SEXUAL DIMORPHISM IN SMOOTH-BILLED ANIS

SEXUAL DIMORPHISM IN A JOINT-NESTING PLURAL BREEDER, THE SMOOTH-BILLED ANI (*CROTOPHAGA ANI*)

By

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Abstract

Sexual selection has been investigated in many different systems but information is lacking in joint-nesting species. Both sexual dimorphism and possible signals of quality were explored in this study for a joint-nesting plural breeder, the smooth-billed ani (*Crotophaga ani*). Sexual dimorphism can be examined for a species in an attempt to reveal particular traits that might be under sexual selection and the strength of sexual selection on them. In mate choice decisions, sexually selected traits may be used by the choosy individual to assess the quality of potential mates. These honest traits need to reveal the condition and genetic quality of the individual accurately. For example, it has been proposed that plumage colour reveals condition, and more specifically parasite resistance in birds. This study explores sexual size dimorphism and dichromatism in smooth-billed anis, as well as the ability of their feather colour to reveal body condition (measured as residuals from a regression of mass versus body size), body size, and parasite numbers. Additionally, because anis are group living birds, this study investigated whether group size had an effect on parasite numbers. Anis from a population in southwestern Puerto Rico were measured for size, their feathers were collected and analyzed using a spectrometer, and ectoparasites were collected by dust-ruffling. For all size traits measured, males were significantly larger than females. Males also had more exaggerated bill depths (relative to body size) than females. There were no differences between the sexes for all colour morphometrics, except tail saturation; females had more saturated tails than males. While no decisive conclusions can be drawn as to why the dimorphic traits are different between males and females, sexual selection may have played a role. The relationship between $\log_{10}$ average lice numbers per group and group size was positive, but not significant. In male smooth-billed anis, plumage colour was not related to lice or mite numbers, or body size and body condition. Plumage colour was also not related to body condition and size in females. Additionally, ectoparasites did not have an effect on male body condition. Plumage may not only be an inaccurate signal of parasite resistance or body size and body condition in the smooth-billed ani, but ectoparasites may not even have a negative effect on their host. The inability to find significant results may have been affected by other factors, such as feather wear and the age of individuals. These results warrant a more detailed look into the social behaviour of the group-living smooth-billed ani.

Preface

This Master's thesis is composed of four chapters. The first chapter is a general introduction and the last, fourth chapter is a conclusion of the entire thesis. The second and third chapters are written in manuscript format to be submitted to appropriate journals. Below are the contributions for each of these chapters:

Chapter 2: Sexual size dimorphism and sexual dichromatism in the smooth-billed ani (*Crotophaga ani*), a plural-breeding joint-nester.

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Contribution: Field work conducted in the 2006 and 2007 breeding seasons were conducted by the candidate with the assistance of various field volunteers and the assistance and guidance of A. Samuelsen and J.S. Quinn. Field data from previous years were collected from past students and volunteers from the Quinn lab. Sexing of anis caught in the 2006 and 2007 season (and some from previous years not already sexed) were conducted by the candidate with the assistance of R. Sivayoganathan. Past sexing was conducted by previous students of the Quinn lab. Feather reflectance measurements of feathers collected from the 2006 and 2007 seasons were conducted by the candidate with the guidance of S.M. Doucette. The reflectance of the feathers from 2003 and 2004 were measured by S.M. Doucette. Statistical analyses were conducted by the candidate with the assistance of S. Dudley. The manuscript was written by the candidate with suggestions from J.S. Quinn, A. Samuelsen and F.J.J. Chain. The research was supervised by J.S. Quinn.

Chapter 3: Parasites, plumage and condition in a joint-nesting plural breeder, the smooth-billed ani (*Crotophaga ani*).

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Chapter 1

General Introduction

Sexual Selection

Sexual selection can act when there is variation in mating success for a species (Andersson 1994). Under this scenario, those traits that increase the mating success of an individual will be positively selected for (Krebs & Davies 2003). In most sexual species, females are more limited than males in how much they can reproduce because of inherent reproduction costs (Bateman 1948; Trivers 1972), therefore limiting a male's opportunity for mating. Males must consequently gain mating access to females either through male-male competition or through female mate choice (Krebs & Davies 2003). Because some males are more successful in obtaining mates and reproducing offspring than others, mating success varies across individuals, which in turn can give rise to sexual selection (Krebs & Davies 2003).

In species where the sexes differ in the degree of competition for mates a difference should arise between the sexes in the traits under sexual selection. These selected traits are under different and greater selective pressure in the sex that has increased competition for mates (usually males). The resulting difference in appearance between the sexes is known as sexual dimorphism (Dunn et al. 2001). Studying sexual dimorphism in a species is beneficial because it gives an idea of what traits may be under selective pressure and may indicate the strength of sexual selection on the traits of interest (Mays et al. 2006).

Across different taxa, extensive research has gone into learning about female mate choice and its associated sexually selected traits (see Andersson 1994). Because females generally have a more limited reproductive rate, it is in their fitness interests to choose mates that will give them the most benefits. The benefits a female may gain from her mate can be direct (such as parental care or territory defense) or indirect (such as good genes for her offspring; Neff & Pitcher 2005). Sexually selected traits (e.g. bright plumage, long tails) are cues to females that allow them to evaluate potential mates accurately (Clayton 1991; Kempenaers et al. 1997). Zahavi's (1975) handicap principle states that sexual selection favours exaggerated traits in the non-choosy sex (normally males) because these traits are an honest signal of the individual's condition and genetic quality. The trait must be a handicap or costly to produce to be honest. Only those males that are of the highest quality can produce the most exaggerated trait. A female may use indices or traits considered to be handicaps to assess males, but she may also use indices that are not handicaps, but are correlated to the fitness and condition of an individual (Maynard Smith & Harper 2003). The main difference between handicaps and other indices is whether they are costly to the individual in situations outside of mating. Handicaps are costly in non-mating contexts, while other indices are beneficial to the individual in non-mating contexts (Maynard Smith & Harper 2003).

Overall, traits assessed by females need to be condition-dependent (Griffith & Pryke 2006). The health, growth and development of an individual are all affected by the environment and genetic quality of that individual, which in turn affect their condition. This condition is ultimately revealed in sexually selected traits (Griffith & Pryke 2006). If a female chooses a male with an extreme sexually selected trait, she is choosing a male in good condition who can provide direct and indirect benefits.

Examples of sexually selected traits

Body Size

There are numerous examples of species with sexual size dimorphism, including many within bird families (Szekely et al. 2007). Some species show extreme size differences between the sexes, such as black skimmers (*Rynchops niger*), a species in which females are two thirds smaller than males (Quinn 1990). Male great bustards (*Otis tarda*) are three times larger than females (Collar 1996). Males and females in other species do not appear to differ in size until they are actually measured and tested statistically. This was the case for both Steere's liocichla (*Liocichla steerii*) and Caspian terns (*Sterna caspia*; Mays et al. 2006; Quinn 1990).

Females may choose larger males because an individual that is large due to good genes and/or high quality environment might signal an ability to obtain many resources and/or efficiently use them to develop a large body size (Bonduriansky 2007). Also, being large might be advantageous in itself by better enabling an individual to attract mates and compete with others for food and resources (Kodric-Brown & Brown 1984; Szekely et al. 2007). In some species, females have been shown to prefer larger males; for example, unmated Darwin medium ground finches (*Geospiza fortis*) are statistically smaller than mated males (Price 1984).

Badges of dominance status

Another way individuals can assess quality of potential mates is by learning their dominance status through badges of status (Mennill et al. 2004; Otter et al. 1998). A study on black-capped chickadees (*Poecile atricapillus*) found that plumage colouration, a sexually dimorphic trait, correlated with dominance status and females preferred more dominant individuals (Woodcock et al. 2005). The females in the study were able to assess dominance of males by observing the plumage of the males and not the interactions between them. Badges of dominance are indices to females that are not costly to produce or develop (Maynard Smith & Harper 2003), but for such signals to be honest and prevent cheaters an associated cost is required to having a trait that signals high dominance status (Tibbetts & Dale 2004). One hypothesis is that the continuous and aggressive confrontations encountered by an individual with a signal of dominance are significantly costly (Johnstone & Norris 1993; Maynard Smith & Harper 2003; Tibbetts & Dale 2004).

Plumage colour

Yet another sexually selected trait in birds is plumage colouration. Similarly to body size, there exists a wide range in the degree of sexual dichromatism observed in birds. Eclectus parrots (*Eclectus roratus*) are an example of extreme sexual dichromatism because females are a bright red and males are green. One important discovery to the study of colour in birds is that birds can see colours within the UV range (Odeen & Hastad 2003). Species may appear monochromatic but are in fact dichromatic at shorter wavelengths that we cannot see. This was the case for the crown feathers of blue tits (*Parus caeruleus*; Hunt et al. 1998) and blue-fronted Amazon parrots (*Amazona aestiva*; Santos et al. 2006).

Colours within the UV range, as well as purples, blues and greens, are considered structural plumage colours, which have received a lot of recent attention. Structural colouration is different from pigment-based plumage signals in that it is produced by the scattering of light off of the reflective feather microstructure (Doucet & Montgomerie 2003). Feather microstructure also produces iridescence, a quick change in colour when a surface is viewed at different angles and illuminations (Prum 2006).

The use of structural plumage colouration as a signal to females choosing mates has been studied in several species. Pied flycatcher (*Ficedula hypoleuca*) females preferred males with increased UV reflectance from their feathers in a manipulative mate choice experiment conducted by Siitari et al. (2002). The opposite test was done for bluethroats (*Luscinia* s. *svecica)* in which male UV reflectance was reduced and females again preferred the males with greater UV reflectance (Andersson & Amundsen 1997). Female starlings (*Sturnus vulgaris*) and zebra finches (*Taeniopygia guttata*) also use UV colours to choose a mate (Bennett et al. 1997; Bennett et al. 1996).

Plumage as a signal of condition

A lot of research on sexual selection in birds has focused on plumage colouration and whether it is a good signal of condition for choosy females. For structural plumage colouration to be a sexually selected trait, it needs to accurately represent the condition of an individual. It may be costly to develop a feather microstructure that produces the sexiest plumage colour, but little is known about this (Osorio & Ham 2002). The light that is scattered and reflected by the microstructure of feathers is affected by the shape of feather barbs, the number and thickness of fine structures and how regular and precise they are (Andersson & Prager 2006; Prum 2006). All of these may result in variation between individuals in the colour their plumage produces, but it is not known if the formation of these structures is dependent on the condition of the individual.

Plumage structural colouration as an honest signal of quality and condition was tested in blue grosbeaks (*Guiraca caerulea*; Keyser & Hill 1999). The study revealed that male grosbeaks with the shortest wavelengths and highest intensity and contrast (measures of structural colour brilliance) had the highest tail feather growth rate. Perhaps those males that could obtain nutrients for fast feather growth were also able to allocate resources to feather microstructure. A separate study found the body size of blue grosbeaks was positively correlated with plumage blueness (Keyser & Hill 2000).

Hamilton and Zuk Hypothesis

Plumage colouration may not only reveal the general condition of an individual, but more specifically, their parasite resistance. Hamilton and Zuk (1982) proposed that females choose to mate with relatively parasite free males and that they are able to choose these mates by assessing sexually selected traits that reveal the health, immunocompetence, or parasite resistance of an individual. Several studies have found that females will choose less parasitized males when given the choice between males with different infection levels (reviewed by Clayton 1991). Hamilton and Zuk (1982) applied their theory specifically to plumage colouration; males with higher quality plumage have a higher resistance or lower exposure to parasites, which may be passed on to their offspring (ie. a "good genes" hypothesis).

Parasites are in a parasitic symbiosis with their hosts, which means the parasite benefits from the relationship while the host suffers a cost (Proctor & Owens 2000). A parasite can be an endoparasite and live within the host, such as blood-bourne parasites, or it can be an ectoparasite and live on the outside of the host, such as on the skin or feathers (Smyth 1994).

Blood-bourne parasites live off the blood of many bird species and can be found within the bloodstream or intracellularly (Atkinson & Van Riper 1991). *Plasmodium, Leucocytozoon*, and *Haemoproteus* are intracellular blood parasites that have received a lot of attention by bird biologists (Atkinson & Van Riper 1991).

The ability of plumage colour to predict blood-borne parasite loads has been tested. Doucet and Montgomerie (2003) looked at the relationship between *Haemoproteus* loads and variation in structural plumage colouration in satin bowerbirds (*Ptilonorhynchus violaceus*). They found that males with brighter plumage (a measure of reflectance) had fewer blood-bourne parasites and concluded that female satin bowerbirds may use plumage brightness as an indicator of endoparasite resistance in males.

Two common ectoparasites that infect birds are lice and feather mites. Feather mites are from the class Arachnida, subclass Acari, suborder Astigmata. The three subfamilies that make up the feather mites are Analgoidea, Freyanoidea and Pterolichoidea. Feather mites rely on birds and live in or on their skin and feathers. Those that live on the surface of the feathers feed on uropygial oil present on feathers, as well as other substances that collect in it, such as fungal spores and pollen (Proctor & Owens 2000; Proctor 2003). Lice are from the order Phthiraptera; those found on birds are from the suborders Ischnocera and Amblycera, both of which are considered chewing lice (Marshall 1981). Both suborders feed on the feathers and skin debris of their host, but Amblycera also feed on blood (Marshall 1981).

Figuerola et al. (2003) tested the Hamilton and Zuk hypothesis by comparing feather mite load to plumage characteristics of serin (*Serinus serinus*). They learned that individuals with lower parasite loads had feathers with greater brightness and chroma. In addition to this correlative study, they manipulated the parasite load of individuals by removing mites from some individuals and found those with the removed parasites had brighter plumage. Harper (1999) looked at the effects of mites on plumage for five different species of birds and found similar results: birds with fewer mites had higher quality plumage.

Parasites and condition

The effects that parasites have on their avian hosts has been a topic of interest because a female should benefit from choosing a male with high parasite resistance. Avian parasites can impose a number of different costs on their hosts such as disease risk (act as vectors), time spent preening, and the consumption or resources in the form of blood and feathers (Brown et al. 2006).

Blood-borne parasites have been found to negatively affect both reproductive success and adult condition. A study by Merino et al. (2000) on blue tits found the fledgling success was greater for individuals that were given medicine to reduce their *Haemoproteus* numbers. These same females that received the medicine also had heavier body masses after the treatment compared to the control group, possibly due to the reduction in *Haemoproteus*.

There is still a lot of controversy in the literature on whether feather mites are even parasitic, but instead commensals, or even mutualists. In the case of commensalism, the feather mite benefits from the symbiotic relationship, while the host is unaffected. Mutualism occurs when both the mite and the host benefit from the interaction (Proctor & Owens 2000).

Feathers mites were found to be parasitic in a study on house finches (*Carpodacus mexicanus*; Thompson et al. 1997). Individuals with higher mite loads had reduced flight feather growth. A negative relationship was also found between feather mite load and plumage colouration (duller birds had more feather mites). Harper (1999) presented results that indicated that feather mites were parasitic in his study on nine different bird species; he found that individuals with lower pectoral muscle scores (ie. lower condition) also had higher mite loads.

Several studies have shown feather mites may be commensals. Pap et al. (2005) conducted an experiment in which they fumigated a group of barn swallows (*Hirundo rustica*), and no difference was found in the breeding performance or tail and wing feather growth between the treatment and control groups. There was also no relationship between feather mite load and body condition for Linnets (*Carduelis cannabina*; Blanco et al. 1999).

Birds may even benefit from feather mites because the mites eat pathogenic fungi and bacteria, as well as reduce the necessity for preening by removing detritus and old oil. Blanco et al. (1997) found a positive relationship between feather mite numbers and body condition in red-billed choughs (*Pyrrhocorax pyrrhocora*). A study done on different colonies of cliff swallows found varying results (Brown et al. 2006); there was an overall positive relationship between feather mite loads and survival, however, two colonies had a negative relationship and four colonies had no relationship.

There have also been conflicting results on whether or not lice harm their hosts. Clayton (1990) observed the effects of Ischnocera lice on rock doves (*Columba livia*) and found they caused considerable damage to the birds' feathers and females chose males that were less lousy. Because the feather damage was only at the distal portion of the feathers and therefore only observed upon close inspection, Clayton (1990) proposed that females chose males with fewer lice because of the greater display rate of these males, possibly due to better thermoregulation compared to those males with damaged feathers. Booth et al. (1993) found that feather chewing lice on rock doves caused an increase in metabolic rate due to reduced thermoregulation, which ultimately resulted in a decline in host mass. However, when male rock dove condition (weight), survival, and fitness were compared to Ischnocera lice loads in an additional study, no relationships were found (Clayton & Tompkins 1995). Amblycera lice, which feed on blood and reside on the skin of their host in addition to their feathers, cause dermatitis and scratching, increasing the amount of grooming required by the host. Amblycera may also act as vectors for endoparasites (reviewed in Clayton 1990).

Study species

Smooth-billed anis (Crotophaga ani) are birds for which little is known about sexual dimorphism or signals of genetic quality and body condition. What makes anis intriguing is their joint-nesting plural-breeding system in which multiple males and females form a breeding group and share a single nest (Vehrencamp & Quinn 2004). Sexual selection is not well studied in joint-nesting breeders, but one study on Taiwan yuhinas (*Yuhina brunneiceps*), another joint-nesting species, revealed a sexual size dimorphism in wing chord length (a measure of body size) and the authors proposed that this may be the result of females choosing larger males (Yuan et al. 2006).

The mean group size of smooth-billed anis in a study population in Puerto Rico ranges from 3.8 to 6.2, depending on the year, but groups can have as many as 17 adult members (Quinn & Startek-Foote 2000; Vehrencamp & Quinn 2004). Groups are mostly made up of unrelated individuals (Blanchard 2000).

Parental care is shared amongst members of a breeding group, including nest building, incubation and nestling food provisioning, but a single male from each group does most of the nocturnal incubation (Quinn & Startek-Foote 2000; Vehrencamp & Quinn 2004). When there are multiple females within a group, competition for optimal egg positions in the nest will result in the tossing and burial of eggs (Quinn & Startek-Foote 2000; Schmaltz et al. 2008).

Although socially monogamous, a previous study has found that female anis do participate in extra-pair copulations and have extra-pair young (Blanchard 2000). When a species pairs monogamously, a female is not necessarily constrained to her social mate because she may mate outside the pair-bond to gain the benefits of a higher quality male (Moller 1992). This may allow for a greater opportunity for sexual selection to occur due to an increase in variance in male reproductive success (Webster et al. 1995).

Smooth-billed anis appear to be sexually monochromatic because both sexes are all black with purple and green iridescence on their feathers. Males are assumed to be larger than females based on field observations, but no tests have been conducted to determine if this is in fact the case. Both sexes have long tails and a distinct crest on the top of their bills, of which the purposes, if any, are unknown.

Goals of Study

The questions to be addressed in this thesis relate to sexual dimorphism and signals of quality and body condition in smooth-billed anis, topics that need to be addressed to learn more about mate choice and sexual selection in a joint-nesting plural breeder. Chapter two of this thesis will explore sexual dimorphism in anis. I investigated whether anis are sexually size dimorphic for several different morphometric measures of size. In addition to these measures, I compared male and female tail lengths and bill-depths, relative to body size. Tail length may indicate condition because feathers are costly to grow and bill crests may be a badge of status because it is likely not costly to produce or develop I also examined sexual dichromatism in anis by analyzing the iridescence produced from the microstructure of their feathers. The third chapter tests the Hamilton and Zuk hypothesis that plumage signals parasite resistance in male smooth-billed anis. It also explores whether feathers signal body size and condition and whether parasites actually have a negative impact on male condition. In addition, because anis live in groups, I looked at the relationship between group size and ectoparasite loads because it has been found that group living may influence the number of ectoparasites infecting individuals (Poulin 1991).

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Chapter 2

Sexual size dimorphism and sexual dichromatism in the smooth-billed ani (*Crotophaga ani*), a plural-breeding joint-nester

Abstract

Studying sexual dimorphism may reveal possible traits under sexual selection. Sexual size dimorphism and dichromatism are particularly well studied among birds. This study investigates both size dimorphism and dichromatism in a joint-nesting plural-breeding species, the smooth-billed ani (*Crotophaga ani*). No extensive quantitative analyses on sexual size dimorphism have been conducted until now and little is known about the characterisitics of the structural plumage colouration in anis. Adult anis from a population in Puerto Rico were caught over several years and size morphometrics and feather samples were collected. We determined plumage reflectance using a spectrometer and brightness, hue, and saturation were calculated for three body regions (head, mantle and tail). Smooth-billed anis were found to be dimorphic in size, with males being larger than females. Males had greater bill-depths relative to body size than females, but this was not the case for relative tail length. Females had greater tail saturation than males, but no other plumage colour measures were significantly dimorphic. Sexual selection may have resulted in the dimorphisms observed, while the monomorphic traits may have been the result of mutual mate choice or natural selection, but a more in-depth look at the mating system of smooth-billed anis is required to better understand these results.

Introduction

Sexual selection has received a lot of attention since Darwin (1871) first proposed his theory that explains sexual dimorphism in species. Birds are particularly interesting for the study of sexual selection and dimorphism because of the variety in the degree of dimorphism across species. Sexual size dimorphism and dichromatism in birds may result from either form of sexual selection: intrasexual competition or mate choice (Andersson 1994). For some species, the possibility that selection may act differently on the sexes is more obvious because of great sexual dimophorism. For example, black skimmer *(Rynchops niger)* females are two-thirds the size of males, with no overlap in linear dimensions between sexes (Quinn 1990). The sexes of a species can also be completely different colours, as in the case of Eclectus parrots (*Eclectus roratus*), where females are a bright red while the males are green (Heinsohn et al. 2005). However, it is not always apparent whether a species is dimorphic or not because differences between the sexes can be subtle. In Steere's liocichla (*Liocichla steerii*), males and females appear the same size when handled, but are slightly dimorphic when actually measured (Mays et al. 2006). In terms of plumage, some species, such as blue tits (*Parus caeruleus*), appear monochromatic until their feathers are observed under a spectrometer (Hunt et al. 1998). Structural plumage colour, like that produced by the crown feathers of blue tits, can occur within the UV range, which most birds can see (Doucet 2002). So, while blue tits were thought to be monochromatic, their crown feathers were revealed to be dichromatic at UV wavelengths.

Studying sexual dimorphism in a species can be extremely useful and informative when the mating system of the species is not well studied and when it is not known whether or not sexual selection is influencing particular traits of the species. Sexual selection may not be apparent in some species because they are mistaken to be monomorphic. Different traits are under sexual selection in different species, so studying which traits are dimorphic for a particular species may indicate those under selection (Mays et al. 2006). Also, the extent of sexual dimorphism in a species may reveal the intensity or strength of sexual selection on the traits in question (Badyaev & Hill 2003; Mays et al. 2006).

Sexual selection has received little attention in many cooperative breeders, species in which social groups cooperatively raise young and one or more individuals are raising young that are not their own (Stacey & Koenig 1990). This is likely because of the general thought that sexual selection is not occurring in cooperative breeding species that appear to be sexually monomorphic (Cockburn 2004). Dunn et al. (2001) looked at sexual dimorphism across different mating systems and showed that cooperative breeders have low sexual dimorphism levels in both size and colour; however, most species included lived in related groups in helper-at-the-nest systems (cooperative groups composed of a breeding social pair and non-breeding helpers) and plumage was not measured using a spectrometer. Due to low sexual dimorphism in some cooperative breeders, sexual selection may be weak in these species, but this is not the case for all cooperative breeders (Pruett-Jones 2004).

One of the most studied cooperative breeding species is the superb fairy wren (*Malurus cyaneus*), which has extreme sexual dimorphism in plumage colouration (Dunn & Cockburn 1999). Female fairy wrens are brown while the breeding plumage of males is a striking black and blue. Superb fairy wrens have a helper-at-the-nest system, but what allows sexual selection to occur in this species is the extraordinary numbers of extra-group fertilizations that occur. Females are choosy and only a small proportion of males within a population are able to obtain extra-pair fertilizations (Dunn & Cockburn 1999).

Although the occurrence of extra-pair copulations in other cooperative species is less extreme than in the superb fairy wren, extra-pair fertilizations may provide the variation in reproductive success needed for sexual selection to work. The presence of extra-pair mating is important with respect to sexual selection because it can increase the variance in mating success among group males, increasing selection pressures on sexually selected traits (Dunn et al. 2001; Webster et al. 1995). Extra-pair fertilizations have been reported in several cooperative breeders (see Pruett-Jones 2004).

While sexual selection and dimorphism in superb fairy wrens have been studied extensively, they are not well studied in joint-nesting cooperative breeders. Joint-nesting is defined as a system in which more than one female lays eggs in a single nest (Koford et al. 1990). Taiwan yuhinas (*Yuhina brunneiceps*) are a joint-nesting species that have been found to be sexually dimorphic in wing chord length, which was proposed to be the result of sexual selection (Yuan et al. 2006). Larger males may be able to maintain a higher breeding status in a group and attract more mates because a female's status depends on her mate's.

Little is known about sexual selection and mate choice in the smooth-billed ani (*Crotophaga ani*), another joint-nesting species. Anis live in groups of mostly unrelated individuals with one or more breeding social pairs that all lay their eggs in the same nest (Blanchard 2000; Vehrencamp & Quinn 2004). Parental care is provided by both males and females and is shared amongst the members of the group, but one male within a group does most of the costly nocturnal incubations (Quinn & Startek-Foote 2000; Vehrencamp & Quinn 2004). Within a group, members are competitive with each other and will toss or bury the eggs of other individuals, creating layers of eggs within the nest, of which only the top layer hatches (Quinn & Startek-Foote 2000; Schmaltz et al. 2008). While anis are thought to be monogamous, there is evidence of extra-pair copulations within groups ((Blanchard 2000; J.S. Quinn unpublished data). No extensive quantitative analyses of different morphometric traits of the anis have been conducted to see if they are sexually dimorphic in size. Both males and females have black plumage with green/purple iridescence - colouration produced by the feather's microstructure (Prum 2006). Because structural plumage colour can also occur in the UV range, anis may appear monomorphic to human eyes while being dimorphic to birds (Doucet 2002). Both sexual size dimorphism and dichromatism were studied in the smooth-billed ani to help determine which traits, if any, may be under sexual selection.

Methods:

Field site

The smooth-billed ani population monitored for this study was located in southwest Puerto Rico, within and around the Cabo Rojo National Wildlife Refuge (17°59'N. 67°10'W). Field work was conducted during the ani's peak breeding times during the wet season (September to January). Each season will be referred to by the year including most of the field season (September to December). Morphological data for this study were collected between 1998-2004 and 2006-2007. Plumage data were collected for the years 2003, 2004, 2006, and 2007.

Adult capturing and processing

Adult anis were captured using three different methods: mist netting, funnel trapping, and nest trapping. Mist-netting involves setting up nets early in the morning or in the evening to catch the birds coming in or out of roost. Occasionally playback and a stuffed decoy ani were used with a mist net during the day. For funnel trapping we used hand-raised anis as lure birds to attract territorial adult anis. One lure bird was placed in a small cage within a larger funnel trap that was designed to allow birds to fly or walk in, but not out ((McClure 1984). The trap was either near a typical foraging site or near an active nest. If the birds were not in sight, playback was sometimes used for about one minute to attract their attention. Nest trapping was conducted at some nests during late incubation of eggs or young chicks (Mock et al. 1999). A small cylindrical trap with a draw down door attached to a string was placed on the nest and observed from a distance using a scope. Once a bird sat on the nest to incubate, the door of the trap was pulled down and the bird was removed from the trap for processing.

Each adult caught received a unique set of three colour bands and one U.S. Fish and Wildlife Service aluminum band, and in the most recent years also received a mark on the bill with non-toxic white nail polish (a unique mark for each member of a group) so that they could be identified easily during field observations.

The morphometric measures taken for each adult included (1) mass, measured to the nearest gram using a spring scale, (2) wing chord, measured to the nearest 1mm with a wing ruler, (3) tail length, measured to the nearest 1mm with a ruler, (4) exposed culmen length, measured to the nearest 0.1mm with calipers, (5) head-tip (a measure from the back of the skull to the front tip of the bill), measured to the nearest 0.1mm with calipers, and (6) maximum bill-depth, also measured to the nearest 0.1mm with calipers.

A small blood sample (50-300ul) was obtained from each adult from either the jugular or brachial vein. The blood was stored at room temperature in approximately 1.5 mls of Queens' lysis buffer consisting of 0.01M Tris, 0.01M EDTA, 0.01M NaCl and 1% n-laurylsarcosine (Seutin et al. 1991).

In addition, feathers from three different sections of each bird were collected: three from the head, three from the mantle (upper back), and one retrix (tail) feather (far right unless missing, in which case the next feather on the right was pulled). These were stored in labeled small zip-lock bags until analyzed.

Molecular Sexing

Anis cannot be sexed easily in the field, therefore each adult bird was sexed using molecular techniques. DNA was extracted from blood using a saturated salt extraction protocol (Sambrook et al. 1989) or 10% chelex (Sigma). For chelex extractions, a small amount of blood and lysis buffer (2ul-4ul, depending on blood concentration) was placed in 300ul of 10% chelex. Samples where vortexed for 15 seconds, centrifuged at 12000g (12000 rpm on Eppendorf 5415 C) for 15 seconds, incubated at 95°C for 20min, vortexed again for 15 seconds, with a final centrifugation at 12000g for 15 seconds. The supernatant was then removed, leaving the chelex beads in the tube, and placed in a new tube for storage. Samples were stored in the fridge if used within 24 hours, otherwise they were stored in a -20° freezer.

Sex was determined for each adult using PCR (polymerase chain reaction) with primers P2 and P8 developed by Griffiths et al. (1998) to sex birds. The primers amplify an intron in the chromobox-helicase-DNA-binding (CHD) gene on each sex chromosome. This intron differs in length for each sex chromosome. Because females are heterogametic in birds, samples taken from females produce two distinct bands when visualized on a gel but samples from males produce only one (Griffiths et al. 1998).

The PCR reaction included 1ul of DNA (~20ng), 0.2uM of each primer (P2 and P8), 2mM of $MgCl_2$, 0.2mM of each dNTP, 1XPCR buffer, 0.375U of taq polymerase and ddH_20 to achieve a final volume of 15ul. For chelex extracted samples, Bovine Serum Albumin (BSA) was added to each reaction to a final concentration of 0.3mg/ml. The cycling conditions consisted of an initial denaturing step for 5 minutes at 94°C, 32 cycles of 51°C for 45 seconds, 72°C for 45 seconds and 94°C for 30 seconds, followed by a final annealing step for 1 minute at 51°C and a final extension step for 5 minutes at 72°C. The final PCR reactions were then run on 2.5% agarose gels by electrophoresis and visualized under UV light so they could be scored as male or female.

Plumage Reflectance

All feathers were taped against matt black cardboard with the three head feathers layered on top of each other, and the three mantle feathers layered on top of each other. The layering is done to better represent the way they would lie on the bird. Reflectance from the feathers was measured by an Ocean Optics USB2000+ Spectrometer connected to a DH-2000 Mikropack light source (Ocean Optics, Dunedin, FL), using methods similar to those of Mays et al. (2006). Measurements were made blind to the individual's sex and morphometric measures. For each feather type, five readings were taken at the same spot on the feathers (iridescent edge of head and mantle feathers, middle of tail feathers). Each of these readings were made up of an average of 50 spectra taken in quick succession using a fiber optic probe in a rubber casing that blocked out all external light and allowed for consistent angle and distance from the feather. The probe was placed at a 90 degree angle, which was found to be the best angle for iridescent feathers because it is the most consistent and produces the most saturated curves (Shawkey et al. 2006). A white standard (100% reflectance; Labsphere, North Sutton, NH) was used to calculate percent reflectance off the feathers. Three variables, brightness, saturation, and hue, were calculated from the reflectance data using the program CLR, version 1.03 (Montgomerie 2008). Brightness, also known as spectral intensity, is the total amount of light being reflected from the surface of the feathers. Saturation, or chroma, is the purity of the light being reflected; the more the colour is made up of only one wavelength, the more pure it is. Hue is the actual colour or the wavelengths that are reflected the most (Figuerola et al. 2003; Montgomerie 2006). Formulas B2 for brightness and S8 for saturation were used for all three feather types (Montgomerie 2008). Formula H1 for hue was used for tail feathers, while formula H3 for hue was used for both head and mantle feathers (explained below; Montgomerie 2008).

$$B_2 = \sum_{\lambda\,\mathrm{min}}^{\lambda\,\mathrm{max}} R_i \Big/ n_w = B_1 / n_w \qquad H_1 = \lambda_{R\,\mathrm{max}}$$

$$S_8 = \left(R_{\mathrm{max}} - R_{\mathrm{min}}\right) / B_2 \qquad H_3 = \lambda_{Rmid} = \lambda_{R50}$$

R_i = Reflectance and wavelength i
n_w = number of wavelength intervals used to calculate B1
R_{max}, R_{min} = maximum and minimum reflectances, respectively, in range of observed wavelengths
λ_{Rmax} = wavelength of maximum reflectance
λ_{Rmid} = wavelength at reflectance midpoint between Rmax and Rmin

The B2 formula is the sum of all the percentage reflectance values for the reflectance spectra produced, divided by the total number of values that were summed. This brightness value, versus B1, is standardized for the number of values summed so that it can be compared to other species ((Montgomerie 2006). S8 is the peak reflectance range divided by brightness, a saturation calculation that can be used for any curve type. H1, the wavelength with the highest reflectance, was used for tail hue because there is a distinct peak in the tail reflectance curve. Because no distinct peak was present for the head and mantle feathers, H3 was calculated. H3 is the wavelength midway between the lowest reflectance value and highest reflectance value, ideal when the reflectance curve does not have a definite peak but instead increases for the entire spectrum, which is the case for the head and mantle curves.

Data analysis

Individual adults caught in multiple years were only represented once (randomly selected year) in each analysis to avoid pseudoreplication. The assumptions for each test were analyzed and $\log_{10}$ transformed when necessary (see results). If the assumptions were not met due to outliers, tests were re-run without outliers to learn of their effects on the data (see results).

All statistical tests were conducted using Statistica 6.1 (StatSoft Inc., Tulsa, OK), except a power analysis performed using JMP 5.0.1a (SAS Institute, Cary, NC). Data are presented as mean ± SD, and the alpha value for all tests was set to 0.05, unless Bonferroni corrected (see results).

Results

Smooth-billed anis were found to be sexually dimorphic in size. There was a significant difference between males and females for all morphometric measures, with males being larger than females (Table 1). There was no difference in means between years and no interaction between year and sex for $\log_{10}$ head-tip length and culmen length. Mean $\log_{10}$ mass and tail length were different between years, but there was no interaction between year and sex. There was, however, a significant difference between years and an interaction between year and sex for maximum bill-depth. Bonferroni posthoc tests revealed that males had a significantly larger bill-depth than females ($p<0.001$) for all years except 2001 ($p=0.091$). Males were larger than females (males ($n=6$) 23.6mm±1.1; females ($n=14$) 22.8mm±0.8), but not significantly, maybe due to the low male sample size (power only 0.4282). A discriminate function analysis including all of the variables also found a significant difference between the sexes (Wilks's lambda = 0.45, $F_{6,246} = 50.21$, $p <0.001$; discriminant score= -3.33*log10 mass-33.466*log10 head-tip-0.134*culmen-0.385*maximum bill-depth-0.070*wing chord-0.002*tail+88.28, where negative scores are classified as males). The analysis could correctly identify 84% of the males and 90% of the females (87% total).

Maximum bill-depth and tail length relative to body size were compared between males and females to determine if males have proportionately higher crests or longer tails. The relative lengths used were the residual values from a regression of each measure versus body size. Because one morphometric measure on its own may not be an accurate measure of overall body size for an individual (Fairbairn 2007), body size was determined from a principle component analysis that included wing chord and culmen length, both of which are used to represent body size in other birds (Bright et al. 2004; Gosler et al. 1998). The measures from all individuals caught were included in the analysis. PC1 explained 72.17% of the variance and was used as an overall measure of body size. A two-factor ANOVA was performed for each trait with year and sex as independent factors and relative maximum bill-depth was significantly different between males and females (N males=156, N females=165; sex $F=15.38$, $p<0.001$; year $F=0.21$, $p>0.9$; year*sex $F=1.38$, $p>0.2$), but tail length was not significantly different (N males=141, N females=158; sex F=0.02, p>0.8; year F=0.05, p>0.9; year*sex F=0.28, p>0.9).

Head and mantle feathers had similar reflectance curves, increasing in reflectance across the spectrum from short to long wavelengths (with a slight drop at the end of the mantle curve), but they were very different from that of tail feathers, which have a distinct peak in the UV/blue region of the spectrum (Figure 1). Only one plumage variable, tail saturation, was significantly different between males and females, with females having a higher saturation than males (Table 2). There was a difference between years for mantle brightness, tail brightness, head saturation, and head hue, but there was no interaction between year and sex for any variable. A discriminate function analysis resulted in a significant difference between males and females (Wilks's lambda = 0.85, $F_{9,128} = 2.42$, p <0.015); however, the only variable that was significant was tail hue (p<0.001, other variables p>0.2). Only 65% of the females and 72% of the males were sexed correctly.

Discussion

Smooth-billed anis are sexually dimorphic in size for mass and all morphometric measures (head-tip, culmen, wing chord, tail length, and maximum bill-depth), with males being significantly larger than females. Maximum bill-depth and tail length measures were significantly different between years, which may be due to measurement error. There were different field assistants each season and both measures are difficult to measure accurately. For maximun bill-depth, it is difficult to hold the calipers at an angle exactly perpendicular to the bill and the highest point of the crest is difficult to determine accurately. The start of the tail measurement (where the tail feathers immerge) can be difficult to determine and the method for holding the ruler at this start position may have changed across years. Mass and tail length may have varied significantly across years because of environmental differences. Mass and feather growth likely fluctuate more than other characterisitics because they are dependent on the amount of current resources available for the individual, which changes each year (Grubb 1991; Wendeln & Becker 1996).

We cannot determine the evolutionary causes of this sexual size dimorphism. Sexual selection, either through female mate choice or male-male competition, is one possible reason for the sexual size dimorphism seen in this species. Females may prefer males that are larger because a larger size may reveal that an individual is in good condition or has a greater ability to compete for food (Kodric-Brown & Brown 1984; Szekely et al. 2007). Size may also be an advantage for males if they must compete or fight to gain access to mates (Szekely et al. 2007). Smooth-billed anis have been observed in aggressive interactions, especially against those trying to join a breeding group (Quinn & Startek-Foote 2000; personal observation).

Natural selection may also be affecting the size of anis and it may be acting differently on each sex because of their different roles, resulting in the dimorphism observed. Females, for example, may benefit from being smaller because a larger body mass may make activities such as flying and foraging more energy consuming (energy which may be more beneficial in egg production; reviewed in Blanckenhorn 2000).

Males may benefit from being large in competitions for resources other than mates, such as territories or food. The selection for larger males may be the result of both natural and sexual selection if females prefer larger males and larger males have an advantage in gaining resources and/or mates.

Two conspicuous traits anis possess for which the purpose is unknown are their long tails and large bill crests. Hypothetically they are used as indicators of quality and condition for mate choice and competitive interactions. A long tail is costly to produce and therefore may reveal the quality of the individual (Zahavi 1975). The bill crest may reveal the dominance status of an individual, assuming it is not costly to produce (Maynard Smith & Harper 2003). To determine if these traits are under sexual selection, their measurements, relative to body size, were compared between sexes. In the case of relative bill-depth, males had higher bill crests, relative to body size, than females. Similarly to body size, this may be the result of both natural and sexual selection acting on the trait. Males with larger bill crests may have an advantage if it is used as a signal in dominance interactions for access to resources or mates (or perhaps bill crests are a tool used in foraging and larger crests are advantageous), and females may prefer these males. Relative tail length was not different between the sexes and therefore not exaggerated relative to body size in males, suggesting this trait is influenced by natural selection and/or mutual mate choice (see below).

The reflectance curves of all feather types resemble those of black birds (Icteridae) and satin bower birds (*Ptilonorhynchus violaceus*), who also have black iridescent feathers produced by feather microstructures (Doucet et al. 2006; Shawkey et al. 2006). Shawkey et al. (2006) and Doucet et al. (2006) found the iridescent colour reflected by these birds' feathers is produced by layers of concentrated melanin granules on the edges of feather barbules that create two or three different interfaces from which light is reflected. Ani feathers likely have similar structural characteristics.

The only plumage colour measure that was significantly different between males and females was saturation of the tail, in which females had greater saturation than males. Greater saturation indicates that the colour reflected is more pure or clean in females than males (Montgomerie 2006). The possible reasons or causes for the dichromatism are the same as those explored for size dimorphism. The tail may indicate the quality of females in female-female competitive interactions for mates and/or resources or even be used as signals by males for mate-choice decisions. Female northern cardinals (*Cardinalis cardinalis*) are aggressive to other female conspecifics and one study found that the darkness and size of their face masks correlate with aggressiveness (Jawor et al. 2004). Doucet et al. (unpublished data) found that females of colonial nesting species that need to compete for nest sites have more conspicuous plumage than non-colonial species. If there is a dominance hierarchy among females within an ani group, which may influence the competition for optimal egg positions in the nest, perhaps their tail is a good indicator of dominance. Amundsen et al. (1997) tested male preference for female throat colouration in bluethroats (*Luscinia s. svecica*) and found males associated longer with and performed more sexual behaviours to females with more colourful plumage. Chroma (or saturation) has been found to correlate with condition in several studies. The spectral curve of blue tit (*Parus caeruleus*) tail feathers peaks in the UV/blue range and the chroma of chick tails has been found to be positively correlated with body condition (Johnsen et al. 2003). The chroma of juvenile Florida scrub-jay (*Aphelocoma coerulescens*) tails is also correlated with body condition (Siefferman et al. 2008). Perhaps tail saturation in female anis reflects condition and therefore be used by males in mate choice decisions. It is possible that saturation is correlated with feather wear (age of feathers; Ornborg et al. 2002)). If this is the case, the observation that females have different tail saturation than males may be the result of differences between the sexes in molting date. Also, plumage colouration may be affected by age, but unfortunately anis cannot be confidently aged beyond hatch year (Keyser & Hill 1999). Potential age-related variation and variation due to age of feathers may also affect our ability to determine differences between males and females for the other colourmetric measures.

Monomorphism of showy traits (such as plumage coloration or conspicuous bill crests) could result because of mutual mate choice (both males and females are choosy) or because of natural selection (competition for resources other than mates; Amundsen 2000). Monomorphic conspicuous traits may have evolved to be used as signals or weapons in aggressive social displays over resources, such as territories or food (West-Eberhard 1983). If both males and females are aggressive when obtaining resources, both can acquire the trait. Whittingham et al. (1992) have suggested that Cuban red-winged black birds (*Agelaius phoeniceus*) have reduced sexual dimorphism because both males and females defend territories against individuals of both sexes (unlike North American populations that are more dimorphic). Anis are very aggressive to individuals trying to join their group and to neighbouring groups at territory boundaries (Quinn &

Startek-Foote 2000; personal observation). While it is unknown whether one sex partakes in these interactions more than another, both sexes are caught in our lure bird traps and therefore are aggressive towards intruders. There is also the possibility of dominance hierarchies within groups for both sexes, which may be important in the reproductive success or food acquisition ability of an individual. Social signals may help in maintaining social stability within the groups by reducing aggressive interactions (Amundsen & Parn 2006; West-Eberhard 1983). Perhaps the iridescent plumage colouration of anis or their long tails are signals of dominance or aggression in both sexes and used in antagonistic interactions to defend their territories or maintain dominance status.

When both sexes provide parental care (ie. the potential reproductive rates are similar), then both should be choosy when deciding on a mate (Amundsen 2000; Parker & Simmons 1996). If this is the case, then traits of both sexes will be under sexual selection. Doucet et al. (unpublished data) found that in species where males provided care in the form of nest building, incubation, and/or feeding, females are more conspicuous than in species where males provide less care. This may be due to the reduced pressures of natural selection to be cryptic and a greater involvement of sexual selection on shaping a female's traits. Both male and female anis provide parental care in all aspects of reproduction (nest building, incubation, and feeding). Perhaps plumage colour or tail length are used as signals of condition in mate choice for both sexes for smooth-billed anis.

The sexual dimorphism observed in smooth-billed anis may indicate that male size in general is under selection pressures either due to female mate choice or male-male competition. Males also have greater bill-depths, relative to body size, than females, a trait that may be a signal of dominance. However, in this species, males do not appear to have exaggerated tail lengths relative to body size and there was not significant difference between the sexes with respect to all plumage characteristics, except for tail saturation. The development of these traits may have been the result of natural selection, for example to be used in aggressive intraspecific interactions, or mutual mate choice. A greater understanding of the ani's mating system would facilitate our understanding of the causes for both their monomorphic and dimorphic traits.

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Table 1. Results of two-factor ANOVAs of smooth-billed ani (*Crotophaga ani*) morphometric measures to determine differences between males and females across eight years.

	Mass[†] (g)	**Head-tip[†] (mm)**	**Culmen (mm)**	**Max bill-depth (mm)**	**Wing chord (mm)**	**Tail (mm)**
	Mean±SD (N)	Mean±SD (N)	Mean±SD (N)	Mean±SD (N)	Mean±SD (N)	Mean±SD (N)
Male	113±11 (135)	54.7±1.6 (160)	33.0±1.3 (161)	23.9±1.1 (158)	151±5 (159)	186±10 (141)
Female	99±13 (140)	51.8±1.8 (167)	31.0±1.0 (167)	22.2±1.0 (167)	146±4 (165)	181±11 (160)
1998	117±14 (13)	54.6±2.8 (13)	32.9±1.8 (13)	23.6±1.9 (10)	151±4 (13)	*
1999	113±11 (23)	52.9±2.4 (27)	32.2±1.7 (27)	23.4±1.7 (27)	148±5 (27)	174±7 (25)
2000	111±12 (14)	52.7±1.8 (58)	32.0±1.5 (58)	23.6±1.3 (58)	148±5 (56)	178±6 (51)
2001	103±13 (20)	52.8±2.6 (20)	32.3±1.3 (20)	23.0±0.9 (20)	147±4 (20)	186±6 (20)
2002	102±13 (49)	53.5±2.1 (49)	32.1±1.5 (49)	23.0±1.5 (49)	149±5 (49)	194±10 (46)
2003	114±14 (23)	53.6±2.2 (25)	31.9±1.6 (25)	22.6±1.2 (25)	149±6 (24)	195±9 (24)
2004	104±12 (41)	53.5±2.0 (41)	31.6±1.4 (41)	22.8±1.2 (41)	149±6 (41)	187±10 (40)
2006	103±13 (42)	52.8±2.1 (45)	31.6±1.6 (45)	22.4±1.1 (45)	148±4 (44)	178±6 (45)
2007	102±14 (50)	53.7±2.5 (49)	31.8±1.4 (50)	23.1±1.1 (50)	150±5 (50)	176±7 (50)
	df	df	df	df	df	df
	10,274	10,328	10,327	10,324	10,323	9, 300
	F, p	F, p	F, p	F, p	F, p	F, p
Year	**5.273, <0.001**	2.264, 0.023[‡]	1.776, 0.081	**6.831, <0.001**	0.976, 0.454	**36.579, <0.001**
Sex	**72.360, <0.001**	**200.119,<0.001**	**181.162, <0.001**	**209.218, <0.001**	**89.442, <0.001**	**28.914, <0.001**
Year*sex	0.437, 0.898	1.020, 0.421	1.191, 0.304	**2.720, 0.007**	1.292, 0.247	0.255, 0.970

*tail length was not measured in 1998

[†] F and p values from log10 transformed data, Mean±SD non-transformed

[‡] Non-significant after sequential Bonferroni correction

Table 2. Results of two-factor ANOVAs of smooth-billed ani (*Crotophaga ani*) colourmetric plumage measures to determine differences between males and females across four years.

	Brightness			**Saturation**			**Hue**		
	Head	**Mantle**	**Tail**	**Head**	**Mantle[†]**	**Tail**	**Head**	**Mantle**	**Tail***
	Mean±SD (N)	Mean±SD (N)	Mean±SD (N)	Mean±SD (N)	Mean±SD (N)	Mean±SD (N)	Mean±SD (N)	Mean±SD (N)	Mean±SD (N)
Male	0.071±0.014 (72)	0.071±0.019 (74)	0.041±0.008 (72)	0.70±0.18 (72)	0.45±0.21 (74)	0.53±0.10 (72)	540.1±36.2 (72)	534.3±61.5 (74)	427.8±64.8 (72)
Female	0.071±0.013 (72)	0.078±0.020 (74)	0.040±0.007 (76)	0.70±0.16 (72)	0.40±0.13 (74)	0.60±0.13 (76)	541.0±30.0 (72)	518.0±65.1 (74)	423.2±54.0 (68)
2003	0.076±0.013 (27)	0.086±0.022 (30)	0.042±0.006 (30)	0.81±0.16 (27)	0.39±0.13 (30)	0.59±0.11 (30)	558.3±22.1 (27)	531.9±63.9 (30)	406.1±41.3 (30)
2004	0.071±0.014 (37)	0.081±0.019 (39)	0.044±0.007 (40)	0.78±0.17 (37)	0.47±0.22 (39)	0.59±0.15 (40)	543.1±29.8 (37)	500.4±63.0 (39)	420.0±62.1 (40)
2006	0.068±0.011 (31)	0.071±0.015 (30)	0.038±0.006 (31)	0.63±0.14 (31)	0.41±0.15 (30)	0.55±0.11 (31)	532.4±39.6 (31)	538.3±67.3 (31)	435.3±58.2 (31)
2007	0.071±0.013 (49)	0.065±0.018 (49)	0.037±0.006 (47)	0.62±0.13 (49)	0.44±0.18 (49)	0.53±0.09 (47)	534.0±33.0 (49)	535.7±57.6 (49)	436.0±65.1 (47)
	df	df	df	df	df	df	df	df	df
	5,143	5,147	5,147	5,143	5,147	5,147	5,143	5,149	5,147
	F, p	F, p	F, p	F, p	F, p	F, p	F, p	F, p	F, p
Year	1.715, 0.167	**10.264, <0.001**	**9.407, <0.001**	**14.499, <0.001**	0.994, 0.398	2.637, 0.052	**4.739, 0.004**	3.463, 0.018[‡]	1.590, 0.195
Sex	0.048, 0.827	6.035, 0.015[‡]	0.343, 0.559	0.164, 0.686	0.753, 0.387	**16.913, <0.001**	0.004, 0.950	4.218, 0.042[‡]	0.481, 0.489
Year*sex	0.324, 0.808	1.534, 0.208	0.619, 0.604	0.182, 0.908	0.480, 0.697	0.984, 0.402	3.169, 0.026[‡]	1.052, 0.317	1.053, 0.371

*outliers present; when outliers removed differences between years become significant, but sex differences and year*sex interaction still n.s.

[†] F and p values from log10 transformed data, Mean±SD non-transformed

[‡] Non-significant after sequential Bonferroni correction

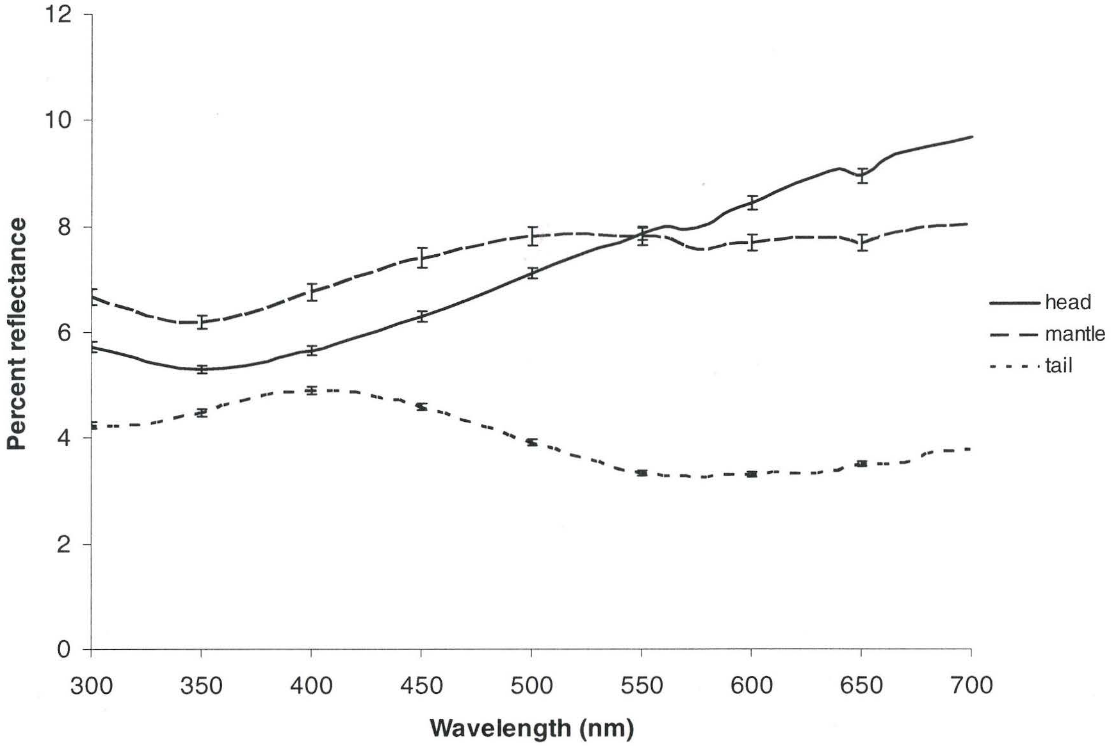

Figure 1. Mean percent reflectance (± SE) of head (N=157), mantle (N=164) and tail (N=162) feathers from smooth-billed anis (*Crotophaga ani*) caught in 2003, 2004, 2006, and 2007.

Chapter 3

Parasites, plumage and condition in a joint-nesting plural breeder, the smooth-billed ani (*Crotophaga ani*).

Abstract:

Sexually selected traits need to accurately reflect the condition and quality of an individual if they are to be used as signals in mate choice interactions. Plumage colouration may reveal an individual's immunocompetence or parasite resistance in some birds, characteristics that can be important in a mate. The ability of male smooth-billed ani (*Crotophaga ani*) plumage to indicate both blood parasite and ectoparasite numbers was explored in this study. Due to conflicts in the literature on whether ectoparasites affect their host negatively, the impact of ectoparasites on the body condition of male anis was also analyzed. We also looked at whether plumage colour was related to body size and condition in both male and female anis to determine if plumage colour is a possible signal of quality. Finally, because anis are cooperative breeders that live in groups, the relationship between group size and ectoparasite numbers was tested. Adult smooth-billed anis from a population in Puerto Rico were caught and dust-ruffled to obtain ectoparasite samples and blood smears were analyzed for the presence of blood parasites. The percent reflectance of feathers from three body regions (head, mantle, and tail) were determined using a spectrometer and brightness, hue, and saturation were calculated for each body region for each individual. Body condition was measured as residuals from a regression of mass versus body size. No blood parasites were found in the blood of the anis observed. There was a positive relationship between $\log_{10}$ average lice numbers per group and group size, though not significant. Feather colour was not a good predictor of ectoparasite numbers in males or body size and body condition in both males and females. Ectoparasites did not have an effect on males for the body condition

measure we analyzed. Our findings suggest that ani plumage colour may not be a good indicator of ectoparasite resistance or condition; however, ectoparasite number may not be an important characterisitic in mate choice decisions because it did not appear to affect male body condition. Other factors may have confounded our results, however, such as age and feather wear.

Introduction

When reproduction is costly, cues such as secondary sexual characteristics allow individuals, normally females, to evaluate potential mates (Clayton 1991; Kempenaers et al. 1997). The trait used to assess these individuals should be costly to honestly reveal the quality and condition of that individual so that the chooser gains benefits, either indirect (good genes) or direct (such as quality resources and parental care; Kodric-Brown & Brown 1984; Neff & Pitcher 2005; Zahavi 1975).

Plumage colouration has been studied extensively in the context of mate choice and sexual selection (reviewed by Hill 2006). Plumage pigmentation has traditionally been the focal point of such analyses, but most recently, with the use of spectrometers, structural plumage colouration has been explored. Structural plumage colouration is different from pigment-based plumage not only in how it is produced (pigment deposition versus feather microstructure) but in what colours are reflected (Doucet & Montgomerie 2003). In addition to blues and greens, feather microstructures can produce colours in the UV range, as well as the phenomenon of iridescence (Doucet 2002; Prum 2006).

Structural plumage colouration has been found to affect female mate choice. For example, Siitari et al. (2002) manipulated the UV reflectance of pied flycatcher (*Ficedula hypoleuca*) plumage and found that females preferred males with increased reflection. Little is known about whether structural plumage colouration is costly to produce (Osorio & Ham 2002), but there is some support for the contention that it relates to body condition. Male structural plumage has been found to correlate with feather growth rates (a measure of condition) in both blue grosbeaks (*Guiraca caerulea*; Keyser & Hill 1999) and blue-black grassquits (*Volatinia jacarina*; Doucet 2002). Females may be able to use feather microstructure to assess the quality and condition of males when making mate choice decisions.

Hamilton and Zuk (1982) proposed that the feathers of males may specifically reveal their exposure level to parasites or parasite resistance to choosy females. Individuals with fewer parasites may be able to produce sexier plumage, and if chosen, may pass on their good parasite resistance genes to their offspring.

The Hamilton and Zuk hypothesis has been studied for both blood parasites and ectoparasites. Doucet and Montgomerie (2003) found a negative relationship between *Haemoproteus* loads and structural plumage brightness in iridescent male satin bowerbirds (*Ptilonorhynchus violaceus*). Figuerola et al. (2003) studied the effects of feather mites on plumage characterisitics in serin (*Serinus serinus*) by manipulating the parasite load of males by removing mites and found those with the removed parasites had brighter plumage. Thompson et al. (1997) conducted a study on house finches (*Carpodacus mexicanus*) in which he tested the effects of feather mites on both plumage colour and body condition. They found a negative relationship between mite load and both plumage color score (a low score means dull plumage) and flight feather growth.

We studied parasite abundance and the signal function of plumage in smooth-billed anis (*Crotophaga ani*). Smooth-billed anis are cooperative, joint-nesting breeders that form groups consisting of one or more pairs that are mostly unrelated (Blanchard 2000; Vehrencamp & Quinn 2004). Anis have black plumage with green and purple iridescence. We conducted this study to learn about the presence and abundance of both blood parasites and ectoparasites that infect smooth-billed anis, and to learn whether male plumage characteristics could signal parasite abundance. To determine whether parasites have a negative affect on male anis, we looked at the relationship between relative parasite numbers and body condition. Finally, we also investigated whether the plumage of male smooth-billed anis could predict body size or condition and therefore be used as a signal of quality in female mate choice. Because it is unknown whether male anis are choosy in mate choice and because female anis have more saturated tails than males (chapter 2), there is the possibility that tail saturation is a sexually selected trait in females; we therefore looked at the relationships between female plumage and body condition and size as well.

Unlike most other studies looking at the relationship between parasites and plumage, ours is unique in that we are studying a species that is group living. The main transmission mode of most mite species is through direct contact with infected individuals (Proctor & Owens 2000) and nightly roosting provides this contact. Lice can also transfer from one individual to another through contact, as well as through the sharing of nests (Marshall 1981). Group living may increase the chances of individuals acquiring and accumulating ectoparasites because of the increase in transmission rates between individuals (Poulin 1991). Poulin (1991) found that group living birds have greater mite prevalence than solitary birds, demonstrating that group living does have an effect on parasite loads. Anis within a group share a communal roost and perch close together each night which may facilitate the transfer of parasites between individuals (personal observation). We therefore also needed to look at the effects of group size on parasite numbers in this species.

Methods

Field site

Smooth-billed anis were studied in southwest Puerto Rico, within and around the Cabo Rojo National Wildlife Refuge (17°59'N. 67°10'W). Field work was conducted during the peak ani breeding period during the wet season (September to January). Each season will be referred to by the year including most of the field season (September to December). Morphological data for this study were collected between 1998-2004 and 2006-2007. Plumage data were collected for the years 2003, 2004, 2006, and 2007.

Adult capturing and processing

Adult anis were captured using three different methods: mist netting, funnel trapping, and nest trapping. Mist-netting involves setting up nets early in the morning or in the evening to catch the birds coming in or out of roost. Occasionally playback and a stuffed decoy ani were used with a mist net during the day. For funnel trapping, anis were hand-raised and used as lure birds to attract territorial adult anis. One lure bird was placed in a small cage within a larger funnel trap that was designed to allow birds to fly or walk in, but not out (McClure 1984). The trap was either near a typical foraging site or near an active nest. If the birds were not in sight, playback was sometimes used for about one minute to attract their attention. Nest trapping was conducted at some nests during late incubation of eggs or young chicks (Mock et al. 1999). A small trap with a draw down door attached to a string was placed on the nest and observed from a distance using a scope. Once a bird sat on the nest to incubate, the door of the trap was pulled down and the bird was removed from the trap for processing.

Each adult caught received a unique set of three colour bands and one U.S. Fish and Wildlife Service aluminum band, and in the most recent years also received a mark on the bill with non-toxic white nail polish (a unique mark for each member of a group) so that they could be identified easily during observations in the field.

The morphometric measures taken for each adult included (1) mass, measured to the nearest gram using a spring scale, (2) wing chord, measured to the nearest 1mm with a wing ruler, (3) tail length, measured to the nearest 1mm with a ruler, (4) exposed culmen length, measured to the nearest 0.1mm with calipers, (5) head-tip (a measure from the back of the skull to the front tip of the bill), measured to the nearest 0.1mm with calipers, and (6) maximum bill-depth, also measured to the nearest 0.1mm with calipers.

A small blood sample (50-300ul) was obtained from each adult from either the jugular or brachial vein. The blood was stored at room temperature in approximately 1.5

mls of Queen's lysis buffer consisting of 0.01M Tris, 0.01M EDTA, 0.01M NaCl and 1% n-laurylsarcosine (Seutin et al. 1991). During the 2003, 2004, 2006 and 2007 breeding seasons a small drop of blood was also placed on a microscope slide and a thin smear was made (~1 cell thick).

In addition, feathers from three different sections of each bird were collected: three from the head, three from the mantle (upper back), and one retrix (tail) feather (far right unless missing). These were stored in labeled small zip-lock bags until analyzed.

During the 2007 breeding season, birds captured with sufficient time to sample before dark were dust-ruffled using a method similar to Walther and Clayton (1997). Dust-ruffling has been found to be an accurate method for determining ectoparasite abundance on live birds (Clayton & Drown 2001). Each bird was dust-ruffled by the same individual (MB), using the same technique. The ruffling occurred in a deep container (to minimize wind effects) lined with a fresh sheet of paper. Each bird was dusted with 2.5ml of Zema Z3 Flea & Tick Powder for Dogs (Zema®, Research Triangle Park, North Carolina), containing 0.1% pyrethins and 1.0% piperonyl butoxide, a formula that is non-toxic to birds and quickly kills ectoparasites (Jackson 1985). After dusting the powder over the entire bird, its feathers were ruffled for five minutes. Ruffling for an arbitrary amount of time versus ruffling until all parasites have been removed provides a relative measure of parasites abundance and is less time consuming (Clayton & Drown 2001). The amount of time each section of the bird was ruffled for was consistent for each bird: 30s for the head, 30s for the tail, 1min for the back, 1min for the dorsal side of left wing, 30s for the ventral side of left wing, 30s for the ventral side of right wing, and

1min for the dorsal side of the right wing. The dust and parasites collected on the paper were then transferred into a tube using a funnel. Any residue left on the funnel was rinsed into the tube using 70% ethanol, in which the parasites were stored. Any loose feathers were ruffled and discarded before transfer of the sample into the tube.

Group sizes were determined by monitoring each group throughout the breeding season. We re-sighted colour bands and bill marks to determine group members as well as counted the number of individuals flying in and out of each groups' roost.

Blood-borne parasite loads

The blood smears produced in the field were fixed and stained using a Hemacolor® stain kit (EMD Chemicals, Gibbstown NJ), and then viewed under a 63X oil immersion objective lens for presence of parasites. An initial scan of 20 individuals from different groups and from both the 2006 and 2007 seasons found no blood parasites so no further observations or analyses were conducted.

Ectoparasite Indices

Due to the time consuming nature of ectoparasite counting, we targeted only samples from males that had feather reflectance analyses performed (see chapter 2 for detailed sexing methods). All samples were counted by the same individual (MB). Nine samples were not done blind to the ID of the individual, but the plumage reflectance and morphometric measurements were unknown. All subsequent samples were analyzed blind by having an additional individual cover the labels on the tubes and assign arbitrary numbers to each.

Each sample of ectoparasites (which included the parasites, dusting powder, and 70% ethanol) was divided up into 8-10 different Petri dishes to thin out the dust for easier observation of the parasites. Each dish had 1-4mm of ethanol. If additional ethanol was required over what was already in the sample, 40% ethanol was added. Each sample was observed under 25X magnification with a dissecting scope. Each dish had a grid on it to facilitate counting lice and feather mites. A subsample of lice and mites was collected for identification.

Plumage Reflectance

Collected feathers were taped against matt black cardboard and percent reflectance was measured for each body region (head, mantle, and tail; see chapter 2 for full details of methods). Three variables, brightness, saturation, and hue, were calculated from the reflectance data using the program CLR, version 1.03 (Montgomerie 2008; see chapter 2 for formulas and reasoning behind their selection). Brightness, also known as spectral intensity, is the total amount of light being reflected from the surface of the feathers. Saturation, or chroma, is the purity of the light being reflected; the more the colour is made up of only one wavelength, the more pure it is. Hue is the actual colour or the wavelengths that are reflected the most (Figuerola et al. 2003; Montgomerie 2006).

Data analysis

For the analyses involving plumage colourmetrics, if an individual bird was caught more than one year a randomly selected year was used to represent that individual to avoid pseudoreplication. The assumptions for each test were analyzed and $\log_{10}$ transformations performed when necessary (see results). If the assumptions were not met due to outliers, tests were re-run without outliers to learn of their effects on the data (see results). All statistical tests were conducted using Statistica 6.1 (StatSoft Inc., Tulsa, OK). Data are presented as mean ± SD, and the alpha value for all tests was set to 0.05.

Results

Five species of ectoparasites were found in the population of smooth-billed anis that we studied. Three species of mites were identified: *Piciformobia ani* (Cerny 1975), *Aniibius drepanophorus* (Gaud & Atyeo 1990), and *Aniacarus* sp. (identified by Proctor, H.). The *Aniacarus* species is possibly *Aniacarus mexicanus* (Gaud & Atyeo 1990), but only one female was examined. Two lice species were also identified: *Osborniella crotophagae* (Stafford 1943) and *Vernoniella guimaraesi* (Thompson 1948; identified by Galloway, T.). Specimens of lice collected have been deposited at the J.B. Wallis Museum of Entomology, Department of Entomology, University of Manitoba (Winnipeg, Manitoba, Canada). There was substantial variation in parasite numbers among the males analyzed, ranging from 31 to 535 mites (mean: 178±128, N=22), and 2 to 28 lice (mean = 8±6, N=22) on a single individual. For details on the plumage measures, including descriptive statistics and percent reflectance curves, refer to chapter 2.

To determine whether lice or mites were more abundant in larger groups than smaller groups, parasite numbers were averaged for each group (if there was more than one sample analysed within a group) and two regressions were performed, one for each ectoparasite type. The dependent variable was either the average mite or lice numbers ($\log_{10}$ transformed) for each group and group size was the independent variable. For lice, the relationship was almost significant (N=10, R=0.62, F=5.02, p=0.055; Figure 1) and for mites it was not significant (N=10, R=0.26, F=0.56, p>0.4).

To determine the effects of ectoparasites on male plumage characteristics, a multiple regression was performed for each colourmetric measure calculated. The dependent variable in each analysis was one of the plumage characteristics (hue, brightness, or saturation) for each feather type while the predictor variables were mite numbers and lice numbers. To control for the date the parasite sample was collected and body size, these variables were also included in the model. Because one morphometric measure on its own may not be an accurate measure of overall body size for an individual (Fairbairn 2007), body size was determined from a principle component analysis that included wing chord and culmen length, both of which are used to represent body size in other birds (Bright et al. 2004; Gosler et al. 1998). The measures from all individuals caught were included in the analysis. PC1 explained 72.17% of the variance and was used as an overall measure of body size. No significant relationships were found between parasite numbers (both lice and mites) and all plumage measures (brightness, hue and saturation) for each body region (Table 1).

An additional multiple regression was used to look at the effects of parasites on body condition. Residuals from a regression of mass versus PC1 scores for body size were used as a measure of body condition. These residuals correspond to the amount of energy and resources an individual can utilize (ie. have not been incorporated into their structural components; Brown 1996). In this case the dependent variable was body condition and the predictor variables were mite and lice numbers. Again, body size and date the individuals were caught were included in the model as controls. Both the number of mites and number of lice could not predict body condition (N=22, mites: $\beta = 0.13$, $p>0.57$, lice: $\beta = 0.08$, $p>0.70$).

A general linear model was performed for each plumage measure to determine if feather reflectance is related to body condition and body size in both males and females. The dependent variable in each model was one of the plumage measures for a particular body region and the predictor variables were body condition (measured as residuals from a regression of mass versus body size) and body size. Because feathers were collected across four years, year was included in the model. There were no significant relationships between plumage measures and body condition or body size for either sex (Tables 2 and 3).

Discussion

The presence of *Haemoproteus* parasites in smooth-billed anis has been reported for one individual in Colombia (Bennett & Borrero 1976), but in three additional studies, nine smooth-billed anis from Colombia were negative for blood pathogens (Basto et al.

2006; Londono et al. 2007; Rodríguez & Matta 2001). We report in this study that no blood parasites were detected for the twenty individuals analyzed from our population in Puerto Rico. The main hypotheses as to why a species is not infected with blood parasites are that the vector is rare, that the host has exceptional parasite resistance, and that the parasite's host-specificity limits the chances of a host encountering a parasite that is capable of inhabiting it (reviewed in Martinez-Abrain et al. 2004). This last hypothesis is especially likely for island-dwelling hosts for which the chances of being exposed to a related species with a parasite that can infect it are rare (Martinez-Abrain et al. 2004). This may be the case for smooth-billed anis in Puerto Rico. Another reason why we may not have encountered any parasites is simply the numbers are too low to detect any (Martinez-Abrain et al. 2004).

The two louse species identified have been found to be parasites of smooth-billed anis in the past (Clayton et al. 1992) and to our knowledge are the only lice found on smooth-billed anis. Two of the mite species have been previously reported to parasitize smooth-billed anis (*Piciformobia ani* and *Aniibius drepanophorus*), but *Aniacarus* sp. has not been (Proctor, personal communication). Both types of parasites, especially mites, were very prevalent and varied considerably in numbers on the male anis in our study. The observed variation may be due to differences in their parasite resistance or ability to avoid or remove parasites, but the fact that anis live in groups adds another factor. Anis are allo-preeners and will preen not just their mates but other members of their group (personal observation). If anis are able to remove mites and lice through preening (which is not known), then other group members may play a role in determining parasite loads for an individual.

There does not appear to be a relationship between mite numbers and group size; however, there was a positive trend observed between lice numbers and group size. Our sample only included 10 different groups and for several of them only one individual represented the groups' parasite number, therefore, perhaps a larger sample size may reveal a significant relationship. As far as I know, there has not been a comparison between mite and lice transmittance, but perhaps lice transfer between hosts more than mites, resulting in the difference observed between the two parasite types.

This study showed that parasites do not appear to affect plumage characteristics of male anis. There were no significant relationships between parasite numbers and any of the measured plumage metrics. Feathers may not be used as indicators of male parasite resistance by female anis. Other studies have found similar results. Blanco et al. (1999) found no relationship between feather redness and feather mite loads in Linnets (*Carduelis cannabina*). Plumage characteristics were not good predictors of ectoparasite numbers in satin bower birds either (Doucet & Montgomerie 2003). Clayton (1990) examined the damage to rock dove (*Columba livia*) feathers caused by lice and found they did not damage the iridescent tips of their feathers, only the distal portions (though he did not test the effects using a spectrometer).

Other factors may have prevented us from seeing a relationship between feathers and parasites, however. To measure accurately the effects of parasites on a bird's plumage the parasite load should be determined *before* the plumage analyzed has fully grown (Figuerola et al. 2003). Figuerola et al. (2003) found a significant change in feather mite abundance on birds during the year he was studying the effects of mites on feather colour in serin (*Serinus serinus*). The parasite numbers analyzed after feather growth do not represent those on the bird when the feathers were developing. Anis are difficult to recapture and their molting schedule is unknown, therefore it would be difficult to apply this method here, but it may affect our results involving parasites. Also, there may be too many additional factors that mask the effects of parasites on plumage coloration (Harper 1999). Effects of age (anis cannot be aged beyond hatch year; Keyser & Hill 1999), and feather wear (age of feathers; Ornborg et al. 2002) may swamp the relationship in smooth-billed anis.

Parasites did not seem to have an impact on the body condition of male smooth-billed anis. Females may not be interested in the ectoparasite loads of potential mates because they may not affect his quality. There have been conflicting results on whether feather mites are parasites. Some evidence, like the results found in this study, suggests a commensal relationship. Pap et al. (2005) found barn swallows that had been fumigated to remove ectoparasites had no difference in breeding performance or tail and wing feather growth from the control (unfumigated) group. Linnet (*Carduelis cannabina*) body condition was also not related to feather mite loads in a study by Blanco et al. (1999). Feather mites may have a beneficial, rather than detrimental, affect on their host by cleaning the feathers of pathogenic fungi, bacteria, detritius and old oil, reducing the need to spend time preening (Blanco et al. 1997). Louse numbers do not appear to have an effect on the condition, survival or fitness of male rock doves either (Clayton & Tompkins 1995).

Parasites do not seem to affect the plumage of anis, but we also found that their plumage may not be good predictors of condition and quality. We found no relationships between plumage characteristics and size or condition in anis. Similar results were found in a study on bluethroats (*Luscinia s. svecica*; Smiseth et al. 2001). Smiseth et al. (2001) looked at the relationship between structural plumage reflectance of the blue patches on bluethroat males and body size (measured as tarsus length) and body condition (measured as residuals from a regression of body mass versus tarsus length) and found no significant results. Structural plumage colour needs to be costly to produce to honestly represent the quality of an individual (Zahavi 1975). The costs of developing feather microstructures that produce the sexiest plumage are not well studied. Unlike pigment produced colour, colours produced by feather structures are not dependent on limited nutrients (Prum 2006). Changes in the shape of feather barbs, the number and thickness of fine structures and how regular and precise they are all affect the light that is reflected off a feather (Andersson & Prager 2006; Prum 2006). Cortex thickness and the concentration of melanin in feathers caused the variation in iridescent plumage reflectance in satin bowerbirds (*Ptilonorhynchus violaceus minor*; Doucet et al. 2006). So, there is variation in structure and particular causes for this variation, but it is still not known whether it is costly to produce the structure with the best reflectance and signal an individual's quality. When we measured the mass of an individual may have affected our ability to see a relationship between plumage colouration and condition; we did not know the condition (mass) of the bird while it was developing its feathers. Finally, factors such as age and wear on the feathers may have masked any relationship.

Knowing that the plumage of anis does not appear to signal condition or body size in both males and females, and knowing that anis are mostly sexually monochromatic (chapter 2), leads us to believe that smooth-billed ani plumage colouration is not sexually selected. Tail saturation is sexually dimorphic in anis (chapter 2), but tail saturation did not correlate with condition or size in either sex. Male ani plumage colour also does not appear to be a good signal of parasite resistance for females and parasites do not affect the amount of useable nutrient and energy resources a male has stored (the measure of body condition we looked at). For future studies exploring parasites and feathers in anis, their molting schedule needs to be explored to determine when parasites should be collected, if their schedule is predictable. The effects of parasites on female quality would be worth while to explore because parasites may have female-specific effects. If this is the case, then a female should prefer males with fewer parasites so that her daughters benefit from his good parasite resistance genes (if parasite resistance is heritable in anis). While the residuals from a regression of body mass and body size did not show a negative correlation with lice, perhaps a different measure of condition or fitness, such as reproductive success, would reveal an impact. If this is the case, because we found a positive trend between lice numbers and group size, perhaps there is a disadvantage to a large group if they attract more lice and increase the risk of transmission between individuals.

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Table 1. Multiple regressions of plumage colour measures of three body regions versus number of mites and lice of male smooth-billed anis (*Crotophaga ani*).

		Number of Mites		Number of Lice	
	N	**β**	***p***	**β**	***p***
Head brightness	20	0.43	0.07	0.1	0.64
Mantle brightness	21	-0.31	0.15	-0.12	0.52
Tail brightness	19	-0.42	0.13	-0.31	0.19
Head saturation	20	0.27	0.32	0.05	0.85
Log_{10} Mantle saturation	21	-0.06	0.80	0.09	0.69
Tail saturation	19	-0.26	0.39	0.31	0.23
Tail hue	19	0.32	0.26	-0.26	0.29
Head hue	20	-0.40	0.17	-0.25	0.35
Mantle hue	21	-0.18	0.46	-0.13	0.59

Date caught and body size controlled for by including as predictors

Table 2. General linear models of plumage colour measures of three body regions versus body condition and body size across four years (2003, 2004, 2006, 2007) of male smooth-billed anis (*Crotophaga ani*).

		Body condition		**Body size**	
	N	***F***	***p***	***F***	***P***
Head brightness	70	0.13	0.72	0.09	0.76
Mantle brightness	72	0.14	0.71	1.17	0.28
Tail brightness	71	0.04	0.85	0.13	0.71
Head saturation	70	1.22	0.27	0.76	0.39
Log_{10} Mantle saturation	72	1.91	0.17	0.05	0.83
Tail saturation	71	0.29	0.59	1.98	0.17
Head hue	70	0.01	0.92	1.51	0.22
Mantle hue	72	0.01	0.92	0.98	0.33
Tail hue	71	0.20	0.66	0.18	0.68

There were no significant interactions with year

Table 3. General linear models of plumage colour measures of three body regions versus body condition and body size across four years (2003, 2004, 2006, 2007) of female smooth-billed anis (*Crotophaga ani*).

		Body condition		Body size	
	N	*F*	*p*	*F*	*P*
Head brightness	68	0.87	0.35	1.21	0.28
Mantle brightness	70	0.43	0.52	2.78	0.10
Tail brightness	72	3.30	0.07	1.48	0.23
Head saturation	68	2.12	0.15	0.18	0.67
Log_{10} Mantle saturation	70	6.30	0.02*	0.12	0.73
Tail saturation	72	1.10	0.30	1.51	0.22
Head hue	68	2.05	0.16	3.08	0.85
Mantle hue	70	0.09	0.77	2.41	0.13
Tail hue	72	0.52	0.48	0.74	0.39

There were no significant interactions with year except with body size for tail brightness (p=0.01) but non-significant after sequential Bonferroni correction

*Non-significant after sequential Bonferroni correction

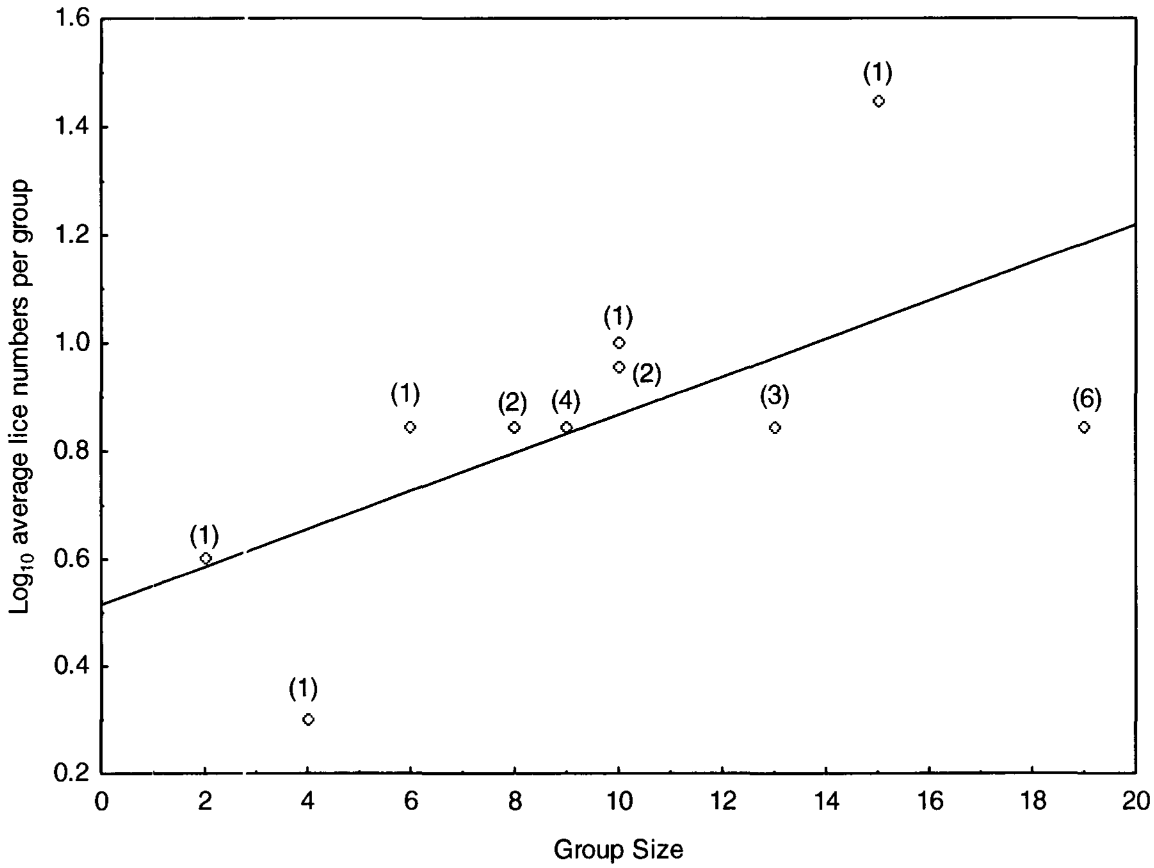

Figure 1. Log_{10} average lice numbers per group (sample size of group in brackets) of male smooth-billed anis versus group size ((N=10, R=0.62, F=5.02, p=0.055).

Chapter 4

Conclusion

This study explored some of the traits that may be involved in the mating behaviour of a cooperative joint-nesting plural breeder, the smooth-billed ani (*Crotophaga ani*). First, sexual dimorphism in size and plumage characteristics was examined because dimorphism may indicate the strength of sexual selection (chapter 2). The population of anis we studied are sexually size dimorphic (males are larger than females), but only sexually dichromatic in tail saturation (females have greater saturation than males). Male anis do not have exaggerated tail lengths, relative to body size, than females, however, they do have more exaggerated bill crests than females. Second, to further explore plumage traits, we looked at the ability of ani plumage to signal parasite resistance, body condition, and body size to conspecifics in smooth-billed anis (chapter 3). Also, due to conflicting results in the literature on the effects of ectoparasites on their hosts, we looked at whether feather mites and lice had a negative effect on male ani condition (chapter 3). Because anis live in groups, we also looked at the effect of group size on ectoparasite numbers (chapter 3). None of the male ani plumage characterisistics measured could predict ectoparasite numbers and ectoparasites did not appear to affect the condition of male anis. For both males and females, plumage colour did not correlate with body size or condition. Lice numbers were positively correlated to group size, but not significantly. There was no relationship between mites and group size.

Body size

The size dimorphism observed in anis may be attributed to sexual selection, but may also be the result of natural selection. It is possible that female preference for larger males resulted in males being larger than females (Kodric-Brown & Brown 1984;

Szekely et al. 2007), or that larger males are better able to fight to gain access to females (Szekely et al. 2007). Natural selection may have played a role in shaping the body size of anis and may have acted differently on each sex due to different roles. Larger males may have evolved by natural selection because being larger is advantages in situations other than mating, such as in competitions for food or in times of food shortages (Szekely et al. 2007). It may be advantageous for females to be smaller; for example, a larger body mass may make activities such as flying and foraging more energy consuming (reviewed in Blanckenhorn 2000). Most likely, both sexual and natural selection play a part in creating the size dimorphism observed. Males that are larger have a competitive advantage for resource acquisition and females choose these males for that reason.

Relative tail length and bill-depth

Males had greater maximum bill-depths, relative to body size, than females. Like body size, the exaggerated bill crest of males may have evolved by natural selection, sexual selection, or both. The bill crest may be used as a badge of dominance in this social species (Maynard Smith & Harper 2003). Those that have higher bill crests and are dominant may be more successful in competitions for resources and mates as well as in attracting more mates. Compared to females, males did not have longer tails, relative to body size, therefore they are not exaggerated in males and likely not a sexually selected trait, unless there is mutual mate choice occurring (Amundsen 2000). It is unknown whether male anis have mate preferences. Males may be choosy in smooth-billed anis because they provide parental care, but females may be choosier. With the opportunity for EPC's by males and the assumed limiting number of eggs females can produce, females should be choosier than males because they have a lower potential reproductive rate (Clutton-Brock & Vincent 1991; Dunn et al. 2001; Webster et al. 1995). However, it is unknown whether there is enough extra-pair fertilizations occurring to cause such an increase in potential reproductive rate in males relative to females. Alternatively, the long tail in anis may have evolved by natural selection for the same purpose in each sex (Amundsen 2000). Perhaps they are used in predatory defense when incubating. Anis wave their tails while incubating at the nest and it is unknown what purpose this has.

Plumage colour

Tail saturation

The dimorphism in tail saturation observed in the anis is more difficult to interpret than size. Like size dimorphism, it may have come about through mate choice, but in this case, male mate choice. Perhaps males prefer females with more saturated tails because it signals higher quality. We determined, however, that tail saturation is not correlated with body condition or size in female anis. Alternatively, low tail saturation may actually indicate high quality and male tails signal quality to choosy females, but, as with females, tail saturation does not signal body size or condition in males and other studies have found that chroma (saturation) is positively correlated with body condition (Johnsen et al. 2003; Siefferman et al. 2008) and that saturation of iridescent feathers decreases for birds under nutritional stress at molt (McGraw et al. 2002). There are several alternative reasons, however, for why the relationships between feather colour and condition and size were not seen. Perhaps tail saturation indicates something other than the amount of useable energy stored by an individual (the measure of condition we used), such as reproductive success (Keyser & Hill 2000). Because mass fluctuates, it is possible that our condition measure was not a good representation of an individual's condition when they were growing the feathers we analyzed, which may have prevented us from seeing a relationship. Or, feather microstructures that produce colour may not be costly to develop, thus no relationship would be seen between feather colour and any measure of condition (Prum 2006).

Another explanation for the sexual dimorphism observed in tail saturation is that natural selection is acting differentially on the sexes and this difference causes their tails to differ in tail saturation. Maybe tail saturation is used as a signal in competitive intersexual interactions in females only and is correlated with dominance and aggressiveness (Jawor et al. 2004). Or, perhaps males and females have slightly different diets and their diets contribute to the saturation of their tails. Finally, plumage colouration may be affected by the age of the individual (which cannot be easily determined in anis; Keyser & Hill 1999) or the wear on the feathers we measured (ie. the age of the feather; Ornborg et al. 2002). If saturation is affected feather age, then differences in molting dates between males and females may also result in the dimorphism observed.

Other colour measures

There was no difference detected between the sexes for all the additional colourmetrics we analyzed, and therefore likely not sexually selected, unless mutual mate choice is occurring; however, the finding that these measures did not correlate with body size or body condition reinforces the idea that these traits are not used as signals of quality used in mate choice (but see tail saturation for possible explanations for not seeing a relationship between colour and condition and size). Additionally, they did not predict ectoparasite numbers in males (see Hamilton and Zuk hypothesis below). Perhaps plumage colouration has evolved by natural selection in both sexes (Amundsen 2000), such as for use as signals of aggression in competitive interactions (West-Eberhard 1983). However, there may have been other factors masking differences between males and females, such as the age of the individuals and the age of the feathers we measured.

Hamilton and Zuk hypothesis

Feathers did not signal ectoparasite numbers in male anis, but this may be because parasites do not affect the body condition of their host and/or feathers do not signal a host's condition (see above). However, perhaps ectoparasites affect another aspect of the host's fitness, such as reproductive success. There may also have been too many other factors affecting our ability to see any relationships. We did not collect parasites numbers while the feathers we measured were growing (Figuerola et al. 2003) and we cannot determine the age of the hosts or their feathers. The size of the individual's group may also affect ectoparasite numbers due to larger groups attracting more parasites and increasing opportunities for parasite transmission (Poulin 1991); however, we did not find significant relationships between ectoparasite numbers and group size and including group size into the models did not change the results (results not presented). There was a positive trend in the relationship between lice and group size, which may reveal a cost to group living in this species if lice have a negative effect on their host (other than our measure of body condition).

Future

The next step would be to determine if certain traits are associated with mating and reproductive success. Perhaps body size, plumage colouration and/or ectoparasite numbers are correlated to the number of mates a male can obtain or the number of offspring he produces. Those traits that are shown to be associated with an increase in mating success or mate acquisition may be under sexual selection. Another possibility is to do an experimental mate choice study to determine if females (or males) are choosing particular traits in their mates. A manipulative experiment that involves enlarging the bill crest of some individuals would also be informative to learn whether individuals with larger crests are more successful at obtaining mates or encounter more challenges from other members of the group. A more in-depth look at the variation in mating success between males and females is also needed to get a better idea of the opportunity for and strength of sexual selection in the species.

Another area of research that needs to be addressed in this species is dominance. The traits analyzed in this study may be related to dominance status and aggressiveness. A method of determining dominance status within groups would be extremely informative. Aggressiveness of individuals to conspecifics would also be revealing. If certain traits are positively correlated to dominance status or aggressiveness, such as male body size or bill-depth and female tail saturation, perhaps they are used as signals in antagonistic interactions.

Finally, the parasite numbers of more individuals from a variety of group sizes need to be collected to get a better picture of whether lice numbers do increase with an increase in group size. It would be interesting to determine whether increased lice numbers are a cost to group living in smooth-billed anis. If lice negatively affect their host, such as in reproductive success, perhaps there is an advantage to living in smaller groups.

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