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Source: Zoological Science, 21(8) : 817-821

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.21.817

ZOOLOGICAL SCIENCE **21**: 817–821 (2004)

Sexual Dimorphism of Acoustic Signals in the Oriental White Stork: Non-invasive Identification of Sex in Birds

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ABSTRACT—Identification of the sex of birds is important for captive breeding of endangered species. In the oriental white stork (*Ciconia boyciana*), an endangered species, both sexes produce an acoustic signal called "clatter" by rattling their mandibles together to generate sounds. We examined the structure of male and female clatter to determine whether clatter is sexually dimorphic. The acoustic structure of the clatter of the two sexes proved to be dimorphic with respect to the fundamental frequency; female clatter had higher fundamental frequencies. The fundamental frequency correlated significantly and positively with bill length, suggesting that bill morphology contributes to the sexual dimorphism of clatter. Sexing can be done by acoustic signals without capturing birds, and thus is useful as a non-invasive sexing method for ecological and conservation studies of birds.

Key words: endangered species, sexual dimorphism, sex identification, acoustic signal

INTRODUCTION

The identification of the sex of birds is of fundamental importance not only for studies in behavioral ecology but also in conservation biology, especially for captive breeding of endangered species. The sex of most avian species can be determined by external morphology and/or DNA-based techniques (Griffiths *et al*., 1998). The wild population of the oriental white stork (*Ciconia boyciana*) in Japan became extinct in 1971, but captive breeding efforts have been successful (Murata, 1997). The oriental white stork is known to have no readily observable sexual differences in external appearance or behavior. On the other hand, there are marked sexual differences in the external measurements of the head region, with males' bills being longer than those of females (Komiya *et al*., 1986; Murata *et al*., 1988; Murata *et al*., 1997). Measuring the bill length of captured birds is used as a reliable method for sex identification at Tama Zoological Park, where captive breeding of the oriental white stork has been successful.

The oriental white stork shows an intriguing acoustic communication behavior. It is a voiceless species, but claps its mandibles together to make a 'kata-kata-kata' sound, known as "clatter". Both sexes of the oriental white stork produce clatter in the context of courtship during the breeding season, and this behavior is also observed in other *Ciconia* species (Kahl, 1971). Clatter is also produced by both sexes in agonistic situations throughout the year. Bill morphology is known to influence the acoustic structure of vocalizations in songbirds (Oscines, Passeriformes) (Podos, 2001). It has been hypothesized that birds with larger beaks have jaws that are adapted for movements requiring strength, such as crushing hard seeds, but that are less able to perform rapid movements (Podos, 2001). Thus birds with such jaws would produce songs with comparatively low repetition rates of the syllables or component sounds of the song. Consistent with this hypothesis, it has been found that among Darwin's finches of the Galapagos Islands, birds with larger beaks and body sizes produce songs with comparatively low rates of syllable repetition (Podos, 2001). Bill morphology should strongly affect acoustic structure in the oriental white stork, which uses the bill as a sound-producing organ. In addition, the existence of sexual differences in bill morphology in the oriental white stork predicts an associated sexual dimorphism of clatter. Sexing birds by acoustic analysis does not require the capture of birds, and thus it could be used as a non-invasive method for the identification of sex in bird species in which the sexes are indistin-

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guishable on the basis of appearance (Taoka *et al.*, 1989; Mizuta *et al.*, 2003).

In this study we examined the acoustic structure of the clatter produced by male and female oriental white storks, both in courtship and non-courtship contexts, to determine whether sexual differences exist. We then conducted a correlation analysis between bill morphology and acoustic structure to gain insight into the causation of the sexual dimorphism in the acoustic signals.

MATERIALS AND METHODS

Subjects and recordings

We examined 7 male and 7 female oriental white storks. All of the birds were housed in outdoor aviaries at Tama Zoological Park (35°38'N, 139°24'E), Tokyo. As a matter of zoo policy, storks above the age of three years are placed in the adult cage, where pair formation occurs. Breeding pairs from the adult cage may subsequently be transferred to breeding cages. The birds recorded in this study were in either the adult or breeding cages. Individual birds were identified by color rings. The birds were fed with smelt and horse mackerel every day.

Clatter was recorded between January 2002 and January 2003 and subjected to sound analysis. All clatter, along with video images of the performing birds, was recorded with a Sony DCR-TRV30 video recorder. There was no systematic difference in recording distance for male and female birds. Both sexes exhibit clattering in two different behavioral contexts; a courtship context and a non-courtship, agonistic context. Our recordings included clatter made in both courtship and non-courtship contexts.

Analysis of acoustic structure

Clatter varies in duration; a single bout of clattering can last from about 1 to 10 s. When a stork stopped clattering for 1 s or more, we considered the clatter bout to have ended. A clatter bout consists of sound elements that are repeated multiple times (Fig. 1A). Each element is produced by one clap of the mandibles. All recorded sounds were digitized (16-bit and 32-kHz resolution) and

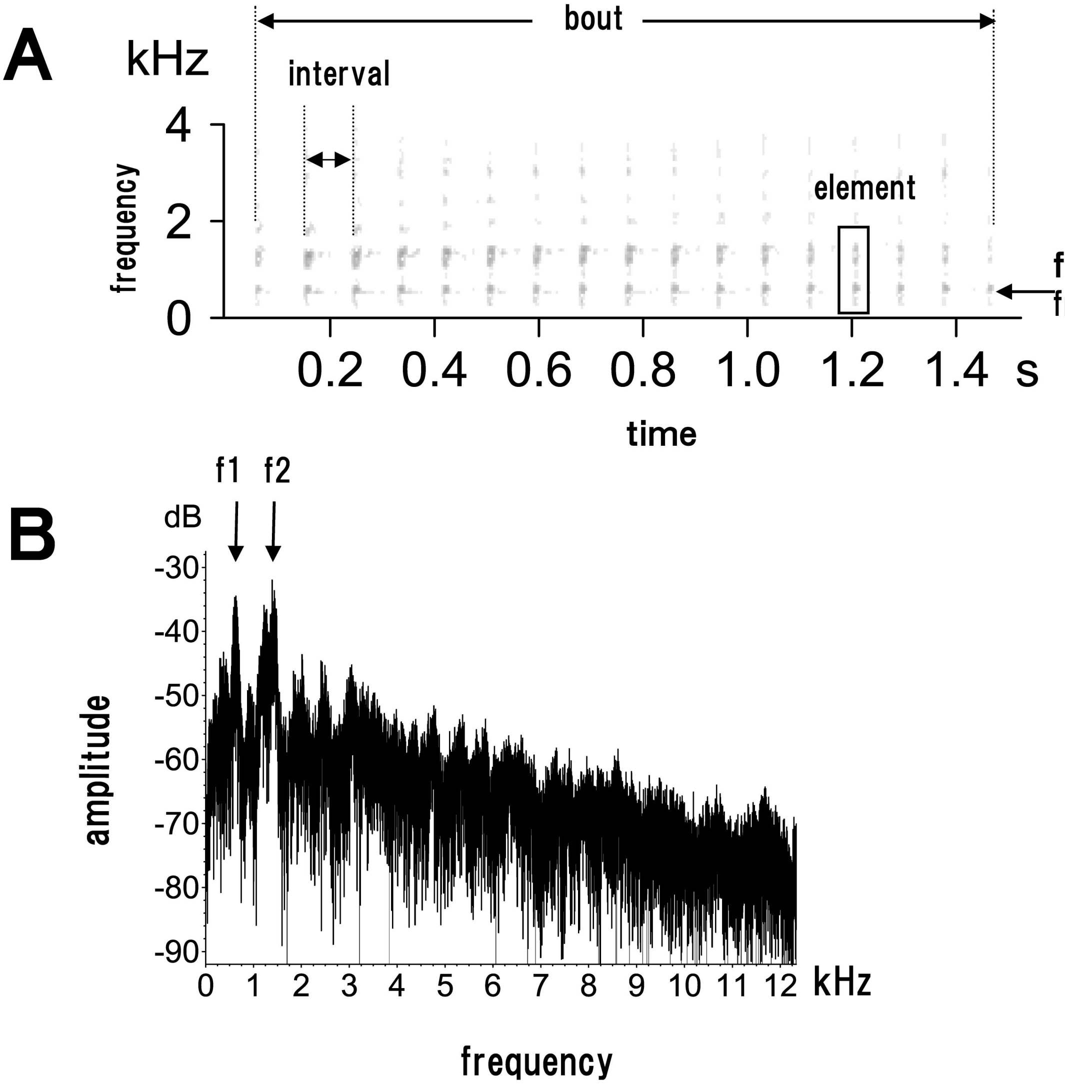

Fig. 1. (A) A sound spectrogram illustrating the structure of a bout of clatter consisting of sound elements. The temporal parameters measured were the interval between adjacent elements and the length of a bout (bout length). (B) A power spectrum generated for the first second of a clatter bout. We measured the frequency at peak amplitude, within the range below 900 Hz, to obtain the fundamental frequency of clatter (marked with f1). The frequency at peak amplitude above 900 Hz was measured for the second harmonic frequency (marked with f2).

analyzed with a computer-based analysis system (Avisoft SAS-Lab Pro. version 3.5, Raimund Specht, Germany). We analyzed four acoustic parameters of clatter: fundamental frequency, second harmonic frequency, interval, and bout length.

To obtain the fundamental frequency of clatter, power spectra (frequency resolution, 1 Hz) were generated for the first second of each bout of clatter in order to control for sampling error (Fig. 1B). The fundamental frequency of each bout of clatter was determined by measuring the frequency at peak amplitude, within the range below 900 Hz, using an on-screen cursor. There are the second harmonics in clatter bouts. The frequency at peak amplitude within the range above 900 Hz was also measured to determine the second harmonic frequency. In our pilot analysis, each of 6 clatter bouts from 6 birds (3 males and 3 females) was measured repeatedly (6 times). The multiple measurements varied by not more than one percent.

The temporal structure of clatter was analyzed using an on-screen cursor with sound spectrogram. A fast Fourier transformation (FFT) length of 128 (temporal resolution=2 ms) was used to produce the plots for temporal and frequency measures. We measured all the intervals between the onsets of adjacent elements for each bout of clatter and calculated the mean value for the clatter bout.

Bill morphology and sex identification

As a matter of zoo policy, storks over 30 weeks of age are captured and bill length (the distance between the bill tip and the point at which the bill meets the frontal bone of the skull) and head bill length (HBL: the distance between the tip of the upper mandible and the back of the head) are measured to the nearest 1 mm. The bill is fully grown by the age of 30 weeks, so bill length and HBL do not change greatly thereafter (Komiya *et al.*, 1986; Murata *et al.*, 1997). In the present study, we used these measurements of the head region for each bird. At Tama Zoological Park, the sex of oriental white storks is reliably determined by the linear discriminant function with HBL that was reported in the previous study (Murata *et al.*, 1997). The sexes of the birds used in this study were determined using the linear discriminant function of Murata *et al.* (1997) (6 males and 6 females) or by observed breeding behavior (position during copulation; one male and one female).

Statistical Analysis and sample clatter

The question addressed in the analysis was whether the measured acoustic parameters differed between the sexes. It was possible that the measured parameters could differ between behavioral contexts (i.e., courtship context and non-courtship context) as well as between the sexes. To separate the influences of sex and behavioral context on the measured parameters, we used a two-way analysis of variance (ANOVA; sex and behavioral context as factors) for fundamental frequency, second harmonic frequency, interval, and bout length, respectively.

The datasets for each individual varied from one to 9 clatter bouts, and the mean value was used for statistical analysis. Analyses identical to those reported here, but using one value randomly chosen from the datasets for each individual, yielded qualitatively similar results.

The values from 7 males and 7 females were log-transformed to satisfy the assumptions of the parametric test (Zar, 1996). The sample sizes for each group of the two-way ANOVA were 4 birds in male and female courtship groups and three in male and female non-courtship groups. The birds used in courtship groups were different from those in non-courtship groups. Data were analyzed using Stat View version 5 (SAS Institute Inc., USA). The level of significance was set at $P<0.05$.

RESULTS

There was no significant difference between the different behavioral contexts (fundamental frequency: F 1, 10=0.228, NS; second harmonic frequency: F 1, 10=0.064, NS; interval: F 1, 10=4.904, NS; bout length: F 1, 10=2.927, NS), nor was significant interaction effect of sex and behavioral context (fundamental frequency: F 1, 10=1.603, NS; second harmonic frequency: F 1, 10=0.129, NS; interval: F 1, 10=1.047, NS; bout length: F 1, 10=0.488, NS). These results indicate that the measured parameters are not influenced by behavioral context.

The fundamental frequency of clatter varied significantly between the two sexes (F 1, 10=67.103, $P<0.0001$, Fig. 2A). There was no overlap in the range of fundamental frequencies for the both sexes; individual means were less than 670

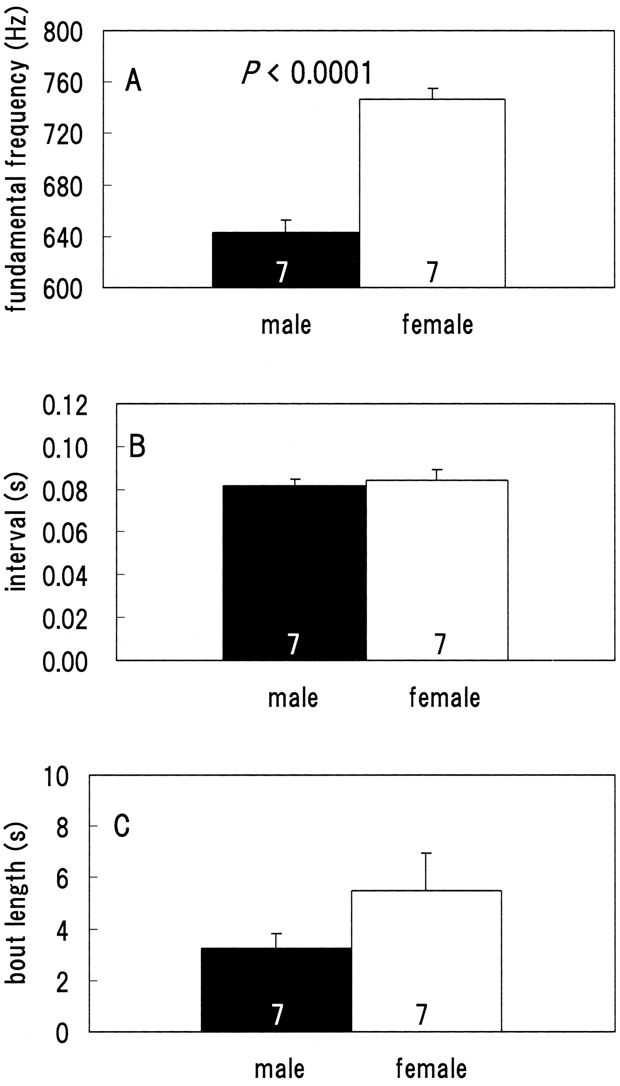

Fig. 2. Comparison between male and female clatter. There was difference between the sexes in fundamental frequency (A), but not in interval (B) or in bout length (C). Values are mean and SE. Number of animals is indicated for each group.

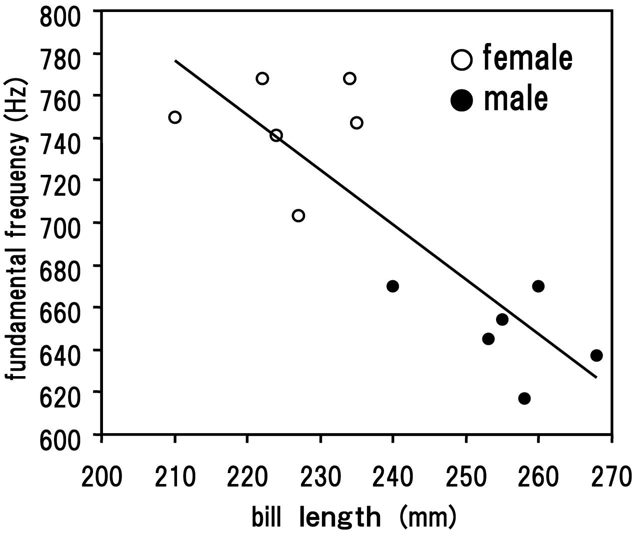

Fig. 3. The relationship between fundamental frequency and bill length of 12 oriental white storks (6 males and 6 females). The line indicates the linear regression.

Hz in males and more than 703 Hz in females. The second harmonic frequency also differed significantly between the sexes (F1, 10=6.417, p<0.03), although there was overlap in the range for the both sexes. Thus, the sex of the oriental white stork can be determined reliably by fundamental frequency. The temporal parameters measured (interval and bout length) did not differ between the sexes (interval: F 1, 10=0.202, NS; bout length: F 1, 10=1.112, NS, Fig. 2B, C).

Figure 3 shows the relationship between bill length and fundamental frequency. There was significant negative correlation between bill length and fundamental frequency (Spearman rank correlation: $r_{s=}$–0.825, P<0.01). This observation suggests that bill length affects the fundamental frequency of clatter.

DISCUSSION

The results of the acoustic analysis in this study show that the clattering sounds of oriental white storks are sexually dimorphic in the frequency parameters, especially in fundamental frequency. Thus, it is possible to sex the oriental white stork non-invasively based on the acoustic structure of the clatter sound. Captive breeding efforts have been made and the reintroduction to the wild of captive-bred storks is planned at Toyooka, where the last wild oriental white stork in Japan was caught and placed in captivity (Murata, 1997). In the near future, determination of the sex of storks in the wild will be needed in Japan. Thus, it is worthwhile to compare the sound sexing method with the methods reported previously in a point of sexing storks in the wild. The capture of birds is required to sex them by external measurements or DNA analysis of blood samples (Itoh *et al.*, 2001). Each individual can be marked on the capture and traced thereafter, which is necessary for ecological and conservation studies of birds. However, in the case of large birds like the oriental white stork, catching and manipulating them requires several handlers and is dangerous for both the birds and the humans. On the other hand, the sound sexing method avoids the capture of birds and should prove quite useful, especially in large birds. It is not difficult to capture and mark storks early in life. It would be more useful to mark them when young for tracing each individual without the difficulty of the capture.

The vocalizations of birds can be generally separated into calls and songs. Sexual dimorphism in the acoustic structure of bird vocalizations has been found extensively in calls, which are regularly used by males and females (reviewed by Ballintijn and ten Cate, 1997). On the other hand, singing is a behavior that is restricted mostly to males in many species found in temperate regions. Recently, however, interest in the songs of females has been growing, because in the tropics females sing regularly. Even in temperate regions, singing by females is probably more common than has often been assumed (Langmore, 1998; Slater, 2003). With growing interest in female song, evidence of sexual differences in acoustic structure has begun to accumulate even in song (reviewed by Brenowitz and Kroodsma, 1996; Yamaguchi, 1998). As such acoustic sexual dimorphism becomes well-documented, it will become a method of determining sex in both conservation research and basic research, such as behavioral biology.

What kind of physiological and/or morphological differences might give rise to the observed acoustic dimorphism in the oriental white stork? Sex differences in acoustic signals may theoretically be explained by differences in the peripheral organs and/or the central nervous system. We found that there was significant negative correlation between bill length and fundamental frequency. In addition to bill length, there are sexual differences in other bill measurement, bill depth and bill width of the oriental white stork (Komiya *et al.*, 1986; Murata *et al.*, 1988) Thus, the difference in bill morphology between the two sexes can account for the sexual dimorphism in fundamental frequency. Bill morphology affects the repetition rates of component sounds of the song in Darwin's finches (Podos, 2001, see Introduction). Before completing the analysis of our recordings of the oriental white stork, we anticipated that the sex difference in bill length would lead to a difference in the repetition rates of sound elements. Contrary to our expectations, we did not find any sexual difference in the interval, which determines the repetition rates of elements. Thus, bill morphology may not affect rapid movement of the jaws in this species.

In birds, vocal activity is associated with testosterone levels (Wada, 1982). Administration of testosterone in Barbary doves increased both the number and the length of call bouts (Groothuis *et al.*, 1993). Consistent with this, males produce longer call bouts than females, which are considered to have lower levels of testosterone (Ballintijn and ten Cate, 1997). Thus, in doves, differences in the circulating levels of testosterone between the two sexes may account for the sex difference in bout length. In oriental white storks, no sexual difference was found in the bout length of clatter. Wingfield and Farner (1993) reviewed comparisons of plasma levels of androgens in males and females during the breeding season among many bird species. The ratio of plasma androgen in the male to that in the female varies widely among species, ranging from 1 to 20. It was suggested that such species differences in male-female androgen ratios might be related to differences in the degree of sexual dimorphism in reproductive behavior. In line with this argument, we predict a low male-female ratio for testosterone levels in the oriental white stork, reflecting the lack of difference in the bout lengths of clatter.

The origins and evolution of communication have been an important theme in ethology. In the process called ritualization, a behavior or movement that is functional in one context acquires a secondary value as a signal that is used in communication. The drumming of woodpeckers against hollow trees clearly shows the ritualization process for a percussive signal (McFarland, 1981). The foraging and nest-building movements of woodpeckers were probably precursors of the drumming that functions as a territorial signal. The origins of clattering in the oriental white stork remain unknown. In captivity and in the wild, close observation of the oriental white stork may provide clues about the bill movements that served as precursors for this ritualized behavior.

REFERENCES

Ballintijn MR, ten Cate C (1997) Sex differences in the vocalizations and syrinx of the collared dove (*Streptopelia decaocto*). Auk 114: 22–39

Brenowitz EA, Kroodsma DE (1996) The neuroethology of birdsong. In "Ecology and evolution of acoustic communication in birds" Ed by DE Kroodsma, EH Miller, Cornell University Press, New York, pp 285–304

Griffiths R, Double MC, Orr K, Dawson RJG (1998) A DNA test to sex most birds. Mol Ecol 7: 1071–1075

Groothuis T, Morimando F, Hutchison RE (1993) Testosterone and the development of postural and vocal displays in the Barbary Dove. In "Proceedings of the international conference on hormones, brain and behaviour" Ed by J Signoret, F Fabre, J Balthazart, Instaprint SA, Tours, pp 81–82

Itoh Y, Suzuki M, Ogawa A, Munechika I, Murata K, Mizuno S (2001) Identification of the sex of a wide range of carinatae birds by PCR using primer sets selected from chicken EE0.6 and its related sequences. J Hered 92: 315–321

Kahl MP (1971) Social behavior and taxonomic relationships of the storks. Living Bird 10: 151–170

Komiya T, Sugita H, Matsushima K (1986) Sex determination in the eastern white stork, *Ciconia ciconia boyciana*, by morphological measurement. J Jpn Assoc Zool Aqua 28: 55–60 (in Japanese with English summary)

Langmore NE (1998) Functions of duet and solo songs of female birds. TREE 13: 136–140

McFarland D (Ed) (1981) The Oxford companion to animal behaviour. Oxford University Press, Oxford

Mizuta T, Yamada H, Lin R-S, Yodogawa Y, Okanoya K (2003) Sexing White-rumped Munias in Taiwan, using morphology, DNA and distance calls. Ornithol Sci 2: 97–102

Murata K (1997) Reintroduction plan for the Oriental white stork (*Ciconia boyciana*) in Japan and its veterinarian's role. Jpn J Zoo Wildl Med 2: 117–122

Murata K, Miyashita M, Nagase K, Komiya T, Matsushima K (1988) Sex determination in the Eastern white stork, *Ciconia c. boyciana*, by bill measurements and discriminant analysis. J Jpn Assoc Zool Aqua 30: 43–47 (in Japanese with English summary)

Murata K, Matsushima K, Sato M, Hosoda T, Nakayama R (1997) Sex determination of captive immature Oriental white storks, *Ciconia boyciana*, by head and bill measurements. J Jpn Assoc Zool Aqua 38: 120–125 (in Japanese with English summary)

Podos J (2001) Correlated evolution of morphology and vocal signal structure in Darwin's finches. Nature 409: 185–188

Slater PJB (2003) Fifty years of bird song research: a case study in animal behaviour. Anim Behav 65: 633–639

Taoka M, Sato T, Kamada T, Okumura H (1989) Sexual dimorphism of chatter-calls and vocal sex recognition in Leach's strom-petrels (*Oceanodroma leucorhoa*). Auk 106: 498–501

Wada M (1982) Effects of sex steroids on calling, locomotor activity, and sexual behavior in castrated male Japanese quail. Horm Behav 16: 147–157

Wingfield JC, Farner DS (1993) Endocrinology of reproduction in wild species. In "Avian biology, vol. 9" Ed by DS Farner, JR King , KC Parkes, Academic Press, New York, pp 163–327

Yamaguchi A (1998) A sexually dimorphic learned birdsong in the northern cardinal. Condor 100: 504–511

Zar JH (1996) Biostatistical Analysis. 3rd ed, Prentice Hall, London

(Received February 18, 2004 / Accepted June 3, 2004)