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Examination of pair-duet stability to promote long-term monitoring of the endangered red-crowned crane (*Grus japonensis*)

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Abstract Acoustic-based monitoring has proved useful for many birds and seems promising for the endangered red-crowned crane. However, its validity in crane conservation is unclear in the absence of knowledge concerning the long-term stability of pair-specific duets. The redcrowned crane is monogamous and long-lived, with stable pair bonding both within and between years. Pair mates perform loud duets-a succession of male and female calls emitted with definite temporal coordination. We examined the stability of duets for five captive pairs over five years (2003–2007) on the basis of analysis of the syllables within the duets. MANOVA showed that the effect of pair identity on syllable characteristics was always stronger than the effect of the year of recording. Cross-validation of duets from 2004, 2005, 2006, and 2007 with discriminant analysis (DFA) functions derived, respectively, from pooled samples from 2003, 2003-2004, 2003-2005, and 2003-2006 resulted in comparably high percentages of correct classification into pairs. The pairs could be reliably identified by their duets and pair-specific differences in syllable characteristics were stable with time. These data suggest acoustic monitoring is a feasible alternative to more invasive methods of identification.

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I. A. Volodin · E. V. Volodina Scientific Research Department, Moscow Zoo, B. Gruzinskaya, 1, 123242 Moscow, Russia **Keywords** Vocal behaviour · Unison call · Call-based monitoring · Pair identity · Conservation · Voice printing · *Grus japonensis*

Introduction

Censuses and monitoring in nature are fundamental aspects of conservation. Acoustic monitoring has proved its use for examination of territory turnover, dispersal, and survival in many bird species (review Terry et al. 2005). It is applied to long-lived territorial birds, for example owls (e.g. Galeotti and Sacchi 2001; Lengagne 2001; Delport et al. 2002; Tripp and Otter 2006), the great bittern (Gilbert et al. 2002; Puglisi and Adamo 2004), the common loon (Walcott et al. 2006), and the red-breasted goose (Volodin et al. 2008).

Acoustic monitoring is preferable to leg, neck, and radio tagging as a noninvasive tool, excluding potentially traumatic capture and handling. Neck collars and other tags reduce survival (Menu et al. 2000; Schmutz and Morse 2000) and are inappropriate for rare birds. For birds living in dense grass (e.g. corncrakes and bitterns), and for nocturnal species, for example owls, acoustic monitoring is often the only appropriate technique. The applicability of this method depends mainly on the presence of individually distinctive vocalizations and their stability with time (Lengagne 2001; Terry et al. 2005; Volodin et al. 2008).

Acoustic monitoring is promising for the red-crowned crane (*Grus japonensis*). About 2,000 red-crowned cranes remain in the wild (Archibald 2000), and this species has endangered status in the IUCN Red List (IUCN 2004). The red-crowned crane is monogamous, long-lived, with stable pair bonding for years (Archibald and Lewis 1996; Masatomi 2000). Pair mates together guard a large breeding territory and share parental care (Viniter 1981; Kitagawa

1982; Archibald and Lewis 1996). Usually, mated pairs occupy the same nesting territories from year to year (Masatomi 2000). Duets, representing a succession of pair mate calls, emitted with temporal coordination, occur in all 15 crane species (Archibald 1976; Archibald and Lewis 1996). Mating pairs perform duets throughout almost the whole year (Archibald 1976; Viniter 1981; Kamata 1994). Red-crowned crane duets are loud and can be recorded with acceptable quality from a distance of 300–800 m (Klenova et al. 2008).

Pair-distinctive duets have been reported for redcrowned cranes (Klenova et al. 2008), for grey crowned cranes *Balearica regulorum gibbericeps* (Budde 2001), and for common cranes *Grus grus* (Wessling 2000). The pair identity encoded in the structure of the duet provides a first prerequisite for acoustic monitoring of cranes. The validity of the vocalization-based methods in crane conservation remains unclear, however, in the absence of a second prerequisite—knowledge of the long-term stability of pair duets. In this study we examined the stability of pair-specific duet features in captive pairs of the red-crowned crane for five years.

Materials and methods

Subjects and study site

Our subjects were five breeding red-crowned crane pairs kept in Crane Breeding Centre of Oka Biosphere State Nature Reserve (Ryazan region, Russia). The pairs were kept in separate enclosures of 100 m^2 per pair, in auditory contact with conspecifics or other cranes. Six of the ten birds were wild-captured, and four birds were raised in captivity. At the beginning of this study, the ages of the crane ranged from ten to nineteen years.

Acoustic recordings

We recorded 349 duets from the five pairs (49–85 duets per pair, mean \pm SD = 70 \pm 13.1, 4–20 duets per pair per year, mean \pm SD = 14 \pm 5.9) in May–August, 2003– 2007. Most of the duets were produced spontaneously during everyday activity or zoo procedures and enclosure cleaning, but in 2006 and in 2007—also in response to playbacks of conspecific duets. The playbacks included one to three duets. We broadcasted no more two playbacks to a focal pair per day, separated by minimum of 30 min. The pair mates performed a duet close to each other; the distance from birds to the microphone was 8–40 m. As pair mates were distinctive by body constitution, plumage colouration, beak shape, etc., we could distinguish between male and female during a duet. We recorded duets with a Marantz PMD-222 cassette recorder (D&M Professional, Kanagawa, Japan) and a Sennheiser K6-ME67 "shortgun" condenser microphone (Sennheiser Electronic, Wedemark, Germany). Playbacks were broadcast with a Marantz PMD-222 and Creative Travel Sound 400 dynamic (Creative Labs, Dublin, Eire).

Acoustic analysis

Both digitizing (22.05 kHz sampling rate, 16 bit precision) and call measurements were made with Avisoft-SASLab Pro v. 4.3 (Avisoft Bioacoustics, Berlin, Germany). The digitized calls were low-pass filtered at 6 kHz and low-sampled to 11.025 kHz. Spectrograms were created with a Hamming window, FFT-length 512 points, frame 50%, and overlap 96.87%, providing time resolution 1.5 ms and frequency resolution 21 Hz.

The red-crowned crane duet begins with an introduction (an unordered sequence of the male and female calls) followed by the main part, containing a few or many regularly repeated syllables (Archibald 1976; Klenova et al. 2008) (Fig. 1a). A syllable may contain one or two male calls and one to four female calls. Two-syllable types (with one male and two female calls or with one male and three female calls) are most common (over 70% of syllables), and at least one of these two types may be heard for any pair (Klenova et al. 2008). We extracted the syllables of these two types to analyse the stability of interpair differences in the duets. If fewer than eight syllables of these two types were available from the given duet, we measured all of

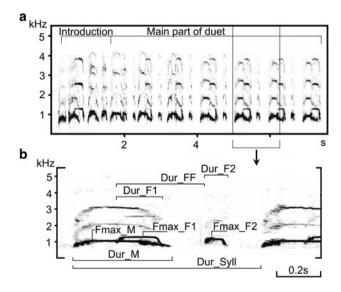


Fig. 1 a Spectrogram of a red-crowned crane pair whole duet. The introduction, main part, and one syllable are shown. b Enlarged section of the duet between the *vertical lines* from the *upper* figure showing the measured characteristics of the duet syllable

them (minimum of two syllables). If more than eight syllables were available, we measured the first eight in order, counting from the beginning of a duet.

For each syllable, we measured eight characteristics (Fig. 1b): the Dur Syll—syllable duration, i.e. the duration from the beginning of a male call of a syllable to the beginning of the male call of the next syllable, Dur Mmale call duration, Dur F1-first female call duration, Dur_F2-second female call duration, Dur_FF-duration from the beginning of a first female call to the beginning of the second female call, Fmax M-male call maximum fundamental frequency, Fmax_F1-first female call maximum fundamental frequency, Fmax_F2-second female call maximum fundamental frequency. All measurements were made from the screen with the reticule cursor and exported automatically to Microsoft Excel (Microsoft, Redmond, WA, USA). We then calculated the mean values of the syllable characteristics within duets. Thus, each duet was represented by eight mean values of temporal and frequency syllable characteristics.

Statistical analyses

Because the distribution of values for all measured acoustic characteristics did not differ from normality for duet samples for each pair (P > 0.05, Kolmogorov–Smirnov test), we could apply parametric tests. We used two-way MANOVA to compare the variability of the values of the eight syllable characteristics between pairs and between years. This analysis compared the effect of factors "pair identity" and "year of recording" on each syllable characteristics contributed most to pair vocal identity.

To classify duets between pairs within years, we used discriminant analysis (DFA) as standard procedure, with eight included characteristics, to determine whether duets could be assigned to the correct pair. We estimated the degree to which each duet characteristic contributed to classification on the basis of DFA Wilks' Lambda values.

To identify pairs in the future years, we used a cumulative approach to prepare samples for the cross-validation (Volodin et al. 2008). We used DFA functions calculated for the pooled samples of calls from a few previous years to discriminate the calls of the following year. For example, to discriminate duets of 2005, we used the DFA function, calculated for duets of 2003–2004. This approach imitated in applied analysis in nature, with the number of calls recorded from individuals or pairs identified by voice growing from year to year (Volodin et al. 2008). This approach enabled better estimation of the variability within the training duet samples recorded for each pair. To compare the mean values of syllable characteristics in the duets of each pair between years we used the Student *t*-test. All statistical analyses were performed with Statistica, v. 6.0 (StatSoft, Tulsa, OK, USA). All the tests were two-tailed and factor influence was regarded as significant when P < 0.05.

Results

With two-way MANOVA, we tested the influence of factors "pair identity" and "year of recording" on the values of the syllable characteristics (Table 1). MANOVA showed significant differences between pairs for all the eight syllable characteristics, but between years only for six of the eight characteristics. Comparison of *F*-ratios showed that the effect of pair identity on syllable characteristics was always stronger than the effect of the year of recording. The first five characteristics with between-pair variability exceeding those within pair were Fmax_F1, Dur_M, Dur_Syll, Dur_F1, and Fmax_M in order of decreasing importance.

Using the cross-validation procedure of DFA we tested the stability of the values of the syllable characteristics between years. First, we calculated DFA functions for five pooled samples, containing all duets recorded in 2003, in 2003–2004, in 2003–2005, in 2003–2006, and in 2003– 2007. The DFA standard procedure, applied separately to each sample of duets with all eight syllable characteristics included in the analysis, resulted in high percentages of correct classification to pair, 96.8–100% between analyses (Table 2). Characteristics that contributed most to discrimination were Fmax_M, Fmax_F1, Dur_M, and Dur_F1. With the exception of Dur_Syll, the same characteristics were revealed by MANOVA.

Cross-validation of duets from the test sets (represented by samples from 2004, 2005, 2006, and 2007) with DFA

 Table 1
 Two-way MANOVA results for effects of pair and year on values of duet characteristics for the red-crowned crane

Duet parameter	Pair effect		Year effec	t	
	$F_{(4,340)}$	Р	$F_{(4,340)}$	Р	
Dur_Syll	503.9	0.000	10.5	0.000	
Dur_M	604.1	0.000	14.2	0.000	
Dur_F1	458.1	0.000	1.6	0.185	
Dur_F2	89.7	0.000	9.0	0.000	
Dur_FF	369.2	0.000	0.9	0.486	
Fmax_M	379.7	0.000	3.0	0.018	
Fmax_F1	605.2	0.000	4.8	0.001	
Fmax_F2	154.4	0.000	4.7	0.001	

Bold text indicates a significant fit

Pair	Discrimination		Cross- validation		Discrimination		Cross- validation		Discrimination		Cross- validation		Discrimination		Cross- validation		Discrimination	
	n (2003)	%	n (2004)	%	n (2003– 2004)	%	n (2005)	%	n (2003– 2005)	%	n (2006)	%	n (2003– 2006)	%	n (2007)	%	n (2003– 2007)	%
1	7	100	4	100	11	100	9	100	20	100	20	100	40	100	9	100	49	100
2	7	100	13	76.9	20	95	13	76.9	33	100	20	95	53	98.1	20	90	73	93.2
3	20	100	20	95	40	97.7	5	60	45	95.6	20	85	65	93.8	20	95	85	92.9
4	19	100	20	100	39	100	10	100	49	100	7	100	56	100	17	100	73	100
5	11	100	7	100	18	100	20	100	38	100	20	100	58	100	11	100	69	100
Total	64	100	64	93.8	128	98.4	57	91.2	185	98.9	87	95.4	272	98.2	77	96.1	349	96.8

Table 2 DFA results for classification of red-crowned crane duets to correct pair for four pooled samples of duets, recorded in one or in a few successive years, and cross-validation results for classification of duets, recorded for each pair over a year

n the number of duets, recorded in one or in a few successive years (in brackets); % percentage of duets assigned to correct pair

Fig. 2 Changes from year to year in the characteristic values of four duet syllables in the five red-crowned crane pairs studied. 03, 04, 05, 06, and 07 are the years of the recordings (2003, 2004, 2005, 2006, and 2007, respectively). Points represent means, whiskers min-max, *P < 0.05, Student *t*-test

Pair 1	Pair 2	Pair 3	Pair 4	Pair 5
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functions derived from the training duet sets (represented, respectively, by samples from 2003, 2003–2004, 2003–2005, and 2003–2006) resulted in high percentages of correct classification to pair, varying from 91.2 to 96.1% between analyses (Table 2). Therefore, pair-specific differences in syllable characteristics were stable between years.

With the Student *t*-test we found that the values of the four duet characteristics which contributed most to discrimination by DFA did not usually differ significantly between successive years for each of the five pairs (Fig. 2), whereas these values did differ significantly between pairs (Table 1).

Discussion

We showed that red-crowned crane pairs can be identified by their duets with very high accuracy both within and between years, by measuring eight time–frequency characteristics. Duets for three of the five pairs examined (pairs 1, 4, and 5) could be classified with 100% accuracy, whereas from 1 to 3 duets of pairs 2 and 3 were misclassified in different years. One-hundred percent accuracy for these two pairs could, however, be achieved by inclusion of an additional characteristic—the percentage of syllables differing in the number of male and female calls. For pair 2, 66.4% of syllables were for two female calls per male call and only 7.3% for one female call per male call. For pair 3, these percentages were 37.9 and 54.8%, respectively (Klenova et al. 2008). Thus, joint use of quantitative and qualitative indicators could provide 100% accuracy of vocal identification for all five pairs.

Although the small sample size (349 duets from five pairs) limits generalization of our results, this is the largest sample size which could be obtained from the same birds of this rare species throughout this long-term period. However, the limited generalization did not prevent the use of the duet-based censuses in conservation the red-crowned crane, at least for the continental population. Nesting populations of this species are rather fragmented and dispersed over a large territory (Viniter 1981; Andronov 1988). Local populations of red-crowned cranes rarely exceed five nesting pairs in a neighbourhood. For example, the nesting population in one of the largest reserves of the red-crowned crane in Russia (1,200 km²), the Khingansky State Nature Reserve (Andronov 1988), does not exceed eleven nesting pairs (Parilov 2002). These cranes are also long-lived, with high fidelity to nesting places (Masatomi 2000). Therefore, our data on duet specificity and stability for five pairs of red-crowned cranes encourages duet monitoring in most localities.

Both MANOVA and DFA selected two frequency (Fmax_M and Fmax_F1) and two time (Dur_M, Dur_F1) syllable characteristics as contributing most to pair identity. Under natural conditions, degradation can affect the time but not frequency characteristics of calls propagated through the environment (Naguib and Wiley 2001). At the same time, the red-crowned cranes nest in relatively open habitats, where the reverberation effects of time characteristics are negligible for identification purposes (Klenova et al. 2008). Thus, we have established that the duet characteristics relevant to pair identity can be correctly measured in duets recorded remotely in natural habitats of the red-crowned crane.

In comparison with other bird species, the temporal stability of calls of the red-crowned crane is remarkable and quite unusual. Use of data from captive birds enabled us to be confident of the identity of examined birds throughout all four years of the study. Instead, studies focussed on retained identity of bird calls mostly use data for unmarked wild individuals (e.g. Gilbert et al. 1994; Galeotti and Sacchi 2001; Delport et al. 2002; Gilbert et al. 2002; Tripp and Otter 2006), on the basis of similarity of call structures, recorded repeatedly in the same home territory.

However, the limited data from marked birds show that some individuals change their calls significantly with time. For example, the "boom" calls of 18 wild marked male great bitterns *Botaurus stellaris* could be classified with 91% accuracy within day, but with only 52% accuracy over two weeks (Puglisi and Adamo 2004). Similarly, 12 of 13 wild male common loons *Gavia immer*, that switched their previous-year territories also changed their individually distinctive "yodel" calls whereas loons that did not switch their previous-year territories retained the same call structures (Walcott et al. 2006). At the same time, both for wild and captive bald eagles *Haliaeetus leucocephalus*, no stable features in chatter calls were found either within or between years (Eakle et al. 1989). Frequency characteristics of calls of the common quail *Coturnix coturnix* also changed strongly over a year (Guyomarc'h et al. 1998). Also, whereas the two-syllable calls of four of five captive male red-breasted geese *Branta ruficollis* enabled identification of individuals with 46–100% accuracy in five different years, for the fifth male retained identity was very low (Volodin et al. 2008).

In contrast, for five captive male eagle owls *Bubo bubo*, 100% of their "hoot" calls were assigned to correct individuals in the next year (Lengagne 2001). Therefore, the sustainability of call features varies strongly both between bird species and between individuals. The red-crowned crane is one of few species with retained long term pair-specificity in duet structure. Therefore, for this endangered species vocalization-based monitoring is especially appropriate, and the present study encourages its use for censuses and conservation. As a noninvasive tool for identification of breeding pairs, vocalization-based monitoring may help to extend knowledge of the biology of this poorly studied species: its home ranges, territory fidelity, and population numbers in different localities.

We do not yet know which changes pair-specific duet structure undergoes in cases of re-mating or of changing a home territory. Nevertheless, our unpublished observations suggest that for the second of these, at least, pair-specific differences in syllable characteristics do not change noticeably. Some of the pairs studied were placed in new enclosures during the study period, and all of these retained their duet characteristics unchanged. Further study is necessary to understand whether individual red-crowned cranes will change their duet contributions after re-mating. Also, further investigations are necessary to examine whether other crane calls besides the duets may be used as markers of individual identity. This is very important for monitoring birds released into nature in relation to active re-introduction programs (Andronov and Rozdina 2002; Andronova and Andronov 2005), in order to estimate their survival and breeding success. Also, additional data on pair-specificity in duet structure and their stability through years is necessary for other crane species to estimate perspectives of the vocalization-based monitoring for the entire group.

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