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Title: Using DNA to verify sex and species identity of dried bird specimens: a tool for correcting specimen records

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The surrounding Kelvingrove Park and nearby River Kelvin, are teaming with wildlife, providing a haven for many garden and woodland birds. Kingfishers, goosanders and sand martins breed along the river and even a pair of peregrines are prospecting to nest nearby.

However, this fantastic wildlife is completely unknown to many of the local people and visitors to the city. Working in close partnership with Culture & Sport Glasgow and Glasgow City Council, this project aims to address this need by better connecting people with their local natural environment and raising their awareness about urban wildlife. Situated in the heart of the city, people from a wide range of backgrounds are able to learn about and appreciate the fragile natural world that surrounds us.

We are doing this by:

- Sensitively installing CCTV cameras at appropriate wildlife sites, to beam live images from nest sites back to the Museum's Environmental Discovery Zone.

- Employing two Information Officers to help interpret the local natural environment and live CCTV images, engaging with over 16000 visitors p/a within the Museum. They lead daily-guided walks through the park, and offer a series of stimulating talks about local wildlife and natural environment in the museum.

- Establishing an environmental field teaching programme we engage with around 3000 school children, all the activities encourage children to get outdoors and experience nature at first-hand.

- We are developing an interpretation trail along the River Kelvin and through the Park, enhancing people's enjoyment of the area and raising their awareness of the wildlife.

Volunteers

We have a team of 15 volunteers who play a vital role in the delivery of this project. They engage with the public within the museum, carry out guided walks and support the Information Officers in delivering a programme of talks in the museum.

Additionally, volunteers help carry out bird surveys in the park and along the River Kelvin, enhancing our understanding of wildlife within the park and assisting the Park Rangers in creating better areas for biodiversity. This has included work to improve the banks of the river, naturalising the duck pond and planting areas of wild meadows to encourage more wildlife to thrive in this urban oasis.

Support

This project has received tremendous support from the local community, Glasgow City Council and Glasgow University. The Heritage Lottery Fund and Scottish Natural Heritage fund the project until March 2009.

Using DNA to verify sex and species identity of dried bird specimens: a tool for correcting specimen records

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Meticulous archiving of biological material in past centuries has left us with a legacy of natural collections. The Natural History Museum's bird collection is an example: it includes a million skins and a million eggs, representing 90% of known species. These are rich sources for research, and archive collections represent a bank of DNA diversity for research. The sex of any given museum specimen has implications for its research potential, since sex is a key variable in a wide variety of studies, and correct identification of specimens is obviously critical for any study. Applying DNA-based tools to museum collections may be a means for checking and correcting species and sex information in specimen records.

PCR (polymerase chain reaction) technology has undoubtedly increased the potential for research using such DNA-based tools as it enables amplification of DNA fragments many thousand-fold from a small quantity of DNA template. However, museum material still present challenges because maximum PCR amplicon size declines significantly with specimen age, and fragments larger than 300 bp are difficult to am-

plify consistently. For example, Lee & Griffiths (2003) used a popular set of avian sexing primers to validate the recorded sex of moorhen skins. This utilizes differences in the length of two introns within the sex-linked CHD1 gene as markers for the Z and W chromosomes, producing amplicons in the region of 300–400 bp.

The oldest sample that could be sexed via this method was collected in 1910 and there was a significant relationship between specimen age and the success of molecular sexing. The effect of specimen age on amplicon length was confirmed using primers to amplify a range of different sized fragments of the mitochondrial cytochrome *b* gene (Lee & Griffiths 2003). Similarly, the 'barcode' method suggested as a standard DNA technique for verifying species identity of birds (Hebert et al. 2004) uses primers targeting a fragment of approximately 751 bp, too large for successful PCR of degraded archive DNA. One possible solution is to reduce the size of PCR amplicon targeted by primers.

Recently I examined the feasibility of extracting DNA from archive blown eggs, and PCR amplification of short DNA fragments (Lee & Prys-Jones 2008), and demonstrated improved DNA sexing methods for use with museum skin samples with primers targeting smaller amplicons than previously (Bantock et al. 2008). For the latter, two different techniques were designed for nonpasserine and passerine birds (Neognathae). The technique for nonpasserines was based on a new primer which, in combination with one existing primer, targeted a smaller amplicon in the CHD1 sex-linked gene than previously. Primers targeting ATP5A1, an avian sex-linked gene not previously used for sex identification, were developed for passerines. Comprehensive testing across species demonstrated that both primer pairs sex a range of different species within their respective taxonomic groups. Rigorous evaluation of each method within species showed that these permitted sexing of specimens dating from the 1850s. For corn bunting museum specimens, the ATP5A1 method sexed 98% of 63 samples (1857–1966). The new CHD1 method was similarly successful, sexing 90% of 384 moorhen specimens from six different museum collections (1855–2001). In contrast, the original sexing method only identified the sex of less than half of 111 museum moorhen samples.

A protocol was developed for extracting DNA from egg membranes and other internal debris recovered from the interior of blown museum bird eggs. A variety of commercially available DNA extraction methods were found to be useful. DNA sequencing of PCR products for a 176-bp fragment of mitochondrial DNA was successful for most egg samples (> 78%) even though the amount of DNA extracted (mean = 14.71 ± 4.55 ng/µL) was significantly less than that obtained for bird skin samples (mean = 67.88 ± 4.77 ng/µL). For PCR and sequencing of snipe (Gallinago) DNA, eight new primers for the 'DNA barcode' region of COI mtDNA were tried. In various combinations, the primers target a range of PCR products sized from 72 bp to the full 'barcode' of 751 bp. Not all possible combinations were tested with archive snipe DNA, but a significantly better success rate of PCR amplification for a shorter 176-bp target compared with a larger 288-bp fragment (67% vs. 39%) was found.

Currently work continues in establishing the minimum amount of sequence data required for species verification among snipe species.

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