



## SHORT COMMUNICATIONS

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### BACTERIA IN THE CROP OF THE SEED-EATING GREEN-RUMPED PARROTLET

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**Abstract.** The Green-rumped Parrotlet (*Forpus passerinus*) feeds almost exclusively on seeds of *Croton hirtus*, (Euphorbiaceae) during reproduction. *Croton hirtus* seeds are low in protein and rich in carbohydrates, particularly starch. We studied the bacterial composition of the crop of the Green-rumped Parrotlet to explore if microbial activity could enhance digestion by this parrotlet. Crop contents were cultured anaerobically and counted by agar dilution. Isolated bacterial strains were identified by membrane fatty acids analysis using gas chromatography. Green-rumped Parrotlet crops contained from  $10^5$  to  $10^{12}$  bacteria per g wet content. Predominant strains included species of *Enterococcus*, *Lactobacillus*, *Streptococcus*, *Propionibacterium*, and enterobacterias. Both *Lactobacillus* and *Streptococcus* produce amylase. Our finding suggests that microbial activity in this species' crop represents an important adaptation to a seed diet by providing enzymes that digest dietary starch. We conclude that crop microbial flora seems to be important in the nutritional physiology of this small psittacid.

**Key words:** *bacteria, crop, fermentation, Forpus passerinus, psittacid, starch digestion.*

#### Bacteria Identificadas en el Bucle de *Forpus passerinus*

**Resumen.** El periquito *Forpus passerinus* se alimenta principalmente de semillas de *Croton hirtus* (Euphorbiaceae) durante su período reproductivo. Las semillas de *C. hirtus* son pobres en proteínas y ricas en carbohidratos, principalmente almidón. Nosotros estudiamos la composición bacteriana del buche de

esta ave para explorar si la actividad microbiana puede ser importante en la digestión de los carbohidratos de la dieta. El contenido del buche fue cultivado anaerómicamente y el número total de colonias viables de bacterias fue obtenido por el método de dilución en agar. Las bacterias fueron identificadas mediante análisis de ácidos grasos de membrana, utilizando cromatografía de gases. Encontramos que el buche está colonizado por  $10^5$  a  $10^{12}$  colonias de bacterias por g de contenido húmedo. Los géneros predominantes son *Enterococcus*, *Lactobacillus*, *Streptococcus*, *Propionibacterium* y enterobacterias. *Lactobacillus* y *Streptococcus* producen amilasa. Nuestros resultados sugieren que la actividad microbiana en el buche representa una adaptación importante para una dieta a base de semillas, al proveer enzimas que degradan el almidón de la dieta. Nosotros concluimos que la microflora bacteriana del buche puede ser importante en la fisiología nutricional de este pequeño psitácido.

Starches are the most abundant polysaccharides in seeds (Robbins 1993, Huntington 1997), but are more difficult to digest than disaccharide sugars because they are complex, they have indeterminate molecular weights, and they are highly insoluble, forming an orderly crystalline structure that hinders the action of enzymes in the aqueous milieu of the gut (Klasing 1998, Stevens and Hume 1998). Nonetheless, multiple adaptations allow herbivores to use starch as an energy and carbon source.

In herbivorous ruminants bacteria occur in both aerobic and anaerobic forms. Fermentation by anaerobic bacteria in the forestomach breaks the substrate into volatile fatty acids that the host can absorb (Kotarski et al. 1992, Robbins 1993). Starches can also be digested, by the sequential action of a variety of enzymes, into their easily absorbed glucose components. Starch-digesting amylase can be produced endoge-

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nously by the salivary glands and pancreas in herbivores and omnivores (Robbins 1993), or can be produced by bacteria in the gut of herbivores (Kotarski et al. 1992). This enzyme attaches to the amylose helix at aqueous surfaces and then sequentially hydrolyzes interior maltose units until the nonreducing end is reached. Maltose, isomaltose, and dextrans are further hydrolyzed by enzymes attached to the membranes of enterocytes (Klasing 1998).

In birds, salivary amylase is either minimal or absent (Robbins 1993); however, amylase activity has been demonstrated in the crop of the chicken (*Gallus domesticus*; Bolton 1965) and turkey poults (*Meleagris gallopavo*; Pinchasov and Noy 1994). Hydration of food during storage in the crop or esophagus facilitates subsequent hydrolysis by pancreatic  $\alpha$ -amylase in the duodenum (Klasing 1998). Not surprisingly, granivorous birds have well-developed crops, which in addition to providing storage space (Klasing 1998), could be the site of extensive hydration of starch-rich seeds. In addition, in granivores such as the pigeon (*Columba livia*; Shetty et al. 1990, Baele et al. 2001) and chicken (Fuller and Turvey 1971), the crop may also work as an organ for microbial fermentation, facilitating the digestion of starch (Ziswiler and Farner 1972).

The most impressive microbial activity ever reported in the crop was found in the Hoatzin (*Opisthocomus hoazin*), a Neotropical folivore (Grajal et al. 1989, Dominguez Bello et al. 1993). This species is the first bird reported to have true foregut fermentation, similar to that found in several groups of herbivorous mammals. However, a paucity of information limits our understanding of crop microbial activity as an adaptation to herbivory in birds.

The Green-rumped Parrotlet (*Forpus passerinus*) is the smallest Neotropical psittacid (25 g). It inhabits savanna, pastures, and forest edges in the central plains (llanos) of Venezuela (Hilty 2003). This parrotlet feeds mostly on the seeds of shrubs, and its nestlings are fed almost exclusively the unripe seeds of the euphorb *Croton hirtus* (Waltman and Beissinger 1992, Pacheco 2000). *Croton hirtus* seeds are low in protein (9–13% dry mass) and high in carbohydrates (84%, Pacheco 2000), mainly starch.

Green-rumped Parrotlet nestlings have a mean digesta retention time of 18–20 hr, and a large portion of this time can be accounted for by residence in the crop. Indeed, 50% of the seeds remain in the crop for 5–9 hr and seeds are still observed in the crop until 10–13 hr after ingestion (Pacheco 2000). This retention time is extremely long for a bird of its size and considerably higher than that of other granivorous birds (Karasov 1990). This unusually long retention time could be an adaptation for digesting starch-rich food, since a long time in the crop should facilitate hydration of the starch or possible fermentation by microbial activity. Presumably, adult Green-rumped Parrotlets have a long retention time also. In this study, we investigated the anaerobic bacteria inhabiting the crop of the Green-rumped Parrotlet.

## METHODS

Four adult and two nestling Green-rumped Parrotlets were captured separately, at Ranch La Fe (8°34'N,

TABLE 1. Mean ( $\pm$  SD) number of colony formation units (CFU) of viable bacteria in the crop of adult and nestling Green-rumped Parrotlets. Means are from triplicate samples.

Specimen	CFU per g of wet content
Female 1	$1.1 \pm 0.4 \times 10^5$
Male 1	$7.3 \pm 2.1 \times 10^{12}$
Male 2	$4.5 \pm 3.3 \times 10^9$
Male 3	$1.7 \pm 1.2 \times 10^7$
Nestling 1	$2.8 \pm 2.0 \times 10^{12}$
Nestling 2	$4.3 \pm 0.4 \times 10^8$

67°35'W), near the town of Corozopando, in the central Venezuelan llanos of Guárico State. Birds were euthanized using an inhalant anesthetic (Halothan®, Roche, S.A., Caracas, Venezuela) and their crops were dissected and transported to the laboratory in liquid nitrogen. Crop contents were cultured in M10 medium (Caldwell and Bryant 1966) under anaerobic conditions (Bryant 1972). Specifically, bacteria were cultured under CO<sub>2</sub> (O<sub>2</sub>-free atmosphere) in Hungate tubes (16 × 125 mm) sealed with butyl rubber stoppers (Bellco Glass, Vineland, New York). The number of colony-forming units (any entity, usually a viable single cell, which can form a colony) were estimated by the agar dilution method, inoculating (in triplicate) with serial dilutions of crop contents in M10 agar-rolled tubes. Incubations for total counts of bacteria were carried out at  $37 \pm 1^\circ\text{C}$  (SD) during 5–7 days. After counting the colonies on agar-rolled tubes (2% weight vol<sup>-1</sup>) of M10, we picked and transferred representative bacteria colonies to tubes of M10 broth. Isolated bacteria strains were identified by microscopic examinations (Gram stain and cell shape) and membrane fatty acid analysis (MFAA) by gas chromatography and were compared to the bacterial database of the Sherlock® Microbial Identification System (Sasser 2001; MIDI, Inc., Newark, Delaware).

Anaerobic bacterial identification at Microbial Identification, Inc., is based on a similarity index that ranges from 0 to 1, and expresses how closely the fatty acid composition of an unknown sample compares with the mean fatty acid composition of the bacteria strains used to create the library. For anaerobes, a single match to a species with a similarity index of 0.35 or higher, and with a difference of at least 0.05 between first and second choices, is considered a good match. Similarity index values lower than 0.30 indicate that although the species may not be in the database, those listed provide a possible genus match. Aerobic cultures or counts of aerobic bacteria were not performed because they are only accurate if performed on fresh samples.

## RESULTS

The crops of Green-rumped Parrotlets (adults and nestlings) were colonized by anaerobic bacteria (Table 1). The bacteria isolated are likely resident in the crop of the Green-rumped Parrotlet, and not accidentally found there, since each bacteria type was found in more than

one individual, and individuals were captured in separate instances.

Bacteria isolated from the crops of Green-rumped Parrotlets were predominantly facultative anaerobic Gram-negative rods and Gram-positive cocci or coccobacilli (Table 2). Predominant strains included species of Gram-positive *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Propionibacterium*, and *Pediococcus*, as well as Gram-negative *Enterobacter*, *Klebsiella*, and *Escherichia coli* (Table 2). There were also unidentified bacteria that might well represent unknown species colonizing an environment that has not been studied previously.

## DISCUSSION

Total counts of viable bacteria in the crop of the seed-eating Green-rumped Parrotlet were similar to those found in the forestomach of several herbivorous mammals, and in the crop of chickens, pigeons and the Hoatzin ( $10^9$ – $10^{10}$  colony-forming units, Stevens and Hume 1998). To our knowledge, no information on the bacterial gut flora of other psittacines is available.

Most bacteria were close to genera that are also commonly found in the crops of other granivorous birds such as pigeons (Shetty et al. 1990, Baele et al. 2001) and chickens (Fuller and Turvey 1971). In the pigeon crop, *Streptococcus* and *Lactobacillus* predominate over coliforms and *Enterococcus*. The mildly acidic condition prevalent in the pigeon crop lumen seems to favor the growth of streptococci (Shetty et al. 1990, Baele et al. 2001). In chickens, the crop is highly colonized by bacteria compared to other regions of the gut (Fuller and Turvey 1971). *Lactobacillus*, a permanent resident of chicken crop mucosa, predominates over streptococci and coliforms, and it has been suggested that they might play a role in the maintenance of the normal microbiological equilibrium of the host (Fuller 1977).

The crops of Green-rumped Parrotlets harbored amylase-producing *Lactobacillus* and *Streptococcus* species in very high densities. These bacteria can degrade starch into maltose, maltotriose, and glucose (Champ et al. 1983, Kotarski et al. 1992). These oligosaccharides and disaccharides are hydrolyzed to monosaccharides by enzymes located in the microvilli, or brush border, of intestinal cells (Stevens and Hume 1998). Glucose could be absorbed through the crop, as suggested by Soedarmo et al. (1961), or fermented by *E. coli*, *Klebsiella* spp. and *Enterobacter* spp., producing volatile fatty acids. Most of these volatile fatty acids are immediately absorbed; they are used as an energy source and to maintain acidity within a tolerable range for the microbial population (Stevens and Hume 1998). Microbial fermentation of starch and sugars in the chicken crop yields lactate, volatile fatty acids (acetic, formic, propionic and butyric acids), and ethanol (Bolton 1965). Although fermentation of starch produces less energy than its conversion to glucose by endogenous enzymes, microbial fermentation could be an advantage to animals on a high-starch diet (Stevens and Hume 1998).

The presence of starch-digesting bacteria in high counts strongly suggests starch degradation in the crop of the Green-rumped Parrotlet. This would allow at

TABLE 2. Most common bacteria isolated from the crops of six Green-rumped Parrotlets.

Gram stain and shape	Total colonies isolated	Species <sup>a</sup>	Similarity index	Colony formation units	Growth	Isolated from	
						Nestlings	Adults
Cocci +	13	<i>Enterococcus durans</i>	0.65	$10^7$ – $10^{10}$	Facultative anaerobe	2	3
Cocci +	6	<i>Streptococcus mitis</i>	0.60	$10^6$ – $10^{10}$	Facultative anaerobe	1	2
Cocci +	3	<i>Pediococcus damnosus</i>	0.41	$10^8$ – $10^{10}$	Facultative anaerobe	1	1
Rods +	5	<i>Propionibacterium acnes</i>	0.80	$10^5$ – $10^{10}$	Anaerobe	1	2
Rods +	5	<i>Lactobacillus delbrueckii</i>	0.55	$10^{10}$	Facultative anaerobe	1	2
Rods—	5	<i>Escherichia coli</i>	0.72	$10^6$ – $10^8$	Facultative anaerobe	1	2
Rods—	10	<i>Enterobacter cloacae</i>	0.89	$10^3$ – $10^8$	Facultative anaerobe	0	2
Rods—	3	<i>Klebsiella oxytoca</i>	0.64	$10^2$ – $10^3$	Facultative anaerobe	0	1
Rods—	4	unidentified	—	$10^{10}$	Anaerobe	1	0
Rods—	2	unidentified	—	$10^{10}$	Anaerobe	1	0
Rods—	2	unidentified	—	$10^7$	Facultative anaerobe	0	1

<sup>a</sup> As identified by the Sherlock® Microbial Identification System, Microbial Identification, Inc., Newark, Delaware (Sasser 2001).

least a partial digestion of *C. hirtus* and other seeds. Importantly, protracted retention of food in the crop should facilitate hydration of starch, as well as enhance the efficiency of the microbial activity. The synergistic effects of a very long retention time and the presence of starch-digesting bacteria appear to be a key adaptation for the processing of an energy rich, but difficult to digest, seed diet. In addition, a significant crop bacterial biomass might also constitute a source of bacterial protein, enhancing the nitrogen budget of Green-rumped Parrotlets, particularly on a protein-poor diet (Pacheco 2000).

Our limited knowledge of microbial activity and composition of the crop microflora of birds precludes a complete assessment of microbes' relevance to the digestion of bird food. The best-documented case is the Hoatzin, which has a well-developed crop and caudal esophagus where fermentation generates high levels of volatile fatty acids (Grajal et al. 1989). Although not an obligate folivore, the Speckled Mousebird (*Colius striatus*), endemic to sub-Saharan Africa, also has volatile fatty acids in its well-developed proventriculus and ventriculus, which are produced by the various bacteria found there, possibly *Planococcus* or *Rumenococcus* and some others that were rod-shaped (Downs et al. 2000). The levels of volatile fatty acids found in Speckled Mousebirds (Downs et al. 2000) are similar to those in Hoatzins (Grajal et al. 1989). In these folivorous birds, symbiotic relationships with fiber-digesting bacteria enable them to utilize otherwise nondigestible plant fiber as an energy and carbon source (Robbins 1993).

A limited amount of pregastric fermentation may also occur in less specialized species, such as pigeons (Shetty et al. 1990) and chicken (Champ et al. 1983). In these species, food remains in the crop for several hours and microbial digestion results in high levels of lactic acid production and low levels of volatile fatty acids. The digestive contribution of this microbial action is minor, and the major nutrients consumed by bacteria are the readily available carbohydrates and amino acids, which are digested easily by the bird without microbial help (Klasing 1998). However, in the chicken, the major contribution of microbial fermentation in the digestion of complex carbohydrates occurs in the ceca (Mead 1989). The Green-rumped Parrotlets do not have ceca; thus, the crop is the most likely place where significant microbial activity associated with starch digestion could be found.

There is indirect evidence of the potential role of the crop microflora in one other parrot. The Kakapo (*Strigops habroptilus*), a herbivorous New Zealand endemic, eats plant roots, rhizomes, bulbs, buds, leaves, flowers, pollen cones, and berries from a variety of species (Powlesland et al. 1997). The crop of the Kakapo is particularly well developed, but existence of crop-associated bacteria have not been studied (Morton 1978).

Our findings suggest that microbial activity in the crop of seed-eating and possibly leaf-eating birds could be a more general phenomenon than currently acknowledged and an important adaptation to a vegetable diet. In summary, we propose that crop-associated bacteria could be important in the nutritional

physiology of this small psittacid by providing starch-digesting enzymes and possibly a source of additional nondietary protein.

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## REST-PHASE THERMOREGULATION IN FREE-RANGING WHITE-BACKED MOUSEBIRDS

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**Abstract.** In White-backed Mousebirds (*Colius colius*), clustering is an important component of rest-phase thermoregulation, particularly at low air temperature. We tested predictions concerning thermoregulation under natural conditions by recording rest-phase body temperature in a flock of free-ranging White-backed Mousebirds in an arid habitat during winter in the Karoo, South Africa. We found that the mousebirds' rest-phase body temperature was relatively labile, but was generally maintained above 32°C, despite air temperatures as low as –3.4°C. Patterns of rest-phase body temperature were highly synchronized among individuals within the cluster. The mousebirds showed no evidence of torpor under natural conditions; a shallow departure from normothermy, during which

body temperature was reduced to 29–33°C, was observed on only one occasion. The observed patterns of thermoregulation supported our predictions, and confirm that thermoregulation in clustering White-backed Mousebirds in the wild is significantly different from that of single birds under laboratory conditions.

**Key words:** behavior, body temperature, *Coliiformes*, *Colius colius*, mousebirds, thermoregulation, sociality.

### Termorregulación en la Fase de Reposo en Individuos de *Colius colius* que se Desplazan Libremente

**Resumen.** En *Colius colius*, la agregación es un componente importante de la termorregulación en la fase de reposo, particularmente a temperaturas bajas del aire. Examinamos predicciones relacionadas con la termorregulación en condiciones naturales registrando la temperatura corporal en la fase de reposo en una bandada de individuos de *C. colius* que se desplazan libremente durante el invierno en un ambiente árido en el Karoo, Sudáfrica. Encontramos que la tempera-

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tura corporal de la fase de reposo de *C. colius* fue relativamente lábil, pero fue mantenida generalmente por arriba de los 32°C, a pesar de temperaturas del aire tan bajas como -3.4°C. Los patrones de temperatura corporal de la fase de reposo estuvieron altamente sincronizados entre individuos dentro del agrupamiento. *C. colius* no mostró evidencia de torpor bajo condiciones naturales. En una única ocasión se observó un pequeño desvío de la normotermia, durante la cual la temperatura corporal fue reducida a 29–33°C. Los patrones de termorregulación observados apoyaron nuestras predicciones y confirmaron que la termorregulación en agrupamientos silvestres de *C. colius* es significativamente diferente de la de aves solitarias bajo condiciones de laboratorio.

The mousebirds (Coliiformes) are a phylogenetically ancient order endemic to the Afrotropics (Sibley and Ahlquist 1990), and patterns of thermoregulation in this group have proven to be of considerable interest. The mousebirds are unusual in that they roost communally (clustering), and also exhibit a pronounced capacity for torpor (Bartholomew and Trost 1970, Prinzing, Göppel and Lorenz 1981, Hoffmann and Prinzing 1984, Brown and Foster 1992, McKechnie and Lovegrove 2001a, 2001b). Hence, they provide a useful taxon within which to investigate the interaction between these two mechanisms of energy conservation. Avian thermoregulation during the rest phase of the circadian cycle typically involves the defense of a body temperature ( $T_b$ ) setpoint by means of metabolic heat production, with rest-phase  $T_b$  maintained within narrow limits during normothermy (i.e., in the absence of any facultative hypothermic response) (Prinzing et al. 1991, Dawson and Whittow 2000). In contrast, most species of mousebirds investigated to date exhibited relatively labile normothermic rest-phase  $T_b$  during laboratory studies (Bartholomew and Trost 1970, Prinzing, Göppel et al. 1981, Brown and Foster 1992, McKechnie and Lovegrove 2001a, 2001b). Because the rest-phase  $T_b$  of mousebirds is atypically variable, patterns of thermoregulation in this order provide insights into the distinction between normothermic thermoregulation and the various categories of avian facultative hypothermic responses traditionally recognized in the literature.

Although mousebirds are well known for their propensity to enter torpor under laboratory conditions, the energy savings associated with clustering likely obviate the need for such facultative hypothermic responses under most natural conditions (McKechnie and Lovegrove 2001a, 2001b). White-backed Mousebirds (*Colius colius*) inhabit the arid western and central regions of southern Africa (Fry et al. 1988). Under laboratory conditions, White-backed Mousebirds did not maintain a constant normothermic rest-phase  $T_b$  setpoint, as typically occurs during avian thermoregulation, but exhibited approximately linear decreases in rest-phase  $T_b$  at rates dependent on air temperature ( $T_a$ ) and the number of birds in a cluster (McKechnie and Lovegrove 2001a). In this study, the lowest rest-phase  $T_b$  observed in a single (nonclustering) bird able to spontaneously arouse was 26°C at  $T_a = 5$ °C. At the same  $T_a$ , four out of nine single birds exhibited non-

regulated, apparently irreversible decreases in rest-phase  $T_b$  near the end of the rest phase, and three of these birds had to be rewarmed using an artificial heat source (McKechnie and Lovegrove 2001a). These observations suggested that the thermoregulatory capacity of single White-backed Mousebirds was limited, and that clustering is an obligatory component of rest-phase thermoregulation at low  $T_a$ . In the present study, we examined the thermoregulatory role of clustering in free-ranging White-backed Mousebirds by recording rest-phase  $T_b$  over a period of two and a half weeks during the coldest time of the year. We predicted that clustering free-ranging White-backed Mousebirds should exhibit considerably less rest-phase  $T_b$  lability, and maintain higher overall rest-phase  $T_b$ , than single birds under laboratory conditions. We also predicted that facultative hypothermic responses such as torpor should be rare or absent entirely.

## METHODS

### STUDY SITE

We conducted the study on the farm Tierberg (33°07'S, 22°16'E; 750 m elevation) near Prince Albert, South Africa, during June 2000. This area is characterized by sparse, dwarf shrubland, with a high proportion of succulent plants (Dean et al. 1993). We conducted the study during June, which is one of the two coldest months at Tierberg, and thus at this time of year the mousebirds experience the highest thermoregulatory costs (Dean et al. 1993). The following climatological data for Tierberg were obtained from the Computing Centre for Water Research, University of Natal: mean annual precipitation: 245 mm; coefficient of variation of mean annual precipitation: 37%; mean daily maximum and minimum temperatures (June): 14.0°C and 1.1°C respectively. At Tierberg, White-backed Mousebirds occur along drainage lines in vegetation consisting predominantly of sweet thorn (*Acacia karroo*), common guarri (*Euclea undulata*), namaqua kuni-bush (*Rhus undulata*), yellow pomegranate (*Rhizogum obovatum*) and *Diospyros* spp. (Dean et al. 1993). Rainfall at Tierberg occurs year-round, and the availability of fruit is not distinctly seasonal (Dean et al. 1993).

### BODY TEMPERATURE MEASUREMENT

Temperature-sensitive FM transmitters (Sirtrack, Havelock North, New Zealand) with an average mass of 1.6 g and frequencies between 146.0 and 146.5 MHz were used to measure  $T_b$  in eight mousebirds (body mass = 40.8 ± 5.7 g). The transmitters were calibrated in a water bath at temperatures between 10°C and 45°C, using a standard mercury thermometer (accurate to within 0.05°C), with an accuracy traceable to the U.S. National Bureau of Standards. We trapped the mousebirds using walk-in traps constructed from wire mesh and baited with a variety of fruits. The transmitters were implanted into the peritoneal cavities of the mousebirds under inhalation anesthesia (isoflurane in oxygen; induction and maintenance, 2.5%; flow rate, ca. 0.5 L min<sup>-1</sup>). Following surgery, the mousebirds typically recovered from anesthesia within 10 min, but we kept them in a holding cage for at least 2 hr to ensure complete recovery. They were then released at the site of capture. The mousebirds were re-

captured at the end of the study so that the telemeters could be removed.

Signals from the transmitters were recorded using a custom-built multichannel data-logger. The data-logger controlled a single side-band mode communications receiver (Model IC-R10, Icom, Bellevue, Washington) and recorded pulse intervals of all eight transmitters in 10-min intervals, using a 6-element Yagi antenna. Air temperature was measured by the data-logger using a monolytic temperature transducer (LM335). Data were stored on an electronically erasable programmable read-only memory (EEPROM) chip and downloaded daily. Electronics and software were developed by the authors. The duration of the study was limited by the battery life of the transmitters (ca. 3 weeks).

#### STATISTICAL ANALYSES

Data collection commenced immediately following implantation, but data recorded within 48 hr of surgery were excluded from the analyses. We obtained a total of 54  $T_b$  traces from 2–8 birds on the nights of 12–28 June. For analyses of rest-phase  $T_b$ , we considered the rest phase to commence immediately following the rapid decreases in  $T_b$  that occurred shortly after sunset and to end immediately preceding the rapid increases in  $T_b$  shortly before departure from the roost site the following morning. Body temperatures were compared using repeated-measures analyses of covariance (Zar 1999), using mean nighttime  $T_a$  and minimum nighttime  $T_a$  as covariates. Results are presented as means  $\pm$  SE.

#### RESULTS

The mousebirds spent the daylight hours foraging and sunning in the vegetation along the drainage line. Late each afternoon, they moved to a specific area along the drainage line, and spent the last half-hour before sunset sunning themselves. While sunning, the mousebirds clung to suitable perches with their underparts oriented toward the sun, with the belly feathers raised to expose the skin. Immediately following sunset, they formed a cluster in a dense tree in the drainage line. The narrow reception angle of the Yagi antenna meant that the time of arrival at the roost site could be determined to within 10 min by the time of the first  $T_b$  measurements. The average time between sunset and the commencement of clustering was <10 min. The group of mousebirds that clustered at this site appeared to consist of 12 individuals. The tree in which the birds clustered was exposed to solar radiation until sunset, but was shaded by a hill until approximately 45 min after sunrise. In the mornings, the birds left the roost while it was still shaded, on average <10 min before sunrise. The same site was used every night, with the exception of the night of 21 June. On this night, the birds clustered in a low bush on the surrounding open plain, 0.95 km from the usual roost site. We suspect that the birds used a different roost site on this night because of the presence of a Pale Chanting Goshawk (*Melierax canorus*), which was observed flying and perching within 50 m of the regular roost site shortly prior to sunset.

The mean nighttime  $T_a$  (sunset – sunrise) was  $3.4 \pm 0.5^\circ\text{C}$  (range  $-0.7$  to  $12.9^\circ\text{C}$ ). The mean minimum nighttime  $T_a$  was  $0.0 \pm 0.5^\circ\text{C}$  (range  $-3.4$  to  $9.2^\circ\text{C}$ ).

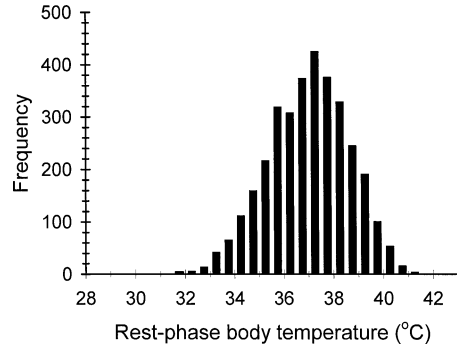


FIGURE 1. Frequency histogram of normothermic rest-phase body temperature recorded in eight clustering White-backed Mousebirds between 12 and 28 June 2000 at Tierberg, South Africa. Data from the night of 21–22 June have not been included, since the birds exhibited a shallow departure from normothermy.

Typically, the temperature reached a minimum shortly before sunrise. On most nights there was little or no wind.

#### BODY TEMPERATURE

Compared to typical avian thermoregulation, the rest-phase  $T_b$  of clustering White-backed Mousebirds was variable and ranged from  $32^\circ\text{C}$  to  $40^\circ\text{C}$  (Fig. 1). Minimum rest-phase  $T_b$  showed a weak but significant positive relationship ( $F_{1,48} = 8.2$ ;  $P < 0.05$ ) with mean  $T_a$  between sunset and sunrise, best described by a linear model (minimum rest-phase  $T_b = 33.93 + 0.237T_a$ ;  $r^2 = 0.15$ ).

The rest-phase  $T_b$ s of the mousebirds decreased gradually during the night (Fig. 2). This observation is confirmed by the fact that in all traces, minimum rest-phase  $T_b$  during the final hour of the rest phase ( $34.8 \pm 0.2^\circ\text{C}$ ) was significantly lower (repeated measures ANCOVA,  $F_{1,90} = 75.1$ ,  $P < 0.05$ ) than minimum rest-phase  $T_b$  during the first hour ( $37.4 \pm 0.2^\circ\text{C}$ ). Hence, rest-phase  $T_b$  decreased on average by  $2.6^\circ\text{C}$  during the course of the rest phase. The mean cooling rate, calculated by fitting linear regressions to rest-phase  $T_b$  as a function of time, was  $0.22 \pm 0.01^\circ\text{C hr}^{-1}$ . This cooling rate is equivalent to ca. 25% of the mean cooling rate exhibited by single birds, and to ca. 40% of that exhibited by clusters of six birds, at  $T_a = 5^\circ\text{C}$  under laboratory conditions (McKechnie and Lovegrove 2001a). Cooling rate was negatively correlated with mean  $T_a$  between sunset and sunrise ( $F_{1,47} = 12.1$ ;  $P < 0.05$ ; Fig. 3). The relationship between cooling rate and mean  $T_a$  was best described by a linear model (Fig. 3). The rest-phase  $T_b$ s of individual mousebirds within the cluster were highly synchronized (Fig. 2, 4), although on some occasions a rest-phase  $T_b$  gradient of up to  $3^\circ\text{C}$  existed between individuals.

With the exception of the night of 21 June, the birds did not show any obvious departures from normothermic thermoregulation. On the night of 21 June, the birds' rest-phase  $T_b$  decreased rapidly to between  $29^\circ\text{C}$  and  $33^\circ\text{C}$  at ca. 03:00 (Fig. 4). Thereafter, rest-phase  $T_b$  returned to normal levels. Even though the night

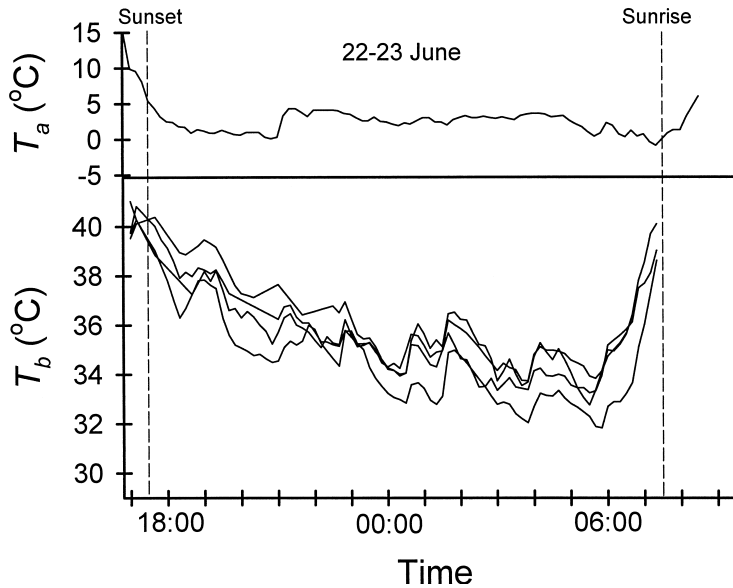


FIGURE 2. Representative traces of body temperature ( $T_b$ ) of four clustering White-backed Mousebirds (lower graph) and air temperature ( $T_a$ ; upper graph), recorded during the night of 22–23 June 2000 at Tierberg, South Africa. The commencement and termination of  $T_b$  measurements coincide with the times that the birds arrived and departed from the roost site, respectively.

was relatively warm, the birds clustered in a more exposed site than usual, and experienced strong wind and rain. We suspect that the decrease in rest-phase  $T_b$  coincided with a bout of heavy rain. Data from the night of 21 June were not included in the above analyses.

The  $T_b$  of the mousebirds immediately prior to departure from the roost in the morning ranged from 36.6°C to 42.5°C, with a mean of  $39.0 \pm 0.2^\circ\text{C}$ . Typically, the birds left the roost site before sunrise, at which time  $T_a$  was still close to the overnight minimum. These observations suggest that the birds increased their  $T_b$  by means of endogenous heat production, and that solar radiation did not play any role in elevating  $T_b$  to active-phase (diurnal) levels.

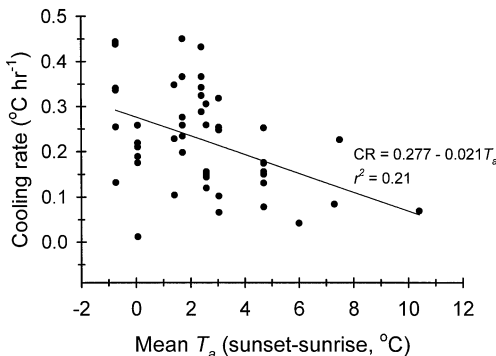


FIGURE 3. Rest-phase cooling rate in eight clustering White-backed Mousebirds, plotted against mean air temperature ( $T_a$ ) between sunset and sunrise.

## DISCUSSION

Consistent with our predictions, clustering free-ranging White-backed Mousebirds exhibited considerably less labile rest-phase  $T_b$  and lower cooling rates than single birds under laboratory conditions. Under laboratory conditions, single White-backed Mousebirds exhibited an average cooling rate of ca.  $0.8^\circ\text{C hr}^{-1}$ , and rest-phase  $T_b$  ranging from 26°C to 38°C (McKechnie and Lovegrove 2001a). The cooling rates we observed in clustering free-ranging birds were lower than those observed in groups of six birds under laboratory conditions (McKechnie and Lovegrove 2001a), most likely on account of the larger cluster size. Our results reiterate the importance of social thermoregulation in White-backed Mousebirds. Whereas clustering free-ranging birds maintained rest-phase  $T_b > \text{ca. } 32^\circ\text{C}$  at air temperatures as low as  $-3.4^\circ\text{C}$  (with one exception, see below), single birds under laboratory conditions exhibited rest-phase  $T_b$  down to 26°C, and in some instances nonregulated, irreversible decreases in rest-phase  $T_b$  at  $T_a = 5^\circ\text{C}$ . Although we found significant relationships between both rest-phase  $T_b$  and cooling rate and  $T_a$ ,  $T_a$  by itself is rarely a meaningful approximation of an organism's thermal environment (Robinson et al. 1976, Mahoney and King 1977, Bakken 1989, Wolf and Walsberg 1996) and is of limited utility in estimating heat fluxes between a bird and its surroundings. The data measured under laboratory conditions by McKechnie and Lovegrove (2001a) provide more convincing evidence for an effect of  $T_a$  on patterns of thermoregulation, since convective and radiative heat fluxes were approximately constant.

Even when clustering, the rest-phase  $T_b$  of the mousebirds was more labile than that of most birds,

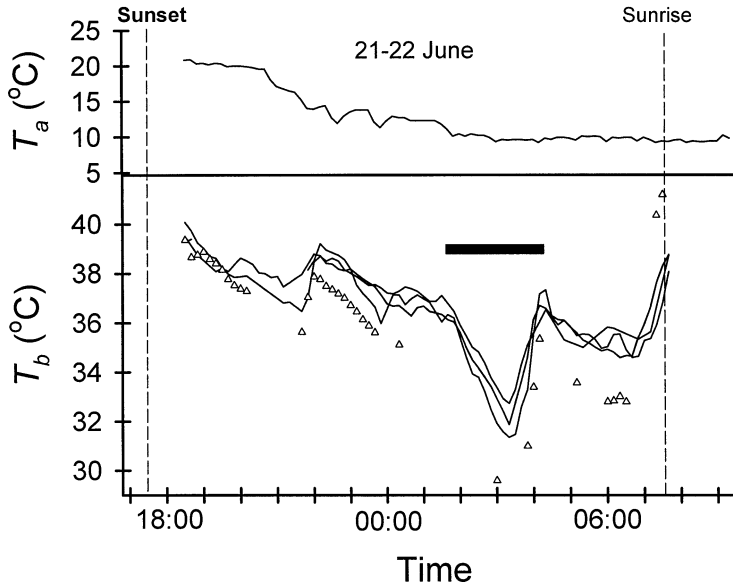


FIGURE 4. Body temperature ( $T_b$ ) of four free-ranging White-backed Mousebirds (lower graph) and air temperature ( $T_a$ ; upper graph) on the night of 21–22 June 2000 at Tierberg, South Africa. The commencement and termination of  $T_b$  measurements coincide with the times that the birds arrived and departed from the roost site, respectively. The triangles represent intermittent data, but have been included because this bird exhibited the lowest  $T_b$  recorded during the study. The shallow departure from normothermy discussed in the text is indicated by a horizontal bar.

and did not appear to involve the defense of rest-phase  $T_b$  within a narrow range with respect to a single set-point  $T_b$ , as typically occurs in birds (Dawson and Whittow 2000; for examples see Maddocks and Geiser 1997, Boix-Hinzen and Lovegrove 1998, McKechnie and Lovegrove 1999). The normothermic rest-phase  $T_b$  range of ca. 8°C exhibited by White-backed Mousebirds is unusual for birds, but is similar to the pattern observed in Puerto Rican Todies (*Todus mexicanus*; Merola-Zwartjes and Ligon 2000), as well as in some mammals (Wooden and Walsberg 2002).

The labile patterns of thermoregulation shown by clustering White-backed Mousebirds in the wild presumably reflect in part an inherited trait, since relatively variable rest-phase  $T_b$  is widespread in the Coliiformes (Bartholomew and Trost 1970, Prinzinger, Göppel et al. 1981, Hoffmann and Prinzinger 1984, McKechnie and Lovegrove 2001a, 2001b). Nevertheless, these labile rest-phase  $T_b$  patterns are likely to have adaptive value in the arid habitats this species occupies. The pattern of gradual rest-phase cooling exhibited by White-backed Mousebirds under laboratory conditions, and to some extent under natural conditions, requires lower energy expenditure than typical avian homeothermy, characterized by the defense of a constant rest-phase  $T_b$  of ca. 38–39°C (Prinzinger et al. 1991). Any avenue whereby rest-phase energy expenditure is reduced is likely to be adaptive in arid areas characterized by unpredictable food supplies (Lovegrove 2000). For instance, Tieleman and Williams (2000) have shown that both basal metabolic rate and field metabolic rate are reduced in birds inhabiting arid

habitats. We argue that the labile rest-phase  $T_b$  of free-ranging White-backed Mousebirds contributes to this species' ability to survive even in the most arid southern African habitats (Harrison et al. 1997).

#### TORPOR IN FREE-RANGING MOUSEBIRDS

The pronounced capacity for torpor usually associated with the Coliiformes was not evident during the study period. Avian torpor typically involves  $T_b$  depression by >10°C below normothermic levels followed by re-warming at the onset of the active phase (Prinzinger et al. 1991, Reinertsen 1996, McKechnie and Lovegrove 2002). Despite June being one of the coldest months at Tierberg, with the amount of time available for foraging reduced due to shorter daylength, we did not observe torpor in White-backed Mousebirds during two and a half weeks of observations. With the possible exception of the night of 21 June, when the mousebirds exhibited a decrease in rest-phase  $T_b$  to between 29°C and 33°C, there was no evidence of facultative departures from normothermy. Our data hence support the argument that in wild mousebirds, the use of torpor is probably rare. Under most conditions, the energy savings mousebirds make by means of social thermoregulation probably obviate the need to employ facultative hypothermic responses such as torpor to reduce energy expenditure.

Currently, there is considerable interest in the degree to which patterns of thermoregulation under artificial, laboratory conditions reflect those that occur in free-ranging birds and mammals in their natural environments (Geiser et al. 2000, Geiser and Ferguson 2001).

The mousebirds provide a striking example of the potential importance of sociality and communal roosting, and offer intriguing insights into the interaction between behavioral and metabolic thermoregulation. Moreover, they appear to represent an exception to the observation that facultative hypothermic responses are generally more frequent and pronounced under natural conditions than in artificial, laboratory environments (Geiser et al. 2000).

#### DEFINING AVIAN FACULTATIVE HYPOTHERMIC RESPONSES

Avian facultative hypothermic responses have traditionally been divided into hibernation, daily torpor, and rest-phase hypothermia (Prinzinger et al. 1991, Reinersen 1996, McKechnie and Lovegrove 2002). Hibernation and daily torpor are readily distinguished on the basis of bout length and minimum  $T_b$  (Geiser and Ruf 1995). However, the distinction between rest-phase hypothermia and torpor is less clear (McKechnie and Lovegrove 2002). The patterns of thermoregulation we observed in free-ranging White-backed Mousebirds, as well as Merola-Zwartjes and Ligon's (2000) observations on thermoregulation in Puerto Rican Todies, highlight an additional problem: distinguishing facultative hypothermic responses from normothermic thermoregulation. Consider the traces of rest-phase  $T_b$  recorded on the night of 21 June (Fig. 4). The transient decrease in rest-phase  $T_b$  at approximately 02:00 could be explained in at least three different ways. First, the increase in cooling rate at ca. 02:00 may reflect a rapid increase in the rate of heat loss from the cluster (e.g., onset of rain). According to this explanation, the rest-phase  $T_b$  of the clustering birds decreased until some threshold  $T_b$  was reached. Once this threshold was reached, the birds increased their metabolic heat production. This increase in heat production returned rest-phase  $T_b$  to normothermic levels at ca. 04:30. Second, the synchronized hypothermic response may have represented a facultative decrease in metabolic heat production. According to this explanation, the mousebirds responded to energetic stress by means of a facultative, regulated decrease in metabolic rate and hence rest-phase  $T_b$ . A third possibility is that at ca. 02:00 the mousebirds were disturbed and abandoned the cluster. The increased heat loss rates may reflect the fact that birds were, for a short period, thermoregulating individually instead of in a cluster. Following this explanation, the subsequent increase in rest-phase  $T_b$  indicates the time at which the cluster reformed.

Richard Dean and Sue Milton kindly allowed us to use Tierberg Research Station for this project. Mike Savage of the Department of Agrometeorology, University of Natal, provided site-specific sunrise and sunset times for Tierberg. The staff of the Computing Centre for Water Research, University of Natal, provided climatological data for Tierberg, and Mark Brigham commented on an earlier version of this manuscript. We sincerely thank all concerned. This study was funded by National Research Foundation and University of Natal Research Fund grants to BGL. The experiments performed in this study were approved by

the University of Natal Animal Ethics Sub-committee and comply with current South African laws.

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## LOW FREQUENCY OF EXTRA-PAIR PATERNITY AND HIGH FREQUENCY OF ADOPTION IN BLACK-LEGGED KITTIWAKES

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**Abstract.** We studied the timing and frequency of extra-pair copulations and extra-pair fertilizations in the Black-legged Kittiwake (*Rissa tridactyla*), a socially monogamous seabird that breeds in dense colonies. We recorded 313 copulations by 82 marked pairs in 25 000 nest-hr over three years. We recorded only two extra-pair copulations, both of which were obtained by opportunistic males that disrupted pair copulations in progress. Pairs performed a mean of 14

copulations per clutch suggesting that males may copulate relatively frequently for paternity assurance. Our parentage analysis based on eight microsatellite markers did not detect any extra-pair paternity in 119 offspring from 86 broods; however we did detect three cases of adoption caused by chicks moving to adjacent nests. The between-nest movement of a minimum of 8% of chicks suggests that escaping siblicide may be an adaptive strategy for some nestlings, despite the risk of falling into the sea and drowning (observed in 11 of 21 chicks).

**Key words:** adoption, Black-legged Kittiwake, extra-pair copulation, extra-pair paternity, paternity assurance, *Rissa tridactyla*, within-pair copulation.

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## Baja Frecuencia de Paternidad Extra-Pareja y Alta Frecuencia de Adopción en *Rissa tridactyla*

**Resumen.** Estudiamos la frecuencia de cópulas y fertilizaciones extra-pareja y el momento en que éstas ocurrieron en *Rissa tridactyla*, un ave marina socialmente monógama que se reproduce en colonias densas. Registramos 313 cópulas por parte de 82 parejas marcadas en 25 000 horas-nido a través de tres años. Registramos sólo dos cópulas extra-pareja, ambas obtenidas por machos oportunistas que perturbaron cópulas que se estaban efectuando entre miembros de una pareja. Las parejas realizaron un promedio de 14 cópulas por nidada, lo que sugiere que los machos estarían copulando frecuentemente para asegurar su paternidad. Nuestros análisis basados en ocho marcadores microsatélites no detectaron ninguna instancia de paternidad extra-pareja en 119 crías de 86 nidadas. Sin embargo, detectamos tres casos de adopción causados por el movimiento de pichones a nidos adyacentes. El movimiento entre nidos de al menos el 8% de los pichones sugiere que escapar del siblicidio podría ser una estrategia adaptativa para algunos de éstos, a pesar del riesgo de caer al mar y ahogarse, lo que se observó en 11 de 21 pichones.

During the past two decades, extra-pair copulations (EPCs) have been shown to be common in socially monogamous species (Birkhead and Møller 1992, Petrie and Kempenaers 1998, Griffith et al. 2002). Females may engage in EPCs to acquire indirect benefits such as good genes (Kempenaers et al. 1992) or genetic diversity (Olsson et al. 1994, Petrie et al. 1998), or direct benefits such as food (Gray 1997), fertilization insurance (Birkhead and Møller 1992, Wagner 1992a) or parental care (Davies et al. 1992). It has also been suggested that extra-pair copulations are more frequent in colonial species (Birkhead et al. 1987, Møller and Birkhead 1993). Colonial breeding provides opportunities for extra-pair copulations because there are many easily accessible potential extra-pair partners (Møller 1987). In addition, males in colonial species are generally unable to guard their mates because one partner must defend the nest while the other partner forages (Birkhead et al. 1987, Birkhead and Møller 1992). Instead, males in non-mate-guarding species typically copulate frequently to assure their paternity (Birkhead et al. 1987, Wagner 1992b, Møller and Birkhead 1993). Various hypotheses predict that the level of extra-pair paternity should be negatively correlated with the degree of paternal investment, the male reproductive lifespan, and the duration of the pair bond (Griffith et al. 2002). However, numerous exceptions to these generalizations exist, making it desirable to increase the number of species under investigation. For example, Waved Albatrosses (*Pheobastria irrorata*) and Adélie Penguins (*Pygoscelis adeliae*), despite fitting the profile of species with low extra-pair paternity, have been reported to have substantial levels of extra-pair paternity (respectively 25% and 9% of young are extra-pair, Huyvaert et al. 2000, Pilastro et al. 2001).

The Black-legged Kittiwake (*Rissa tridactyla*) is a well-studied pelagic colonial seabird with a monogamous mating system (Coulson and Thomas 1983). Although extra-pair copulation and extra-pair paternity frequencies are known to be lower in seabirds than in passerines and other groups (Westneat and Sherman 1997, Birkhead et al. 2001), kittiwakes could be expected to exhibit at least some level of EPC and extra-pair paternity for the following reasons. First, kittiwakes breed in highly dense colonies (Cramp 1985) where extra-pair copulation opportunities are numerous. Moreover, as in many species where males feed their mates prior to egg laying (Birkhead et al. 1987), female kittiwakes are largely unattended by their mates during their presumed fertile period when they wait at the nest to be fed (Helfenstein 2002, Helfenstein, Wagner et al. 2003). Second, several studies have suggested that individual kittiwakes vary widely in their intrinsic genetic quality (Coulson and Wooller 1976, Coulson and Thomas 1985, Cam et al. 2002). In this context, female kittiwakes may be expected to seek extra-pair copulations from high-quality extra-pair males to obtain extra-pair fertilizations. Courtship feeding may lead to increased clutch size (Helfenstein, Wagner et al. 2003), and female kittiwakes could accrue direct benefits by trading extra-pair copulations for food as in some other species (Wolf 1975, González-Solís et al. 2001). The primary aim of this study was to examine the frequencies and seasonal timing of within-pair and extra-pair copulations and to identify which individuals might engage in EPCs. We used eight microsatellite markers (Tirard et al. 2002) to analyze parentage on a set of 86 families, and estimated the frequency of extra-pair paternity.

Siblicide has been reported in kittiwakes by Dickens and Clark (1987), and we observed that sibling aggression may lead to heavy injuries and death (Roberts and Hatch 1994). Our other goal was to document the occurrence of adoption that may result from nestlings moving to adjacent nests to escape sibling aggression (Roberts and Hatch 1994).

## METHODS

The study was conducted in Cap-Sizun, Brittany, France (48°50'N, 4°35'W) from 1999 to 2001. This kittiwake population has been extensively studied since 1979, and more than 15 000 individual birds have been color banded either as chicks or adults (Danchin and Monnat 1992, Helfenstein, Wagner, et al. 2003). We observed one cliff comprising more than 250 breeding pairs every year. In this cliff, we focused on a subsample of 14 nests in 1999, 29 in 2000, and 39 in 2001, in which both males and females were color banded. The disposition of this open, vertical cliff allowed us to observe all the nests from a single observation point approximately 30 m away. We made observations from the early copulation period (mid-March) to the end of egg laying (early June). During daily observation sessions of 1 to 10 hr, we recorded all copulation attempts involving individuals of the focal pairs. Copulations were classified as successful if we observed mountings with cloacal contact, or unsuccessful if we observed mountings without cloacal

contact. We recorded the identities of the copulating birds or noted when one of them was unmarked.

As part of a broader study on life-history traits in kittiwakes (Danchin and Monnat 1992, Danchin et al. 1998, Cam et al. 2002), we also monitored the entire colony daily. We scanned all nests and recorded the laying date of the first egg, the number of eggs laid, and the number of chicks hatched. During the monitoring routine, we were able to observe occasions when a chick moved from its nest to an adjacent one. All means are reported  $\pm$  SD.

#### BLOOD SAMPLING AND GENETICS

In addition to behavioral observations, we collected blood samples from chicks (ca. 0.2 mL) and adults (ca. 0.4 mL) for subsequent DNA extraction and genotyping. Blood was collected from the brachial vein and stored in Tris-EDTA. Chicks were sampled during banding, when they were 8 to 25 days old. For all nests, the complete brood ( $1.5 \pm 0.6$  chicks, range 1–3 chicks,  $n = 86$  families) was sampled. Adults were caught on the nest using a metal hook fixed at the end of a 4-m fishing rod. Microsatellite genotypes were obtained from each individual at seven loci developed from kittiwakes (Tirard et al. 2002) and one locus developed from Common Murres (*Uria aalge*; Ibaruchi et al. 2000). DNA extractions, PCR amplifications, and profiles were obtained using the method described in Tirard et al. (2002). All genotypic profiles were scored by CT.

#### RESULTS

##### BEHAVIORAL DATA

We observed 313 successful copulations involving 52 marked males and 56 marked females in three years and 800 hr of observation (223 hr in 1999, 67 hr in 2000 and 512 hr in 2001, a total of ca. 25 000 nest-hr). The vast majority of the copulations (90%) occurred between 21 days before and 2 days after the laying of the first egg (day 0) and peaked at day  $-5$  (Fig. 1). The remaining 37 copulations occurred earlier, between days  $-54$  and  $-23$ . Copulation rate began to increase at 21 days before laying (Fig. 1), which was also the mean starting date of the pairs' nest-building activity (Helfenstein, Wagner, et al. 2003). Nest building requires coordination between partners and occurs after pair formation (Cam et al. 1998, Helfenstein, Wagner, et al. 2003). We therefore considered only copulations occurring between days  $-21$  and  $+2$  to be postpairing copulations. The mean sperm storage duration in birds varies widely (Birkhead and Møller 1992). The amount of time that female kittiwakes can store sperm is not known, but we conservatively assumed that any sperm transferred between 21 days before and 2 days after laying may fertilize the eggs. Mean copulation frequency in this period was  $0.04 \pm 0.03$  copulations  $\text{hr}^{-1}$  which totals  $14.1 \pm 10.6$  copulations per clutch ( $n = 79$  pair-years) on a 14-hr daylight basis. Of 394 copulation attempts (comprising both successful and unsuccessful copulations) six (1.5%) were EPC attempts. Three unsuccessful EPC attempts were made by the same male with the same female in one year. We observed two successful extra-pair copulations, which represents  $<1\%$  of the 313 suc-

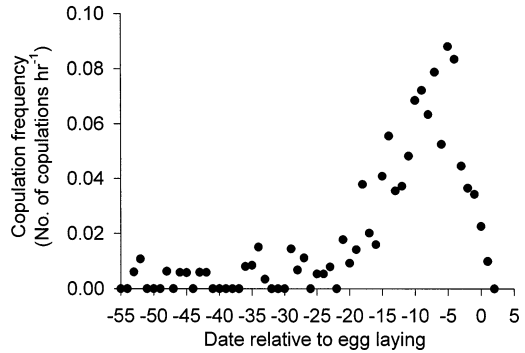


FIGURE 1. Seasonal variation in copulation frequency in Black-legged Kittiwakes. Copulation frequency is the mean number of copulations per hour per pair. It was computed from observation of  $47.0 \pm 16.6$  pairs per day and is reported relative to the first day of egg laying for each pair (day 0). Copulations commenced more than 50 days before the laying of the first egg of the pair but remained very low until day  $-21$  and peaked on day  $-5$ . No copulations were observed more than 2 days after the laying of the first egg.

cessful copulations. All six EPC attempts were initiated by the extra-pair male flying to the female. Males either forcibly mounted the female or disrupted a copulation in progress by displacing the pair male. After disrupting the copulations, extra-pair males were able to replace the resident males on the females' backs and one extra-pair male eventually achieved cloacal contact. The second successful EPC was initiated by a neighboring male that was attending a first-time-breeding female's nest. Successful and unsuccessful EPCs were performed between  $-17$  and  $-3$  days before egg laying (mean  $9.7 \pm 5.5$  days). The two successful EPCs occurred at days  $-9$  and  $-3$ .

During our routine daily observation of the colony we observed that, in multiple-chick broods, the first-hatched chicks were often very aggressive toward their younger siblings and repeatedly attacked them with their bills. Younger chicks sometimes tried to avoid sibling aggression by walking into an adjacent nest, but often (11 of 21 chicks) missed and fell into the sea where they drowned. In some cases however, the escaping chicks reached the neighboring nest and were accepted and fed by the adults even when they were still incubating their own eggs. Overall, 10 adoptions occurred in 76 nests of color-banded pairs comprising 119 chicks, which is nearly identical to the 8% adoption frequency found by Roberts and Hatch (1994) in an Alaskan population.

##### PARENTAGE ANALYSES

Exclusion probabilities were calculated for each marker (Jamieson 1994) based on allele frequency from samples of 63–97 presumably unrelated adults. The combined exclusion probability for the set of 8 markers was 0.98 (range 0.15–0.78). A total of 45 putative fathers, 50 putative mothers, and 119 chicks out of 86 families were genotyped and analyzed. We obtained both behavioral records and genotypic profiles for 25

TABLE 1. Genotypic composition of the five mismatched sets of putative parents and offspring from a total sample of 86 Black-legged Kittiwake families and 119 chicks. Each letter represents a unique allele at a given microsatellite locus. The probability of false inclusion corresponds to the probability that the parent's genotype can match the offspring's by chance (Jeffreys et al. 1992). Table entries in boldface highlight the loci at which the chick's genotype did not match the genotype of one or both putative parents. Loci are named according to Ibaruchi et al. (2000) and Tirard et al. (2002). Two of three chicks in which microsatellite data indicated adoption were observed moving between nests prior to sampling.

Family	Individual	Locus								Probability of false inclusion	Interpretation
		K6	K16	K31	K32	K56	K67	K71	Ulo-12a12		
1	Putative father	<b>E/E</b>	A/C	A/C	F/H		A/B	<b>A/A</b>	A/B	0.02	Adoption
	Putative mother	<b>E/E</b>	A/A	A/A	F/G		B/B	<b>A/A</b>	A/A	0.12	
	Offspring	<b>C/F</b>	A/C	A/C	F/H		B/B	<b>B/B</b>	A/B		
2	Putative father		A/B	<b>C/C</b>	<b>D/G</b>	A/B	B/B	B/B	A/B	0.06	Adoption
	Putative mother		C/C	<b>C/E</b>	<b>D/E</b>		A/B	A/B	A/B	0.35	
	Offspring		A/C	<b>A/D</b>	<b>A/G</b>	A/B	B/B	B/B	A/B		
3	Putative father	<b>D/E</b>	<b>A/B</b>	<b>C/E</b>	<b>B/J</b>		B/B	A/B	A/A	0.05	Adoption
	Putative mother	<b>E/E</b>	<b>A/A</b>	A/C	<b>A/I</b>		B/B	A/B	A/B	0.44	
	Offspring	<b>A/B</b>	A/C	A/A	<b>D/J</b>		B/B	B/B	A/A		
4	Putative father	D/E	A/A	A/A	<b>C/D</b>		B/B	A/A	A/A	0.26	Mutation or genotyping error
	Putative mother	E/E	A/C	C/E	<b>C/C</b>		A/B	A/B	A/A	0.12	
	Offspring	E/E	A/C	A/E	<b>B/B</b>		B/B	A/B	A/A		
	Putative father	<b>D/E</b>	A/C	<b>A/C</b>	G/H	A/A	B/B	A/B	<b>A/A</b>	0.02	
	Putative mother	<b>B/F</b>	<b>A/A</b>	<b>A/B</b>	G/G	A/B	A/B	A/B	<b>A/B</b>	0.06	
	Offspring	<b>D/E</b>	<b>A/B</b>	<b>A/E</b>	G/G	A/A	B/B	A/B	<b>B/B</b>		Undetermined

pairs. Out of the 119 chicks of the genetic sample, 114 had no mismatching alleles in any loci with either the putative mother or the putative father. The average paternal probability of false inclusion (i.e., the probability that the genotype of the putative father matched the genotype of the offspring by chance, Jeffreys et al. 1992) was  $0.04 \pm 0.05$  (range 0.00011–0.24) for these chicks, indicating that they were likely to have been fathered by the pair male. Based on the mean probability of false inclusion, we estimated that, within a 95% confidence interval and assuming a normal distribution, up to 5% of the 114 chicks could be the result of an undetected extra-pair fertilization.

In one case, both parents were excluded at two different loci (Table 1, family 1). The probability of false inclusion on the remaining loci was 0.02 for the putative father and 0.12 for the putative mother and this chick was considered as an adoption. Two other chicks did not match both putative parents' genotypes at one locus and either the putative mother or the putative father at one other locus (Table 1, family 2, 3). Our observations of chicks moving from their nest to a neighboring one confirmed that these two chicks were adoptions. One chick neither matched the paternal nor the maternal alleles at the same single locus (Table 1, family 4). This may be due either to mutation or to adoption. The nest in which the chick had been reared was physically isolated from any other nests, making it impossible that the flightless chick could have transferred itself into a different nest. We therefore considered the mismatches to be due to mutations or genotyping errors. One last chick (Table 1, family 5) did

not match the putative father at one locus and the putative mother at another locus. At two other loci, despite mismatches, shared alleles between parents did not allow us to exclude either the putative father or mother. We were therefore unable to determine the origin of this chick.

For one of the two females that we observed copulating with an extra-pair male, no genetic data were available to analyze the paternity of the offspring. For the other female, the offspring genotype did not show any mismatch with the genotype of the social father. The probability of false inclusion of the social father was quite high (0.07), but no genetic data were available to test the paternity of the putative extra-pair male. Hence, we could not distinguish whether this extra-pair copulation was simply unsuccessful in achieving fertilization or whether we lacked sufficient power to exclude the social father and detect an extra-pair fertilization.

## DISCUSSION

Female kittiwakes were never seen seeking extra-pair copulations. In 20 years of population monitoring, copulations have never been observed away from the nesting colonies, making it unlikely that EPCs are performed in other locations (J.-Y. Monnat, unpubl. data). Consistent with these behavioral observations, our genetic analysis did not detect any extra-pair paternity. Our 4% probability of false inclusion does not allow us to conclude that extra-pair paternity is absent in kittiwakes. However, our markers did allow us to detect cases of adoption that had been verified by direct

observations. This latter result, combined with our large sample size of 119 offspring, suggests that if extra-pair paternity is not absent in kittiwakes it is likely to be close to zero.

It is now well established that extra-pair copulations and paternity are common in socially monogamous species (Birkhead and Møller 1992, Petrie and Kempenaers 1998, Griffith et al. 2002). Therefore, as Griffith et al. (2002:2195) have noted, "levels of extra-pair paternity below 5% of offspring are now considered worthy of explanation." Seabirds and many other non-passerines with high levels of paternal care generally exhibit low frequencies of extra-pair paternity (Birkhead et al. 2001). In kittiwakes, biparental care is crucial, and males and females share equally in nest building, incubation, and chick feeding (Coulson and Wooler 1984, Coulson and Porter 1985, Coulson and Johnson 1993, Roberts and Hatch 1993). It has been proposed that a female's decision to engage in extra-pair copulations depends on the balance between the benefits of this reproductive strategy and the costs of male responses to cuckoldry (Birkhead and Møller 1996, Gowaty 1996). Moreover, two comparative analyses (Møller 2000, Møller and Cuervo 2000) have found that the frequency of extra-pair paternity was negatively related to male parental care and to reduction in female reproductive success caused by the removal of the mate. This suggests that high levels of paternity confidence may be a prerequisite for paternal care and that the more females depend on their mates to achieve reproductive success the less likely they may be to engage in extra-pair copulations (Trivers 1972, Fitch and Shugart 1984, Xia 1992). Given the high investment of male kittiwakes in their progeny, this hypothesis may explain why female kittiwakes do not seek EPCs. The pursuit of EPCs may also have other costs for females. Females are unlikely to seek EPCs at another male's nest because material from their unattended nest is likely to be stolen, and this can delay reproduction and reduce reproductive success (FH, unpubl. data). Accepting an extra-pair male on the nest may also have a cost. Nest acquisition is often achieved by prospecting and squatting (Danchin 1987, 1988, Cadiou et al. 1994) and the acquisition of the nest by a new male may lead to divorce or dispersal (Chardine 1987, Danchin and Cam 2002). These potential costs to females may explain why only males initiated EPCs.

We observed males forcing EPCs by interrupting a copulation in progress. Interference in copulations by extra-pair males has been observed in another kittiwake population (Chardine 1986) but without being followed by EPC attempts. The presence of males that try to force copulations may create conditions for the evolution of paternity assurance strategies (Helfenstein, Wagner, and Danchin 2003). Males copulate more ( $14.1 \pm 10.6$  copulations per clutch) than is likely to be needed to achieve fertilization. They also perform a mean of  $3.4 \pm 1.8$  ( $n = 82$  pairs) cloacal contacts during a single copulation, which leads to an average of 47.6 cloacal contacts performed per clutch. Not all cloacal contacts result in sperm transfer (Hunter et al. 1996) and we cannot exclude the hypothesis that relatively frequent copulation with multiple cloacal con-

tacts may have other functions, such as strengthening the pair bond (Wagner 1996, 2003). However, this high number of cloacal contacts may also indicate that males inseminate their mates with more sperm than is necessary if their paternity were not at all at risk (Birkhead et al. 1987). Within-pair copulations reached a peak in frequency five days before egg laying, suggesting that copulations were timed to match female fertility (Birkhead et al. 1987). All these factors combined (cost of retaliation, direct cost of EPCs, paternity assurance strategies) might explain the low level of EPCs and extra-pair fertilizations in kittiwakes.

The behavior of flightless chicks leaving their nests, apparently to seek adoption in a neighboring nest, may occur when the risk of travel between nests (falling into the sea) is lower than the risk of starvation or siblicide. This intriguing phenomenon, reported in several species (Holley 1981, Carter and Spear 1986, Plissner and Gowaty 1988, Morris et al. 1991), raises the question of why parents have not evolved recognition cues or simply forbid access to their nests to outside offspring. A high degree of relatedness between foster parents and the neighboring chicks could explain why individuals adopt stray chicks. Close relatedness could either be due to a high level of natal philopatry or a high level of EPC between neighbors. On the basis of our observations and paternity analysis, we can at least exclude the hypothesis that EPC rate between neighbors is promoting adoption in our study species.

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

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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 signaling.

### 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 *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 podría oscurecer la evaluación visual del tamaño de la bolsa por las hembras. Investigamos si el tamborileo provee la misma información sobre el 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 podrían hacer una mejor evaluación del tamaño de la bolsa gracias a la combinación entre las señales visuales y auditivas.

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 Székely, 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. Noncourting males do not inflate the gular pouch, and it shrinks and changes to skin color 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 behavior, with short inspections only eliciting relatively few sequences of drumming (VM, pers. obs.).

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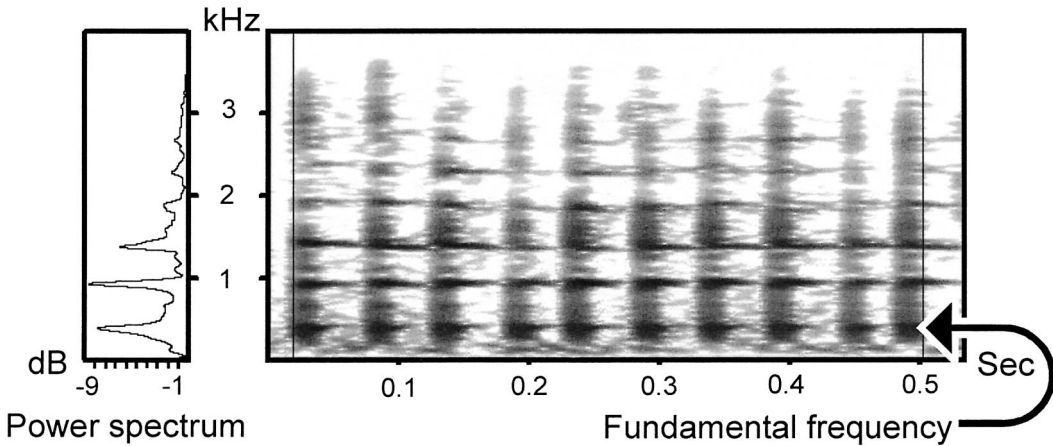


FIGURE 1. Spectrogram of a Magnificent Frigatebird drumming sequence of 10 sound elements (right) and mean power spectrum for the area delimited in the spectrogram (left). The power spectrum indicates six frequency bands. The lowest frequency band, called the fundamental frequency, has a frequency of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

The 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 12 000–14 000 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 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 endeavoring 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 digitized (16 bit, 8 kHz sampling frequency) and analyzed 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 (Fig. 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 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  $1.3 \times 2.0$  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 (Fig. 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

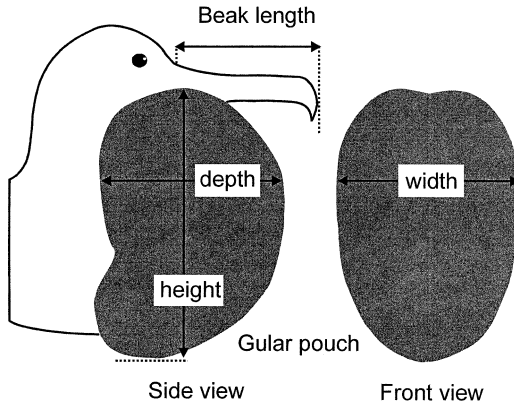


FIGURE 2. Method for gular pouch and beak length measurements taken on 32 male Magnificent Frigatebirds.

with the average beak length for the population. Within the population the beak length varies only slightly between males (mean beak length =  $10.6 \pm 0.03$  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 (Fig. 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).

In exploratory analyses aimed at determining the required number of sequences and sound elements there-

in needed for representative results, we analyzed 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 analyzed (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 analyzed to a maximum of 25. Following that, we included 43 of the 69 males recorded in the final analysis, with  $16.6 \pm 0.9$  (SE, range 5–25) sequences analyzed 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  $24 \pm 0.66$  cm, range 14–31 cm; depth  $11 \pm 0.42$  cm, range 8–15 cm; width  $15 \pm 0.49$  cm, range 12–18 cm; pouch volume  $4206 \pm 438$  cm<sup>3</sup>, range 1783–7962 cm<sup>3</sup>).

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  $\times$  depth  $\times$  width:  $r_s = -0.82$ ,  $P < 0.01$ ,  $n = 18$ ; Fig. 3).

Six sound-recorded, wing-tagged males 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:  $r_s = 0.43$ ,  $P = 0.40$ ; Fig. 4). The mass of the males was significantly, but positively, correlated with fundamental frequency (mass:  $r_s = 0.89$ ,  $P < 0.05$ ; Fig. 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

TABLE 1. Physical features of Magnificent Frigatebird drumming, obtained from analysis of spectrograms (Avisoft 2002). Individual male averages were calculated from averages obtained per sequence. Differences in sample sizes were caused by other sounds, like juvenile begging calls, in some cases hampering anything but registration of the fundamental frequency. Mean number of analyzed sequences per male:  $16.6 \pm 0.9$  (SE, range 5–25).

	Fundamental frequency (Hz)	Frequency band interval (Hz)	Element duration (sec)	Interelement interval (sec)
Number of individual males	43	40	40	40
Mean $\pm$ SE of individual averages	$459 \pm 7$	$513 \pm 15$	$0.026 \pm 0.001$	$0.060 \pm 0.001$
Range of individual averages	390–566	374–848	0.019–0.036	0.052–0.072
Range of SE for individual averages	0–15	4–72	0–0.003	0.001–0.005

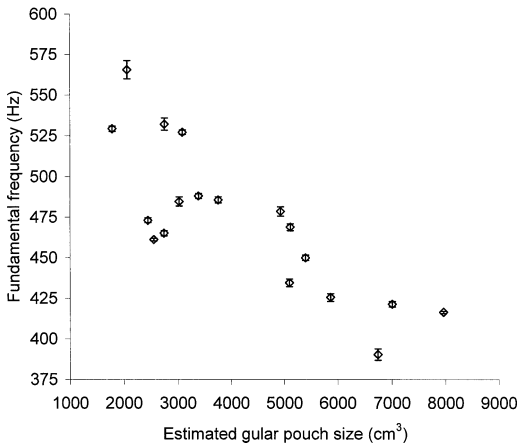


FIGURE 3. Mean  $\pm$  SE fundamental frequency of drumming produced by male Magnificent Frigatebirds as a function of gular pouch size. The gular pouch size was calculated as height  $\times$  depth  $\times$  width (Fig. 2), after all measures had been standardized by beak length.

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  $\times$  width  $\times$  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 be-

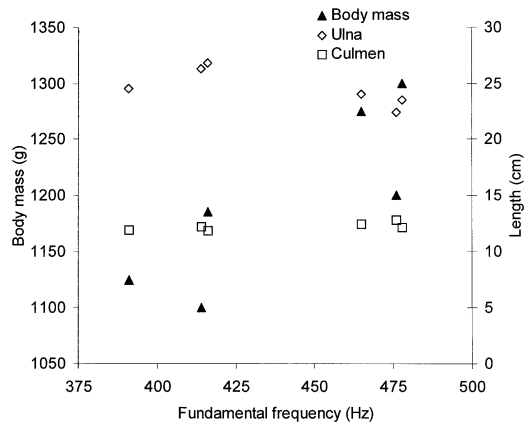


FIGURE 4. Magnificent Frigatebird body size (as indexed by ulna and culmen length) and mass plotted against the fundamental frequency of courtship drumming. The positive relationship of frequency with body mass indicates that the gular pouch signals some quality other than simple body size.

tween 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 signaling 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 signaling of a single trait. The observed bimodal signaling of gular pouch size suggests that pouch size is very important in mate attraction, either through signaling 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.

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