

Cooperative breeding and possible multiple paternity in the supposedly monogamous White-crowned Robin-Chat *Cossypha albicapillus*

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Summary

We report a case of cooperative breeding and putative multiple paternity in a nest of the White-crowned Robin-Chat *Cossypha albicapillus*. These phenomena have not been reported previously in this species and closely-related robin-chats. In a nest found in The Gambia we observed at least five adults feeding or visiting the young. We genotyped four adults (one female, three males) and the two young using a microsatellite marker previously developed for another species. The genotype data were consistent with the female being the mother of the two nestlings, but paternal alleles suggested two different males as fathers of the nestlings. Our observations show that cooperative breeding occurs in the White-crowned Robin-Chat, and that monogamy may not be as universal in this species as previously assumed.

Résumé

Reproduction coopérative et possible paternité multiple chez le Cossyphé à calotte blanche *Cossypha albicapillus*, supposé monogame. Nous rendons compte d'un cas de reproduction coopérative et paternité multiple putative dans un nid de Cossyphé à calotte blanche *Cossypha albicapillus*. Ces phénomènes n'ont pas jusqu'ici été rapportés pour cette espèce et les cossyphes proches. Dans un nid trouvé en Gambie, nous avons observé au moins cinq adultes de cette espèce nourrissant ou visitant les jeunes. Nous avons analysé le génome de quatre adultes (une femelle, trois mâles) et des deux jeunes en utilisant un marqueur microsatellite développé pour une autre espèce. Les données des génotypes étaient cohérentes avec l'hypothèse que la femelle était la mère des deux poussins, mais les allèles paternels suggéraient que deux mâles différents étaient les pères des poussins. Nos observations

montrent qu'il peut y avoir une reproduction coopérative chez le Cossyphé à calotte blanche, et que cette espèce n'est pas toujours monogame comme on le supposait.

Introduction

A historical northern bias, due to the geographical origin of researchers, has led to a relative neglect of aspects of avian life history in the tropics, such as duetting and cooperative breeding (Arnold & Owens 1998, Slater & Mann 2004). This bias limits our understanding of bird biology, since the different ecological conditions in tropical areas may lead to strong differences in life history patterns (Ghalambor & Martin 2001).

We report here a case of cooperative breeding and multiple paternity in a nest of the White-crowned Robin-Chat *Cossypha albicapillus*, phenomena that appear not to have been reported previously in this genus (Keith *et al.* 1992). Robin-chats (*Cossypha* and the closely related *Cossyphicula*, *Dessonornis* and *Oreocossypha*) typically inhabit forest areas and are restricted to sub-Saharan Africa. All robin-chats on which observations have been made are considered territorial and socially monogamous (Keith *et al.* 1992), though many are somewhat secretive and live in dense vegetation, so few details of their breeding biology are available. In The Gambia, the White-crowned Robin-Chat has adapted well to open landscapes, gardens and orchards with human habitation (Barlow *et al.* 1997).

Methods

In September 1997, we discovered a White-crowned Robin-Chat nest in a large Mango tree *Mangifera indica* outside a farmhouse in Kamboujay, near Brikama, in The Gambia. The nest was near the end of a low branch, *c.* 3 m from the ground. It was coarsely built of thick twigs and Mango leaves with some plastic scraps. There were two chicks in the nest. Their eyes were closed during the week we were able to observe them. They had dark skin, with rusty-ginger natal down. There were no mouth markings, their flanges were pale yellow and gapes dirty yellow. Since we observed more than two adults visiting the nest, we surrounded the area with mist nets to catch and mark all individuals so as to be able to genotype the birds. We caught five adults in total, and marked them with colour rings and patches of coloured dye on the tail feathers. On 17 September, we conducted a 4-h watch on the nest to assess the contribution of each adult to the rearing of the chicks. Observations were performed through binoculars from *c.* 50 m distance.

Blood samples were taken from the chicks and four of the five adults, and extracted by a phenol procedure. We used PCR to amplify several microsatellites

previously developed for other bird species. We tried the amplification of the following: PCA μ 7 and PCA μ 9 from the Blue Tit *Cyanistes caeruleus* (Dawson *et al.* 2000), MME12 from the Song Sparrow *Melospiza melodia* (Jeffrey *et al.* 2001), HrU2 from the Barn Swallow *Hirundo rustica* (Primmer *et al.* 1995), MCY μ 4 from the Superb Fairy-wren *Malurus cyaneus* (Double *et al.* 1997), and PDO μ 5 from the House Sparrow *Passer domesticus* (Griffith *et al.* 1999). Of these markers, PCA μ 7 was monomorphic, PCA μ 9 and PDO μ 5 showed persistent multiple banding, and MME2 was homozygous in all the genotyped individuals. However, MCY μ 4 presented five different alleles, allowing us to examine the paternity of the chicks, with due caution given the low power of a single marker.

The 20 μ l PCR reaction for MCY μ 4 contained 1 μ l of DNA, 2 μ l Bioline 10x buffer, 1.25 mM MgCl₂, 200 μ M of each dNTP, 1 pM of each primer and 0.4 units of Bioline Taq made up to 20 μ l with distilled water. Amplifications were performed in a PCT-100 Thermal Controller (MJ Research Inc) with an initial step of 95°C for 4 min. followed by 30 cycles of 95°C for 10 s., 53°C for 30 s. and 72°C for 30 s. The programme was finished with a final extension step of 72°C for 5 min. Sizing of final fragments was performed on 6 % acrylamide gels (1500 v. for 2–3 hours), with a 10-base ladder (Invitrogen) to size alleles. Alleles were visualized by silver staining following Promega's protocol and applications guide. Birds were sexed using the P2 and P8 primers for the CHD gene, which amplify sex-specific products for most bird species (Griffiths *et al.* 1998).

Results

Five alleles were found for the MCY μ 4 locus in the four adult robin-chats (Table 1). Both chicks showed alleles that may have originated from the female that was caught feeding them. The putative paternal allele in chick 2 could have come from male 2.

Table 1. Genotypes and nest observations of the family group of White-crowned Robin-Chat. A feeding visit was when the bird was seen perched on the nest while delivering food to the chicks. Other visits comprise occasions where the bird perched on the nest without feeding the chicks.

Bird	Alleles at MCY μ 4	Feeding visits	Other visits	Vocalisations
Female	158, 167	4	6	Long and chatting calls
Male 1	160, 162	6	1	None recorded
Male 2	158, 167	10	0	Song
Male 3	162, 167	1	6	Song
Unsexed adult	not sampled	0	0	None recorded
Chick1	164, 167	-	-	-
Chick 2	158, 158	-	-	-

However, the putative paternal allele of chick 1 was not shared by any of the three males seen at the nest from which we had samples. Therefore, this chick is likely to have been fathered by either the additional bird that was seen feeding the chicks but which we could not catch, or by another male external to the group. Reliance on a single marker precludes a definitive conclusion, as genotyping errors or germ-line mutations in the juvenile cannot be excluded (Jones *et al.* 2010).

Feeding observations show that most feeding visits were done by three birds (Table 1), which were later genetically identified as a female and two males. Of the two males that were observed feeding the chicks, male 2 had fathered one nestling, whereas neither male appeared to have fathered the other. Regrettably, the limitations of the genotype data do not allow us to draw conclusions about the genetic relatedness between the adults.

Two of the three males that were seen feeding the chicks produced full song during the observations, whereas the female uttered only calls.

Discussion

Cooperative breeding used to be considered uncommon among birds (Arnold & Owens 1998), but recent analyses show that is more widespread (Cockburn 2003). Our observations show that co-operative breeding can occur in the White-crowned Robin-Chat, and that monogamy may not be as universal in this species as assumed, at least in our study area. However, we stress that a single marker is weak evidence, and further studies are needed to confirm this phenomenon. Most studies of robin-chats to date have been conducted in southern Africa, including the Cape Robin-chat *Dessonornis caffra* (Rowan 1969) and Red-capped Robin-Chat *Cossypha natalensis* (Farkas 1969), and there is a need for additional work on species elsewhere in Africa.

It was surprising to discover that two different males produced full song, as one might expect this only to be produced by the territorial male. It is possible that male song may have additional functions in a cooperative context, but to our knowledge no study of this topic has yet been carried out.

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