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## **Seasonal Changes of Sexual and Territorial Behaviour and Plasma Testosterone Levels in Male Lesser Sheathbills (*Chionis minor*)**

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*With 3 figures*

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### **Abstract**

Lesser Sheathbills *Chionis minor* (Aves: Charadriiformes) have a brief annual breeding season but under certain circumstances territoriality persists outside the breeding season. Seasonal increases in plasma testosterone levels in free-living adult males coincided with increases in the mass of testes, the seasonal peak in mutual pair displays and the occurrence of nest-building and copulation. Territorial aggression outside the breeding season was not dependent on high testosterone levels.

### **Introduction**

Most studies of the endocrine basis of avian behaviour have been analyses of the effects of exogenous hormones, or correlations between endogenous hormones and behaviour of birds in laboratories (reviews by FOLLETT 1973; LOFTS and MURTON 1973; MURTON and WESTWOOD 1977). Recently, circulating levels of hormones have been studied in the field in relation to episodic (HARDING and FOLLETT 1979) or seasonal changes in behaviour (TEMPLE 1974; LISANO and KENNAMER 1977; WINGFIELD and FARNER 1978; BERRY, MILLAR and LOUW 1979). These studies have the advantage of using free-living birds exposed to a full range of external stimuli, but involve problems of relating changes in a specific behaviour pattern to changes in levels of a particular hormone. This is particularly so in studies of seasonal events, since territori-

ality, nest-building and courtship, which are all known to be affected by hormones, are restricted to the same time of year in most birds (MURTON and WESTWOOD 1977).

We report on seasonal changes in territorial and sexual behaviour and plasma testosterone levels in free-living, adult male Lesser Sheathbills at Marion Island (46°54'S, 37°45'E). Lesser Sheathbills are omnivorous charadriiform shorebirds, resident on four sub-Antarctic island groups (BURGER 1979). Breeding in this species is restricted to a brief season in summer (95% of a sample of 94 eggs were laid between 4 and 31 December; BURGER 1979), and all breeding birds are territorial. Territoriality is not, however, necessarily restricted to the breeding season. This characteristic thus affords an opportunity to study the separate role of hormones in territorial as distinct from sexual behaviour.

Pairs of Lesser Sheathbills maintain territories only within penguin colonies and virtually all the food eaten by territorial adults and their chicks is derived from penguins (BURGER 1979). Territorial tenure is dependent on the presence of relatively abundant food supplies while the penguins are present. In colonies of King Penguins *Aptenodytes patagonicus*, which are present on Marion Island all year, the sheathbills remain territorial all year, but in colonies of Rockhopper Penguins *Eudyptes chrysocome* and Macaroni Penguins *E. chrysolophus*, which desert the island for the austral winter (May to October), the sheathbills are territorial only during the summer, November to April (BURGER 1980b).

## Methods

Blood samples were collected from living birds, via brachial veins, or from the hearts of birds which had been shot, within 10 min of death. The procedures were deemed comparable since in laboratory rats mean brachial vein plasma testosterone was not significantly different from mean cardiac plasma testosterone. The heparinised blood was immediately centrifuged at 2000–2500 r.p.m. for 15 min, the plasma aspirated, transferred to Eppendorf reaction vials and stored at –15 °C until assay.

Blood samples were collected between 12.30 and 16.30 h (local time) in an attempt to obviate possible diurnal fluctuations in testosterone levels (BALHAZART 1976). Three samples collected from roosting birds at night (at about 21.00 h) were, however, also included since the testosterone levels in these samples were similar to those in plasma collected between 12.30–16.30 h at the same time of year (see Fig. 3). Birds were observed for 20–30 min prior to sampling to determine whether they were territorial or not and to record displays. All of the sampled birds were adult males which were known to have held territories in either King or Rockhopper Penguin colonies. Some of the birds had, however, temporarily abandoned their territories in Rockhopper Penguin colonies.

Plasma testosterone concentration was estimated in duplicate by radioimmunoassay of ether extracts of samples using an antiserum raised against testosterone-3-carboxy methyl oxime-bovin serum albumin conjugate. The antiserum was highly specific for testosterone and exhibited less than 5.1% cross-reaction with dihydrotestosterone and minimal cross-reaction with other naturally occurring steroids (MILLAR and KEWLEY 1976). Intra-assay and inter-assay coefficients of variation were 5.4% and 9.9% respectively.

Behavioural data were collected at a colony of King Penguins occupied by 12 pairs of territorial adult Lesser Sheathbills and variable numbers of immatures and non-territorial adults. All the territorial birds and most of the others had been sexed, aged (BURGER 1980a)

and colour-ringed. The frequencies of conspicuous displays performed by the Lesser Sheathbills were recorded for 30-min periods, at the same time of day as the blood was sampled, and at intervals of about 10 days between June 1976 and May 1977. Observations were made from an exposed vantage point and weather conditions, (cold, wind and rain) limited observation to 30 min. Additional incidental observations were made between January—November 1974 and April 1976—May 1977.

## Results

### Seasonal Variation of Territorial Behaviour

The maintenance of territories in penguin colonies by Lesser Sheathbills included behaviour with three apparent functions: to maintain boundaries between neighbouring territories; to evict intruding conspecifics; and to advertise the presence of the territorial pair. These objectives were attained with the use of a variety of displays, which are described and analysed elsewhere (BURGER 1980b).

Boundaries between neighbouring territories in penguin colonies were maintained by ritualised boundary disputes, usually involving only males, which occasionally led to fighting. Boundary disputes and fighting occurred at any time of the year in the King Penguin colony but were always infrequent (Fig. 1; A, B).

The eviction of non-territorial intruders, and very rarely also territorial birds, was achieved by overt chasing and through use of the Run-and-Call display. Chasing and Run-and-Call displays occurred at the King Penguin colony throughout the year (Fig. 1; B, C), and the frequency of occurrence of both activities correlated with the numbers of potential intruders present at the colony ( $r = 0,71$  and  $r = 0,54$  respectively,  $p < 0,01$ ,  $n = 31$ ).

Advertisement by the territorial pair was largely achieved through a visually and audibly conspicuous mutual pair display, the Bob Call display. This display was also important in maintaining tolerance of the members of the pair for each other's presence in the territory with the use of alternating elements of aggression and appeasement (BURGER 1980b). Bob Call displays occurred at the King Penguin colony throughout the year (Fig. 1, E) but were most common from mid-September to mid-December, which was when other adults were prospecting for territories and courtship and nest-building was in progress.

The frequency of occurrence of the above displays is evidence that the Lesser Sheathbill pairs in the King Penguin colony actively advertised and defended their territories all year.

### Seasonal Occurrence of Sexual Behaviour

Copulation was a rare event among Lesser Sheathbills. During two full years of observations at many parts of the island, copulation or precopulatory behaviour were seen only 18 times, between 21 October and 30 December (Fig. 2). Copulation was not important outside the breeding season as a means

of re-inforcing pair bonds. Copulation was apparently only associated with fertilization and occurred at the time of year when adult males had enlarged testes (Fig. 2).

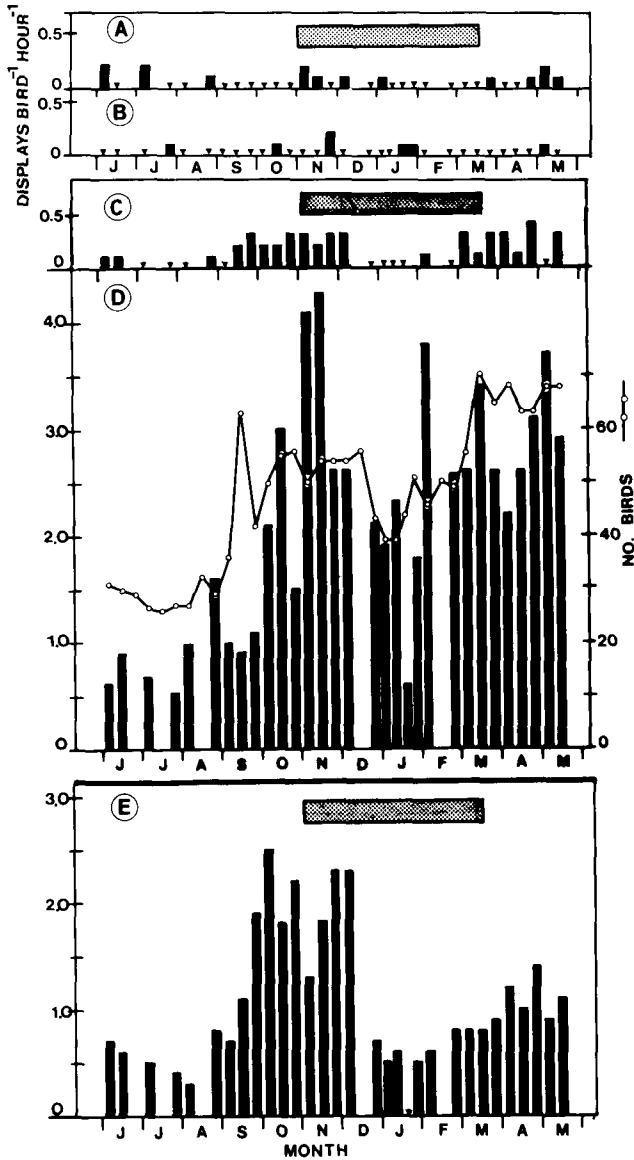
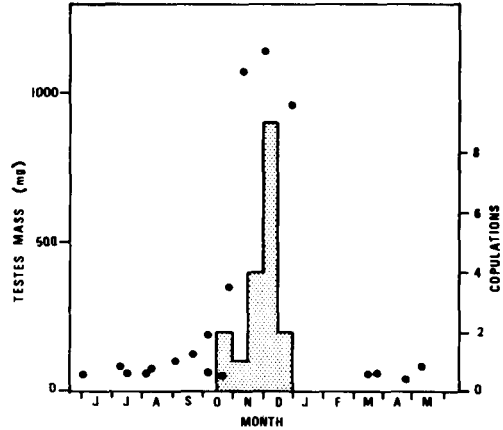


Fig. 1: The frequencies of occurrence of displays by Lesser Sheathbills within a King Penguin colony (solid bars). Triangles indicate no displays recorded in an observation period, the stippled bars delineate the Lesser Sheathbill's breeding season and the open circles the numbers of Lesser Sheathbills counted at the penguin colony. A: Boundary disputes; B: Fighting; C: Run-and-Call displays; D: Chasing; E: Bob Call displays

Fig. 2: Seasonal variations in the combined mass of the two testes of individual males (dots), and the incidence of copulation attempts (stippled) in adult Lesser Sheathbills. Data from two full years of observations from many parts of Marion Island



Plasma Testosterone Levels

Plasma testosterone levels in adult male Lesser Sheathbills ranged from  $< 0,1$  to  $7,5 \text{ nmoles l}^{-1}$  and showed a seasonal trend (Fig. 3). Testosterone levels in four months preceding laying (August to November) were significantly higher than at any other time of the year (t-test,  $p < 0,01$ ). The mean testosterone levels in three males which were incubating (sampled late December) or rearing chicks (February and mid-March) were not significantly different from those in 9 non-breeding males sampled from mid-March to July (t-test,  $p > 0,05$ ).

Seasonal variations in testosterone levels did not correspond to changes in territorial behaviour. In winter (April to September) when adult Lesser Sheathbills in the King Penguin colony were actively defending and advertis-

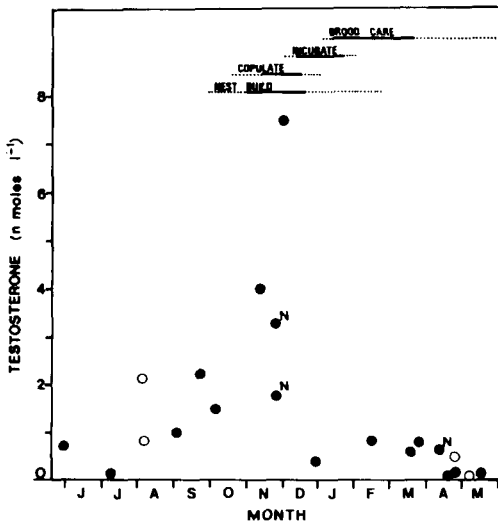


Fig. 3: Levels of testosterone in blood plasma of adult male Lesser Sheathbills which were defending territories (dots) and not defending territories (open circles). The maximum duration (dotted lines) and periods of maximum activity (solid lines) of reproductive events are also shown. Three blood samples collected from birds roosting at night are also indicated (N)

ing territories, testosterone levels in territorial birds were not higher than in those birds showing no territorial behaviour (Fig. 3).

Testosterone levels were, however, highest at the time of year when nest building and copulation occurred and when the Bob Call pair display was most frequently given. Plasma testosterone levels correlated significantly with the combined masses of the testes in the Lesser Sheathbills which had been shot ( $r = 0,74$ ,  $p < 0,01$ ,  $n = 14$ ).

*Table 1:* Comparison of plasma testosterone levels (nmoles l<sup>-1</sup>) in male Lesser Sheathbills which had performed a Bob-call pair display within 20 min of being sampled, and those which had not

	Reproductive activity	Display performed			No display performed		
		Mean	Range	N	Mean	Range	N
Mid-March - July	None (Wintering)	0,4	0,2 - 0,8	3	0,4	0,1 - 0,7	6
August - October	None (Pre-breeding)	1,9	1,5 - 2,2	3	0,9	0,8 - 1,0	2
November - December	Nest building and courtship	5,8	4,0 - 7,5	2	2,6	1,8 - 3,3	2
January - mid-March	Incubation and brood care	0,7	0,6 - 0,8	2	0,4	-	1

Plasma testosterone levels appeared to vary according to the bird's behaviour immediately prior to sampling. Males which had performed a Bob Call display shortly before being sampled had higher testosterone levels than those males which had not displayed (the ranges did not overlap), in all seasons except the winter (Table 1). This conclusion remains tentative since the data were insufficient for rigorous statistical testing and pooling data from different seasons to increase the sample sizes was not acceptable, due to the seasonal variations of testosterone in this study levels. The highest concentration of plasma testosterone (7,5 nmoles l<sup>-1</sup>) was from a male which had copulated 10 min before its blood was sampled.

### Discussion

Plasma testosterone levels in male Lesser Sheathbills were above 1,0 nmoles l<sup>-1</sup> only between August and December. The occurrence of nest-building, copulation, the maximum frequencies of mutual pair displays and the increase in the mass of the testes, which is an index of active spermatogenesis (MURTON and WESTWOOD 1977), all coincided with high levels of plasma testosterone, but incubation and brood-care did not. This suggests that testosterone is important in mediating physiological and behavioural events leading up to egg production in Lesser Sheathbills. Other hormones may also be involved. Testosterone is known to play an important role in spermatogenesis in birds (LOFTS and MURTON 1973; MURTON and WESTWOOD 1977). Injections of testosterone propionate have been demonstrated to modify nest-building, courtship and mating behaviour in several bird species (CROOK and

BUTTERFIELD 1968; HUTCHISON 1970; ADKINS and PNIEWSKI 1978; BALTHAZART and HENDRICK 1978; DEVICHE 1979), and the seasonal occurrence of these behaviour patterns coincided with high endogenous levels of plasma testosterone in several species (BALTHAZART and HENDRICK 1976; LISANO and KENNAMER 1977; WINGFIELD and FARNER 1978; BERRY et al. 1979).

It has been suggested that the endocrine system might be important in modulating minute to minute behavioural responses of animals during social interactions (HARDING and FOLLETT 1979). We tentatively suggest that short-term increases in testosterone levels in male Lesser Sheathbills coincided with the performance of Bob Call displays and copulation. This is in accordance with experiments on a variety of species in captivity, in which the concentration of testosterone in males' plasma was higher following sexual stimuli, such as copulation or exposure to the females (reviewed by HARDING and FOLLETT 1979). We cannot conclude whether the altered hormone level or the behaviour was the causal factor in Lesser Sheathbills (see BALTHAZART 1976). Exogenous testosterone is known to affect behaviour and by inference increased endogenous production probably induces behavioural changes. HARDING and FOLLETT (1979) have shown, however, that experimentally induced aggression caused significant changes in circulating levels of hormones, including testosterone, within 19 min in free living male Red-winged Blackbirds *Agelaius phoeniceus*.

Territorial aggression in Lesser Sheathbills was not restricted to the time of year when testosterone levels were high. The year-round availability of food in King Penguin colonies permitted territoriality to persist amongst adult Lesser Sheathbills living in such a colony in winter. Between mid-March to July all adult males sampled had low testosterone levels, whether they were actively defending and advertising territories or not. This indicates that either territorial aggression could be stimulated by testosterone at very low levels, or more probably, that territorial aggression was not influenced by plasma testosterone concentration in this species.

There is conflicting evidence on the role of testosterone in aggressive behaviour in birds. Aggressive territorial defence has often been attributed to the effects of androgens (DAVIS 1963; LOFTS and MURTON 1973), but as DAVIS (1963) pointed out, this was probably due to the seasonal coincidence of territoriality with courtship, nest-building and mating, behaviour which was known to be influenced by testosterone. In laboratories, some authors observed increased aggression following exogenous testosterone treatment (ETIENNE 1964; SELINGER and BERMANT 1967; ARNOLD 1975), but others found very little or no change in aggression (DAVIS 1957; VOWLES and HARWOOD 1966; BALTHAZART 1974; DEVICHE 1979).

It has been suggested that androgens stimulate aggression in birds in "reproductive" situations, when males compete for females or nest sites, but that aggression in other contexts, such as for food in wintering flocks, might not be controlled by testosterone (CROOK and BUTTERFIELD 1968; ARNOLD 1975). Our data tend to support this hypothesis. The main objective of territorial behaviour in Lesser Sheathbills was the defence of food resources in

penguin colonies. Although breeding was ultimately dependent on the acquisition of a territory (BURGER 1979), males did not compete directly for females, nest sites or other objectives of immediate sexual significance.

Since territorial aggression in Lesser Sheathbills appeared to be independent of high testosterone levels, this behaviour might be influenced by other hormones. Exogenous progesterone, perhaps acting indirectly, was found to increase aggressiveness towards conspecifics in breeding males of two species of birds (VOWLES and HARWOOD 1966; MURTON, THEARLE and LOFTS 1969). Several studies have suggested that luteinising hormone, rather than testosterone, mediates intermale aggression in passerine birds (DAVIS 1963; MATHEWSON 1961; CROOK and BUTTERFIELD 1968), although this view has been challenged by ARNOLD (1975). Recently luteinising hormone-releasing factor was found to directly influence behaviour in rats (MOSS and McCANN 1976) and courtship behaviour in one bird species (CHENG 1977) and might be considered to affect aggression in other birds, as it is produced in the central nervous system and affects neural function (NEMEROFF and PRANGE 1978).

In conclusion, it appears that while high testosterone levels in Lesser Sheathbill males might stimulate reproductive activities, high levels were not essential for territorial aggression to occur.

#### Summary

At Marion island in the sub-Antarctic all breeding activities of Lesser Sheathbills *Chionis minor* were restricted to a brief summer season and all breeding adults had territories within penguin colonies. Pairs with territories in colonies of King Penguins *Aptenodytes patagonicus* remained territorial in the winter but those in colonies of other penguin species did not.

Plasma testosterone levels in adult male Lesser Sheathbills were significantly higher in the four months preceding laying than at any other time of the year. Nest-building, copulation, the peak frequencies of mutual pair displays and the seasonal increase in testes masses all coincided with high testosterone levels. Boundary disputes, territorial fighting, eviction of intruders and advertisement of the territory by Lesser Sheathbills occurred throughout the year in a King Penguin colony and were independent of high testosterone levels. In winter both territorial and non-territorial adult males had very low testosterone levels.

The data suggest that in this species high testosterone levels might stimulate reproductive activities but high levels were not essential for territorial aggression to occur.

#### Zusammenfassung

Die Brutaktivität der Scheidenschnäbel *Chionis minor* auf der subantarktischen Marion Insel beschränkt sich auf eine kurze Sommersaison. Alle brütenden adulten Vögel hatten Territorien in Pinguinkolonien. Paare mit Terri-



torien in Kolonien des Königspinguins *Aptenodytes patagonicus* zeigten auch im Winter Territorialverhalten, im Gegensatz zu solchen mit Territorien in Kolonien anderer Pinguinarten.

Der Testosteronspiegel im Blutplasma adulter männlicher Scheidenschnäbel war in den vier Monaten vor der Eiablage bedeutend höher als zu anderen Jahreszeiten. Nestbau, Kopulation, das Maximum der Paar-Rituale und die saisonbedingte Zunahme an Testismasse fielen zeitlich zusammen mit hohem Testosteronspiegel. Grenzstreitigkeiten, Territorialkämpfe, Vertreibung von Eindringlingen und die Revieranzeige kamen das ganze Jahr über vor, unabhängig von hohem Testosteronspiegel.

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