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Stable Isotope Analysis of Northern Pintail (*Anas acuta*) Feathers Wintering on Junam Reservoir†

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For the first time in Korea, stable isotope ratio mass spectroscopic analysis method was introduced as an alternative method to track the movement of the migratory birds. Hydrogen isotope ratio (δ^2H) and oxygen isotope ratio ($\delta^{18}O$) of the feather samples were collected from northern pintail, which were captured in Junam Wetland Park and were measured using a continuous flow-isotope ratio mass spectrometer. The δ^2H values of all feathers show a bimodal frequency distribution with a wide range of δ^2H values (-30 ‰ to -120 ‰), while the range of $\delta^{18}O$ values was relatively very small (*ca.* 6-18 ‰). The difference between the two modes in the frequency distribution of δ^2H values for male was more than that for female. Although the two-dimensional plot of δ^2H *versus* $\delta^{18}O$ can provide the information on the breeding ground of the migratory wild birds. The northern pintails captured in Junam Wetland Park have more than two geographical origins.

Keywords: Isotope ratio mass spectrometry, Migratory birds, Hydrogen isotope ratio, Oxygen isotope ratio, Origin.

INTRODUCTION

Full-scale studies investigating the geographical origins of migratory birds by using stable isotope ratios were initiated in 1997[1,2]. The elements in the water and food, which are utilized by migratory birds during hatching and growth, were introduced and immobilized in the feathers among the biological tissues of migratory birds. The habitats can be investigated by using isotope ratios, particularly with respect to hydrogen and oxygen, which vary among regions. The International Atomic Energy Agency (IAEA) and the World Meteorological Organization (WMO) have established over 800 observatories in 101 countries since 1961 and have built a database of the measured results of hydrogen and oxygen isotope ratios in rainwater. According to this database, the isotope fractionation makes the values of δ^2H and $\delta^{18}O$ in the rainwater smaller in regions nearer to the North Pole, in mountainous regions than in marine regions, in inland regions than in coastal regions and in winter than in summer. Likewise, because the values of hydrogen (δ^2H) and oxygen ($\delta^{18}O$) isotopes vary among regions, the information on the habitats could be determined based on the hydrogen and oxygen isotope ratios in the feathers of migratory birds.

In this study, to measure the hydrogen and oxygen isotope ratios in the feathers of migratory birds, northern pintails were selected among various species of migratory birds that arrive in each region of Korea every year. The Junam Reservoir, which is one of the major domestic habitats for winter migratory birds, such as the Han river, Nakdong river, Cheonsu Bay, Geum river and Dongjin river, has been recently evaluated as a region of high ecological value and on average, receiving over 10,000 winter or summer migratory birds of over 50 species each year. In this study, feather samples were collected from the migratory birds captured alive in the Junam Reservoir and the δ^2H and $\delta^{18}O$ in the feather samples were analyzed using an isotope ratio mass spectrometry (IRMS). To our best of knowledge, this is the first time that these isotopes were measured in domestically captured northern pintails.

EXPERIMENTAL

Collection of samples and pre-treatment: Nineteen northern pintails, consisting of 8 females and 11 males, were captured at a single site at the Junam Reservoir, which is located in Dong-eup, Uichang-gu, Changwon-si, Gyeongsangnam-do, at 35º18240.322N latitude and 128º40252.322E longitude (World Geodetic System 1984) on March 9, 2009. The water-

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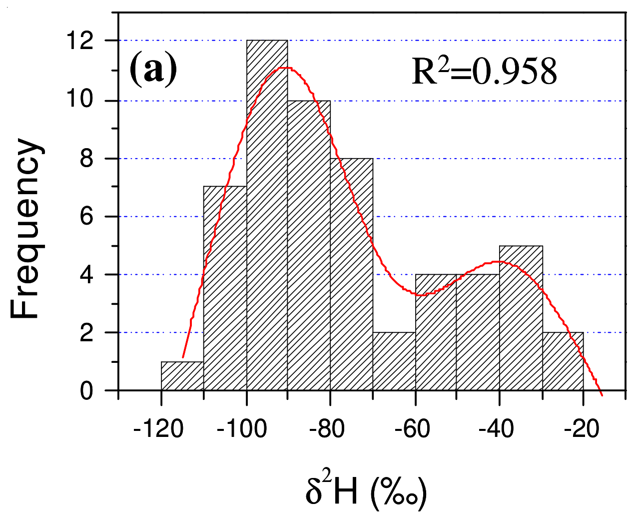
soluble or fat-soluble contaminants on the surface of the feathers were removed using a Branson ultrasonic cleaner and dried in the hood at room temperature. The dried rachises were cut from the top for mass spectroscopic analysis, weighed on a microbalance (Mettler Toledo XP205V) and those with weights within the range of 0.3-0.5 mg were selected for analysis. Two to three samples were cut from the top from each feather; one feather was used for hydrogen isotope ratio analysis, whereas the other was subjected to oxygen isotope ratio analysis.

Stable isotope ratio mass spectroscopic analysis of feathers: IsoPrime IRMS (UK, Micromass IsoPrime) equipped with a high-temperature pyrolyser (Germany, HEKAtech) was used for the analysis of feather samples. The feather samples, which were injected into the carbon tubular reactor in the high-temperature pyrolyser, decomposed into hydrogen (H_2), carbon monoxide (CO) and nitrogen (N_2) at a high temperature of 1400 ºC. These three gases moved together with helium gas used as a carrier gas, were passed through the gas chromatography column installed in the 60 ºC oven inside the elemental analyzer and decomposed into respective pure components and injected into the mass spectrometer. The feather samples were prevented from coming into contact with water vapor during the analysis by compressing and sealing the silver capsule containing the feather samples, manufactured by EuroVector and loading it onto the zero-blank autosampler, equipped with the high-temperature pyrolyser.

RESULTS AND DISCUSSION

The frequency distribution of hydrogen and oxygen isotopes in the feathers of the northern pintail as detected by IRMS is presented in Fig. 1. The δ^2H values of the hydrogen isotopes showed negative values, whereas the $\delta^{18}O$ values of the oxygen isotopes generated positive values. The histogram of the δ^2H values of the hydrogen isotopes from 55 samples decomposed into two Gaussian distribution curves, which had average values of -92 and -39 ‰, respectively (Fig. 1(a)). At this time, the R^2 value for the curve fitting by the two Gaussian distribution curves was 0.958 and this showed a high possibility that the northern pintails reproduced in the two regions that had different δ^2H values and migrated to the Junam Reservoir to pass the winter.



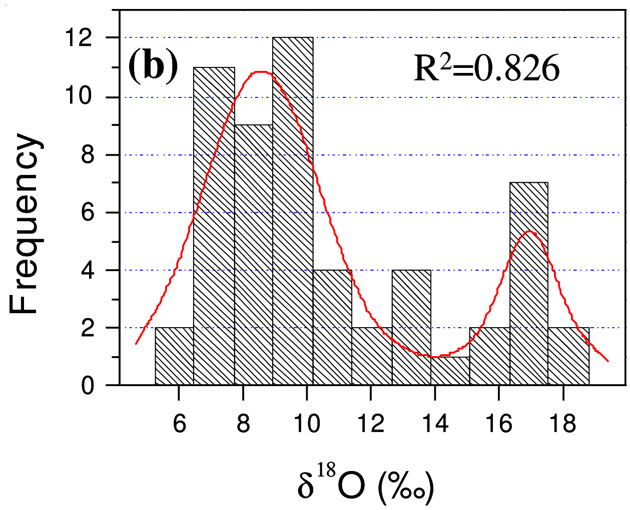


Fig. 1. Frequency distribution of the stable isotope ratio of all feathers from the northern pintail. (a) Hydrogen isotope ratio (δ^2H) and (b) oxygen isotope ratio ($\delta^{18}O$)

Furthermore, to examine differences in migration characteristics according to sex, two separate histograms (Fig. 2) were drawn for males and females, respectively. There were many cases in which the migration characteristic of the females differed from that of the males, such as the case where males arrived at the breeding ground earlier than females, the case where females and males inhabited different parts of the wintering place, or the case where after wintering, females returned to their original breeding ground, whereas males dispersed to different regions[3,4]. When the frequency distribution curve for the δ^2H data was distributed into two Gaussian distribution curves according to sex and the two curves were fitted, the R^2 values for the males and females were 0.920 and 0.921, respectively, which were almost identical to each other and because the difference between the two modes of males was much greater than that of females, it was assumed that the males had a higher possibility of their having reproduced in the two regions where the difference in isotope ratios was high.

According to the annual average value of the isotope ratios calculated at the location of the Junam Reservoir (35º18'40.3"N latitude and 128º40'52.32.3"E longitude) by the method suggested by Bowen and Revenaugh[5], the δ^2H value of the Junam Reservoir was -54 ‰ relative to the Vienna Standard Mean Ocean Water (VSMOW) reference value. Because the δ^2H values were within a wide range between -67 and -176 ‰, measuring the δ^2H values in the feathers in the wintering place can provide information on the location of its breeding ground. Likewise, although the location of breeding ground of the migratory birds in question can be investigated by comparing the δ^2H values in the rainwater and those in the feathers in each breeding ground, the δ^2H values in the rainwater cannot be directly correlated with those in the feathers because *in vivo* metabolism incurs differences between the δ^2H values in the rainwater ingested by migratory birds and those in the feathers of the migratory birds. One of the methods for correcting this difference is to use the discrimination factor. The discrimination factor can be defined as the y-intercept values in the linear regression curves of the δ^2H values in the feathers and in the rainwater according to geographical location. Various

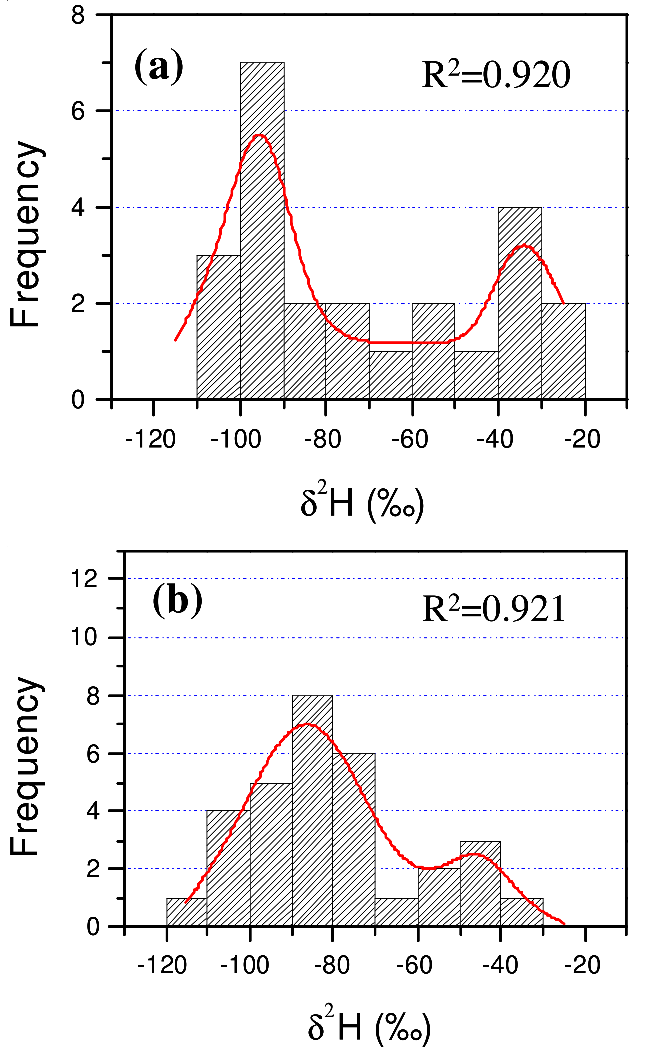


Fig. 2. Effect of gender on the hydrogen isotope ratio (δ^2H) of the feathers from (a) male and (b) female

values such as -25, -27, -28 and -34 ‰ have been presented so far according to the species of migratory birds and their regional differences[1,6-8]. Consequently, there are limitations in using the fixed discrimination factor values for different species of migratory birds and regions. To obtain reliable discrimination factor values, the measured δ^2H values in the rainwater in a specific range and those in the migratory birds, which reproduce in the region, have to be secured. However, the discrimination factor for the northern pintails has not been investigated to date. Likewise, in the case that the precedent-measured data is insufficient, 25 ‰ can be approximately applied as the discrimination factor[9] and the northern pintails captured in the Junam Reservoir in March 2009 can be assumed to have the possibility of having reproduced near in region 43ºN 132ºE. However, it is just one example of the estimation of the geographical origin of the migratory birds. For a more exact assumption, the base map that represents the regional distribution of stable isotope ratios of various breeding grounds as well as the measurement precision and accuracy of mass spectroscopic analysis results have to be established first.

To estimate the actual breeding ground, the measurement precision and accuracy with respect to hydrogen elements have to be established and several cases are present in which the isotope ratios of other elements are measured additionally. In this study, oxygen, which is one of the major component elements of water, was selected for isotope ratio measurements in 56 feather samples and the results are shown in Fig. 1(b). The frequency distribution of the δ^{18}O values shows a bimodal frequency distribution similar to that of the δ^2H values. However, the R^2 value for the curve fitting by the two Gaussian distribution curves was 0.826, which was much smaller than that for δ^2H.

The distribution characteristics of isotope ratios (δ^2H and δ^{18}O values) from 19 northern pintails are shown in Fig. 3. Although identical feather samples were collected from the same individual, the standard deviation values of δ^2H were generally greater than those of δ^{18}O; in some samples (sample A, B, G, H, N, O, Q, R and so on), the standard deviation values were > 10 ‰. Some samples showed high standard deviation values of δ^2H but very small changes in the δ^{18}O

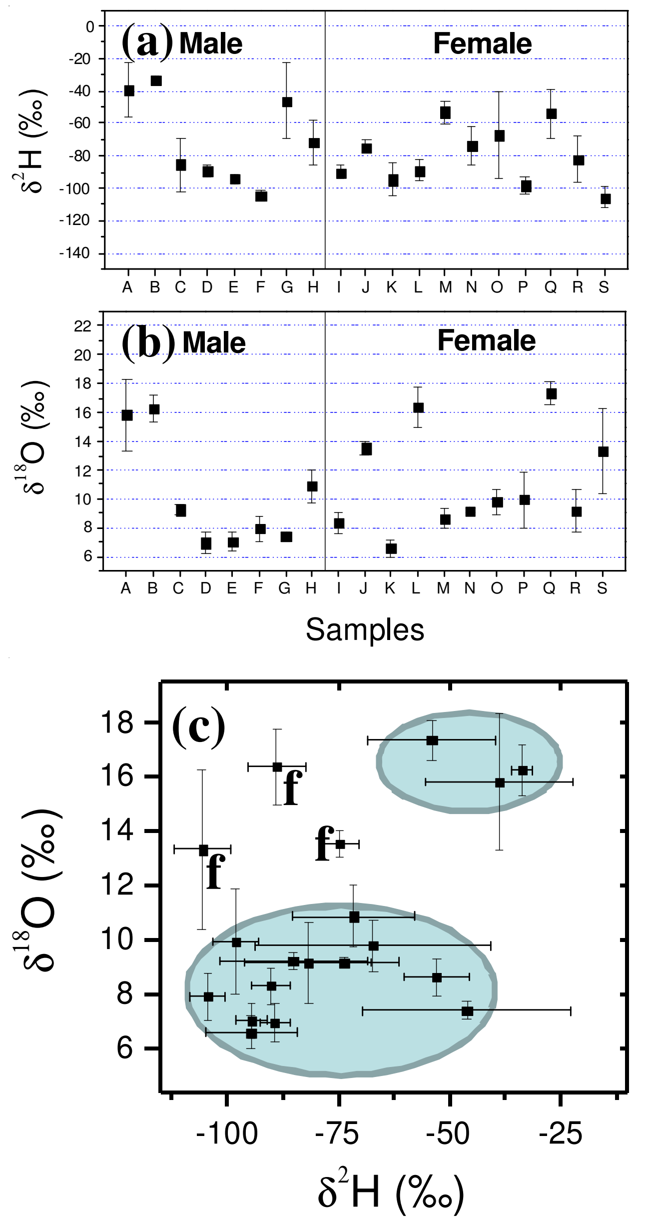


Fig. 3. Inter- and intra-sample homogeneity of the stable isotope ratio of the feathers. (a) Hydrogen isotope ratio (δ^2H), (b) oxygen isotope ratio (δ^{18}O) and (c) a two-dimensional plot of δ^2H *versus* δ^{18}O

values and generally, because the measured density of δ^2H varies greatly with characteristics of the sample itself, pretreatment of sample and the level of mass spectroscopic analysis technique, additional studies are required to determine whether these resulted from the non-uniformity of δ^2H distribution in samples or between samples according to rachises or the measurement precision issue of stable isotope ratio mass spectroscopic analysis. The two-dimensional plot of δ^2H *versus* $\delta^{18}O$ is presented in Fig. 3(c). The entire data set could not be broken down in the two shaded groups because of the measured data marked "f" and can only be assumed to have reproduced in some regions of the northern hemisphere.

Conclusion

To track migratory birds, various analytical techniques have been conducted to investigate the correlation between breeding grounds and the habitats of endangered migratory birds in order to develop effective conservation programs. This study serves as the first attempt to predict the origins of migratory birds by measuring the stable isotope ratios of feathers collected from captured birds. The results of mass spectroscopic analysis of hydrogen and oxygen isotope ratios showed that the migratory birds visiting the Junam Reservoir reproduced in two different regions. Our results showed that it is premature to make a definite conclusion on the breeding grounds of the northern pintails based on the data collected from the present study. Consequently, to track the origins and migration routes of migratory birds, it is imperative that the precision and accuracy of measurements of isotope ratios in the feathers be improved. Furthermore, because there have been no recent data on stable isotope ratios for the rainwater in each domestic region since 2004, the basic data required for drawing the base map are absolutely insufficient. Consequently, the hydrogen and oxygen isotope ratios in the domestic rainwater have to be determined through additional studies.

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