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Vertebral Formula in Red-Crowned Crane (*Grus japonensis*) and Hooded Crane (*Grus monacha*)

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ABSTRACT. Red-crowned cranes (*Grus japonensis*) are distributed separately in the east Eurasian Continent (continental population) and in Hokkaido, Japan (island population). The island population is sedentary in eastern Hokkaido and has increased from a very small number of cranes to over 1,300, thus giving rise to the problem of poor genetic diversity. While, Hooded cranes (*Grus monacha*), which migrate from the east Eurasian Continent and winter mainly in Izumi, Kagoshima Prefecture, Japan, are about eight-time larger than the island population of Red-crowned cranes. We collected whole bodies of these two species, found dead or moribund in eastern Hokkaido and in Izumi, and observed skeletons with focus on vertebral formula. Numbers of cervical vertebrae (Cs), thoracic vertebrae (Ts), vertebrae composing the synsacrum (Sa) and free coccygeal vertebrae (free Cos) in 22 Red-crowned cranes were 17 or 18, 9–11, 13 or 14 and 7 or 8, respectively. Total number of vertebrae was 47, 48 or 49, and the vertebral formula was divided into three types including 9 sub-types. Numbers of Cs, Ts, vertebrae composing the Sa and free Cos in 25 Hooded cranes were 17 or 18, 9 or 10, 12–14 and 6–8, respectively. Total number of vertebrae was 46, 47, 48 or 49, and the vertebral formula was divided into four types including 14 sub-types. Our findings clearly showed various numerical vertebral patterns in both crane species; however, these variations in the vertebral formula may be unrelated to the genetic diversity.
KEY WORDS: cranes, vertebrae, wild animals.

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The Red-crowned Crane (*Grus japonensis*) is one of the rarest crane species. The total wild population in the world is only about 2,800. There are two separated populations: a migratory population in the east Eurasian Continent of north-east Asia (continental population) and a non-migratory population in eastern Hokkaido, Japan (island population) [15, 18, 19]. The island population has steadily grown from a very small number (estimated at around 60) in the 1950s to over 1,300 birds mainly due to artificial feeding in winter [17]. On the other hand, the Hooded Crane (*Grus monacha*) is migratory and winters in Izumi, Kagoshima Prefecture, Japan, and its population exceeds 10,000, which is about eight-time larger than that of the Hokkaido population of Red-crowned cranes [5]. These two species of cranes and the White-naped Crane (*Grus vipio*) are major species in Japan, and these birds and their habitats are designated as special natural treasures of Japan, because of their scarcity, preciousness and beauty.

Recently, based on results of mitochondrial DNA analysis, it has been shown that only two haplotypes exist in the island population, whereas seven types are present in the continental population of Red-crowned cranes [10]. On the other hand, ten types have been detected from Hooded cranes dead in Izumi [1]. Thus, the island population of Red-crowned cranes is severely deficient in genetic diversity compared to the continental one and Hooded cranes, possibly due to a "bottleneck" effect [10, 17]. There is concern about the scarce diversity, which might lead to the serious declines of immunoreactivity, adaptability and other properties in Red-crowned cranes in Hokkaido [17].

The number of vertebrae varies greatly among different vertebrate species, ranging from as few as six in some frogs to as many as several hundred in some snakes and fish [8]. Almost all mammals have seven cervical vertebrae (Cs), a number that remains remarkably constant [6]. On the other hand, various numbers of C have been reported even in domestic birds: for example, 12 in pigeons, 14 in chickens and ducks and 17 in geese [21]. However, little is known about the numbers of thoracic vertebrae (Ts), lumbar vertebrae (Ls), sacral vertebrae (Ss) and coccygeal vertebrae (Cos) as well as the pattern and variation of vertebral formula in domesticated birds [16, 21], and there has been a few report about the vertebral formula in wild birds including cranes.

We collected Red-crowned and Hooded cranes, found dead or moribund in each field, and observed skeletons with focus on the vertebral column. In this study, we clarified the vertebral formulas for two crane species, which showed numerical variation of each part of the vertebrae.

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MATERIALS AND METHODS

In the period from 1982 to 2008, 22 Red-crowned cranes were found dead or moribund in fields in eastern Hokkaido, and they were carried to Kushiro Zoo in Kushiro and necropsied there immediately after death. After necropsy, cranes were kept in a freezer until they were sent to Rakuno Gakuen University, Ebetsu, Japan. Thawed carcasses were skinned, soft tissues including muscles were removed, and skeletons were prepared by boiling and/or bacterial maceration in warm water at 37–50°C. If necessary, macerated bones were soaked in ethyl-ether and ethanol (1:1) for removal of fat and in 5% H_2O_2 for bleaching. Necropsy and freezing of 25 Hooded cranes were performed at Kagoshima University, Kagoshima, Japan. The following process and methods were the same ones used for Red-crowned cranes. All cranes were identified as adults by the color of feathers. Data from both sexes were included together, since no sexual difference in vertebral formula was observed.

Different from mammals, for which the vertebral column can be easily divided into Cs, Ts, Ls, Ss and Cos, it is difficult to describe the vertebral formula in birds because, (1) some caudal Cs have rudimentary ribs and may therefore be mistaken as Ts, (2) some Ts fuse with each other to form a single notarium or some caudal Ts join the synsacrum (Sa), and (3) differences among Ts, Ls, Ss and Cos composing the Sa are not clear [7]. In this study, we defined the term Ts as vertebrae carrying obvious costal and transverse costal foveae articulating with the heads and tubercles of well-developed ribs, according to Nickel *et al.* [21]. Moreover, as the number of vertebrae composing the Sa, we counted the vertebral bodies between adjoining vertebral foramina without dividing into Ls, Ss and Cos as Sa.

RESULTS

In both Red-crowned and Hooded cranes, overviews of whole vertebral column and shapes of the individual vertebrae were very similar; however, all vertebrae of the Red-crowned crane were slightly larger than those of the Hooded crane. There were 17 cervical vertebrae with one and five exceptional cases having 18 in Red-crowned and Hooded cranes, respectively (Table 1 and Figs. 1 and 2). Cervical vertebrae (Cs) of the middle parts had long-bodied and well-developed cranial and caudal articular processes.

Red-crowned cranes had 9, 10 or 11 thoracic vertebrae (Ts), and Hooded cranes had 9 or 10 Ts. They showed considerable variation in number and order of Ts joining with each other or fused into the Sa. Among the 3rd to 6th Ts, two or three vertebrae were fused to form a single notarium (*Os dorsale*) (Fig. 3), and the last one to three Ts were fused to compose the Sa in both species (Fig. 4).

The synsacrum (Sa) joining with the pelvis consisted of the last one to three Ts, all Ls, all Ss and some cranial Cos

Table 1. Variation of vertebral formula in Red-crowned and Hooded cranes

Species(N)	Types	Number of cases	Number of vertebrae				
			Cervical	Thoracic (*)	Synsacrum**	Free coccygeal	Total
Red-crowned(22)	type17-10-a	2	17	10 (9th,10th)	13	7	47
G. japonensis	type17-10-b	5	17	10 (9th,10th)	13	8	48
	type17-10-c	1	17	10 (8th,9th,10th)	13	7	47
	type17-10-d	8	17	10 (9th,10th)	14	7	48
	type17-11-a	1	17	11 (9th,10th,11th)	13	7	48
	type17-11-b	1	17	11 (9th,10th,11th)	13	8	49
	type17-11-c	1	17	11 (10th,11th)	14	7	49
	type17-11-d	2	17	11 (9th,10th)	14	7	49
	type18-9	1	18	9 (8th,9th)	14	7	48
Hooded(25)	type17-9-a	1	17	9 (8th,9th)	13	7	46
G. monacha	type17-9-b	1	17	9 (9th)	14	7	47
	type17-9-c	1	17	9 (8th,9th)	14	7	47
	type17-10-a	1	17	10 (9th,10th)	12	7	46
	type17-10-b	1	17	10 (9th,10th)	12	8	47
	type17-10-c	1	17	10 (8th,9th,10th)	12	8	47
	type17-10-d	1	17	10 (9th,10th)	13	6	46
	type17-10-e	6	17	10 (9th,10th)	13	7	47
	type17-10-f	1	17	10 (8th,9th,10th)	13	7	47
	type17-10-g	4	17	10 (9th,10th)	13	8	48
	type17-10-h	1	17	10 (9th,10th)	14	7	48
	type17-10-i	1	17	10 (9th,10th)	14	8	49
	type18-9	4	18	9 (8th,9th)	13	6	46
	type18-10	1	18	10 (9th,10th)	13	6	47

*: Ordinal number(s) in parentheses show the thoracic vertebrae fused into the synsacrum. **: Each numeral shows the number of vertebrae except for one to three thoracic vertebrae (*).

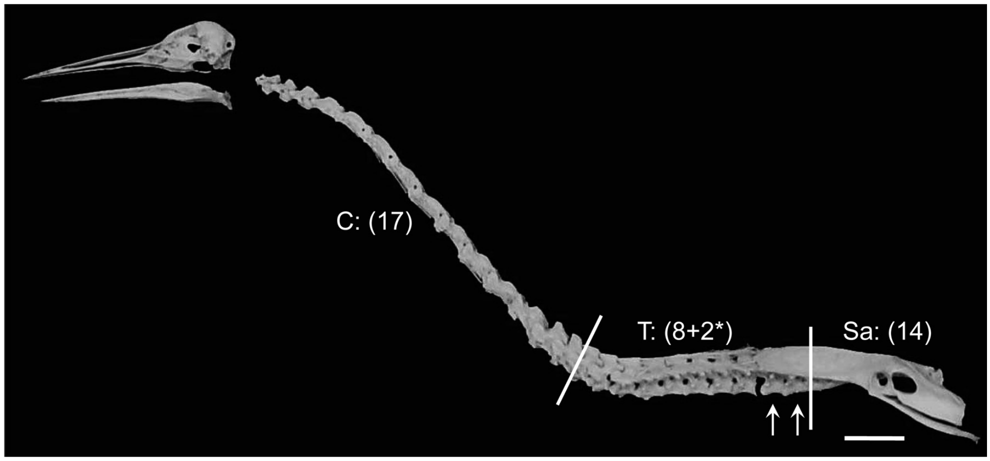

Fig. 1. Lateral view of the vertebral column, including the cranium and pelvis without free caudal vertebrae, of a Red-crowned crane belonging to the major type of 17-10-d (Cs: 17, Ts: 10, Sa: 14 and Cos: 7). *: Last two thoracic vertebrae (9th and 10th shown by arrows) joining the synsacrum (Sa). White lines show boundaries between cervical vertebrae (Cs) and thoracic vertebrae (Ts) and between Ts and Sa. Bar=5 cm.

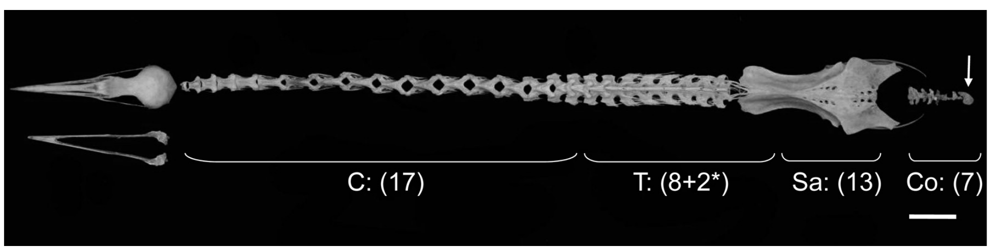

Fig. 2. Dorsal view of the vertebral column, including cranium and pelvis of Hooded crane belonging to the major type of 17-10-e (Cs: 17, Ts: 10, Sa: 13 and Cos: 7). *: Last two thoracic vertebrae (9th and 10th) joining the synsacrum (not seen due to cover of the pelvis). Arrow shows the pygostyle. Bar=5 cm.

(Fig. 4). Owing to the difficulty in distinguishing among segments, the number of vertebrae fusing to compose the Sa was counted on the basis of vertebral bodies between separate transverse processes and vertebral foraminae. Since Ts fusing to the Sa were not counted as Sa but into Ts (Table 1), the exact numbers of vertebrae composing the Sa were 15–16 in Red-crowned cranes and 14–16 in Hooded cranes.

The coccygeal vertebrae (Cos) were divided into fused Cos, which composed a part of the synsacrum, and free Cos. The numbers of fused Cos were not clear as mentioned above, while the numbers of free Cos could be easily counted and were 7 or 8 in Red-crowned cranes and 6 to 8 in Hooded cranes (Table 1). The last Co was pygostyle as a result of fusion of a variable number of individual Cos (Fig. 2).

In the 22 Red-crowned cranes, the numbers of Cs, Ts, vertebrae composing the Sa and free Cos were 17 or 18, 9–11, 13 or 14 and 7 or 8, respectively. Based on the total number of vertebrae and vertebral formula, we divided the 22 Red-crown canes into three types (47, 48 and 49 types) and 9 sub-types (Table 1). In Hooded cranes, on the other hand, the numbers of Cs, Ts, vertebrae composing the Sa and free Cos were 17 or 18, 9 or 10, 12 to 14 and 6–8, respectively, and 25 cases were divided into four types (46, 47, 48 and 49 types) and 14 sub-types (Table 1). Type 17–10, which possessed 17 Cs and 10 Ts, was a common major type seen in 16 of the 22 Red-crowned and in 17 of the 25 Hooded cranes.

DISCUSSION

Almost all mammals consistently have seven Cs with a few exceptions in sloths having eight [3, 20] and manatees having six [23], while the numbers of vertebrae in other regions (Ts, Ls, Ss and Cos) are slightly variable [6]. In birds, reptiles and amphibians, however, the number of vertebrae of each region varies considerably [6]. Moreover, the total number of vertebrae in birds varies both between and within species, reported ranges being from 40 to 64 [25] and from 40 in finches to 59 to 61 in ratite birds, such as cassowaries and emus [24]. In this study, the numbers of vertebrae were 47–49 in Red-crowned cranes and 46–49 in Hooded cranes. Therefore, both cranes have medium numbers of vertebrae among birds examined.

Unlike mammals, it is very difficult to summarize the vertebral formula in birds. Even in chickens, vertebral formulas described in veterinary textbooks [16, 21] include Cs: 14, Ts: 7 (T2-5 being fused together and T7 joining the Sa), Sa:

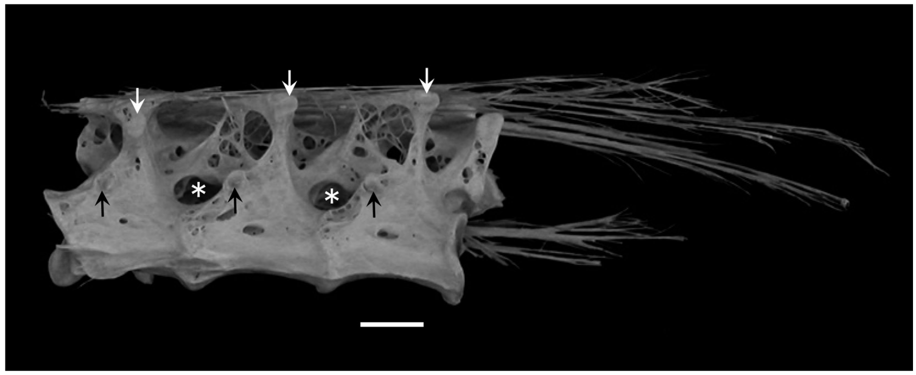

Fig. 3. Ventrolateral view of a notarium of a Red-crowned crane. In this case, three (3rd, 4th and 5th) thoracic vertebrae were fused to form a notarium. Each vertebra possesses costal (black arrows) and transverse costal foveae (white arrows) for the head and tubercle of a rib, respectively. Asterisks show intervertebral foraminae. Bar=1 cm.

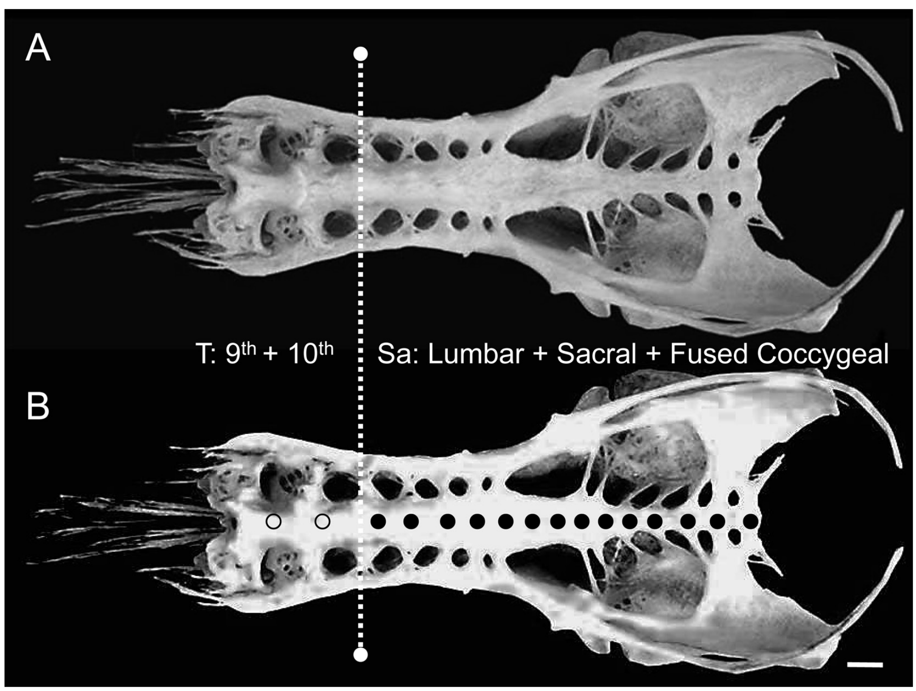

Fig. 4. Ventral view of the synsacrum fused with the pelvis (A) and their scheme (B) in a Red-crowned crane belonging to type 17-10-d (major type). Circles on the median plane of scheme show individual vertebral bodies of fused thoracic vertebrae (open ones) and lumbar, sacral and fused coccygeal vertebrae (solid ones) between adjoining vertebral foraminae. White dotted line shows boundary between last thoracic and first lumbar vertebrae. Bar=1 cm.

14–15 [21] or 15–16 [16] and free Cos: 5, and there is no description about variations of Cs, Ts and Cos in chickens, nor is there any mention about vertebral formulas for other species. In this study, we clearly showed that Red-crowned and Hooded cranes had numerical variations of Cs, Ts, vertebrae composing the Sa and free Cos. Depending on the total number of vertebrae and vertebral formula, we divided Red-crowned cranes into three types including 9 sub-types and Hooded cranes into four types including 14 sub-types.

In birds, the numbers of Cs are 11 in macaws [14], 12 in pigeons [16, 21], 13 in swifts [25] and penguins [9], 14 in chickens [16, 21], Japanese quails [27] and ducks [16, 21], 15 in rheas [11], 17 in geese [16, 21] and ibises [12], 18 in ostriches and emus [11] and cormorants [22] and 19 in grebes [29]. Moreover, Woolfenden [28] examined the vertebral formulas of many water birds and reported that the numbers of Cs were 16–20 in many species and that the largest number of Cs (21–25) was found in swans. In this study, we found that the number of Cs in almost all Red-crowned and Hooded cranes was 17 with a few cases showing 18. Compared with previous reports for other species, the number of Cs in cranes was not so large considering their long neck. The fact that not only swans but also grebes and cormorants possess more Cs than those in cranes with very long neck suggests that the number of Cs depends on the degree of curvature of the neck rather than its length, as has been suggested in some textbooks [2, 4, 13].

The numbers of Ts have been reported to be 7 in fowls and pigeons and 9 in ducks and geese [21]. Other textbooks have shown the number of Ts only for the chicken: 4 to 6 [7] or 7 [4, 16]. In this study, Red-crowned cranes had 9, 10 or 11 Ts and Hooded cranes had 9 or 10 Ts, and both species of cranes showed considerable variations in the number and order of Ts fusing into the notarium or the Sa. Moreover, two or three Ts of middle parts (T3-6) fused to form a single notarium in both species of cranes. Storer [26] defined the notarium (*Os dorsale)* as a group of fused Ts and reported its existence in birds of ten orders, including *Order Gruiformes*. The family Gruidae was shown to have a highly variable pattern of fusion of Ts and to have the unfused vertebrae between the notarium and Sa [26].

There is great variation in number of vertebrae forming the Sa in chickens (15–16) and in other birds (9–22) [16]. The last one to three Ts fusing into the Sa were counted as T in the present study according to several textbooks except for the textbook by Getty [7], resulting in total numbers of vertebrae composing the Sa being 15–17 in Red-crowned cranes and 14–16 in Hooded cranes (Table 1) with the numbers of free Cos being 6 to 8 in both species.

It has been reported that the island population of Red-Crowned cranes has only two haplotypes of mitochondrial DNA [10], though Hooded cranes have ten types [1]. In this study, however, the extent of variation in the vertebral formula in the two species was almost the same. This result indicates that genetic diversity has little influence on numerical variations of vertebrae. Different from mammals, variations of vertebral formulas in birds might be derived from a factor other than poor genetic diversity, although the cause is unknown. Further investigations are required to elucidate the mechanism of variation in vertebral patterns in both cranes as well as in other birds.

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