggests that unequal sex proportions, due to varying characteristics, such as birth group size, birth year, birth order, and maternal age, are common among certain cooperative breeding species (Bales et al 2000; Bull and Charnov 1988; Komdeur et al 1997; Rapaport et al 2013). These trends are seen under various circumstances, such as type and condition of environment, presence of local competition, and/or the social status of the parents.

Empirical evidence from several studies has shown that animals with chromosomal sex determination can adaptively adjust their offspring sex ratio (Komdeur and Pen 2002). The adjustment is generated by multiple variables, both pre and post-hatch mechanisms, including the external environment, seasonal changes, paternal quality, social environment, or physical size. These variables have suggested bias in sex ratios favoring the sex that has the most beneficial outcomes and least amount of caretaking costs to the breeder, thus drastically improving the offspring’s chance for survival (Komdeur and Pen 2002). Such a bias in sex ratio is commonly seen in cooperative breeding species. Cooperative breeding occurs when there are more than two individuals contributing to the care of offspring (Cockburn 2004). These individuals can
be comprised of multiple breeders of either sex in addition non-breeding helpers, often offspring, which stay behind at the nests to help feed the next generation (Ligon and Burt 2004). To qualify as a true helper, behaviors must include: 1) any type of actions generated directly to an individual that is not their offspring, 2) the behavior must have a cost to the individual performing such action, and 3) the behavior must be beneficial to the receiver of the action (Gilchrist 2006). Numerous helpers directly improve survival rates and future and/or present reproductive success rates in breeders because helpers can increase the condition and survival of the young through direct actions toward the young and lighten the work-load of the breeders (Gilchrist 2006). These behaviors may increase the probability that parents are viable to breed again and/or increase the productivity in each brood (Emlen and Wrege 1991).

Yet, it is important to note that the initial birth sex ratio does not predict the adult sex ratio of the population. Rather, a skew in sex ratio may emerge during the period of life when individuals aim to obtain a breeding position within their community (Komdeur et al. 2017). One variable that contributes to this skew is preferential care. Parental investment may skew the sex ratio in favor of the sex that sustains a higher mortality rate after birth to help increase the chance for survival. For example, lone golden lion tamarin (Leontopithecus rosalia) females will recruit one to two unrelated males to inhabit a territory, resulting in small groups where reproduction occurs for the first time. With a small group of inhabitants, it is suggested that females give birth to more sons, which typically become non-breeding helpers (Rapaport et al 2013, Bales et al 2000). A linear relationship is also prominent between group size at the time of birth and offspring survival, suggesting that a smaller group size positively influences offspring survival.
because there are more communal resources and opportunities available. This is especially seen among males who play an important role in the community as helpers (Rapaport et al 2013, Bales et al 2000). Furthermore, the Seychelles warbler (*Acrocephalus sechellensis*), a species where females are helpers, have a skew in their sex ratio based on territory condition. If breeding pairs reproducing in low-quality territories (areas that lack food availability) are unhelped, the birth sex ratio is skewed towards males by 77%, whereas unhelped breeding pairs reproducing in high-quality territories, have sex ratios skewed towards females with only 13% males born (Komdeur et al 1997). This trend is expected to be a result of the benefits the offspring receive based upon the quality of territory. For example, helpers (females) born in high-quality territories improve their parents’ reproductive success, in return gaining experience, heightening inclusive fitness and co-breeding opportunities. However, helpers (females) born in low-quality territories decrease their parents’ reproductive success due to the competition for limited resources (Komdeur et al 1997). Hence, to alleviate the chance for a decrease in reproductive success and reduce competition for limited resources, the sex ratio is skewed toward the non-helpers (males).

Patterns of birth sex ratio bias due to quality of territory and competition within the community may be explained through two different processes – local resource competition and local resource enhancement. Local resource competition occurs when individuals of one sex within a species is a dispersing sex and the other a philopatric sex – remaining natal. The competition among philopatric individuals over access to local resources provides support for bias in birth sex ratios toward the dispersal sex (Hamilton 1967, Clark 1978, Silk 1984). A study conducted by Johnson has suggested local
resource competition intensity and birth sex ratio linkage in primates (Johnson 1998). Intensity and birth sex ratios were linked in seven primate genera and in the same study, Johnson showed birth sex ratios with more male-bias in nine primate genera with female philopatry when compared to less male-bias birth sex ratios from seven other primate genera without female philopatry (Johnson 1988). Another study conducted by Faust and Thompson (2000) showed a significant difference in male births when compared to female births in thirteen species showing male helper patterns. On the contrary, local resource enhancement occurs in cooperative breeding species when differences in effectiveness of males and females are seen. Birth sex ratios are expected to show bias towards the more helpful sex if the benefits derived from helpers do not vary significantly across parental groups (Gowaty and Lennartz 1985, Emlen et al. 1986, Pen and Weissing 2000).

Despite affirmative results of bias in birth sex ratios in studies of other species, a general explanation among avian species is lacking. Therefore, the need for additional studies is crucial to understand sex ratio variation and the potential implications it has on breeding status success among avian species (Cockburn et al. 2002). In some birds, a selective advantage occurs for breeders who vary parental investment in their offspring based on prevailing conditions (Trivers and Willard 1973). Specifically, if maternal condition can impact offspring fitness within a community, mothers in good condition should show bias towards males due to their higher variance in reproductive success. By skewing the ratio in favor of the philopatric sex (males), female breeders are able to keep their breeder status by not competing for resources with additional females and gain an abundance of non-breeding helpers, mitigating their energy costs (Rapaport L.G. et al
2013). These themes are most closely related to local resource competition and local resource enhancement, especially since the latter has been seen in other cooperative breeding species, such as the Seychelles warbler.

Local resource competition and local resource enhancement models are particularly important to cooperative breeders who vie for the ability to become the breeders of the community to continue their ancestry. Some cooperative breeding avian species, such as the Seychelles warbler (Komdeur et al. 1997), the laughing kookaburra (*Dacelo novaeguineae*; Legge et al. 2001), the bell miner (*Manorina melanophrys*; Ewen et al. 2003), and the green woodhoopoe (*Phoeniculus purpureus*; Ligon and Ligon 1990), show that breeding pairs without helpers reproduce more of the helping sex to generate additional helpers, thus skewing their sex ratio.

In a study of the laughing kookaburra, offspring sex varied with hatch rank and the type of social group. It showed male-biased clutch and fledging sex ratios when the social groups had female helpers, especially if all of the helpers were female. In contrast, social groups with unassisted or male-only helpers showed female-biased clutch and fledging sex ratios. Production of more male eggs was a facultative response to an increase in female helpers within a group. The biases shown in this study occur when breeding females limit the recruitment of daughters because unlike male helpers, female helpers depress the breeding success of their parents (Legge et al. 2001). Additionally, this same study showed that across all nests, two-thirds of the first-hatched young were male, two-thirds of the second-hatched young were female, and an equal ratio among the third-hatched young (Legge et al. 2001). Therefore, a corollary relationship found
between hatch rank and sex sequence was non-randomly distributed, resulting in the avoidance of hatching a daughter first followed by a son (Legge et al. 2001).

In the bell miner, males are the philopatric and helping sex and increase the reproductive success of their parents. Facultative control was again seen in a study of the female bell miner breeders. For example, newly founded colonies, characterized by low food availability, were shown to have a female-biased primary sex ratio. In contrast, colonies over one year old, characterized by an increase in food availability, were shown to have a male-biased primary sex ratio. The increase in food availability is due to a cycle this avian species abides by – they aggressively defend their territory from interspecific competitors, thus greatly increasing the availability of food resources. However, the increase in phytophagous Psyllidae insects (major component of the bell miner diet) results in a decrease of tree health and/or tree death due to the secretion of a carbohydrate-rich coating on the trees (Ewen et al. 2003). Hence, the bell miners disperse to new areas with low psyllid abundance and continue the cycle. Thus, providing evidence that female breeders adjust the sex ratios toward the dispersing sex when food resources are low and toward the philopatric sex when there is an abundance of food availability (Ewen et al. 2003).

Finally, a study on the sex ratios of the green woodhoopoe, a species where females help, showed a female-bias sex ratio in the first nests of new female breeders when compared to experienced female breeders that had few helpers (Ligon and Ligon 1990). With nestling mortality low (~5%) and restricted predation of entire broods, it was concluded that the bias was not due to differential starvation of either female or male chicks. Rather, the possible explanation given for female-bias sex ratios was
modification at laying: female eggs weigh almost 20% less at laying and are therefore less expensive to lay and then raise post-hatching than male eggs. As such, young female breeders may discriminate (actively decide to discard eggs) based on egg size to have more females who help contribute more in this species (Ligon and Ligon 1990). Additionally, Komdeur and Pen (2002) have suggested that mortality of sex-specific eggs may occur before laying. Emlen (1997) has argued a potential mechanism of maternal sex ratio control is through sex-specific resorption of eggs. This means the mother would resorb the larger egg (male) she does not want, but Emlen argues this would be quite costly because it would take more time to replace the egg with the preferred sex (female). Furthermore, this mechanism may cause a later laying date, which may compromise the offspring survival and may cause a more pronounced hatching asynchrony (Klomp 1970). Other suggested mechanisms for sex-ratio bias include the influence of maternal hormones (Krackow 1995). Although both of these mechanisms are theories as to how sex ratios may be skewed, they offer a starting point to continue studies on additional mechanisms for sex ratio bias.

Unlike other avian cooperative breeding species, the chestnut crowned babbler (Pomatostomus ruficeps) does not have much research supporting or negating sex ratio variation at the population level. This passerine bird is approximately 50 grams and native to arid and semi-arid regions of southeastern Australia. In this species the males are the helpers and philopatric sex, whereas females are the dispersal sex. They are found living in large cohesive social groups ranging from three to twenty-three individuals (Portelli et al. 2009). Each social group is comprised of one to three breeding pairs with a variable number of non-breeding helpers of both sexes, with primarily male
helpers (Portelli et al. 2009). Both breeding and non-breeding helpers are responsible for building the breeding nests and providing food for the hatchlings (Portelli et al. 2009). If sex ratio variation occurs among chestnut crowned babblers as it does in other cooperative breeders, we should find an overall population sex ratio bias towards the more philopatric and helping sex - males. With this information, studies may be further performed to identify the cause(s) of this sex ratio bias and the possible implications this bias may have in the breeding of the Australian chestnut crowned babbler. For instance, one may be able to predict the social status or reproductive success of future generations, both vital characteristics in a cooperative breeder’s success.

Hypothesis

Due to the cooperative breeding status of the Chestnut Crowned Babbler and the implications this type of breeding suggests in other avian species, I expect the ratios to be skewed in each individual clutch to favor the philopatric sex, males. A lower social status for the parents will result in a more balanced sex ratio or a skew towards females due to the lack of environmental resources and lack of potential reproductive benefits produced by males. The females will thus disperse due to the lack of resources and reduce competition. In contrast, I predict in a clutch having high social status parents the sex ratio will be skewed more towards the males who increase parental reproductive success and gain valuable helping experiences.
CHAPTER TWO

Materials and Methods

Field Sampling

A 7 x 8 km study site within the University of New South Wales’ Arid Zone Research Station at Fowlers Gap (31.3642° S, 141.6788° E) in western New South Wales, Australia was used to study a ringed population of chestnut crowned babblers (Figure 1).

Figure 1. Map of New South Wales, Australia. Red star marks site of University of New South Wales’ Arid Zone Research Station at Fowlers Gap.
Chestnut crowned babbler chicks were captured from the nest between hatching and fledging during the breeding seasons (July-December) of 2007-2015. Chicks were sampled on days 0, 10, and 15 after hatching. Using a 26-gauge needle, blood samples (~50 µl) from a prick in the brachial vein were collected with a capillary tube and emptied into ~300 µl ethanol. Earlier samples were only used if the chick did not survive to fledging.

**DNA Extractions and Concentration Measurements**

DNA was extracted from whole blood using Puregene DNA extraction kits (Qiagen, USA). Before extraction, blood cells were digested using the following procedure: labeled Eppendorf Safe-Lock tubes received six to eight 0.9-2.0 mm stainless steel beads and five to 10 µl of blood from each individual; 200 µl of cell lysis solution was added to the blood and bead mixture and beat for 30 seconds to ensure the blood was well homogenized; finally, 3 µl of proteinase-K was added to each tube and tubes were put in the shaking dry bath overnight at 50°C and 950 rpm.

Once digested, samples were put on ice for 5 minutes when 100 µl of protein precipitation solution was added, vortexed, and returned to ice for 5 minutes. Samples were then centrifuged for 5 minutes at 4°C at full speed. The supernatent from each sample was carefully removed and 300 µl of isopropanol added and mixed until DNA was precipitated. The tubes were centrifuged for 1 minute at 4°C. Tubes were carefully removed to not disrupt pellet, all isopropanol was removed from each tube and 300 µl of 70% ethanol was added to tube by swirling around the edges to wash the tube and DNA,
trying to dislodge the pellet if possible. Tubes were placed in the centrifuge for 1 minute at 4°C at high speed. Tubes were carefully removed to not disrupt the pellet and the ethanol was completely removed by pipette and evaporation. Once completely dry, 10-50 µl of DNA hydration solution was added to rehydrate the DNA pellet.

The concentration and purity of each sample was determined spectrophotometrically and then placed in the freezer to preserve the DNA. The DNA concentration and purity of each sample were established by placing 1 µl of DNA sample on each loading surface of the spectrophotometer tray. The tray was loaded in the spectrophotometer and protocol was run to isolate the DNA concentration, 260nm/280nm, and 280nm/30nm purity values.

**PCR and Gel Electrophoresis**

Many bird species, including the chestnut crowned babbler, are sexually monomorphic in adults. Hence, sexing in this bird is performed through DNA and genetic markers primarily located on the chromodomain helicase DNA binding (CHD) gene (Doutrelant et al 2004). This gene is located on the W chromosome with the homologous pair on the Z chromosome (Doutrelant et al 2004). Female birds are the heterogametic sex with one W chromosome and one Z chromosome, whereas male birds have two Z chromosomes (Figure 2).
Figure 2. Karyotypes for avian species. The right karyotype shows female, indicated by one W and one Z heterogametic chromosome, whereas the left shows male, indicated by two Z homogametic chromosomes.

PCR was conducted with an Invitrogen mastermix, P2 and P8 primers, and ~100-200ng of DNA (Griffiths et al 1998). P2 and P8 primers were specifically used to locate the sex-specific DNA of the CHD gene for amplification in PCR. These primers surround the fragment of the gene and intron, allowing for discrimination between the Z and W chromosomes on a gel. The P2 primer target DNA sequence is 5’-TCTGCATCGCTAAATCCTTT-3’ and the P8 primer target DNA sequence is 5’-CTCCCAAGGATGAGRAAYTG-3’ (Griffiths et al 1998). Samples were then run on a Biorad Thermal Cycler as follows: 1.5 minutes at 94°C; then 45 cycles at the following temperatures: 46°C for 45 seconds, 72°C for 45 seconds, and 94°C for 30 seconds; finally, 48°C for 1 minute, 72°C for 5 minutes, and kept at 4°C until gel electrophoresis.
Gel electrophoresis was run on a 2% agarose gel at 50 V until the loading dye bands were visibly dispersed. The gel was transferred to a gel imaging system where the gel was exposed to ultraviolet light, illuminating the bands on the gel to determine the sex of each chick. When observed on the gel, the distinct banding patterns of either one band (male) or two (female) and size of the CHD gene displays the sex of the bird (Figure 3). These results were then used to analyze the sex ratios presented in the subject pool.

Figure 3. Agarose gel shows the sex of each sample after gel electrophoresis was completed. The four samples on the far left are females, indicated by two bands and the four samples to the right are males, indicated by one single, brighter band (Bilodeau, GJ, et al. 2007)
Data Analysis

Using the program R, statistical analyses were performed to determine the sex ratios and if they were significantly different than 1:1 (R Core Team 2013, JJ Allaire et al. 2015). The population level sex ratio was first examined, followed by nestlings only. The overall sex ratio of the sample population was compared using a chi-square test for 2,016 individuals (n=2016). Nestling sex ratios were compared using a chi-square test for 115 different breeding groups, and a total of 463 individuals (n=463). Chi-square tests were used to evaluate the overall sample population and individual chick population level because this test compares an experimental count versus deviation from expected results. Because the data was not distributed normally, sex ratios were log-transformed. Using the log transformed results, they were then evaluated along with six different variables (hatch date, brood size, breeding unit, number of helpers, year, and attempt) to determine if there was any significance or discrepancies within each variable.

Hatch date was characterized by the day each individual chick within the brood hatched. To quantify these results, a numerical number (1-154) was given to each day corresponding to the days throughout the breeding/hatching season (July-November), despite the year of hatching. For example, July 1st was given the number one and each day following, despite month or year, was given the subsequent number through November 30th (day 154). Chicks were born between July 23rd (day 23) and November 6th (day 130) across nine years. Brood size was characterized by the number of offspring in the clutch at time of laying. Breeding unit was characterized by the social status of the breeding female (i.e. primary breeding group or secondary breeding group). Number of helpers included the number of helpers for each individual group. This data was tracked
using a small (2 x 12mm) Trovan passive integrated transponder (PIT) tag that was injected into all captured individuals within the population. Each tag contained a unique alphanumeric code that registered the date and time to a copper coil antenna linked to a LID-650 PIT-tag reader that was fitted to the entrance of the babblers’ enclosed, dome-shaped nests. This ensured that all birds must pass through the antenna and reader in order to access the nest. The year was characterized by the calendar year each brood was born in. Finally, attempt was characterized by the number of broods produced in each nest/breeding unit.

A single t-test was used to calculate the significance for breeding unit, whereas a linear model was used to calculate the significance for hatch date, brood size, number of helpers, year, and attempt. The t-test was used on breeding unit because this variable was a continuous variable with two treatments that was measured, whereas the linear models measured a continuous variable tested against more than two treatments. Following these calculations, boxplots were produced to show the distribution of the log transformed sex ratio among the different variables. This enabled both confirmatory and exploratory questions to be answered at once to either confirm or refute the hypothesis.
CHAPTER THREE

Results

Population Level Sex Ratios

The sex ratio was calculated and determined at the population level to be 48.9:51.1, females to males, using a chi-square test; this sex ratio was not significantly different from 50:50 ($\chi^2 = 987$, df = 2015, p-value >0.1).

Chick Population Sex Ratios

At the nestling level, there was also no significant difference between males and females ($\chi^2 = 15.162$, df = 114, p-value >0.1).

Hatch Date vs. Sex Ratio

There was not a significant difference between hatch date and sex ratio when using a linear model (F-statistic = 0.7587, df = 70,43, p-value >0.1). There is no difference in sex ratio across a five-month window throughout a nine-year time span.
There was not a significant difference between brood size and sex ratio when using a linear model (F-statistic = 0.9967, df = 1, 112, p-value >0.1; Figure 4).

Figure 4. Boxplot showing no statistical significance between the brood size and sex ratio.
Breeding Unit vs. Sex Ratio

There was not a significant difference between breeding unit and sex ratio when using a paired t-test ($t = <0.01$, $df = 197.21$, $p$-value $> 0.1$; Figure 5).

Figure 5. Boxplot shows no statistical significance between the number in the breeding unit and the sex ratio.
**Number of Helpers vs. Sex Ratio**

There was not a significant difference between the number of helpers and the sex ratio when using a linear model (F-statistic = 0.004723, df = 1, 112, p-value >0.1; Figure 6).

Figure 6. Boxplot shows no statistical significance between the number of helpers and sex ratio.
**Year of Birth vs. Sex Ratio**

There was not a significant difference between the year of birth and sex ratio when using a linear model (F-statistic = 0.2395, df = 1,112, p-value >0.1; Figure 7).

Figure 7. Boxplot shows no statistical significance between the year born and sex ratio.
Number of Attempts vs. Sex Ratio

There was not a significant difference between the number of attempts and sex ratio when using a linear model (F-statistic = 0.1705, df = 1,112, p-value >0.1; Figure 8).

Figure 8. Boxplot shows no statistical significance between the number of attempts performed and sex ratio.
CHAPTER FOUR
Discussion and Conclusion

In this study, I found that the overall population sex ratio and the individual chick population levels were both non-significant. Additionally, no consistent bias was detected across any of the six variables tested – hatch date, brood size, breeding unit, number of helpers, year of birth, or number of attempts.

In this analysis presented here, the non-bias sex ratio is in accordance with Fisher’s Principle. This principle predicts that the sex ratios of populations are expected to be identical due to the equal contribution of parental genes, regardless of their rarity, including alleles determining sex (Carvalho et al. 1997). As seen in other cooperative breeding species, I found that the bias in sex ratio did not conform to the predictions of the helper repayment hypothesis. This hypothesis predicts – 1) an overall bias towards the helping sex in a population; and/or 2) an overproduction of the helping sex at the individual level for breeders without helpers (Doutrelant et al. 2004).

The results presented here, also do not conform to the predictions derived from local resource enhancement models, as in other cooperative breeding species. This model predicts a birth sex ratio bias towards the more helpful sex when the benefits received do not significantly vary across parental groups (Gowaty and Lennartz 1985, Emlen et al. 1986, Pen and Weissing 2000). With the birth of more helpers (males), it allows the opportunity to repay the cost of their own production during breeding seasons by raising
the offspring. This experience allows for the heightening of inclusive fitness and co-breeding opportunities, which in turn increase the parental reproductive success.

However, the non-significant population sex ratio combined with the non-significant results of the six variables indicate the need for additional study on this species, especially among a cooperative breeding species. The implications of this study have significance in cooperative breeding because they do not conform to the normal implications of cooperative breeding, such as a skew in sex ratio. A non-bias sex ratio in the cooperative breeding chestnut crowned babbler may be explained by the costs versus benefits available within the arid environment. If there are no benefits to helping or dispersing and all offspring receive the same parental care, there would be no reason to skew the sex ratio. In addition, because this avian species is found only in Australia, the species is exposed to the same environmental conditions. Therefore, it is possible to exclude most environmental factors, such as temperature or available resources. With an equal territory condition across the nation, this avian species does not have any additional benefits or costs on territory or environment.

Without additional benefits or costs, one might question why helpers would still help other breeding groups and offspring that are not theirs with no additional benefits. Because some chestnut crowned babbler helpers will never become breeders themselves (stay helpers for their lifetime), they need to find a way to potentially continue their genetic information to increase survival of the species. They do this by helping raise offspring that is closely related to them in order to pass on genetic information that is closely related to their own if direct offspring are not an option. Therefore, this avian species is able to preserve their gene pool and reproductive success rates. Hence, as
suggested in previous background information, a skew in sex ratio is commonly visible toward the helping sex in cooperative breeders. However, with non-significant values determined in this study, additional research should be conducted to discern if a different variable may be responsible for a non-bias or if one of the variables is excluded from their environment, would a possible sex ratio bias emerge?

Due to the insignificance of the six variables examined in this study, one may predict that cooperative breeding in the chestnut crowned babbler is subjected to other variables. Therefore, further studies should focus on the variables that define cooperative breeding and if each cooperative breeding species must display specific variables or a skew in their sex ratio to be classified as a cooperative breeder. In addition to these questions, further research should be performed to determine if there is a potential hatch rank interaction in chestnut crowned babblers, as seen in the laughing kookaburra? Do first-born males only occur in younger females? Therefore, maternal age, hatch rank, and territory quality should also be investigated in future studies on the cooperative breeding chestnut crowned babbler.
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