UNIVERSIDAD NACIONAL AUTÓNOMA
DE MÉXICO

POSGRADO EN CIENCIAS BIOMÉDICAS
INSTITUTO DE ECOLOGÍA

FEMALE MATE CHOICE IN THE MAGNIFICENT FRIGATEBIRD (*FREGATA MAGNIFICENS*)

TESIS
QUE PARA OBTENER EL GRADO DE
DOCTORA EN CIENCIAS
PRESENTA
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Preface

The present thesis is the result of my Ph.D. project at the Laboratorio de Conducta Animal, Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México. My project was funded by a four-year Ph.D. scholarship from the DGEP, Universidad Nacional Autónoma de México from 1999-2003, and by three years of funding from the Danish Research Agency from 2000-2003. Further financial assistance was received from the private Danish fund "Torben and Alice Frimodts Fond".

My supervisory comity consisted of my supervisor Dr. José Luis Osorno Cepeda, Dr. Robyn Hudson and Dr. Constantino Marcia Garcia. After the death of José Luis Osorno, Dr. Alejandro Córdoba Aguilar was appointed my supervisor and entered my supervisory committee as such.

The aim of this Ph.D. project was to investigate the basis of female mate choice in the magnificent frigatebird (*Fregata magnificens*). In this thesis I present field observational studies of male courting behaviour and mating success together with laboratory analyses of collected samples, aimed at distinguishing between features of mated and unmated males.

This thesis consists of a summary in English and Spanish, a theoretical introduction, an introduction to the study species and project, plus general results, discussion and conclusion. Thereafter follow five article manuscripts and nine appendices. Article II was published in Condor in February 2004, article III has been submitted to American Naturalist for a special edition on feather colouration, and article V has been submitted to Behavioral Ecology.

Vinni Madsen

2005

Acknowledgements

My family in Denmark never questioned my "crazy" notion of going to Mexico to study a big black bird on a small deserted island in the Pacific Ocean, and they were always there for me with encouragement and support. In Mexico, "La Señora" Aurora Granados took me into her home, took care of me, and came to be my Mexican family.

José Luis Osorno supported and assisted me maximally up to his much too early death in April 2004. We were two persons with very different personalities and with very distinct cultural backgrounds. This led to some clashes, the need for some polishing of rough edges on both parts, but also fruitful discussions about a variety of different topics.

I sincerely wish to thank Alejandro Córdoba for his great support after José Luis' death. He provided invaluable help with the writing up of my thesis, and general support when I need it the most.

Thanks are also due to Robyn Hudson and Constantino Macías Garcia, for their support and help throughout my project.

Torben Dabelsteen helped me in numerous ways, but most of all through his practical, constructive and/or supportive advice, for which I am truly grateful.

My "roommate" at the institute, and now friend, Marina de la Vega was throughout an immense help. She provided not only moral support, but also lots of practical help. This included depositing my scholarship into my bank account, enabling me to buy food for my fieldwork volunteers during my fieldwork. She also read a first draft of this thesis and gave my some excellent advice.

The fieldwork was carried out in the Mexican National Park "Isla Isabel" and I thank the office of the Secretaria del Medio Ambiente y Recursos Naturales (SEMARNAT) in México City for permitting this work. I am truly grateful for the absolutely essential logistic support provided by the local SEMARNAT office in San Blas and by the Mexican Navy. This included transport of equipment and personal at the beginning and at the end of each fieldwork season, together with purchase and forwarding of food and fresh water twice a month.

The project was done in collaboration with a lot of very engaged people to whom I am truly indebted.

Gular pouch drumming:
  Torben Dabelsteen and Thorsten J. S. Balsby, Department of Animal Behaviour, Zoological Institute, University of Copenhagen, Denmark
  Equipment for the recording and later analysis was kindly provided by the Center for Sound Communication, Department of Animal Behaviour, University of Copenhagen.

Feather iridescence:
  Daniel Osorio, School of Life Sciences, University of Sussex, United Kingdom

Blood parasites:
  Gediminas Valkiunas, Institute of Ecology, University of Vilnius, Lithuania
  Anders Permin, University of Agriculture, Denmark

Hormone analysis:

Marco Sánchez, Universidad Autónoma de Queteraro, México

Re-sightings of marked males:

Monica Gonzalez, CICESE, México

This project would never have succeeded without the enthusiastic help from my multi-national group of fieldwork volunteers - here presented in alphabetic order:

Alberto Gomez, Anne-Mette Norup, Anja Laursen, Bent Albæk, Bianka Yoko, Christian Schlögl, Christiane Füldner, Chris Smith, Cynthia Lira, Ditte Guldager Christiansen, Emma Kelman, Eva Garde, Frederik Cundin Joelving, Gitte Madsen, Hanne Skovgaard, Jonas Norgaard Mortensen, Jonas Schoubo Jensen, Jordi Prieto, Keld Hedegaard, Klaus Kevin Kristensen, Kristine Vesterdorf, Kristoffer Nielsen, Leticia Hernandez, Lone Munk, Louise Ann Nordtorp Jørgensen, Louise Poulsen, Manuela Broechin, Maria Ribeiro, Monica Gonzalez, Nancy Gonzalez, Niclas Bacher, Nini Skovgaard Jensen, Severino Garcia, Trine-Maria Frederiksen, and Vicente Lopez.

I especially wish to thank Christian Schlögl, who worked with me on the island during two fieldwork seasons. Christian is an exceptionally dedicated and serious fieldworker, who has the great gift of foreseeing things that need to be done, and doing them, often before I even realized the need for them. In the 2000 fieldwork season Christian stayed on the island for an additional 4 months, during which period he continued the registrations of my focal birds, providing me with invaluable data. During the 2002 fieldwork season, Christian took over a great part of my work, after I had a very bad fall, working himself to exhaustion.

Special thanks are also due to Frederik Cundin Joelving, who assisted me with the sound recordings of the male courtship behaviour during the 2001 fieldwork season. Sitting in the open under a hot Mexican sun for hours on end, waiting for one particular male to be active, was absolutely unnerving, but Frederik kept up his part of the work till the (bitter) end.

Kristine Vesterdorf and Eva Garde became so involved in the project that they volunteered to analyse part of the data, which were presented as an independent report at the University of Copenhagen.

At the institute in México City I received endless help and support from Carolina Espinosa, Dr. Rodolfo Dirzo and Dr. Alfonzo Valiente from the "Oficina de Posgrado". Especially Carolina helped me time and time again with the administrative requirements. She always did this with great patience and a never failing helpfulness.

A lot of help and support were also received from numerous of the other students at the "Laboratorio de Conducta Animal".

Throughout the years the fishermen from San Blas, who come to the island for fishing, provided good company, possibilities for going fishing, and great opportunities for parties. The fishermen were also tireless in tutoring me in my endeavours to learn Mexican Spanish, which has had the quite strange side effect that I am now among one of the very few non-locals that can actually understand the local "lengua".

Summary

Female mate choice, as a sexual selection force in natural selection, has been intensively studied. However, few studies of birds have included seasonally expressed soft parts.

This thesis investigated aspects of female mate choice in the magnificent frigatebird (*Fregata magnificens*). Magnificent frigatebird males bear two striking morphological sexual ornaments, namely the seasonally inflatable and red gular pouch plus the black plumage with iridescence at head, back and two breast patches. Furthermore, males perform a striking courtship display, consisting of visual, acoustic and tactile stimuli.

My research focused on differences within the natural variation of male ornamentation between successfully and unsuccessfully mating males. This was facilitated by a heavily male-biased operational sex ratio during the yearly mating season, resulting in only 57.5% (average over four years) of males obtaining a mate. Males were compared with regard to physical features, mainly secondary sexual characters, as well as internal features, such as hormone level and blood parasite prevalence. Male ornamentation was thus investigated through a combination of field observations and laboratory analyses.

After arrival at the breeding colony an unmated male seeks out a suitable display site (Article 1). After selecting a site the male commences with a low intensity courtship, consisting of inflating the red gular pouch and/or wing display, whilst scanning for over-flying inspecting females (Article I). Neither plumage iridescence amplitude nor gular pouch colouration seemed to be directly selected for through female mate choice, as no differences were found with male mating status (Article II & III). The plumage iridescence and gular pouch coloration may, however, assist mate-seeking females in locating and homing in on the right sex, age and mating status. In a densely populated breeding colony, this might result in a substantial reduction of mate search-related costs, and therefore be of direct benefit to the female. The female preference for iridescence of lower wavelength (Article II) could thus also be for contrasting colouration to the green background foliage. Females may also obtain a direct benefit by mating with a male of the same species.

When an mate-seeking female circled or hovered over a a male, he performed high intensity courtship, through adding movements and sound production to the low intensity display (Article I). The feathers of the back and two breast patches have high levels of directional iridescence amplitudes (Article II). The iridescence might thus function as an amplifier of the courtship movements, with the feather movements resulting in reflections of the sunlight in short, sharp flashes of light. During high intensity courtship the male arches backwards and throws the gular pouch from side to side, whilst producing beak-clackings (Article I). From the beak-clacking sporadic bouts of drumming are produced. The drumming reflects the size of the gular pouch, with the fundamental

frequency being negatively correlated with size (Article IV). The fundamental frequency decreases with age, as a function of an increase in gular pouch size with age, whereas the drumming speed and constancy of the drumming rhythm remain stable (Article V). Females preferred males drumming at lower fundamental frequencies, indicative of them having larger gular pouches, and being older (Article V). If older males are also more experienced males, females may obtain a direct benefit, as experienced males may be less nest through nest usurpation by unmated males (Article I). The indirect benefit females may obtain through preferring older, more viable males, is the possible higher genetic quality of such males. Females also preferred to mate with males that drummed at a fast and constant rhythm (Article V). This might signal present male quality, as reflected in his stamina (Article V). Through such preference females could avoid mating with older, previously viable malesof recently impoverished quality or males that have reached senescence.

For further inspection, a female lands by a male, and the courtship character changes. During the first couple of days the male inflates the gular pouch less and thereby also drums less, as require an inflated gular pouch for resonance. Instead the male engages in more 'body shaking' of the female (Article I). Pair formation usually takes about one week, after which the pair starts constructing the nest at the display site (Article I). During this period the gular pouch changes colour and the level of testosterone decreases (Article III).

Male morphology changes with age, as expressed by the increase in tail- plus tail fork-length, iridescence ratio, short-term condition (expressed as size/weight ratio), gular pouch size and gular pouch colour saturation (Articles II, III, & V). I was, however, unable to correlate the features of preferred adult males, i.e. quick and constant drumming, large gular pouches, and plumage iridescence of lower wavelength with measures of male quality (Articles II, III, & V). Mated adult males did not differ from unmated males in blood parasite prevalence, weight/size condition, or testosterone levels (Article III).

The prognostic value of the investigated variables, as checked in a discriminant function analysis, was high (100%) with regard to age-class classification of males. With regard to the mating status I reached 90% correctly classified adult males. This indicated that one or more additional variables need to be included, in order to completely understand how females choose among the males. One such variable could be the 'body shaking' of the visiting female (Article I).

In conclusion, this study seemed to have identified some or most of the characteristics of adult males correlated with female choice, but further investigations are needed to determine which male qualities are expressed by the preferred traits, i.e. the possible direct and/or indirect benefits females may obtain through such choice.

Resumen en español

La elección femenina, como una fuerza de selección sexual en la selección natural, ha sido estudiada intensivamente. Sin embargo, pocos estudios en aves han incluido las partes blandas que se expresan estacionalmente (revisado por Andersson 1994; Ligon 1999).

En esta tesis se investigaron aspectos de la elección femenina en la fragata magnífica (*Fregata magnificens*). Los machos de la fragata magnífica presentan dos notables ornamentos sexuales morfológicos: la bolsa gular roja que inflan estacionalmente y el plumaje negro iridiscente de la cabeza, el dorso y en dos parches en el pecho. Más aún, los machos efectúan un llamativo despliegue de cortejo con efectos táctiles, acústicos y visuales.

Mi investigación se enfocó en las diferencias dentro de la variación natural de la ornamentación entre machos exitosos y no exitosos en aparearse. Esto se vio facilitado dado un marcado sesgo hacia los machos en la proporción sexual operativa durante la temporada de apareamiento anual, cuyo resultando es que sólo un 57. 5 % (promedio de 4 años) de los machos obtienen pareja. Los machos fueron comparados considerando sus características físicas, principalmente caracteres sexuales secundarios, así como características internas como los niveles hormonales y la prevalecía de parásitos en sangre. De este modo, la ornamentación de los machos fue investigada a través de una combinación de observaciones de campo y colecta de material para análisis de laboratorio.

Después de llegar a la colonia reproductora, los machos no apareados buscan un sitio adecuado de despliegue (Artículo 1). Después de decidirse por un sitio, los machos comienzan un cortejo de baja intensidad, el cuál consiste en inflar la bolsa gular roja y/o en ejecutar exhibiciones con las alas, mientras buscan visualmente a las hembras que hacen sobrevuelos de inspección (Artículo 1). Ni la amplitud de la iridiscencia en el plumaje ni la coloración de la bolsa gular parecieron ser directamente seleccionados ya que no se encontraron diferencias asociadas con el estado de apareamiento del macho (Artículo III y IV). El plumaje iridiscente y la coloración de la bolsa gular podrían, sin embargo, ayudar a las hembras en búsqueda de pareja, en localizar y dirigirse a individuos de sexo, edad y estado de apareamiento correctos. En una colonia reproductora densamente poblada, esto podría resultar en una reducción sustancial de los costos asociados a la búsqueda de pareja y por lo tanto beneficiar directamente a las hembras. De este modo, la preferencia de las hembras por una iridiscencia de longitud de onda baja (Artículo III) podría ser por una coloración contrastante con el fondo verde del follaje. Otra posibilidad es que a través de dicha preferencia las hembras obtengan un beneficio directo al aparearse con machos de la especie correcta.

Cuando una hembra muestra interés en un macho, el macho cambia de un cortejo de intensidad alta, añadiendo movimientos y produciendo sonidos, al cortejo de intensidad baja (Artículo I). Las plumas del dorso y del pecho de los machos, tienen niveles altos de amplitudes iridiscentes más direccionales (Artículo III). La iridiscencia podría, de esta manera, funcionar como un amplificador de los movimientos de cortejo, dado que los movimientos de las plumas reflejan la luz solar en cortos y nítidos destellos. Durante el cortejo de alta intensidad los machos se arquean hacia atrás y lanzan la bolsa gular de un lado a otro, mientras castañean con el pico (Artículo I). El castañeo del pico da lugar a una secuencia esporádica de tamborileo. El tamborileo refleja el tamaño de la bolsa gular, pues su frecuencia fundamental se correlaciona negativamente con el tamaño de la bolsa (Artículo II). La frecuencia fundamental del tamborileo disminuye con la edad, dado que la bolsa gular crece con esta última, mientras que la velocidad del tamborileo y la constancia permanecen estables (Artículo V). Las hembras prefirieron tamborileos de frecuencia fundamental baja, los mismos que indican una bolsa gular más grande y de machos de mayor edad (Artículo V). Si los machos de mayor edad son también machos más experimentados, las hembras podrían obtener un beneficio directo, ya que los machos más experimentados podrían ser menos susceptibles a perder su nido por usurpación por parte de machos no apareados (Artículo 1). Las hembras podrían obtener beneficios indirectos al preferir machos maduros de más edad y más viables si la calidad genética de estos machos es más alta. Las hembras también prefirieron machos que tamborilearon con un ritmo rápido y constante (Artículo V). Esto podría señalar la calidad actual del macho, reflejada por su resistencia (Artículo V). A través de dicha preferencia las hembras podrían evitar aparearse con machos de mayor edad, anteriormente más viables, pero cuya calidad se haya empobrecido recientemente, o con machos que hayan alcanzado la senescencia.

Cuando una hembra se interesa en un macho cortejante, aterrizan junto a él y el carácter del cortejo cambia. Durante el primer para de días, el macho infla menos la bolsa gular y, por lo tanto, tamborilea menos. En lugar de ello, el macho se ocupa más en "sacudir" el cuerpo de la hembra (Artículo 1). La formación de la pareja usualmente toma alrededor de una semana, después de la cual la pareja empezará a construir el nido en el sito de despliegue (Artículo 1). Durante este periodo la bolsa gular cambia de color y el nivel de testosterona del macho disminuye (Artículo IV).

La morfología del macho cambió con la edad, lo cual se expresó en el incremento del largo de la cola más la bifurcación de la cola, el largo de la horquilla de la cola, la razón de iridiscencia, la condición a corto plazo, el tamaño de la bolsa gular y la saturación de la coloración de la bolsa gular (Artículo II y IV). Sin embargo, no me fue posible correlacionar las características de los machos adultos preferidos, es decir, el tamborileo rápido y constante, la bolsa gular más grande y por lo menos una tendencia hacia la preferencia del plumaje iridiscente de baja longitud de onda con medidas de la calidad del macho (Artículos III-V). Los machos adultos apareados no difirieron de los machos no apareados en la prevalecía de parásitos en sangre, en la condición de peso/tamaño o los niveles de testosterona (Artículo IV).

El valor de pronóstico de las variables investigadas, evaluadas en un análisis de funciones de discriminantes fue muy alto (100%) con respecto a la clasificación de clases de edad de los machos. Con relación al estatus de apareamiento alcancé un 90% de clasificaciones correctas de los machos adultos. Esto indica que es preciso incluir una o más variables con el propósito de entender por completo cómo las hembras eligen a los machos. Una de dichas variables bien podría ser el "sacudimiento" del cuerpo de la hembra visitante (Artículo I).

En conclusión, este estudio identificó algunas o la mayoría de las caracteristicas de los machos correlacionadas con la elección de las hembras, pero es necesario hacer más investigaciones para entender mejor por qué las hembras eligen de la manera en que lo hacen. Esto involucra estudios donde se esclarezca de manera exacta qué cualidades de los machos expresan los atributos preferidos; es decir, los posibles beneficios directos y/o indirectos que las hembras pueden obtener a través de dicha elección.

Introduction

Sexual selection

The idea of sexual selection was presented by Darwin (1859) and developed further by him (1871). Darwin's concept was that selection arising from differences in male mating success drives the evolution of many of the differences between the sexes and often results in ornamented males. It was thus an attempt to explain why males of many animals have evolved conspicuous ornaments. It was also an attempt to explain why such ornaments, that probably reduce survival and thereby should be selected against by natural selection, still could be beneficial to the male.

The source of sexual selection has traditionally been referred to as the difference in initial parental investment between the sexes (Trivers 1972). The basis of this is that the pre-mating investment of females is generally higher than that of males, as eggs are more costly to produce than sperm. Females will, therefore, only have a limited fitness gain from deserting their brood (Maynard Smith 1977). In contrast, deserting males will have a high fitness gain, as their fitness increases proportionally with the number of mates (Bateman 1948). Recently, it has been shown that spermatogenesis is far from limitless and cheap, and that males have evolved mechanisms to maximise their lifetime reproductive success by allocating finite numbers of sperm (reviewed by Wedell et al. 2002). Males, however, still possess a generally higher reproductive potential than females, which may lead to competition for attracting as many females as possible. In contrast, females, through having a lower reproductive potential than males, should be choosy with regard to mate(s). However, it is the actual and not the potential reproductive rate that is of importance in the evolution of parental care systems and it is anisogamy, i.e. the unlike size and thereby investiment in gametes, that generates the conditions for sexual selection (Kokko & Jennions 2003).

The strength of mate competition and thereby intensity of sexual selection is also dependent on the system of parental care. Prolonged female parental care thus leads to fewer receptive mates for the males to compete over and thereby increased competition. This has been expressed in the operational sex ratio, i.e. the number of sexually active males divided by the number of sexually receptive females (Emlen & Oring 1977). When the rate is higher than one the competition among males becomes intense, whereas a rate below one indicates a relaxed competition. Lately, it has been suggested that a way to measure sexual selection is the sex difference in the variance in fitness (Shuster & Wade 2003). Variance in fitness will result from the exclusion of some individuals from mating and the magnitude of the variance in fitness between sexes will determine the possible degree of divergence between sexes.

Sexual selection mechanisms and occurrence

Darwin (1859; 1871) suggested two processes by which sexual selection takes place: male competition and female choice. Traditionally, the mechanisms of sexual selection were thus defined as different forms of competition for access by one sex to the other sex (Andersson 1994; Andersson & Iwasa 1996), occurring during different phases of the mating, i.e. pre-, syn- or post-mating (see below). Recently, it has, however, been shown that sexual selection may also involve a mechanism of conflict of interest between males and females, i.e. sexually antagonistic evolution (Rice & Holland 1997) and the chase away hypothesis (Holland & Rice 1998). This may result in characteristics that enhance the reproductive success of one sex, but reduce the fitness of the other sex, as when females are enticed into mating sub-optimally with males bearing extreme traits (Holland & Rice 1998, 1999; Holland 2002; Moore et al. 2003). It may, however, be difficult to tease a-part sexual conflict and traditional sexual selection processes (reviewed by Andres & Morrow 2003; Cordero & Eberhard 2003; Pizzari & Snook 2003.

Pre-mating sexual selection

Pre-mating sexual selection in males may occur in the form of contest competition, scramble competition, endurance rivalry, or male mate choice. In females, pre-mating sexual selection occurs as female mate choice.

Male contest competition is direct male/male interactions, such as threats or fights, through which mating success is determined, either directly through winning the right to females, as in pre-copulatory mate-guarding (Grafen & Ridley 1983), or through obtaining possession of resources essential to breeding, like territories or nest sites. Male scramble competition is a strategy in which males search competitively for receptive females, which might result in early male maturation, where males become reproductively active before females, or in more developed sensory and/or locomotory abilities of males (Thornhill & Alcock 1983; Legrand & Morse 2000). Male endurance rivalry is when mating success depends on the duration males can remain reproductively active, which may be daily or over the entire mating season. Male mate choice is when male fitness depends on the quality or number of female(s) accepted by the male as mate(s). Female ornaments may thus be maintained through sexual selection (Jones & Hunter 1993; Johnstone et al. 1996; Johnstone 1997; Jones & Hunter 1999; Amundsen 2000; Kokko & Johnstone 2002), and reflect condition (Domb & Pagel 2001; Jawor et al. 2004), which may be assessed and preferred by males (Hunt et al. 1999; Roulin 1999; Amundsen & Forsgren 2001 Berglund & Rosenqvist 2001; Romero-Pujante et al. 2002, but see Wolf et al. 2004). Another aspect of male mate choice may be the avoidance of mating with previously mated females (reviewed by White 2004).

Female mate choice is the non-random choice of mate(s) made by females, based on male ornaments. Male ornaments are here taken to include morphological traits, visual, tactile, auditory and volatile signals, plus display behaviours. Female mate choice will be dealt with in more detail below.

Syn-mating sexual selection

Syn-mating sexual selection may be in the form of sperm competition or competition avoidance in males and cryptic mate choice in females.

Sperm competition is the competition for egg fertilization and may occur when females are promiscuous (Parker 1970; Birkhead & Møller 1998; Simmons 2001), leading to the evolution of a higher proportion of motile sperm, enhanced sperm survival, and larger ejaculate volumes (reviewed by Wedell et al. 2002). Sperm competition avoidance may be through selection for genitalia developed for emptying the female spermathecas during mating (reviewed by Birkhead & Hunter 1990). Another form may be active behaviours as when the male aedeagus in damselfly (*Calopteryx haemorrhoidalis*) during copulation seems to mimic the movement of the eggs down the reproductive tract, whereby previously stored sperm is ejected from the spermatheca (Córdoba-Aguilar 2002). Another example is the pre-copulatory cloaca pecking of male dunnocks (*Prunella modularis*) resulting in female ejaculation of stored sperm (Davies 1983).

Female cryptic mate choice is differential sperm choice by promiscuous females in fertilization of the egg(s) (Thornhill 1983; Thornhill & Alcock 1983; Eberhard 1996; Pizzari & Birkhead 2000; Jones 2002; Pilastro et al. 2004), which can have important fitness consequences (Gowaty 1997; Jennions & Petrie 2000; Calsbeek & Sinervo 2004), with the possible genetic benefits outweighing the costs of promiscuity (Yasui 1997). Differential fertilization success of male gametes may also result from active female behaviour during or after copulation with a less desirable male in the form of quick re-copulation, delayed oviposition, premature copulation termination, failure to store sperm, or removal of sperm.

Post-mating sexual selection

In males, post-mating sexual selection may occur in the form of mate guarding and differential paternal investment, and in females in the form of extra-pair copulations.

Male mate guarding involves guarding the female against copulation attempts from other males and/or deterring the female from seeking extra-pair copulations (Parker 1970; Björklund & Westman 1983; Davies 1985; Møller 1987; Hatchwell & Davies 1992; Wagner 1993). Male parental care may be graded, as determined by a trade off between present and future reproductive success (Roff 1992; Stearns 1992), which may result in a conflict of interest within a pair (Houston & Davies 1985). This influences the extent of possible sexual selection on male traits, if lower investment of males than females results in a male biased operational sex ratio (Emlen & Oring 1977; Kvarnemo & Ahnesjö 1996) with a likely higher variance in male fitness (Shuster & Wade 2003). An example of this is the system of the magnificent frigatebird, where deserting males may breed successfully annually, whereas females may only breed successfully biennially (Osorno 1999).

Females may introduce sexual selection pressure through engaging in extra pair copulations, if female mating bias for extra-paternity males coincide, as this will result in a higher variance in reproductive success for males relative to that of females. Such difference has been suggested as an explanation for sexual dimorphism in species with a socially monogamous mating system (Møller & Birkhead 1994; Owens & Hartley 1998). The existence of extra pair copulations is widespread among birds, but the rate may vary both between and within species (Birkhead 1998; Griffiths et al. 2002). This may be based in differences in fundamental life history parameters, such as parental care and reproductive lifespan (Mauck et al. 1999; Arnold & Owens 2002), or be determined by genetic benefits of alternative reproductive strategies (Kempenaers et al. 1992; Hasselquist et al. 1996; Sheldon et al. 1997), and the ecological opportunities to engage in them (Dunn et al. 1994; Stutchbury & Morton 1995; Thusius et al. 2001; Bennett & Owens 2002). Females engaging in extra pair copulations may gain direct benefits through fertilization insurance, increased attractiveness of male offspring, and/or indirect benefit through increased offspring fitness due to genes obtained from the extra-pair male (Kempenaers & Dhondt 1993; Jennions & Petrie 2000).

Female mate choice

The basis of female choice is the non-random choice of mate made by females based on male traits (e.g. size), male possessions (e.g. territories and/or nest sites), male ornaments (e.g. exaggerated morphological traits or visual, tactile, auditory and volatile signals), or display behaviours. Female choice may occur pre-mating, i.e. traditional female choice of mate through preferences for certain male traits. Cryptic female choice may occur both during copulation through differential use of sperm stored in spermathecas, and after mating through seeking extra-pair copulations. The present study investigates the potential for pre-mating female choice.

Female preference for certain male ornaments may bias the probability that females mate with a non-random sub-set of males (Kirkpatrick & Ryan 1991; Kokko et al. 2003). Directional selection favours extreme ornaments that deviate from the population mean, and might result in both smaller and larger ornaments. In several taxa less elaborate sexual displays seem to have evolved from more elaborate forms (reviewed by Wiens 2001). However, the most common outcome seems to be selection for exaggerated ornaments, like higher display rates, longer display durations, greater display intensities and greater complexity (reviewed by Ryan & Keddy-Hector 1992).

Female mating preferences are supposed to have arisen from male ornaments exploiting pre-existing sensory biases in females, which may result from sensory evolution in other contexts than mating, such as foraging and predator avoidance (Enquist & Arak 1993; Endler & Basolo

1998). An example is the female mating preference found for acoustic or morphological male features that are not present in their own species. In frogs, *Physalaemus coloradorum* females show preference for a chuck call that is only included in the advertisement call of the tungara frog (*P. pustulosus*; Ryan 1990; Ryan et al. 1990). The direction of the signals may vary between populations as a result of specific aspects of the environment, i.e. sensory drive (Endler & MacLellan 1988; Endler 1992).

Selective mating by females entails costs (reviewed by Reynolds & Gross 1990; Gibson & Langen 1996; Kirkpatrick & Barton 1997) in the form of either direct costs or opportunity costs (Real 1990). Direct costs include for instance increased risk of predation (Arak 1988; Gibson & Bachman 1992) and increased time-expenditure whilst sampling potential mates (Slagsvold et al. 1988; Gibson & Bachman 1992; but see Slagsvold & Dale 1991). Inspecting a high number of males may also delay commencement of breeding, which often affects breeding success negatively (Alatalo & Lundberg 1984). Opportunity costs include loss of previously encountered mates due to emigration or change of mating status (Dale et al. 1992). In order to avoid or reduce the costs of mate search, females may adopt secondary tactics such as fidelity to their former mating site (Lill 1974; Gibson et al. 1991), fidelity to their previous partner or learned partner phenotype (Rintamäki et al. 1995; Uy et al. 2000; Qvarnström et al. 2004) or copying the choices of others (reviewed by White 2004).

Through their choice behaviour females may gain benefits and/or avoid costs (Møller & Alatalo 1999; Kokko et al. 2003). The benefits of a particular mating preference may come in the form of direct benefits to the females themselves or indirect benefits via their offspring (Kirkpatrick & Ryan 1991; Wiley & Poston 1996; Kokko et al. 2003).

Female choice for direct benefits

Direct benefits of female choice may be realised through increased fitness, fecundity, and survival or through cost avoidance (Kokko et al. 2003). Females may increase their fitness through choosing males of the right species, thus avoiding the possible costs of hybridisation (Mayr 1963). Females may be able to enhance their fecundity by choosing to mate with males that provide a higher fertilisation potential (Brown et al. 1996; Savalli & Fox 1998). Fecundity benefits might also be gained via the nutrients that the male provides, like the nuptial gifts given to females during egg-production in insects (reviewed by Thornhill & Alcock 1983), and the feeding of females during courtship, egg-laying and/or incubation in birds (Nisbeth 1973; East 1981; Wiggins & Morris 1986). The amount of food the male provides for the female and the offspring may be correlated with the quality of the male's territory or the quality of the nesting site (Radesäter et al. 1987; Alatalo et al. 1990). Males may also vary in the level of parental care or investment they provide for the female and the offspring, as well as in their ability to defend the brood (Norris 1990; Forsgren et al. 1996; Forsgren 1997). This 'good parent process' has been proposed to drive the evolution of male traits indicating their ability to provide parental care (Hoelzer 1989). Females might also be choosing certain types of male with the purpose of decreasing the costs of mating, which might be in the form of harassment during or after mating, as seen in mallards (*Anas platyrhynchos*; McKinney 1986; Grafe 1997).

Female choice for indirect benefits

Most of the theoretical and also empirical testing of female mate choice has concentrated on establishing the advantages of female choice when the benefits are indirect. From this two main theories have emerged: the 'Fisherian process' and the 'indicator mechanisms' (Fisher 1958; Zahavi 1975, 1977; Weatherhead & Robertson 1979; Lande 1981; Kirkpatrick 1982; Kodric-Brown & Brown 1984; Andersson 1986; Pomiankowski 1987; Grafen 1990a, b; Iwasa et al. 1991; Pomiankowski et al. 1991). The main distinction between these two theories lies in the way they consider the female preferences to have originated and to be maintained.

'Fisherian process'

In the Fisherian theories, an initial preference for an ornamented trait may be selected for if other females show the same preference. If both the preference and the trait continue to evolve positive feedback will take place. Fisher (1958) showed mathematically that a genetic correlation is likely to arise in both characteristics. The benefit for females that choose males exhibiting these traits will be to have more successful 'sexy' male offspring in terms of attracting females (Weatherhead & Robertson 1979). The genetic preference for these male characteristics will produce a "run-away process" that will maintain both the preference and the male trait (Kirkpatrick 1982; Pomiankowski & Iwasa 1993; Hall et al. 2000; Day 2000).

'Indicator mechanisms'

The theory of indicator mechanisms assume that females are able to detect the genetic quality and viability of the bearer, through the variation of phenotypic expression of male ornamentation. Thus, females choosing males of high genetic quality would gain indirect benefits in the form of increased viability of their offspring (Zahavi 1977; Kodric-Brown & Brown 1984; Andersson 1986). The models thus include a trait indicating male genetic quality (e.g. viability) that is correlated with the expression of the male trait (Shluter & Price 1993: Iwasa & Pomiankowski 1999) or with female mating preference (Kirkpatrick 1986; Iwasa et al. 1991; Iwasa & Pomiankowski 1994).

'Fisher-Zahavi model'

The Fisherian process and the indicator mechanisms may, however, not be mutually exclusive (Pomiankowski 1987), and have been combined under the name of the 'Fisher-Zahavi model' (Eshel et al. 2000). The rationale for this that is the benefits from each process may form a continuum depending on how mating success trades off against survival (Kokko et al. 2002), and that attractive ornaments indicative of fitness must also become genetically correlated with male quality (Eshel et al. 2000; Kokko et al. 2002; but see Cameron et al. 2003). Indicator mechanisms may therefore work alongside an inevitable Fisherian process (Mead & Arnold 2004).

Male ornaments

Female choice of mate may be based on variation in the expression of one or more male secondary sexual characters. Male secondary sexual ornaments, as defined by Andersson (1994), are attributes that result in differences in reproductive success, through the expression of the ornament in competition over mates. These traits may be morphological, visual, auditory, tactile plus chemical signals, behaviour patterns and combinations of two or more of the above (see Andersson 1994).

Condition-related male ornament

One of the key predictions in the hypotheses of mate choice for indirect benefits is that the expression of orna-ments should reflect condition, i.e. general health and vigour (Pomiankowski 1987; Grafen 1990a, b; Iwasa & Pomiankowski 1994). Female mating preferences should thus have evolved for male ornaments that signal male genetic quality through being condition dependent. Any effect on overall condition of a male will therefore affect the expression of his ornaments. However, a recent review surprisingly concluded that there is little support for the claim of male ornaments reflecting physical condition (Cotton et al. 2004).

Male ornament variation

One paradox in sexual selection is the fact that variance in genetically based sexually selected male ornaments seemingly persists. Female directional selection should rapidly exhaust genetic variance and drive the variation in ornament expression to fixation. *Also fitness-related traits would be expected to have a low heritability because of the strong selection acting on anything related to fitness (Falconer 1989).* In the "lek paradox" (Taylor & Williams 1982; Reynolds & Gross 1990; Kirkpatrick & Ryan 1991; Pomiankowski & Møller 1995; Rowe & Houle 1996), females of lekking species are still selective with regard to mates even though females obtain no paternal care from males (Gibson & Bradbury 1985; Alatalo et al. 1991; Gratson 1993; Höglund et al. 1997).

One suggested solution of the paradox isthat modification of the phenotypic effect of one locus by another locus, may result in high levels of additive genetic variation remaining in traits, and the amount of variation may even be greater among the sexual than the non-sexual traits (Pomiankowski & Møller 1995). The estimates of the genetic variation of sexually selected traits based on this argument have been revised and the effect of sexual selection on fitness traits appears to be of a low, although significant magnitude (Møller & Alatalo 1999). Another suggested solution is the 'genic capture' hypothesis (Rowe & Houle 1996). This is due to sexually selected ornaments being based on the expression of a large part of the genome, whereby condition has a high genetic variance, and such ornaments show strong condition dependence (Tomkins et al. 2004). This has been modelled based on two different pathways, which both lead to the evolution of costly male sexual traits and female mating preference. The first model (Lorch et al. 2003) is based on the idea that there might be variation in the optimal phenotype, thus fluctuating selection may occur. Such variation may be expressed in time, for instance in cycles of host-parasite co-evolution (Hamilton & Zuk 1982; Westneat & Birkhead 1998), or in space (Jia et al. 2000; Amos & Balmford 2001). The second model (Houle & Kondrashov 2002) is based on the mutation-selection balance, where the rate of new mutations is thought to generate variation as quickly as it is eroded by selection.

Multiple ornaments

A male might display an array of different secondary sexual ornaments. This has been suggested to function as either "multiple messages", where ornaments provide information about different aspects of male quality, or "backup signals", where the same information about male quality is provided by two or more ornaments (Møller & Pomiankowski 1993; Johnstone 1995a; 1996). Models of multiple ornaments indicate that even with direct selection on the preferences, a Fisherian process may lead to evolution of multiple ornaments and preference (Pomiankowski & Iwasa 1993). A good genes model showed that one indicator ornament might block the evolution of other indicators, suggesting that multiple ornaments probably may not all be indicator traits (Iwasa & Pomiankowski 1994). Multiple ornaments may, however, function as a composite ornament instead of partitioned ornaments, and may thus be evaluated more as a whole. An example is mate choice trials in the California quail (*Callipepla californica*), where females, when presented with a natural range of plumage variation, chose primarily on the basis of a composite trait, where two or more traits together elicit a stronger response (Calkins & Burley 2003). Multiple traits might also persist due to differences among females with regard to mating preference. In this respect, it has been shown that female preferences change with age in the satin bowerbird (*Ptilonorhynchus violaceus*; Coleman et al. 2004).

Honest male ornamentation

An important assumption of the indicator models is that the male phenotypic traits must be honest; in other words, the production and maintenance of these traits must be costly for males (Zahavi 1975, 1977; Grafen 1990a, b; Iwasa et al. 1991). Furthermore, for the trait to operate as an honest handicap, these costs should be highest for the lowest quality males (Zahavi 1977; Grafen 1990a, b; Iwasa et al. 1991). This is because males that are in better phenotypic condition should be better able to bear the costs of maintaining the trait. Empirical testing of the costs of sexual traits is difficult, as reflected by the paucity of studies where such differential costs have been empirically proven (Møller 1989; Møller & de Lope 1994; Mappes et al. 1996; Kotiaho et al. 1999).

The genetic indicator mechanism has gained support in studies with several species (von Schantz et al. 1989; Reynolds

& Gross 1992; Bakker 1993; Norris 1993; Møller 1994; Petrie 1994; Hasselquist et al. 1996; Sheldon et al. 1997). In the grey tree frog (*Hyla versicolor*), for example, call length operates as an indicator of male genetic quality. The offspring of males that produce longer calls perform better at each stage of their development than the offspring of males producing shorter calls (Welch et al. 1998). There are, however, also several studies in which no evidence for the effects of male genetic quality upon offspring performance has been found (Boake 1985; Woodward 1986; Mitchell 1990; Howard et al. 1994; Semlitsch 1994).

Several characteristics of offspring performance have been used to examine the possible indirect benefits of female mate choice. Since an individual's fitness is ultimately defined as its success in transfering its own genes to the next generation, the indirect benefits of female mate choice should be revealed as viability and reproductive success of the offspring. Viability of the offspring has been shown to be enhanced with greater levels of male sexual advertisement in peacocks *Pavo cristatus* (Petrie 1994), great tits *Parus major* (Norris 1993), fruit flies (*Drosophila melanogaster*; Taylor et al. 1987) and barn swallows (*Hirundo rustica*; Møller 1994). In many cases, however, offspring quality is only measured using characters closely related to fitness, such as growth rate (Reynolds & Gross 1992; Welsh et al. 1998), development time (Moore 1994) and fledgling condition (Sheldon et al. 1997), and not directly on the fitness of the offspring.

The genus *Fregata*

The genus *Fregata* consists of five species of frigatebirds spread out over both sides of the Equator in the tropics and subtropics. All species are strictly marine birds of both coastal and pelagic waters. They are characterized by long, slender and hooked beaks, slender bodies, long narrow wings and deeply forked tails.

Frigatebirds are designed for a predominantly aerial life, and are eminent gliders and flyers with a high capacity for long distance movements (Dearborn et al. 2003; Weimerskirch et al. 2003), and a high degree of manoeuvrability (Gonzalez-Jaramillo 2000). They have a wingspan of about 2 meters, but are lightweight, with an average weight of about 600-1600 g. This results in the lowest recorded wing-loading (weight/wing area) for any bird species (Nelson 1975; Weimerskirch et al. 2003). Weight reduction is obtained through highly pneumatic bones that weigh less than 5% of the total body mass (Orta 1992). To add strength to the skeleton, the pectoral girdle is fused together, which is a trait unique to frigatebirds (Nelson 1975).

The plumage is not water proofed and the legs are small for the size of the bird (only a few cm). This means that frigatebirds cannot land on the water surface, as their plumage rapidly gets water-logged making them too heavy for take-off (Mahoney 1984). They are also vulnerable on land, as they have difficulties walking, and therefore try to "swim" through the vegetation (van Tets 1965), often leading to lesions of the wings or them becoming entangled in vegetation (pers. obs.).

Foraging is exclusively on pelagic marine resources and is done from/in the air, either through surface dipping (Diamond 1975; Schreiber & Hensley 1976), kleptoparasitizing other marine bird species (Gibbs 1987; Osorno et al. 1992; Gilardi 1994; Vickery & Brooke 1994; Cummins 1995; Le Corre & Jouventin 1997), or through taking floating leftovers from fishing boats (Calixto-Albarrán & Osorno 2000).

The diet varies between populations depending on the extent of each foraging method. In populations where dipping is the most common method, the diet is dominated by flying fishes, jacks and squid (Diamond 1975; Schreiber & Hensley 1976; Harrison et al. 1983). From kleptoparatizing other marine birds the diet may include pelagic prey items (Calixto-Albarrán & Osorno 2000). Finally, bottom foraging/dwelling fish species may dominate the diet of frigatebirds mainly feeding on the leftovers from fishing boats (Calixto-Albarrán & Osorno 2000).

Frigatebirds are long-lived, with great frigatebirds (*F. minor*) reaching ages of over 37 years (Dearborn 2001). Unlike other long-lived birds, frigatebirds do not mate for life but seem to pair with a new mate in every breeding attempt (Dearborn 2001; pers. obs.).

All frigatebird males are ornamented, with an iridescent plumage, a gular pouch, which is red and inflatable during the mating season only, and a conspicuous courtship display. Females share none of these traits. A phylogenetic analysis of frigatebirds and related taxa (13 families totalling 237 species) showed that sexual dimorphism is only present in the five species of frigatebirds (Dearborn et al. 2001). This indicates possible sexual selection pressure on frigatebird males either through male-male interactions or female mate choice. In a study of the monogamous great frigatebird, Dearborn and Ryan (2002) did not find any indication of female mating preferences for redder gular pouches or higher iridescence levels when comparing early and late mating males. Apart from male ornamentation, the plumage of females is generally more black/brown and has larger areas of white than the blacker males. Females are also 2-15% larger and heavier than males (Dearborn 2001; Osorno 1999).

Vocalization is a widespread phenomenon in frigatebirds, and differs both with age and sex. Among the vocalization types are juvenile begging calls, sound produced during male courtship, and females' calls to the juveniles or mates.

Breeding colonies are located on small oceanic islands, and frigatebirds seem to have a high degree of natal philopatry, i.e. a tendency to stay or return to the area of hatching. A study of the genetic differentiation between three breeding colonies of great frigatebirds showed a high degree of differentiation despite the fact that frequent re-sightings of marked birds in other colonies showed them to move extensively between the colonies (Dearborn et al. 2003).

In all frigatebird species the clutch size is unusually low, and consists of one egg only. Incubation lasts approximately 8 weeks, and the following brooding period approximately 4-6 weeks (Nelson 1975). The nestling period lasts about 5 months, but the total juvenile-dependency period is exceptionally long and in some species lasts until the juvenile is 2 years old (Diamond 1973; Nelson 1975; Trivelpiece & Ferraris 1987; Reville 1988, 1991; Osorno 1999). Frigatebird chicks grow at a very slow rate, which probably is an adaptation to an impoverished pelagic habitat. Also, the extended post-fledging period is probably required for the fledglings to acquire the specialized skill necessary for foraging (Nelson 1975). Breeding success is generally low, and of eggs laid only 15-25% yield a fledged young. This is followed by fledgling mortality, mortality at independence, and the 3-5 years prior to breeding (Nelson 1975). One of the major mortality risks for eggs and nestlings is nest usurpation by unmated males, a general strategy for frigatebirds (Nelson 1975; Reville 1988, 1991).

For nest building the male brings all or the main part of the nesting material (Stonehouse & Stonehouse 1963; van Tets 1965; Diamond 1975; pers. obs.). Males prefer to collect nesting material above the ground, but may also pick up material from the ground/sea surface whilst hovering (Nelson 1975; Fairchild et al. 1985). The female constructs the nest at the male display site (Nelson 1975). The male and female share nest guarding, i.e. one bird has to be on the nest, as the nest material or the nest otherwise will be usurped by other males. In all species, males and females also share incubation and brooding of the young chick. In some species it is, however, only the female that tends the juvenile for the entire dependency period. Great frigatebird males in general desert during the last two months of the 12-month parental care period (Dearborn 2001), whereas magnificent frigatebird males in general desert after approximate 2 months of care, leaving an additional 9-12 months of care to the female only (Diamond 1973, 1975; Trivelpiece & Ferraris 1987; Osorno 1999; Osorno & Székely 2004). The mating system thus ranges from strictly monogamous to sequentially monogamous.

Frigatebirds seem to be both socially and genetically monogamous, as only very low levels of extra-pair paternity have been found (1 out of 92 chicks for the great frigatebird; Dearborn et al. 2001, and 0 out of 34 chicks for the magnificent frigatebird; M. Serrano pers. comm.).

The magnificent frigatebird of Isla Isabel

The magnificent frigatebird is the largest of the five species, with a wingspan of over 2 meters (Nelson 1975; Harrison 1983; Orta 1992). The distribution includes both the Atlantic and the Pacific shores of Mexico (Harrison 1983; Orta 1992). The most important breeding colonies in Mexico are in the Pacific: Isla Isabel, Isla Margarita, Islas Marias, and in the Atlantic: Isla Contoy.

The investigation of the magnificent frigatebird on Isla Isabel was initiated by José Luis Osorno in the 1990's, and studies of the same population have been on-going ever since. The breeding population was estimated to consist of 3,600 nests in 1993-4 (Osorno 1996). During the present study, a total population estimate of 35,600-53,400 birds was calculated (Appendix I).

The breeding colony covers most of the vegetated part of the island. The nest density is, however, rather variable (pers. obs.). The most common position of male display/nest sites is in the branches of *Crataeva tapia*. The height of these trees is relatively moderate from less than 1 m to about 5 m. Fallen over trees and branches on the ground may also form the basis of display/nest sites, so the height of the nests may be from a few cm to around 5 m, but always off the ground.

The yearly breeding cycle begins in September, when males start their courtship. The mating season lasts about four months. The first chicks usually hatch late November to early December. Males generally stay in the colony tending their chick until around the beginning of April, when they desert, abandoning the island, and leave the 1-4 months old chick in the care of the female for an additional 9-12 months (Osorno 1999; Osorno & Székely 2004). The female feeding pattern is distinct before compared to after male desertion. Before desertion females feed the chick slightly more often than the males, but after male desertion females nearly double their feeding rate (Osorno & Székely 2004). The growth rate of chicks, therefore, does not vary before and after male desertion. This has lead to modelling of whether chicks may be better off with parental care from one instead of two parents (McNamara et al. 2003). The enhanced parental effort of the females after male desertion does not seem to result in female weight loss (Lee 2004). This indicates that females may be adjusting their investment in the current chick for maximum lifetime reproductive success.

Between-year differences in breeding patterns and breeding success may result from natural causes. Hurricanes late in October 1994 and 2002 resulted in complete (1994; Osorno 1996) or partial (2002; pers. obs.) loss of established nests. The "El Niño" of 1998 resulted in higher biparental feeding rates, later male desertion, and chicks fledging at an earlier age in comparison with 1994, when food availability seemed more limited (Solares 2002). In 2001 a rainstorm on 1st and 2nd of March killed most of the 1-2 month old chicks (L. Lee pers. comm.). These chicks were no longer brooded and thereby protected against the rain and cold by the parents. In many species there is an advantage to mating early in the season; otherwise the best mates, the best sites, or the best seasonal conditions may be missed (Perrins 1970, Hunt and Hunt 1976, Hunt 1980). As the above-mentioned examples have shown this may not apply within this colony. For great frigatebirds on Aldabra, no significant difference in breeding success of birds laying at different stages of the season was found (Reville 1988). Most importantly, there was a surplus of males available each season (Reville 1988), as also within the Isla Isabel population (unpubl. data), so females could delay settlement and yet be certain of finding a mate. Contrary to this Dearborn & Ryan (2002) found reproductive success to decline over the season in a population of great frigatebirds on Tern Island, Hawaii. There may therefore be marked annual differences in whether early breeding results in increased breeding success.

Individual recognition between parents and the chick starts when the chick is about 40 days old and is fully developed at 71 ±30 days of age. When the chick is fully developed, 87% of the parents are able to localise their own chick, even when it has been moved to another nest, with females being better than males in making such distinctions (Lecona 1998).

The diet is dominated by opportunistic foraging on the leftovers from prawn-fishing boats. There are no differences in the size of fish taken by adult females and males, but the diet of flying juveniles contains more fish from surface dipping, indicating an age-related difference in foraging methods (Calixto-Albarrán & Osorno 2000). Kleptoparasitic foraging is mainly performed by females and juveniles on blue-footed boobies (*Sula nebouxii*). Such chases lead only to regurgitation of food in 6% of the cases, and food is only successfully captured by the frigatebird in 67% of these (Osorno et al. 1992). Kleptoparasitic behaviour does not, therefore, seem to be a major foraging method for frigatebirds from this colony.

Research on the frigatebird aerodynamics found that males could modifywingspan and tail-area further than females, resulting in a larger performance envelope. The smaller size of males makes them more agile. The males' lower wing loading and high aspect ratio, i.e. ....

reduces drag, give them a higher climbing rate, smaller turning radii, and a smaller sinking speed. Females' higher wing loading and aspect ratio reduces drag and gives them a lower stalling speed while gliding (Gonzalez-Jaramillo 2000)

From fitting 5 frigatebirds with satellite radio transmitters it was found that males after deserting travelled as far as Central America, whereas females that were still tending a young chick did not travel more than 150-200 km from the island. Movements of both males and females tended to be along the coastline and not over open sea (Osorno unpubl. data)

Ongoing investigations are presently studying the growth rates of chicks in relation to abundance and availability of food (E. Tobon); population genetics and dispersion patterns (M. Gonzalez-Jaramillo); begging calls of nestlings and the ability of the parents to distinguish between individual chicks (C. Wolf); levels of extra-pair paternity (M. Serrano) and the presence of spermathecas in females (N. Palomo).

Male ornamentation of magnificent frigatebird

The magnificent frigatebird is a good model for the study of sexual selection in the form of female mate choice.

First, male desertion results in males being available for breeding every year, whereas females may only breed successfully every second year. As a consequence the operational sex ratio is heavily male biased and the average male mating success 57.5% only over the period 1999-2002 (Appendix III – Table AIII 1). The intensity of sexual selection may therefore be high in this sequentially monogamous species. The biased sex ratio also permits us to investigate female choice based on the apparent natural variation of male ornaments between the non-mating and the mating males. Trait differences between the two male groups are a likely result of female mating preferences, as male – male competition seems to be negligible in this species (Article I).

Secondly, males only bear few morphological ornaments, namely the iridescent plumage and the red inflatable gular pouch. This is an advantage as it limits the number of possible variables and combinations of variable included in the mate choice situation. The plumage ornamentation consists of lanceolated green iridescent head-feathers and "purple" iridescent back- and breast-feathers. The breast-feathers are arranged in two patches on either side of the gular pouch. I was unable to get permission to bring out whole specimens from the national park. The plumage colouration could therefore only be visually inspected on site. I, therefore, do not know if any plumage areas are coloured within the ultraviolet spectrum, which is within the visible spectrum for birds (e.g. Hart 2001; Vorobyev 2003), and of importance in mate choice situations (Bennett et al. 1994). The gular pouch is a very striking skin ornament that is only seasonally expressed, as the colour and size change after mating (Reville 1991; pers. obs.). The remaining part of the year it is therefore only discernable underneath the beak as a strip of skin. It may therefore not be selected for outside the mating season. The mechanism behind gular pouch inflation is still unknown (Appendix IV).

Thirdly, within the theory of multiple ornaments, several male ornaments can either represent ways of signalling different dimensions of male quality ("multiple messages") or be different ways of signalling the same dimension of male quality ("back-up signals"). The two morphological ornaments in frigatebirds are likely to represent two different condition indicators. The plumage may indicate long-term condition, if the birds have continuous moult (Appendix V), or otherwise condition at time of moulting. The gular pouch characteristics, on the other hand, may be indicative of short-term condition, i.e. condition during the mating season.

Fourthly, frigatebirds breed in large colonies and have an extended mating period of approximately three months, which assists in the study of many individual birds.

Aims

The first aim of this investigation was to study the characteristics (ornaments) of males associated with female mate choice. The second aim was to try and relate such male characteristics with some male quality.

Hypotheses

Males may obtain mates differentially in relation to ornamental traits or combination(s) of ornamental traits. In species with no or little male-male competition, female mate preferences may thus be expressed through differences in male ornamental traits with male mating success. In magnificent frigatebirds such ornamental traits could be courtship behaviour, gular pouch size, gular pouch colour, plumage iridescence and/or courtship acoustics. Differential expression of such ornamental traits may be based in underlying differences in male quality, with high quality males being able to have better trait expression than low quality males.

General methods

Study site

Isla Isabel is a small (2 km^2) volcanic island in the Pacific Ocean, situated 70 km off the coast of the Mexican state of Nayarit (21°52'N, 105°54'W) (Figure 1).

Apart from magnificent frigatebirds, several species of marine birds breed on the island, such as red-billed tropicbirds (*Phaethon rubricauda*), brown pelicans (*Pelecanus erythrorhynchos*), brown boobies (*Sula leucogaster*), blue-footed boobies (*S. nebouxii*), red-footed boobies (S. *sula*), brown noddies (*Anous stolidus*), and very sporadically sooty terns (*Sterna fuscata*).

Study area

The fieldwork camp was established in the "Casona", which is located on the plateau at the southern part of the island at the edge of my study area. The study area was the plateau and the lighthouse cliff (Figure 1). Subdivision of the study area into eight smaller areas was done on the basis of differences in substrate and vegetation.

Fieldwork periods

1999: 16th October – 16th December
2000: 22nd September – 31st December
2001: 23rd September – 31st December
2002: 3rd October – 16th December

Marking

Marking of birds with wing-tags and later with metal rings was also initiated by José Luis Osorno, and up to now approximately 2000 birds have been marked. The wing-tags are of reinforced yellow vinyl and have a size of 20.5 x 8.0 cm. The weight is approximately 11 grams, which is less than 1% of the weight of the lightest male. Each bird is supplied with one wing-tag, attached around the ulna of the left wing in a manner that still allows the bird to preen underneath it (Osorno 1999). The closure around the ulna is also small enough not to leave any opening in the secondary wing feathers. The wing tags are for individual identification within and between years, as the numbercode can be read at a distance. The durability of the wing tags seems to be in the range of 5 years. In order to be able to follow the individuals over longer periods, lately the birds have also been given an engraved metal ring. This ring is supposed to last close to the birds' expected lifetime, so individuals may be identified upon re-capture even after the wing-tag has fallen of.

In this study a total of 454 non-juvenile frigatebirds were captured on nights with no moon, through taking them by hand from nests, trees and bushes.

They were measured, feather and blood samples collected, weighed and fitted with a wing-tag with a unique number/colour, and/or letter/number combination for individual recognition. On the right leg they were also given a metal ring with an engraved number and the address of the institute.

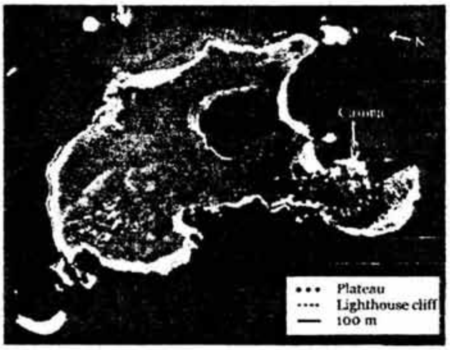

Figure 1. Isla Isabel with the area of the plateau and lighthouse cliff indicated

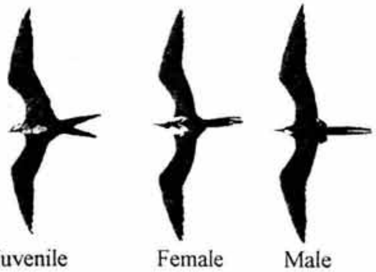

Figure 2. Juveniles have a white head and ventral area. Females have black/brown heads that extend into the white breast, and adult males have all black plumage (e.g. Harrison 1983).

Sexing

Adult magnificent frigatebirds can be easily sexed by the distinct pattern of black/brown and white in females and the dominant black plumage in males (Figure 2; e.g. Harrison 1983). Young birds cannot be sexed by plumage features (e.g. Harrison 1983).

Only male plumage has structural coloration in the form of green and "purple" iridescence (Appendix VI – Figure AVI 3A-C).

In total 55 females and 399 males were marked.

Age-classification

Juvenile birds can be distinguished from adults by the different pattern of black/brown and white (Figure 2; e.g. Harrison 1983). Age-classification of non-juvenile birds was done on the basis of the extent of white in the plumage (Appendix VII, Figure AVII 1-3). Females were divided into two classes and males into four, the exact ages relating to these sub-divisions is, however, still unknown (Appendix VII). In total 55 adult females plus 6 juvenile/sub-adults, 25 (juvenile)/sub-adults, 102 sub-adults and 266 adult males were marked. For male attributes by age (means ± SD) see Appendix VIII.

Biometric measures

The length of the left ulna was measured (±0.5 mm; ♀N=26, ♂N=390), together with the beak-length (±0.5 mm; ♀N=26, ♂N=387) and beak-width (±0.05 mm; ♀N=0, ♂N=203). The birds were weighed (±25 gram; ♀N=25, ♂N=375). A condition measure was calculated as the residual of a linear regression between weight and size (ulna and culmen lengths). This was used as an indication of short-term condition (Articles II-V).

The deeply forked tail consists of 12 feathers – six on each side of the tail-fork. The length of the outer rectrices was measured together with the depth of the tail-fork (±0.5 mm; ♂N=378). The age of the feathers of the tail was noted (numbered 1-12 from right to left; ♂N=197), with old feathers being distinguished on the basis of general abrasion and wear, resulting in them being more round-tipped and bleached by sunlight (Jenni & Winkler 1994). From this the moult rate of the tail was calculated (Appendix V). The moult rate was used as an indication of long-term condition (Article V).

Blood samples

Immediately upon capture a maximum of 1ml was taken from the under-wing vein. One drop of the fresh blood was used for making slides for analysis of blood parasites (♀N=19, ♂N=257; Article IV). The remaining sample was left to coagulate for minimum 30 min before being centrifuged at 3000 rmp/10 min and the serum separated from the plasma. For estimation of the haematocryte ratio all samples were weighed after separation (♀N=18, ♂N=301). Both the serum and the plasma were then stored in liquid nitrogen. The serum sample was later analysed for testosterone levels (♀N=10, ♂N=219; Article IV).

Feather samples

In total six feathers were taken from both males and females. These were two head-feathers, two wing-feathers, and two back-feathers (♀N=10, ♂N=214). From some males only, one feather from each of the two breast patches was also taken (♂N=43) (Article III).

Gular pouch colour

Through visual observations I found that the colour of the non-extendable skin between the mandibles of the under-beak was indistinguishable from the colour of the inflated gular pouch. I therefore measured the colour of this part. It was done by using a Minolta Colorimeter recording in the L a* b* colour space (♂N=133; Article IV).

Sample sizes

Sample sizes varied due to, for instance, some equipment only being available some years, difficulties in getting blood samples from some males, and some samples being spoiled during transportation. Also, some birds had to be released early due to evident stress, and most birds with eggs or chicks were not measured nor sampled.

Re-sights

Throughout the fieldwork period, observations of marked birds were registered. This included registration of sex, age, mating status, behaviour and location within the study area (Article I).

Mating status

Mating status upon marking was divided into three categories. Mated, when with nest, egg or chick. Visited/visiting, when sitting together with a bird of the opposite sex. Non-visited, when an unmated male was sitting alone (females were not captured when sitting alone).

Final overall mating status was assigned at the end of the mating season based on the re-sightings (Appendix III). Males were either categorized as mated, unmated or of unknown mating status (Appendix III). For male attributes (means ±SD) by mating status see Appendix IX.

Focal observations

Fifthy-eight marked males were observed at their display site. Different components of the behaviour were registred as either event (e.g. the drumming) or duration (e.g. gular pouch inflation, movements) registrations (Article I).

Sound recordings

The courtship of 69 males was sound recorded and analysed for relation between gular pouch size and sound production (Article II). Also, the drumming of the courtship was recorded for 56 marked males and the acoustic components analysed in relation to male mating success (Article V).

Manipulations

In order to test some of the findings of this study, various manipulations were carried out. None of them, however, was successful.

Artificial nest sites

To test the importance of the display/nest site quality, artificial nest sites were constructed. The sites attracted males and females the first few days after construction, but were then ignored.

That a nest is a strong attractant to females was confirmed by the observation

Figure 3. The gular pouches of five males were punctured by the use of a blow-dart.

that sometimes a female arrived first with the male arriving shortly afterwards. As the female accepted that the male landed by her, the two birds were probably familiar with each other, and they therefore seemed to be a couple that moved together at the initiative of the female.

Female attraction to red

Recently dead males were collected and placed in trees. Different coloured balloons were positioned in the place of the gular pouch. The colours were red, orange and green. No females showed any interest in these males.

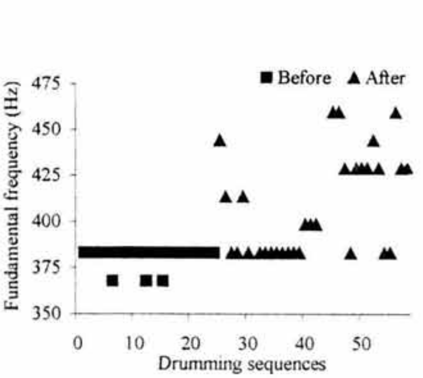

Figure 4. The fundamental frequency before and after the gular pouch was punctured by use of a blow-dart. After the puncturing the male did not succeed in keeping the fundamental frequency as low as before, i.e. he was unable to inflate the gular pouch to the same size as before puncturing. The sound was recorded and analysed in accordance with the Method of Article II.

Gular pouch inflation

To check the importance of the inflated gular pouch, the gular pouches of five courting males were punctured using a blow-dart (Figure 3). The males generally left immediately after the puncturing, and only returned after a week or more, when the gular pouch was again intact. One male did, however, stay for about 30 min after the puncturing, and we got sound recordings both before and after gular pouch puncturing (Figure 4).

Gular pouch colour

To test the influence of gular pouch colour on the female choice of mate, gular pouches were painted using water-soluble theatre make-up. Focal observations were made of unmarked males for up to 5 hrs before sunset. The males that remained at the display site after sunset were captured, marked and painted red (resulting in more red than natural), white (resulting in a pink gular pouch colour) or just painted with water, at random. The idea was to follow the same males the next day, and to compare the interest before and after treatment, of mate-seeking females. The males, however, left or stopped displaying after having been painted.

Model plane

In order to standardize the stimulus given to courting males by over-flying females, I contacted a very enthusiastic model plane operator. Drawings were made for adapting a model plane representing the shape and colouration of an inspecting female magnificent frigatebird. The project, however, had to be abandoned due to lack of success in obtaining necessary funding.

Articles

Article I - Male courtship behaviour

Madsen, V., and Osorno, J.L. The courting behaviour of male magnificent frigatebirds (*Fregata magnificens*). To be submitted to Wilson Bulletin or similar journal

THE COURTING BEHAVIOUR OF MALE MAGNIFICENT FRIGATEBIRDS (*FREGATA MAGNIFICENS*)

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Abstract. A description of male magnificent frigatebird (*Fregata magnificens*) courting behaviour was made from observations of marked individuals at the breeding colony on Isla Isabel, Mexico. Males actively search for a suitable display site. Display site behaviour was divided into alert/non-alert and non-courting/courting periods. Male activity categories were 'non-courting', 'courting – non-visited' and 'courting – visited'. Non-courting behaviours were 'preening', 'aggression' and 'sitting alone'. Courting behaviour was divided into basic courting, consisting of 'gular pouch inflation' 'wing display', and 'sitting visited', and courtship display elicited by female presence, consisting of 'courtship movements', which was one or two of the behaviours 'beak clackings', 'head movements' and 'wing movements' performed simultaneous, or 'full courtship', when all three behaviours were performed simultaneously, plus 'body shaking' of a visiting female. Included in the courting behaviour were occasionally produced 'drumming' bouts. Male alertness ranged between 49-89% of total observation time, with 'courting – non-visited' males being more alert. Preening was performed 4-25% of total observation time, with 'non-courting' males preening more. Levels of aggression ranged 0.4-3.6% of total observation time only, with 'courting – visited' males being more aggressive. Males performed the basic courting behaviour 68-82% of total observation time, whereas courting behaviour was only performed 2-6% of total observation time. 'Courting – visited' males were more active in the basic courting, whereas 'courting – non-visited' males included more 'drumming' in courtship events. In the late phase of female visit, i.e. just before pair formation, males became less active in both the basic courting and courtship display. The less time spent inflated was also reflected in less events with 'drumming'.

Keywords. Courting behaviour; Courtship display; Fregata magnificens; *Secondary sexual traits*

The sexual ornamentation of male frigatebirds, especially the red and inflatable gular pouch, is a widely used illustration of the often-striking results of sexual selection (e.g. Attenborough 1998, p. 193; Ligon 1999, p. 75; *Nature* 2002, vol. 415, p. 255). Paradoxically, although the male frigatebird courtship behaviour is "the most spectacular of any seabird" (Orta 1992), it has only been superficially described and the role of the gular pouch is virtually unstudied.

The five species of frigatebirds (all congeners) include the Ascension frigatebird (*Fregata aquila*), Christmas frigatebird (*F. andrewsi*), great frigatebird

(*F. minor*), magnificent frigatebird (*F. magnificens*), and lesser frigatebird (*F. ariel*). Traditional morphological classification placed frigatebirds within the order Pelecaniformes, composed of birds with all four toes connected by a web. The six genera included in this order were tropicbirds, pelicans, gannets/boobies, cormorants, anhingas/darters and frigatebirds. Lately, data from DNA-DNA hybridisation (Sibley & Ahlquist 1990), rRNA sequences (Hedges & Sibley 1994), and sequencing of three mitochondrial genes (Siegel-Causey 1997) have shown that these six genera are not monophyletic. The DNA-DNA hybridisation and rRNA sequencing both resulted in frigatebirds being grouped with penguins, storks, New World vultures, shearwaters, pelicans, shoebill and loons (Sibley & Ahlquist 1990). Whereas the results of the sequencing of three mitochondrial genes still placed frigatebirds close to anhingas/darters, cormorants and gannets/boobies, but also close to albatrosses, whilst tropicbirds were grouped with shearwaters and New World vultures, and pelicans with shoebills. Due to the uncertainty of the exact phylogeny of frigatebirds, caution might be needed before comparisons are made between the courtship behaviour of frigatebirds with that of other genera, like those originally included in Pelecaniformes (van Tets 1965).

Male courtship display has been described in some detail for three of the five frigatebird species. For the magnificent frigatebird, the courtship was described by van Tets (1965) as a 'rattling display', during which the gular pouch was inflated and the wings spread laterally and fluttered back and forth. The acoustic component of the display was a rattling together of the mandibles, with the gular pouch possibly acting as a resonator. A male and female sitting together would both do the 'rattling display'. In an other study, Diamond (1973) described the courtship of male magnificent frigatebird, as consisting of out-stretched wings, raised well above the substrate, and vibrated rapidly back and forth ('wing-fanning'), at the same time the head was thrown back, the scapulars raised and the pouch fully inflated. The accompanying vocalizations were 'drumming', when the gular pouch was fully inflated, 'reeling', which was independent of gular pouch inflation and also alternated with drumming during display, and the rarely heard 'purring'. After a female had landed, the male continued the display alternating with 'head-shaking' with the female, to which she responded with a twittering sound, touching the male and 'mutual head-shaking'. Diamond (1973) also found that a low intensity display seemed directed towards over-flying males.

The display of great frigatebird males, as described by Nelson (1975), consisted of a 'gular presentation' and 'mutual head-waving'. The 'gular presentation' was an upward presentation of the crimson inflated pouch, with the head thrown back and turned from side to side, trembling of the spread wings, and accompanying *whoo-whoo-whoo* vocalization. When a female landed by the male, the display became more exaggerated and disjointed, and the pair would do 'mutual head-waving', with bills clashing and heads passing under or over the neck of the partner. In 'mutual head-waving' both sexes vocalized. According to Dearborn et al. (2001) and Dearborn & Ryan (2002), the courtship behaviour of great frigatebird consisted of inflating the red throat pouch, erecting the iridescent ruff, tilting and wagging the head and throat pouch, extending and shaking the wings, and vocalizing. When a female landed next to a male for closer assessment, the male usually would intensify his display behaviour.

The courtship display of Ascension frigatebird, as described by Stonehouse & Stonehouse (1963), took place on the ground of a non-vegetated island. Courting males, as distinguished by their inflated gular sacs, would respond violently to females passing overhead through leaning back on the tail, with the body almost upright, the beak pointing vertically into the air and the wings fully extended along the ground. At the same time the male produced a rhythmically throbbing sound, shaking from side to side, vibrating the wings and clapping the beak noisily. After landing by the male, the female bobbed her head repeatedly in front of the male, pressing forward into his chest or axilla and becoming enfolded by his wings. The two birds clapped beaks, shuffled and circled on the nest site, picking up and dropping nesting material and lunging with their beaks at neighbours.

In summary, the courtship of frigatebird males may be described as consisting of wing and gular pouch display plus vocalization. The display has, however, only been described in very general terms and with often-inconsistent terminology. Furthermore, descriptions were mostly of display in response to female inspection or immediately upon landing of a visiting female, with no descriptions of male display before arrival of the female or later in the often-extended pre-mating visit.

Here, we provide a detailed and quantitative description of male courting behaviour in the magnificent frigatebird, as observed in the breeding colony on Isla Isabel in the Mexican Pacific Ocean. We also describe the changes in male display with female presence and during female visit.

METHOD

Fieldwork was conducted in the September/October to December mating season during four years (1999 to 2002) at a breeding colony of approximately 15,000-16,000 magnificent frigatebirds (Madsen 2005) in the Isla Isabel National Park (21°52'N, 105°54'W), about 70 km off the west coast of Mexico. Since 1994, approximately 2,000 frigatebirds have been tagged with individually numbered yellow rubber strips around the ulna of the left wing within the same area of the colony. Focal observations of display site search behaviour were performed from the end of October to the beginning of December in 2001. Focal observations of display site courting behaviour were performed from mid October to the beginning of December in 2002.

Male search for display site

The movements of unmated, non-courting, wing-tagged males within the study area were observed from a 10-meters high vantage point, overlooking the entire area. Each observation period lasted three to four hours. An observation team consisted of two to five persons, one of whom recorded the behavioural observations on the identified focal males, as dictated by the observers. Onsets and terminations of when the focal was stationary (sitting in the vegetation) together with its take-offs, flight pattern over the study area, and landings were recorded. The precision of recording was ± 0.5 sec. For each male, observations were made for as long as visual contact could be kept. Flying males were often lost to sight momentarily, but observation resumed when they re-appeared. When the same male was observed during more than one observation period, the records were pooled to yield one data point per bird, and only males observed for more than 30 minutes in total were included in the analysis. Durations of stationary and flying behaviour were calculated, and total durations, expressed as a percentage of total observation time, were calculated for each male. The mean total duration for all males of sitting and flying was calculated together with the standard error (mean ± SE).

Unmated males' behaviour at a display site

Males' behaviour at display sites was recorded simultaneously at two different sites for three hours in the morning and three hours in the afternoon. Males chosen for observation were wing-tagged, unmated (i.e. neither with nest nor nest building), and observable from the ground at distances of 15-25 meters without disturbing the male or the colony. At these distances acoustic signals could be heard. When possible, males were chosen so that up to three marked males were in the same visual field. Wing-tagged males, arriving within the same visual field during an observation period, were included in the observations, if less than three birds were already being observed. Efforts were made to obtain 9 hours of observation for each male.

During each 3-hour observation period, each pair of the three pairs of observers observed for two hours, in two to three observation bouts of 30-60 minutes duration. One member of the pair of observers continuously dictated the focal individuals' changes in behaviour and posture to the other member of the pair. Recording precision was ± 0.5 sec, and minimum recorded duration 1 sec. The presence of an inspecting female, i.e. a mate-seeking female circling or hovering over the male, or a visiting female, i.e. a female that had landed by a certain male and was sitting in bodily contact with him, was also registered. Durations of each behaviour were calculated from onsets and terminations, and total durations, expressed as a percentage of total observation time, were calculated for each male. In general, only observations of more than 30 minutes of total observation were analysed. The mean total durations for all males per activity category (Table 1; see below) were calculated together with the standard error (mean ± SE).

Drumming was event recorded, i.e. it was noted when drumming was included in a behaviour, and the number of events, in which drumming was included, expressed as a percentage of total events. The mean percentage of events with drumming was calculated for all males per activity category (Table 1; see below) together with the standard error (mean ± SE).

Characterization of behaviour types

Within each category, several distinct behaviours were distinguished, as outlined below, and divided into alert and non-alert periods, defined by whether or not a male was participating in the events of the colony. During non-alert periods a male would not move the head or eyes in response to events, such as agonistic interactions between other birds or over-flying females. Also the male could close its eyes, revealing white eyelids, and the head might droop below the branch of the display site. The 'preening' and 'body shaking' (see below) were categorized as non-alert behaviours, as the male's head was often out of sight, under the wing or under the female. Non-alert periods also occurred during courting behaviour, as a male might remain with the gular pouch inflated and the wings extended.

Non-courting

In the 'non-courting' category three types of behaviour were identified in accordance with the description in Table 2. The behaviours were: 1) 'sitting alone', 2) 'preening', which was only observed as auto-preening, and 3) 'aggression' in the form of "snapping". During extended aggressive interaction the male might erect the greenish iridescent lanceolated feathers of the head. The "snapping" was interpreted as a threat posture, directed towards the other individual, which could be a juvenile, female or male, either sitting or flying overhead. This behaviour seemingly corresponded to the "bill-snapping" mentioned by Diamond (1973), who interpreted it as male defence of the area around his display site. Dearborn & Ryan (2002) described a similar behaviour in great frigatebird, but limited it to male-male interactions in the form of vocalization and "snap/bite", during which feathers could also be erected.

Courting

In the 'courting' category eight types of behaviour were identified in accordance with the description in Table 3. The behaviours were: 1) 'gular pouch inflation, which is a general trait of male frigatebird courting behaviour. 2) 'wing display', which we found to be independent of the acoustic components of courting behaviour, and therefore an extended behaviour in comparison with the lateral extension of the wings during the 'rattling display', as mentioned by Van Tets (1965). Van Tets further compared the male wing display with the begging display of the juveniles, which we did not consider to be similar, as juveniles let the wings hang down from the body instead of

spreading them out. 3) 'beak clackings', which seemed to correspond to part of the 'rattling display', as described by van Tets (1965), and the 'reeling', as described by Diamond (1973). Stonehouse & Stonehouse (1963) also mentioned that lesser frigatebird males were clapping their beaks nosily. According to van Tets such feature has also been reported in great frigatebird, but neither Nelson (1975) nor Dearborn et al. (2001) or Dearborn & Ryan (2002) included such acoustic element in their general descriptions. 4) 'drumming', which seemed to correspond to another part of the acoustic component of the 'rattling display', as described by van Tets (1965), as he also mentioned that the gular pouch could act as a resonator. Diamond (1973) also described drumming as one of the acoustic signals included in male courtship. The 'drumming' was, however, a very distinct acoustic signal compared with the acoustic component of the male great frigatebird display, presented by Nelson (1975) as a *whoo-whoo-whoo* sound. 5) 'wing movements', which seemed to correspond to the wing fluttering of van Tets (1965) and the 'wing-fanning' by Diamond (1973). Also this display seemed to correspond to part of the male great frigatebird 'advertising display', as presented by Nelson (1975), and the extending and shaking of the wings, as described by Dearborn et al. (2001) and Dearborn & Ryan (2002). 6) 'head movements', which seemingly corresponded to the head movements of the male great frigatebird 'advertisement display', as presented by Nelson (1975), and the wagging of the head and throat pouch, as described by Dearborn et al. (2001) and Dearborn & Ryan (2002). 7) 'body shaking', which differed from the mutual 'rattling display', as described by van Tets (1963), and the 'head shaking', as described by Diamond (1973). It also does not seem to correspond with the 'mutual head-waving' described by Nelson (1975) for great frigatebird. 8) 'sitting visited'.
The behaviours 'beak clackings', 'wing movements' and 'head movements' were performed either individually or in combination. The 'full courtship' was defined when all three were performed together, while individual behaviours or a combination of any two behaviours were termed 'courtship movements'. The 'drumming' behaviour may accompany any of the 'courtship movements', 'full courtship', or 'body shaking'. During courtship and 'body shaking' a male would often erect the "purple" iridescent lanceolated back-feathers, as also mentioned in the general description of the courtship behaviour of great frigatebird (Dearborn & Ryan 2002).

Courting sub-categories
The 'courting' category was divided into the two sub-categories; 1) 'courting – non-visited' and 2) 'courting – visited' (Table 1). For a 'courting – non-visited' male the basic courting behaviours were 'gular pouch inflation' and/or 'wing display'. Upon the approach of a female he seemed to test her interest by adding the behaviours 'solo drumming' and/or 'courtship movements', accompanied by some 'drumming' bouts. If the female showed interest in the male by circling or hovering over the male, he would perform 'full courtship', accompanied by some 'drumming' bouts. For a 'courting – visited' male the basic courting behaviours

were 'sitting visited', 'gular pouch inflation' and/or 'wing display'. To this he could add 'courtship movements', 'full courtship' and/or 'body shaking', all accompanied by some 'drumming bouts'. The 'courting – visited' category was divided into three sub-categories, i.e. 1) '<50%', 2) '≥50%', and 3) '≥100%' visited (Table 1). The distinction between the categories was the total duration of female visit, expressed as percentage of total observation time. The '≥100%' visited males were in the late phase of a female visit, i.e. when the female visitor would stay at the display site even if the male left, and allowed him to land again at the site. This phase preceded pair formation, after which the male started bringing nesting material and copulations took place.

Statistical analyses

Data was analysed in the statistical program Statistica release 4.3, using a non-parametric Kruskal-Wallis test for non-paired observations, otherwise a Wilcoxon matched pairs test.

RESULTS

Display site search

In total 44 males were observed, with a mean observation time of 123.3 ± 13.6 min, and these sat in the vegetation on average 43 ± 5% and flew 57 ± 5% of the observation time. Often males took off, flew low over the study area, and returned to the same sub-area or even to the same site in a tree or bush, or flew low over the colony and landed briefly at several different sites. Once a male settled for a particular display site, he inflated his gular pouch and started scanning for over-flying inspecting females. Display sites were elevated branches at a minimum distance from neighbours of about one wingspan, i.e. about 1 meter (cf. 0.9 ± 0.1 meter for magnificent frigatebird; Trivelpiece & Ferraris 1987, and 0.5-1.0 meter for great frigatebird; Reville 1988). Males usually sat with their backs to the sun.

Display site behaviour

In total 58 males were observed for a total of 265.3 hours (mean observation time 274.5 ± 3.6 min). These males belonged to the 'adult' (N=48) and 'sub-adult' (N=10) age-classes, where 'adults' have an all black plumage, and 'sub-adults' have some white breast feathers only (Madsen 2005). Both age-classes were included in the analyses, as males of both age-classes succeeded in obtaining mates (Madsen 2005), and as no significant differences with age-class were found in any of the following analyses.

For comparisons per activity category, male activity was categorized as 'non-courting', 'courting – non-visited' and 'courting' (Table 1) per each 3-hour observation period. Three males were only observed as 'non-courting'. Eight males had different 3-hour observation periods categorized as belonging to either activity category, of which the 'non-courting' observations were included in comparisons of non-courting behaviours, and the 'courting' in the comparisons of courting behaviours. A total of 55 'courting' males were observed. Of these males, 28 males were never observed with a female visitor, and were therefore classified as 'courting – non-visited'. Of the remaining 27 males, 7 males were only observed with a female visitor, and were therefore classified as 'courting – visited', whereas 20 males were observed both with and without a female visitor, of which only the 'courting – visited' observations were included. The analyses of non-courting behaviour were, therefore, performed for 11 'non-courting' males (mean observation time 114.0 ± 20.7 min), 24 'courting – non-visited' males (mean observation time 123.6 ± 14.1 min), and 23 'courting – visited' males (mean observation time 232.9 ± 31.5 min). The analyses of courting were performed for 28 'courting – non-visited' males (mean observation period 129 ± 16.8 min) and 27 'courting – visited' males (mean observation time 239.7 ± 28.1 min).

For comparisons of courting behaviours (Table 3) per female visit category, observations of 'courting – visited' males were categorized in accordance with duration of the female visit as '<50%', '≥50%', and '≥100%' (Table 1) per 3-hour observation period. Observations per category of less than 30 minutes were included in the analysis. Eleven males had different 3-hour observation periods categorized as belonging to different activity categories. The observations of longest observation time were chosen for analyses. The resulting sample sizes in the analyses were 10 '<50%' males (mean observation period 31.7 ± 8.1 min), 11 '≥50%' males (mean observation period 183.7 ± 26.4 min), and 10 '≥100%' males (mean observation period 240.7 ± 40.2 min).

Non-courting behaviours

Analyses showed that time spent preening differed significantly with male activity category ($H_{2,58}$=18.4, P<0.001), with 'non-

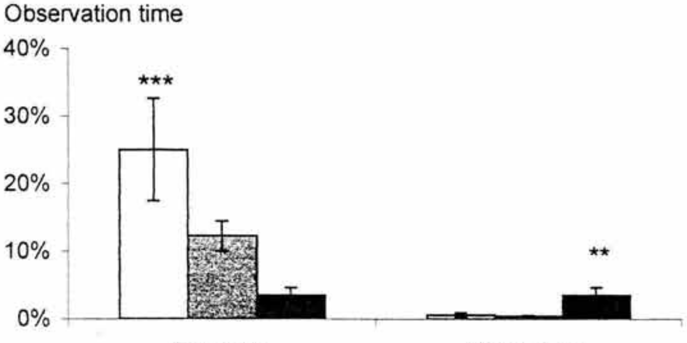

Figure 1. Duration of 'non-courting' behaviour ('preening' and 'aggression', as a percentage of observation time (mean ± SE), by different categories of male activity: 'non-courting' (□, n=11); 'courting – non-visited' (▨, n=24); 'courting – visited' (■, n=23).

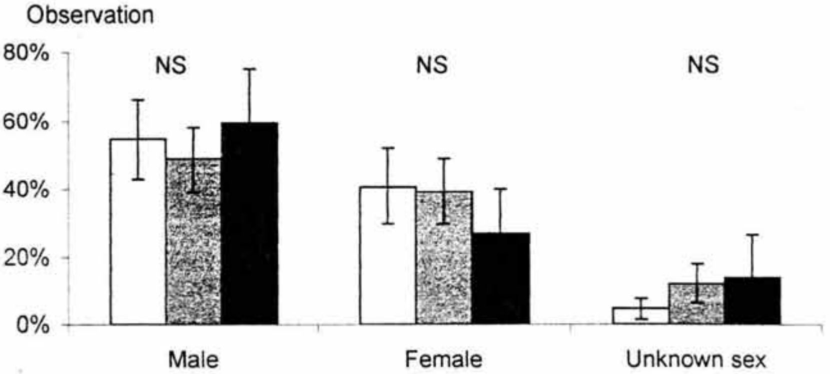

Figure 2. Duration of 'aggression' as a percentage of total aggression time (mean ± SE) directed towards males, females or individuals of unknown sex, by different categories of male activity: 'non-courting' (☐, n=8); 'courting – non-visited' (▨, n=19); 'courting – visited' (■, n=16).

courting' (mean 25 ± 8%) and 'courting – non-visited' males (mean 12 ± 2%) preening more than 'courting – visited' males (mean 4 ± 1%; $H_{1,34}$=12.6, P<0.001; $H_{1,47}$=12.2, P<0.001, respectively; Figure 1). The 20 males that were observed both with and without female visitor, did not differ significantly with regard to preening in the absence or presence of a female visitor ($Z_{1,20}$=1.3, P=0.20), which indicated that preening might be an individual trait. A comparison between 'courting – non-visited' males (N=48) that were (N=20) or were not (N=28) observed with a visiting female, showed that males that obtained female visitors preened significantly less (3 ± 1% versus 13 ± 2%; $H_{1,48}$=14.2, P<0.001). This indicated that preening might reflect some male quality, like ecto-parasite load, that females included in their decision of which males to visit.

The male activity categories also differed significantly in time spent on aggressive encounters ($H_{2,58}$=12.9, P<0.01), with 'courting – visited' (mean 3.6 ± 1.1%) being involved in more aggression than both 'non-courting' (mean 0.6 ± 0.4%) and 'courting – non-visited' (mean 0.4 ± 0.1%) males ($H_{1,34}$=6.0, P<0.05; $H_{1,47}$=11.4, P<0.001, respectively; Figure 1). The 20 males that were observed both with and without female visitor, engaged in significantly more aggression, when visited compared to when not visited ($Z_{1,20}$=2.1, P<0.05) and when compared with other 'courting – non-visited' males not visited ($H_{1,48}$=6.3, P<0.05). There were no significant differences with male activity category in the sex of the counterpart of the aggressive encounters (Figure 2).

Courting

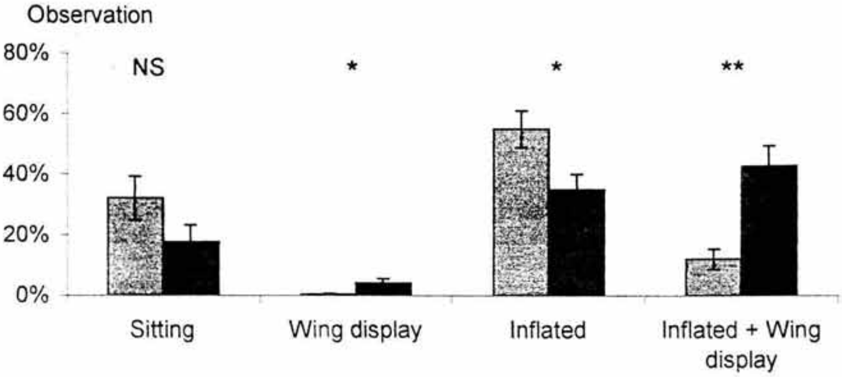

Figure 3. Duration of basic courting behaviours as a percentage of observation time (mean ± SE) by different categories of male activity: 'courting – non-visited' (▨, n=28); 'courting – visited' (■, n=27).

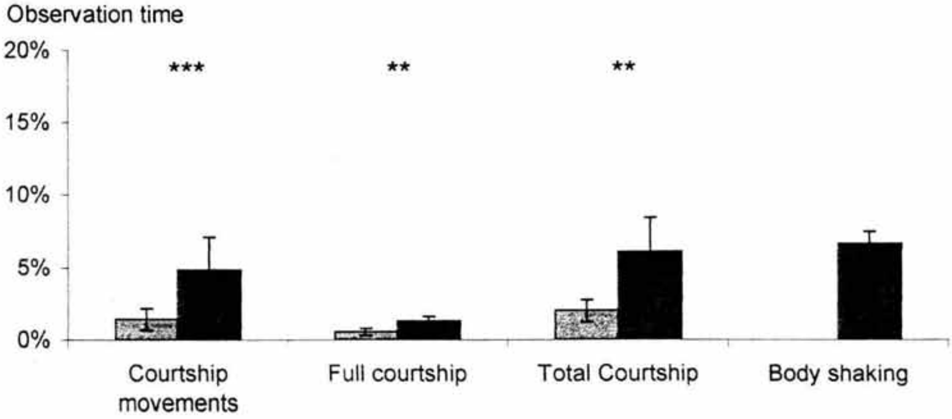

Figure 4. Duration of courtship behaviour in response to female presence as a percentage of observation time (mean ± SE), by different categories of male activity: 'courting – non-visited' (▨, n=28); 'courting – visited' (■, n=27).

The 'courting – non-visited' males were active in the basic courting behaviours, i.e. non-sitting, for a total of 0.4+55+12=67.4% of the observation time (Figure 3), whereas courtship behaviours, in response to the presence of an inspecting female, was only performed during 2% of the observation time (Figure 4). This meant that the time 'courting – non-visited' males performed basic courting behaviour greatly exceeded the time of female presence.

Analyses of the basic courting behaviour per male activity category showed that 'courting – non-visited' spent significantly more time being only 'inflated' than 'courting – visited' males ($H_{1,55}$=5.4, P<0.05; Figure 3). In contrast, 'courting – non-visited' spent significantly less time 'inflated' + 'wing display' and 'wing display' than 'courting – visited' males ($H_{1,55}$=15.6, P<0.001; $H_{1,55}$=4.6, P<0.05; Figure 3). Time spent on the basic courting behaviours did not differ significantly with the presence of a female visitor in the 20 males that were observed both with and without female visitor. This indicated that male basic courting behaviour did not change with female visit, but that females visited more active males, which was also confirmed through comparing the 'courting – non-visited' behaviour of males that were also visited with that of males that were not observed with a visitor, as the former spent significantly more time 'inflated' + 'wing display' (mean 40 ± 6% versus 12 ± 3%; $H_{1,48}$=15.8, P<0.001).

For the courtship behaviours, 'courting – non-visited' males displayed significantly less than 'courting – visited' males ('courtship movements', $H_{1,55}$=12.9, P<0.001; 'full courtship', $H_{1,55}$=8.7, P<0.01; total courtship, $H_{1,55}$=10.5, P<0.01; Figure 4). This pattern was similar in the 20 males observed with and without a female visitor, as males performed significantly more total courtship

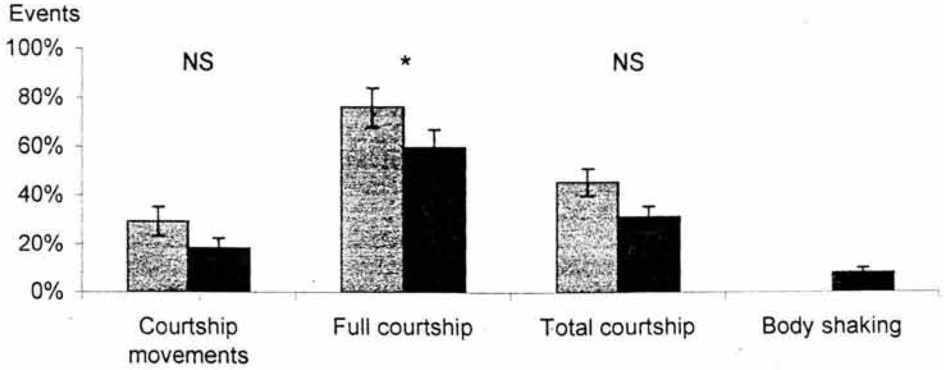

Figure 5. Percentage of courting events including 'drumming' (mean ± SE), by different categories of male activity: 'courting – non-visited' (▨, n=15); 'courting – visited' (■, n=24).

behaviour when visited (mean 7.4 ± 3.0% versus 3.6 ± 1.3%; $Z_{1,20}=2.6$, $P<0.01$), but also the behaviour of the 'courting – non-visited' males that were observed with a female visitor was significantly higher than the males that were not observed with a visiting female (total courtship; mean 3.6 ± 1.3% versus 2.0 ± 0.7%; $H_{1,48}=5.0$, $P<0.05$). Males visited by a female, furthermore, performed the courtship behaviour 'body shaking' so total time spent courting reached 12.8% of the observation time.

The 'courting – non-visited' included drumming in the 'full courtship' more often than 'courting – visited' males ($H_{1,40}=4.0$, $P<0.05$; Figure 5). For all drumming included in courtship behaviour, there were, however, no significant difference (Figure 5). Comparing the courtship behaviour of 'courting – non-visited' males that were observed with a visitor, with that of the males that were not, significantly more drumming was included in the courtship behaviour of the former (65 ± 4% versus 46 ± 6%; $H_{1,43}=8.6$, $P<0.01$). Comparing the same 20 males with and without a female visitor, significantly more drumming was also included during courtship behaviour when non-visited (62 ± 5% versus 39 ± 4%; $Z_{1,20}=3.1$, $P<0.01$). The 'courting – visited' males only infrequently included drumming in the 'body shaking' (Figure 5).

Courting sub-categories

The durations of female visit in the three sub-categories 'courting – visited' males were: '<50%' mean 15 ± 3%, '≥50%' mean 81 ± 4%, ≥100%' mean 111 ± 8%. Dividing the 'courting – visited' category into three sub-categories in accordance with time spent with visiting female, resulted in significant differences in basic courting behaviours ('sitting', $H_{2,31}=19.6$, $P<0.001$; 'wing display', $H_{2,31}=15.0$, $P<0.001$; 'inflated' + 'wing display', $H_{2,31}=9.5$, $P<0.01$; Figure 6). These differences were mostly caused by changes in the behaviour of the category '≥100%', which represented the behaviour

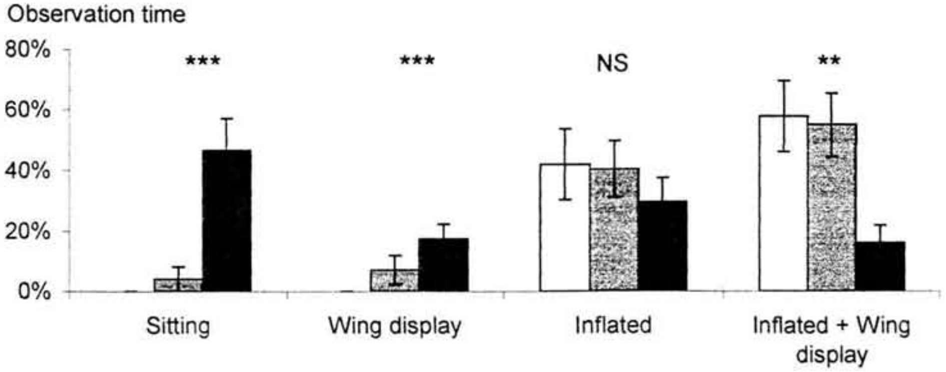

Figure 6. Courtship behaviour forms as a percentage of observation time (mean ± SE), by different categories of female visit activity: '<50%' (□, n=10); '≥50%' (▨, n=11); '≥100%' (■, n=10).

of males in the late phase of female visiting, i.e. just before pair formation. Comparing this category with the other two categories joined confirmed this ('sitting', mean 47 ± 11% versus 2 ± 2%, $H_{1,31}$=19.5, P<0.001; 'wing display', mean 18 ± 5% versus 4 ± 3%, $H_{1,31}$=11.7, P<0.001; 'inflated' + 'wing display', mean 16 ± 6% versus 56 ± 8%, $H_{1,31}$=9.5, P<0.01). Also total time spent inflated, i.e. time spent 'inflated' plus 'inflated' + 'wing display', was significantly less in the '≥100%' category (mean 46 ± 11% versus 98 ± 2%, $H_{1,31}$=12.1, P<0.001). The '≥50%' category showed intermediate behaviour, as also spending more time in the low activity behaviours 'sitting' and 'wing display' than the '<50%' category ('sitting', mean 4.1 ± 1.2% versus 0.0%; 'wing display', mean 7.1 ± 2.2% versus 0.0%; Figure 6).

No significant differences were found between the three categories in time spent in the courtship behaviours (Figure 7).

The amount of courtship events with 'drumming' were significantly different between the three visiting categories ('courtship movements', $H_{2,29}$=11.3, P<0.01; 'full courtship', $H_{2,27}$=16.2, P<0.001; 'body shaking', $H_{2,29}$=7.0, P<0.05; Figure 8). This was mostly caused by a decrease in events with 'drumming' in the category '≥100%'. Comparing this category with the other two categories joined confirmed this ('courtship movements', mean 3 ± 2% versus 32 ± 6%, $H_{1,29}$=11.2, P<0.001; 'full courtship', mean 20 ± 9% versus 82 ± 5%, $H_{1,27}$=15.4, P<0.001; 'body shaking', mean 3 ± 2% versus 19 ± 6%, $H_{1,29}$=5.2, P<0.05; 'courting total', mean 4 ± 3% versus 38 ± 6%, $H_{1,31}$=11.6, P<0.001). This corresponded well with '≥100%' males also presenting less gular pouch inflation (Figure 6). The category '≥50%' again showed intermediate tendencies (Figure 8), but did not differ significantly from the '<50%' category.

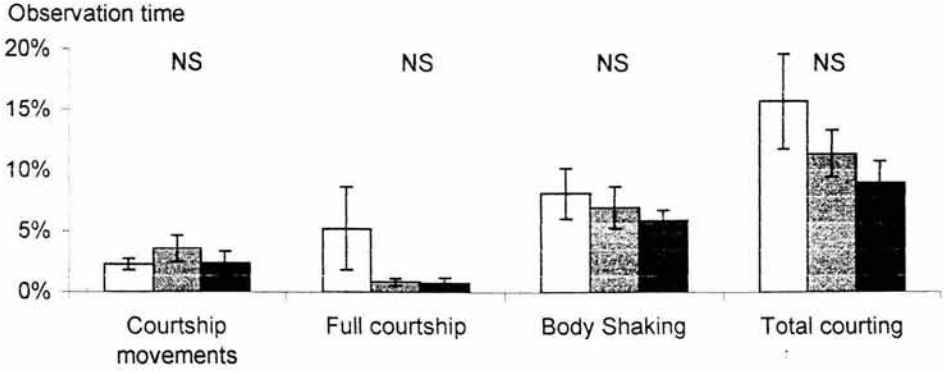

Figure 7. Duration of courtship behaviour as a percentage of observation time (mean ± SE), by different categories of female visit activity: '>50%' (□, n=10); '≥50%' (▨, n=11); '≥100%' (■, n=10).

Alertness

The level of alertness was significantly different with male activity category ('non-courting', mean 54% ± 9%; 'courting – non-visited', mean 79 ± 3%; 'courting – visited', mean 64 ± 6%; $H_{2,58}=6.5$, $P<0.05$), with 'non-courting' males being significantly less alert than 'courting – non-visited' males ($H_{1,35}=6.0$, $P<0.5$). Alertness decreased significantly with longer duration of female visit ('<50%', mean 89 ± 3%; '≥50%, mean 81 ± 5%; '≥100%', mean 39 ± 9%; $H_{2,31}=15.7$, $P<0.001$), with '<50%' and ''≥50%' both being significantly higher than '≥100%' ($H_{1,20}=12.9$, $P<0.001$; $H_{1,21}=9.2$, $P<0.01$).

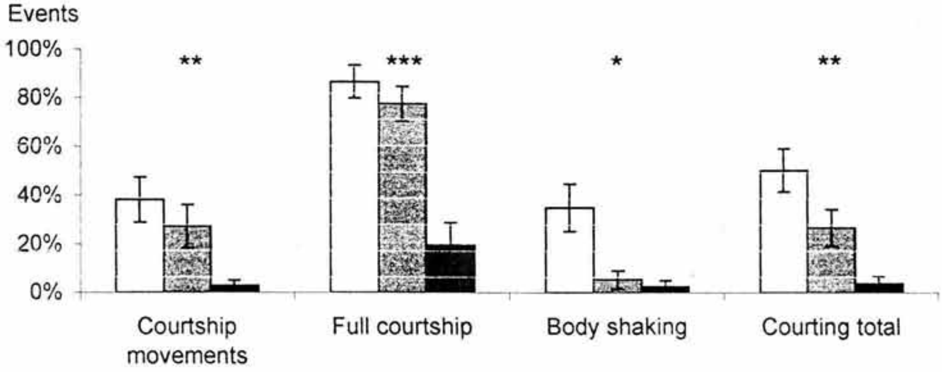

Figure 8. Percentage of courting events including 'drumming' (mean ± SE), by different categories of female visit: '<50%' (□, n=7); '≥50%' (▨, n=8); '≥100%' (■, n=10).

DISCUSSION

The mating season behaviour of magnificent frigatebird was more complex than previously described, with a total of eleven distinct behaviours divided into three 'non-courting' and eight 'courting' behaviours. The eight courting behaviours were further divided into the basic courting display and the actual courtship display that was added to the basic display in the presence of an inspecting or visiting female. The unmated males were divided into three activity categories, based on their behaviour and female presence, with visited males furthermore being subdivided into three categories in accordance with total duration of female visit.

Comparing the different courting behaviours identified in this study with the 'rattling display', as described by van Tets (1965), the latter was divided up into several distinct courting behaviours. Van Tets (1965) did not, however, see the 'rattling display' as a courting behaviour only, but stated that it "apparently serves as a mutual recognition display, as a male advertising and sex-recognitions display, and as an announcement of imminent arrival at the nest". This would suggest that the 'rattling display' covered most, if not all, of the acoustic communication between males and females during the entire breeding season. However, we suggest that a thorough investigation of the acoustic components of frigatebird behaviour may result in the identification of several distinct acoustic signals. In our study population, the nest approach signal sounded distinct from the 'beak clacking'. This was supported by the fact that the nest approach signalling was also performed by males while carrying nest-building material in the beak that would prevent beak clacking. We also found the 'beak clacking' to be distinct from the low "squeaking" sound of the nest-release signal made by males arriving at the nest for their turn in incubation. Van Tets (1965) also stated that visiting females performed a display similar to that of the males, which was not supported by our observations, as we only found that the female responded to the 'body shaking' by rubbing her head against the male and vocalizing. Diamond (1973) also did not register any female display that resembled the male display.

The division of the acoustic components of the courting behaviour of magnificent frigatebird by Diamond (1973) into 'drumming', 'reeling' and 'purring' was more or less supported by our observations, as the reeling probably was the equivalent of the 'beak clacking'. The purring, which should be given to males, females and juveniles alike, did not, however, concede with any of our observations. The description of the full display to females as "outstretched wings rapidly vibrated, head thrown back, and pouch fully blown out" resembled the behaviours we characterized as 'wing movements', 'head movements' and 'gular pouch inflation'. Diamond (1973), however, stated that a low intensity version of the display was also given to over-flying males, which we did not find during our observations. Diamond (1973) further described the behaviour during the female visit as continued full display and 'mutual head shaking', but the 'body shaking' described in this study did not take the form of head shaking only. The difference might be due to lack of detail in the description provided by Diamond (1973) or because differences in the courtship display might exist on a population level.

When comparing the display of the magnificent frigatebird, as described in this study, with the description of the 'advertisement display' for great frigatebird by Nelson (1975) and the general description by Dearborn (2000) and Dearborn & Ryan (2002), there seemed to be great similarities in the display of the two species. Two of the three elements, i.e. gular pouch presentation through head movements and trembling of the wings, seems to correspond well with the 'gular pouch inflation', 'head movements' and 'wing movements' identified in this study. There were, however, subtle differences in the display of the two species, as exemplified by the display of one male great frigatebird visiting our colony for two weeks in 2002, the behaviour of which, identified in video recordings, has been confirmed by D. Dearborn (pers. comm.) to be fairly typical of the species. Head movements of this great frigatebird male were more side wards "snaking" movements, whereas in magnificent frigatebird the head and gular pouch were thrown from side to side. The wing display also differed, in which the great frigatebird male only trembled the wings, whereas the magnificent frigatebird males shook the wings vigorously. The courtship vocalization was also very distinct, with the great frigatebird producing the ghost-like *whoo-whoo-whoo* sound, as described by Nelson 1975, whereas male magnificent frigatebirds produce the drumming sound. Finally, the great frigatebird does not appear to conduct the 'body shaking' display (D. Dearborn pers. comm.). The great frigatebird equivalent to this display might thus be the 'mutual head-shaking display', as described by Nelson (1975).

The behaviour of the mate-seeking frigatebird female has been little described, but seems to be similar for all species (Diamond 1973; Reville 1988; Reville 1991). In our study colony of magnificent frigatebird, a mate-seeking inspecting female would fly low over the colony. In response to male courting behaviour she might circle or hover over the displaying male before either continuing the flight or landing by the male. A female visit, where the male and female sat in close proximity at the display site, might last from seconds up to several days, as also described for great frigatebird (Dearborn & Ryan 2002). Only extended visit periods resulted in mating. At the end of the visit period, the pair started the nest building and copulations took place.

The 'non-courting' males seemed actively searching for a suitable display site, as they moved extensively within the colony and spent about half of their time flying over the colony or from site to site. When sitting in the vegetation they had relative low levels of alertness and spent more time than any other male activity category preening. The 'non-courting' males did, however, not spend more time engaged in aggressive encounters than 'courting' males, which indicated that, even though a display site was essential for a male, it was not a resource over which males fought extensively. This was also supported by 'non-courting' males not engaging in more aggressive encounters with males than any of the other male activity groups. In contrast, the time spent in aggressive encounters by 'courting – visited' males (3.6%) was greater than 'non-courting' and 'courting – non-visited' males (0.4-0.6%). This result was in accordance with the finding of Nelson (1975), who reported that only upon mating did frigatebirds start expressing territorial behaviour in the form of defending their nest site and nest.

The general very low levels of aggressive behaviour observed in this study corresponded well with the 0.3% reported by Dearborn and Ryan (2002) for great frigatebird, based on the behaviour of courting males only. Trivelpiece & Ferraris (1987) also found very low levels of male magnificent frigatebird aggression (34 events during 126 hours of observation). However, in contrast with this study, they found that aggressive interactions were mainly conducted between males (88%). Reville (1991) reported that no instances of threat or fighting was seen between courting lesser frigatebird males, whereas great frigatebird males frequently engaged in treat and fighting. Finally, van Tets (1965) did not register any intraspecific threatening or fighting during two weeks of observation of magnificent frigatebird. We did not observe any fights during the focal observations either, and only two incidents of fighting were ever observed in unmated birds. Both of these fights were between two females sitting by the same male at his display site. The fighting was very fierce with the females trying to grab the other by the head, which, with the hooked beak of frigatebirds, looked like it might cause serious eye damage. Otherwise they went for the neck, which looked like one female was trying to strangle the other. The displaced female was both times thrown off the branch, ending up on the ground.

The 'courting – visited' males were more active than 'courting – non-visited' males in the basic courting behaviour, as they spent most time with both the gular pouch inflated and the wings in display. The 'courting – visited' males also spent more time on courtship displays than 'courting – non-visited' males. When considering the stimuli received by the males in the form of female presence, the 'courting – visited' males might, however, also be much more stimulated than 'courting – non-visited' males. For 'courting – non-visited' males, an inspecting female was only present during the total time of courtship display (2%), whereas for 'courting – visited' males a visiting female was present all of the time. This corresponded to 'courting – visited' males displaying in the presence of a female about 50 times more than 'courting – non-visited' males. One aspect of the display of 'courting – visited' males that was not included in this investigation was the display that took place at night. When walking through the colony at night it was very noticeable that the activity did not cease at sundown, as 'beak-clacking' and female vocalization in response to 'body shaking' could be heard from the sites of 'courting – visited' males. The 'courting – visited' males did therefore seem to perform both day and night, whereas 'courting – non-visited' males could rest, drink or maybe even forage during the night. The intensity of the courtship display might thus be better expressed by the percentage of events with drumming included, where 'courting – non-visited' males included more drumming in the 'full courtship'. For great frigatebird it was reported that males spent 29.3% of their time performing courtship displays (Dearborn & Ryan 2002), which seems to be about twice as much as the time used by magnificent frigatebird. However, it is difficult to make comparisons without exact definitions of which behaviours and male categories were included in the observations.

When comparing the different durations of the female visit, 'courting – visited' males reduced the intensity of the display during the last phase, as less time was spent with the gular pouch inflated, and more time was spent just sitting and with wing display, than in the other categories. This was also reflected by a marked drop in the number of events with drumming, as only males with the gular pouch inflated were able to produce the drumming sound. The marked differences in male courting behaviour with activity category and duration of female visit, showed that caution is required, if one wish to relate differences in male courtship behaviour with actual mating success. This was also emphasized by the fact that even within the group of males that were observed in the last phase of courting, only four out of ten males were later observed nest building or with a nest, showing that the female might end up leaving a male again, even after having spent considerable time with him.

With this detailed description of the courting behaviour of male magnificent frigatebird we hope to have confirmed that the behaviour is as spectacular as stated by Orta (1992), and that their most striking ornament, namely the large red inflatable gular pouch is an important element of the courtship display, as almost all of the courting behaviour was conducted with an inflated gular pouch. Investigations into the importance of the gular pouch in mate selection situations have, however, resulted in a bit more diverse picture. The gular pouch coloration in itself does not seem to be of key importance to females, as redness was not found to differ between early and late mated males of great frigatebird (Dearborn & Ryan 2002), nor between successfully and unsuccessfully mating males in magnificent frigatebird (Madsen 2005). Redness of the gular pouch may instead function as a means of attracting female attention, either through enhanced contrast with the green background, or through signalling the mating status of the male, as only unmated males have a red and inflatable gular pouch. Alternatively, in magnificent frigatebird the size of the gular pouch was reflected in the drumming sound produced during courting behaviour (Madsen 2005) and successfully mating males had larger gular pouches than unsuccessful males (Madsen 2005). Finally, the drumming rhythm constancy and speed also differed between successfully mating and unsuccessful males, where males that mated drummed at a more constant and faster rhythm than unsuccessfully mating males (Madsen 2005). The gular pouch therefore seemed to play both a visual and an acoustic role in the display of magnificent frigatebird of which only the latter seemed to be directly involved in the mate choice decisions of the females.

ACKNOWLEDGEMENTS

This manuscript was completed after the much too early death of José Luis Osorno. Hugh Drummond and Alejandro Córdoba very kindly read and provided very constructive criticism to early drafts of the manuscript. Raul Ortiz-Pulido assisted very constructively with the statistics. We further wish to thank the administrators of the national park Isla Isabel (SEMARNAT) for permissions and logistic support. VM was funded by a Ph.D. fellowship from Universidad Nacional Autónoma de México, and the practical expenses were funded by the Danish Research Agency. Some support was also received from the Instituto de Ecología, UNAM and CONACyT grant (No. 34899-V). Various international volunteers assisted very enthusiastically in the fieldwork.

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Article II - Plumage iridescence

Madsen, V., Osorio, D., & Osorno, J.L. submitted ms. Iridescent coloration and other plumage features as predictors of age and mating success in male magnificent frigatebirds (*Fregata* magnificens). Submitted to American Naturalist for a special number of plumage coloration.

IRIDESCENT COLORATION AND OTHER PLUMAGE FEATURES AS PREDICTORS OF AGE-CLASS AND MATING SUCCESS IN MALE MAGNIFICENT FRIGATEBIRDS (FREGATA MAGNIFICENS)

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Abstract. Compared with pigment colours it is difficult to make consistent and meaningful measurements of iridescent spectra, which may account for the paucity of work on the subject. Here we obtain replicable reflectance spectra from magnificent frigatebird (*Fregata magnificens*), by adjusting feather orientation to maximize reflectance. Back- and breast-feathers are brightly iridescent, with an approximately sinusoidal three-peaked spectrum (300 to 750nm), directed over an angle of 20°. For analyses relating coloration to age and mating success we chose three types of variables to represent the spectra, namely: amplitudes and wavelengths of reflectance maxima/minima, and the ratios between reflectance peaks and troughs. From each of these sets of variables we calculated one composite variable. Iridescent spectra from 214 males did not vary significantly with age, but the proportion of each feather bearing iridescent coloration, body condition, plus tail and tail-fork length all increased significantly with age. Mated males had back-feather iridescence with slightly, but significantly, shorter reflectance wavelength spectra than non-mated males. No other feather features or condition varied significantly with mating status. A discriminant function analysis, correctly classified 66% of males by age or status, but it seems that iridescent coloration is at most weakly correlated with other biological variables.

Key words: *age-class*; Fregata magnificens; *iridescence*; *mate choice*; *plumage features*; *structural feather coloration*.

Birds often use many separate signals in their mating displays, from specialized calls and movements to flesh and feather designs and coloration (Andersson 1994; Ligon 1999). To interpret multi-component signals it is necessary to establish what information each component conveys to receivers, and how they are used. For instance different types of feather pigmentation may signal different aspects of condition or quality (Gray 1996; Owens and Hartley 1998; Badyaev and Hill 2000; Senar et al. 2003). Thus the extent of melanin pigmentation can signal social status (Rowher 1975, 1982; Roper 1986; Møller 1987; Senar et al. 1993; McGraw et al. 2003), and melanin deposition is (fairly) independent of nutritional limitations (Gonzalez et al. 1999; McGraw et al. 2002), or parasite load (McGraw and Hill 2000) at moult. In contrast, carotenoids are often used in mate choice, and the quality and/or extent of carotenoid coloration reflects nutritional condition (Hill and Montgomerie 1994; Hill 2000;

Johnsen et al. 2003; Tschirren et al. 2003), and parasite load (McGraw and Hill 2000; Figuerola et al. 2003) at time of feather growth.

Although structural coloration is probably widely used in signalling it has been less studied than pigmentation. Structural colours are produced by interference between light waves reflected from more or less regularly spaced refractive index boundaries within composite structures, in feathers the materials are keratin, melanin and air. Owing to their dependence on nanoscale order (Land 1972; Fox 1976; Dyck 1976; Prum et al. 1998, 1999; Andersson 1999; Land and Nilsson 2002; Zi et al. 2003) structural colours could be a rich source of information. Hue, brightness, colour purity, and their directionality might all reflect condition during or after growth. Larger scale variations across feathers could reflect developmental stability.

Some structural colours are iridescent, in that their wavelength changes as they move, and these are often directional, resembling coloured mirrors. Others are non-iridescent, with directional properties more like ordinary pigmented surfaces. In practice there is a continuum between these two types (Prum et al. 1998; Osorio and Ham 2002). Relatively non-iridescent structural colours in feathers of blue grosbeaks (*Guiraca caerulea*; Keyser and Hill 1999), brown-headed cowbirds *(Molothrus ater*; McGraw et al. 2002), and blue tits (*Parus caeruleus*; Johnsen et al. 2003) are affected by nutritional status during feather growth. Furthermore, features of structural coloration reflect male quality (Keyser and Hill 2000; Siitari and Huhta 2002; Doucet and Montgomerie 2003; Siefferman and Hill 2003; but see Smiseth et al. 2001; Perrier et al. 2002). Non-iridescent structural coloration seems to be used in courtship displays (Hausmann et al. 2003), and to influence mate choice by bluethroats (*Luscinia s. svecica*; Andersson and Amundsen 1997; Johnsen et al. 1998) and blue tits (Andersson et al. 1998; Hunt et al. 1999), but not blue grosbeaks (*Passerina caerulea*; Ballentine and Hill 2003). Iridescent colours are self-evidently used in sexual signalling, as on the peacock's tail, but remain little studied. An exception is for the starling (*Sturnus vulgaris*), where iridescent coloration correlates with mate preference (Bennett et al. 1997). By comparison, iridescence in the UV did not correlate with mating status and other features preferred by females in barn swallows (*Hirundo rustica*; Perrier et al. 2002).

The paucity of studies of iridescent plumage may partly be due to the difficulty in obtaining meaningful measurements. These mirror-like surfaces are highly directional - in some cases a rotation of one degree can cause a 2-fold change in brightness (Osorio and Ham 2002; unpublished observations). Consequently it is difficult to document their reflective properties. No one viewing geometry (Figure 1*A*) gives the maximum - or even a non-zero – reflectance from all iridescent feathers (Osorio and Ham 2002). Subjective appearance may also be misleading, because their multi-peaked reflectance spectra (Figure 2) mean that iridescent colours may have different appearances for humans and birds

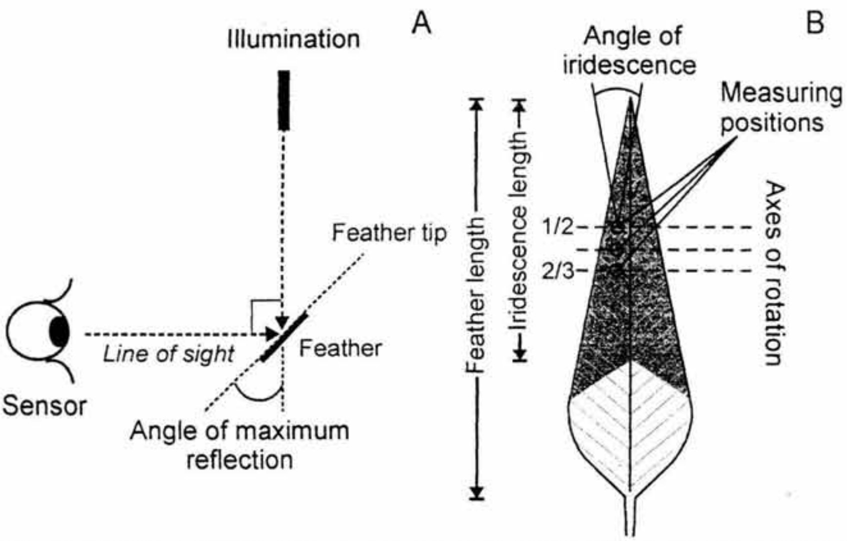

Figure 1. *A*: Viewing geometry. The line of sight was horizontal and the light source placed vertically above the feather, at an elevation of 90°. The feather was rotated to maximize reflectance, and spectra measured at this angle and 5° either side (see also Osorio and Ham 2002). *B*: A sketch of a lanceolated male back-feather, with shading indicating area of iridescence. Shown are the three locations where iridescent spectra were measured. Axes of rotation were in the plane of the main vane and ran through the viewed point.

(Bennett et al. 1994; Hart 2001; Vorobyev 2003), often being more saturated (i.e. colourful) for the birds. For instance, spectra that look grey to us, may present saturated non-spectral hues for a bird (Osorio and Ham 2002).

In this study of magnificent frigatebirds (*Fregata magnificens*) the aim was to investigate the relationships between iridescent coloration, and other measures of body and plumage condition – such as tail and tail-fork lengths -, age and mating success of the male frigatebirds. In order to collect replicable measurements of iridescent reflectance spectra, a method using adjustable viewing geometry was developed. We then established that measures were similar for different feathers from a given bird, and hence can characterize coloration an individual. This meant it was meaningful to investigate the relationships of coloration for between male comparisons of male age and mating success.

Frigatebird biology

Frigatebirds are sexually size and colour dimorphic seabirds. Females are larger than males, and have drab blackish-brown plumage. Adult males of all five species are strikingly black, ranging from totally black (e.g. the magnificent frigatebird) to having some white/lighter areas (e.g. lesser frigatebird, *F. ariel*). All males have iridescent lanceolated feathers, which look purple and greenish in the magnificent

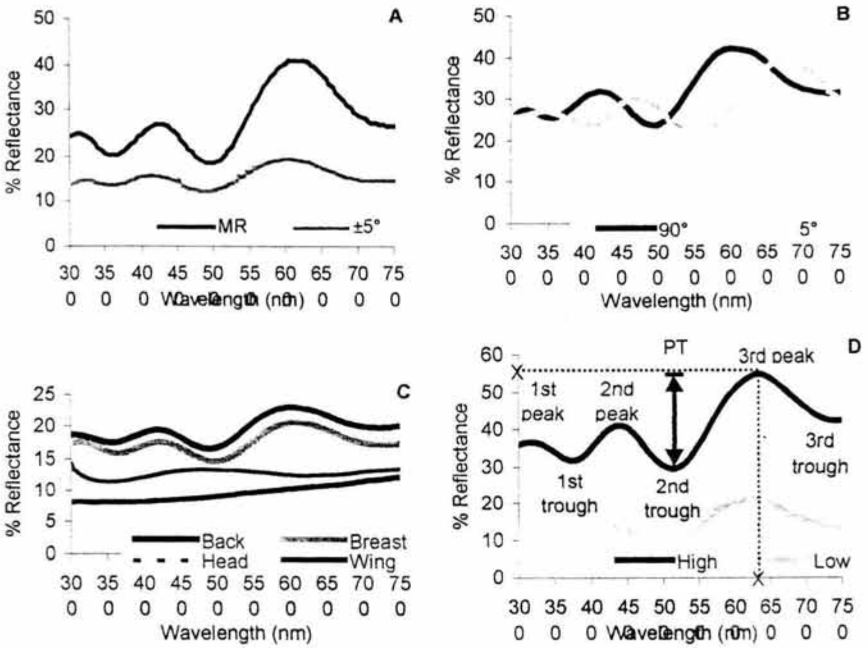

Figure 2: Reflectance spectra, relative to a barium sulphate standard. *A*) Spectra at the angle of maximum reflectance, and the average with the feather rotated 5° either side of the maximum. *B*) Effects of varying the elevation of the light from 5° to 90° above the line of sight. As expected the reflectance curve is blue shifted as the angular separation of the viewer and light source increases (Osorio and Ham 2002). *C*) Spectra at the angle of maximum reflectance for back, breast, head and wing feathers from a male magnificent frigatebird. The first three had iridescent coloration. The separate peaks in violet/blue and red parts of the human visible spectrum account for the purplish colour of the back feathers. *D*) Examples of relatively high and low reflectance back-feathers at the angle of maximum reflectance, and the amplitudes and locations of the peaks and troughs used to characterize the spectra. Analyses did not use the 1st peak, as owing to noise in records this is not always detectable. The remaining five data-points were highly correlated, for reflectance (r_S: 0.91-0.99, N=25, P<0.001), wavelength (r_S: 0.75-0.98, N=25, P<0.001), and peak/trough ratio in reflectance (r_S: 0.72-0.82, N: 25, P<0.001).

frigatebird (Harrison 1985; V. Madsen personal observation).

All frigatebirds are monogamous, but, in contrast to other species, the female magnificent does most of the chick rearing. Magnificent males participate fully in incubating and brooding the young chick, but then desert (Diamond 1973; Osorno 1999; Osorno and Szekély 2004). Magnificent females may therefore breed successfully only every second year, whereas males may breed annually. Consequently the operational sex ratio is male biased; on average only 57.5% of magnificent males pair each year (V. Madsen, unpublished data). Some males attract a mate most years, whereas others consistently fail (V. Madsen, unpublished data). This creates a basis for relatively strong sexual selection. Of the mechanisms of mate competition mentioned by Andersson (1994), sexual selection in the magnificent frigatebird seems to be exerted mainly through female choice. This is so as male-male aggressiveness is very low (V. Madsen, unpublished data) and as no or only extremely low levels of extra-pair paternity exist (M. Serrano, pers. comm.).

Male frigatebirds have two conspicuous morphological sexual traits, the inflatable red gular pouch, which after mating shrinks and reverts to normal skin colour (Reville 1991; V. Madsen, pers. obs.), and iridescent plumage (Harrison 1985; Orta 1992; Dearborn and Ryan 2002). To attract a mate, males select a display site, inflate the pouch and scout for airborne females. The display site will also be the later nest site. The female shows interest by flying, circling or hovering over a male, who then erupts into an intense courtship display (Diamond 1973; Nelson 1975; Dearborn and Ryan 2002; Madsen & Osorno unpubl. ms.). Magnificent males waggle the wings vigorously, arch the back, throw the gular pouch from side to side, clack the beak, and from time to time produce drumming calls involving the gular pouch as a resonator (Madsen et al. 2004; Madsen & Osorno unpublished manuscript).

METHODS

Magnificent frigatebirds were studied in a breeding colony of approximately 15-16,000 birds at the Mexican national park Isla Isabel (21°52'N, 105°54'W) during October to December 2000 to 2002. Two hundred and fourteen non-juvenile male frigatebirds were captured by hand at nights with no moon, and issued with a wing tag that gave a unique number and colour code. Sexing was by characters given in a field guide (Harrison 1985). Age-classification of males was by the extent of white in the plumage; completely black males were aged as adults, sub-adults had some white feathers on the ventral part only, juvenile/sub-adults had some white head feathers. The correctness of this age-classification was confirmed by re-sightings of marked males, over a period of two to four years, where the age score increased in accordance with our assumptions, i.e. males developed a plumage with less white with age (V. Madsen, unpublished data).

Feather samples consisting of two feathers from the head, wing covert, and back, respectively, were collected from the 214 males. The two feathers were taken at random from two different positions within the area, under light-conditions (head-lamps only) that did not allow for prior assessment of iridescence levels. From 43 males samples of one feather from each of the two breast patches situated on either side of the gular pouch were also collected. The iridescent coloration is delicate and was never handled, i.e. only the non-iridescent part of the feather was ever touched. The length of the left ulna and culmen was measured (±0.5mm) and the bird weighed (±25g). The deeply forked tail consists of 12 feathers, and the length of the outermost tail-feathers and the tail-fork were measured (±0.5mm). Mating status of marked males was recorded throughout the field season, and mating success assigned at the end of December, when the mating season was practically over. Males that were registered as mated two or more times during the field work period were classified as 'mated'. Males that were still observed as unmated in December were classified as 'unmated'. Males that were not observed in December, or had not been registered as mated, were classified as of 'unknown mating status'.

Feather samples
For spectral measurements the feathers were first manually straightened, and mounted on matte black cards. The length of the feather and the iridescent part, when present, were measured (±0.5mm; Figure 1*B*). This gave the proportion of the total feather length bearing iridescent coloration. Another piece of card was then placed over the feather with a 7 mm diameter aperture over the area to be measured. For wing-feathers this was left of the vane and 5-6 mm from the tip, for head-feathers it was centred on the vane and 9-11 mm from the tip, and for breast- and back-feathers it was left of the vane and within the iridescent part. The more detailed analysis of iridescence on breast- and back-feathers averaged three locations in the iridescent part along the left side of the vane. Averaging was done to minimize any colour variation due to growth bars (e.g. Murphy et al. 1989; Machmer et al. 1991). The three locations were I: ½, II: midway between ½ and ⅔, and III: ⅔ down the iridescent part from the feather-tip (Figure 1*B*).

Apparatus
The recording apparatus allows adjustment of the position of the light source, and the orientation of the feather surface relative to the recording line-of-sight (Figure 1*A*); it is described in more detail by Osorio and Ham (2002). The mounted feather was placed in a 160 mm radius Cardan arm perimeter, and illuminated by a 6 mm (i.e. 2.1°) UV transmitting liquid light-guide lit by a 400W Xenon arc. The spectrum from 300 to 750 nm was recorded by focusing light from a 1 mm diameter spot on the feather surface with a quartz lens onto a 0.2 mm light-guide connected to a spectrometer (S2000, Ocean Optics). A matte-white teflon reference was used for calibration, and reflectance spectra of this reference measured relative to a standard of freshly pressed (medical-grade) barium sulphate. Before each feather was measured the set-up was recalibrated by taking dark and white references. To establish the stability of the apparatus a series of nine repeated measurements of the white reference at six wavelengths at the approximate values of the peaks and troughs of the feather spectra was performed. Reflectance spectra measurements were stable at these six wavelengths, with coefficients of variance ranging from 0.0045-0.0070%.

Viewing geometry
Measurement of iridescent coloration greatly benefits from having a well-defined and adjustable geometry between the light source, reflective surface and

viewer. The feather was aligned so that the lateral (i.e. width ways) axis of the blade was normal to the line of sight, and its orientation relative to the line-of-sight could be varied by rotation about this axis (for details see figure 1*A* and Osorio and Ham, 2002). Two possible viewing geometries were tested. Firstly with the line of sight and light source nearly co-axial (separated by 5°), as if the sun were almost directly behind the viewer. Secondly, with the light source at the pole, elevated 90° above the line of sight, as if male and female frigatebirds were are at same level, and the sun was directly overhead. As expected reflectance spectra shift to shorter wavelengths as the angle between viewer and light sources increases (Figure 2*B*; Land 1972; Osorio and Ham 2002). For the main study a 90° angle between the light source and the line-of-sight was used.

Analysis of reflectance spectra

After recording, reflectance spectra were smoothed by convolution with a Gaussian (SD approximately 3nm), and reflectance amplitudes plotted at 5nm intervals from 300 to 750 nm (Figure 2*C*). Smoothing removes high frequency noise, but otherwise does not affect the data. Wing-feathers lacked iridescent coloration, and head-feathers had one small peak (Figure 2*C*). We therefore concentrated on back- and breast-feathers, and characterized their spectra by locating wavelengths and amplitudes of reflectance peaks and troughs (Figure 2*D*). Spectra had three peaks and three troughs in the 300 to 750 nm range (Figure 2*D*; see below). Sometimes 1st peaks were not discernible, due to poor sensitivity at short wavelength, and although their location and amplitude can be accurately predicted from longer wavelength measures they were ignored in analyses.

Maximizing reflectance

With an ordinary specular (i.e. mirror) surface, where the angle between illumination and viewer is 90°, the mirror looks brightest at an orientation of 45°. With the iridescent frigatebird feathers the angle of maximum reflectance (MR) was close to but not exactly at 45° to plane of the feather blade. The deviation from 45° gives the angle of tilt in the iridescent reflector relative to the feather surface (using the terminology of Osorio and Ham 2002). For back-feathers tilt ranged from -1° to -8° (N=25). We used measurements with the feather surface oriented to maximize the reflectance, and 5° either side of this maximum. To check the stability of measurements at the angle of maximum reflectance, one back-feather from ten individuals, chosen at random, was measured twice at this angle. Individual means from repeated measurements at three positions were very highly significantly correlated (reflectance: mean r_S=0.99, range: 0.94-1.00, P<0.001, wavelength: mean r_S=1.00, range: 1.00-1.00, P<0.001; peak/trough: mean r_S=1.00, range: 1.00-1.00, P<0.001). Using the angle of maximum reflectance rather than a fixed geometry has two advantages. First, the maximum reflectance is probably of most biological relevance, as this would be the maximum stimulus possible, and secondly, it is easier to obtain consistent measurements. By comparison orienting the feather at a fixed angle introduces a substantial variation in reflectance amplitudes, which is unlikely to be biologically meaningful. Measuring ten back-feathers at both the fixed angle of 45° and the angle of maximum reflectance resulted in a mean difference in reflectance amplitudes of 36% (range 13-63%).

It should be noted that one would never normally measure reflectance spectra of ordinary pigment based coloration with this viewing geometry. This is because the geometry maximizes spectrally unselective specular reflectance, so minimizing the saturation of the pigment colour. However, frigatebird iridescent feather surfaces, as in other species, abolish this non-spectrally selective highlight, so that the colour's brightness and saturation are maximized simultaneously (Osorio and Ham 2002). This unusual quality helps give iridescent structural coloration its striking visual effect.

Characterizing iridescent reflectance spectra

The sinusoidal form of the reflectance spectra immediately suggest that they can be described by a small number of parameters, which are probably determined by feather nanostructure (see Introduction). We chose three variables to describe these spectra, which were almost entirely independent of one another, and account for most of the variation in reflectance spectra (Figure 3). The three variables were based on reflectance amplitude (*RF*), wavelength *(WL*), and peak to trough ratio (*PT*). These measures correspond roughly to brightness, hue and saturation in human colour perception. It is, however, especially difficult to make interferences about colour appearance of multiply peaked spectra (Figure 3*A*), and as this paper is not concerned with modelling appearance either for humans or birds, we will not discuss this matter further.

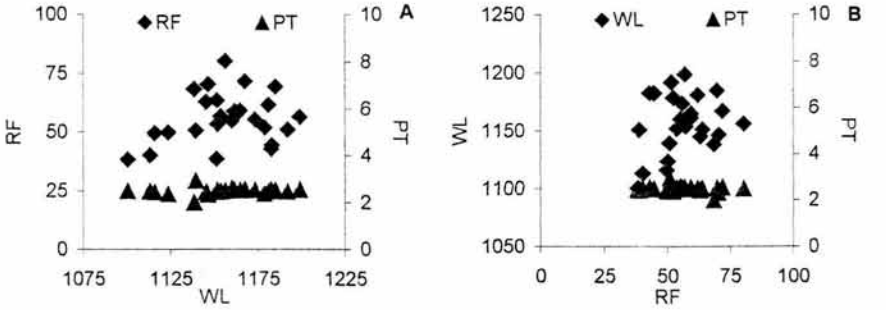

Figure 3. The data points for the three features describing the colour spectra were reduced to one variable each by use of principal component analysis (see Method). The resulting new variables *RF*, *WL*, and *PT* were only weakly correlated with one another (r_S: 0.16-0.25, N=25, P: 0.22-0.44).

1) *Reflectance amplitudes*: The reflectance of the peaks and troughs were strongly correlated to each other (r_S range: 0.91-0.99, $N=25$, $P<0.001$). One Principal Component (PC) was therefore extracted based on correlations and the resulting eigenvectors used to reduce the 5 data points (2 maxima and 3 minima) to one variable (*RF*) for reflectance per individual. The PC extracted explained 96.5% of the original variation. 2) *Reflectance wavelength*: The wavelengths of peaks and troughs were significantly correlated (r_S range: 0.75-0.98, $N=25$, $P<0.001$). One PC was extracted based on correlations and this variable (*WL*) representative of the entire spectrum was calculated per individual. The PC explained 92.2% of the original variation in peak and trough wavelengths. 3) *Peak/trough ratio*: The ratios of peak to trough reflectance across the spectrum were correlated (r_S range: 0.72-0.82, N: 25, $P<0.001$). One PC was extracted based on correlations and one variable (*PT*) representative of the entire curve calculated per individual. The PC explained 94.9% of the original variation in peak to trough ratios.

The variables (*RF, WL, PT*) were only weakly correlated (r_S range: 0.16-0.25, $N=25$, P range: 0.22-0.44; Figure 3*A*,*B*). All three variables were, therefore, included in subsequent analyses.

Within male variation

Before making between-males comparisons it was essential to establish that measurements of a single iridescent feather were representative of the individual. We therefore tested two feathers from each of 25 males and found spectral variables to be highly correlated (*RF*: $r_S=0.81$, $p<0.001$; *WL:* $r_S=0.93$, $p<0.001$; *PT*: $r_S=0.97$, $p<0.001$; $N=25$). We further found that the within male variation was significantly smaller than the between male variation (*RF*: $F_{24,25}=5.11$, $P<0.001$; *WL*: $F_{24,25}=3.21$, $P<0.01$; *PT*: $F_{24,25}=1.97$, $P<0.05$).

Directionality

Apart from the reflectance at the angle of maximum reflectance, an important feature of iridescent coloration is its directionality. This is likely to reflect order in the feather structure, and may influence the appearance of a display. To measure directionality as a function of feather orientation (i.e. the angle between line-of-sight and the sample; Osorio and Ham 2002) we compared reflectance at the angle of maximum reflectance with the mean for 5° either side. Directionality was calculated by dividing the mean reflectance at ±5° by reflectance at the angle giving the maximum reflectance.

Statistics

Analyses used the statistical programs Statistica release 4.3 (StatSoft Inc. 1993) and JMP version 3.2.1 (SAS Institute 1989-1997). Correlations used the non-parametric Spearman rank-correlation. All data was tested for within-group normal distribution using the Shapiro-Wilk's *W*-test. Variables that were not normally distributed, even after transformation, were tested using the non-parametric Kruskal-Wallis ranks test, normally distributed variables was tested using the parametric ANOVA test and included in a discriminant function analysis.

RESULTS

We have described methods for making consistent measurements of iridescent plumage spectra from magnificent frigatebird, and shown that measurements from individual feathers will characterize an entire bird. Four measures were used to describe this coloration: three for the spectral reflectance at the angle of maximum reflectance, *RF*, *WL* and *PT* - which correspond roughly to brightness, hue and saturation -, and one for the proportion of the feather bearing iridescence. Spectra from 43 back-feathers and breast-feathers were significantly correlated (r_S range: 0.42-0.59, N=43, P range: 0.01-0.001). The spectra showed substantial variation, indicative of systematic differences between individuals (Table A1).

Correlation with age-class

For 214 male back-feathers spectral variables *RF*, *WL* and *PT* did not vary significantly with age. Adults and sub-adults had practically identical values, while juvenile/sub-adults had a non-significant tendency to lower iridescence (Table A2 Part I). The similarity of adults and sub-adults is consistent with the field observation that iridescence of back-feathers is not useful for age-classifying birds. Nonetheless, the proportion of the feather bearing iridescence (Figure 1*B*) did increase significantly with age ($F_{2,208}$=6.28, P<0.01; Table A2 Part I). The condition of the males (calculated as the residuals from a linear regression between weight and ulna/culmen length), tail length (represented by the longest of the two outer tail-feathers), and tail-fork length all increased significantly with age ($F_{2,210}$=7.44, P<0.001, $F_{2,207}$=4.07, P<0.05, $F_{2,207}$=12.73, P<0.001 respectively; Table A2 Part II). When entering the back-feather variables in a step-wise forward discriminant function analyses for age, all but the three spectral variables were included in the model, resulting in a total of 66.7% correctly classified males. The main problem was the small difference between adults and sub-adults (Table A2 Part I and II), resulting in very few sub-adults (4.8%) being correctly classified (Table A2 Part III).

Correlation with mating success

Records of mating success were obtained for 172 males. None of the 22 re-sighted juvenile/sub-adults obtained a mate, whereas 7 out of 29 sub-adults and 52 out of 121 adults were successful. To test the role of the variables on male mating success we analysed data for the full adults only. This avoided confounding possible age-class related differences with differences between preferred and non-preferred males.

Spectral data for 121 adult male back-feathers showed a significant relationship in the parameter *WL* with mating status ($F_{1,119}$=4.66, P<0.05; Table A3 Part IA). Mated males had shorter wavelength spectra than unmated. The level of significance was not high enough to still be significant after a Bonferroni correction (Rice 1989), wherefore we cannot exclude the possibility of the significant difference with status resulting from chance. No other spectral variables varied significantly with status (Table A3 Part I). Nor did any of the size, weight or tail feather measurements differ significantly with mating status (Table A3 Part II). When entering the variables in a discriminant function analysis, tail-fork length and iridescence ratio were also included in the model, with mated males on average having shorter tail-forks and lower iridescence ratios than non-mated males. The discriminant function analysis resulted in a total of 65.2% being correctly classified (Table A3 Part III).

Condition

Condition was non-significantly and very weakly correlated with back-feather reflectance spectra variables (r_S range: -0.11-0.08, P range: 0.11-0.26, N=213), back-feather iridescence ratios (r_S=0.06, P=0.38, N=210), and tail-lengths (r_S=0.12, P=0.08, N=210). Condition was weakly, but significantly, correlated with tail-fork length (r_S=0.18, P<0.01, N=210).

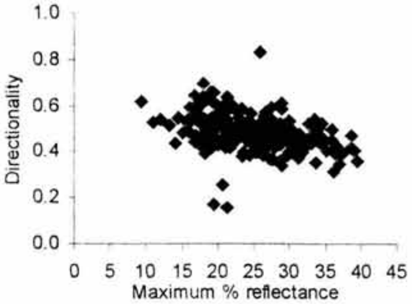

Figure 4. Directionality is measured as the ratio of reflectance at the angle of maximum reflectance to the mean reflectance at 5° either side of this maximum (Figure 2*A*). The 5° rotation reduces reflectance by 50% ±8% (N=214). Directionality is inversely related to brightness (r_S=-0.44, P<0.001, N=214).

Directionality of iridescence

The mean ratio of reduction in reflectance at ±5° to the angle of maximum reflection was 0.5 ±0.08 (N=214). This halving of reflectance for a 5° rotation of the feather (Figure 2*A*), implies that light from a point source is directed into an ellipse approximately 20° across its vertical axis (Figure 1*B*). Iridescence is, therefore, visible only within a restricted angle, otherwise feathers are blacker than soot (<4% reflectance).

One might expect that the angle over which light is directed to be inversely related to the maximum reflectance, so that the total amount of light reflected is relatively constant. It was therefore surprising to find that the reverse is true, the mean reduction in reflectance was negatively correlated with mean reflectance (r_S=-0.44, P<0.001, N=214; Figure 4). Thus feathers with the greatest maximum brightness tend also to reflect light over a wider angle, paradoxically this comparatively low directional selectivity might reduce the overall visual effect of the display produced by the brightest feathers.

DISCUSSION

By using a well-defined and adjustable viewing geometry (Figure 1*A*) we have obtained consistent measurements of the iridescent feather coloration for 214 male magnificent frigatebirds. Although there is substantial variation between birds, there is little evidence that any measure is a good predictor of age-classification, condition or mating success.

There are a number age dependent changes in plumage and body condition. These include the proportion of iridescent coloration on individual back-feathers (Figure 1*B*; Table A2), the length of the longest outermost tail-feather, tail-fork length, and condition, measured as the residual to a linear regression between size and weight measures, all of which increased significantly with age. Thus the lack of difference in reflectance spectra with age was not due to a general lack in age-related differences in plumage features and condition. Checking the prognostic value of the spectra, feather feature and condition variables in a discriminant function analysis, none of the spectra variables were included in the model. The model correctly classified 66.7% of the males by age-class, but with a heavy bias between age-classes, as only very few (4.3%) of sub-adult males were correctly classified.

Mated males had back-feather iridescence of slightly, but significantly, shorter reflectance wavelength than non-mated males, but no other feather features or condition varied significantly with mating status. A discriminant function analysis resulted in 65.2% of the full adult males being correctly classified by mating status, but this included two variables that vary significantly with age, namely tail-fork length and iridescence ratio. Although a larger portion of adult males than sub-adult males obtained a mate, some sub-adults did succeed in obtaining a mate, even in the presence of still unmated adult males. The apparent insignificance of iridescent coloration reported here is consistent with observations of great frigatebirds from Hawaii (Dearborn and Ryan 2002). Feather reflectance was scored as dull, moderate or bright under standardized light conditions at night. Also total length of the iridescent back-feathers was measured and gular pouch colour scored. The prediction was that earlier mated males would be of better quality (i.e. having higher scores) than later mated males. In practice none of the measured features differed between early and later mated great frigatebirds. For magnificent frigatebird such a comparison would not be appropriate, as males arrive at the colony throughout the approximately 3-month mating period (V. Madsen, unpublished data), and no consistent pattern of enhanced breeding success for early breeders seems to exist (V. Madsen, pers. obs.). Females therefore may choose among males available at any given time, and matings occur over a 3-months period.

Amongst seabirds, frigatebirds are unusual in being sexually dimorphic, with males being ornamented with the colourful gular pouch and iridescent plumage. Magnificent females do probably not take any cues from the colour of the gular pouch in their choice of mate, as we have been unable to find any preference for colour features (V. Madsen et al. unpublished manuscript). This was also is in accordance with the findings of Dearborn and Ryan (2002) who also failed to find any difference in gular pouch colour between first and later mated males. We have, however, clear evidence that the features of the drumming, which the pouch is involved in producing (Madsen et al. 2004), are good predictors of mating success. Females clearly prefer males that have larger gular pouches (lower fundamental frequency of the drumming), which drum at a more constant rhythm and that have a higher frequency of drumming (V. Madsen et al., unpublished manuscript). Combining age-related and female preferred gular pouch features related to the courtship drumming with the plumage variables from this study in discriminant function analyses, improved the prognostic value of the discriminant function considerably, leading to a total of 90% (N=25) were correctly classified according to mating status (V. Madsen et al., unpublished manuscript). So in concordance with other studies (Omland 1996a, b) female frigatebirds seem to base their choice on a combination of traits, which may signal different aspects of male qualities (Møller and Petrie 2002; Doucet and Montgomerie 2003; Jawor et al. 2003). By comparison, none of the four parameters we have used to describe iridescent coloration - three spectral and the length - are good predictors of mating success, either singly or jointly.

The apparent insignificance of iridescent coloration resembles that found for swallow UV iridescence (Perrier et al. 2002), but differs from other work on structural coloration and mate choice. At present the reason for our finding is unclear, but it would be of interest to know more about the costs of producing and maintaining iridescent coloration, and understanding how it is used in mating displays.

ACKNOWLEDGEMENTS

We are grateful to the administrators of the national park Isla Isabel (SEMARNAT) for permissions and logistic support. V. Madsen was funded by a Ph.D. fellowship from Universidad Nacional Autónoma de México, and the practical expenses were funded by the Danish Research Agency and the private Danish fund "Torben og Alice Frimodts Fond". Some support was also received from the Instituto de Ecología, UNAM and CONACyT grant (No. 34899-V). Numerous volunteers from Denmark, England, Germany, Mexico, Portugal, Spain and Switzerland entered into the work with enthusiasm and adventurous spirits. The fishermen of Isla Isabel were great company, always helpful and willing to understand us.

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Article III - Gular pouch colour, blood parasites & testosterone

Madsen, V., Valkiūnas, G., Iezhova, T., Mercado, C., Sánchez, M., and Osorno, J.L. unpubl. ms. Gular pouch colouration in the magnificent frigatebird (*Fregata* magnificens); the effects of testosterone levels, blood parasite prevalence, age-class and condition. Journal of Avian Biology or similar journal.

GULAR POUCH COLOURATION IN THE MAGNIFICENT FRIGATEBIRD (*FREGATA MAGNIFICENS*): THE EFFECTS OF TESTOSTERONE LEVELS, BLOOD PARASITE PREVALENCE, AGE-CLASS, AND CONDITION

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Abstract. We studied magnificent frigatebirds (*Fregata magnificens*) over four consecutive mating seasons to identify factors that influence female mate choice. Male frigatebirds possess a seasonally expressed skin ornament, namely the red inflatable gular pouch. The objective of this study was to investigate possible female preference for and trait expression of gular pouch colour, as a function of age-class, blood parasite prevalence, testosterone levels, and condition. Analysed frigatebirds were only infected by one blood parasite (*Haemoproteus iwa*), with an average prevalence rate of 14.9%. Prevalence rate increased with male age-class. Testosterone levels were lower in females and mated males than in males in courtship, and higher in older and in visited males plus in males infected with *H. iwa*. Short-term condition (residuals of size to weight) and long-term condition (tail-feather moult rate) were compared for males by age-class and *H. iwa* prevalence. Only short-term condition increased with age-class. Gular pouch colouration, as measured in the L*a*b* colour space, was lighter plus less red and saturated in mated males. For courting males, gular pouch colour saturation increased with age-class. Courting males infected with *H. iwa* had lighter gular pouches. Gular pouch lightness and redness was highly correlated, but only redness was correlated with testosterone levels. Both redness and lightness was correlated with short-term condition. With regard to final mating success of males, more adult males obtained a mate but no differences were found in final mating status of adult males in *H. iwa* prevalence, testosterone levels, condition or gular pouch colouration.

Key words: age-class; condition; Fregata magnificens; *Haemoproteus* iwa; *mate choice; testosterone levels; sexual trait expression*

Hamilton & Zuk (1982) hypothesized that sexual traits function in parasite-mediated sexual selection through being revealing handicaps (Zahavi 1975; Iwasa et al. 1991) of parasite infection. In this respect, females should choose to mate with males that exhibit good health, or condition, based on a genetically based resistance to parasites or other pathogens, as signalled by bright plumage. Folstad and Karter (1992) presented a possible feedback mechanism for the H&Z hypothesis, the immuno-competence hypothesis, suggesting that the development of elaborate testosterone-dependent ornaments are costly through a physiological trade-off, where elevated testosterone levels result in lowered immune competence. Despite the immunosuppressive nature of testosterone has been questioned (Hillgarth and Wingfield 1997a; Braude et al. 1999). recent studies in birds have generally confirmed the interaction between testosterone levels and immuno-competence (e.g. Saino and Møller 1994; Saino et al. 1995; Zuk et al. 1995; Verhulst et al 1999; Duffy et al. 2000; Evans et al 2000; Peters 2000; Poiani et al. 2000; Castro et al. 2001; Lindström et al. 2001; Buchanan et al. 2003; but see Ros et al 1997; Hasselquist et al. 1999; Buttemer and Astheimer 2000). Wingfield et al. (1990, 2000) proposed the challenge hypothesis, as an explanation of the changes in male testosterone levels though the different life history stages. They suggested that testosterone reaches maximum elevated levels due to behavioural actions when territory establishment and/or mate guarding challenges the male during the breeding season. Notably, their data showed that only males exposed to such behavioural challenges rose above the elevated breeding baseline testosterone secretion, induced by environmental cues, to the level of maximum testosterone secretion. Wingfield et al. (2001) further suggested the social modulation hypothesis as a possible explanation of short-term variations in testosterone secretion. According to this theory, males would be able to maintain a baseline testosterone secretion most of the time, and only increase secretion when necessary, e.g. in response to male-male aggressive interactions.

The haemosporidian blood parasites (order Haemosporida) generally found in birds, are from the three genera, i.e. *Plasmodium*, *Leucocytozoon*, and *Haemoproteus*. They have frequently been used to test the Hamilton-Zuk hypothesis (Møller 1997). The phases of infection in avian hosts are: 1) pre-patent (parasites develop in internal organs, and do not appear in the blood), 2) acute (parasites appear in the blood, and parasitemia markedly increases), 3) crisis (peak of the parasitemia), 4) chronic (a few parasites present in the blood), and 5) latent (the parasites persist in internal organs, but are not present in the blood (Atkinson and van Riper 1991; Valkiūnas 1997). The haemosporidians are therefore discernable in blood samples during phases 2-4 only. Infections may persist in birds for years up to lifetime and may relapse due to changes in hormone levels or environmental and physiological stresses, such as are likely to incur at the onset of the breeding season (e.g. Haberkorn, 1968, Weatherhead and Bennett 1991, Hatchwell et al. 2000, Schrader et al. 2003). During relapses the infection is again detectable in the blood.

In birds, testosterone dependent sexual traits are most commonly structures like spurs, combs and wattles (Owens and Short 1995). Of these, seasonally expressed traits are especially suitable for investigations of testosterone-mediated sexual selection, as they may reflect hormone presence/levels and condition at the time of trait expression (Buchholz 1995). Also they are not selected for outside of the breeding season. Soft parts, like fleshy ornaments, are present in a wide variety of birds (Holder and Montgomerie 1993), and are often involved in sexual competition and mate choice (Stokkan 1979, Ligon et al. 1990, Zuk et al. 1990a,b, Buchholz 1997).

Magnificent frigatebirds males possess only two morphological sexual traits, i.e. an iridescent plumage plus the seasonally red and inflatable gular pouch. Especially gular pouch colour features have the potential to be important indicator traits of male quality, as the gular pouch is only reddish during the courtship period, and between-male variation is pronounced, with colours varying in lightness, hue and saturation from pink over orange/red to red. Another significant trait of male magnificent frigatebird behaviour is that males desert after approximately 6 months out of the total chick-rearing period, which lasts almost 2 years (Diamond 1973; Osorno 1999; Osorno and Szekély in press). This makes the magnificent frigatebird a very suitable species for investigations of sexual selection, as the operational sex ratio during the yearly mating season is heavily male biased. On average only 57.5% of the courting males obtain a mate each year (VM unpubl. data). Competition for mates, as defined by Andersson (1994), seems mainly to be through female mate choice, as male/male aggressive and endurance competition (VM unpubl. data) and fertilization competition (M. Serrano pers. com.) is practically non-existent. Female mate preferences may thus be investigated through comparisons between mated and unmated males.

Here we investigate blood parasite prevalence by sex, age-class and mating status in magnificent frigatebirds of the Mexican national park Isla Isabel. Testosterone levels were analysed by sex, age-class, mating status and blood parasite prevalence. Male short-term condition, as represented by the residuals of a linear regression of weight to size, and long-term condition, as represented by the moult rate of the continuously moulted tail-feathers, were analysed for differences with age-class, mating status and parasite prevalence. The gular pouch colour measurements were also analysed for differences with age-class, mating status and parasite prevalence. The possible relationships between the expression of the seasonally sexual trait, i.e. gular pouch colouration, and simultaneous levels of testosterone, condition and blood parasite prevalence were investigated through correlational analyses.

METHOD

General

The study was performed in a breeding population of approximately 15-16.000 magnificent frigatebirds (VM unpubl. data) in the Mexican National Park "Isla Isabel" situated in the Pacific Ocean (21°52'N, 105°54'W), about 70 km off the coast. The fieldwork was conducted from September/October to December during the years 1999 to 2002. In total 454 non-juvenile birds were captured by hand at nights with no moon. Each bird was marked with a yellow rubber wing-tag with an individually distinct combination of numbers and/or letters, which enabled individual recognition at a distance and between years. The marked birds were sexed (55 females and 399 males) in accordance with a field guide (Harrison 1985). All marked females were adults. Males were age-classed according to the extent of white in the plumage as "juvenile/sub-adults" (with white feathers on the head and ventral area, N=31), "sub-adults" (with white feathers on the ventral area, N=102), and "adults" (all black, N=266). The ageing criteria were confirmed through re-sightings of marked individuals, with observed changes over the years being in accordance with our criteria.

Upon marking the mating status of the males was registered. Males that were mated, i.e. nest-building or with a nest, were classified as 'mated', all other males, i.e. unmated males, were classified as 'courting'. Of the marked males, 30 were 'mated' and 369 'courting'. The 'courting' males were sub-divided into 'visited', i.e. with a female present, or 'non-visited', i.e. without a female present. Of the courting males 224 were 'non-visited' and 140 'visited'. At the end of the each mating season each 'courting' male was assigned with a final mating status in accordance with the registrations made during the same fieldwork period. The final mating status was 'unmated→mated' for males that had at least commenced nest building. Males that were still unmated at the end of the mating season were classified as 'unmated'. Final mating status could be assigned to 320 of the marked males.

The birds were measured (ulna and culmen lengths; measuring error ±0.5mm) and weighed (measuring error ±25 gram). The deeply forked tail consists of 12 feathers, which have a continuous moult cycle of approximately 3 years (VM unpubl. data). For 194 males, registrations were also made of the age, i.e. 'old' or 'new', of each of the 12 tail-feathers. The distinction was done on the basis of general abrasion and wear, which resulted in 'old' tail-feathers being more round-tipped and bleached by sunlight than 'new' tail-feathers (Jenni and Winkler 1994). Blood samples of up to 1 ml were taken by puncturing the brachial vein. The blood was analysed for testosterone levels and blood parasites prevalence (see below). Finally, gular pouch colour was measured (see below).

Testosterone

Samples were collected from October to December during four fieldwork seasons and analysed in the spring of 2001, 2002 and 2003. In total, 229 samples, from 10 females and 219 males, were taken for testosterone analysis. After collection, the samples were left to coagulate before being centrifuged for 10 minutes at 3,000 rpm. Once the serum was separated from the plasma, all samples were stored at a temperature well below zero. Before analysis, the samples were thawed slowly until room temperature was reached. The analyses were done by use of IMMULITE® with an operating temperature of 19-30°C and a Photon Counting Photomultiplier Tube as luminometer. The range of calibration was 0.1 – 20.0 ng/ml testosterone.

Blood parasites

One blood smear was prepared from 19 females and 257 males. Blood films were air-dried, fixed in methanol, and stained with Giemsa. In accordance with recommendations by Weatherhead and Bennett (1991), and Valkiūnas (2001), the slides were examined for blood parasite prevalence for 10-15 min at low magnification (× 400), and then at least 100 fields were studied at high magnification (× 1,000). Haemosporidian parasites were identified according to Valkiūnas (1997).

Gular pouch colour measurements

It was not possible to measure colour of an inflated gular pouch during the daytime. It was, however, observed that the colour of the non-extendible part in between the two sides of the under-beak, was visually indistinguishable from the inflated gular pouch colour. Colour measurements were, therefore, taken from this area at night by pressing the Colorimeter gently against the skin, whilst the male was lying on its back with the beak extended backwards (Figure 1). All measurements were done by the same person (VM) using a Minolta Colorimeter and D_{65} standard illuminant. The D_{65} illuminant corresponds to average daylight, including the ultraviolet wavelength region. The colorimeter was calibrated before each nightly measuring session. Males were measured five times each at slightly varying positions and an average calculated. The colour was measured using the L*a*b* colour space, which corresponds to the human CIELAB (CIE 1971) colour psychometrics. In the L*a*b* colour space the lightness is given by L* (range: 0 to 100), where 0 is black and 100 light. The a* and b* (range: ±60) are the chromaticity coordinates, where +a is red, -a is green, +b is yellow and –b is blue. The L*a*b* colour space thus represents a colour sphere with an achromatic centre, where a* and b* are equal to zero. Colour saturation is therefore the radius of the circle centred in the centre of the colour sphere to the point coordinate given by a* and b*. Hue is indicated by the slope of the line going from the centre to the point coordinate given by a* and b*. Here we used the inverse of the slope, as higher values then indicated a redder colour, which was intuitively more useful (Figure 2).

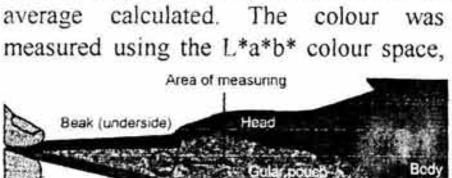

Figure 1. Measuring of gular pouch colour was done at night. The area measured was the non-extendable skin between the two sides of the under-beak. The males they were lying on the back and the beak extended back-wards.

Colour vision in birds is different from human colour vision, as the range over

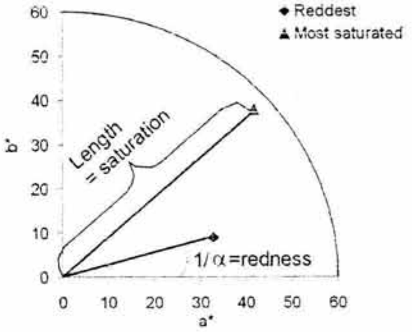

Figure 2. The red (a*) and yellow (b*) part of the L*a* b* colour sphere. Colour saturation in this space is the length of the line from the centre of the sphere to the point of (a*,b*). Colour hue is indicated by the α of the line from the centre to the point of (a*,b*), in order for the values to indicate redness we used the 1/α of the line. An example of a very saturated and very red gular pouch, respectively, is shown in the figure.

which birds may register colour extends into the ultraviolet (300-400 nm) (Bennett et al. 1994, Hart 2001). Also birds generally have four colour cones (humans have only three) each with a specific oil-droplet type that narrows the wavelength span of each cone further (Hart 2001; Vorobyev 2003). Using a Colorimeter, which corresponds to the sensitivity of the human eye, may therefore not give us exact information on how colour is perceived by the birds, but will provide us with good indications about the colour differences, which are also perceivable by birds.

Condition scores

Two different condition scores were calculated. The first was the residuals to a linear regression on weight versus size as represented by ulna and culmen lengths. This score was representative of the immediate short-term condition. The second measure was the rate of moult of tail-feathers, calculated as the ratio of new feathers out of the total of 12 tail-feathers. Eventually missing feathers were included in the count by a factor ½. This score was, due to continuous moulting, representative of the general long-term condition.

Statistical tests

Normality of the data was tested using the Shapiro-Wilks test. When data, even after transformation, did not comply, the non-parametric Kruskal-Wallis rank-test was used. Otherwise a parametric ANOVA was used. All correlations were calculated using the Spearman correlation. Data was analysed using Statistica software release 4.3 (StatSoft Inc. 1993). In analyses of age-class effect the smaller sample sizes of younger males and low prevalence of parasite infection resulted in the sub-division of males into three age-classes not always being feasible; the two younger age-classes were therefore joined into one age-class called "non-adults" throughout. Only adult males were included in the testing for differences with final mating status, in order to avoid confounding the analyses with possible age-class related differences.

RESULTS

Mating status

Mating status was assigned to a total of 320 males. Of 25 juvenile/sub-adults, none succeeded in obtaining a mate. Of 83 sub-adults and 212 adults, 25 and 101 were classified as 'mated' or 'unmated→ mated', respectively. Significantly more adult males obtained a mate than non-adult males (χ^2_1=17.0, P<0.001).

Blood parasites

Only one species of blood parasite, *Haemoproteus iwa*, was found. The overall prevalence of infection was 14.9% (Table 1). There were no significant differences in infection prevalence adult sex (χ^2_1=0.73, P=0.39). The prevalence of infection increased with age-class in males from 7.8% to 20.1% (χ^2_2=7.33, P<0.01). There was no significant difference in infection prevalence between mated and unmated males (χ^2_1=2.72, P=0.10).

Testosterone levels

Samples from 229 individuals were analysed (Table 2, Figure 3). In 38 samples the concentration of testosterone was outside the range of calibration (25 below 0.1 ng/ml and 13 over 20.0 ng/ml). The 38 samples were included in the data set as 0.10 and 20.0 ng/ml, by which they would be given equal low and high scores, respectively. The range of testosterone levels was very large, but testing the variance from samples of same status individuals (e.g. adult non-visited courting males and females) there was no significant differences in the within year variance compared to the between year variance (adult non-visited courting males: $F_{3,61}=0.27$, $P=0.85$; females: $F_{2,7}=0.59$, $P=0.58$).

Courting males had significantly higher testosterone levels than females ($H=6.2$, $P<0.05$, $N=199/10$) and mated males ($H=16.0$, $P<0.001$, $N=199/20$). Testosterone levels increased significantly with age-class ($H=6.6$, $P<0.05$, $N=75/12$). Males that were visited by a female upon marking had significantly higher levels than non-visited males ($H=4.2$, $P<0.05$, $N=59/65$). No significant differences were found with final mating status for adult males ($H=0.01$, $P=0.91$, $N=37/70$). Finally, courting males infected by *H. iwa* had significantly higher testosterone levels than non-infected males ($H=13.9$, $P<0.001$, $N=23/112$).

Condition scores

The short-term condition scores from 371 individuals were analysed (Table 3, Figure 4). The scores did not vary significantly with mating status upon marking ($F_{1,364}=3.29$, $P=0.07$, $N=348/18$). Condition scores increased significantly with age-class ($F_{1,346}=23.81$, $P<0.001$, $N=128/219$). This was also significant when comparing adults and non-adults within the non-infected group ($F_{1,198}=12.83$, $P<0.001$, $N=92/108$). No differences in short-term condition scores were found with final mating status for adult males ($F_{1,175}=2.71$, $P=0.17$, $N=72/105$). The long-term condition

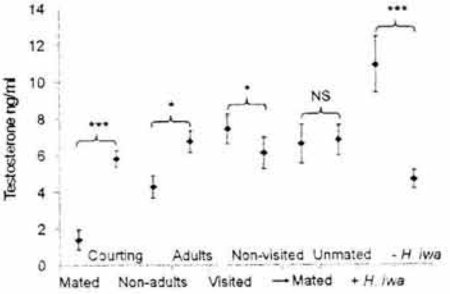

Figure 3. Mean testosterone levels ± SE for mated (N=20), courting (N=199). non-adult (N=75), adult (N=124), visited (N=59), non-visited (N=65), unmated→mated (N=37), and unmated males (N=70). Significance levels

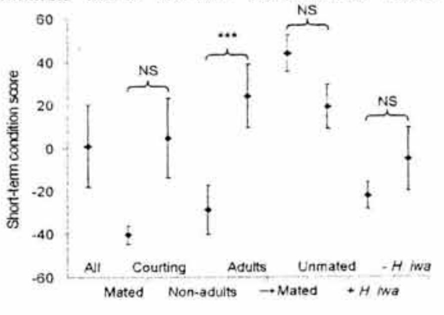

Figure 4. Mean short-term condition scores ± SE for all (N=371), mated (N=18), courting (N=348), non-adult (N=128), adult (N=220), unmated→mated (N=72), unmated (N=105), infected (N=39), and non-infected males (N=213). Significance levels are: * P<0.05, ** P<0.01, *** P<0.001, and NS=not significant.

scores did not vary significantly with age-class (H=1.6, P=0.21, N=61/137), final mating status of adult males (H=1.3, P=0.26, N=47/70), or *H. iwa* prevalence (H=0.0, P=0.98, N=19/89).

Gular pouch colour measurements

Colour measurements were obtained from 138 males (Table 4, Figure 5). The observed change in gular pouch colouration after mating was confirmed by all colour components being significantly different between mated and courting males. The lightness of the gular pouch increased significantly after mating ($F_{1,136}$=33.10, P<0.001, N=6/132), whereas redness and saturation decreased significantly after mating ($F_{1,136}$=11.47, P<0.001, $F_{1,136}$=47.40, P<0.001; N=6/132). Saturation increased significantly with age-class ($F_{1,130}$=11.15, P<0.01, N=31/51), whereas lightness and redness did not differ significantly (Lightness: $F_{1,130}$==3.53, P=0.06; Redness: $F_{1,130}$=1.59, P=0.21; N=31/51). No significant differences were found with mating status for adult males (Lightness: $F_{1,80}$=0.15, P=0.70, Redness: $F_{1,80}$=2.25, P=0.14, Saturation: $F_{1,80}$=0.01, P=0.90; N=31/51). Comparing males with and without *H. iwa* prevalence showed that males with infection had gular pouches that were significantly lighter ($F_{1,65}$=6.51, P<0.05, N=13/54). Redness and saturation did not vary with *H. iwa* prevalence (Redness: $F_{1,65}$=1.94, P=0.17, Saturation: $F_{1,65}$=0.01, P=0.92; N=13/54)

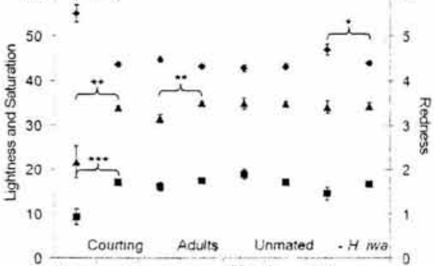

Figure 5. Mean gular pouch colour lightness, saturation and redness ± SE for mated (N=6), courting (N=132), non-adult (N=37), adult (N=95), unmated→mated (N=31), and unmated (N=51), infected (N=13), and non-infected males (N=54). Significance levels are: * P<0.05, ** P<0.01, *** P<0.001, and NS=not significant

Correlations

Correlations between the different variables were investigated; here only significant results are shown. Testosterone levels were correlated with gular pouch redness (all: r_S=0.27, P<0.01, N=105; ÷*H. iwa*: r_S=0.45, P<0.001, N=50). Testosterone levels were also correlated with short-term condition (all: r_S=0.23, P<0.001, N=198, ÷*H. iwa*: r_S=0.32, P<0.001, N=111). Short-term condition and gular pouch lightness was modestly/weakly correlated (all: r_S=-0.49, P<0.001, N=124; ÷*H. iwa*: r_S=-0.39, P<0.01, N=54). Short-term condition and gular pouch redness were modestly correlated (all: r_S=0.46, P<0.001; N=124). Finally, lightness was strongly correlated with redness (all: r_S=-0.84, P<0.001, N=132; ÷*H. iwa*: r_S=-0.74, P<0.001, N=54).

DISCUSSION

The blood of magnificent frigatebirds was only infected with *Haemoproteus iwa* (Work and Rameyer 1996) belonging to the family Haemoproteidae (Valkiūnas

1997). *Haemoproteus* infections have been considered to be relatively benign (Atkinson and van Riper 1991; Bennett et al. 1993), but lately studies have found some pathogenic effects of *Haemoproteus* prevalence. Great tits (*Parus major*) infected with a *Haemoproteus* sp. showed increased immuno-system activity, a probable result of host defence against the infection (Ots and Hõrak 1998), and higher *Haemoproteus* infection intensities, which was not part of this study, have been shown to result in increased mortality in collared flycatchers (*Ficedula albicollis*; Nordling et al. 1998) and pigeons (*Columbia livia*; Sol et al. 2003).

The overall prevalence of the *H. iwa* infection of 15% was equal to that of H. sp. for nestling great and lesser frigatebirds (*F. minor* and *F. ariel*) on Aldabra (Lowery 1971), but lower than overall prevalence of *H. iwa* of 35-37% in Hawaiian adult and juvenile great frigatebirds (Work and Rameyer, 1996). The Hawaiian birds were captured in March, which is also during their mating season (e.g. Dearborn 2001), so conditions were similar with respect to possible hormone and/or stress induced relapse (Haberkorn, 1968, Weatherhead and Bennett 1991, Hatchwell et al. 2000, Schrader et al. 2003). It therefore seems that differences in prevalence may exist between species and/or colonies.

H. iwa prevalence did not differ with sex, which was in accordance with the results from great frigatebird (Work and Rameyer 1996) and findings in purple martins (*Progne subis*) infected with *H. prognei* (Davidar and Morton 1993), pied flycatcher (*F. hypoleuca*) infected with *Haemoproteus* sp. (Dale et al. 1996), and red-bellied woodpeckers (*Melanerpes carolinus*) infected with *H. velans* (Schrader et al. 2003). *H. iwa* prevalence increased with age-class (from 8% to 20%) in non-juvenile males, which was contrary to the results from great frigatebird, where adults and juveniles were equally infected (Work and Rameyer 1996), but in accordance with the results from Purple martins, where infection prevalence in first year birds was 17% and 37% in older birds (Davidar and Morton 1992), and in pied flycatcher, where infection prevalence in first year birds was 34% and 81% in older birds (Dale et al. 1996). The increase in prevalence with age may be due to higher mortality in young immunologically naïve birds at initial *Haemoproteus* infection, due to resulting higher infection intensity (Sol et al. 2003), as has been found in purple martins (Davidar and Morton 1993), collared flycatcher (Nordling et al. 1998), and in pigeons (Sol et al. 2003). The increase in prevalence with age-class might also be due to deterioration in immune function with age-class, as in collared flycatcher (Cichon et al. 2003), or finally, be due to increased exposure to the vector with time. The vectors of *Haemoproteus* transmission are various biting dipterans of the families Hippoboscidae ("louse/flat flies") and Ceratopogonidae ("biting midges") (Atkinson and van Riper 1991). We collected several Hippoboscid flies from the plumage of birds during handling, so they seem the likely vector in this colony. Hawaiian and Aldabran frigatebirds were also infested by a Hippoboscid fly (Lowery 1971, Work and Rameyer 1996).

Neither short- nor long-term condition differed with parasite prevalence, which was in accordance with the lack of difference found for tarsus length and body mass in pied flycatcher (Dale et al. 1996), but contrary to the decrease in condition found in red-bellied woodpeckers (Schrader et al. 2003), and the increase in body mass found in some groups of Great tits (Ots and Hõrak 1998). These findings indicate that pathogenic effect(s) of being (chronically) infected by a *Haemoproteus* parasite might not always be measurable directly in the condition of infected birds.

Testosterone levels were significantly higher in courting males infected with *H. iwa*. This seemed to be in accordance with one prediction from the immunocompetence hypothesis (Folstad and Karter 1992), stating that higher testosterone levels should be expected to lead to higher infection risk due to the simultaneous depression of immune system function by testosterone. The *Haemoproteus* infection is, however, chronic, so we were more likely to measure testosterone levels from males that were experiencing relapses in infection, than from males with initial infections. In accordance with another prediction of the hypothesis we should thus have expected males to reduce levels of testosterone in order to have a more efficient immune system response to the reoccurring infection. Another possibility is that males with higher testosterone levels were more prone to relapses, but we have been unable to find any literature in support of this. Also there is the possibility that the infection itself increases testosterone levels, but we have only been able to find that some parasite infections seem to lower testosterone levels (reviewed by Hillgarth and Wingfield 1997a). Finally, infected males may need very elevated testosterone levels to develop the sexual trait and/or to perform at levels equal to non-infected males. Folstad and Karter (1992) also predicted that parasite infection should result in reduced expression of the testosterone dependent sexual trait. In accordance with this we found that the gular pouch colouration of infected males was lighter. So even though frigatebird males did not show any effect of the parasite prevalence in the form of reduced condition, they were not completely untouched either, as testosterone levels were higher and gular pouch colouration was lighter than in non-infected birds.

Females generally preferred older males over younger (sub-adult) males, and as *H. iwa* prevalence was higher in males of the oldest age-class, this did not indicate avoidance of infected males as a form of female mate choice. Similar results have been found in studies of purple martins (Davidar and Morton 1993) and pied flycatchers (Dale et al. 1996), where preferred older birds also had higher parasite prevalence rates. In purple martins infected birds had significantly higher breeding success and equal return rate after winter migration as non-infected birds (Davidar and Morton 1993), and in the study of pied flycatchers parasite prevalence did not influence breeding success, and infected males had higher return rate after the winter migration (Dale et al. 1996). This could indicate that birds that had survived the initial infection were of general high quality, and it might, therefore, even be beneficial to females to mate with infected older males. Females did, however, not seem to select directly for males infected with *H. iwa*, as mated and unmated males did not differ in lightness of the gular pouch colouration, which would have been expected, if females preferred the infected males, as they had lighter gular pouches than non-infected males.

Testosterone levels were in general very variable, but higher in adult males and in males with a female visitor. The social modulation hypothesis (Wingfield et al. 2001) suggests that males may modulate testosterone secretion in response to social interaction, and thereby increase levels above the already elevated breeding baseline level, when required. The social interactions/challenges envisioned by Wingfield et al. (1990, 2001) were male-male aggressive interactions and mate guarding. We would like to extend such social challenges to include courtship in response to female presence in magnificent frigatebirds, as sexual displays in general also are thought to be testosterone-dependent (e.g. Adkins-Regan and Pniewski 1978; Balthazart 1983; Wingfield 1994, Wingfield et al. 1997; Fusani et al. 1997). In frigatebirds a mate-seeking female will fly low over the colony. When approaching a non-mated male, he will commence courting her. If the female is interested, she will circle or hover over him, and he will burst into a high intensity courtship, which the female sometimes responds to by landing by him. The female may stay with the male from seconds up to several days, through out which period the male will continue courting her. In accordance with the hypothesis, we suggest that non-mated males may have an increased breeding base-line testosterone level that results in development of the gular pouch and relevant behaviour, such as seeking out a display site, inflating the gular pouch, and scanning for approaching mate-seeking females. Female presence could then be the appropriate social signal for modulating testosterone levels, whereby males would avoid having the elevated testosterone level required for the active courtship, during the long periods of no female presence. Upon female presence, male frigatebirds will, however, need to perform at their utmost due to the fierce competition for mates, where only just over half of the males succeeding in obtaining a mate each year. This could then explain the higher levels of testosterone in males with a female visitor. It might also explain the higher testosterone levels in the oldest age-class, as males within this group were more likely to obtain a mate and may therefore also have received more female attention. Wingfield and Wada (1989) found that the effect of a social challenge could be measured within 10-30 minutes, but the duration of the effect might last hours. Such a prolonged duration of the effect could explain some of the variability found in testosterone levels, as males might have had to respond to very different levels of social interactions during the day, before they were captured at night. Finally, the variable levels of testosterone might have resulted from some males being mis-categorized. A male sitting alone at night might actually be a nest-building male in the early stage, where the gular pouch is still reddish, a presently non-visited male might have been visited all day, and finally may a *H.*

iwa infection have been missed, if the presence in the blood stream yet was too low to be registered.

Males that were mated upon marking, i.e. either nest-building or incubating, had lower levels of testosterone and gular pouches were lighter, less red and less saturated than non-mated males. This confirmed the visually observed changes in gular pouch colouration upon mating from red to skin-coloured. Further it indicated a testosterone dependency of the trait similar to the testosterone-mediated colouration of the red frontal shield of moorhens (*Gallinula chloropus*). In moorhens the shield is small and dull red outside the breeding season, but grows and changes colour during the breeding season (Petrie 1988). Implants of testosterone induced similar changes, with implanted birds having bigger plus significantly darker and less dull red shields than control birds, i.e. lightness decreased and saturation increased with increased testosterone levels (Eens et al. 2000). In a pilot study the change of gular pouch colouration in the magnificent frigatebird was also found to be reversible, as mated males implanted with testosterone again developed red gular pouches (J. L. Osorno, unpubl. data). Our results did not show a general graded response of gular pouch colouration to levels of testosterone, as only redness was in anyway correlated with testosterone levels. The correlation was, however, not straight forward, as it was positive and significant for non-infected males, whereas for infected males the correlation was negative and non-significant. This resulted in a positive significant correlation for all males. Our findings, therefore, pointed more in the direction of a threshold-like response of gular pouch colouration to levels of testosterone. Another possibility is that the development of the secondary sexual character might be dose dependent at lower levels of testosterone, but that elevated levels are required in other social contexts (Hillgarth and Wingfield 1997b), which could be in connection with the courtship behaviour, as suggested above.

In males that were not mated upon marking, the oldest age-class had higher short-term condition scores. The tendency held true for both *H. iwa* infected and non-infected males, with only the latter, however, reaching significance. Short-term condition was negatively correlated with gular pouch lightness and positively correlated with gular pouch redness. Gular pouch lightness and redness was negatively correlated for both infected and non-infected males, but testosterone levels were correlated with gular pouch redness only. Finally, the oldest age-class had gular pouches of more saturated colouration, which was not found to correlate with any other of the investigated variables. We did not find any indications of female preference for any feature of gular pouch colouration, as mated and unmated males did not differ in any of these. This went against the general findings in galliforms, where female mate preferences are based on the colour and size expression of condition associated and testosterone mediated soft-parts (e.g. Ligon et al. 1990; Zuk et al. 1990a,b; Holder & Montgomerie 1993; Buchholz 1995). Our finding was, however, in accordance with the result of an investigation of great frigatebirds on the Hawaiian Tern Island, which also failed to find any female preference for gular pouch colour, as scored by use of Munsell cards at day at standardized angles to the light (Dearborn and Ryan 2002). The colour spectrum visible to birds includes the ultraviolet colours, which none of the two investigations included, as gular pouch colours in this study were measured with a colorimeter adjusted to the human visual range, and in the study of great frigatebirds comparisons were based on human visual observations. This should, however, not affect our results, as a pilot study, where a spectrometer was used for measuring gular pouch colouration, did not find indications of any significant reflectance peaks within the UV range (J. L. Osorno unpubl. data).

It may therefore seem that there is no apparent female preference in frigatebird for the expression of two male sexual traits, namely gular pouch colouration and feather iridescence (Dearborn and Ryan 2002, Madsen et al. unpubl. ms). The red gular pouch colouration is, however, probably easily distinguishable against the green vegetation, and with iridescence amplitudes of over 50% and a very narrow angle of iridescence (20°) any feather movements or movements of individuals in relation to the feathers will result in sharp flashes of light. In a densely populated frigatebird breeding colony increased detectability of individuals of correct sex, age-class and mating status may assist males in attracting female attention and assist females to easier home in on the individuals of interest. Gular pouch colouration and plumage iridescence might therefore have been selected for through male competition for attracting females and/or through reduced search cost to females reacting to the signals.

In conclusion, this study raised a number questions that need further investigation. One is whether the prevalence of the blood parasite *H. iwa* fluctuates between colonies and frigatebirds species, and the possible underlying ecological factors for such differences. Another is whether female presence and courtship behaviour may result in social modulation of testosterone secretion. And finally, whether infection by *H. iwa* may cause elevated testosterone levels or whether these also may be an effect of social modulation, as infected males need more testosterone to compete with non-infected males.

ACKNOWLEDGEMENTS

This manuscript was finished after the much too early death of José Luis Osorno, the supervisor of VM. We are grateful to the administrators of the national park Isla Isabel (SEMARNAT) for permissions and logistic support. VM was funded by a Ph.D. fellowship from Universidad Nacional Autónoma de México, and the practical expenses were funded by the Danish Research Agency and the private Danish fund "Torben og Alice Frimodts Fond". Some support was also received from the Instituto de Ecología, UNAM and CONACyT grant (No. 34899-V). Anders Permin from the Danish University of Agriculture helped establish the contact between VM and GV and provided practical help throughout, for which we are truly grateful. Numerous volunteers from Denmark, England, Germany, Mexico, Portugal, Spain and Switzerland entered into the work with enthusiasm and adventurous spirits. The fishermen of Isla Isabel were great company and always willing to provide whatever help needed.

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Article IV - Gular pouch size

Madsen, V., Balsby, T.J.S, Dabelsteen, T., & Osorno, J.L. 2004. Bimodal signalling of a sexually selected trait: gular pouch drumming in the magnificent frigatebird. Condor, 106: 157-161.

BIMODAL SIGNALING OF A SEXUALLY SELECTED TRAIT: GULAR POUCH DRUMMING IN THE MAGNIFICENT FRIGATEBIRD

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Manuscript received 27 February 2003; accepted 16 October 2003.

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Abstract. Female magnificent frigatebirds (*Fregata magnificens*) search for mates while flying and by visiting males at their display sites. Males respond with an intense courtship display that includes making a characteristic drumming sound and presenting the inflated gular pouch, a likely sexually selected trait. Viewing angle, male posturing, and proximity might, however, limit females' ability to appraise pouch size exactly. We investigated whether drumming provides the same information about pouch size and indeed found fundamental frequencies to be negatively correlated with estimated pouch sizes. Probably, the gular pouch functions as a resonance chamber. Females' assessment of gular pouch size could therefore be enhanced by the combination of visual and auditory signals.

Key words: courtship display, Fregata magnificens, *magnificent frigatebird, secondary sexual trait, visual and acoustic signalling.*

La Señalización Bimodal de un Atributo Seleccionado Sexualmente: El Sonido de la Bolsa Gular en *Fregata magnificens*

Resumen. La búsqueda de pareja en las hembras de la fragata magnifica (*Fregata magnificens*) incluye la inspección durante el vuelo y visitas a los machos en sus sitios de despliegue. Los machos responden con despliegues de cortejo intensos que incluyen un sonido característico de tamborileo y la presentación de la bolsa gular inflable, un atributo probablemente seleccionado sexualmente. El ángulo de visión, la postura del macho y la proximidad podria obscurecer la evaluación visual del tamaño de la bolsa por las hembras. Investigamos si el tamborileo provee la misma información sobre tamaño de la bolsa y encontramos que las frecuencias fundamentales del tamborileo estuvieron negativamente correlacionadas con los tamaños estimados de las bolsas gulares. Probablemente la bolsa gular funciona como una cámara de resonancia. Las hembras podrian hacer una mejor evaluación del tamaño de la bolsa gracias a la combinación entre las modalidades visual y auditiva de la señal.

Competition for mates often involves elaborate secondary sexual traits that have no direct function in reproduction. Such traits are usually only expressed in one sex and function in mate competition either as weapons or as displays that convey information about individual quality, such as strength, size, attractiveness, or genetic make-up, to same-sex opponents or prospective mates. Trait exaggeration, under sexual selection, is expected whenever mating success increases with increased trait expression. Thus a correlation is expected between the quality of the individual and the degree of expression of the trait (Andersson 1994, Zahavi and Zahavi 1997, Ligon 1999).

Magnificent frigatebirds (*Fregata magnificens*) form monogamous pairs each breeding season, but the male deserts the female and the chick after approximately 6 months, leaving the female to provide parental care for another 18 months (Osorno 1997, 1999, Osorno and Szekély, in press). One obvious result of the male desertion is a heavily male-biased operational sex ratio, also reflected by the fact that in our study population only about 55% of our marked males successfully obtained a mate (VM, unpubl. data). There is therefore scope for strong sexual selection on male attributes in this species.

Male magnificent frigatebirds possess two such attributes: the red inflatable gular pouch and the iridescent plumage. The gular pouch is only used by courting males in mate attraction. Non-courting males do not inflate the gular pouch, and it shrinks and changes to skin colour after the male has obtained a mate (VM, pers. obs.) At long range the inflated gular pouch may attract females to available males. Upon approaching a courting male, the female makes a close-up inspection by flying or hovering over the male and possibly landing by the male at his display site. During close-up inspection females are likely to assess the size of the inflated gular pouch, but visual size assessment might not be straightforward since viewing angle, male posturing, and proximity affect and obscure perceived size. Female inspection makes the male intensify gular pouch presentation. The male twists, probably to make the gular pouch look as large as possible, and produces sequences of deep, loud drumming sounds. The duration of this intense courtship depends on the female behaviour, with short inspections only eliciting relatively few sequences of drumming (VM, pers. obs.).

The aims of this study are first to describe the acoustic characteristics of the drumming, as this has not previously been done. Second, we test whether the gular pouch could function as a resonance chamber for the drumming, thereby producing an auditory size-related signal, as a backup for visual size perception. This was done by investigating whether the fundamental frequency and visually estimated gular pouch size are negatively correlated, as would be predicted by acoustics (e.g., Fletcher 1992).

METHODS

The study was conducted from 19 October to 1 November 2000 in a breeding colony of 12000–14000 magnificent frigatebirds in the national park Isla Isabel (21°52'N, 105°54'W) situated off the west coast of the state of Nayarit, Mexico. We recorded

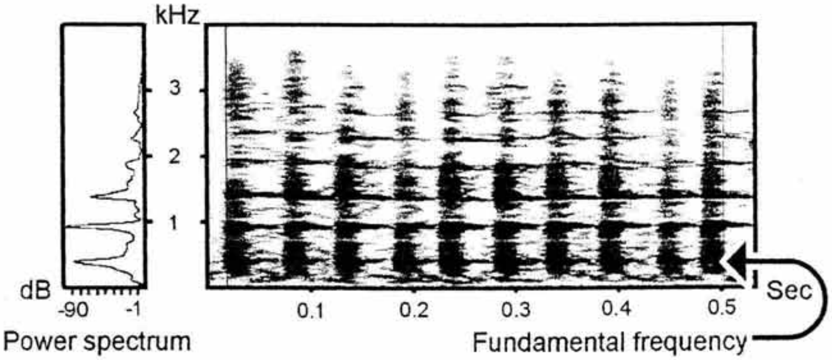

Figure 1. Spectrogram of a magnificent frigatebird drumming sequence of 10 sound elements (right) and mean power spectrum for the area delimited in the spectrogram (left). The power spectrum indicates six frequency bands. The lowest frequency band, called the fundamental frequency, has a frequency of about 450 Hz.

the drumming produced during intense courtship by 69 males at different display sites. Males were chosen on the basis of who was active and accessible at the time we went out to record. All recordings were made between 05:30 and 14:00, of males with fully inflated gular pouches. Each male was recorded during one recording session at close range (1.5 to 12 m) with a Sony WM-DC6 tape recorder and a Sennheiser MKH70 P48 directional microphone. Immediately after the sound recordings, photographs were taken of the fully inflated gular pouches at close range (5–10 m) using a Pentax Espio 105WR camera equipped with a 38–105 mm zoom lens. For each individual several photos were taken endeavouring to get the pouch (1) exactly from the front and (2) from the side, with the beak in the frame. We obtained good photos of 32 of the sound-recorded males.

The drumming sounds were digitised (16 bit, 8 kHz sampling frequency) and analysed in Avisoft version 4.15 (Avisoft 2002) using the spectrogram window (FFT 512, frame 100%, Hamming-window, frequency resolution 15 Hz, overlap 98.43%, time resolution 1 msec). We reduced ambient noise using the lower cut-off frequency filter option set at 0.3 kHz. For each sequence we obtained frequency measures from the mean power spectrum of the sequence, and time measures for each sound element of the sequence using the automatic parameter measures option. The frequency bands of the drums were identified by visual inspection of the spectrograms (Figure 1), ensuring that only frequency bands uncorrupted by background noise had their frequencies measured. The peak frequency (frequency with maximum amplitude) was measured for each band and sequence and used to calculate individual male averages. From the average peak frequencies for each band we calculated frequency band intervals. The settings for the automatic parameter

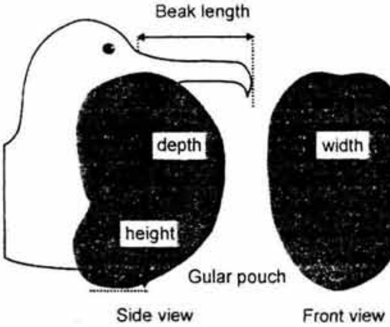

Figure 2. Method for gular pouch and beak length measurements taken on 32 male magnificent frigatebirds.

measures (threshold −10 dB, hold time of 0.001 seconds) resulted in most drumming elements being correctly identified. Elements that were not correctly separated (e.g., because of background noise) were abandoned. From the automatic measurements we obtained the number of sound elements in each sequence, interelement intervals, and element durations. Average interelement intervals and element durations were calculated per sequence and used to calculate individual male averages.

In order to estimate gular pouch sizes the photos of individual males were enlarged to a size of 1.3 × 2.0 m to maximize the picture. From the enlarged pictures we took the following measures with a folding ruler (to the nearest mm): (1) *gular pouch height*, the maximum height seen directly from the side, (2) *gular pouch width*, the maximum width seen directly from the front, (3) *gular pouch depth*, the maximum depth seen directly from the side, and (4) *beak length*, the maximum length seen directly from the side (Figure 2). As the pictures of the different males were taken at variable distances, the measures obtained were not directly comparable. To achieve comparable measures, we first divided each pouch measure of a male by the length of its beak obtained from a picture taken at the same distance and magnified by the same factor as the enlargement, and then multiplied this reduced measure with the average beak length for the population. Within the population the beak length varies only slightly between males (mean beak length = 10.6 ± 0.03 cm, n = 207; VM, unpubl. data).

Nonparametric Spearman rank correlations were used for calculating correlation coefficients and ANOVA was used for testing differences in variances of normally distributed data. All tests were calculated in SAS version 6.12 (SAS Institute 1990).

RESULTS

Spectrograms showed that a drumming sequence consisted of two or more distinct sound elements in close continuation, with less than 0.1 sec between the start of successive sound elements (Figure 1). Each sound element within a drumming sequence contained several frequency bands, the peak frequencies of which were not harmonically related, as they were not multiples of the peak frequency of the lowest frequency band (hereafter called the fundamental frequency). Descriptive acoustic features of drumming sequences were fundamental frequency (Hz), frequency band interval (Hz), element duration (sec), and interelement interval (sec; Table 1).

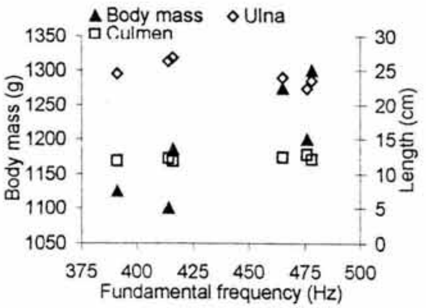

Figure 4. Magnificent frigatebird body size (as indexed by ulna and culmen length) and mass plotted against the fundamental frequency of courtship drumming. The positive relationship of frequency with body mass indicates that the gular pouch signals some quality other than simple body size.

In exploratory analyses aimed at determining the required number of sequences and sound elements therein needed for representative results, we analysed between 31 and 60 drumming sequences from each of the 7 males from which we obtained the largest number of sound recordings. The variation of the fundamental frequency (expressed as the coefficient of variation) over a sequence was low (0–2.2%) and independent of the number of sound elements in the sequence. Neither did it vary much (0.3–3.4%) with number of sequences analysed (the first 5, 10, 15, 20, 25, or 30 sequences) from each male. We therefore decided to include all males with more than 5 sequences in our analysis and limit the number of sequences analysed to a maximum of 25. Following that, we included 43 of the 69 males recorded in the final analysis, with 16.6 ± 0.9 (SE, range 5–25) sequences analysed per male.

Individual variance of the fundamental frequency was significantly less than the variance within the population (ANOVA: $F_{42,659} = 380.9$, $P < 0.001$). Estimations of gular pouch height, width, and depth were obtained for 32, 26, and 19 males, respectively, and of all three measurements for a total of 18 males. These measures also showed a substantial variation between males (height 24 ± 0.66 cm, range 14–31 cm; depth 11 ± 0.42 cm, range 8–15; width 15 ± 0.49 cm, range 12–18 cm; pouch volume 4206 ± 438 cm^3, range 1783–7962 cm^3).

Individual average fundamental frequency was significantly negatively correlated with the gular pouch size measures (Spearman rank correlation: height: $r_s = -0.75$, depth: $r_s = -0.77$, width: $r_s = -0.81$; all $P < 0.01$) and with estimated gular pouch size (height × depth × width: $r_s = -0.82$, $P < 0.01$, $n = 18$; Figure 3).

Six sound-recorded, wing-tagged males

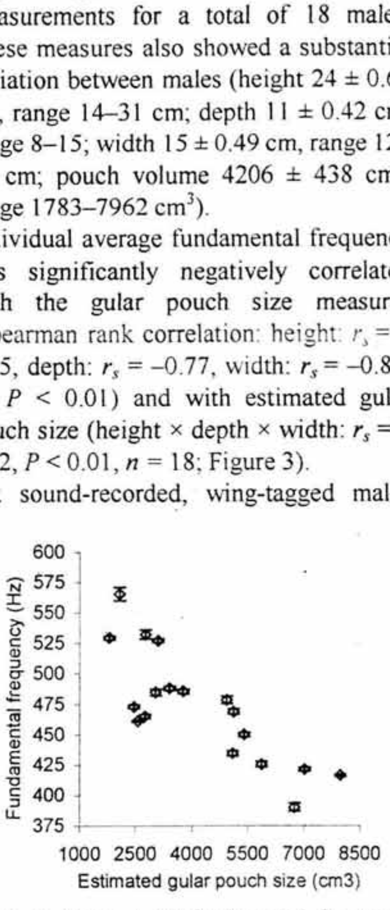

Figure 3. Mean ± SE fundamental frequency of drumming produced by male magnificent frigatebirds as a function of gular pouch size. The gular pouch size was calculated as height × depth × width (Figure 2), after all measures had been standardized by beak length.

were measured and weighed during the same field season. No significant correlation was found between the size of these males as expressed by ulna and culmen length and the fundamental frequency of their drumming (ulna: $r_s = -0.71$, $P = 0.11$; culmen: $r_s = 0.43$, $P = 0.40$; Figure 4). The mass of the males was significantly, but positively, correlated with fundamental frequency (mass: $r_s = 0.89$, $P < 0.05$; Figure 4).

DISCUSSION

In this study we found a significant negative correlation between estimated gular pouch size and the fundamental frequency of the drumming produced during male courtship in response to female presence and inspection. The correlation was not perfect, probably because the estimated gular pouch size was represented by the smallest box (height × width × depth) into which the pouch would fit. This means that a box of a certain volume might represent gular pouches of slightly varying volumes, as some pouches are a bit more heart-shaped than others. The birds with the largest gular pouches produced the lowest fundamental frequencies, which supported our hypothesis that the gular pouch acts as a resonance chamber in the production of the drumming sound. The actual mechanism behind the sound production is still unknown, but our observations and video recordings (VM, unpubl. data) indicate that it may be some form of specialized beak clacking.

For species where sound is produced by a larynx or laryngeal apparatus, like in mammals and anurans, it has generally been found that the fundamental frequency is negatively correlated with individual body size (Martin 1972, Morton 1977, Davies and Halliday 1978, but see Bee et al. 2000, Reby and McComb 2003) and mass (Friedl and Klump 2002). Some fish also vocalize (Ladich 1997), and for the croaking of gouramis (Teleostei), which is produced by the pectoral fins, it has been shown that the dominant frequency was negatively correlated with body mass and length (Ladich 1998). In birds the fundamental frequency of song and calls depends on properties and adjustments of the syrinx (Goller and Suthers 1996a, 1996b), and might be decreased by increasing size and thickness of the syringeal membranes (Fitch 1999). For the drumming produced by male magnificent frigatebirds, however, this is not the case. We did not find any indications that fundamental frequency was negatively correlated with size of males, as represented by measures of the ulna and culmen length. On the contrary, there was a significant, positive correlation between weight and fundamental frequency, which is the opposite of what acoustics would predict if fundamental frequency simply correlated with body size.

Male frigatebird courtship display consequently includes signalling of a secondary sexual trait by two different modalities: visual and auditory expression of the gular pouch size. Especially the auditory part seems to provide honest information about gular pouch size because, unlike visual presentation of the gular pouch, males are unable to manipulate the fundamental frequency of drumming. We expect that only when the trait-dependent difference in mating success is large will there be sufficient selection pressure for males to increase the courtship expenditure into bimodal signalling of a single trait. The observed bimodal signalling of gular pouch size suggests that pouch size is very important in mate attraction, either through signalling important qualities of the male or through arbitrary female preferences.

Sexual selection in the magnificent frigatebird seems to be through female mate choice only, as male frigatebirds do not engage in scramble competition, because females are the active mate-seeking sex. Neither is there any endurance rivalry, as males only mate successfully once each breeding season, extra-pair copulations seem to be very rare (M. Serrano, pers. comm.), and courting males only engage in low and equal levels of male-male and male-female aggression (VM, unpubl. data).

This study demonstrated a high consistency of acoustic trait expression within males and significant differences between males, which provide females with a range of stable signals for use in mate choice situations. To what extent female magnificent frigatebirds actually base their choice of mate on gular pouch size, or take cues from the other signals provided by the drumming, is presently being investigated.

We would like to thank the Center for Sound Communication at Department of Animal Behaviour, University of Copenhagen, for support with equipment for fieldwork and analyses. We are grateful to the administrators of the national park Isla Isabel (SEMARNAT) for permissions and logistic support. VM was funded by a Ph.D. fellowship from Universidad Nacional Autónoma de México, and the practical expenses, including travel for TJSB, were funded by the Danish Research Agency. Some support was also received from the Instituto de Ecologia, UNAM and CONACyT grant (No. 34899-V). Different international volunteers assisted with the fieldwork.

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Article V - Courtship acoustics

Madsen, V., Dabelsteen, T., & Osorno, J.L. submitted. Gular pouch drumming in the magnificent frigatebird (*Fregata magnificens*); age-class and mating status related differences in acoustic components. Behavioral Ecology.

GULAR POUCH DRUMMING IN THE MAGNIFICENT FRIGATEBIRD (*FREGATA MAGNIFICENS*); AGE-CLASS AND MATING STATUS RELATED DIFFERENCES IN ACOUSTIC COMPONENTS

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Abstract: The courtship display of magnificent frigatebird (*Fregata magnificens*) males includes an acoustic signal, the drumming sound, which is probably produced by specialized beak-clackings that are resonated by the inflated gular pouch. We investigated how three non-correlated acoustic components of this drumming correlate with age, mating status and age-correlated ornaments. The fundamental frequency of the drumming sound varied with age-class, being lowest in the oldest males indicating that these males have the largest gular pouches. Among the oldest age-class, the adults, all three acoustic components varied with mating status. Successfully mating males had larger gular pouches, plus a quicker and more constant drumming cadence than males that did not succeed in mating. By choosing a male with a large gular pouch the female would thus predominantly choose an older male who has proven his viability and may have more breeding experience. A faster and more constant drumming cadence are likely indicators of present male stamina. By choosing males of high stamina female would probably benefit through obtaining a fit partner. The prognostic value of the acoustic components in predicting age-class and mating status was considerably improved when combined with aspects of visual ornaments such as feather iridescence and gular pouch color. The implications for multiple signaling theory are discussed.

Key words: acoustic communication; age-class; female preference; *Fregata magnificens*; gular pouch drumming; secondary sexual trait expression

Competition for mates has resulted in the evolution of exaggerated sexual ornaments, which are often expressed in elaborate courtship displays that may include various acoustic signals (Andersson, 1994). Many of these signals seem to be highly energetically demanding (review by Ryan and Kime, 2003) and thus comparable to other costly sexually selected traits that have been shown to be important in assessment of male quality by females (Andersson, 1994). In birds, the most common acoustic signals are in the form of song and calls (Catchpole and

Slater, 1995). However, in some species drumming, consisting of mechanically produced bouts of similar sound elements in close continuation, seems to have similar functions as song. For instance, woodpeckers use drumming, which is produced by both sexes through rapid series of striking the beak against a tree trunk, in territorial establishment as well as in mate attraction and pair bond maintenance (Short, 1982; Stark et al., 1998; Wilkins and Ritchison, 1999; Dodenhoff et al., 2001). Similarly, males of the ruffed grouse (*Bonasa umbellus*) use tree logs for drumming during the spring breeding season for both territorial signaling and mate attraction (Lovallo et al., 2000; Naidoo, 2000). In the magnificent frigatebird (*F. magnificens*) drumming, which is probably produced by specialized beak-clackings being resonated by the inflated gular pouch (Madsen et al. 2004), is strictly used in male courtship display.

Magnificent frigatebirds form monogamous pairs with asymmetric investment in parental care by the two sexes. Males will desert after some months of tending to the one chick (Diamond, 1973; Osorno, 1999; Osorno and Székely, 2004), whereas females will tend to the chick/juvenile for approximately 18 months and thus only breed every second year (Nelson, 1975; Osorno, 1999). This results in a heavily male biased breeding season operational sex ratio, with only about 57.5% (average over four years; Madsen, 2005) of the courting males obtaining a mate each year leaving scope for relatively strong sexual selection in this species. Male frigatebirds possess two conspicuous morphological secondary sexual traits: the red inflatable gular pouch and the black iridescent plumage. The gular pouch is a seasonally expressed trait that is fully developed and red only during the mating season (Reville, 1991; Madsen et al., manuscript in preparation). Mate-seeking males will select a display site, inflate the gular pouch, and commence scanning for over-flying mate-seeking females. When a female shows interest in a male, by circling or hovering over him, he will respond with a high intensity display, which includes occasional bouts of drumming. The drumming seems produced by specialized beak-clackings being resonated by the inflated gular pouch. The fundamental frequency of the drumming elements reflects gular pouch size, as larger gular pouches have lower fundamental frequencies (Madsen et al., 2004). The duration of the high intensity male display is determined by the behavior of the female. A male will keep up the display as long as the female is circling or hovering over him, and only stop when she moves away. Females seem to choose a mate on the basis of the display, and as males do not use the display in the rarely occurring aggressive encounters (Madsen and Osorno, manuscript in preparation) female choice seems to be the main process of sexual selection in this species.

The objective of this study is to investigate how non-correlated features of the drumming vary with age-class and mating status of the males. The drumming features may represent different aspects of male quality, e.g. size of the gular pouch (fundamental frequency of elements), how fast the male is drumming (element interval), and the constancy of its drumming rhythm (variance of element interval; Fig. 1). We compare these results with the expression of other secondary sexual traits, i.e. gular pouch color and plumage iridescence, to identify the best predictors of age-class and mating success. Our predictions were that older and successfully mating males would be more ornamented than younger and unsuccessfully mating males, in accordance with the theories of age- and condition-dependent expression of sexual traits, which state that adult males and males of higher quality should have a higher degree of trait expression.

METHODS

Study area

Magnificent frigatebirds were studied from September/October to December 1999-2002 in a breeding colony of approximately 17,000 birds (Madsen, 2005) in the Mexican national park "Isla Isabel" (21°52'N, 105°54'W), located off the West Coast of the state of Nayarit.

Sound recording and analysis

The drumming of 86 wing-tagged males was recorded at close range (1.5 to 15 meters) using a SONY WM-DC6 tape recorder and a Sennheiser MKH70 P48 directional microphone. The drumming was digitized (16 bit, 8 kHz sampling frequency) and analyzed in *Avisoft*© version 4.15 (*Avisoft*© SASLab Pro, 2002) using the spectrogram window (FFT 512, frame 100%, Hamming-window, frequency resolution 15 Hz, overlap 98.43%, time resolution 1ms). We reduced ambient noise using the lower cut-off frequency filter option set at 0.3 kHz. In accordance with Madsen et al. (2004) a drumming sequence was defined as two or more sound elements in close continuation, i.e. with less than 0.1 second between the start of each sound element. Minimum 5 sequences and maximum 25 sequences were analyzed per male (mean 18.4 ± 0.86 sequences). Each sound element within a drumming sequence contains several frequency bands, of which the lowest frequency band's peak frequency, hereafter called the fundamental frequency, is negatively correlated with gular pouch size (Madsen et al., 2004). Descriptive acoustic components of a drumming sequence were fundamental frequency (Hz), frequency band interval (Hz), sound-element duration (sec), element interval (sec) (Figure 1), and the coefficient of interval variance. The peak frequency (frequency with maximum amplitude; Figure 1) for each frequency band was measured in the power spectrum, as the average for the sequence. Individual male averages were calculated per frequency band over all sequences analyzed, and frequency band intervals calculated by subtracting these frequency band averages. Sound element duration and element intervals in seconds for each sound element in a sequence were obtained from the 'automatic parameter measures' (threshold -10 dB, hold time of 0.001 seconds). The few elements that could not be correctly separated (e.g. because of background noise; Figure 2) were excluded from subsequent analyses. Average sound element durations and element intervals were calculated per sequence and individual male averages calculated together with the coefficient of

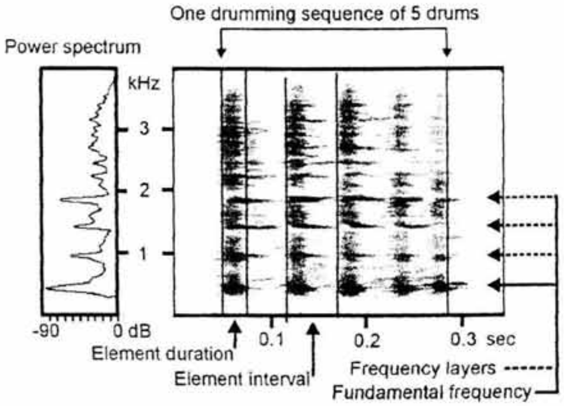

Figure 1. At the right hand side an un-filtered spectrogram of a drumming sequence of five sound elements, at the left hand side the mean power spectrum for the area delimited in the spectrogram. Four frequency bands can be identified. The lowest frequency band, called the fundamental frequency, has a frequency of about 440 Hz. Element duration and element interval are indicated.

interval variance over all sequences analyzed.

Frigatebird captures and measurements

A total of 56 of the sound-recorded males were captured at night for wing tagging and measuring of size (culmen plus ulna length, accuracy: ±0.50 cm), and weight (accuracy: ±25 grams). Only a sub-sample of 18 was actually marked in the same year as they were sound-recorded, but because morphologic size was not expected to change after the juvenile stage, we included all males in a correlation test between fundamental frequency (gular pouch size) and morphologic size and weight.

Frigatebird age-class classification

Age-class classification of non-juvenile males was based on the extent of white in the plumage, with completely black males being classified as 'adults', 'sub-adults' had some white feathers on the ventral part only, and 'juvenile/sub-adults' had also some white feathers on the head (Madsen, 2005). The actual age-span within each age-class is, however, not known.

Mating success

Registrations of mating status were made throughout the fieldwork period. Final overall mating status was assigned to each male at the end of the mating season in December. Only mated males build a nest. Males that had been observed nest building and/or with a nest were therefore classified as 'mated'. Males that had been observed throughout the field period and not been nest building or with a nest, and that were observed at least once as unmated at the end of the mating season were classified as 'unmated'. Males that had not been observed nest building and/or with nest during the fieldwork period, and which were not observed at the end of the

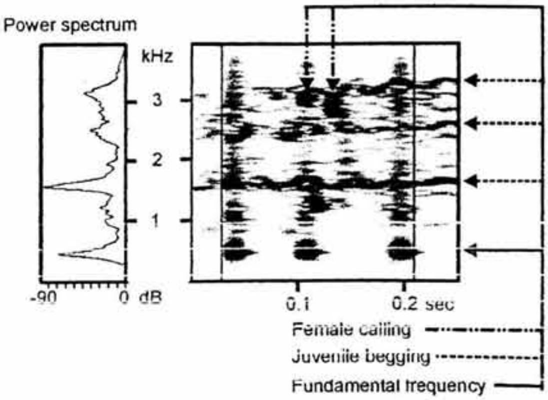

Figure 2. Spectrogram and power spectrum of a drumming sequence consisting of three sound elements, as indicated by their fundamental frequency. The second element of the drumming sequence is partly (above 1 kHz) masked by a female call. Juvenile begging calls overlap the entire drumming sequence.

mating season were classified as being of 'unknown mating status'.

Condition measures

Two different measures of condition were calculated. As a measure of probable short-term condition the residuals to a linear regression between weight and size (ulna and culmen length) were calculated (Jakob et al., 1996). Molting of feathers constitutes a considerable energetic and nutritional expense (King, 1980; Murphey and King, 1991; Ellis and Gabrielsen, 2002). As male frigatebirds have been found to have a continuous molt cycle of the tail-feathers of approximately 3 years (Madsen, 2005), a measure of probable long-term condition would therefore be the ratio of new to old feathers in the tail.

Statistical tests

Analyses used the statistical program Statistica release 4.3 (StatSoft Inc., 1993). All data was tested for within-group normal distribution using Shapiro-Wilk's *W*-test. Normally distributed variables with homogenic variances were tested using parametric students t-test, ANOVA and a stepwise forward discriminant function analysis (DFA). Correlation analyses used Pearson product-moment correlation for normally distributed data with homogenic variances; otherwise Spearman *r*-correlation was used. When males were recorded more than once the same year only the recording with most drumming sequences was included in the analyses for differences with age and mating status. The acoustic values of the same male recorded at different time in the same year were highly significantly correlated (r. 0.57-0.63, $p<0.01$, $n=19$). If there was no difference in number of drumming sequences, one recording was chosen at random. For males that were re-recorded the following year, the recording from the first year was generally used in the analyses for differences with age and mating status. The only exception was one male, for whom mating status could only be assigned the second year, wherefore the second year recording was included in the analyses.

RESULTS

The five acoustic components of the drumming differed between the 86 males analyzed (Table 1). It was always possible to measure the fundamental frequency whereas it was only possible to obtain values for the other variables from 81-82 males (Table 1) due to frequency and/or time interference by background noise from conspecific vocalizations (Figure 2). The most prominent background noise in the colony was the extensive communication above 1 kHz between the fledglings from the previous breeding season and their mothers. With fundamental frequencies of the drumming being below 1kHz, these would always be discernable to an inspecting female (Figure 2).

A Pearson product-moment correlation analysis found three of the five variables to be significantly correlated, i.e. fundamental frequency with frequency band distance ($r=0.64$, $p<0.001$, $n=81$), fundamental frequency with sound element duration ($r=-0.38$, $p<0.001$, $n=81$), and sound element duration with frequency band distance ($r=-0.30$, $p<0.01$, $n=81$). In the following analyses only three non-correlated components, i.e. fundamental frequency, element interval and coefficient of interval variation, were included. These three variables were representative of the size of the gular pouch, how fast a male could drum, and how well he could keep the rhythm of the drumming.

In total 24 males were recorded twice during the same field season. The fundamental frequency increased significantly with recording date (mean 429.6 to 451.7Hz, $t_{paired}=-2.76$, $p<0.05$, $n=24$), whereas neither element interval nor coefficient of interval variation changed significantly between recordings (interval: $t_{paired}=0.36$, $p=0.72$, $n=21$; coefficient: $t_{paired}=0.38$, $p=0.71$, $n=21$. Recording date was therefore included as a covariate in the continued analyses of the fundamental frequency. The fundamental frequency differed significantly with age-class, with the fundamental frequency of 'adult' males being lower than that of younger males (Table 2). A post-hoc Tukey test showed that the significant difference was between 'adults' and 'sub-adults' ($p<0.01$), whereas 'juvenile/sub-adults' did not differ significantly from any of the other two groups. This was probably due to the low sample size ($n=3$) of 'juvenile/sub-adults' from whom it was difficult to obtain recordings of drumming. Element interval and coefficient of interval variation did not vary with age-class (Table 2). In total 11 males were re-recorded two consecutive years and fundamental frequencies were significantly higher the first (mean 462.2 Hz) than the second (mean 411.0 Hz) year ($t_{paired}=4.86$, $p<0.001$, $n=11$), without controlling for within season chances. Neither element interval nor coefficient of interval variation changed significantly between years (interval: $t_{paired}=-0.75$,

$p=0.48$, $n=8$; coefficient: $t_{paired}=0.43$, $p=0.68$, $n=8$).

We checked the prognostic value of the drumming components by entering them in a stepwise forward discriminant function analysis for age-class. Through a step-wise forward analysis, the model first included the variable that explained most of the difference between groups; hereafter followed the variable that second-best described the difference, and so forth. The variables included in the model are presented in this order (Table 3, part I). The maximum percentage of correctly classified males of 87.2% was reached with only the fundamental frequency in the model (Table 3, part I). None of the 'juvenile/sub-adults' and few of the 'sub-adults' were correctly classified. The model was tested on the same set of variables from which it was derived due to the low sample sizes of younger birds.

From other parts of this investigation significant age-differences have been found for feather features, with younger males having shorter tail length and tail fork-length (joined in one principal component, which explained 92.2% of the original variation), lower ratio of iridescence to total feather length in back-feathers, and lower short-term condition (Madsen et al., manuscript submitted). Also gular pouch coloration has been found to be lighter and less saturated in younger birds (Madsen et al., manuscript in preparation). When including these parameters with the drumming components in the DFA, the samples sizes decreased further. This was especially because not all of the handled birds were sound-recorded, and because only birds handled and recorded in the same or adjacent years were included in the analyses. The model incorporated five of the above-mentioned variables, i.e. short-term condition, fundamental frequency, tail- and tail fork-length, feather iridescence ratio and gular pouch lightness, by which a 100% correct classification of age-class was reached (Table 3, part II).

Mating status was assigned to 78 of the 86 sound-recorded males. Of these, 23 of 65 'adults' and 2 of 10 'sub-adults' and none of 3 'juvenile/sub-adults' succeeded in obtaining a mate. In order not to confound age-class related differences with mating status-related differences, only the age-class of 'adult' males were included in consequent analyses of differences with mating status. For the 65 'adult' males, all three sound parameters differed significantly with mating status. Mated males had lower fundamental frequency, lower element interval, and lower coefficient of variation of element interval than unmated males (Table 4), which meant that males preferred by females had bigger gular pouches plus drummed at a faster and a more constant rhythm.

We checked the prognostic value of the drumming components by entering them in a discriminant analysis for mating status. The result was that all three drumming components were included in the model, which reached a maximum percentage of correctly classified males of 83.9% (Table 5, part I). From other parts of this investigation a correlation with mating status has been found for the reflectance wavelength of the iridescent back-feathers, where males with iridescence spectra of slightly lower wavelengths (nm) were preferred (Madsen et al., manuscript submitted). The prognostic value increased when this variable was included in the DFA, and resulted in a total percentage of correctly classified males of 90.0% (Table 5, part II). No significant differences with mating status have been found with regard to gular pouch coloration (Madsen et al., manuscript in preparation).

Correlation analysis between the three acoustic components and size plus weight measures resulted in very weak to weak correlations only (Table 6). The two most extreme values did reach uncorrected significance (p=0.03) but were not significant after a sequential Bonferroni correction (Rice. 1989). The size of the males did therefore not seem to have an affect on the acoustic components of the drumming. This is concordant with a previous study (Madsen et al. 2004). Short-term and long-term condition measures were only very weakly correlated with the drumming components (Table 6). The two condition measures were not correlated with each other (r=0.29, p=0.07, n=40).

DISCUSSION

In this study we found that the drumming sound included in the male courtship display in magnificent frigatebirds varied between individuals in five acoustic components, of which three were non-correlated: fundamental frequency (indicative of gular pouch size, Madsen et al. 2004), element interval (indicative of drumming cadence), and coefficient of interval variance (indicative of drumming cadence constancy). The fundamental frequency was lower in 'adult' than 'sub-adult' males and decreased within an individual from one year to another. This indicates that the size of the gular pouches increases with age. A female that assesses male age and gular pouch size on the basis of fundamental frequency of the drumming sounds would come to the correct assessment in 87% of all cases as shown by step-wise forward discriminant function analysis (DFA). If she combines the drumming components with other variables known to vary significantly with age-class, i.e. feather features and short-term condition (Madsen et al., manuscript submitted) and gular pouch color variables (Madsen et al., manuscript in preparation), the percentage of correct assessments would increase to 100%.

Females seem to utilize this option since among the adult age-class, successfully mating males differed from unsuccessful males with respect to all three non-correlated acoustic components. The drumming of successfully mating males had lower fundamental frequencies, i.e. larger gular pouch, and they drummed at a faster and more constant cadence than unsuccessful males. As we had also found gular pouch size to be an age-class related trait, the female preference for lower frequencies indicated that, even within the adult age-class, females might prefer older males. The actual age-span of this age-class is unknown, but the report of some great frigatebirds being 37 years old (Dearborn et al., 2001) does indicate that the age-span might be considerable. We hypothesize that by choosing an older male a female may obtain a direct benefit, if older males, perhaps through more breeding experience, have higher breeding success. In frigatebirds, the breeding success is greatly reduced due to nest usurpation by yet unmated males leading to loss not only of the nest, but also of the egg or the chick (Reville, 1988; 1991; Madsen V, personal observation). In our breeding colony, nest usurpation happened frequently during the last one and a half months of the mating season, when nests were most abundant (Madsen V, unpublished data). It is possible that experienced males lose the nest less frequently than inexperienced males. The indirect benefit a female may obtain through choosing an old male may be superior genes, as indicated by the higher viability of older males (i.e., an age-based indicator mechanism; Trivers, 1972; Manning, 1985; Kokko and Lindström, 1996; Kokko 1997; 1998, but see Brooks and Kemp, 2001).

Successfully mating males also drummed with a higher cadence than unsuccessful males. In another seabird, the blue petrel (*Halobaena caerulea*), signaling cadence mirrored male condition, as expressed by the weight of the male. The call cadence thus provided females with information about male condition, as heavy birds produced calls of high cadence and light individuals of low cadence (Genevois and Bretagnolle, 1994). Likewise, female swamp sparrows (*Melospiza georgiana*) showed more solicitation response to more physically demanding vocal performance, which included trills at a high cadence (Ballentine et al., 2004). In the strawberry poison frog, call cadence, among other traits, was negatively correlated with mating success, but as call cadence decreased with age this might reflect a female preference for older males and not for the signal cadence per se (Pröhl, 2003).

Mated males also drummed at a more constant cadence than unmated males. Lambrechts and Dhondt (1986) found that male quality in great tits (*Parus major*), as indicated by winter dominance and survival, was negatively related to cadence shifts during the song, with only high quality males being able to maintain a constant cadence throughout the song. We therefore hypothesize that the constancy of the drumming cadence in magnificent frigatebirds might reflect male quality in the form of stamina. Together with the female preference for older males, the preference for high stamina would probably benefit the female through securing that the present condition and/or quality of the male also was sufficiently high. This way, males that had reached senescence or had recently become sick would not be chosen as mates.

The lack of correlation between the acoustic components and the long-term condition measure (expressed as the molt rate of tail-feathers) was more or less expected, as the gular pouch is a seasonal trait, and therefore more likely to reflect short-term condition. The lack of correlation between short-term condition (expressed as the residuals of weight versus size), acoustic components of the drumming, and mating success might result from this measure not being an adequate measure of male quality (Cotton et al., 2004). In some passerines it has been found that fat scores are lowest in dominant birds, as they have more predictable access to food (Cuthill et al., 1997), and that higher quality males may sustain larger proportional weight-loss than males of inferior quality, as found in elephant seals (*Mirounga angustirostris*; Deutch et al., 1990). Also blue tit (*Parus caeruleus*) males that did not lose paternity, and even cuckolded other males, had lower short-term condition scores than males that lost paternity. This seemed to be the result of the capability of successful males in investing more, as they did not experience reduced survival probability (Kempenaers et al., 1997). Another possibility is that the measure does not really reflect male condition, as it might vary considerably with the amount of fish presently in the male. When marking and weighing a male we did not know whether he had just returned from or whether he was just about to leave on a foraging trip. From at study of the diet of magnificent frigatebird chicks within the same colony, it was found that each regurgitation weighed on average 104.6 ± 4.3 gram (Calixto-Albarrán and Osorno, 2000). This would mean a difference in average male weight of 8-9%, which would have a serious effect on the short-term condition measure. The short-term condition measure did, however, increase significantly with age-class, which would be in accordance with a general increase in foraging efficiency and in energy conservation through learning, as also found in other seabird species, like Manx shearwater (*Puffinus puffinus*; Brooke, 1990) and wandering albatross (*Diomedea exulans*; Weimerskirch, 1992).

The prognostic value of the three acoustic components of the drumming was an 84% correct classification of mating status. When including reflectance wavelength of the iridescent back-feathers, which is the only other variable that has been found to vary significantly with mating status, the percentage of correctly classified males increased to 90%. This indicates that the four variables included in the model might explain most of female mate preference, but that we still seem to miss some variables. One such variable may be aspects of the male courtship display, or more specifically, the courting during the pair formation period, which was not included in this study, but during which period the female might still decide to leave the male.

The increase in accuracy of mating status classification when more variables were included in the DFA model, indicated that females evaluated males based on multiple traits. The multiple ornament theory states that signals might function either as back-up signals (signal same aspect of male quality) or multiple messages (signal different aspects of male quality; Møller and Pomiankowski, 1993; Johnstone, 1995, 1996). In magnificent frigatebirds, two of the acoustic components, i.e. the cadence and constancy of cadence, were probably both indicators of present male quality/condition, as expressed through his stamina. The plumage feature was more likely a signal of male quality/condition at time of molt, which would be a very long-term condition measure, if back-feather molt were continuous, as observed in the tail-feathers. Otherwise it would be an indicator of condition outside the mating season, namely during the period of molt. Finally, the third acoustic component, the fundamental frequency, could be a likely indicator of overall male viability, as it decreased with age-class. Different traits signaling different aspects of male quality have also been found in other bird species like feather ornament and song in barn swallow (*Hirundo rustica*; Møller et al., 1998), plumage and territory in yellow-browed leaf warbler (*Phylloscopus inornatus*; Marchetti, 1998), bill and plumage colors in northern cardinals (*Cardinalis cardinalis*; Jawor et al., 2003), and plumage and bower features in satin bowerbirds (*Ptilonorhynchus violaceus*; Doucet and Montgomeri, 2003). The manner in which females evaluate several traits could be through composite trait preference, as found for feather features in California quail (*Callipepla californica*), where females showed preferences for several feature features in combination compared to the same features separately (Calkins and Burley, 2003). Lately, it has been suggested that males have a multitude of different traits because different aged females evaluate the males differentially (Coleman et al. 2004). In our study population this could not be investigated as almost exclusively females in adult plumage mated, such that no age-class distinctions could be made in female preferences.

ACKNOWLEDGEMENTS

This manuscript was finished after the much too early death of José Luis Osorno, the supervisor of V.M. We thank the "Center for Sound Communication" at Department of Animal Behaviour, University of Copenhagen, for support with equipment for fieldwork and analyses. Don Dearborn and Thorsten J. S. Balsby provided fruitful ideas and comments to earlier drafts of this ms. We are especially grateful to the numerous international volunteers very enthusiastically assisted V.M. with her fieldwork through out the years, with special mentioning of Frederik Cundin Joelving, who assisted with the recordings in 2001, and Christian Schlögl, who assisted twice in the fieldwork, and who supplied invaluable data from the spring period during his first 6 months stay. We thank the administrators of the national park Isla Isabel (SEMARNAT) for permissions and logistic support, and the fishermen of San Blas for their company and support on the island. V.M. was funded by a Ph.D. fellowship from Universidad Nacional Autónoma de México, and the practical expenses were funded by the Danish Research Agency. Some support was also received by J.L.O. from the Instituto de Ecología, UNAM and CONACyT grant (No. 34899-V).

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General discussion

In this study, male mating success was found to correlate with the expression of some male ornamental traits, whereas other ornametal traits were not found to differ with mating success. Successfully mating males differed from unsuccessfully mating males primarily in the expression of the acoustic element, i.e. the drumming, included in the courtship display. Secondly, mating succss differed with male age-class, as males from the oldest age-class were generally more successful in securing a mate than younger age-class males. This respect, marked differences were found with age-class in male ornamentation and condition, as also reflected in the higher mating success of males drumming at low fundamental frequencies, mirroring gular pouch size, an age-related trait. Thirdly, the coloration of the ornamental back-feathers in successfully mating males, differed from that of unsuccessful males in being of a slightly lower "purple" hue.

Among the ornamental traits that were found not to correlate with male mating success, were all aspects of gular pouch coloration, i.e. colour saturation, hue and brighness, together with iridescence amplitude.

The aim of relating male ornamental traits that correlated with female mate preference to male quality only succeeded partially. The female preference for males of the oldest age-class could indicate male quality as expressed in viability. On the other hand, no ornamental trait could be correlated with male condition, nor did females seemingly select against bloodparasite prevalence. In the following the implications of these findings will be discussed in more detail.

Age-related mating preference

Although not shown experimentally, female magnificent frigatebirds seem to prefer to mate with males from the oldest age-class, with only some males from the sub-adult age-class succeeding in mating (Articles II, III, & V). Within the oldest age-class the characteristics of preferred males varied in some traits that also differed with age-class, such as gular pouch size (Article V) and some feather features (Article II). This indicated that even within the oldest age-class, where the age-span could be as much as 20 years, females might have a mating preference for older males. In many long-lived species, the expression of male traits as well as mating success increases with age (Manning 1985; Johnstone 1995b). This does not, however, imply that females always exert a direct choice for age, it might rather be that the expression of a preferred trait is correlated with age.

The indirect benefit a female may obtain from a preference for an older male could be that the male, through having survived that long, has proven his high genetic quality for viability, also called the age-based indicator mechanism (Trivers 1972; Manning 1985; Kokko & Lindström 1996; Kokko 1997; 1998). In this respect, modelling has shown that if adult survival is important in relation to fitness, then preferences for older males might evolve

(Kokko & Lindström 1996; Beck & Powell 2000; but see Hansen & Price 1995). Also modelling of the relationships between age, life history trade-offs and viability indicators through optimal lifetime advertisement strategies, has shown that age-dependent sexual advertisement on average may be honest (Kokko 1997). In a recent review of the models of age-based indicator mechanisms it was, however, concluded that it might be questionable whether older males indeed are adaptively superior to younger males, and therefore may confer genetic benefits on their offspring (Brooks & Kemp 2001). The main reason for this was that an equally possible strategy for males of high genetically quality is to invest so heavily in advertisement that they survive for shorter periods, but such males may still have the same, or higher, lifetime reproductive success as long-lived males (Grafen 1990a, b; Eshel et al. 2000; Kokko 2001).

The direct benefits a female may obtain through a mating preference for an older male may be in the form of an older male being more experienced than a younger male. In many bird species breeding performance seems closely related to reproductive experience and age (e.g. in seabirds: Coulson 1966; Wooller et al. 1990). Females may thus identify male experience through its association with age-dependent maximal expression of sexual traits, like the age- and experience related size of song repertoire in male red-winged blackbirds (*Agelaius phoeniceus*; Yasukawa et al. 1980, Yasukawa 1981). Age-related experience may be in the form of having better foraging skills, as found in sparrowhawks (*Accipiter nisus*; Newton et al. 1981) and song sparrows (*Melospiza melodia*; Nol & Smith 1987). Experience might also be in the form of older males being more efficient breeders, as found in great tits (*Parus major*; Perrins & McCleery 1985) and short-tailed shearwaters (*Puffinus tenuirostris*; Wooler et al. 1990). In frigatebirds, an experienced male might be better at preventing nest usurpation by yet unmated males, which is a major cause of egg or chick loss (Reville 1991; pers. obs.). Another possibility is that an experienced male may be more reliable with regard to returning in time for his incubation turn, whereby the female may not be forced to abandon the nest due to starvation. An experienced male might also provide better or more parental care, such as better provisioning of the chick, as for example found in Tengmalm's owl (*Aegolius funereus*; Korpimäki 1988) and antartic fulmars (*Fulmarus glacialoides*; Weimerskirch 1990).

Finally, a female may choose an older male merely because he is more experienced in economizing his energy and thus may provide the right level of and/or more stimuli throughout the long and complex courting and mating period (Article I). If the courting is not also functioning to communicate how good the male is at providing resources to the female, this would be comparable to the sensory trap hypothesis, where female choice is not related to any benefits provided by the male. One of the things that young males seem to need to learn is whom to court. Young males were sometimes observed courting juveniles and other age-classes, which, like the female, have some white in the plumage. Also some young males were observed to produce very active courtship displays in response to inspecting females. When a female landed by them, they, however, performed practically no courting or 'body shaking'. This could either be because they did not know how to court a visiting female, or because they had expended too much energy in courting the flying female. The higher mating success of older males might therefore be because older males are better courting males, a trait that is probably correlated with but not necessary dependent on age. This could also explain why some sub-adults succeeded in mating, even when older males within the population were still unmated, if such males already had learned how to court a female most efficiently and effectively.

Female preference for acoustic traits

Magnificent frigatebird females preferred males that drummed at a fast and constant rhythm (Article V). Such traits are likely stamina dependent and therefore good indicators of present male quality. In combination with female preference for older males, this may be a way of securing that the choice will not be based on past viability alone. This may assist females in avoiding mating with older, but presently sick males, or males that have reached senescence, as none of these would be able to keep up such a stamina-dependent display. Choosing a male of present high quality might be of direct benefit to the female if such males also have a higher degree of stamina during incubation and brooding. Individual incubation/brooding turns last for several days, when birds are prone to loose weight due to starvation and water evaporation. The lack of correlation between the acoustic components and condition measures based on weight/size residuals and moult rate may be because such measures do not accurately mirror male quality. Males of higher quality might actually be the males that can tolerate larger weight losses (Deutsch et al. 1990) or invest more (Kempenaers et al. 1997). Males of higher quality may also be those that do not need large fat reserves, as they are more efficient foragers (Cuthill et al. 1997).

Female preference for multiple ornamentation

Overall male evaluation by females, may be accomplished through combining several male traits, as indicated by the higher prognostic value when more variables were included in a discriminant function analysis for mating status. The multiple ornament theory states that signals might function either as back-up signals for the same aspect of male quality or multiple messages for different aspects of male quality (Møller and Pomiankowski 1993; Johnstone, 1995a, 1996). In the magnificent frigatebirds two of the acoustic components, i.e. the drumming cadence and constancy of cadence, were probably both indicators of present male quality, as expressed through his stamina. The plumage feature was more likely a signal of male condition at time of moult, i.e. a long-term condition measure if back-feather moult is continuous, as observed in the tail-feathers (Appendix V). Otherwise it would be an indicator of condition outside the mating season, namely during the period of moult. Finally, the third acoustic component, the fundamental frequency, could be a likely indicator of overall male viability as it decreased with age-class.

Different traits signalling different aspects of male quality have also been found in other bird species like feather ornament and song in the barn swallows (*Hirundo rustica*; Møller et al. 1998), plumage and territory in yellow-browed leaf warblers (*Phylloscopus inornatus*; Marchetti 1998), bill and plumage colours in northern cardinals (*Cardinalis cardinalis*; Jawor et al. 2003; Jawor & Breitwisch 2004), and plumage and bower features in satin bowerbirds (*Ptilonorhynchus violaceus*; Doucet & Montgomeri 2003). The manner in which females evaluate several traits could be through composite trait preference, as found for feather features in California quail (*Callipepla california*), where females show preferences for several features in combination compared to the same features separately (Calkins & Burley 2003). Resently, it has been suggested that males have a multitude of different traits since females may evaluate males differentially, like shifting mate preferences with age (Coleman et al. 2004). In our study population this could not be investigated as almost exclusively females in adult plumage mated, so that no age-class distinctions could be made in female preferences.

Female preference and parasite load

Mating preference by female magnificent frigatebirds was not correlated with blood parasite prevalence, in the form of *Haemoproteus iwa*, even though prevalence was reflected in the significantly lighter gular pouch colour of infected males (Article III). This was in concordance with the findings in other bird species, like sage grouse (*Centrocercus urophasianus*; Gibson 1990) and pied flycatchers (*Ficedular hypoleuca*; Dale et al. 1996), but contrary to the general finding of females preferring lesser-parasitized males (Andersson 1994).

The best strategy for the female, with regard to ensuring the genetic quality of the offspring in relation to genetic parasite defence, might be to ensure high levels of heterozygosity (Brown 1997), as found with the major histocompatibility complex in mice (*Mus*; Potts et al. 1991; Potts & Wakeland 1993). Other studies have also linked increased heterozygosity to higher viability (e.g. Allendorf & Leary 1986; Lynch & Walsh 1998), and resistance to infectious diseases (e.g. Briles et al. 1983; Han et al. 1992). A study of red-winged blackbirds (*Agelaius phoeniceus*) did not, however, find successfully mating males to be more heterozygotic than unsuccessfully mating males. Neither were more heterozygotic males in better condition nor less infected by parasites than more homozygotic males (Weatherhead et al. 1999). In frigatebirds, a recent study has found that relatedness of mates was higher than in the population as a whole (Cohen & Dearborn in press), which might instead lead to increased homozygocity

The seeming lack of preference in magnificent frigatebird females for males without *H. iwa* prevalence might be because such males, after having survived the initial infection, were no worse than non-infected males. Another possibility is that such males actually were of higher quality, as demonstrated through their ability to survive and cope with the parasite (Davidar & Morton 1993). A combination of high quality of males that survive and cope with parasite infection and high parasite prevalence rate could thus, hypothetically, lead to a preference for infected males. No indication of such preference was found either, but this may be because of the relatively low *H. iwa* prevalence within this colony.

Prognositc value of identified differences in ornamental traits with male mating success

The prognostic value of the three acoustic components of drumming together with the reflectance wavelength of the iridescent back-feathers resulted in 90% correctclassification of males by mating status. This indicated that the four variables included in the model might explain most of female mate preference, but that one or two more variables might still be missing. One such possible variable could be the quality of male courtship display, or more specifically, the courting during the pair formation period, which was not included in this study, but during which period the female may still decide to leave the male.

Age related differences in male characteristics

Marked differences were, in general, found in the characteristics of non-juvenile males with age-class. Younger males were as such of lower short-term condition (Articles II, III, & V), their plumage was generally less developed (Article II), and their gular pouches were smaller (Articles IV & V) and of less saturated coloration (Article III), even in spite of them having lower blood parasite prevalence (Article III).

The lower short-term condition of younger males could indicate that the specialized foraging method of frigatebirds has an effect beyond the juvenile stage. The generally less developed morphological traits of younger males could, therefore, be a direct expression of low condition in condition-dependent traits (Rowe & Houle 1996). Another possibility could be differences in resource allocation between current and future reproductive effort with age (Roff 1992; Gustafsson et al. 1995). The residual reproductive value is generally higher for younger than older males (Williams 1966; Schaffer 1974). The strategy of younger males might, therefore, be to allocate more resources to survival and less to sexual advertisement, whereas the strategy of older males might be to allocate more resources to sexual advertisement and reproduction than to survival (Wetton et al. 1995; Mountjoy & Lemon 1996; Sundberg & Dixon 1996; Richardson & Burke 1999). A comparable pattern is that of delayed plumage maturation found in other bird species (Rohwer et al. 1980; McDonald 1989). Finally, the costs of breeding might be disproportionably higher for younger birds, and for long-lived species, it has thus been suggested that the reproductive interest of the individual should be to minimize the risk of mortality due to breeding, especially during the early phases of their reproductive life (Curio 1983). Long-lived birds should, therefore, postpone investment in sexual ornaments and thereby first breeding attempt until an increase in skills and efficiency ensures that breeding performance does not increase their mortality disproportionally (Lack 1968; Nur 1984).

Ornamental traits apparently not under direct selection

No female preference was found for feather iridescence amplitudes (Article II) or for gular pouch colour saturation or redness (Article III). The signal function of these sexual ornaments, therefore, did not seem to be part of female evaluation of male quality and not under direct selection by the female. Several possibilities exist, however, for indirect selection for iridescence levels and gular pouch coloration. First, back-feather iridescence amplitudes may function as display amplifiers (Hasson 1989, 1991). Through the use of amplifiers, male display may improve female discrimination power, increasing the female's tendency to accept better males and to reject males of lower quality. The display amplifiers are thus not under direct selection, as they by themselves are not attractive to females. The increased precision of female choice may result in a decrease in the time required to assess males, thus providing females with more time for more detailed assessment of the same or more males, or allowing females to allocate more resources to reproduction. In the magnificent frigatebird one male quality aspect preferred by females seems to be male vigour, as indicated by the preference for high and constant drumming cadence (Article V). The high amplitudes of iridescent reflectance, together with the strong angular dependence of its intensity (Article II), means that over-flying females will see male iridescent plumage as short flashes of strong light. The light effects will be switching on and off according to the actual direction of each feather or area of feathers to the sunlight and the angle between the male and female. The effect of the males' high intensity courtship during female inspection, where the males vigorously shake the wings and the body, might therefore be enhanced by the iridescence. That plumage iridescence might function as a signal-amplifier has also been suggested by Fitzpatrick (1998). Ornaments that function as enhancers of behavioural traits, on which females base their choice, have also been found in studies of lekking species like the sage grouse (*Centrocercus urophasianus*; Gibson & Bradbury 1985), and black grouse (*Tetrao tetrix*; Höglund et al. 1997).

Secondly, gular pouch colouration and plumage iridescence might function as efficacy signals (Marchetti 1993; Endler & Théry 1996; Andersson et al. 1998; Andersson 2000). Efficacy signals are characterized by providing a better spectral contrast to the background, and are as such conspicuous rather than exaggerated, and favoured through better detectability. In this context the male plumage in magnificent frigatebirds may function as an efficacy signal, as black plumage with "purple" light-effects probably provides a sharp contrast to the background of green foliage and grass. The redness of the gular pouch may also enhance the contrast. Contrast is of importance to males' mating success, and leads to sexual selection for such a trait, as has been shown for canaries (*Serinus* *canaria*), where females associate more with males that contrast strongly with the background, and not with a particular background colour (Heindl & Winkler 2003). Background contrast to the background may also determine how a receiver perceives a signal colour (Brou et al. 1986; McFadden 1992; Luo et al. 1995).

Thirdly, the seasonally expressed gular pouch coloration may be interpreted as a status signal. Usually, badges of status refer to social hierarchies of dominance (Rohwer 1975; Dawkins & Krebs 1978), but I suggest that the term might also be used with badges of mating status. In this respect, the frigatebird male may be signalling his unmated status, and thereby availability, through having a red gular pouch, as the colour of the pouch changes after mating (Article III), and the rest of the year is only discernable as a small stripe of skin under the beak (Reville 1991; pers. obs.).

For frigatebirds breeding in densely populated colonies, increased ability to distinguish between sexes, between mature/immature birds, and between mated/unmated males might reduce the search costs for mate-seeking females substantially. In this respect, the black plumage of adult males is distinctly different from the brown/black and white plumage of females and juveniles. Also, among non-juvenile males, adults might be distinguished by their completely black plumage and higher areas of iridescence. The striking black plumage with "purple" light-effects probably also contrasts strongly with the background of green foliage and grass, assisting females in zooming in on the sex and age of interest. The male iridescent and black plumage plus the redness of the gular pouch might therefore have been developed mainly as a means for males to attract and assist females, with a resulting reduction in search costs. In this respect, Schluter and Price (1993) have shown how female preferences and signal traits can diverge in response to the combined effect of 'detectability' (related to search cost) and 'honesty' (related to direct benefits).

Furthermore, no female preference was found for gular pouch coloration, nor was any correlation found between gular pouch colouration and testosterone levels (Article III). Testosterone levels might, however, be better correlated with male courtship behaviour, as sexual displays in general are testosterone-dependent (Adkins-Regan & Pniewski 1978; Balthazart 1983; Wingfield 1994, Wingfield et al. 1997; Fusani et al. 1997). In this respect, the testosterone levels were indeed higher in courting adult males with a female visitor than in courting adult males without a visitor (Article III). This led to a suggestion of extending the 'social modulation hypothesis' (Wingfield et al. 1990, 2001) to include the possibility that males may modulate testosterone secretion in response to the female presence, through increasing the testosterone level above the breeding baseline level, when required to perform the appropriate courting behaviour. Female presence might thus function as the social signal inducing increased testosterone levels. In this way, males may avoid having an elevated testosterone level during the extended periods without female presence (Article I). The 'social modulation hypothesis' might also explain the higher levels of testosterone in the oldest age-class, as males within this group usually have more mating success, and might, therefore, also be more likely to attract female attention. Another possibility is that the oldest age-class invests more in courtship.

General conclusion & future work

This study provides an understanding of the factors that relate to male mating success in the population of magnificent frigatebirds on Isla Isabel. Further investigations are required in order to obtain a better understanding of female benefits resulting from their choice of mate.

Thorough investigations of different aspects of possible male quality are required. This should include possible age-related differences in condition and breeding success, as well as the possible effect of male stamina. The magnificent frigatebird may be an excellent study species for investigation on evolutionary stable strategies of age-dependent sexual advertisement, as modelled by Kokko (1997).

Female mate choice could also be investigated further by studying the importance of male courtship during the visiting/pair formation period, with special emphasis on the body-shaking behaviour (Article I).

Playback experiments could be performed in order to investigate the importance of gular pouch drumming *versus* visual size appraisal (Article IV) in the female gular pouch size assessment. Playback experiments could also be used to test the role of the acoustic components of the drumming. This can be done through trying to enhance the possible attractiveness of a male through simultaneous playback of drumming at a faster and more constant rhythm. Playback experiments may also provide females with supernormal stimuli, i.e. lower fundamental frequencies, faster and more constant drumming rates than normally experienced within the population, in order to test the limits of female preferences (Article V).

Very little is yet known about possible effect of physiological constraints on iridescence amplitude and wavelength (Article II). This may be difficult to investigate in frigatebirds, but might be investigated using other bird species that may be kept in aviaries and manipulated under controlled circum-stances. The preference for 'purple' iridescence of low wavelength could be investigated through blocking the reflectance in the ultraviolet spectra. For the birds, this would result in a very different colour of much higher wavelength.

This investigation also raised some interesting questions about the possible relationship between testosterone and infection risk and/or relapse of the blood parasite *Haemoproteus iwa* prevalence (Article III). A study of this relationship would be difficult to investigate in the colony at Isla Isabel, as the prevalence rate was as low as 15%. During a stay at the magnificent frigatebird breeding colony on Isla Margarita, I noted that the frigatebirds there presented a greater incidence of infection by black fly (*Olfersia* sp.). This colony may, therefore, have higher blood parasite prevalence, which would facilitate a comparative study.

The social modulation hypothesis of testosterone levels could be tested in relation to challenges, such as female presence, in connection with the courtship display (Article III).

Another interesting avenue for future investigations would be a comparative study of two or more frigatebird species. In this respect, the acoustic components of the courtship differ greatly between magnificent and great frigatebirds (pers. obs.; D. Dearborn pers. comm.). Magnificent frigatebirds drum, whereas great frigatebirds produce a 'whooing' sound. It may, therefore, be of interest to investigate how the same 'instrument', i.e. the inflated gular pouch, may be used such different sound production. Iridescence also differs, with great frigatebirds only having green and no 'purple' iridescence. There are also various other subtle differences in the courtship of the two species. A third species that may be included is the lesser frigatebird, which would be of interest as it's distribution overlaps with the great frigatebird.

Finally, magnificent frigatebirds use many different types of vocal communication, which could be investigated, especially with regard to individual recognition within the pair.

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Appendices

Appendix I – Frigatebird population size

Introduction

The exact population size of magnificent frigatebirds on Isla Isabel is not known. In 1996 a count was made of the number of nests visible from viewpoints and paths, which resulted in a total of approximately 3,600 nests, equal to an annual breeding bird population of approximately 7,200 birds (J. L. Osorno unpubl. data).

I, however, wished to have an indication of the total population size and especially the number of available males within the plateau and adjacent areas, so initiated the below investigation.

Method

Counts of over-flying birds were made during afternoons on 6 days in 2001 and on 16 days in 2002 at Playa de Ocaso. The wind pattern of the island is such that during the afternoon the wind freshens from that direction, wherefore the soaring birds pass low over the beach, before going round the light-house cliff.

On each afternoon 5 counts of 1 minute duration were made with an interval of 10 minutes. The over-flying birds were divided in to the following categories:

Males	marked/unmarked
Females	marked/unmarked
Unknown sex	marked/unmarked

The birds of unknown sex were the youngest birds (Juveniles and Juvenile/(Sub-adults) (see Ageing above). A marked bird was a bird with a wing tag.

For each afternoon session the totals of observed birds were calculated for each category.

At the end of the fieldwork season, the total number of different wing-tags observed that season within the same categories were counted, and the total number of birds within each category and in total estimated from the rates of marked to not-marked birds counted and re-sighted.

Results

The numbers in *italics* were calculated on the basis of the ratios between counted and re-sighted marked birds to the ratios of counted marked and unmarked birds. An example: 1144 unmarked males / 95 marked males * 523 re-sighted males = *6298* estimated unmarked males. The numbers in **bold** are the totals of re-sighted marked males and calculated number of unmarked males. An example: 523 marked re-sighted males + *6298* calculated unmarked males = **6821** males. The population estimate for the plateau and adjacent areas is therefore a total 15,432 to 20,136 birds or on average of 17,784 birds (table AI 1).

Conclusion

Due to a high degree of site fidelity, and the fact that birds have only been marked at the plateau and the lighthouse cliff, this population estimate is probably a quite accurate estimate of the birds within the plateau, lighthouse cliff and adjacent areas. The number of females in 2001 seems a bit over-estimated, as also only based on very few sightings of wing-tagged birds. On average the number of females is also a bit higher than the number of males, but as the estimation of the number of males is based on a larger sample size, I think it is more realistic. The male breeding population at the plateau and adjacent areas was about 7,800 birds. Based on the size of this area to the rest of the frigatebird breeding area on the island, the total number of frigatebirds would probably be in the region of 2-3 times this number, i.e. about 35,600-53,400 birds, with a yearly male breeding population of roughly 15,600-23,400 birds. These numbers are only very rough estimates, but with an increased effort, and making counts at the same time at different parts of the island, I believe that this method will be able to provide a good estimation of the total population of Isla Isabel.

Appendix II – Frigatebirds on Isla Isabel

In the below table is presented a summary of the known aspects of the biology of the frigatebirds on Isla Isabel.

Appendix III – Overall male mating success

Introduction

Male magnificent frigatebird desertion (Osorno 1999, Osorno & Székely 2004) may allow males to mate every year, whereas females may only mate successfully every second year. This was suspected to result in a heavily male biased operational sex ratio and a resulting low overall male mating success.

I, however, wished to investigate the overall mating success in more detail, in order to get an indication of the possible level of sexual selection in this sequentially monogamous species.

Method

Frigatebirds have been wing-tagged within the same area of the colony on Isla Isabel since 1994. The wing-tag is a numbered yellow rubber strip placed around the ulna of the left wing, which allows for individual identification of the marked bird. At the beginning of this study in 1999 approximately 1000 frigatebirds had been marked and in the years 1999-2002 additionally approximately 800 were marked.

Throughout each season, records were made of re-sighted individuals, each containing the date, age, sex, mating status, behaviour and location of the bird. Birds were aged by the pattern and extent of white in the plumage (Appendix VI). Non-juvenile birds were sexed by plumage pattern (Harrison 1983). Mating status of males was registered as mated if the gular pouch colouration was not red plus the gular pouch texture was firm and/or if nest-building, incubating or tending to a chick. When a male was observed with a red and loose gular pouch and/or in courtship display the mating status was registered as unmated.

At the end of each season all records of re-sighted males were analysed for overall mating success. This resulted in a division into three categories; 1) those that had been observed as mated; hereafter called "mated"; 2) those that had not been observed as mated, and were still observed as unmated at the end of the mating season; hereafter called "unmated", and 3) those that had not been observed as mated nor observed at the end of the mating season, which were classified as being of unknown over-all mating success.

Results

Male mating status could be assigned to a total of 210 males in 1999, 318 males in 2000, 324 males in 2001 and 307 males in 2002. This resulted in percentages of successfully mating males in the range of 53.7-63.2% or an over-all mating success over all the years of 57.5% (Table AIII 1).

Conclusion

The result of the re-sightings confirmed the expected male biased operational sex ratio, with an overall average male mating success of 57.5% only. This indicated an operational sex ratio of 1.74 and thereby a likely intense competition between males for a mate.

According to Schuster & Wade (2003), the source of sexual selection is the exclusion of some individuals from mating. The strength of the sexual selection is thus proportional to the variance in fitness, and the sex difference in the variance determines the magnitude of the divergence between sexes. Here males obviously have a higher variance in mating (some obtain one female other no female), whereas the variance in females should be zero (all females probably obtain a mate). The selection intensity is, however, not that simple to calculate, as some males may obtain a mate (almost every year) and some males may only mate very infrequently, if at all. I am therefore in contact with Steve Shuster, who is presently trying to work this out.

Appendix IV – Gular pouch inflation mechanism

Upon opening the gular pouch of a recently dead male, I found that at the back of the gular pouch, up against the trachea, was a large double membrane (Figure AIV 1A). When lifting the membrane there were channels coming into the gular pouch (Figure AIV 1B). However, when following one of these channels it only lead to the body cavity (Figure AIV 1C). Going through the membranes, I might, however, have missed a possible connection with an air sac.

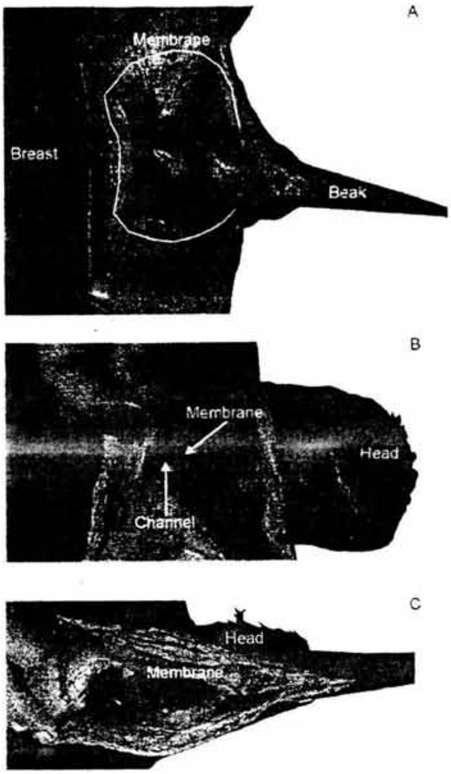


Figure AIV 1. When opening a gular pouch a "double"-membrane was found against the throat (A). Channels were leading into the gular pouch (B), but when following one only the body cavity was reached (C).

Appendix V – Tail feather moult

Introduction

The deeply forked tails of frigatebirds seem to invite an investigation based on the theory of fluctuation asymmetry (Møller and Höglund 1991, Bjorksten et al. 2000). This theory suggests that male ornaments are costly to produce and any the asymmetry in growth of ornaments like tail feathers will therefore mirror male quality.

Method

The age of the feathers of the tail was noted (numbered 1-12 from right to left; ♀N=0, ♂N=197), with old feathers being distinguished on the basis of general abrasion and wear, resulting in them being more round-tipped and bleached by sunlight (Jenni & Winkler 1994). For calculating the individual number of old feathers in the tail, eventually missing feathers were included in the count by a factor ½.

Results

The mean number of old feathers out of the 12 feathers in the tail of 162 individuals was 4.5±0.13 (range 1-10). For three recaptured adult males the number of feathers that had changed status from old to new and new to old were exactly the same and were 3, 4, and 4 tail-feathers respectively. This indicated a general turn-over rate of change 0.31 of the tail-feathers per year, whereas the average number of old feathers in the tail indicated a bit higher turn-over rate, namely between 0.37 and 0.39 for all ages.

The moulting pattern of the tail-feathers in males therefore seems to be one of continuous moult. Continuous moulting strategies have also been found in other species that depend on their soaring ability, like vultures, which have been found to have a moult cycle of about 3 years (Houston 1975).

Conclusion

The theory of fluctuation asymmetry (Møller and Höglund 1991, Bjorksten et al. 2000), which is based on the simultaneous growth of feathers, so that they are responding to the same conditions and are of approximately the same age, is therefore not applicable in this species. Continuous moult may lead to asymmetries caused by several factors like; the feathers not being of same age, i.e. have been subjected to different degrees of abrasion, the youngest feathers might still be growing, or that they were grown under different conditions.

Appendix VI – Plumage differences by sex

Introduction

Within their clade frigatebirds are exceptional through being sexually dimorphic (Dearborn et al. 2001). This is a probable effect of the sexual selection pressure exerted by females within these five species in their choice of mate. The difference in plumage coloration is obvious when observing the birds, and accurate sexing can be done easily from the plumage patterns and coloration (e.g. Harrison 1983).

As part of the investigation of the male iridescence (Article IV), I did, however, choose to analyse some female feathers from the same regions as those analysed for the males to show the difference in reflectance spectra.

Method

The feathers were collected and analysed in accordance with the Method described in Article IV.

Results

The feathers from the females generally reflected less light than the male feathers from the same body regions. Male head- and back-feathers reflected light in the form of iridescence, i.e. structural coloration. Male head-feathers had one peak within the green range and the back-feathers had three peaks, which through colour summation within our visual range resulted in what we see as "purple" iridescence. No iridescence was found in any of the feather samples from females.

Conclusion

The sexual dimorphism of plumage coloration was confirmed, as no iridescence was found in females, whereas both male head- and back-feathers reflected the light as colour iridescence as an effect of structural coloration.

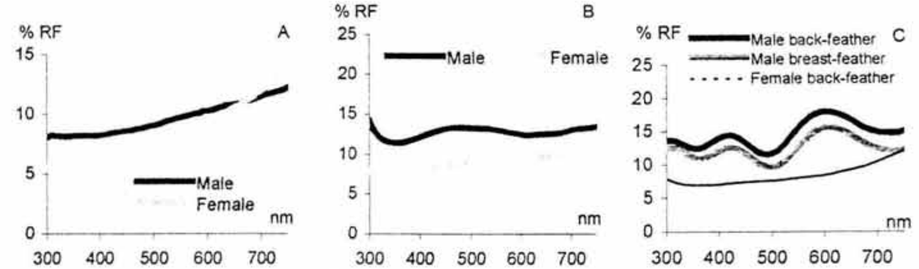


Figure AVI 1. Mean colour spectra curves from 24 males and 10 females measured at an angle of 45° to the light-source. A: Wing feathers, i.e. over-wing coverts. B: Head feathers, where males had a slight peak in the green section of the spectrum (~480nm). C: Back and breast feathers, where males had three marked peaks at ~310nm (UV), ~420nm, and ~600nm. Females had no marked peaks.

Appendix VII – Age-classification

Introduction

Non-juvenile magnificent frigatebirds have varying degrees of white in the plumage. I tentatively, and in accordance with tendencies found for other species, used the level of "whiteness" as an indicator of age. This was to be confirmed by registrations of changes in the same marked individuals with time.

Method

Age-classification rules:

- Non-sexable:
  - Chicks (Figure AVII 1A)
    - Downs and black back-feathers
  - Juveniles (Figure AVII 1B)
    - White head
  - Juvenile/(Sub-adults)(Figure AVII 1C)
    - Very few black feathers in the white on head and neck
- Females:
  - Sub-adult (Figure AVII 2A&B)
    - Some white/light feathers on head
  - Adults (Figure AVII 2C)
    - All black-brown on head
- Males:
  - Juvenile/Sub-adults (Figure AVII 3A)
    - More than 50% white feathers on head.
    - White feathers on ventral area
  - (Juvenile)/Sub-adults (Figure AVII 3B)
    - Less than 50% white feathers on head
    - White feathers on ventral area.
  - Sub-adults (Figure AVII 3C)
    - Some white feathers on ventral area
  - Adults (Figure AVII 3D)
    - No white feathers

Wing-tagged frigatebirds were sexed and tentatively age-classified upon marking. The same birds were then observed later the same year and/or the following years. At every re-sight the age-classification was noted again.

Results

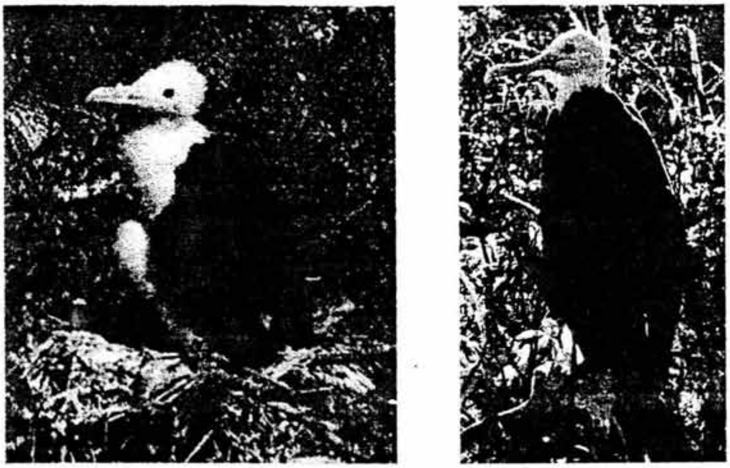
Only from marked males did I get enough data for an analysis of the direction of changes in the plumage colouration. This analysis showed that the changes were in accordance with my ageing-rules, i.e. males changed from having some white to less and less white (table AVII 1).

The number of +1 registrations of sub-adults might be a bit over-estimated as last-stage sub-adults can be difficult to distinguish from adults from a distance.

None of the males marked as adults were registered as being of lower age-class.

Conclusion

It seems that the age-classification rules are valid for age-classifying magnificent frigatebirds. The different classifications did not, however, span the same period in years. In data analysis, younger age-classes were often joined, in order to even the age-span some. We still do not have sufficient data to try and add real age to the different age-classes. The continued registrations, made by Monica Gonzalez, will hopefully be able to fill this void within the next couple of years. That young males are less ornamented and may have delayed plumage maturation and go through several sub-adult plumages is also found in species like the long-tailed manakin (*Chiroxiphia linearis*; McDonald 1989), and in over 30 species of North American passerines (Rohwer et al. 1980).

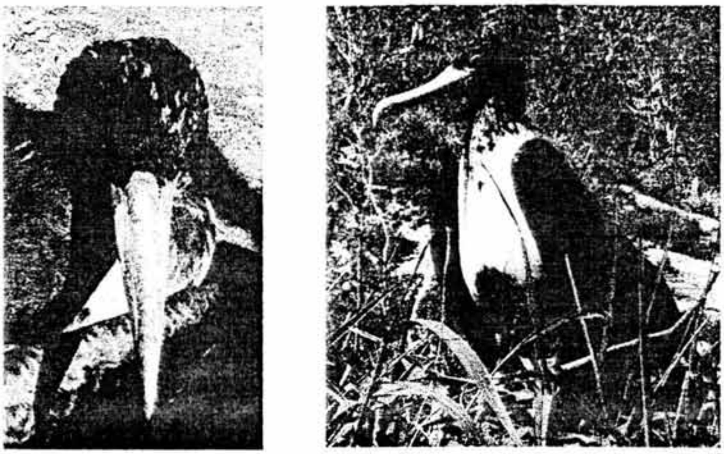


A: Chick
Downs and back-feathers only

B: Juvenile (Fledgling)
White head

C: Juvenile/(Sub-adult)
Very few black feathers on head or neck

Figure AVII 1. Non-sexable age-classes were chicks, juveniles and juvenile/sub-adults)

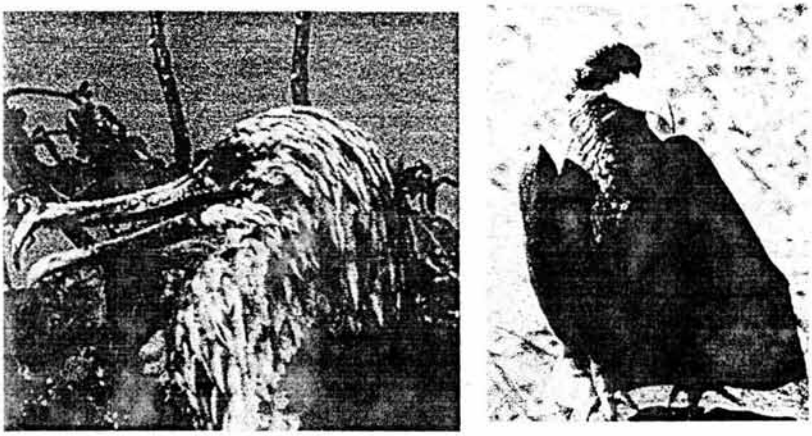


A: Sub-adult
Some white/light feathers on
head

B: Sub-adult
Some white/light feathers on
head

C: Adult
All black-brown on head

Figure AVII 2. Females were divided into two age-classes. The sub-adults (A & B) had some white/light feathers on the head, whereas the adults (C) had all black-brown heads.



A: Juvenile/Sub-adult
More than 50% white feathers on head
White feathers on ventral area

C: Sub-adult
No white feathers on head
Some white feathers on ventral area

B: (Juvenile)/Sub-adult
Less than 50 % white feathers on head
White feathers on ventral area

D: Adult
No white feather

Figure AVII 3. Non-adult males were divided into four different age-classes (A-D) in accordance with the amount of white in the plumage.

Appendix VIII – Male characteristics per age-class

Appendix IX – Male characteristics per mating status

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