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Behavioral and Hormonal Basis of Polygynous Breeding in Male Bush Warblers (*Cettia diphone*)

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[1]Plasma levels of testosterone and corticosterone were measured in free-living male bush warblers captured on their breeding ground at different times of the breeding season. Their territoriality was also estimated from their singing response to song playbacks. The pattern of change detected in the levels of plasma testosterone was different from that of "typical" monogamous species but similar to that of polygynous species. In "typical" monogamous species, plasma testosterone levels elevated during territory settlement and courtship behavior and then declined to low, stable levels during incubation. In bush warblers, plasma levels of testosterone were already high (1–2 ng/ml) upon arrival in late March and peaked (2.5–4 ng/ml) in early June. They then decreased but relatively high levels were maintained until early August. In late August the testosterone concentration was 0.03 ng/ml or less. Plasma levels of corticosterone also showed a seasonal change, being highest in May to July and declining in late August. Territoriality showed clear seasonality, reflecting the levels of circulating testosterone. Upon arrival, latency periods for responses to song playback were long and singing activity was rather low but this behavior was soon stabilized and a high degree of territoriality was maintained to late August. These results suggest that high levels of circulating testosterone and corticosterone allow males to pursue a polygynous breeding strategy, to hold a territory, and to maintain breeding activity for a prolonged period, characteristics which are likely to be adaptations to dense bushes with high rates of predation and brood parasitism of this species.

Key Words: testosterone; polygyny; breeding strategy; corticosterone; male behavior; bush warbler; territoriality.

The annual breeding cycle of a bird is the result of adaptation to its habitat and is precisely regulated by hormonal events (Wingfield and Farner, 1980). Gonadotropin secretion triggered by environmental stimulating cues initiates the process of gonadal maturation and promotes gonadal steroid hormone secretion (see for review Wingfield and Farner, 1994). Steroid hormones influence the reproductive behavior, which then may influence the behavior of other individuals (see for review Balthazart, 1983; Wada, 1985). However, the interrelationships among environmental stimuli, hormone levels, and behavior are not well understood. In the last two decades, progress has allowed us to monitor circulating levels of steroid hormones in free-living birds and to associate them with reproductive behavior (see for review Wingfield and Farner, 1994). However, most such studies have focused on monogamous species.

Testosterone stimulates certain types of behavior, such as singing, and is directly related to the intensity of short-term aggressive behavior. Testosterone may

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not directly trigger aggressive behavior but may facilitate the frequency and intensity of such behavior. In the monogamous songbird species so far studied, testosterone levels are high during territory establishment and again during a period of mate-guarding and then decline sharply (Wingfield and Moore, 1986). In males of bigamous pied flycatchers, *Ficedula hypoleuca* (Silverin and Wingfield, 1982), and brood parasitic brown-headed cowbirds, *Molothrus ater* (Dufty and Wingfield, 1986), the seasonal profiles of circulating testosterone are significantly different from the typical patterns of monogamous breeders. Testosterone levels are elevated for a prolonged period, associated with sequential or continuous mate-guarding behavior.

The red-winged blackbird (*Agelaius phoeniceus*) and yellow-headed blackbird (*Xanthocephalus xanthocephalus*) are the only polygynous species in which steroid hormone levels have been estimated in free-living populations (Beletsky *et al.*, 1989, 1990). The studies of these resource-defense polygynous species showed that in red-winged blackbirds there was no peak in the average value of testosterone levels early in the breeding season and a 5-week-long high plateau during the period when most nests were initiated and that in yellow-winged blackbirds testosterone levels of territory owners remained high during the first 8 weeks of the breeding season when most males established territories. However, more data are required before a meaningful comparison of different breeding strategies can be made.

Bush warblers (*Cettia diphone*) occur along the eastern rim of the Eurasian continent and the Japanese islands and are one of the best known avian species in Japan. They are residents or short-distance migrants on Honshu (main) island. In early spring, males begin to sing an unmistakable song in wintering sites, such as bushes, parks, and backyards of lowland areas, and most, if not all, birds migrate to highland areas and breed in bamboo bushes or grass meadows. Males sing from late March through August in the breeding area and emigrate from the breeding areas in September to October.

However, scientific studies on this species are very sparse and only a few papers concerning ecological observations, the role of songs, and adrenocortical response to stress have been published (Haneda and Okabe, 1970; Hamao, 1992, 1993; Wingfield *et al.*, 1995). Haneda and Okabe (1970) studied the breeding ecology of a population in the Togakushi highlands in central Honshu and reported no participation of males in incubation and rearing of the young, suggesting a polygynous breeding strategy in the species. Hamao (1992) studied the species in the Myoko highlands, also in central Honshu, confirmed the observations of Haneda and Okabe (1970), and presented convincing data of polygyny. He focused on one territory and searched for nests. Seven nests of different females were found in the territory of one male, some simultaneously and the others sequentially from May to June, of which only one nest was successfully fledged (Hamao, 1992). In his study site, he found another two territories that had at least two nests simultaneously. Clear sexual size dimorphism was also observed; males are larger than females by 18.8% in wing length and 70.4% in body mass (Hamao, 1992). Male bush warblers are very territorial and keep their territory during the whole breeding season, defending it with territorial songs, but do not participate in any parental care (Haneda and Okabe, 1970; Hamao, 1992). This breeding strategy is presumed to be a result of habitat adaptation to bamboo bushes with a good food supply but a high rate of nest predation and brood parasitism from little cuckoos (*Cuculus poliocephalus*). Therefore, the seasonal profiles of steroid hormones in this species should be valuable for understanding the relationship between hormones and mating system, especially regarding testosterone and aggression.

Corticosterone is involved in regulating energy metabolism as a glucocorticoid in birds and levels are therefore high when energy demands are high. Corticosterone is also thought to regulate adaptive changes in behavior, when individuals are exposed to stressful environmental conditions. During the breeding season, even without major deterioration of weather or nutritional conditions, holding a territory may be energy demanding and stressful. Owners continuously advertise to attract mates, guard their territories, and defend their nests against a variety of predators. Therefore, we predicted that corticosterone levels of the males would be consistently higher during the breeding season than during the nonbreeding season. In a previous experiment (Wingfield *et al.*, 1995), we observed the adrenocortical response to acute stress in two free-living populations of male bush warblers to test the hypothesis that populations living in severer habitats with shorter breeding seasons are likely to suppress the response during the nesting phase. However, the initial levels of corticosterone and the response to the acute stress of the Furano (Hokkaido) population living in the northern area were higher than those of the Chichibu (central Honshu) population. We concluded that stress modulation may not be a simple consequence of shorter breeding season and severer climate, but other ecological factors not yet known are involved. We collected the samples only from June to July and did not determine the levels for other breeding phases. We noted in both populations relatively high corticosterone levels compared with those reported in other passerine species (e.g., Astheimer *et al.*, 1994 for white-crowned sparrows; Beletsky *et al.*, 1989 for red-winged blackbirds; Schwabl *et al.*, 1991 for garden warblers; Silverin *et al.*, 1997 for willow warblers; Silverin and Wingfield, 1998 for pied flycatchers).

In this study, we attempted to measure changes in levels in testosterone and corticosterone during the entire breeding season and associated territorial behavior in a successfully breeding population of male bush warblers. Hormonal data indicate that high levels of circulating testosterone and corticosterone allow a male to hold a territory for a prolonged period, which may be an adaptation to dense bushes with high rates of predation and brood parasitism.

MATERIALS AND METHODS

Study Site and Capture Technique

The study site was in the ‘‘Forest areas 28, 29’’ and their vicinity (elevation 1100 to 1200 m) of the University Forest of the University of Tokyo at Chichibu (35° 55′ N and 138° 52′ E), Honshu (main) island of Japan. We visited the study site from April to October, 1995 and from March to November, 1996 (Table 1). The area consists of mountain forest of deciduous and coniferous trees in which forest beds are covered with dense bamboo grass, *Pseudosasa purpurascens*. The climate at Chichibu is that of the Pacific side of Japan, cool winters with little snow fall and warm and humid summers. At the study site, we measured ambient temperature at intervals. The collected data indicate that, in late March and early April, ambient temperature was between 0 and 10°C (in 1996 it decreased to below 0°C in early April). From late April, the range of temperature in a day became wide, from 10 to 25°C, and the minimum temperature gradually increased until July, so that the range became narrow accordingly. From late July to early August, the ambient temperature began to decrease.

Male bush warblers were captured with Japanese mist nets. They were attracted into a net by song playback. In capture trials in 1995, a mist net (36 mesh, four shelves, 12 m wide) was erected in an expected territory area along a work road or a walk in the woods and a tape recorder was placed next to the net at the middle of the poles. Tape-recorded songs of bush warblers (reproduced from commercial sources and the private collection of Dr. H. Momose) were played for 30 min as one session. In capture trials in 1996, songs were played for 5 min in advance in an expected territory area to normalize the distance between the sound source and the territory owner at the beginning of a session and then a mist net was erected. Tape-recorded songs of bush warblers were played for 30 min as one session.

When captured, birds were removed from the net and blood samples were collected from the wing vein within 5 min after capture. After blood sampling, body mass was measured with a Pesola scale to the nearest 0.1 g, and wing length was measured with a scale to the nearest 0.5 mm. Fat deposits in the furculum and abdomen were assessed using an arbitrary scale (Wingfield and Farner, 1978) of 0–5, in which 0 = no fat and 5 = grossly bulging fat bodies. After measurement, the birds were color banded and released at the capture site.

Observation of Behavior

To estimate territoriality, singing behavior was observed and recorded after playback until capture or the end of the session if no bird was captured. As an index of territoriality, the following was recorded: (1) the latency to respond, i.e., the time before the first song after playback; (2) the number of songs during a session; and (3) the number of long calls (continuous songs) during a session. There are two types of songs (H and L types) distinguishable by sonagraphic analysis (Hamao, 1993), but in this study we did not distinguish the two types but considered the song as a whole. If there was no response by song in a session, latency was 30 min. Numbers of songs and long calls were expressed as counts per min.

Sampling Procedures

All birds were removed from the mist net as soon as possible and a blood sample (50–100 μl) was collected from a wing vein into a heparinized capillary tube after venipuncture. Capillary tubes were sealed with molding clay and stored on ice until return to the station. The blood was centrifuged, and the plasma was harvested and stored in a refrigerator. Samples were brought to the laboratory in an ice-cool container and stored at −20°C until assayed.

Hormone Assay

Without any extraction, plasma levels of testosterone and corticosterone were estimated by a direct radioimmunoassay. Since standard radioimmunoassay methods for steroid hormones employ an extraction by organic solvents and chromatographic separation for sample preparation (for example, Wingfield and Farner, 1975, 1978; Wingfield *et al.*, 1992), our procedures and validation are briefly described.

The assay was carried out using polypropylene vials with a conical tip (MILLI-3; Lumac, the Netherlands). The buffer solution was that used for peptide hormone assay, i.e., 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS; 0.01 M phosphate, 0.14 M NaCl, 0.1% sodium azide, pH 7.5). The assay protocol was a two-incubation double-antibody method which is briefly described as follows.

On the first day, the following solutions were introduced into the vials: 100 μl 1% BSA–PBS; 100 μl of standard series (testosterone: 20 to 5120 pg / ml in 1% BSA–PBS; corticosterone: 0.1 to 25.6 ng / ml in 1% BSA–PBS) or samples; 100 μl of antibody diluted to ×2000 (testosterone) and to ×500 (corticosterone) in 1% NRS–EDTA–PBS; 100 μl of labeled antigen, 1,2,6,7[^{3}H]testosterone (Amersham; TRK-402, 37 MBq / ml) and 1,2,6,7[^{3}H]corticosterone (Amersham; TRK-406, 37 MBq / ml), respectively, about 25,000 to 35,000 dpm in 100 μl 1% BSA–PBS (about 1 μl of labeled hormone into 10 ml 1% BSA–PBS). On the second day, 250 μl of second antibody in EDTA–PBS was added and incubated overnight after agitation. On the following day, all the tubes were spun, the supernatant was discarded, and the precipitates were dissolved in 100 μl of 0.1 N NaOH. Two milliliters of liquid scintillator (Aquasol-2; DuPont) was added into the polypropylene vials and counted on a Beckman scintillation counter.

A series of 10, 20, 40, and 80 μl of quail control plasma gave a parallel inhibition to the standard curves of testosterone and corticosterone. To test the validity of assay without extraction, 8, 16, 32, 64, and 128 pg of testosterone was added to 10 μl of quail control plasma and volume-adjusted to 100 μl with 1% BSA–PBS. Ten microliters of each aliquot were assayed in triplicate as mentioned above. For corticosterone, 40, 80, 160, 320, and 640 pg were added to 10 μl of control plasma. The obtained regression line for testosterone was $y = 0.87x + 23.43$ ($R^2 = 0.999$) and for corticosterone $y = 1.003x + 149.44$ ($R^2 = 0.991$).

To test validation further using physiological parameters, plasma samples were obtained from Japanese quail kept on long-day (LD) photoperiods (16L8D) with fully developed cloacal protrusions (295 ± 25.8 mm^2) and on short-day (SD) photoperiods (8L16D) with regressed cloacal protrusions (35 ± 3.0 mm^2) (four birds each). The samples were assayed in duplicate as mentioned above. Circulating testosterone levels were 6.4 ± 0.93 ng / ml in LD birds and 0.023 ± 0.023 ng / ml in SD birds.

All data indicated that plasma in a small sample volume without extraction gave specific and reliable results in accordance with physiological parameters. Thus, plasma concentrations of testosterone and corticosterone were estimated in a 10-μl plasma sample volume in duplicate with intraassay variations of 5.7 and 12.19%, respectively, and interassay variations of 23.0 and 23.2%, respectively.

Statistics

Where sample size was adequate and equality of variance criteria was met, parametric tests [analysis of variance (ANOVA) and Fisher's least significant test (PLSD) as post-hoc tests] were preferentially applied using StatView (SAS International, Inc.) running on Windows95. Differences were considered significant when $P < 0.05$. Throughout the text, behavioral parameters and hormone concentrations are the mean ± SE.

Hormonal data of juveniles captured in September and October were not included in statistical analyses.

RESULTS

General Description

Table 1 shows the dates of visits to the study site and the numbers of trial sessions and birds captured. In 1995, when we started observations in April, birds had already arrived at the site and were singing. In 1996, when we visited the site on March 14–15, no bird responded to the playback, although one bird was seen and another's singing was heard at a lower elevation (650 m) around the lodging station. From April in 1995 and late March in 1996 through August, adult bush warblers could be captured by song playback. In September and October, adult birds were not captured but the young were. However, until at least early October we heard nonreproductive short songs in bushes and sometimes saw adult birds flying. It was very difficult to capture the same individual repeatedly; the data presented below was collected from a population living at the study site. These observations indicate that male bush warblers arrived at the study site in late March, stayed until October, and then departed for wintering grounds.

TABLE 1
Dates of Visits to the Study Site and the Numbers of Playback Sessions and Birds Captured

1995			1996		
Date	Sessions	Birds	Date	Sessions	Birds
April 12–14	12	3	March 14–15	—	0
May 18–20	35	7	March 28–29	7	2
June 7–9	29	3	April 11–12	15	4
June 28–July 1	26	3	April 24–26	23	10
July 13–15	22	7	May 9–11	23	8
August 3–5	18	3	May 23–24	23	8
August 24–26	10	3	June 6–8	23	7
September 25–27	12	0	June 19–21	20	3
October 25–27	25	12^{a}	July 3–5	17	6
Total	189	41	July 17–19	17	6
			August 1–2	16	9
			August 20–21	17	5
			August 29–30	19	2
			September 12–13	16	1^{a}
			September 26–27	15	4^{a}
			October 10–11	8	8^{a,b}
			October 24–25	10	26^{a,b}
			Total	269	109

[a] Juveniles.

[b] More than one bird captured in a session.

We did not try to find nests on the study site; we wanted to leave the territories as undisturbed as possible. Thus, we do not know rates of breeding success and predation. Since we often observed little cuckoos flying over, brood parasitism may not be uncommon.

Behavioral Observations

Figures 1 and 2 show three indices of territorial behavior of male bush warblers during the period that we visited the study site (Table 1). The numbers of songs and long calls in April, 1995 are not available since we recorded only the latency time. Behavioral responses of male bush warblers to song playback were typical of those of other passerine species. Over an observation session, males responded with songs and long calls and approached the sound source after a certain latency. These parameters significantly changed according to time of year in 1995 ($F(8, 171) = 27.009$, $P < 0.01$ for the latent period; $F(7, 167) = 10.897$, $P < 0.01$ for the number of songs; $F(7, 167) = 6.081$, $P < 0.01$ for the number of long calls) and in 1996 ($F(15, 430) = 271.626$, $P < 0.01$ for the latent period; $F(15, 437) = 39.665$, $P < 0.01$ for the number of songs; $F(15, 437) = 7.001$, $P < 0.01$ for the number of long calls).

At the beginning of the breeding season, latency to song playback was longer with greater individual variation compared to the rest of the season while the birds were responsive to playback. After an initial high level, the latency decreased significantly ($P < 0.05$ between April 12 and June 6 in 1995, $P < 0.01$ between April 11 and April 24 in 1996) and remained low until the end of the breeding season (Figs. 1 and 2, top). At the end of the breeding season, latency increased significantly.

In contrast, the number of songs was already 1.5 counts/min at the beginning of the breeding season and gradually increased until May (Fig. 2, middle). It began to decrease ($P < 0.01$) in late August and was zero in late September. The number of long calls was

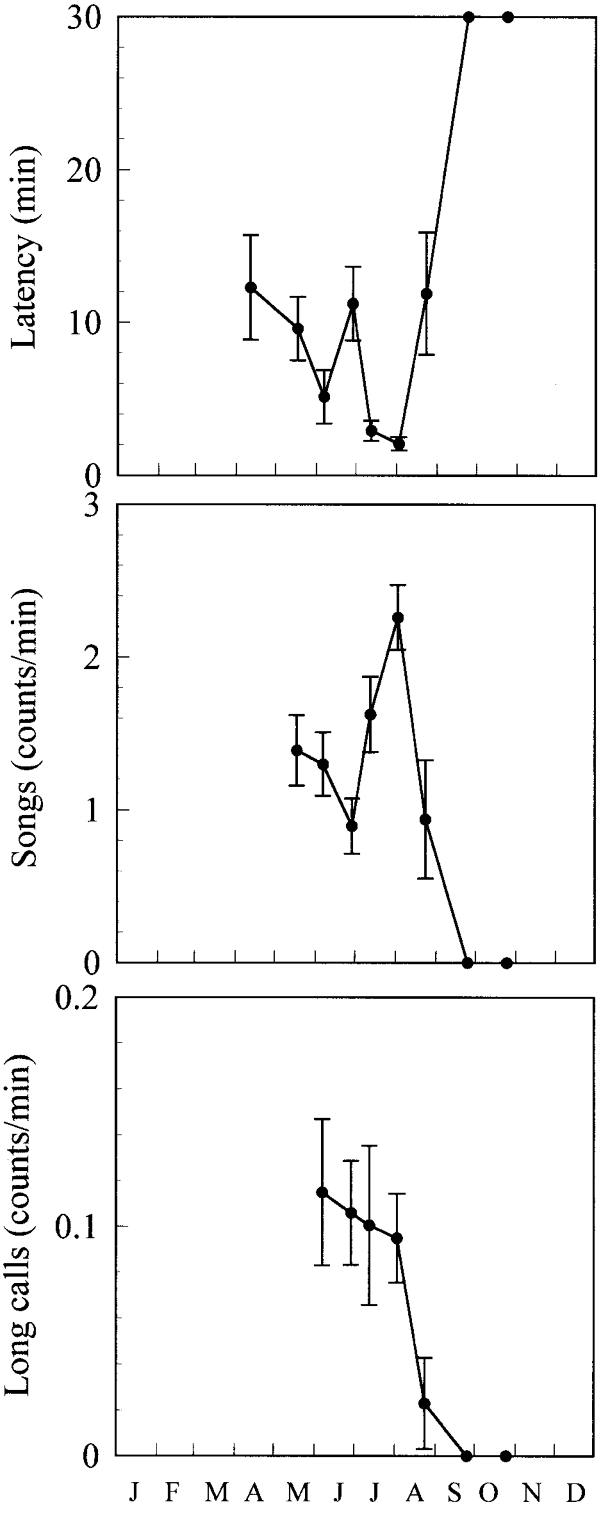

FIG. 1. Changes in territoriality of male bush warblers measured by latency to playback by singing (top), number of songs per minute (middle), and number of long calls per minute (bottom) in 1995. The sample size for each plot is listed as the number of trial sessions in Table 1 and the vertical bars give the standard error of the mean.

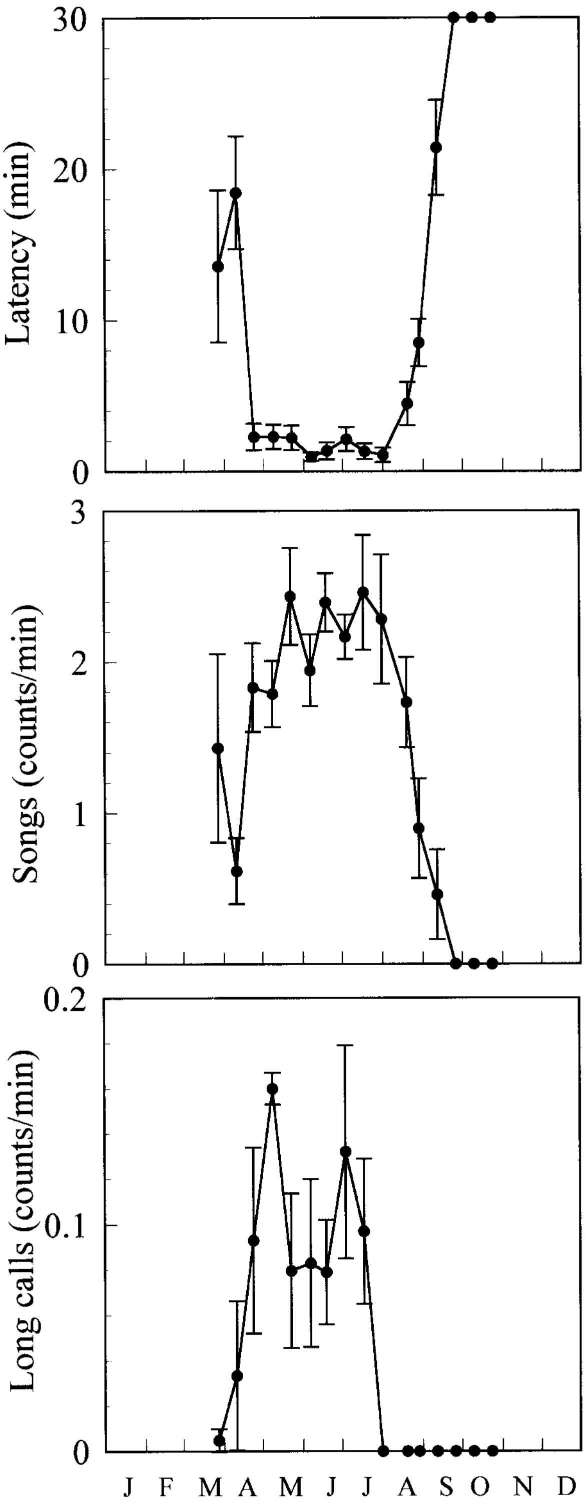

FIG. 2. Changes in territoriality of male bush warblers measured by latency to playback by singing (top), number of songs per minute (middle), and number of long calls per minute (bottom) in 1996. The sample size for each plot is listed as the number of trial sessions in Table 1 and the vertical bars give the standard error of the mean.

about one-tenth that of songs, was more variable, and decreased earlier (Fig. 2, bottom).

Changes in Body Weight and Circulating Hormones

Latency from the start of song playback until capture was different in each session. However, duration from song playback to capture had no significant effect on hormonal levels (Spearman rank correlations: $z = 0.586$, $P = 0.558$ for corticosterone 1995; $z = 0.037$, $P = 0.9708$ for corticosterone 1996; $z = 1.088$, $P = 0.2768$ for testosterone 1995; $z = -1.112$, $P = 0.2662$ for testosterone 1996). Thus, no correction according to the duration was made in the analysis below.

Figures 3 and 4 show changes in body mass and in circulating levels of testosterone and corticosterone in male bush warblers during the study period (Table 1). Body mass and plasma levels of testosterone and corticosterone in adult birds changed significantly during the observation periods in both 1995 ($F(6, 21) = 3.141$, $P < 0.05$ for body weight; $F(6, 22) = 4.071$, $P < 0.01$ for testosterone; $F(6, 21) = 5.751$, $P < 0.01$ for corticosterone) and 1996 ($F(11, 56) = 18.627$, $P < 0.01$ for body weight; $F(11, 58) = 2.127$, $P < 0.05$ for testosterone; $F(11, 58) = 3.572$, $P < 0.01$ for corticosterone).

The body mass was lowest upon arrival at the study site (breeding ground) and had increased significantly ($P < 0.01$), by 20% in early May and by 35% in early June in 1996. Even in 1995, when the data on arrival were not available, body mass increased significantly from April to May ($P < 0.01$). These high values were maintained from May to July, and in August the body mass decreased significantly ($P < 0.01$ between July 13 and August 24 in 1995 and $P < 0.01$ between August 1 and August 20 in 1996).

Plasma levels of testosterone changed seasonally, were already high upon arrival at the breeding ground, and had further increased by June in 1995 ($P < 0.05$ between May 18 and June 7) and by late May in 1996 ($P < 0.05$ between April 11 and May 23). Circulating testosterone levels then decreased sharply in 1995 and gradually in 1996 until early August (Figs. 3 and 4, middle). The circulating levels of testosterone were 0.65–0.97 ng/ml at that time. The testosterone levels had significantly decreased to less than 0.2 ng/ml by late August ($P < 0.01$ between June 6 and August 24 in 1995 and $P < 0.05$ between July 4 and August 20 in 1996). In young birds captured in September and October, circulating testosterone was at the basal level.

Plasma levels of corticosterone also changed clearly according to season (Figs. 3 and 4, bottom). Upon arrival, the levels were already high (around 30 ng/ml) and further increased to 70 to 80 ng/ml in April–May. Then, plasma levels of corticosterone decreased gradually, reaching the arrival levels by July and a low in late August ($P < 0.05$ between July 13 and August 24 in 1995; $P < 0.05$ between July 18 and August 20 in 1996). The corticosterone level peaked in April, earlier than the testosterone level. In juveniles, plasma corticosterone levels were low in September and had increased to 25 ng/ml by October.

DISCUSSION

A 2-year study of circulating hormone levels and behavior of male bush warblers indicates that the seasonal profile of plasma testosterone levels differed from that of a typical monogamous species in which there is an early peak associated with territory establishment. In bush warblers, plasma testosterone levels were already high on arrival at the breeding grounds and had increased further by May–July. They tended to remain at high levels through the end of July to early August and then decreased to basal levels in late August. Reflecting this sustained high level of plasma testosterone, territorial behavior estimated by responses to simulated intrusion was observed throughout the entire breeding season.

Changes in Circulating Testosterone

The profile of circulating testosterone in male bush warblers resembles those of polygynous red-winged blackbirds (Beletsky *et al.*, 1989) and brown-headed cowbirds, a brood parasite (Dufty and Wingfield, 1986). Males of these two bird species do not participate in parental care but guard female partner(s) while building nests and/or from other males. Levels of plasma testosterone remained elevated throughout the breeding season. The prolonged peak in male bush warblers can be explained by the multiple, sequential nest initiations in male territories which are dispersed in time and by the fact that males must guard mates

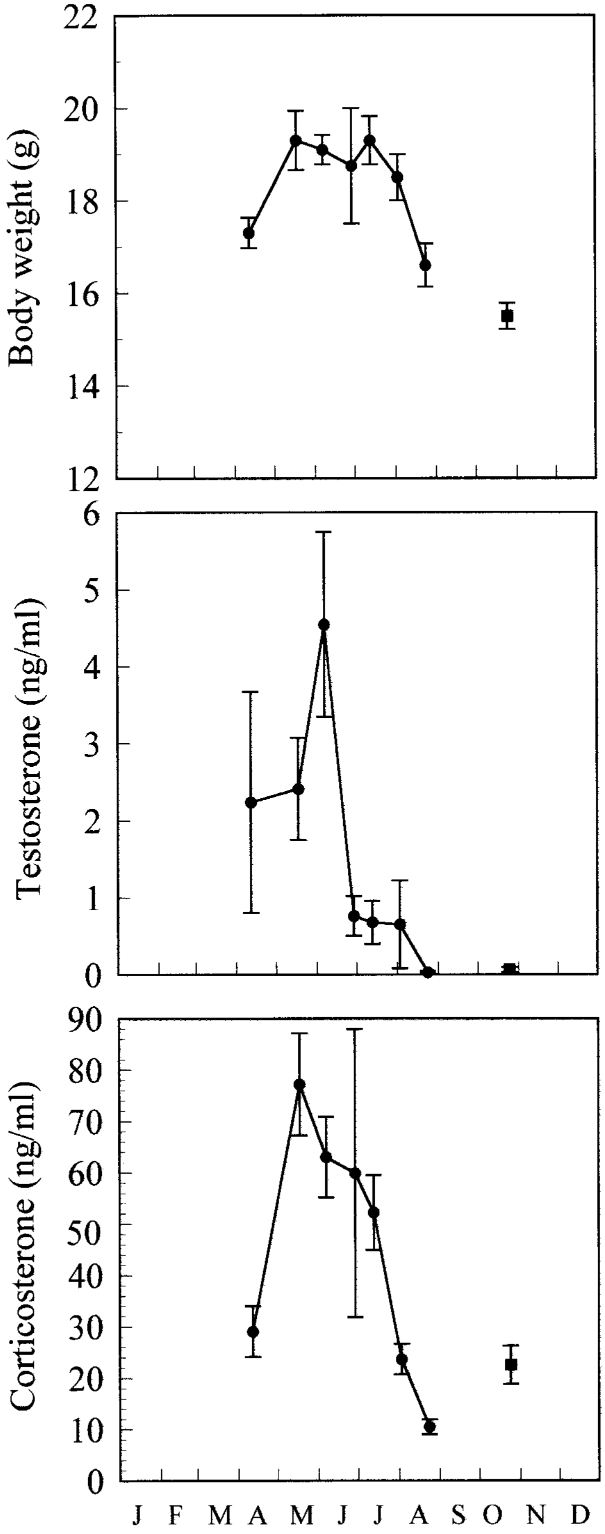

FIG. 3. Changes in body mass (top) and plasma levels of testosterone (middle) and corticosterone (bottom) in 1995. The sample size for each plot is listed as the number of birds captured in Table 1 and the vertical bars give the standard error of the mean. Solid circles designate data of adults and solid squares those of juveniles.

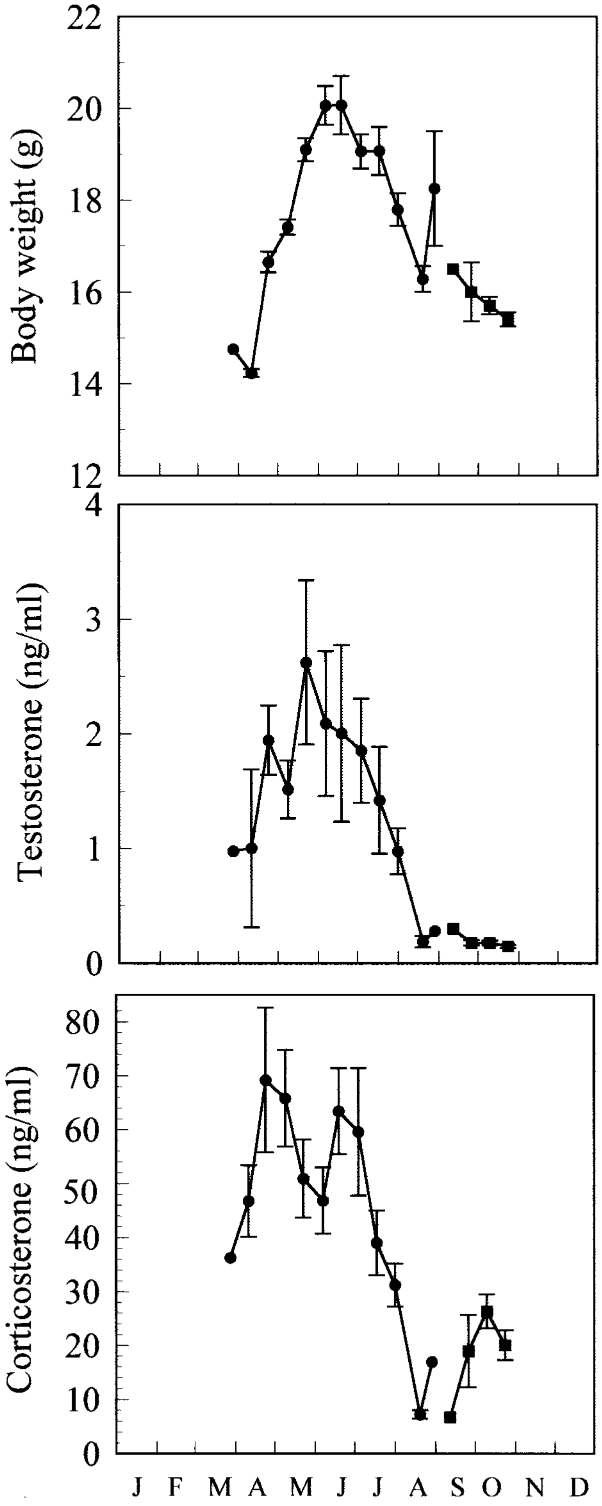

FIG. 4. Changes in body mass (top) and plasma levels of testosterone (middle) and corticosterone (bottom) in 1996. The sample size for each plot is listed as the number of birds captured in Table 1 and the vertical bars give the standard error of the mean. Solid circles designate data of adults and solid squares those of juveniles.

during their fertile periods, which is also the case for male red-winged blackbirds. This interpretation is supported by data from pied flycatchers. Monogamous males exhibited seasonal profiles of plasma testosterone concentrations similar to those found in other monogamous breeders, but bigamous males, mating sequentially with two females, maintained high testosterone levels until the second female was incubating (Silverin and Wingfield, 1982).

Research suggests that elevated testosterone concentrations are physiologically incompatible with parental care activities, such as feeding young (Silverin, 1980; Hegner and Wingfield, 1987). Yellow-headed blackbirds, another polygynous species, showed high levels of plasma testosterone at the beginning of the breeding season but the levels had declined to near basal by May to early June because the males of this species feed nestlings in the nest of their primary female (Beletsky *et al.,* 1990). The extended high levels of plasma testosterone are characteristic of polygynous males who do not participate in parental care but only guard the territory. This was directly demonstrated by an experiment in which testosterone was administered to monogamous white-crowned sparrows and song sparrows to maintain high levels during the parental phase. The treatment induced polygyny in these species, influencing males to have more than one mate in an expanded territory (Wingfield, 1984). Testosterone-implanted male pied flycatchers and white-crowned sparrows spent much time singing and patrolling the territory (Silverin, 1980; Moore, 1984).

The present physiological data are consistent with the ethological observations that bush warblers employ a polygynous breeding strategy and do not participate in parental care. Hamao (1992) observed seven nests of different females built on one territory in his study site, some simultaneously and the others sequentially. The difference in behavior from that of red-winged blackbirds and yellow-headed blackbirds was a prolonged breeding activity. Bush warblers maintain breeding activity until early August, but red-winged blackbirds terminate it in June. Prolonged high testosterone levels may affect social relations with the females and the young in the territory and in advertising ownership for the next year. In many avian species, including passeriformes and anseriformes, there is a transitory rise of circulating testosterone and sexual activity such as singing without an increase in testes weight (Campbell *et al.,* 1978; Donham, 1979; Hirschenhauser *et al.,* 1999; Lincoln *et al.,* 1980; Paulke and Haase, 1978; Peczely *et al.,* 1993). The role of these increases are not well studied but are suggested to be preparatory for reproduction and sexual behavior during the early phases of the next breeding season. Most birds terminate breeding activity in June due to photorefractoriness and show transitory elevation of sexual activity in autumn in advertisement for the next breeding season. Bush warblers may have evolved to avoid photorefractoriness and sustain breeding activity as long as possible, resulting in the continuation of sexual activity to an autumnal preparatory activity. Further studies are required to validate this notion.

The question arises as to what environmental cues induce the termination of breeding activity in late August in this species. As described under Materials and Methods, ambient temperature decreased to 20°C in August to September, when testosterone plasma concentrations decreased (Figs. 3 and 4). Temperature may be a possible environmental cue which acts as supplementary information to daylength. In Japanese quail kept under natural outdoor conditions, temperature was also suggested as an environmental cue for termination of the breeding activity (Wada *et al.,* 1992). Experimentally, temperature decrease together with short days induced termination of breeding activity in Japanese quail (Tsuyoshi and Wada, 1992; Wada, 1993).

Behavioral Observations

Since bush warblers adapt to bamboo bushes and are always hidden or on the move, it is difficult to study their behavior, other than singing. Latency should be measured by the initiation of both exploring and singing behavior, but here we designated latency as time to the first song and not to exploring behavior for the reason given above. Actual latency may be shorter than what we presented. We also did not provide the closest approach to the sound source here for the same reason mentioned above.

The longer latency at the beginning of the breeding season may be due to mobility before stabilization of the territory boundaries. Differences in latent periods in 1995 and 1996 are due to the methods of measurement. In 1995, we started song playback without preplayback but in 1996 we recorded behavioral responses after 5 min preplayback. The preplayback attracted the owner and this resulted in measured latencies in 1996 that were shorter and more stable than those in 1995. We also did not incorporate exploratory behavior after song playback. Song playback sometimes induced exploratory behavior without singing. We often observed birds flying over the mist net without any song. Therefore, the numbers of songs that we counted and present here may underestimate the actual singing activity in spontaneous states. Some birds also jumped into the mist net without any songs and long calls after song playback. These birds were not counted in this study and this is the reason why the sample size is different in the statistical analysis from hormonal ones.

Changes in Plasma Corticosterone and Body Weight

Plasma corticosterone levels also showed a clear change according to season. This supports the prediction that territory owners were probably under greater energetic demands and continuous stress. A rise in average corticosterone levels in late April and consistently high levels through July was notable, because this was the period when territorial challenges probably declined in frequency and obtaining food became easier. One stressful activity in which all territorial males engaged regularly was nest defense. Nest predation rates might be high throughout the breeding season, although we did not measure this. The relationship between plasma hormone levels and antipredator behavior is largely unknown. However, Beletsky *et al.* (1989) suggest that the positive relationship between breeding success and relative concentration of this hormone may influence breeding success in red-winged blackbirds.

It is also interesting to note the clear seasonal changes in body mass. The profiles shown in Figs. 3 and 4 reflect actual changes in body mass in this free-living population, since other physical parameters, such as wing length, are stable. Fat scores (data not shown) upon arrival were 0 to 1 and had increased to 1 to 2 by May to July. This profile indicates a considerable increase in body mass after arrival in the breeding grounds, suggesting that the area provides good quantity and quality of food for this species. Since possession of a good quality territory is a prerequisite for breeding success, holding such a territory for as long as possible and advertising it may be advantageous to the owner in returning the next season.

Other Technical Considerations

Since we captured the birds by song playback, there is a possibility that the results on seasonal hormonal changes obtained in this study do not reflect the actual circulating levels in the field but instead indicate a response to simulated intrusion. In song sparrows, challenging by song playback with a live decoy induces an acute testosterone increase (Wingfield and Wada, 1989), but male bush warblers did not show any increase in plasma levels of testosterone in response to the playback of tape-recorded songs (Wingfield *et al.*, 1995). The degree of response may vary according to the mating system and breeding strategy. It was proposed that monogamous species in which males provide most of the parental care would be most responsive in terms of testosterone increase to song playback and the responses of males that show no parental care would be least (Wingfield *et al.*, 1995). It is possible that the increase is too acute to detect the basal level with this capture method. If this is the case, our results reflect the changes in difference in sensitivity to song playback challenge. Beletsky *et al.* (1989) captured red-winged blackbirds with grain traps in which birds are not stressed until approached to remove them and yet still found seasonal changes in hormonal levels. This may indicate that our results also reflect actual levels in circulation. Further studies are required to clarify this issue.

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