Using stable isotopes to unravel and predict the origins of great cormorants (*Phalacrocorax carbo sinensis*) overwintering at Kinmen

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The Food and Agricultural Organization of the United Nations and the World Organization for Animal Health have called for a better understanding of the role that migrating birds may play in spreading H5N1 highly pathogenic avian influenza (HPAI). Bird banding, traditionally used in studies of migration, is limited by low recapture rates. Telemetry can only be applied to larger species and a limited number of birds. We show that analyses of multiple stable isotopes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ and δD) can provide an understanding of the number of breeding populations represented at large congregations of wintering birds, probable locations of these breeding populations, and which breeding populations do not contribute migrants to a wintering site. As Asia is thought to be the origin of many HPAI strains and the center of their evolution, and as bird migration is poorly understood in this part of the world, we recommend that, in addition to banding, satellite, and VHF telemetry, the stable isotope analysis of migration patterns should become a part of long-term surveillance studies. Copyright © 2008 John Wiley & Sons, Ltd.

The risk to human health of the H5N1 highly pathogenic avian influenza (HPAI) is widely acknowledged, and efforts are underway to prepare for a possible pandemic.¹ Discoveries of the survival of infected birds,^{2–5} as well as outbreaks of HPAI among migratory wild birds in 2005 and 2006,^{6,7} have led the Food and Agricultural Organization of the United Nations and the World Organization for Animal Health to call for a better understanding of the role that migrating birds may play in spreading HPAI.⁸ In particular, they call for a more precise understanding of flyways and congregation sites where large numbers of birds may mix and spread HPAI.

Naturally occurring stable isotopes found in feathers can be an important investigative tool to examine potential links between the breeding and wintering grounds of migratory birds.⁹ Proteins formed during the growth of the feather become metabolically inert. They then form a permanent record in which stable isotopes of hydrogen and oxygen reflect the local environmental conditions in which

the feather was grown, while stable isotopes of carbon and nitrogen are influenced by the local diet. Therefore, the origin of a bird can often be inferred from knowledge of the isotopic values of feathers compared with the isotopic signatures of the local environment and diet.^{9,10}

We chose to study the migration of *Phalacrocorax carbo sinensis* (the great cormorant) because of its potential to bring HPAI to Taiwan. Although HPAI has not yet been detected in Taiwan,¹¹ individuals of *P. c. sinensis* infected with HPAI have been found elsewhere.^{12,13} Kinmen Island, which lies about 5 km off the coast of China (24°44'N, 183°33'E, 150 km², Fig. 1) and is administered by Taiwan, is the location of one of the two largest congregations of wintering *P. c. sinensis* in East Asia. These wintering *P. c. sinensis* are also a focal attraction for ecotourism to Kinmen. The large number of cormorants wintering at Kinmen (greater than 10 000 individuals), and the likelihood that they come from many different breeding populations, suggest the possibility that they could be a vector for bringing HPAI to Kinmen.

Although δD values of feathers have typically been compared with the δD of precipitation to infer migration,^{14,15} the distributions of δD and $\delta^{18}\text{O}$ in precipitation across Asia are not as precisely known as in North America and Europe. This is because there are fewer data collection sites and the frequency of data collection is more sporadic in Asia.¹⁶ This makes it more difficult to infer the origins of birds by comparing the δD values of precipitation and of feathers in Asia alone. Consequently, we sought to infer breeding

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Figure 1. East Asia breeding range and Kinmen wintering sites of *Phalacrocorax carbo sinensis*. The locations marked by black circles indicate where cormorants formerly bred but where none were found during our July 2005 visit. The site with breeding cormorants that we did not visit is marked by a plus sign. The sites with breeding individuals and where feathers were collected are marked with grey triangles. These three sites are Qinghai Lake, Ussuri River and Chita Peninsula. Ci Lake, Ti Lake and Ling Shui Lake are the roosting sites at Kinmen in winter and are indicated by black squares.

locations of *P. c. sinensis* wintering at Kinmen based on the direct comparison of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ and δD values of feathers collected at both wintering and breeding sites.

EXPERIMENTAL

Feather collection

An extensive literature review revealed at least 19 sites where *P. c. sinensis* have been known to breed in East Asia (Table 1,

Fig. 1). While *P. c. sinensis* breeding sites are also likely to occur in Siberia (Dr Sergey Surmach, personal communication), we did not visit these sites because little information has been published on them and because of the difficulty of obtaining visas to travel to these sites. However, we visited 10 of the 19 sites above (Fig. 1) in July 2006 and found breeding *P. c. sinensis* at four sites only: Qinghai Lake (a saline lake), E Ling Lake, Ussuri River in China, and Chita Peninsula in Japan.¹⁷ This suggests a significant loss of *P. c. sinensis* breeding sites. We

Table 1. Possible breeding sites of great cormorants (*Phalacrocorax carbo sinensis*) in East Asia

Place name	Position	Habitat type	Sources
Tang Wang River	Tang Yuan Co., Hei Long Jiang Province	Swamp	1
Yalu River	Fu Yuan Co., Hei Long Jiang Province	Swamp	1
Qin De Li Swamp	Fu Yuan Co., Hei Long Jiang Province	Swamp	1
San Jiang Nature Reserve*	Fu Yuan Co., Hei Long Jiang Province	Swamp	1
Hong He Nature Reserve*	Fu Yuan Co., Hei Long Jiang Province	Swamp	1
Qi Hu Lin River and A Bu Xin River Swamp	Hu Lin Co. and Rao He Co., Hei Long Jiang Province	Swamp	1
Xing Kai Hu Nature Reserve*	Mi Shan City, Hei Long Jiang Province	Lake and swamp	1
Zha Long Nature Reserve*	Qiqihar City, Hei Long Jiang Province	Swamp	1, 2
Ussuri River*†	The boundary river of Northeast China and south of the Russian Far East, Hei Long Jiang Province	River	1
Hulun Lake*	Hulun Buir Grassland, Inner Mongolia Autonomous Region	Swamp	1
Wu Liang Su Hai	Ba Yan Zhao Er City, Inner Mongolia Autonomous Region	Lake	1
Ha Su Hai	Hu He Hao Te City, Inner Mongolia Autonomous Region	Lake and swamp	1
Hu Tu Bi	Dzungaria, Xinjiang Uyghur Autonomous Region	Reservoir	3
Qinghai Lake*†	Qinghai Province	Lake	1, 2
Zha Ling Lake*, E Ling Lake*†	Ma Duo Co and Qu Ma Lai Co., Qinghai Province	Swamp	1
Ri Tu, Ge Er	South Part of Tibet Autonomous Region	—	1
Chita Peninsula*†	Japan	Lake	4

*Indicates breeding sites visited in July 2005.

†Indicates breeding birds found.

1: China Swamps/Wetlands Database.

2: Liu JS, *et al.* *Wild Animal* 1994; 5: 19.

3: Fan XS, University Bulletin of Shihezi University 1999; 3: 1.

4: Johnsgard PA. *Cormorants, Darters, and Pelicans of the World*, Smithsonian Institution Press: Washington and London, 1993.

were unable to collect feathers from E Ling Lake in China because of the difficulty accessing the site. We also collected feathers once a month from *P. c. sinensis* wintering at Kinmen from December 2005 to February 2006.

Rather than capturing and removing feathers from live adult birds, we chose to collect newly dropped feathers found lying on the ground¹⁸ including primary, secondary and tail feathers at breeding sites and at Kinmen. We are confident that all the collected feathers were newly dropped because these feathers were neither weathered, nor damaged, nor covered by a layer of dirt or dust. We ensured that the collected feathers were from different individuals by collecting them under widely separated nests at breeding sites and under separated roosting trees (*Casuarina equisetifolia*) at Kinmen. We also collected body feathers or flight feathers from dead nestlings which had fallen out of their nests at the breeding sites. Since the nestlings had not yet migrated, the isotopic signatures of their feathers must reflect that of the local habitats.^{19,20} We collected feathers from a total of 19, 21 and 48 birds from breeding sites at Qinghai Lake, Ussuri River and

Chita Peninsula, respectively, for stable isotope analysis. At Kinmen we also collected a total of 39, 27 and 32 feathers from Ci Lake, Tai Lake and Ling Shui Lake, respectively.

Feather cleaning and sample preparation

Before cleaning, we removed the vane of each feather and excluded 2 cm of the base (proximal) portion of the rachis including the calamus because this part is close to the subdermal portion and has been shown to differ isotopically from the rest of the feather.²¹ We then sonicated the feathers in distilled water for 30 min, followed by sonication in petroleum ether for an additional 30 min to remove contaminants from the surface of the feathers. They were then air-dried in a fume hood.

Once cleaned, we cut the samples from both the tip and the base of each feather. Each sample was placed in a separate tin capsule for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis and in a pre-combusted silver capsule for $\delta^{18}\text{O}$ and δD analysis and then weighed. The sample weights ranged from 0.7 to 0.8 mg for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis and from 0.135 to 0.165 mg for $\delta^{18}\text{O}$ and δD analysis. All samples and

Figure 2. $\delta^{13}\text{C}$, δD , $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of the tip and base of the *Phalacrocorax carbo sinensis* feathers from breeding sites.

the standards for $\delta^{18}\text{O}$ and δD analysis were stored in plastic culture trays, loosely covered with the lid, and were allowed to equilibrate at room temperature with ambient moisture in the air for 5 days prior to analysis.

Isotopic analysis

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the feathers were analyzed using an elemental analyzer (Costech ECS 4010, Valencia, CA, USA) coupled to a Delta V isotope-ratio mass spectrometer (Finnigan, Bremen, Germany). External standards used for carbon were UCLA Carrera (a working standard from Ian Kaplan's laboratory, UCLA $\delta^{13}\text{C} = 2.52\text{‰}$) and LSVEC (NIST, $\delta^{13}\text{C} = -46.48\text{‰}$). For nitrogen, we used USGS 25 (NIST, $\delta^{15}\text{N} = -30.4\text{‰}$) and USGS 26 (NIST, $\delta^{15}\text{N} = 53.5\text{‰}$) as external standards.

For $\delta^{18}\text{O}$ and δD analysis, silver capsules containing feather samples were analyzed using a high-temperature conversion elemental analyzer (TC/EA, ThermoFinnigan, Bremen, Germany) connected to a Finnigan Delta V isotope-ratio mass spectrometer. We analyzed samples of the tips and bases of feathers of 6–12 birds from each site at the Stable

Isotope Research Facility (SIRFER) at the University of Utah using their in-house standards. We then ran the remaining feathers at Brigham Young University using the feathers that we had run at SIRFER as in-house standards.

All stable isotope values of the samples are expressed in 'delta' (δ) notation. Delta values for carbon and nitrogen are expressed relative to Pee Dee Belemnite (PDB) and atmospheric nitrogen, respectively.^{22,23} Delta values for hydrogen and oxygen are expressed relative to V-SMOW (Vienna Standard Mean Ocean Water).²⁴ The overall precision of the isotope measurements was $\pm 0.05\text{‰}$ for carbon and nitrogen, $\pm 0.1\text{‰}$ for oxygen, and $\pm 1\text{‰}$ for hydrogen.

Data analysis

We designed our statistical analyses to answer the following questions: (1) can we use stable isotopes to infer breeding sites, (2) are there isotopic differences between feathers from different breeding sites with regard to age and feather type, (3) could *P. c. sinensis* wintering at Kinmen have come from the breeding sites we sampled, and (4) if they did not migrate

Figure 3. $\delta^{13}\text{C}$, δD , $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of the tip and base of the *Phalacrocorax carbo sinensis* feathers from Ci Lake, Ti Lake and Ling Shui Lake roosting sites at Kinmen in winter.

from these breeding sites, from which locations could they have possibly migrated? We first analyzed feathers that we collected to establish that their stable isotopes could be used to infer breeding sites. This was done by using a paired t-test of the tips and bases of the rachis of all the feathers. We found no significant difference in these two values of each feather (see below) and we, therefore, averaged the isotope values of tip and base of the rachis to represent the value of each feather for further comparison. We then used site (Qinghai Lake, Ussuri River and Chita Peninsula), age (adult and nestling) and feather type (body feather, primary feather, secondary feather and tail feather) as factors in a single full model analysis of variance (ANOVA) to compare feather delta values for each isotope from breeding sites and used a Tukey's post hoc test to determine differences within factors. We used a post hoc t-test to test the difference of the isotopic values between the feathers of the nestlings and adults within a breeding site. To determine whether it is possible that *P. c. sinensis* wintering at Kinmen could come from breeding sites at Qinghai Lake, Ussuri River, or the Chita Peninsula, we computed the multivariate normal distribution for $\delta^{13}\text{C}$, δD , $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ for each breeding site and then computed a Hotelling's t -squared test for each site and observation. *P. c. sinensis* were assumed to come from the specific breeding site if $T^2 < 9.49$, $X^2_{0.95,4}$; otherwise they were assumed to come from sites other than the three from which we collected feathers. Finally, to answer the question of where these *P. c. sinensis* breed and roughly how many breeding populations winter at Kinmen, we used cluster analysis to objectively group wintering *P. c. sinensis* based on the stable isotope values of their feathers. We used a hierarchical approach based on the mean distance.²⁵ We tested this approach using the data we collected from birds at our three breeding sites.

RESULTS

Isotopic signature of feathers from breeding sites

Our data indicate that the feathers we collected were grown on the breeding grounds. First, we found no statistically significant differences in the isotopic values of base and tip of the rachis from the same feathers for $\delta^{13}\text{C}$, δD , $\delta^{15}\text{N}$ or $\delta^{18}\text{O}$ values (Table 2, Figs. 2 and 3). This indicates that the entire feather was grown at a single location. Second, $\delta^{13}\text{C}$, δD , $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values were significantly different between breeding sites (Tables 3 and 4). Third, there were no significant differences in $\delta^{13}\text{C}$, δD , $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ between different feather types (Table 3 and Fig. 4). Finally, the feathers from nestlings were not significantly different from

Table 2. Paired t-tests of isotopic values of base and tip from the same feathers ($n = 186$). Means ± 1 SE is shown. The significant level is $P < 0.05$

Isotope value (‰)	Tip	Base	t-value (Df)	P
$\delta^{13}\text{C}$	-18.68 ± 0.38	-18.71 ± 0.39	0.42 (185)	0.67
δD	-86.68 ± 2.26	-87.11 ± 2.22	0.66 (185)	0.51
$\delta^{15}\text{N}$	11.50 ± 0.32	11.57 ± 0.33	-0.45 (185)	0.65
$\delta^{18}\text{O}$	15.46 ± 0.19	15.50 ± 0.18	-0.74 (185)	0.46

Table 3. Effects of sites (Qinghai Lake, Ussuri River and Chita Peninsula), ages (adult and nestling) and feather types (body feather, primary feather, secondary feather and tail feather) on isotopic values of feathers ($n = 88$) collected at breeding sites

Effect	Df for numerator	Df for denominator	F	P
$\delta^{13}\text{C}$				
Place	2	85	485.79	<0.0001
Age	1	76	0.61	0.44
Feather type	3	76	1.02	0.39
δD				
Place	2	85	102.43	<0.0001
Age	1	76	0.47	0.49
Feather type	3	76	0.33	0.81
$\delta^{15}\text{N}$				
Place	2	85	256.88	<0.0001
Age	1	76	0.02	0.88
Feather type	3	76	1.18	0.32
$\delta^{18}\text{O}$				
Place	2	77	115.93	<0.0001
Age	1	76	0.88	0.35
Feather type	3	77	2.02	0.66

the dropped feathers of adults in $\delta^{13}\text{C}$, δD , $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values within a breeding site (Fig. 4, Table 5). Since the feathers of nestlings must have grown at the breeding site, this strongly indicates that the adult feathers did also.

Origin of *P. c. sinensis* wintering at Kinmen

Our results show that it is very unlikely that *P. c. sinensis* wintering at Kinmen could come from breeding sites at Qinghai Lake, Ussuri River, or the Chita Peninsula. There is a possibility that three of the 98 *P. c. sinensis* sampled at Kinmen may have spent the breeding season at Qinghai Lake, two on the Ussuri River and two at the Chita Peninsula. However, it reasonably excludes the possibility that 91 of the *P. c. sinensis* summered at the above locations (Table 6).

Having determined that most *P. c. sinensis* wintering at Kinmen do not breed on either Qinghai Lake, the Chita Peninsula, or at the Ussuri River, we next questioned where these *P. c. sinensis* breed and roughly how many breeding populations winter at Kinmen. When we tested cluster analysis on our known data, it reproduced the breeding populations quite closely (Table 7). When we applied this analysis to the data collected from the *P. c. sinensis* wintering

Table 4. Tukey's between site comparisons of the isotopic values (‰) of feathers from breeding sites. Least-square means ± 1 SE is shown

Isotope value (‰)	Breeding sites		
	Qinghai Lake ($n = 19$)	Ussuri River ($n = 21$)	Chita Peninsula ($n = 48$)
$\delta^{13}\text{C}$	-21.65 ± 0.37^a	-26.34 ± 0.35^b	-13.90 ± 0.23^c
δD	-33.20 ± 4.71^a	-124.96 ± 4.48^b	-70.66 ± 2.97^c
$\delta^{15}\text{N}$	14.90 ± 0.24^a	11.57 ± 0.23^b	17.71 ± 0.15^c
$\delta^{18}\text{O}$	16.34 ± 0.53^a	6.96 ± 0.45^b	13.51 ± 0.41^c

Different letter superscripts within a row indicate significantly different values ($P < 0.05$).

Figure 4. $\delta^{13}\text{C}$, δD , $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of *Phalacrocorax carbo sinensis* feathers from nestlings and adults of breeding sites. The statistical results are shown in Tables 2,3,4 and 5.

Table 5. Post hoc t-test between the isotopic values (‰) between the feathers of the nestlings and adults within a breeding site. The level of significance is $P < 0.05$

Isotopes	<i>P</i> values between nestlings and adults		
	Qinghai Lake	Ussuri River	Chita Peninsula
$\delta^{13}\text{C}$	0.68	0.24	0.96
δD	0.66	0.99	0.17
$\delta^{15}\text{N}$	0.81	0.54	0.26
$\delta^{18}\text{O}$	0.62	0.07	0.06

Table 6. Number of *P. c. sinensis* wintering at Kinmen (three different locations) which might reasonably be inferred to have spent the summer at either Qinghai Lake, the Ussuri River, or Japan's Chita Peninsula based on Hotelling's t-squared test

	Breeding sites				Total
	Qinghai Lake	Ussuri River	Chita Peninsula	Other sites	
Kinmen					
Ling Shui Lake	2	1	1	28	32
Tai Lake	0	0	1	26	27
Ci Lake	1	1	0	37	39
Total	3	2	2	91	98

at Kinmen, the analysis produced nine clusters plus two outliers. Assuming these represent distinct breeding populations, we suggest that approximately 65% of the *P. c. sinensis* wintering at Kinmen come from four of 9–11 breeding populations (Table 8, bold italics), while 25% come from the remaining five breeding populations. The two outliers may represent additional populations. Our data also suggest that approximately 70% of the wintering *P. c. sinensis* at Ling Shui Lake were from breeding populations three and four, and that approximately 60% of the *P. c. sinensis* at Tai Lake were from breeding population three. Likewise, about

Table 7. Analysis of feathers collected at three breeding sites. Cluster analysis correctly assigned 97% of breeding cormorants to the correct breeding site based on stable isotope analysis of feathers

Cluster name	Breeding sites			Total
	Qinghai Lake	Ussuri River	Chita Peninsula	
1	0	0	48	48
2	16	0	0	16
3	0	21	0	21
4	2	0	0	2
5	1	0	0	1
Total	19	21	48	88

Table 8. Cluster analysis of feathers collected at three wintering sites in Kinmen. The clusters in the table represent inferred breeding populations to which wintering *P. c. sinensis* were assigned

Cluster name	Wintering sites			Total
	Ling Shui Lake	Tai Lake	Ci Lake	
1	0	1	14	15
2	3	0	10	13
3	14	3	1	18
4	9	16	2	27
5	0	0	6	6
6	0	0	4	4
7	3	3	0	6
8	2	1	1	4
9	0	3	0	3
10	1	0	0	1
11	0	0	1	1
Total	32	27	39	98

Note: Cluster analysis clearly separated cormorants into 11 possible breeding populations: four major populations (bold italics), five minor populations, and two questionable populations based on one bird each. The major breeding origins of cormorants of each wintering site at Kinmen are indicated in bold. The numbers of feathers of each cluster do not imply the exact numbers of individuals coming from the breeding populations.

60% of the *P. c. sinensis* at Ci Lake were from breeding populations one and two (Table 8).

Using $\delta^{13}\text{C}$, δD , $\delta^{15}\text{N}$ or $\delta^{18}\text{O}$ values, we were able to largely exclude the possibility that cormorants wintering at Kinmen came from breeding populations at Qinghai Lake, Ussuri River or the Chita Peninsula. Cluster analyses using these isotopes also allowed us to infer the number of probable breeding populations present at Kinmen. However, because we did not collect feathers from breeding populations in Russia, we determined general locations of possible breeding sites of our hypothesized populations based on the comparisons of feather δD values with the mean δD of precipitation during the breeding season according to the best available δD isocline maps of Asia.^{16,26} The precipitation collecting sites used to create these maps are not continuously active, and the map is derived from spatially and temporally discontinuous data. We assumed no diet-tissue fractionation^{27,28} and that the δD values of the precipitation are directly reflected in the feathers. This comparison suggests that approximately 50% of the *P. c. sinensis* wintering at Kinmen come from five breeding populations that may be located in the region around Lake Baikal or an area encompassing the Amur, Khabarovsk and Primorsky regions of Russia (Fig. 5, clusters 2, 4, 6, 7, and 9). It also

Figure 5. Inferred breeding region for each cluster of *Phalacrocorax carbo sinensis* feathers at Kinmen. The color of each cluster in (a) matches the color of the inferred breeding area in (b). Areas of the same colors in (b) have the same δD values for mean June precipitation (adapted from Ref. ⁷¹). We chose precipitation δD map in June as the example of our inference; due to the patterns of precipitation δD are similar among the months of molting periods, and consequently does not affect our inference (compare (b) with (c) and (d)). The percentage of feathers of each cluster is indicated in parentheses. The totals are given in Table 6. The percentages of feathers of each cluster do not imply the exact percentage of individuals coming from the breeding populations.

suggests that approximately 40% may originate from four breeding populations that are probably found in Mongolia, the region just south of the Ussuri River, and possibly parts of Siberia (Fig. 5, clusters 1, 3, 5, and 10). Finally, approximately 5% of the *P. c. sinensis* may come from populations further south, possibly representing two distinct breeding populations (Fig. 5, clusters 8 and 11). The δD values of the feathers from these individuals suggest the possibility that these *P. c. sinensis* may have come from breeding populations in China, Korea, or Japan, although these breeding sites could also be located in Mongolia or Siberia.

When the results of both the Hotelling's *t*-squared test and the cluster analysis are compared, the evidence suggests that it is unlikely that 96 of the 98 *P. c. sinensis* sampled at Kinmen summered at either Qinghai Lake, the Ussuri River, or the Chita Peninsula. It is possible that one of the remaining two may have migrated to Kinmen from the Ussuri River while the second may have come from the Chita Peninsula.

DISCUSSION

Molting pattern

Although the molting patterns of *P. c. sinensis* in East Asia have not been studied, many cormorants molt their flight feathers gradually during the months of late summer and autumn.²⁹ However, some cormorants show a relatively complex and extended molt, meaning that some cormorants not only molt at their breeding sites in summer, but also extend their molt to non-breeding sites in winter.^{30,31}

Our data suggest that most *P. c. sinensis* in East Asia molt flight and tail feathers *before* they migrate, i.e. over a 3–4 month period while they are still on their breeding grounds. First, we found no differences between the isotopic values of base and tip of the same feather rachis, indicating that the entire feather was grown at a single location. Second, since the feathers of nestlings must have grown at the breeding site and the feather isotopic values of adults are not different from those of nestlings within the same site, it is highly probable that the adult feathers have also grown at the breeding site. Third, we found that feather isotope values are primarily consistent within breeding sites. Studies on cormorants (*Phalacrocorax carbo*)^{18,30} and other migratory bird species^{26,32,33} show that within a species the feathers grown at the breeding areas have distinctly different isotope signatures from those at the wintering areas or intermediate locations. Therefore, if the *P. c. sinensis* in East Asia has an extended molt,³⁰ we should find large variations in isotopic values within single feathers and/or between feathers from birds at the same breeding sites.

Moreover, even if we apply the published fractionation factor,¹⁵ it is very difficult to make the case that the *P. c. sinensis* grew these feathers at Kinmen. At Fuzhou, the place nearest to Kinmen with long-term records of the δD of the precipitation, the mean δD value of the precipitation from October to February is -25‰ ,³⁴ whereas the δD values of sea water usually range between -5 and 5‰ , regardless of latitude.^{35–37} Applying the -25‰ fractionation factor measured between feather and water of red-wing black birds (*Agelaius phoeniceus*)¹⁵ to the δD value of sea water and wintertime precipitation at Kinmen suggests that feathers

grown there should have a mean δD value between -20 and -50‰ . Instead, most feathers collected at Kinmen had δD values ranging primarily from -70 to -120‰ . This strongly suggests that these feathers were grown much further north, probably on the breeding grounds.

Our field observations also indicate that *P. c. sinensis* molt their flight and tail feathers before they migrate to Kinmen. We surveyed their roosting sites monthly and searched for feathers intensively at Kinmen. At a main roosting site (Ci Lake), several thousands of *P. c. sinensis* stayed 12–18 h daily from November to March. However, over the whole roosting site we were only able to collect a few dozen primary, secondary and tail feathers each month. In addition, none of the *P. c. sinensis* we collected (alive or dead) at Kinmen had any developing feathers. These observations, along with feather isotopic comparisons, provide strong evidence that most *P. c. sinensis* drop and regrow their flight feathers and tail feathers at breeding sites.

Isotopic differences between feather isotopes of breeding populations

Our study shows that stable isotopes can be used to differentiate the breeding populations of *P. c. sinensis* in East Asia. For nitrogen, feathers from Chita Peninsula had the highest $\delta^{15}N$ values, followed by feathers from Qinghai Lake and Ussuri River. However, it is hard to interpret what the differences in $\delta^{15}N$ values mean, since they can be caused by differences in the isotopic signature of the diets,^{24,38,39} trophic levels,^{40,41} and also by fasting or nutritional stress.^{42,43}

However, we can interpret the meaning of differences in $\delta^{13}C$ values.^{9,41,44–46} It is generally accepted that animals with an entirely marine diet have higher $\delta^{13}C$ values (-12‰ to -19‰) than animals with a terrestrial or freshwater diet (-20‰ to -27‰).^{18,30,38,44,46–54} The $\delta^{13}C$ values of feathers (range: -12.8 to -18.8‰ ; Figs. 2 and 4) from Chita Peninsula suggest a strong marine dietary influence. This is also supported by our field observations which show that this population, although nesting around freshwater ponds, feeds mainly in Mikawa Bay and Ise Bay during the breeding seasons (Tzung-Su Ding, personal observation).

The $\delta^{13}C$ values suggest that Qinghai Lake cormorants are mostly freshwater feeders while the comparatively negative $\delta^{13}C$ values of Ussuri River cormorants strongly suggest that they are *exclusively* freshwater feeders (Figs. 2 and 4). Qinghai Lake cormorants were reported to *mainly* feed on Przewalski's naked carps (*Gymnocypris przewalskii*), an endemic species of Qinghai Lake.^{55,56} The Przewalski's naked carp is considered a 'salmonid analogue', with adults making annual spring migrations from Qinghai Lake into freshwater rivers to spawn. The adults then return to the lake in the summer.^{57,58} Although $\delta^{13}C$ values of Przewalski's naked carps have not been reported, $\delta^{13}C$ values of the Pacific salmon *Oncorhynchus* spp.^{30,59,60} tend to be more negative than those of marine fish,^{18,30,47,52} and more positive than those of freshwater fish.^{18,47,51,54,59,61,62} This provides a possible explanation for the pattern of $\delta^{13}C$ values for the Qinghai Lake population. It is also worth noting that one feather collected at Qinghai Lake had a high $\delta^{13}C$ value (10.51‰ , Figs. 2 and 4). This suggests that this cormorant and

possibly other individuals may prefer to feed on prey species found in Qinghai Lake.

The δD and $\delta^{18}O$ values of feathers from Qinghai Lake were the highest, followed by those from Chita Peninsula and Ussuri River. It is reasonable that the feathers collected from Ussuri River have lower δD and $\delta^{18}O$ values than those from Chita Peninsula and Qinghai Lake since the spatial distribution of δD and $\delta^{18}O$ in the precipitation generally decreases with increasing latitude and increasing distances from the ocean in Asia.³⁴ An interesting isotopic pattern occurs in Asia in which the δD values and $\delta^{18}O$ values in the precipitation at Qinghai Lake and surrounding areas are much higher than that of precipitation in the coastal regions of China and Japan in summer.³⁴ This may be due to the contribution to precipitation of evaporated groundwater that diffuses through the soil or is transpired by plants in this semi-arid region.⁶³ Therefore, a high δD and $\delta^{18}O$ in the precipitation may cause the high feather δD values and $\delta^{18}O$ values of Qinghai Lake cormorants.

Although the variance in individual foraging behavior may cause differences in isotope patterns of individuals at the same site,⁹ the small variances in feather $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values within the same breeding population suggest that cormorants of the same breeding population feed on similar prey species. This corresponds with the observed feeding practices of *P. c. sinensis*, which are colonial birds and usually form a foraging flock with hundreds to thousands of individuals taking schooling fish.⁶⁴ Thus they are probably feeding on similar prey.

Origin of *P. c. sinensis* wintering at Kinmen

Our analysis suggests that the *P. c. sinensis* gathered at Kinmen come from 9–11 different breeding populations over a large geographic range. However, the data suggest that most of these cormorants do not breed at Qinghai Lake, the Chita Peninsula, or Ussuri River. It also appears that cormorants roosting at Kinmen segregate according to breeding population.

Our inferences concur with observational data. *P. c. sinensis* banded at Qinghai Lake were recovered near the Bay of Bengal, India (Lai-Xing Lee, personal communication). *P. c. sinensis* at Chita Peninsula have long been considered a resident bird species, overwintering locally.¹⁷ Thus both our isotopic data and previous observational studies suggest that it is unlikely that Qinghai Lake and Chita Peninsula populations overwinter at Kinmen.

It is possible that Kinmen *P. c. sinensis* could come from North East China. Banded *P. c. sinensis* from Zha Long Nature Reserve, Hei Long Jiang Province, were recovered at Mingjiang Estuary, Fujian, in 1988,⁶⁵ a place close to Kinmen. However, our visits suggest that Ussuri River is the only existing breeding site in North East China and few *P. c. sinensis* could come from this region. Our inference of origins based on δD alone suggests that *P. c. sinensis* wintering on Kinmen primarily come from further north than the Ussuri River. Dr Sergey Surmach's (personal communications) field observation shows that the breeding *P. c. sinensis* can currently be found at Lake Baikal and in an area encompassing the Amur, Khabarovsk and Primorsky regions. Our inferences of origins using δD alone seem not only to tally

with Dr Surmach's observation, but also suggest some other potential breeding sites.

Although the utilization of δD in precipitation patterns to study the movements of migratory birds has become common,^{9,66} the degree of fractionation of δD between precipitation and feathers is still controversial. Previous studies have shown mixed results, with some showing enrichment of feathers,^{67–69} while others suggesting none.^{27,28} Since the diet of *P. c. sinensis* is aquatic, with very little or no terrestrial input, we assumed that there is little or no fractionation between the δD of precipitation and the δD of the feathers.

In addition, the accuracy and precision of such studies depend on the accuracy and precision of the δD values in precipitation maps. Since the continental patterns of δD in precipitation across Asia are not as precisely known as in North America and Europe, and the influences of food and drinking water on δD values of *P. c. sinensis* are not well understood, caution should be used when inferring breeding origins of *P. c. sinensis* wintering at Kinmen based on patterns of δD in precipitation alone.

CONCLUSIONS

Our study shows that the stable isotope approach has the potential to provide migratory information at the population level, to uncover the possible existence of previously unknown breeding populations, and to make coarse inferences and predictions concerning bird migration and possible future transmission of HPAI in East Asia. In contrast, satellite telemetry and VHF telemetry can only be applied to a limited number of individuals, but has a very high spatial and temporal resolution, which allows the precise assessment of bird movements. As Asia is thought to be the origin of many HPAI strains and the center of their evolution, and as migration is poorly understood in this part of the world, we therefore recommend that, in addition to banding, satellite and VHF telemetry,⁸ stable isotope analysis of migration patterns become a part of long-term surveillance studies. Moreover, combining multivariate analyses of stable isotopes with analyses of the phylogenetic trees of birds and HPAI may provide an opportunity to better understanding the interaction of bird migrations and the spread of HPAI.^{10,70}

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