



## The use of intrinsic markers to study bird migration

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Bird ringing is a vital tool for elucidating patterns of bird migration. Nevertheless, only a small proportion of ringed birds are ever recovered and, in recent years, new techniques such as intrinsic markers have afforded new insights into the ecology and evolution of migration. Stable isotope ratios (SIRs) in particular have shown enormous potential as a tool for studying bird migration, but their interpretation can be complicated by geographical, temporal and seasonal climatic variation, as well as anthropogenic factors. Combining different types of intrinsic markers, such as SIRs and genetic markers, may be a more powerful approach than using a single technique in isolation. Intrinsic marker techniques increase the value of conventional ringing, but new studies, particularly in remote areas and in combination with techniques for tracking individuals remotely, are necessary for realising the power of this approach.

Much of our understanding of bird migration has been gained over the last 100 years, largely as a result of observations and the ringing of birds on a large scale (Marchant 2002). Nonetheless, despite intensive mark–recapture effort in which millions of birds have been ringed, relatively few are ever recovered (Webster *et al* 2002). Furthermore, modern tracking techniques, such as satellite telemetry, currently have severe limitations when applied to some species, particularly small birds travelling long distances (Kelly *et al* 2002). An alternative approach that is increasingly being explored is the use of intrinsic markers, such as stable isotope ratios (SIRs), trace elements and genetic markers, which have the potential to complement ringing and enhance our ability to answer key questions concerning avian migration. However, much of the existing research exploring the utility of intrinsic markers, particularly SIRs, for tracing migratory origins has taken place in the Americas and there are important questions concerning the applicability of this approach for birds breeding across other continents. In this review, we summarise some of the groundbreaking studies employing intrinsic markers for the study of avian migration, as well as more-recent applications of these techniques, with a particular focus on studies of Palearctic–African migrants.

### INTRINSIC MARKERS

#### Stable isotopes

Isotopes are atoms of the same element with different numbers of neutrons, and therefore unique atomic masses. Unlike radioactive isotopes, stable isotopes persist for long periods of time. The proportions, or ratios, of the different stable isotopes of naturally occurring elements such as carbon (C), nitrogen (N), and hydrogen (H) vary across the environment, often in systematic ways. The stable isotope ratio of an element is expressed, in delta ( $\delta$ ) notation, as the ratio of the rarer, heavier form to that of the more common, lighter form (McKinney *et al* 1999). Perhaps the simplest example is hydrogen, which has two common stable isotopes: <sup>2</sup>H (also called deuterium) and <sup>1</sup>H, with the lighter isotope (<sup>1</sup>H) being more abundant (West *et al* 2006). In at least some parts of the world, there is a strong latitudinal gradient in the ratio of hydrogen isotopes in precipitation and groundwater, with higher latitudes generally having lower isotopic ratios. SIRs in the local environment are incorporated into plants during nutrient uptake and are transferred through the food web, so that SIRs in the body tissues of an animal are related to SIRs in the environment where those tissues were grown (Kelly & Finch 1998, Webster *et al* 2002). Measuring the SIRs of body tissues can therefore provide important clues about the diet, origins and environments of animals in the field (Kelly & Finch 1998). The choice of tissue is a critical element in studies employing SIRs because the rate at which isotopic ratios of the diet are incorporated into the body (the isotopic turnover rate) differs according to the tissue type.

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Typically, metabolically inert tissues such as hair, feathers, and claws are used to determine the origins of migrants.

Two of the earliest studies to demonstrate the potential of SIRs for linking breeding and non-breeding grounds of migratory birds were those of Hobson & Wassenaar (1997) and Chamberlain *et al* (1997). Hobson & Wassenaar (1997) found a strong correlation between feather hydrogen SIRs ( $\delta^2\text{H}_f$ ) of passerines sampled at breeding locations across North America, and those in precipitation ( $\delta^2\text{H}_p$ ), thus showing that the stable isotope signatures in feathers can reflect those of the environment where those feathers were grown. Similarly, Chamberlain *et al* (1997) demonstrated that carbon and hydrogen SIRs in feathers of Black-throated Blue Warblers *Dendroica caerulescens* grown on North American breeding sites varied systematically along a latitudinal gradient. In both studies, the most likely breeding origins of birds caught in wintering areas could be predicted. SIRs, particularly those of hydrogen, also vary with altitude and can be used to investigate altitudinal migration (eg Hobson *et al* 2003).

The use of SIRs for migration studies requires data on their environmental distributions and in recent years researchers have begun to map environmental isotopic patterns across the globe. Studies by Hobson *et al* (2004) and Bowen *et al* (2005) suggest that the range of  $\delta^2\text{H}_p$  values is narrower, and the correlation between  $\delta^2\text{H}_p$  and  $\delta^2\text{H}_f$  in passerines weaker, in Europe than in North America, which indicates that there may be less potential for using SIRs to determine the origins of individual birds in Europe than in the Americas. Nevertheless, two recent studies used hydrogen SIRs to investigate the source areas of irruptive migrants into northern Europe. Feathers of northern Bullfinches *Pyrrhula pyrrhula pyrrhula* from an invasion into Scotland and Denmark had a wide range of  $\delta^2\text{H}$  values, suggesting that these birds originated from a wide area (Newton *et al* 2006). In contrast,  $\delta^2\text{H}_f$  variation in multiple Common Crossbill *Loxia curvirostra* irruptions into Britain was greater between than within years, suggesting that irruptions in different years originated in widely separated regions (Marquiss *et al* 2008). These contrasting results may be due to differences in foraging ecology between the two species: Common Crossbills specialise on the seeds of the Norway Spruce *Picea abies* and irruptions may be driven by localised food shortages, involving different regions in different years, whereas northern Bullfinch have a more varied diet and irruptions of this species may be driven by depletion of resources across vast areas (Marquiss *et al* 2008). However, it is also possible that the more varied diet of Bullfinches may result in greater variation in  $\delta^2\text{H}_f$  values in this species; the effect of diet on the extent of SIR variation is a question that remains to be explored.

In addition to helping us to understand migratory strategies, SIRs can play an important role in revealing mechanisms underlying population genetic changes and species differentiation. Using  $\delta^2\text{H}$  values in claw material, Bearhop

*et al* (2005) inferred the winter quarters of Blackcaps *Sylvia atricapilla* breeding in southern Germany and Austria. This important study showed that birds from the same wintering sites also tended to mate together on their breeding grounds. Blackcaps began overwintering in Britain & Ireland only in the past 50 years, and this 'assortative mating' may be the mechanism behind the rapid change in the species' migratory behaviour (Bearhop *et al* 2005). Carbon and nitrogen SIRs of Pied and Collared Flycatchers *Ficedula hypoleuca* and *F. albicollis* on the Swedish islands of Gotland and Öland revealed that, rather than wintering in an area between that of the two parent species, hybrid first-year birds winter in the same area as the Pied Flycatcher. First-year flycatchers undertake their migratory journey without accompanying adults and these results suggest that the genes controlling the migratory route of Pied Flycatchers are dominant over those of the Collared Flycatcher (Veen *et al* 2007). SIRs are also relevant to ornithologists interested in vagrancy now that Fox *et al* (2007), using hydrogen SIRs, have shown that a Baikal Teal *Anas formosa* shot in Denmark was likely to have been a genuine vagrant from breeding grounds in eastern Russia, rather than an escaped European captive.

An important issue for any study of migration is the extent to which individuals from the same breeding area all migrate to the same wintering area, and vice versa. This has been termed 'migratory connectivity' (Webster *et al* 2002). SIRs in feathers of Swallows *Hirundo rustica* breeding in England and Switzerland suggest that the two study populations winter in different parts of Africa (Evans *et al* 2003). More recently, Procházka *et al* (2008) combined SIRs and ring recoveries of Reed Warblers *Acrocephalus scirpaceus* to confirm strong connectivity between breeding and non-breeding grounds, with birds breeding on either side of the migratory divide of southwest- and southeast-migrating populations wintering in different areas. Three breeding populations with an unknown migratory direction could then be assigned to their most probable non-breeding areas based on their feather SIRs (Procházka *et al* 2008).

Migratory connectivity is a key concept for conservation biology because populations with restricted ranges and high site fidelity (*ie* strong migratory connectivity) may be especially vulnerable to environmental change (Pain *et al* 2004). The finding, from SIR data, that populations of the globally-threatened Aquatic Warbler *Acrocephalus paludicola* from different European breeding populations are segregated on their African moult sites (Pain *et al* 2004) argues for effective conservation measures to be undertaken throughout their range. A recent study in which SIR data for the Cerulean Warbler *Dendroica cerulea* in North America show that breeding populations with the strongest migratory connectivity have also experienced the most severe declines (Jones *et al* 2008) emphasises the importance of SIR techniques for conservation biology.

SIR data can also provide information on the quality and characteristics of habitats in different areas used by migratory birds; this is important because events occurring during one stage of the annual cycle are likely to influence subsequent stages. Habitat-specific carbon SIRs suggest that winter habitats of American Redstarts *Setophaga ruticilla* determine their physical condition and spring departure dates, which in turn affect both time of arrival on the breeding grounds and body condition on arrival (Marra *et al* 1998). This influential study has paved the way for further SIR-based studies of seasonal interactions or 'carry-over effects' in this and other species. In an elegant European study, carbon SIRs of colour-marked Black-tailed Godwits *Limosa limosa islandica*, a highly philopatric subspecies, showed that the selection of winter and breeding habitats were strongly linked: individuals from high-quality breeding sites in Iceland also use high-quality wintering sites further south in Europe (Gunnarsson *et al* 2005). Whether this results from winter site selection by early-fledging chicks from good habitats, or breeding site selection by early-returning birds from good winter habitats, is not known. Nevertheless, this 'seasonal matching' of habitat quality may have important consequences for the fitness of individual birds and for our understanding of how individual choices affect evolutionary processes at a population level.

SIRs can also be used to uncover aspects of migratory strategies such as moult, site-fidelity and movement patterns. For most migratory species, the timing and extent of summer moult are relatively well described, but winter moult remains poorly studied (Jenni & Winkler 1994). In Savi's Warblers *Locustella luscinioides*, isotopic signatures of feathers grown in Europe differed significantly from those grown in Africa, suggesting that SIRs can be used to describe the extent of winter moult (Neto *et al* 2006). Feather SIRs suggest that several trans-Saharan migratory passerine species tend to return to the same or similar stopover areas, where they are thought to undergo a partial or complete moult before resuming their southward migration (Yohannes *et al* 2007). In a subsequent study, the feather isotope profiles of Great Reed Warblers *Acrocephalus arundinaceus* in Sweden during the spring were consistent between years, indicating that this species may show high fidelity to moulting areas (Yohannes *et al* 2008). Finally, by relating SIRs to capture dates, it may be possible to assess the relationship between migration timing and breeding origins. In Wilson's Warblers *Wilsonia pusilla*, for example, the relationship between capture date and  $\delta^2\text{H}_f$  values is consistent with a pattern of 'leapfrog' migration, whereby individuals that breed at southern latitudes migrate earlier than those breeding at more northerly latitudes (Paxton *et al* 2007).

While these diverse studies suggest that SIRs have enormous potential as a tool for studying bird migration, it is important to be aware that geographical, temporal and seasonal climatic variation can complicate the interpretation of isotope studies, as can anthropogenic factors (Rubenstein & Hobson 2004). Furthermore, although it is often assumed that all individuals in a sampling area have similar tissue isotope ratios, this is not necessarily the case. Juvenile birds, for instance, may consume a different diet or feed at a different trophic level to adults (Rubenstein & Hobson 2004) and nutritional status and water stress may have confounding influences on tissue SIRs (eg Kelly 2000, Cherel *et al* 2005).

There are vast areas of the world where a lack of data on environmental variation in SIRs makes it difficult to assign species to their most likely origins, except on a broad geographic level. For example, Chamberlain *et al* (2000) used feather SIRs to investigate a migratory divide between two subspecies of Willow Warbler, *Phylloscopus trochilus trochilus* and *P. t. acredula*. Although mean stable isotope signatures suggested that the two subspecies wintered in different sites in Africa, it was not possible to identify the locations.

Evidence from several recent studies suggests that the use of SIRs to trace migratory movements may not be applicable for all migration systems or bird species, especially on relatively small spatial scales (Rocque *et al* 2006). In particular, concerns have arisen over the general applicability of  $\delta^2\text{H}$  values (eg Rocque *et al* 2006, Langin *et al* 2007) and there may be physiological anomalies in some species, particularly raptors, which preclude using  $\delta^2\text{H}$  values to identify origins (eg Smith *et al* 2008). Moreover, a recent study reported poor reproducibility of  $\delta^2\text{H}_f$  values between laboratories (Smith *et al* 2009) suggesting that, in some cases, it may be difficult to compare results from different studies.

Using multiple stable isotopes, or combining these with other data types (see Future developments, below) may be more informative than using a single stable isotope. Sulphur and strontium may be useful additional stable isotopes for tracking movements (eg Kelly *et al* 2005, Font *et al* 2007, Sellick *et al* 2009), although their general applicability has yet to be fully explored.

### Trace elements

Trace elements are chemical markers, analogous to SIRs in that the patterns of trace elements in feathers are ultimately derived from diet, which in turn is strongly influenced by surface geology (Hobson 2005). Analytical techniques now allow numerous trace elements in feathers to be measured routinely (Hobson 2005) and these markers may be useful for identifying origins of birds. Their potential has been

demonstrated in a study by Parrish *et al* (1983), who were able to correctly assign all of 29 nestling Peregrine Falcons *Falco peregrinus* to three different natal areas in Alaska and western Greenland, using 14 trace elements. However, Donovan *et al* (2006) were unable to identify natal locations of several passerine species with high accuracy on the basis of trace elements of feathers and there is little evidence that trace elements vary across the environment in systematic and predictable ways. Rather than determining origins of migrants, this approach might be more suitable for elucidating movement patterns at a finer geographic scale, such as dispersal patterns (Poesel *et al* 2008) or for testing similarity among groups of individuals in their use of particular moulting areas (Szépl *et al* 2003).

### Genetic markers

Populations of most, if not all species show some levels of genetic structuring (Balloux & Lugon-Moulin 2002). If genetic variation of populations is structured geographically on the breeding grounds, then individuals sampled at overwintering sites could potentially be assigned back to their breeding locations (Webster *et al* 2002, Clegg *et al* 2003). Genetic markers such as mitochondrial DNA (mtDNA) have been used for tracing the origins of migratory birds but the degree of success has been variable. MtDNA sequences of Dunlins *Calidris alpina* link breeding and wintering/staging regions at a broad geographic scale (eg Wennerberg 2001, Lopes & Wennerberg 2006). In contrast, a comparable study of White-rumped Sandpiper *C. fuscicollis* demonstrated little evidence for mtDNA differentiation between breeding populations (Wennerberg *et al* 2002) and studies of five Neotropical passerine species showed genetic structure in mtDNA only at the broadest continental scale (Kimura *et al* 2002, Ruegg & Smith 2002, Lovette *et al* 2004).

An alternative to mtDNA markers is to use polymorphisms in sequences of nuclear DNA, ie DNA fingerprinting. Such techniques have been used to identify population-specific markers for three shorebird species (Haig *et al* 1997) and to obtain genetic markers distinguishing two subspecies of Willow Warblers with different migratory behaviour (Bensch *et al* 2002).

Another type of nuclear marker is microsatellites (short sequences of repetitive DNA that can differ in length between individuals), which have become one of the most popular molecular markers mainly because they are highly polymorphic. The major drawback of microsatellite markers is that considerable development time is often required for identifying and characterising markers for new study species (Zane *et al* 2002). Nevertheless, a number of studies have found varying levels of genetic differentiation within avian populations using these markers. To date,

however, the only published study using microsatellites to investigate migratory connectivity is that of Clegg *et al* (2003), who used eight microsatellites (along with hydrogen SIRs) to reveal broad-scale genetic variation between western and eastern populations of Wilson's Warblers in North America.

### FUTURE DEVELOPMENTS

Combining different types of intrinsic markers, such as SIRs and DNA markers, offers the potential for tracing migratory movements at multiple scales (Webster *et al* 2002) and may be a more powerful approach than using a single technique in isolation (eg Coiffait 2007). However, this approach is at an early stage and initial studies have had mixed success. Using mtDNA,  $\delta^{13}\text{C}_f$ , and morphological measurements, there were only limited differences between Arctic breeding sites of the White-rumped Sandpiper and no relationships between markers (Wennerberg *et al* 2002). Complementary geographical variation across the American continent has been reported in microsatellite DNA and  $\delta^2\text{H}_f$  values of Wilson's Warbler populations, but these two data types have not been integrated (Clegg *et al* 2003). Wintering Northern Yellow Warblers *Dendroica petechia*, *aestiva* group, were assigned to a broad region of origin in North America by overlaying maps of mtDNA and  $\delta^2\text{H}$  data, although this required a relatively large data set of several hundred individuals (Boulet *et al* 2006). Most recently, feather SIRs have been combined with trace elements, genetic markers (mtDNA and DNA fingerprints) and biometrics to assign seabird species to their breeding origins (Gómez-Díaz & González-Solis 2007). In this study, genetic markers were of limited use in detecting geographic structuring, but combining SIRs, trace elements and biometrics was more successful than using a single technique in isolation. Perhaps the most successful (in terms of accuracy and robustness of assignments) integration of different intrinsic markers to date was reported by Kelly *et al* (2005), who used a combination of mtDNA,  $\delta^2\text{H}_f$  and  $\delta^{34}\text{S}_f$ , to assign Swainson's Thrushes *Catharus ustulatus* to site-specific origins with 76–80% accuracy.

Given the number of factors affecting environmental and tissue variability in SIRs, rigorous statistical approaches to analyse stable isotope data are still lacking (Hobson 2005) and a major challenge for the future is to combine data of different types, such as chemical and genetic markers, within a single framework (Norris *et al* 2006). A number of studies are following the lead of Royle & Rubenstein (2004), by integrating SIRs with likelihood probabilities based on population abundance data, and this should lead to improved accuracy of assignments. Similar Bayesian statistical approaches have already proved to be a useful

means of examining genetic population structure (see Beaumont & Rannala 2004 for a review).

While intrinsic markers can serve as an additional and powerful tool for studying migration and migratory strategies, there are important caveats, particularly with SIRs. We need better data on geographical, environmental and temporal variation in SIRs and the relationship of these factors to tissue SIRs in individuals before this technique can be used to its full potential. Furthermore, the requirement for expensive equipment, such as mass spectrometers for SIR and chemical tracer studies and extensive laboratory facilities for DNA-marker analysis, means that the applications of these techniques are limited by their high costs and availability of resources. It is clear that, despite the considerable potential of these new techniques for addressing important biological questions in the future, ringing will continue to play a vital role in bird migration research for decades to come. The application of intrinsic marker techniques is firmly rooted in the capture of birds for ringing and the skills of ornithologists in identifying, ageing and sexing individuals in the hand. New ringing studies, particularly in remote areas and in combination with new technologies for remotely tracking individual birds, are necessary for confirming and realising the power of intrinsic marker data.

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