# Egg and skin collections as a resource for long-term ecological studies

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#### Summary

Well labelled and reliable series of bird skins and eggs collected over long time-spans offer opportunities to determine environmentally induced changes in parameters of ecological interest, such as geographical range, the age structure of populations, clutch-size, and the timing of breeding and migration. They also reveal environmentally induced changes in morphology related to pollution and a changing food base. Finally, they can be used for various chemical analyses to determine the presence and effects of certain environmental pollutants and even, through stable isotopes, the geographic origins of particular birds and changes in their diets over time. The value of museum collections for these purposes is sufficiently high for us to recommend the resumption of the systematic accumulation of avian specimens for long-term ecological research, but this should only be done if adverse effects on conservation status can be avoided. One example is the long-term scheme to collect specimens of birds of prey found dead by the public in the U.K., which has resulted in several valuable conservation-oriented applications.

### Introduction

Museum collections have been used most frequently for research on taxonomy, phylogeny and geographical distribution. However, avian museum collections contain much information relevant to other areas of biological research, such as ecology, behaviour and physiology (Ricklefs 1980). Of particular interest to ecologists are the long series of specimens collected over 100–200 year spans which offer opportunities to study long-term changes in morphology, phenology and chemical composition, and in this paper we focus on the use of avian museum collections for such long-term ecological studies.

Avian specimens are preserved in museum collections as skins, skeletons and as whole specimens in alcohol or formalin, eggs and nests. Of these, usually only skins and eggs are collected in large enough numbers to give sufficient material for ecological long-term studies which require large data series. Natural history museums, with their commitment to long-term preservation and documentation of specimens, provide an excellent resource for ecologists. The value of a long-term ecological study is difficult to predict in advance, so relatively few continue for decades. Museum collections are a resource for retrospective long-term studies and especially for monitoring and hypothesis testing.

We identify three ways in which ornithological collections can provide information for long-term ecological studies. These are: the use of data from labels; morphological measurements; and chemical analysis. We present examples of each of these types of research and indicate technical difficulties and prospects for progress.

# Distribution, life history and phenology from collection label data

The data held on a specimen usually include information on species, collecting date and approximate locality. Sometimes there is also information on age, sex, moult, stage of development of gonads, skull ossification, clutch size, stage of development of embryos, habitat and precise locality (latitude/longitude or national grid reference). To obtain these data it may be necessary to examine the label attached to the specimen or to consult accompanying registers, notebooks, data-cards or computerised databases. Large amounts of data can be accumulated quickly. This information can be used for studies of distribution, life history and phenology.

#### Distribution

Data from egg and skin collections can make a valuable contribution to establishing the range of a bird species. The use of clutch and nest localities is preferable for establishing the breeding range if there is a possibility of migration or dispersal. Museum collections can reveal long-term changes in distribution, especially the contraction of range. Evidence from museum specimens for the expansion of range is likely to be less reliable because the earlier lack of specimens from an area might reflect lack of collecting effort. Contraction of range can be checked by new survey work.

#### Life-history studies

Some indication of long-term changes or geographical differences in the demographic rates of birds may be available from museum collections. Proportions of skins attributable to different age categories may reflect the balance of recruitment and mortality in the sampled population. Snow (1956) used the proportion of first years to adults in museum collections to estimate annual mortality rates of Blue Tits *Parus caeruleus* in different parts of Europe. The colouration (green or blue) of the wing-coverts allows one-year-old adults to be distinguished from older birds. Mortality rates varied among seasons and regions within Europe. However, for some species, the methods used to obtain specimens could influence the age distribution, so results should be interpreted with caution.

Clutch sizes from museum egg collections can be used to investigate changes over time. Rodgers (1990) showed that the clutch size of North American Wood Storks *Mycteria americana* had not significantly declined from 1875 to the late twentieth century. Therefore reduction in clutch size cannot account for the population decline observed in Florida. In contrast, the clutch size of the Snail Kite *Rostrhamus sociabilis* in the Florida Everglades decreased significantly over 100 years, during a period of change in hydrology (Beissinger 1986).

A problem with the use of records of clutch size from museum collections is that egg collectors were probably selective with respect to clutch size. Lack (1946) showed for the European Robin *Erithacus rubecula* that clutches in collections were 0.52 eggs larger compared to field observations. The distribution of clutch size was skewed

towards larger clutches for data from oological collections (Lack 1946). Some egg collectors may have made up large false clutches to impress their fellow collectors, but it is thought that these can usually be identified (see for example Beissinger 1986).

#### Phenology

Phenology investigates the timing of naturally recurring events and the relationship of their timing with biotic and abiotic variables. Examples include arrival and departure dates of migratory birds and the timing of egg-laying (Sparks & Crick 1999). One hypothesis is that the observed and recorded event is correlated with climate.

Several recent field studies have shown that birds are laying eggs earlier in north temperate areas than in the past (Crick *et al.* 1997) and that egg-laying dates are correlated with spring temperature (McCleery & Perrins 1998, Crick & Sparks 1999) and the North Atlantic Oscillation (Forchhammer *et al.* 1998). Most of these studies are based on data from nest record cards from the British Trust for Ornithology, which are available for only the last 30–50 years. Egg collections on the other hand provide longer-term data, going back over 150 years. The oldest dated specimen in the egg collection of the Natural History Museum, Tring, is a Great Bustard *Otis tarda* from 1801 (N. J. Collar pers. comm.; see also Walters 1993), but large numbers of specimens are available from 1850 onwards. Dates of collection of clutches can easily be recorded from labels and long-term changes and relationships with climate investigated. Egg-laying dates can be estimated if a record was made of the stage of development of the embryos (McNair 1987), but this information was often not recorded. Another problem is that the terminology used to describe development is variable and specific to the collector. Although eggs containing large embryos often have a large hole through which the egg contents were removed, the diameter of this hole is not always a good indicator of incubation stage (Storer 1930; see McNair 1987).

Rodgers (1990) used data from museum labels from North American Wood Stork eggs collected in Florida to study geographic and temporal variation in laying dates. He showed that there is a significant north–south difference in the main period of egg-laying. Storks in southern Florida lay eggs in October and December to June, whereas birds in central Florida lay between February and May. Laying dates in southern Florida changed from being concentrated in one month (January) in the nineteenth century to a wide spread of laying months observed in the twentieth century. It was suggested that this change might be caused by changes in the hydrology of southern Florida.

Byrkjedal & Thompson (1998) used the results of an extensive compilation of the dates and distribution of localities where museum skins of tundra-breeding plovers *Pluvialis* were collected to describe the phenology of long-distance migration. This approach is widely applicable to comparative studies of the timing of migration and could be used to study changes in migration over time.

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#### Problems with label data

Caution has to be taken when using data from labels. In addition to the problems already mentioned, there is the possibility of accidental or deliberate inaccuracy. Eggs may be especially likely to be associated with falsified data in countries where egg collecting has been illegal in recent decades, because collectors fear prosecution and therefore may falsify collection dates (review of legal aspects in Sutcliffe 1993).

# Morphological measurements

Museum specimens represent an immense resource of morphological data for comparative studies among regions and over long periods of time. The length and breadth of eggs and the weight of the shell are commonly recorded for eggs in museum collections. Other possible measurements include blowhole diameter, which is useful for adjustment of shell weight for the missing piece of shell in calculation of eggshell indices. Multiple measurements or photogrammetry can be used to measure egg shape, and eggshell thickness can be measured directly using a modified micrometer (see Green 1998). A variety of measurements of the bill, legs, wings and feathers can be taken from skins.

#### Long-term changes in eggs associated with environmental pollution

The detection of eggshell thinning as an effect of contamination of birds of prey with DDE (a metabolite of the insecticide DDT) relied heavily on the use of eggs in museum collections (Newton 1979). The most frequently used method is to weigh the eggshell and divide the weight by an index of the surface area of the egg calculated from the length and breadth. By comparing recently taken eggs with older museum specimens Ratcliffe (1970) showed that eggshell thinning in Peregrine Falcons *Falco peregrinus* in Britain had begun at the same time as the first widespread use of DDT in 1947 as a stepwise change. The correlation between introduction of DDT and eggshell thinning was observed in several species of raptor and fish-eating bird in Britain (Table 5 in Ratcliffe 1970) and around the world (Anderson & Hickey 1972, Newton 1979, Risebrough 1986). The eggshell thickness of most species recovered, once organochlorine pesticide use had been reduced or phased out (Newton 1986, Risebrough 1986, Ratcliffe 1993).

A recent study by Green (1998) found that eggshell thickness of four species of thrush has declined over the past 150 years. This decline was evidently not caused by organochlorine pollution, because it began before the introduction of DDT and a step-like decline is not observed. One possible hypothesis is that acid deposition has caused a reduction in the availability of calcium and that this reduced the quality of eggshells laid by thrushes. Such a mechanism is suggested by short-term experimental work on Great Tits *Parus major* in the Netherlands (Graveland 1998).

The extent to which differences in the methods used to prepare and store eggs might contribute to differences in indices or direct measurements of eggshell thickness is still uncertain. The use by collectors of chemicals and different mechanical techniques to remove egg contents, the application of preservatives and the accumulation of dust might all affect the measurements. As with all museum material, the selection of eggs on the basis of size, shape or other characters might also lead to bias.

#### Evolutionary changes in morphology

The detection of small changes in morphology resulting from natural selection is rendered more feasible by the use of museum specimens because it extends the time period over which they can be measured. Smith *et al.* (1995) showed that the bill size of a Hawaiian honeycreeper, the Iiwi *Vestaria coccinea*, was different for museum specimens collected before 1902 than for live birds measured in the 1990s. The proportion of birds with the longest upper mandibles had declined, leading to a reduction in mean length. The Iiwi formerly used its decurved bill to take nectar from the long curved corollas of flowers of lobelioids, which have declined greatly in abundance; the birds now feed mainly from flowers that do not have long tubular corollas. Hence it may be that natural selection has caused the observed change in bill length. The possibility that the apparent change might be caused by post-mortem alterations in the bill morphology of the museum specimens was evaluated in several ways. Smith *et al.* (1995) showed that bill measurements of a related species did not change significantly and that, although post-mortem changes in bill shape occurred, they could not account for the observed change in upper mandible length.

Tornberg *et al.* (1999) measured a large sample of museum specimens of Northern Goshawk *Accipiter gentilis* from northern Finland to test the hypothesis that a longterm decline in the abundance of grouse, one of their most important prey types, would change the pattern of natural selection for body size. They suggested that males, which are the smaller sex, should experience increased selection for small size because grouse are at the upper end of the size range of their prey spectrum and smaller prey have become relatively more important as the grouse declined. Conversely, the much larger females should experience increased selection for large size because their main alternative prey, mountain hare *Lepus timidus*, is larger than grouse. Hence, males were predicted to become smaller and females larger during a period in which grouse populations and their importance in the diet of Goshawks were both declining. Both of these predictions were supported by a multivariate analysis of 14 skin and skeletal characters. The fact that measurements of the two sexes changed in opposite directions clearly (a) rules out spurious trends caused by post-mortem changes and (b) renders implausible the possibility that the size of full-grown birds might have changed in response to changes in prey availability at the nestling stage.

#### Fluctuating asymmetry of morphological characters

Small random differences between the right and left sides in characters that are approximately bilaterally symmetrical are referred to as fluctuating asymmetry (FA). Variation among individuals in the degree of FA has genetic and environmental components and may increase with decreasing genetic variability and deteriorating environmental conditions. Comparative studies of FA may allow changes over time in genetic variability and/or environmental stress to be monitored in populations. Measurements of FA in museum specimens can extend the period of such studies. Lens *et al.* (1999) studied FA in the tarsus length of forest passerines living in fragments of cloud forest in Kenya. They compared FA of museum specimens collected in 1938–1948 with that of birds mist-netted in 1996–1998. FA increased substantially for five out of the six species studied in a small (50 ha) and recently disturbed forest fragment, but showed no marked change over time for the same species in a larger (220 ha), less disturbed fragment. Studies of this type could be used more widely to identify the occurrence of environmental stress caused by habitat loss or deterioration and the effects of loss of genetic diversity. Parallel studies of changes in genetic diversity by DNA analysis from museum specimens, like that of Groombridge *et al.* (2000), might be a valuable adjunct in disentangling the relative importance of environmental stress and genetic diversity.

## Changes in feather growth rates from ptilochronology

Some feathers show transverse markings consisting of alternating light and dark bars. These bars represent daily increments in the length of the feather during growth (Grubb 1989) and can be used after growth has ceased to measure the rate of extension of the feather. The technique is called ptilochronology. Measurements of growth bars can be made on feathers on museum skins, and changes in feather growth rates can then be compared over long time periods or among regions. Since feather growth may be affected by nutrition and environmental factors, such studies are of potential value in assessing long-term changes in habitat quality.

Carlson (1998) measured growth bars on the rectrices of White-backed Woodpeckers *Dendrocopos leucotos* from northern Europe. The growth bars on the feathers of museum skins collected between 1832 and 1942 were significantly wider than those of birds captured in Sweden during 1990–1992. It was also found that the width of the growth bars of birds in the recent sample was positively correlated with the density of dead trees. Since dead wood provides food for woodpeckers it seems plausible that variation in growth-bar width reflects differences in nutritional status. The difference between the growth bars of museum specimens and the recently trapped birds might then suggest that habitat conditions were less favourable in Sweden in the 1990s than they had been in the past.

Measurements of growth bars on museum skins appear to have potential value in long-term studies. However, it may be important to account for age and sex differences in feather growth rates and for differences in the areas in which specimens were collected in different time periods. A further problem is that growth bars are often indistinct and difficult to measure. Measurement methods could be improved if the precise physical basis of the bars was better understood.

#### Problems with morphological measurements

Researchers should be aware of several problems with morphological measurements of museum specimens. Shrinkage of various parts of bird skins occurs and may lead to distortion of shape as well as changes in linear measurements. Experiments conducted by Väisänen (1969) indicated that measurements of eggs are unlikely to be much affected by storage in museum collections. However, egg preparation techniques might change over time, thin-shelled eggs could break selectively, or

dust may accumulate on the specimens. More detailed investigations are required to evaluate these problems.

The specimens in museum collections do not necessarily represent a random sample. Egg collectors in particular are known to wish to include abnormally coloured eggs in their collections (Lack 1958). Such eggs might also be atypical in other respects. Changes in such biases over time might then lead to spurious trends in measured parameters. This might invalidate Fisher's (1937) conclusion that eggs of abundant species are more variable in size than those of uncommon species. However, this observation could also be explained by collecting habits, since collectors might select for diversity in abundant species and keep all specimens of rare species.

## **Chemical analysis**

Chemical analyses of museum specimens allow the tracking of changes in pollutants and the determination of birds' movements and diets. Museum specimens are ideal for monitoring because long time series are available, acting as temporal controls (i.e. specimens collected before the introduction of the pollutant) and spatial controls (specimens from areas where the pollutant is absent). Most chemical analysis methods require samples, such as feathers or pieces of eggshell, to be taken from the museum specimens. However, recent advances in sampling techniques and analysis methods require smaller sample amounts, which will reduce the impact on specimens in museum collections and allow large-scale, long-term data collection. Sampling is becoming less destructive, and more chemical analyses can be done. These advances have opened up a new field of museum-based research.

#### Pollutants

Chemical analyses of avian specimens from museum collections have shed light on many environmental problems. Birds accumulate heavy metals from their food and secrete them into their growing feathers during moult. Measurement of heavy metal concentrations in feather samples from live-trapped birds and museum specimens offers a method for monitoring long-term changes and spatial variation in contamination. Studies of mercury concentrations in seabird feathers provide an example of this approach. The concentration of mercury in feathers is related to that in the diet (Furness 1993). The mercury is stably bound to the feather keratin in organic form (Applequist *et al.* 1984). The presence of inorganic mercury added by post-mortem treatment of museum skins with inorganic mercury and organic mercury and organic mercury concentrations separately (Monteiro & Furness 1997).

Analysis of contour feathers taken from live and museum specimens of four species of seabirds from the north-east subtropical Atlantic Ocean revealed substantial increases in mercury concentration over a period of more than 100 years (Monteiro & Furness 1997). The rates of increase observed were in accord with those predicted from knowledge of anthropogenic emissions of mercury. A potential complication is that species of fish and marine invertebrates vary considerably in their mercury concentration (Monteiro *et al.* 1998). Therefore, a change in diet could lead to a

change in the mercury concentration in feathers that might be misinterpreted as reflecting a change in mercury contamination of the ecosystem.

The chemical analysis of pollutants in eggshell membranes was instrumental in establishing the association between the widespread use of DDT and eggshell thinning in raptors. Peakall (1974) used ether extraction to remove DDE residues from the inner eggshell membrane without destroying the specimens. Eggs collected before 1947 did not contain any DDT, whereas later eggs had high levels of DDT in their membranes.

More recent work has investigated the heavy metal concentration in eggshells directly. Flores & Martins (1997) compared metals in eggs laid near coal-based power plants to eggs laid 100 km away. Using graphite furnace atomic absorption spectrometry they determined cadmium, lead and fluoride concentrations in the eggshell and egg contents. The eggshells from polluted areas contained higher concentrations of cadmium, lead and fluoride. The development of these methods, combined with a newly developed laser ablation sampling technique, which requires only minute samples (about 50µm diameter), will facilitate large-scale sampling from egg collections and might help to evaluate the effects of pollutants on breeding birds and retrospectively assess long-term changes.

#### Tracing bird movements using stable isotopes

Most elements that occur in biological materials are found as at least two stable isotopes (Hoefs 1980 in Schaffner & Swart 1991). Isotope ratios vary geographically because of differences in geochemistry and various fractionation mechanisms during element cycling. These differences are reflected in the tissues of animals. The differences in isotope ratios in tissue can be used as natural ecological tracers, providing information on the regional origins and movements of individuals. However, isotope ratios, unlike ringing recoveries, cannot give very precise locations. The stable isotope ratios in an animal's tissues are correlated with the ratios in its diet. Elements commonly used are carbon, nitrogen, hydrogen, oxygen and strontium taken up through the food web. Tissues available for sampling from museum specimens of birds include bone, eggshell and feathers. Requirements for the size of samples are becoming small.

Chamberlain *et al.* (1997) and Hobson & Wassenaar (1997) found that stable isotope analysis of tissue from North American insectivorous songbirds could provide information on their breeding or natal locations. Hobson & Wassenaar found that a gradient in the relative abundance of deuterium in rainfall during the plant-growing season across North America was reflected in the deuterium content of feathers grown by birds at various sites. Hence, analysis of the deuterium content of feather samples taken from birds trapped on migration or in their winter quarters could be used to identify their origins. Chamberlain *et al.* (1997) similarly reported that gradients in deuterium and <sup>13</sup>C could be used to identify the probable region of origin of migratory warblers. They also found that bone samples varied in <sup>87</sup>Sr content, reflecting geographic variation in the abundance of this isotope among drainage basins with different underlying rocks. Their results suggest that using the relative

abundances of several isotopes in a multivariate approach is likely to improve the precision with which areas of origin can be located.

An important issue for the application of stable isotope methods to museum specimens is the stability with which the element being studied is bound within the tissue being sampled. A fraction of the hydrogen and deuterium in feathers can exchange with the environment and so could be influenced by storage conditions. Chamberlain *et al.* (1997) found that 13% of the hydrogen in songbird feathers exchanged with that in the environment. Equilibration of feather samples with ambient water with a known standard relative deuterium abundance allows this potential problem to be overcome.

#### Monitoring changes and spatial differences in diet using stable isotopes

Stable isotope ratios in the tissues of animals are affected by the habitats they use for feeding. For example the relative abundance of <sup>18</sup>O in water and aquatic animals differs between freshwater and oceanic systems. Using the isotope signatures of eggshells, Schaffner & Swart (1991) showed that Western Grebes *Aechmophorus occidentalis* feed in freshwater habitats whereas tropicbirds *Phaethon* are oceanic feeders, having low  $\delta^{18}$ O and high  $\delta^{18}$ O respectively. In theory a long-term study could determine changes in feeding patterns (from freshwater to saltwater) based on isotope signatures of eggshells.

Isotope signatures in bird tissues are also affected by the trophic level of the organisms they eat. Hence, changes in diet can be detected by the analysis of museum specimens. Thompson *et al.* (1995) found that the relative abundance of <sup>15</sup>N in feather samples from Northern Fulmars *Fulmarus glacialis* in the north-east Atlantic declined between the early and late nineteenth century. The relative abundance of <sup>15</sup>N tends to increase with increasing trophic level, so this change implies that Fulmars have changed their diet to include a higher component of animals of low trophic level. Thompson *et al.* suggested that this might be due to the cessation of commercial whaling within range of the sampled colonies, although changes in the species or age of fish eaten might also explain the change in isotope ratio.

Nitrogen isotope signatures of eggshells can also be used to determine the diet of birds, as has been shown by feeding experiments (Hobson 1995). This technique could be applied to eggshells in museum collections to determine changes in diet over time. Emslie *et al.* (1998) suggested that eggshell fragments found in the sediment of abandoned penguin colonies could be used to determine changes in the prey items from different trophic levels (e.g. krill *vs.* squid *vs.* fish).

# Limitations of museum collections in future ecological long-term studies

For ecologists to make use of avian collections in long-term studies they require information about available specimens. Ideally ecologists would have information on the number of specimens, localities and date of collection. Currently only a few readily available databases of avian collections exist. Examples include an inventory of major egg collections in the United States (Kiff 1979, Kiff & Hough 1985), and the online database of Manchester Museum (http://www.museum.man.ac.uk). Online databases appear more flexible and allow easy access to large datasets. Databases are needed to make ecologists aware of the potentially available data currently hidden in museum collections.

Few avian museum collections have had new specimens added to them in recent decades at rates that even approach those of the late nineteenth and early twentieth centuries (Remsen 1995, Winker 1996). Hence, future studies of long-term change will increasingly suffer from a gap in the data between the early spate of collecting and recent material, most of which has been specially obtained for a specific study. Indeed, for this reason many of the studies we have referred to above involve a comparison of measurements or analyses of old museum specimens with recent data from live-trapped birds. This problem obviously calls for careful storage and use of existing material, but a case can also be made for a revival in the accumulation of avian specimens as a resource for long-term ecological studies, provided that this does not have a negative impact on the conservation status of the bird species.

In some cases representative samples could be collected for a limited set of species at fixed intervals in time. This would involve a carefully coordinated collection effort with detailed recording of ancillary data on every specimen and the preservation of a range of tissues. An alternative or supplementary approach would be to encourage members of the public and amateur ornithologists, in particular ringers and nest recorders, to contribute dead birds and deserted and addled eggs they find incidentally to museums to a much greater extent than they do at present. Although this approach would not permit the use of stratified random sampling, it would have the advantage that specimens from a wide range of species could be obtained. Our perception is that this does not happen at present, at least in western Europe, because both the public and amateur ornithologists are not aware that new specimens are useful for research purposes or that museums are the places that can use them.

The potential of this latter approach is illustrated by the scheme to collect and analyse carcasses of raptors administered by the Centre for Ecology and Hydrology in the U.K. Advertisements for carcasses were placed in bird journals. This resulted in the collection of thousands of specimens over a period of more than thirty years, even though the population sizes of the species concerned are not particularly large. Although the primary objective was to obtain tissue samples for the analysis of pesticide residues (e.g. Newton *et al.* 1993), the specimens have also been used for long-term studies of population ecology (e.g. Newton *et al.* 1999, Wyllie & Newton 1999). Hence, we suggest that availability of new specimens need not be an impediment to the accumulation of a continuous series. A more pertinent question is probably whether museums now have sufficient resources, especially of staff, to prepare, document and store increased numbers of specimens.

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