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SEX DETERMINATION OF THE WHOOPING CRANE BY ANALYSIS OF VOCALIZATIONS[1]

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Much work has been done with Whooping Cranes (*Grus americana*), officially listed as an endangered species under the Endangered Species Act of 1973. However, very few studies have been concerned with Whooping Crane vocalizations.

This study was initiated as a result of observations by Dr. Rod Drewien of the University of Idaho. In his work with the experimental, cross-fostered population of Whooping Cranes that migrates between Grays Lake, Idaho and Bosque del Apache National Wildlife Refuge, New Mexico, Drewien noticed that the pitch of the *Guard Call* may differ between sexes in the Whooping Cranes (R. Drewien, pers. comm.). No reliable sexual dimorphism exists in Whooping Cranes, and at the time of Drewien's observations the preferred method of determining the sex of Whooping Cranes was by karyotyping of mitotic cells from developing feathers. Because birds must be captured, this method is impractical. Potentially, call analysis could provide an easier method of determining the sex of Whooping Cranes (R. Drewien, pers. comm.). In addition, significant differences between sexes exist in pitch of the *Guard Call* in the closely related Greater Sandhill Crane (*Grus canadensis*) (Weekley 1985). Differences in calls between sexes have also been found in Manx Shearwaters (*Puffinus puffinus*) (Brooke 1978).

Archibald (1977) had shown sex-related frequency differences in the *Unison Call* of Whooping Cranes, and pairs also exhibited sex-specific postures during this call (Archibald 1977, pers. observ.). *Unison Calls,* however, are performed only by pair-bonded, adult cranes and, in addition, are rarely observed in wild Whooping Cranes (T. Stehn, pers. comm.; R. Drewien, pers. comm., pers. observ.). *Unison Calls,* therefore, do not provide a reliable means of sex determination for adults, and no means of sex determination for subadults.

Alternatively, *Guard Calls* are produced by all nonjuvenile Whooping Cranes, whether paired or not, are given by sub-adults as young as one year of age (pers. observ.), are heard very frequently, and are easily induced without great distress to the cranes (T. Stehn, pers. comm.; R. Drewien, pers. comm., pers. observ.). In addition, *Guard Calls* are loud and, unlike lower amplitude vocalizations given by Whooping Cranes, can be recorded from a distance.

We quantified features of *Guard Calls* of Whooping Cranes from all populations, both captive and wild, and compared these features between sexes to determine if analysis of vocalizations could provide an alternative method of sex determination in Whooping Cranes.

METHODS

Recordings of Whooping Crane Guard Calls were made from May 1990 through May 1991. Recordings of members of the captive flock of Whooping Cranes at the Patuxent Wildlife Research Center (Patuxent) in Laurel, Maryland were made from 7-14 May 1990 and again from 17-24 November 1990. Recordings from the same birds made during these two periods were compared to check for seasonal variation in calls which may result from varying hormone levels (G. Gee, pers. comm.; D. Ellis, pers. comm.). Additional recordings of captive Whooping Crane calls were obtained from a second captive flock at the International Crane Foundation (ICF) in Baraboo, Wisconsin from 9-17 December 1990. Calls from wild Whooping Cranes were obtained from 2-16 January 1991 at the Aransas National Wildlife Refuge (Aransas), Texas. Calls of Whooping Cranes from the Grays Lake, Idaho experimental population were recorded at various times between April 1990 and May 1991.

Whooping Crane calls were recorded using a Marantz model PMD-221 monaural portable cassette recorder with a Marantz RB430 rechargable nickel-cadmium battery pack. The microphone was a Shure 579SB-LC dynamic omnidirectional microphone with a Roché 75 cm fiberglass parabolic reflector. The signal passed through a Shure model A85F line-matching transformer prior to entering the cassette deck. Calls were recorded onto Denon HD8 high bias, metal particle audio cassette tape. A reference tone was recorded onto the tape at the beginning of and during each recording session to allow for proper tape speed calibration during playback for analysis.

An experiment was also conducted at Patuxent to test effects of varying stimulus intensity on Whooping Crane *Guard Calls,* since the potential existed for *Guard Call* features to be graded signals (e.g., Barklow 1979). On three successive days of similar weather conditions at approximately 10:30 hours, two Whooping Cranes were presented a stimulus of a purple flag. The flag was

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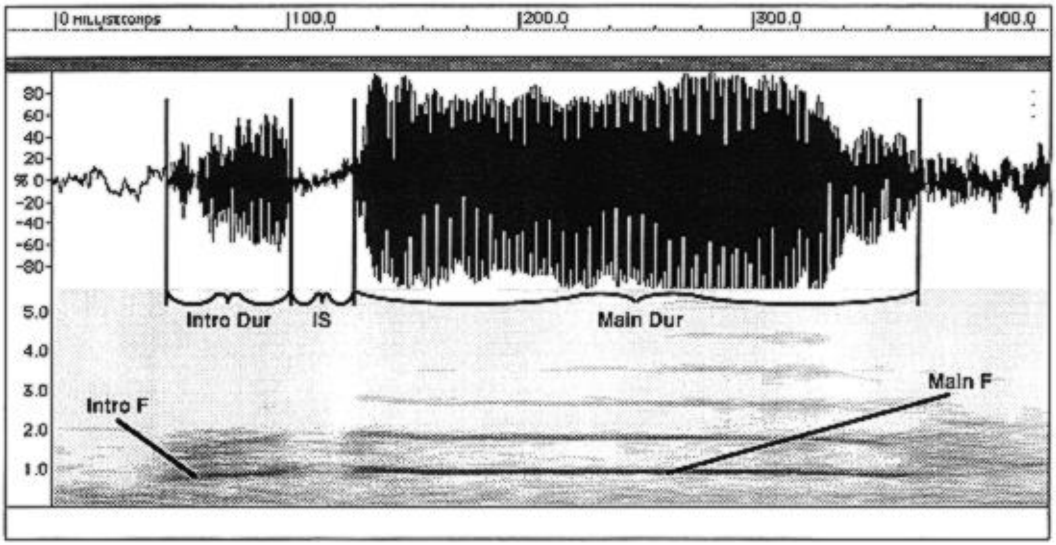

FIGURE 1. Waveform (top) and spectral display (bottom) of Whooping Crane *Guard Call* from SoundEdit Pro showing features measured for analysis. Intro F = Introductory Syllable Frequency, Intro Dur = Introductory Syllable Duration, IS = Inter-Syllable Distance, Main F = Main Call Body Fundamental Frequency, Main Dur = Main Call Body Duration. The vertical axis of the waveform represents relative amplitude, while the vertical axis of the spectral display represents frequency in kilohertz. The horizontal axis in both displays represents time in milliseconds.

shaken vertically adjacent to the crane's pen. The flag pole was 6 m tall on the first day, 4 m tall on the second day, and 2 m tall on the third day. Calls obtained normally, without the pole stimulus, were used as a fourth, lowest intensity level. The sequence of pole lengths which was used was chosen to potentially amplify any graded responses by the cranes. By subjecting the cranes to the strongest stimulus first, and weaker stimuli on successive days, any habituation to the stimulus by the cranes should make any graded response appear more noticeable. Measurements obtained from calls in this experiment were tested for relationships with stimulus intensity by multiple regression. No significant effects were found in call features as a result of varying stimulus intensity.

During recording, spoken identifications of the birds giving calls were recorded onto the tape along with the calls. Captive cranes were identified by pen number, wild cranes were identified by leg bands or territory name. While sub-adult Whooping Cranes range over relatively large distances at Aransas (Bishop 1984, pers. observ.), pairs of adults and family groups defend exclusive winter territories by which they can be identified (Stehn and Johnson 1986; T. Stehn, pers. comm.).

Actually identifying a specific call as coming from a specific crane often proved difficult, even with captive birds. However, in the case of captive birds the pen from which a call came was readily determined. With wild cranes, only one pair of birds was usually within recording distance and so the identity of the pair was readily determined for these birds as well. On the 103 occasions in which calls were seen to come from a specific bird of a pair, either penned or wild, 101, or 98.1%, of the calls coming from the male of the pair was lower in pitch. These calls came from Whooping Cranes at Patuxent, ICF, and Aransas, and showed that these sex-related pitch differences were consistent across locations. The two occasions when males were classified as having a higher pitched voice occurred at Aransas with wild cranes at a distance of well over 100 m, and the classification was less than certain. In addition, calls from unisexual groups at Patuxent (two female only pens and one male only pen) were significantly different (unpaired t-test, two-tailed $P = 0.0001$) in the fundamental frequency of the main portion of the call, with females being higher-pitched than males. Therefore, for all other calls in which the male and female of a pair called in very close temporal proximity, the lower-pitched call was assumed to have been given by the male of the pair and the higher-pitched call was assumed to have come from the female. We made this assumption with confidence. If a bird from a pair called by itself and the call was not visually determined to have come from a specific bird, the call was not used for analysis.

Calls were analyzed using Apple Macintosh IIfx and Apple Macintosh SE/30 computers in conjunction with SoundEdit version 2.0.2, a beta version of SoundEdit Pro, and the MacRecorder sound digitizer, all from Farallon Computing, Inc., Emeryville, California. We used SoundEdit 2.0.2 and SoundEdit Pro in combination for analysis. Sounds were sampled at a rate of 5 kHz, since this sampling rate provided the maximum possible frequency resolution of 5 Hz within the range of frequencies in which measurements were being made, and provided a maximum time resolution of 0.2 msec,

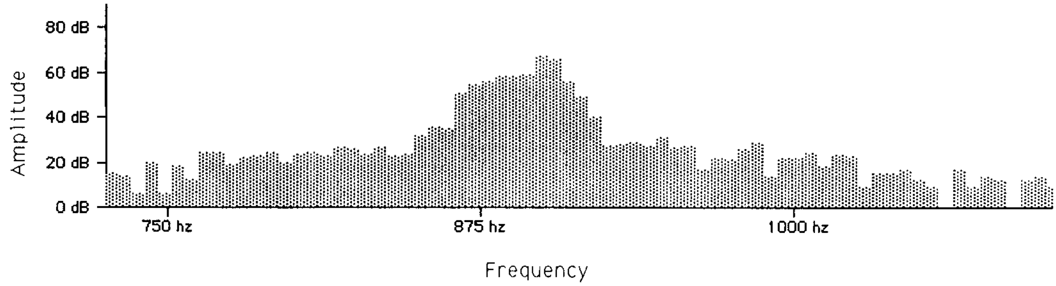

FIGURE 2. Spectrogram, or power spectrum, display from SoundEdit 2.0.2. Shown here is a measurement for Main Call Body Fundamental Frequency of a male Whooping Crane, which in this example is 895 Hz.

although the time resolution used was 0.4 msec. Sonogram display was computed using the Hamming algorithm.

We considered a recorded call to be usable for analysis if it did not overlap a call from another crane and if the call was subjectively determined to be sufficient amplitude for all call features to be visible on waveform and spectral displays of SoundEdit Pro.

Guard Call features that were measured are shown in Figure 1. Following is a description of each feature used for analysis.

(1) *Introductory Syllable.* The pulse immediately before the main portion of the call which was clearly separated from the main portion of the call.

(A) *Introductory Syllable Frequency (Intro F).* The lowest dominant frequency above 500 Hz which was not less than third highest in amplitude or lower than 20 db in amplitude.

(B) *Introductory Syllable Duration (Intro Dur).* The length, in milliseconds (msec), of the Introductory Syllable.

(2) *Inter-Syllable Distance (IS).* The time, in msec, between the Introductory Syllable and the Main Body of the call.

(3) *Main Body.* The call portion following the Introductory Syllable.

(A) *Main Body Fundamental Frequency (Main F).* The peak frequency of the first harmonic band of the main portion of the call (Fig. 2).

(B) *Main Body Duration (Main Dur).* The time, in msec, from the beginning of the Main Body of the call to the end of the call.

In all cases, variation was tested by one-factor or two-factor analysis of variance (ANOVA) using Statview SE+ Graphics by Abacus Concepts, Inc. on an Apple Macintosh IIfx or Apple Macintosh SE/30 computer. Significant differences by multiple comparison were determined using Fischer PLSD at a significance level of $\alpha = 0.05$. This method of multiple comparison was used because the other two multiple comparison methods provided by StatView SE+ Graphics, Scheffe and Dunnett-t, were inappropriate for the hypotheses being tested in this study (Zar 1984).

For the determination of sex, these same features were analyzed by discriminant analysis using CSS by StatSoft, Inc., on a Hewlett Packard Vectra RS-120 computer. Discriminant analysis for sex identification was done using the backward stepwise method, as this provided both the best discrimination and the fewest number of discriminant functions.

RESULTS

Guard Calls of Whooping Cranes from the Grays Lake population possessed characteristics which were, in general, so different from those of other Whooping Crane populations that the Grays Lake birds were excluded from analysis (see Carlson 1991 for a more thorough analysis of *Guard Calls* from the Grays Lake Whooping Crane population).

Whooping Cranes from Patuxent were tested for variation between May and November. A two-factor ANOVA showed that significant differences existed for some features of both male and female *Guard Calls* between May and November (see Table 1). For all of the possible combinations of sex and month, most features exhibited significant differences between sexes. Comparisons between males and females which showed no difference are for features, IS and Main Dur, which were removed from the discriminant analysis for sex determination.

TABLE 1. Results from 1-factor ANOVA for comparisons of measured *Guard Call* features of Patuxent birds between May and November. Sig = significant by Fischer PLSD multiple comparison at 95% significance level, NS = not significant.

Comparison	Intro F	Intro Dur	IS	Main F	Main Dur
Male May vs. Male November	NS	NS	NS	Sig	Sig
Mal May vs. Female May	Sig	Sig	Sig	Sig	NS
Male May vs. Female November	Sig	Sig	Sig	Sig	NS
Male November vs. Female May	Sig	Sig	Sig	Sig	Sig
Male November vs. Female November	Sig	Sig	NS	Sig	Sig
Female May vs. Female November	Sig	NS	Sig	NS	NS

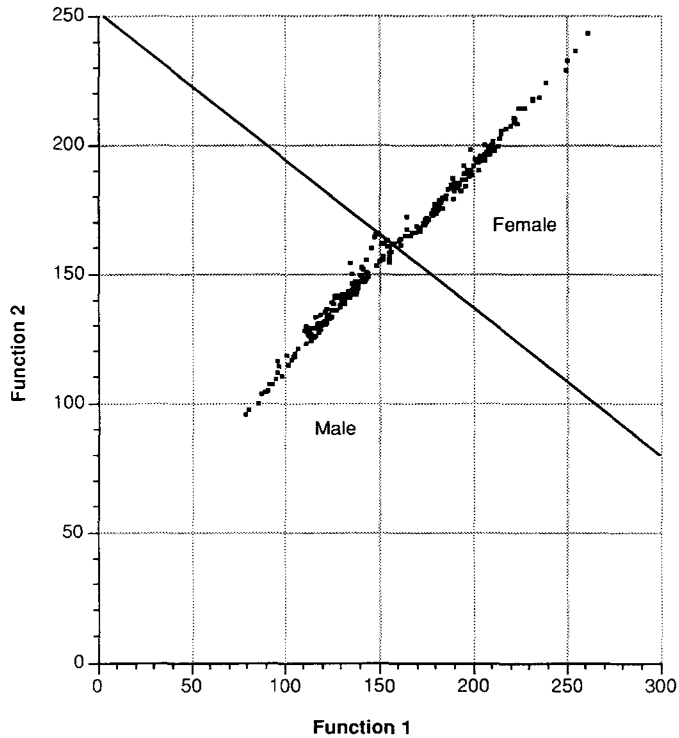

FIGURE 3. Discriminant territories for sex determination showing all individual cases ($n = 259$).

None of the correlation coefficients (R) between call features were large enough to warrant removal of any variables from discriminant analysis ($R = -0.621$ to 0.636). Discriminant analysis provided an overall success rate of 98.8% ($n = 259$) in classifying Whooping Cranes by sex. One-hundred percent of females ($n = 118$) and 97.8% of males ($n = 141$) were classified correctly. The best discrimination was obtained by removing IS and Main Dur from the analysis using the backward stepwise method. Discrimination was then achieved by the following functions:

$$\text{Function 1} = -200.268 + 0.165(\text{Intro F}) + 0.248(\text{Intro Dur}) + 0.217(\text{Main F})$$

$$\text{Function 2} = -139.295 + 0.130(\text{Intro F}) + 0.482(\text{Intro Dur}) + 0.178(\text{Main F})$$

A plot of all the individual cases and the discriminant territories for each sex is illustrated in Figure 3. Cutting scores for each function were determined by the following equation from Hair et al. (1987):

$$F_{CU} = (N_A F_{MA} + N_B F_{MB})/(N_A + N_B)$$

where F_{M*} equals the centroid for each group and N_* equals the number of cases in each group. For Function 1, then, the cutting score is 160.960 and for Function 2 the cutting score is 161.404. In both cases males are below the cutting score and females are above.

Very similar results were obtained by using descriptive statistics. For the main syllable frequency, the mean for all male calls combined was 946 Hz with 99% upper confidence limit at 961 Hz. For all female calls combined the mean was 1115 Hz with 99% lower confidence limit at 1101 Hz. This separation potentially should allow for discrimination of sex by ear.

DISCUSSION

The seasonal variation observed in calls of birds from Patuxent did not hinder sex identification, as the only non-significant differences between any combination of sex and month were in features which were removed from discriminant analysis for sex determination. Sex determination by discriminant analysis proved highly successful. The 98.8% successful classification rate is comparable to that for karyotype analysis. Peter Van Tuinen, a cytogeneticist who performs Whooping Crane sexing by karyotype for ICF, stated that (pers. comm.) while 100% accuracy is achieved for readable karyotypes, approximately 98% of birds sampled produce these readable karyotypes. Also, some errors occur due to samples becoming confused during collection, shipment, processing, and record transcription. These errors are possibly evidenced by two cranes which were removed from analysis because of ambiguities in behavior and vocalizations. We recommend that sexing procedures for Whooping Cranes include vocal analysis, together with or replacing karyotyping.

In addition to the success rate of discriminant analysis, the 99% confidence limits for male and female main syllable frequency are clearly separated. In the field, a person with good frequency discriminating ability should be able to determine the sex of Whooping Cranes by ear. Even given the differences previously described for Grays Lake Whooping Cranes, the sex of these birds can probably be determined by this method as well. The fundamental or dominant (see Gaunt 1983) frequency in the main portion of *Guard Calls* from the Grays Lake cranes seemed to fit into the two frequency groups described for males and females from other locations.

Sex determination by ear could be aided by behavioral and morphological differences in paired Whooping Cranes that would make sex determination easier. Males of a pair were observed to spend much more time than females in the alert posture and usually positioned themselves closer to an observer than females (pers. observ.; T. Stehn, pers. comm.). Also, wild cranes almost exclusively sort themselves by size when pairing, with males of a pair being larger than the female (G. Archibald, pers. comm.; S. Swengel, pers. comm.). Our observations at Aransas National Wildlife Refuge supported this hypothesis (pers. observ.). This size dimorphism can be very subtle, however, and difficult to visually determine from a distance. Also, sub-adults or unpaired adults of the same sex but different body size may occur together. Therefore, size dimorphism should be used as a secondary check of the sexes of birds in a pair and not a primary determining factor.

The site fidelity exhibited by adult Whooping Cranes should also provide a means of identifying unbanded wild birds. The sexes of cranes returning to specific sites can be determined by vocal analysis, with minimal disturbance, and no injury to members of this endangered species.

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