Genetic monogamy in Von der Decken's and Northern Red-billed hornbills

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Abstract

*Avian monogamy is usually characterized by social monogamy, a cooperative pair bond rather than genetic monogamy, a pair bond accompanied by fidelity. Hornbills (Bucerotidae, Bucorvidae) have a range of monogamous social systems, but only one species, Monteiro's hornbill (*Tockus monteiri*) has been confirmed to be genetically monogamous. We examined paternity patterns for two Tockus species, Northern Red-billed hornbill (*T. erythrorynchus*) and Von der Decken's hornbill (*T. deckeni*). We collected blood from females and chicks in nestboxes, and the putative father delivering food to the nestbox, or accompanying juvenile birds. Paternity was determined using a double-digest restriction site-associated DNA sequencing (ddRAD-seq). All males delivering food for both species (n = 35 males). For males accompanying juveniles, 14 of 15 putative fathers were identified as the genetic father. Our results extend the occurrence of genetic monogamy to three species of Tockus hornbills.*

Keywords*:* genetic monogamy, *Tockus* hornbills, pair bonds, fidelity

Introduction

Avian monogamy has been a topic of interest for many decades (Lack 1968). However, only recently has social monogamy been distinguished from genetic monogamy. Social monogamy (85% of bird species) is defined as an exclusive living arrangement involving a pair bond between a male and female, often characterized by cooperation in resource acquisition and parental care (Bennett and Owens 2002). Genetic monogamy is defined as an exclusive pair bond accompanied by exclusive parentage or fidelity (Gowaty 1996, Reichard 2003). Social and genetic monogamy are not always equivalent; on average, 12.5% of offspring of socially monogamous species result from extra-pair copulations (Bennett and Owen 2002).

Asian hornbill species exhibit four monogamous social systems including nomadic pairs, part-time territorial pairs, year-round territorial pairs, and territorial family groups (Kinnaird and O'Brien 2007). Among African hornbills, savanna species including ground-hornbills (*Bucorvus* spp.) and Tockus hornbills (*Tockus* spp.) are the best studied (Kemp 1995). Ground-hornbills are cooperative breeders on large territories and savanna Tockus hornbills are monogamous pairs on part-time or year-round territories.

Only one study has been published on genetic monogamy in a hornbill species, Monteiro's Hornbill (*Tockus monteiri*) in Namibia (Stanback et al. 2002), where blood was obtained from 38 hornbill families including 138 chicks. No evi-

dence of extra-pair copulations was found and all chicks were assigned to their putative father. In this paper, we report on the results of a paternity analysis of chicks of two African savanna hornbill species, Von der Decken's hornbill (T. deckeni) and Northern Red-billed hornbill (T. erythrorhynchus) in northern Kenya.

Methods

This project was conducted at the Mpala Ranch and Research Center, Laikipia County, Kenya (0.284 – 0.5248 N and 36.828 – 36.8838 E). Mpala Ranch is a 200 km^2 cattle ranch and wildlife conservancy. Topographically, Mpala Ranch consists of rolling hills, an uplifted plateau, granitic inselbergs, and is bordered by rivers along more than half of its boundary. Annual rainfall averages 594 mm in the south and 430 mm in the north, with rains typically occurring during April-May and October-November. Droughts are sporadic, but increasing in frequency (Franz 2007). The landscape is covered by bushland dominated by *Acacia mellifera*, *A. etbaica*, *A. brevispica*, and *Grewia tenax*, and by *A. drepanolobium* open woodland. Hornbill species observed on the ranch include Eastern yellow-billed (*T. flavirostris*), Crowned (*T. alboterminatus*), Northern red-billed (*T. erythrorynchus*), Von der Decken's (*T. deckeni*), African grey (*T. nasutus*) and Silvery-cheeked hornbills (*Bycanistes brevis*). Only Von der Decken's and Northern red-billed hornbills have been observed to breed on Mpala.

We used three strategies to capture hornbills. First, we used walk-in traps, baited with peanuts and set around the Mpala Research Center from 2011 to 2015. Second, we deployed 90 nest boxes during the breeding seasons of March – July 2012 through 2015. Boxes were 25 x 20 x 50 cm with a 6 cm diameter entrance, a perch below the hole, and a lockable side door for examining the female and chicks (Fig. 1). Boxes were placed in trees or on 4 m poles along service roads. Boxes were examined every 4 days. Blood samples were obtained from females in nestboxes after chicks were hatched, and from chicks 2 weeks after hatching. Finally, males delivering food to nest boxes were captured using mist nests placed in front of nest boxes and using recorded male hornbill calls as an audio lure. All hornbills were tagged with a National Museums of Kenya metal identification leg band and colored plastic leg bands.

Genetic analysis was conducted at the Cornell Laboratory of Ornithology to identify the genetic parentage of chicks. They used a double-digest restriction site-associated DNA sequencing (ddRAD-seq: Puritz et al. 2014) analysis. This approach simultaneously locates single nucleotide polymorphisms (SNP) and genotyping steps and is optimized to return a statistically powerful set of SNP markers (typically 150-600 after stringent filtering) from large numbers of individuals (up to 240 per run). For full details of the analysis, see Thrasher et al. (2017).

Results

We monitored 10 Northern red-billed hornbill (NRBH) successful nesting events in 2012 (n = 2), 2013 (n = 3), and 2015 (n = 5) involving nine females and 19 chicks, and captured four young juvenile birds with putative fathers in walk-in traps. For Von der Decken's hornbills (VDDH), we monitored 27 successful nesting events in 2012 (n = 2), 2013 (n = 9), 2014 (n = 6) and 2015 (n = 10) involving 26 females and 40 chicks, and 11 capture events involving young juveniles traveling with an adult. For chicks banded and bled at the nest site, we had 100% assignment of social father to genetic father for both species. For four cases of juvenile NRBH captured with an associated adult male, in all

Fig. 1. Male Von der Decken's hornbill delivering a beetle to female in nestbox.

cases, the adult male was the genetic father. For 11 cases of juvenile VDDH captured with an associated adult male, in 10 of 11 cases, the male was identified as the genetic father.

Discussion

Our results extend the finding of genetic monogamy in Montiero's hornbill (Stanbeck et al. 2002) to two additional *Tockus* species, Northern red-billed and Von der Decken's hornbills. *Tockus* hornbills exhibit several characteristics of long-term monogamy including year-long associations, territoriality, courtship feeding and sperm storage. Mulder et al. (1994) argued that any tendency toward extra pair copulations in birds would be a compromise between females seeking genetically superior males and her dependence on male provisioning during nesting. Gowaty (1996) also believed that the female should always seek a genetically superior male and that socially bonded males should attempt to restrict access to females through mate guarding or other constraints. Gowaty believed that the threat of withholding food from females was sufficient to constrain her, since female *Tockus* hornbills generally molt tail and wing feathers during incubation and are unable to fly, and male desertion would doom the female and her chicks. Such conflict-mediated reproductive strategies assume that costs incurred are tolerable for (at least) one sex and that one sex gains at a cost to the other (Mock and Forbes 1992).

Stanback et al. (2002) note that mate guarding is not particularly strong in Montiero's hornbills and Finnie (2012) found the same in Southern yellow-billed hornbills (*T. leucomelas*). This loose guarding, combined with sperm storage, allows for the possibility of extra-pair copulations prior to entering the nest. Purple Sandpipers (*Calidris maritima*) are long-lived, socially monogamous sandpipers also characterized by strong mate and territory fidelity, high male parental investment and loose mate guarding, that rarely seek extra-pair copulations (Pierce

and Lifjeld 1998). Both Stanback et al (2002) and Pierce and Lifjeld (1998) argue that, when male paternal investment is high, the females have little to gain from extra-pair copulations and should resist extra-pair copulations, making male attempts energetically expensive. Stanback et al. (2002) also argue that the complete dependence of female and chicks on male provisioning results in a strong overlap in reproductive interests of males and females, resulting in cooperation rather than conflict.

For seven females (NRBH = 1 and VDDH = 6) that nested at least twice during our study, we found that the same male was the father after two to four years, indicating extended pair bonds in both *Tockus* species. Based on the observation of extended pair bonds in hornbills, overlap in reproductive interest, and high male parental investment, we agreed with Stanback et al. (2002) that mate choice by females is based on provisioning skills rather than phenotypically expressed genetic superiority (Kinnaird and O'Brien, 2008). Females should try to retain a good male provider. Since male provision is equal to female reproductive success, and inferior male providers will either kill the female and chicks or force the female to abandon the chicks, females should only engage with untested males under three conditions; a female mating for the first time, a female who has abandoned an inferior male provider, or a female who has lost a mate. Females abandoning inferior males may be a common feature in monogamous systems with extended pair bonds where females use paternal investment as a signal of male quality.

A distinction between our ideas and those of Stanback et al. (2002) is the order of events in the development of monogamy in hornbills. Stanback et al. (2002) assume that nest-sealing evolved as an anti-predator behavior which in turn led to long-term sperm storage, high male investment, and cooperation over conflict. We argue that if nest sealing was an effective anti-predator behavior, it should have evolved more often among cavity nesters (Kinnaird and O'Brien, 2008). Although hornbills have higher nesting success than other cavity nesting species, the result is not significantly different. We believe that female nest sealing evolved as a female strategy to ensure the cooperation of the male.

Our study strengthens the argument that *Tockus* hornbills are genetically monogamous as well as socially monogamous. It also leads us to question whether the pattern of genetic monogamy will hold for Asian hornbills and the forest hornbills of Africa. Given the increasing use of nest boxes to augment hornbill nesting, there is a greater opportunity to test for paternity within other hornbill genera.

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