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### High Migratory Fuel Loads Impair Predator Evasion in Sedge Warblers

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During migration, many species of birds rely on stored fat for fuel. The extra mass taken on for migration entails costs (Witter and Cuthill 1993). Time and energy must be devoted to foraging to build up fat loads, and increased feeding may increase the risk of being attacked by predators. An additional cost of increased fuel loads may be higher predation risk owing to reduced ability to take off, maneuver, and climb. Mass-dependent predation risk has been the focus of several recent theoretical studies (McNamara and Houston 1990, Hedenström 1992, Witter and Cuthill 1993, Brodin 2000). In species that depend on flight to escape from predators, takeoff ability is crucial because once the prey are airborne, the success rate of predators diminishes (e.g. Rudebeck 1950, Kenward 1978, Lindström 1989, Cresswell 1993).

Within the natural range of body mass of nonmigratory birds (ca. 10% diurnal increase in mass), mass seems to have no measurable effect on takeoff ability (Kullberg 1998, Kullberg et al. 1998, Veasey et al. 1998, van der Veen and Lindström 2000). In migratory birds, fuel loads of 20 to 30% of lean mass are common (Alerstam and Lindström 1990), and fuel loads may exceed 100% of lean mass when passerines are about to cross wide barriers (e.g. Fry et al. 1970, Finlayson 1981). Although fat storage is the most common explanation for mass changes in birds, mass may change because of other reversible processes, e.g. by increases or decreases in muscle mass and in various internal organs (Piersma and Lindström 1997).

To date, only two species of migrants have been studied with respect to takeoff ability in a predator-escape situation. Kullberg et al. (1996) calculated that Blackcaps (*Sylvia atricapilla*) carrying 60% of lean body mass as fuel would have an angle of ascent that was 32% lower and a velocity that was 17% lower

than Blackcaps that were carrying no fuel load. European Robins (*Erithacus rubecula*) carrying a fuel load off 27% took off at an angle that was 17% lower than robins carrying no fuel load, whereas velocity remained unaffected (Lind et al. 1999). The main aim of the study we report was to investigate how takeoff ability in Sedge Warblers (*Acrocephalus schoenobaenus*) is affected by large migratory fuel loads. In contrast to earlier studies, we studied wild Sedge Warblers just prior to a trans-Saharan crossing, thereby studying natural fuel loads versus fuel loads achieved in captivity.

**Methods.**—This study was conducted in October 1997 on the Island of Lesbos (39°01'N, 26°33'E) in the eastern part of the Greece archipelago. Sedge Warblers that breed in Europe winter in tropical Africa (Moreau 1972). In western Europe, Sedge Warblers feed heavily on reed aphids (*Hyalopterus pruni*) during autumn migration, accumulating very large fuel loads in preparation for trans-Saharan passage (Bibby et al. 1976, Bibby and Green 1981).

We trapped migrant first-year Sedge Warblers in mist nets between 0600 and 1200. Nets were checked every 20 min. After capture, birds were banded, weighed, and various morphometric measures collected. Visual fat scores were estimated following a scale modified from that of Pettersson and Hasselquist (1985), which ranged from zero (no visible fat) to six (whole belly covered with fat). Because many of the birds we trapped also had stored fat covering their breast muscles, we extended our scale to include three more stages. A bird with a fat score of nine had the whole abdomen (including belly and breast muscles) covered with fat. In total, we used 30 Sedge Warblers in the experiments. Birds were kept singly in small textile bags for 30 to 60 min before being released into the experimental cage. Each bird was only used once in the experiments, and all birds were successfully released back into the wild directly after each trial.

The experimental setup was similar to that of Kullberg et al. (1996) and Lind et al. (1999). However, because we conducted experiments under field condi-

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tions, we constructed a cage that could be easily transported. The experimental cage was a cubic tent-like construction (1.35 m long  $\times$  0.7 m wide  $\times$  1.35 m high), with three sides and the floor covered by thick unbleached cotton and the roof covered by thin white cotton to provide good light inside the cage. Aluminum pipes attached to the outside supported the cage, thereby avoiding any structures inside the cage. The only side that was covered by netting faced an additional tent that was attached to the experimental cage to provide protection from visual disturbance and wind. In the far upper end of this additional tent, a cardboard model of a Merlin (*Falco columbarius*) was hidden in a box. The model was three-dimensional and painted so that it closely resembled a gliding Merlin.

We regard the model as a very good general predator stimulus, and it has proven to elicit strong and immediate escape behaviors in Sedge Warblers and other species we have investigated. The fact that many birds elicited alarm calls after takeoff strongly suggests that they interpreted the model as a raptor. We placed a bent twig on the floor of the experimental cage to attract the bird to perch in a position where it sat facing the opposite side from where the Merlin would "attack." After release into the experimental cage, birds flew around for a few minutes before landing on the floor or on the twig. When a bird sat at the top of the bent twig and in the correct position, the falcon model was released in a standardized way and came gliding in an angle of 35° and at an average speed of 7 km per h. It took 1 s ( $\pm$  SE of 0.02 s) for the model to travel from the starting point inside the box until it stopped just in front of the netting.

We recorded takeoffs with two Hi-8 video cameras. One camera was placed perpendicular to the line of takeoff outside the tent. Because the video camera had to be about 30 cm from the tent wall to cover a sufficient area, we covered the area between the tent and the camera lens with cotton. The opening in the tent wall (40  $\times$  50 cm) was furthermore covered by transparent fiberglass. To record any side movements of the birds, a second camera was placed along the line of takeoff just outside the far end of the tent (a small hole in the cotton permitting video recording from outside). Side movements in takeoffs deviating from the center trajectory would give errors in the analysis owing to incorrect perspective given by the lens of the perpendicular camera. However, none of the takeoffs deviated more than 5 cm from the center trajectory when analyzing the video from the second camera, so no corrections had to be made in the analyses.

By analyzing the videos from the perpendicular camera, we measured velocity, acceleration, and angle of ascent of each bird in the same way as has been done previously (Lind et al. 1999). Analysis of the angle of ascent and velocity at 60 cm from the start was

made possible by videotaping an arc (at 60 cm distance) and every 5th angle from the horizontal plane drawn on a screen, which afterwards were redrawn from videotapes and used on the TV screen during analysis (see Lind et al. 1999). We measured velocity at the very start of the takeoff between the two first video frames when the bird was in the air. Because one video frame covered 0.02 s, the velocity (m per s) between two frames was calculated as the distance between two frames divided by 0.02. Acceleration (m per s<sup>2</sup>) between the first measured velocity ( $V_1$ ) and at 60 cm from the perch ( $V_2$ ) was calculated  $V_1 - V_2$  divided by the time in seconds between the two measured velocities. We recorded angle of ascent of each bird by measuring the angle from the perch to the point where the bird passed the 60cm distance from the perch.

A useful estimate of flight capacity in birds is wing loading. Pennycuik (1989) used N per m<sup>2</sup> for wing loading, but we use g per cm<sup>2</sup> because we find it more informative. This measurement takes into account each individual's wing area and body mass. We measured each experimental bird's body mass ( $\pm$  0.1 g), wing length ( $\pm$  1 mm), and wing span ( $\pm$  1 mm) and traced the outstretched left wing on a piece of paper. Wing area was later measured using Leica Q500IW image analysis equipment linked to a Hamamatsu C5810-10 ccd camera. These measurements enabled us to calculate wing loading according to the method used by Pennycuik (1989). Furthermore, to estimate fuel load of each bird, we used data on size-specific fat-free body mass related to wing length for 73 Sedge Warblers with no visual subcutaneous fat caught in southern Sweden from 1991 to 1994 (lean body mass [g] =  $-0.593 + 0.169 \times$  wing length [mm]; B. Peterson unpubl. data). Wing lengths of our birds varied from 63 to 69 mm, and estimated lean body mass ranged from 10.1 to 11.1 g. We estimated fuel load as a percentage of lean body mass by subtracting size-specific lean body mass from total body mass, dividing this by size-specific lean body mass, and multiplying by 100. We use the term "fuel load" as the increase in body mass that results from migratory fat and the eventual change in muscle mass and various internal organs that cannot be distinguished here (Piersma and Lindström 1997).

*Results.*—We subjected 30 Sedge Warblers to a simulated predator attack by the model Merlin. Half of them tried to escape by taking off in a straight line away from the "attacking" Merlin, whereas 13 others darted sideways at an angle of almost 90° from the model. These two groups of birds did not differ significantly in wing length ( $t = -1.15$ ,  $P = 0.25$ ), body mass ( $t = 0.79$ ,  $P = 0.43$ ), or fuel load ( $t = 1.11$ ,  $P = 0.27$ ; all  $df = 26$ ). The two remaining birds did not fly at all. These two birds and the 13 that flew away in a trajectory that deviated more than 5 cm from a straight line were excluded from further analysis because estimations of flight speed and angle of ascent

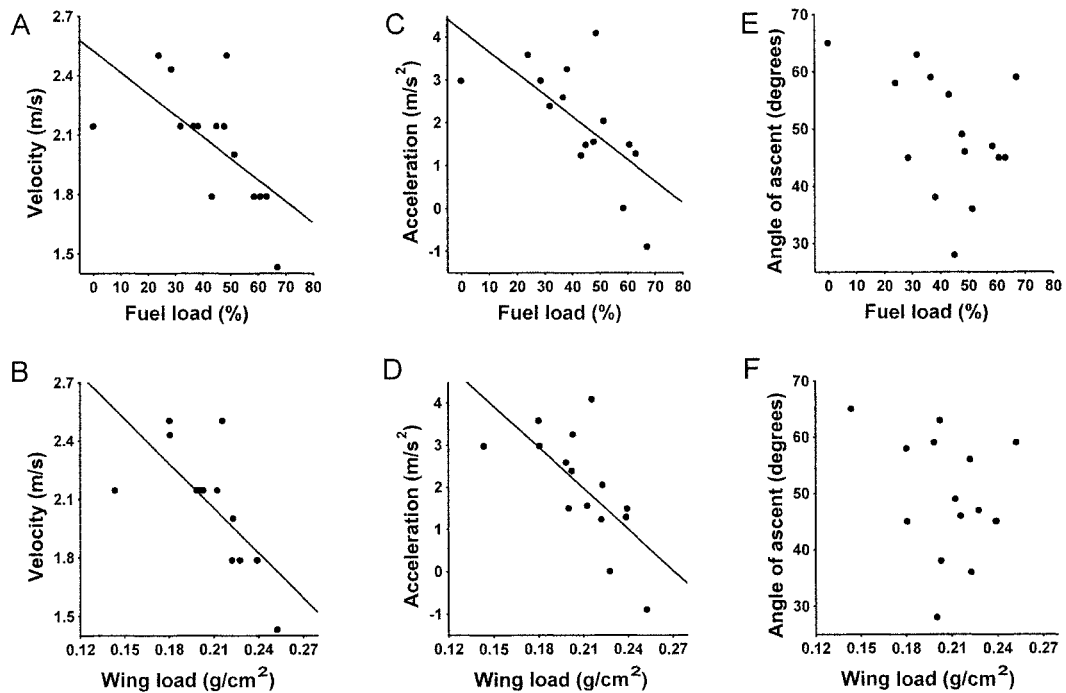


FIG. 1. Velocity (m per s) at 60 cm from the start, acceleration (m per s<sup>2</sup>) from the first measurable velocity to 60 cm from the start, and angle of ascent (degrees) at 60 cm from the start in relation to fuel (A, C, E) and wing loading (B, D, F) of experimental Sedge Warblers.

were impossible. Most Sedge Warblers emitted alarm calls when attacked.

The 15 Sedge Warblers under study varied in body mass from 10.8 to 18.2 g, in fuel load from 0 to 67% of lean body mass, and in wing loading from 0.14 to 0.25 g per cm<sup>2</sup>. Some of them had large amounts of subcutaneous fat that almost totally covered their breast muscles. Accordingly, fat score was strongly correlated with body mass ( $R^2 = 0.76$ ,  $b = 0.7$ ,  $n = 30$ ,  $P < 0.001$ ), indicating that much of the variation in body mass resulted from fat storage.

Elevated body mass impaired takeoff ability of Sedge Warblers. Birds that carried a large fuel load, and thus had heavier wing loading, had a lower velocity at 60 cm from the perch than did lighter birds (fuel load,  $R^2 = 0.35$ ,  $b = -0.01$ ,  $n = 15$ ,  $P = 0.01$ ; wing loading,  $R^2 = 0.45$ ,  $b = -7.6$ ,  $n = 15$ ,  $P = 0.004$ ; Figs. 1A and 1B). The same pattern also occurred for acceleration (fuel load,  $R^2 = 0.38$ ,  $b = -0.05$ ,  $n = 15$ ,  $P = 0.007$ ; wing loading,  $R^2 = 0.40$ ,  $b = -32.5$ ,  $n = 15$ ,  $P = 0.006$ ; Figs. 1C and 1D), whereas fuel load had no significant effect on takeoff angle (fuel load,  $P = 0.10$ ; wing loading,  $P = 0.30$ ; Figs. 1E and 1F).

According to the relationship we found, increasing fuel load from 0 to 60% reduced flight velocity by 26%, and increasing wing loading from 0.14 to 0.25 g per cm<sup>2</sup> reduced flight velocity by 32%. When they crossed the line 60 cm from the perch, the fastest bird

had attained a velocity of 2.5 m per s, and the slowest bird was flying at 1.4 m per s.

**Discussion.**—Sedge Warblers often carry very large fuel loads for their migration between Europe and Africa. This is evident during both legs of migration: Sedge Warblers have been recorded with body masses up to 21.7 g during autumn in Britain (Gladwin 1963) and with masses up to 20 g in spring at Lake Chad in Africa (Fry et al. 1970). This indicates that Sedge Warblers regularly need to handle an increased predation risk owing to large fuel loads. Sedge Warblers with large body masses are caught less often at banding sites (Bibby et al. 1976), indicating that they behave in a more secretive way and probably are more reluctant to fly when they are heavier. Judging from prey remains at a colony of Eleonora's Falcons (*Falco eleonora*) off Crete, Sedge Warblers are the most common prey species among the *Acrocephalus* warblers that migrate through the area (Ristow et al. 1986 in Handrinos and Akriotis 1997). However, Eurasian Reed-Warblers (*A. scirpaceus*) are much more commonly observed during autumn migration in Greece, indicating that Sedge Warblers are more susceptible to predation, maybe because of a different migratory strategy (Handrinos and Akriotis 1997).

Our results provide strong support for an increase in predation risk with increasing fuel loads in mi-

gratory Sedge Warblers. We found that increasing fuel load from 0 to 60% reduced flight velocity by 26% in Sedge Warblers. In a similar study on takeoff ability in Blackcaps, the same increase in fuel load reduced flight velocity by 17% (Kullberg et al. 1996). Interestingly, we were unable to demonstrate an effect of fuel load on angle of ascent in Sedge Warblers, whereas angle of ascent in heavy Blackcaps was affected to a larger extent than flight speed. Studies of European Starlings (*Sturnus vulgaris*) with artificially increased body mass and on gravid females indicate that heavier birds have a lower angle of ascent while maintaining the same velocity (Witter et al. 1994, Lee et al. 1996). A similar effect occurred in European Robins, where a 27% increase in fuel load lowered the angle of ascent by 17%, but takeoff velocity was unaffected (Lind et al. 1999). These results could indicate that takeoff decisions differ between species because a tradeoff occurs between angle of ascent and takeoff speed (Witter and Cuthill 1993, Kullberg et al. 1998).

As noted by Hedenström (1992), turning radius increases with increased body mass. Thus, an alternative explanation for our results is that heavy birds chose a low flight speed to maintain maneuverability. However we believe that in the very initial phase of the escape, it is of utmost importance for the bird to get fully airborne before initiating other evasive strategies, such as trading speed for maneuverability. It is important to note that we measured only the initial phase of takeoff (60 cm), and the birds could fly as much as 1.8 m before having to maneuver (reaching the top of the tent). In fact, most birds did not maneuver but flew straight into the cotton ceiling.

Another interesting result is that a high proportion (43%) of Sedge Warblers took off almost perpendicular to the model's attack trajectory. Placed in the same experimental setup, only 1 out of 73 Blackcaps chose a similar strategy (C. Kullberg et al. unpubl. data). For Sedge Warblers, which live close to the ground in dense bushes or reeds, darting off at a sharp angle to a predator's line of attack may be adaptive because the probability of disappearing from the predator's view is high in dense vegetation. Blackcaps often live in a microhabitat that is more open and thus may be less prone to use this escape strategy. We expect to find an array of different escape tactics depending on a species' habitat, but at present, little is known about species-specific escape strategies (Pulliam and Mills 1977, Lima 1993).

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## Versatility from a Single Song: The Case of the Nightingale Wren

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Versatility in song production of birds has drawn considerable attention since its description by Hartshorne (1956), who suggested that birds vary their vocal output to avoid habituation in listeners, especially if singing is extensive. The best-known route to song versatility involves creating permutations and combinations of song elements learned from neighbors or relatives, combined with improvisations (Nowicki et al. 1999). Birds may learn whole songs or individual song elements, which then may be arranged in novel ways.

Versatility might be achieved in other ways besides acquiring numerous song types. For example, individuals could shift the tempo of their songs by altering internote or intersong intervals. Alternatively, birds might sing the same pattern of notes but shift their frequency range. Black-capped Chickadees

(*Poecile atricapilla*) shift the frequency of their whistled songs, which has been suggested to function as a repertoire-enlarging strategy (Horn et al. 1992). Without changing the order of song elements, shifts in tempo or frequency might change the perception of the song sufficiently to prevent habituation.

Here, we describe songs of three Nightingale Wrens (*Microcerculus philomela*), which are residents of tropical lowland forests from southern Mexico to central Costa Rica (AOU 1998). The song of this species has a peculiar quality that has struck some observers as being "random" because it is difficult to discern a clearly recurring pattern (Howell and Webb 1995). This distinctive song has been the primary justification for splitting *M. philomela* from *M. marginatus*, the Scaly-breasted Wren (Slud 1958; Stiles 1983, 1984).

*Methods.*—Recordings of Nightingale Wren songs were made by DWL at La Selva Biological Station, Costa Rica (10°26'N, 83°59'W), using a Sony TCM-

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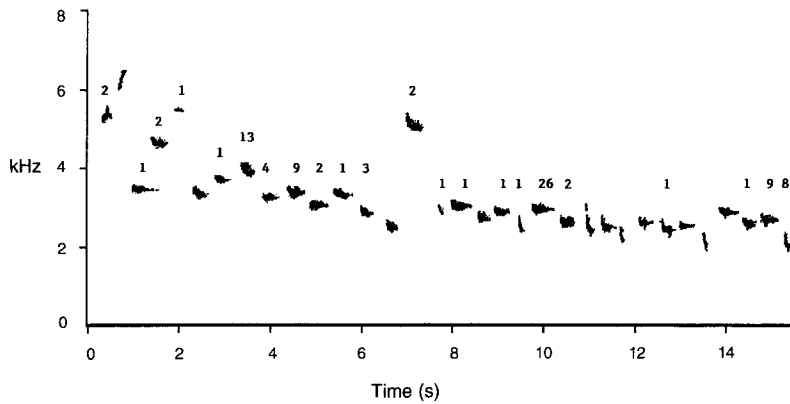


FIG. 1. Sonogram of a 33-note Nightingale Wren song recorded at La Selva Biological Station, Costa Rica. Numbers above each note indicate how many of the 1998A songs were terminated after the bird sang that note.

5000EV recorder and Sennheiser ME67/K6 microphone. One recording was made on 13 March 1998 and is 17 min 47 s in duration. The second recording was made on 14 March 1998 and is 3 min 48 s in duration. These recordings, which probably are of different individuals, are referred to below as 1998A and 1998B, respectively; copies are archived at the Borror Laboratory of Bioacoustics (numbers 25923 and 25924).

Very few archived recordings are available for *M. philomela*, but we obtained nearly all of the holdings of this species (indexed as *Microcerculus marginatus*) from the Library of Natural Sounds (LNS) at Cornell University. One of these recordings (LNS 28138) was made by A. van den Berg at La Selva on 29 May 1981. It is the only archived recording of sufficient duration (4.5 min) to permit significant analysis. It is referred to below as 1981.

Recordings were digitized using Canary 1.2.1 software on a Macintosh computer at a sampling rate of 44 kHz. We used Canary to extract measures of highest frequency, lowest frequency, and peak frequency of each note. The three measures proved to be highly intercorrelated, so we report only peak frequency here because it was the easiest to measure reliably. The reliability of the measure was estimated by having a second person re-measure the notes on 20% of the sonograms ( $n = 455$  notes). Across all re-measured notes, the average similarity was 99.9%.

**Results.**—Listening to a singing Nightingale Wren does not provide a clear sense of when a song begins and ends. Singing may go on more-or-less continuously for minutes with what seems to be a random output of clear, short, whistled or slurred notes given at a rate of about two notes per s. Nonetheless, our analyses revealed that each bird sang a highly stereotyped sequence of notes. The apparent randomness of the song results from stoppages of singing at almost any point, followed by a brief pause and then

resumption of singing with the same notes that were given at the beginning of the sequence.

Songs began with a pair of high-frequency notes, the second of which was higher and softer than the first and was upslurred (Fig. 1). The starting pair of notes occurred whenever a pause of more than 0.5 s occurred during a singing bout (inter-note intervals were about 0.1 s). There were 94 songs in the 1998A recording, 17 in 1998B, and 25 in 1981. Five songs in 1998A and seven in 1998B had their starting pair of notes preceded by one or two "stuttered" notes. The 1981 bird used stuttered notes 21 times, and the stutters were up to four notes long. Because stuttered notes were not given in all songs, we do not include them in the analyses that follow.

The number of notes per song was highly variable for all three individuals, but all three had maxima of either 32 or 33 notes. However, these maxima rarely were achieved because the birds stopped singing at almost any point in the song (Fig. 1). The number of notes per song averaged  $17.7 \pm \text{SD of } 9.4$ ,  $23.3 \pm 10.3$ , and  $14.5 \pm 9.0$  for 1998A, 1998B, and 1981, respectively. We found no evidence that the longer songs consisted of repetitions of any subset of notes.

The sequential patterning of notes was highly stereotyped for each bird. For example, we took a random sample of 10 songs from 1998A that terminated after 21 notes (the most common stopping point). A correlation analysis of the peak frequencies of the notes for each pair of songs in the sample ( $n = 45$ ) yielded a mean  $r$  of 0.99 ( $P < 0.01$ , all  $df = 19$ ), indicating that the frequency relationships of the notes of any song were repeated almost exactly in other songs.

Although the patterning of notes was stereotyped, birds sang their songs across a range of frequencies. For instance, the lowest peak frequency of the first note in any 1998A song was 4.85 kHz, but the first notes of the other songs were as much as 21% higher

TABLE 1. Pearson correlations (df in parentheses) among song variables for the three recordings of Nightingale Wren songs.

Variables	1998A	1998B	1981
Number of notes and frequency index of same song	0.39 (75)*	0.57 (13)	0.30 (22)
Number of notes and following ISI	-0.46 (83)*	-0.65 (9)	-0.67 (21)*
Number of notes and preceding ISI	0.17 (81)	0.24 (11)	0.07 (21)
Number of notes and number of notes of next song	-0.28 (89)	-0.31 (12)	-0.31 (21)
Number of notes and frequency index of next song	-0.54 (77)*	-0.62 (12)	-0.76 (21)*
Frequency index and frequency index of next song	0.43 (70)*	-0.03 (11)	0.06 (21)
Frequency index and preceding ISI	0.64 (74)*	0.91 (8)*	0.85 (21)*
Frequency index and following ISI	0.26 (71)	-0.25 (9)	0.08 (21)

\*  $P < 0.05$  after applying Bonferroni procedure.

than that, up to a maximum of 5.9 kHz. The other notes had similar ranges. The high correlation of peak frequencies among songs means that birds shifted frequencies in almost exactly the same way.

The notes of longer songs tended to be sung in higher frequency ranges. To obtain an index of song frequency, we summed the peak frequencies of the first eight (1998A and 1998B) or seven (1981) notes, because most songs had at least that many notes. The correlation between the number of notes in a song and the song's frequency index was positive in all three recordings (Table 1).

We measured the intersong intervals (ISI) between 92 song pairs for the 1998A recording. The mean ISI was  $3.4 \pm 6.6$  s, but 36 of the ISIs lasted less than 1 s. Several ISIs were much longer, including four that exceeded 18 s. If these lengthy ISIs are excluded (because they were at least 2.5 z-scores above the mean and probably were not associated with singing [Kroodsmma 1977]), the mean ISI was 2.2 s. Mean ISIs for 1998B and 1981 were 3.9 and 3.3 s, respectively.

Because of the often lengthy songs and usually brief ISIs, the percentage of time in which the bird was actually singing (i.e. the sum of song durations divided by total recording time) was very high. In our three recordings, singing time ranged from 71 to 80% of total recording time.

All three birds organized their singing bouts in predictable ways. When they sang a longer song, the following ISI was brief, and the next song tended to be shorter and had a lower frequency index. Interrelationships among these variables are summarized in Table 1.

Longer songs were followed by shorter ISIs in all three recordings (Table 1). In contrast, no significant correlation existed between song length and duration of the ISI that preceded the song (Table 1).

Lengthier songs generally were followed by shorter songs that were sung in a lower frequency. The correlations between the number of notes in successive songs were negative (although not significant), and those between the number of notes in a song and the frequency index of the next song were strongly

negative (Table 1). No consistent trend occurred across recordings for the frequency indices of successive songs (Table 1). The higher the frequency index of a song the longer the pause that preceded the song; however, no systematic trend existed between the frequency index of a song and the duration of the ISI that followed it (Table 1).

The 1998A and B songs were remarkably similar. For example, we performed a correlation analysis of the peak frequencies of the four 21-note songs from 1998B with the corresponding notes of four 21-note songs drawn randomly from 1998A. The minimum  $r$  for these four correlations was 0.97 ( $P < 0.01$ , all  $df = 19$ ). Furthermore, a correlation analysis of peak frequencies of four randomly selected 32-note songs from 1998B with those of randomly selected 32-note songs from 1998A yielded a minimum  $r$  of 0.95 ( $P < 0.01$ , all  $df = 30$ ), indicating that the notes of each bird's song bore nearly identical frequency relationships to the other's song. We conclude that the two recordings came from neighboring birds that shared song types. The 1981 La Selva songs were much like those in 1998. The main difference was that the 1998 songs had a series of three notes near the beginning of the song (notes 3 to 5 or 6 to 8 in Fig. 1) that was not present in 1981.

*Discussion.*—The Nightingale Wren has achieved versatility in its song output despite each bird having only one song in its repertoire. This versatility is achieved by terminating the song at virtually any point in its sequence. Furthermore, songs seldom were completed. Taking all of the La Selva data together, 134 songs were initiated, but only 19 (14.2%) were completed. Songs were terminated after as few as one note or as many as 32 (of 33) notes, and at many points in between. Because the length of a song (i.e. number of notes) and the length of the song that followed it were weakly correlated, listeners were "kept guessing" about what would come next.

Birds sometimes abort their songs before completing them, but incomplete songs have received little attention. Winter Wrens (*Troglodytes troglodytes*) have a complex song and often terminate their songs with-

in or between song sections (Kroodsma 1980, Kroodsma and Momose 1991). Chaffinches (*Fringilla coelebs*) respond to playback of full song by singing about 10 songs that are incomplete to various degrees, after which they resume full song (Heymann and Bergmann 1988). Chaffinches sing incomplete songs when raptors fly overhead or when humans approach. Sage Sparrow (*Amphispiza belli*) songs begin with the same syllables but sometimes are terminated after a variable number of syllables (Rich 1983). Similar observations have been reported in White-throated Sparrows (*Zonotrichia albicollis*; Borror and Gunn 1965) and Harris's Sparrows (*Zonotrichia querula*; Shackleton et al. 1991), but neither study provided data on the commonness of incomplete songs. In Chaffinches and Sage Sparrows, however, incomplete songs are clearly the exception, whereas in Nightingale Wrens they are the rule.

Another mechanism for increasing song versatility is frequency shifting. Nightingale Wrens sing a highly stereotyped sequence of notes, but the frequency of the first note varies between 4.9 and 5.9 kHz. Frequency shifting is well studied in the whistled song of Black-capped Chickadees. The relatively simple song of this species consists of two notes at different frequencies, and chickadees maintain the frequency relationship between the two notes regardless of the absolute frequency of the first note (Hill and Lein 1987, Horn et al. 1992). Borror and Gunn (1965) also noted frequency shifting by White-throated Sparrows, and Morton and Young (1986) reported that Kentucky Warblers (*Oporornis formosus*) shift frequencies to approximate those of playback songs, although these authors did not describe frequency shifting in other contexts. Frequency shifting may be a method of increasing song diversity in birds that have small repertoires.

The Nightingale Wren is noteworthy, at least among wrens, for its extensive singing time. The relative amount of time spent singing ranged from about 21 to 50% in North American wrens (Kroodsma 1977), and *Thryothorus sinaloa* and *T. felix* in Mexico had values of 28% and 17%, respectively (Brown and Lemon 1979). With more than 70% of the time spent singing, the Nightingale Wren clearly is exceptional in this regard, although data are lacking on other wrens.

The song bouts of Nightingale Wrens are organized in such a way that the length, frequency range, and pauses between songs are varied but nonetheless predictable. When birds sing a lengthy song, they take only a short pause afterward. However, shorter pauses tend to be followed by shorter songs that have a lower frequency range. These observations are consistent with both motivational and performance-constraint approaches to song performance (Lambrechts 1996, Podos 1997). Our observation that longer songs tend to be sung in a higher frequency range and to be followed by shorter pauses is consistent with a

motivational account of song variation during a bout. However, singing long songs with a high frequency range may be a difficult task, and despite high motivation, the short pause that follows may not be sufficient for these birds to immediately repeat the performance; thus, longer and higher-frequency songs tend to be followed by shorter and lower-frequency songs (and vice versa), which may enhance the perceived variation in song output.

In conclusion, the combination of varied song lengths, frequency shifting, and short intersong intervals creates the perception of extremely long and rambling note sequences in the Nightingale Wren. This species seems to have created a versatile song performance through the use of relatively uncommon modifications of a single song.

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## Molecular Evidence for Extrapair Paternity and Female-Female Pairs in Antarctic Petrels

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Considerable interspecific variation exists in the frequency of extrapair fertilizations (EPFs) in birds. In general, EPFs are more common and occur at higher frequencies in passerines than in nonpasserines (Westneat and Sherman 1997). Lower rates of EPFs are typical for territorial nonpasserines as well as those that breed colonially (Westneat and Sherman 1997). This seems to contradict Birkhead and Møller's (Birkhead and Møller 1992, Møller and Birkhead 1993) hypothesis of intense sperm competition in colonial birds. Their arguments were based on the assumption that the need for nest defense in dense aggregations restricts the ability of males to guard their mates, and that the high number of potential extrapair mates available in colonies selects for a high rate of extrapair copulations (EPCs).

In contrast, Westneat and Sherman (1997) found no correlation across species between the frequency

of EPFs and nesting dispersion, local breeding density, or breeding synchrony, although EPFs were related to nesting density within species. This suggests that EPC rates are not informative regarding EPF rates in colonial birds (Westneat and Sherman 1997), or that the pattern reported by Møller and Birkhead (1993) does not hold true when more species are included. The conflicting evidence regarding the relationship between extrapair activities and breeding density calls for more empirical studies, especially among colonial nonpasserines.

Social monogamy is the predominant mating system in the Procellariiformes (Warham 1990). Several aspects of their breeding biology may, however, provide favorable opportunities for extrapair sexual activity. First, colonial breeding provides ample opportunities for EPCs because many potential partners are available at close range (Birkhead and Møller 1992, Møller and Birkhead 1993). Second, when the sexes are spatially and/or temporally separated, as may be the case in procellariiforms where

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adults seek food far from the colony, males have few cues to assess whether their mates have been unfaithful. Hence, few reasons exist to expect a facultative decrease in male parental investment if cuckolded, in contrast to the case for many territorial species where a male may have more reliable cues to his mate's unfaithfulness (e.g. female disappearance, high intrusion rate, etc.). Accordingly, colonial breeding may facilitate EPCs for both sexes, and colonial species may be expected to display high rates of EPC.

Paternity studies require error-free sex determination of adults. This is straightforward for clearly dimorphic species, or if sex determination can be done from genitalia during the fertile period. But if fieldwork can be performed only during the nestling period, sex determination may be problematic for largely monomorphic species, and indirect methods must be used.

In the Antarctic Petrel (*Thalassoica antarctica*), the two sexes differ slightly in mean body size. Lorentsen and Røv (1994) used this difference to determine the sex of Antarctic Petrels by discriminant function analysis (DFA). The procedure correctly determined the sex of 92% of the birds in a sample from the same year. This does not necessarily imply a similar resolution if the discriminant function is adopted for samples from other years, or if data are collected by other observers. Moreover, although useful for many purposes, 92% resolution in sex determination is insufficient for paternity studies. Therefore, we performed molecular sexing of all breeding adults according to the PCR-based method of Griffiths et al. (1998).

The main aim of our study was to analyze whether extrapair paternity occurs in a colonial procellariiform, the Antarctic Petrel. We also tested the robustness of morphological sex determination (from DFA) across seasons relative to that obtained from molecular techniques.

*Study area and methods.*—The study was conducted at Svarthamaren (71°53'S, 05°10'E), Mühlig-Hofmannfjella, Dronning Maud Land, Antarctica, during the austral summer of 1993–1994. About 250,000 pairs of Antarctic Petrels bred on a northeast-facing mountain slope at 1,650 m elevation, more than 200 km from the nearest open sea (Mehlum et al. 1988, Røv et al. 1994). Antarctic Petrel nests were relatively evenly spaced at a density of 0.76 nests per m<sup>2</sup> (Mehlum et al. 1988). The topography of the colony was relatively flat and open. Thus, neighboring pairs often interfered with each other, indicating that many opportunities for EPCs were available.

The Antarctic Petrel is an open-nesting, medium-sized petrel (500 to 675 g) and lays a single egg after a prelaying exodus of 20 to 23 days (Pryor 1968, Lunders 1977). Both parents incubate (Lorentsen and Røv 1995), and hatching occurs synchronously within the colony in the middle of January (Haftorn et al.

1991, Amundsen et al. 1996). After the egg hatches, parents brood their chick constantly for 9 to 13 days (Bech et al. 1988, Røv et al. 1994) before it is left unattended. Chicks at Svarthamaren are fed by both parents, which on average return from feeding areas in the open sea every second day with 80 to 250 g of food (Haftorn et al. 1991, Lorentsen 1996).

We marked 75 nests containing an egg on 5 January 1994. For 42 (56%) of these nests, we obtained blood samples for DNA fingerprinting from both parents and the chick. We also obtained blood from seven additional pairs that were used for molecular (and morphological) sex determination. The adult on duty was banded, measured (wing length,  $\pm 1$  mm; head plus bill length,  $\pm 0.1$  mm; bill depth,  $\pm 0.1$  mm), and dyed on the back with highly diluted picric acid. Measurements were taken by the same person as in 1992 (N. Røv) to control for effects of the measurer (Lorentsen and Røv 1994) and hence, to specifically test for temporal reliability of morphological sex determination.

We collected about 50  $\mu$ L of blood in capillary tubes and immediately suspended the sample in 1 mL of Queen's lysis buffer (Seutin et al. 1991). When the mate returned to the nest, the same procedure except dyeing was repeated. All adult birds were caught during incubation or early in the brood-rearing period. Blood samples were collected from chicks a few days after hatching, using the same methods as for adults. The samples were deep frozen until analysis. The sex of adults was determined using the discriminant function of Lorentsen and Røv (1994), and by molecular techniques (see below). When using the discriminant function, the pair member with the lowest discriminant score was considered to be the female.

DNA isolation, gel electrophoresis, and Southern blotting followed the protocol of Krokene et al. (1996), with some minor adjustments (see Bjørnstad and Lifjeld 1997). Briefly, DNA was isolated through a standard procedure of proteinase K digestion, phenol/chloroform washes, and isopropanol precipitation. DNA was cut with the restriction enzyme HaeIII, electrophoresed in 0.8% agarose with TBE-buffer, and blotted onto Hybond Nfp (Amersham) nylon membranes according to the manufacturer's protocol. The minisatellite probe *per* (Shin et al. 1985) was radioactively labeled with Redivue ( $\alpha$ -32P)dCTP (Amersham) using the Prime-a-Gene labeling kit (Promega). The hybridization procedure followed the manufacturer's protocol for the Hybond Nfp membrane. Filters were autoradiographed with or without an intensifying screen at  $-80^{\circ}\text{C}$  for one to seven days using Kodak BioMax MS film.

Scoring of fingerprint bands was done by marking each nestling band on an acetate overlay with a specific color according to whether a similar band was in the fingerprint of the father, the mother, both, or neither of them. Bands not present in either parent,

i.e. novel bands, may arise through scoring error, mutation, or mismatched parentage. Scoring was carried out by KA and JTL. Only a few disagreements occurred between scorers (ca. 20 bands), and in these cases the scorings of JTL were used. Because mutations of minisatellite DNA typically occur randomly at a frequency of 1 in 100 to 300 bands (e.g. Burke and Bruford 1987, Westneat 1990, Lifjeld et al. 1993), chick fingerprints normally will contain none or only a few mutated bands, depending on the number of bands. In the present study, nestling fingerprints contained either 0 to 1 or 8 or more novel bands. We consider cases of a single novel band to be the result of mutation, and those with eight or more novel bands to result from mismatched parentage.

Band sharing was calculated by the formula of Wetton et al. (1987). Parentage was excluded when band sharing was lower than the lowest value recorded for chicks related to both parents and within the limits for band sharing between mates, which we assume are genetically unrelated.

The sex of adults was determined by PCR amplification of the CHD-Z and CDH-W genes on the sex chromosomes (Z and W) using the primer pair P2 and P8 described by Griffiths et al. (1998). These primers typically amplify one band in males and two bands in females, because the female is the heterogametic sex in birds. Analyses were out carried according to the protocol of Griffiths et al. (1998), except that we radioactively labeled the P2 primer with (gamma 32P) ATP (Amersham) and electrophoresed the products on 6% polyacrylamide gels. Products were visualized by autoradiography.

**Results.**—On average, we counted  $30.9 \pm \text{SE of } 0.9$  (range 17 to 43,  $n = 42$ ) scorable bands per nestling; 38 nestlings (90%) had a fingerprint profile that matched (0 to 1 novel bands) both putative parents. Band-sharing coefficients averaged  $0.57 \pm 0.01$  (range 0.37 to 0.72) with the putative mother and  $0.58 \pm 0.01$  (range 0.39 to 0.73) with the putative father, and we considered 0.37 to be the lower limit for band sharing with a genetic parent.

The remaining four nestlings had between 8 and 14 novel bands (Fig. 1). Each chick had high band sharing with one parent (range 0.40 to 0.77) and low with the other (0.15 to 0.19). The latter values fall within the range of values observed for paired birds (0.00 to 0.28;  $\bar{x} = 0.13 \pm 0.01$ ,  $n = 42$ ). The putative father was excluded from being the genetic father in three cases, and the excluded parent was one of two female parents in the fourth case. In summary, 3 of 41 nestlings (7%) resulted from EPFs (excluding the young of the female-female pair).

We did not actively seek among fingerprinted males to find possible extrapair fathers. However, we happened to find the genetic father of one of the extrapair chicks among the males fingerprinted on the same gel: he was the male that bred at the closest of

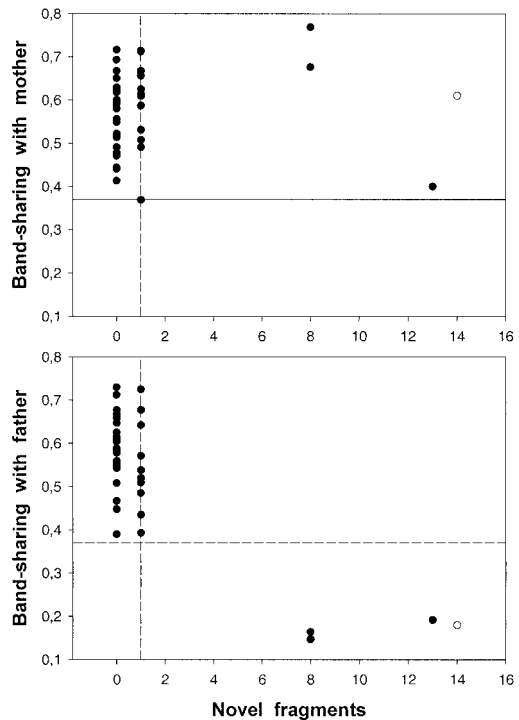


FIG. 1. Relationship between band sharing with putative mother (above) and putative father (below) and number of novel fragments in offspring fingerprints of Antarctic Petrels from Svarthamaren, Dronning Maud Land (filled circles). Open circles show band-sharing coefficients in the female-female pair. Dashed lines indicate the criteria for excluding parentage.

the neighboring nests. This male also was the father of the chick in his own nest.

Mean discriminant scores based on morphology were  $0.97 \pm \text{SD of } 1.06$  (range  $-2.06$  to  $2.93$ ,  $n = 49$ ) for individuals assessed as males and  $-1.05 \pm 1.11$  (range  $-3.11$  to  $1.18$ ,  $n = 49$ ) for individuals assessed as females. Discriminant scores from 1992 (Lorentsen and Røv 1994) did not differ significantly from those of 1994 (males,  $t = 1.47$ ,  $df = 116$ ,  $P = 0.14$ ; females,  $t = 0.97$ ,  $df = 103$ ,  $P = 0.33$ ).

Using molecular sexing, the P2/P8 primers gave one band (ca. 350 base pairs) present in all adults ( $n = 98$ ) and a slightly larger band present in 51 adults. Hence, this pattern agrees with the sex-specific pattern described by Griffiths et al. (1998) that individuals with one band are males, and those with two bands are females. The female-specific band was the larger one. Among the 49 pairs analyzed, two (4%) consisted of two females.

The DFA correctly determined the sex of 88% of the males and 92% of the females ( $\bar{x} = 90\%$ ). Discriminant scores for one of the two female-female

pairs assessed by the molecular method clearly indicated that both were females. For the other pair, discriminant scores indicated that the pair members were of opposite sex. The mean discriminant score of males whose sex was determined incorrectly ( $-0.09 \pm 1.15$ ) was significantly lower than that of males whose sex was determined correctly ( $1.09 \pm 0.96$ ;  $t = 2.32$ ,  $df = 45$ ,  $P < 0.05$ ). For females, mean discriminant scores for the two groups did not differ significantly ( $-0.43 \pm 1.59$  and  $-1.10 \pm 1.08$  for incorrect and correct, respectively;  $t = 1.48$ ,  $df = 49$ ,  $P = 0.15$ ).

**Discussion.**—The main findings of our study were: (1) three nestlings (7%) were sired by extrapair males; (2) two pairs (4%) consisted exclusively of females; and (3) DFA based on morphological measurements correctly determined the sex of 88% of the males and 92% of the females, as confirmed by molecular sexing.

Our study is the second to demonstrate extrapair paternity in a species of procellariiform. In the Short-tailed Shearwater (*Puffinus tenuirostris*), Austin and Parkin (1996) found that 11% of the chicks were sired by extrapair males. This value is close to that found in our study of Antarctic Petrels (7%). Studies of three other procellariiforms (Cory's Shearwater [*Calonectris diomedea*], Swatschek et al. 1994; Leach's Storm-Petrel [*Oceanodroma leucorhoa*], Mauck et al. 1995; Northern Fulmar [*Fulmarus glacialis*], Hunter et al. 1992) failed to find evidence for EPFs. This does not necessarily imply an absence of extrapair sexual relationships. In Northern Fulmars, 2.4% of all copulations were extrapair, but these apparently were unsuccessful in fertilizing the eggs. Taken together, it seems clear that extrapair paternity is relatively infrequent among procellariiforms, including the Antarctic Petrel. However, the apparent absence of EPFs in certain species should be interpreted cautiously because of the limited power in detecting rare EPFs with small sample sizes.

The relatively low incidence of EPFs found in procellariiforms is inconsistent with the prediction of high rates of EPC and EPF for colonial birds as suggested by Birkhead and Møller (Birkhead et al. 1987, Birkhead and Møller 1992, Møller and Birkhead 1993). Several reasons may account for this apparent contradiction. First, increasing density (i.e. colonial breeding) increases the encounter rate with other males and at the same time often prevents males from guarding their mates. Hence, colonial species generally have high levels of within-pair copulations (Birkhead et al. 1987; Møller and Birkhead 1991, 1993), which may serve as an effective paternity guard (Møller and Birkhead 1991, Hunter et al. 1992). Second, a large potential for sperm competition may not imply that sperm competition actually occurs. For instance, Wagner (1991, 1992) found evidence for female control of EPCs in Razorbills (*Alca torda*). This was achieved by females resisting EPCs

during the fertile period, allowing EPCs when the probability of fertilizing the egg was low during the prelaying period, and possibly by expelling extrapair sperm. Thus, empirical studies on colonial seabirds suggest that EPC rates are low and that EPCs can be ineffective in fertilizing eggs (cf. Scwhartz et al. 1999).

We found the genetic father of one of the extrapair chicks to be the male at the closest neighboring nest. This male also was the genetic father of the chick in his own nest, so we know that his social status at the neighboring nest was determined correctly. Neighboring males often are the genetic fathers of extrapair offspring (Björklund and Westman 1983, Alatalo et al. 1984, Westneat 1990, Yezerinac et al. 1995, Johnsen et al. 1998).

If a surplus of females exists in the population, "pairing" with another female may be a favorable strategy for some females because it allows them to get established in the colony. The experience with a site gained through forming a female-female pair may greatly improve chances for future successful breeding for the non-genetic parent. Female-female pairs are frequently observed in gull and tern populations with female-biased sex ratios (Nisbet and Hatch 1999). Despite the short-term costs, joining another female and taking care of her young may lead to increased fitness.

Austin and Parkin (1995) found that the majority (75%) of unrelated adults caught in nest burrows of Short-tailed Shearwater were females. They attributed this finding to breeding birds entering the wrong burrow, or prebreeding or failed breeders entering burrows at random. Similarly, Swatschek et al. (1994) found that about 30% of all Cory's Shearwaters captured entering burrows at night were nonbreeders or unrelated breeders entering the wrong burrows. Approaching alien nests apparently is not uncommon in procellariiforms, especially those that enter their burrows at night. Such behavior can be a means of prospecting for future breeding sites. However, a considerable difference exists between burrow-nesting procellariiforms where prospectors might have to enter the burrow to check whether it is occupied, and open-nesting species (like the Antarctic Petrel) where prospectors can observe nest sites from a distance. In our study, we could not completely rule out that the two female-female pairs included a female that only visited the nest for a short time period because we only handled the adults when blood samples were taken. On the other hand, blood samples were taken during late incubation or early in the brood-rearing period, at which time it is relatively unlikely that alien individuals should take over. Moreover, the unrelated females provided care (incubation and/or brooding) for the chick, a type of behavior that would not be expected from prospectors.

Agreement between the results from DFA and the

molecular sexing procedure confirms that DFA provides a relatively reliable means of assessing the sex of individuals (Lorentsen and Røv 1994). The relatively low discriminant scores of incorrectly classified males, and the relatively high scores of incorrectly classified females, show that sex determination by DFA of morphological data may fail for small males and large females. Also, molecular sexing revealed two female-female pairs. Although DFA may detect such cases, a general assumption when using such tools is that pair members are of opposite sexes. Consequently, if female-female pairs are a regular phenomenon, as observed in several gull and tern species, then results from DFA must be interpreted with caution.

Our findings are in line with the statement of Westneat and Sherman (1997) that EPF rates generally are low for colonial birds. Infrequent extrapair fertilizations may be due to little or inefficient extrapair sexual activity. EPCs may be infrequent among many colonial seabirds because of difficulties in assessing the quality of potential extrapair partners. Moreover, the few cases of EPC may have limited influence on realized paternity because of efficient paternity guards, or because females selectively reject sperm from such copulations. The finding that both adults attending two nests were females suggests that prospecting females use "adoption" of eggs or chicks laid by other females as a means of establishing themselves in the colony. Further studies are needed to answer whether such adoptions are only short term, or whether they include taking on full parental duties, including the feeding of nestlings until independence.

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