

Short communication

Using stable isotope analysis of multiple feather tracts to identify moulting provenance of vagrant birds: a case study of Baikal Teal *Anas formosa* in Denmark

TONY (A. D.) FOX,^{1*}

THOMAS KJÆR CHRISTENSEN,¹

STUART BEARHOP² & JASON NEWTON³

¹Department of Wildlife Ecology and Biodiversity, National Environmental Research Institute (NERI), University of Aarhus, Kalø, Grenåvej 14, DK-8410 Rønde, Denmark

²School of Biology & Biochemistry, Queen's University Belfast, MBC, Lisburn Road, Belfast BT9 7BL, UK

³NERC Life Sciences Mass Spectrometry Facility, Scottish Universities Environmental Research Centre, Rankine Avenue, Scottish Enterprise Technology Park, East Kilbride G75 0QF, UK

The stable hydrogen isotope ratio (D/H or ²H/¹H, conventionally expressed as δ²H) in feathers of birds correlates strongly, through local diet, with that of precipitation in the areas where the feathers are grown (Hobson & Wassenaar 1997, Kelly *et al.* 2001, Meehan *et al.* 2001, Rubenstein *et al.* 2002). Because deuterium depletion in precipitation occurs with increasing distance from atmospheric recharge source, strong gradients exist both in continental North America (Hobson & Wassenaar 1997) and in Europe (Hobson *et al.* 2004) after adjusting for the effects of topographical relief that are known to affect precipitation isotope ratios. The stable isotope ratios of oxygen (δ¹⁸O) in rainfall also correlate with those of δ²H (Craig 1961), although these show less robust correlations with ratios of feathers grown locally (Hobson *et al.* 2004). Such relationships enable the chemical establishment of migratory connectivity of birds between different areas in the annual life cycle of an individual without the need physically to follow that individual in time and space (Bearhop *et al.* 2005, Newton *et al.* 2006). Isotope ratios in feathers of long-distance migratory waterfowl are of particular interest (Mehl *et al.* 2004, 2005); the distribution of energetically

costly events throughout their annual cycle, to avoid temporal overlap of demanding processes, means that different feather tracts are replaced at different times of the year. Hence, feathers may be grown in highly contrasting isotopic environments, such that different parts of what appears to be a uniform plumage may show strikingly different isotope signatures when present simultaneously on a bird, reflecting those isotope ratios present in the food source at the point of specific feather replacement.

In this short communication, we apply this theory to test whether a first-winter Baikal Teal *Anas formosa*, mistakenly shot as a Teal *Anas crecca* in the vicinity of Skælskør, southeast Zealand, Denmark (55°12'N, 11°18'E), on 24 November 2005, was more likely to be an escaped individual of European captive origin or a genuine vagrant from at or beyond the nearest breeding areas along the Yenisey River (85°E). The specimen was submitted to the National Environmental Research Institute as part of the Danish wing survey scheme that assesses the species, age and sex composition of the Danish hunting bag (Christensen 2005). Baikal Teal nest in the tundra and taiga across eastern Siberia and Kamchatka and winter in Korea and China (Kear 2005). After dramatic declines in the 20th century, the population was thought to number fewer than 40 000 birds in the 1980s, but exploitation of newly created rice paddies in South Korea has apparently led to significant increases, with more than 400 000 counted in a single flock in November 2003 (Kear 2005). Such an increase could explain the recent increase in records in Europe, where there have been some 83 documented reports of birds in the wild since 1906. However, as many European countries follow the United Kingdom in classifying the species as one that only occurs as an 'introduction, transportee or escapee', an objective assessment of the true number of reports of sightings throughout Europe cannot be made. The species is an attractive one, widely prized in captivity, imported into Europe from China 'in thousands' during the first half of the 20th century, when many birds were not pinioned and were therefore free to escape (Delacour 1954). Although less common in collections since 1945, imports have continued and there are thought to be at least 26 breeding pairs in captivity in the UK (B. Hughes pers. comm.). There have been two previous sight records from Denmark, one bearing colour rings on both legs and another with a metal ring, suggesting that both were of captive origin. If the shot Danish bird was genuinely wild, we predicted that feather tracts grown on the natal area would show stable isotope ratios characteristic of some of the most extreme continental conditions in the Northern Hemisphere, with very low values of both δ²H (< -110‰) and δ¹⁸O (< 12‰), based on data presented from a broad range of avian species collected throughout western Europe (Hobson *et al.* 2004). This was expected to be the case for the upper and lower wing coverts and the primary feathers, which are grown on the fledging areas and not replaced before the following post-breeding wing moult. Moreover, because first-winter *Anas*

*Corresponding author.
Email: tfo@dmu.dk

species undergo successive tail moult and partial body moult from late autumn through the winter, we also compared stable isotope ratios in these two elements in newly grown feathers (distinguished by intensity of colour and the degree of wear) in the tail and on the body flanks with worn feathers on the tail and neck, which were the conspicuous remnants of the first post-fledging plumage.

METHODS

The specimen was identified as a first-winter bird by the presence of worn, notched tail feathers alongside new grown ones (adults replace all tail feathers simultaneously during the post-breeding moult). Each feather was cut into small pieces, ground and homogenized in a pestle and mortar. Hydrogen and oxygen isotope analyses were performed simultaneously by continuous-flow isotope ratio mass spectrometry (CF-IRMS), involving a Costech ECS 4010 elemental analyser and HTG-02 induction furnace interfaced to a ThermoElectron Delta XP Plus mass spectrometer. A 0.75-mg aliquot of each ground feather was weighed into a clean silver capsule, folded up and pyrolysed over hot graphite. Because a proportion of organic hydrogen (mainly that involving N–H and O–H bonds) readily exchanges with ambient water vapour (Schimmelmann *et al.* 1999), we used keratinous standards prepared and measured using an off-line equilibration method at Environment Canada in Saskatoon (Wassenaar & Hobson 2000). Assuming constant $\delta^2\text{H}$ of ambient water vapour during a single 6-h experiment, and a uniform proportion of exchangeable hydrogen in animal keratin (19–20%; Wassenaar & Hobson 2000), then a linear correction equation using three samples of known non-exchangeable hydrogen isotope ratio can be applied to the raw hydrogen isotope data (Wassenaar & Hobson 2003). The three keratinous standards used, CFS, BWB-II (see Wassenaar & Hobson 2000) and ISB, had $\delta^2\text{H}$ values of –138, –108 and –72‰, respectively. Replicate analyses on the internal laboratory standard (ISB – homogenized Icelandic Kittiwake *Rissa tridactyla* feather, ground using a liquid nitrogen freeze grinder) suggest a $\delta^2\text{H}$ reproducibility (standard deviation for three replicates) of 1‰. Oxygen isotope data were normalized using the international standards IAEA-CH6 (sucrose), IAEA C3 cellulose and Ennadai cellulose. Replicate $\delta^{18}\text{O}$ analyses of ISB suggest that the $\delta^{18}\text{O}$ reproducibility is 0.60‰. All stable isotope ratios are reported in permil (‰) using the δ notation: $\delta_{\text{sample}} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, where δ_{sample} represents the isotope ratio of the sample relative to the standard, and R_{sample} and R_{standard} are the fractions of heavy (^2H or ^{18}O) to light (^1H or ^{16}O) isotopes in the sample and standard, respectively.

RESULTS

Individual stable isotope ratio values obtained for the Baikal Teal feather samples are shown in Figure 1 in comparison

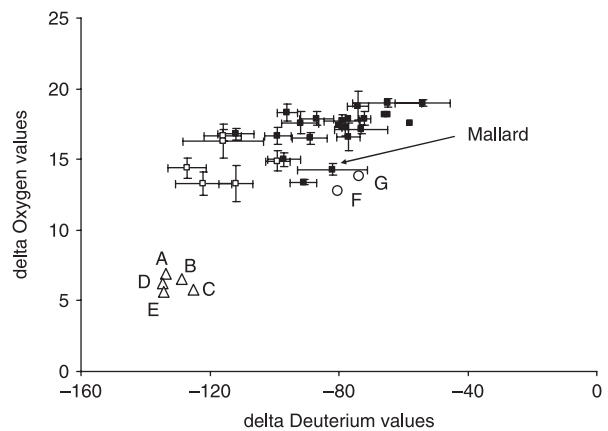


Figure 1. Plot of stable isotope ratios of various bird feathers from a range of sedentary European breeding species (solid squares) and grouse (Tetraonidae) species from Scandinavia (open squares) showing mean values \pm sd (from Hobson *et al.* 2004). The value for European Mallard samples is indicated by the arrow. Also shown (A to G) are individual feather samples from the Baikal Teal shot in southeast Denmark in November 2005. Feather samples come from: A, neck feather; B, secondary wing feather; C, old un-moulted tail feather; D, primary upper-wing covert; and E, secondary under-wing covert (all grown on the natal area), compared with: F, new body feather; and G, newly grown tail feather adjacent to C (all grown on the wintering site).

with mean (\pm se) values for a range of sedentary European species sampled in Europe (taken from table 1 in Hobson *et al.* 2004). Among the feathers known to have been grown on the natal area (primary and secondary wing coverts, secondary feather and worn neck feathers), the mean $\delta^2\text{H}$ was –131.3‰ (\pm 3.7‰; 95% CI) and $\delta^{18}\text{O}$ was 6.3‰ (\pm 0.5‰), compared with –77.1‰ (\pm 6.6‰) and 13.3‰ (\pm 1.0‰), respectively, among newly grown feathers in the tail and body, initiated in late autumn and early winter. These values are highly significantly different for both elements for both sets of feather tracts ($P < 0.001$ based on *t*-tests).

DISCUSSION

The isotopic data presented here strongly suggest that the two generations of feathers were grown in different climatic regions. The stable isotope ratios of feathers produced during development of its first complete plumage whilst on the natal areas (notably samples from among flight feathers and worn body feathers) corresponded to those expected if the bird had subsisted on dietary items available in a highly continental environment. The stable isotope ratios of fresh feathers initiated after departure from the natal area are typical of those expected from a

bird feeding on dietary items characteristic of the more near-coast isotopic signatures prevailing in Europe. Moreover, the stable isotope ratios in both elements in these newly grown feathers closely resembled those of European Mallard *Anas platyrhynchos* presented by Hobson *et al.* (2004), which are typical of aquatic species in having slightly less negative $\delta^2\text{H}$ and particularly low $\delta^{18}\text{O}$ values compared to avian species with terrestrial trophic relationships. In stark contrast, the feathers originated on the rearing area showed very low $\delta^2\text{H}$ values, which can only originate in areas subject to rainfall with very low $\delta^2\text{H}$ values. Such conditions only prevail in the northern Rocky Mountains, boreal forest and tundra areas of North America, where this species does not occur (Hobson & Wassenaar 1997, Bowen *et al.* 2005), and in the extreme continental boreal forest and tundra regions of mid continental Russia, where the species does occur (Hobson *et al.* 2004, Bowen *et al.* 2005). The alternative unlikely explanations for obtaining such extreme values are (1) that the bird was raised in captivity within the breeding range or (2) that it was fed in captivity on artificial foods containing stable isotope ratios that characterize such areas. This latter explanation requires that foodstuffs were manufactured from raw materials the organic content of which was either derived from these northern continental areas or was artificially created to contain such isotope ratios. Moreover, around 30% of the hydrogen in feathers comes from drinking water (Hobson *et al.* 1999). These alternative interpretations are therefore highly improbable. We therefore conclude that this individual was reared to fledging in continental conditions characteristic of the natural breeding range of the species. The use of stable isotope analysis is not sufficiently sensitive to pinpoint with any accuracy the precise natal area of this individual. Nevertheless, the contrast between the stable isotope signatures of feathers grown in winter and those grown on the natal site are sufficient to demonstrate that the bird was almost certainly not raised in captivity in western Europe (without the need for recourse to destructive sampling of museum specimens for calibration purposes) and therefore reflects genuine vagrancy in this species. This individual therefore constitutes the first authenticated occurrence of wild-reared Baikal Teal in Denmark and Western Europe, suggesting that other observations of the species in Europe may also relate to genuine vagrancy of wild individuals.

This analysis confirms the power of using a relatively simple analytical technique to determine the migratory patterns undertaken by individual birds in the absence of other means of corroboration. However, we would caution that this technique does not provide a universal panacea for confirming patterns of vagrancy among bird species. We contend that the use of stable isotopes to track movements of individuals requires the careful testing of specific hypotheses, rather than wide general application, before it can be used to maximum benefit. This necessitates the fulfilment of several criteria before the method can be

applied successfully. First, the bird must be available in the hand to confirm the age, sex and condition of feathers from specific tracts. Secondly, some knowledge of the bird's moulting provenance is essential in relation to the recent historical migration route taken by that individual in time and space to account for its arrival at the time and point of capture at that locality. Thirdly, it is essential that patterns of feather moult in the age and sex category of the species concerned are known in sufficient detail to know when (and hence where) each feather was likely to have been grown during the last moult. Finally, the technique must exploit one or more stable isotope ratios that are most likely to provide information (such as that presented here) that enables confirmation of predictions generated by the original hypotheses. In situations where these criteria are fulfilled, retrospective analysis of museum specimens of bird species of unknown origin could prove fruitful in confirming or disproving vagrancy.

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