

<b>Institution:</b> University of Glasgow
<b>Unit of Assessment:</b> Unit 5, Biological Sciences
<b>Title of case study:</b> Development of a simple test that enables reliable sexing of birds
<p><b>1. Summary of the impact</b></p> <p>Half of the world's bird species cannot be sexed by their physical appearance. This posed a major problem for conservation breeding, which is dependent upon identification of the birds' sex for mating birds, as well as ensuring an equal sex ratio of birds for reintroduction into the wild. Researchers at the University of Glasgow developed a simple DNA test to determine the sex of birds. The test has been adopted by commercial companies in the UK and USA, one of which includes Avian Biotech (USA), who perform approximately 50,000 tests a year for commercial, conservation and private breeders, generating revenues of around £618,000. The test is available to a broad range of international groups, including zoos and conservation organisations, where it has been fundamental to the management of captive breeding of some of the world's rarest bird species.</p>
<p><b>2. Underpinning research</b></p> <p>More than half of all adult and almost all juvenile birds have no easily detectable external characteristics that allow the identification of their sex. Early approaches to sex determination by surgical means posed a risk to the bird due to infection or complications of anaesthesia. Likewise, the visual inspection of sex chromosomes extracted from blood was labour-intensive and expensive. Research by Dr Richard Griffiths at the University of Glasgow has developed a rapid, low cost DNA-based sex test that can be applied to a broad range of bird species, without risk to the birds.</p> <p>A sex-specific test requires a diagnostic marker present in one sex and not the other; the source of such a marker in birds is the W sex chromosome, as this occurs only females (ZW) and not in males (ZZ). While at the University of Oxford (1987–1995), Griffiths identified a gene called the chromo-helicase-DNA-binding gene (CHD1), which is present in the sex chromosomes of all bird species. Although a copy of this gene is present on both male 'Z' and female 'W' sex chromosomes, there are sufficient differences between the male and female genes to use CHD1 as a sex-specific diagnostic marker. Griffiths successfully demonstrated that a DNA test based on the detection of the CHD1 gene could be used to identify the sex of a bird. The test was based on a routine laboratory technique, polymerase chain reaction (PCR), which was used to amplify a short fragment of CHD1 from DNA extracted from a drop of bird's blood, feathers or egg shell. However, these CHD1 fragments were of similar size in both male and female birds so, to differentiate the sex specific fragments, the PCR product must be cut with a restriction enzyme that cuts at a specific DNA sequence within the female but not the male. This adds an extra step to the test, increasing both the time required and the cost; furthermore, due to slight variations in the DNA sequence of the CHD1 fragments between bird species, the particular choice of restriction enzyme had to be optimised for each new bird species tested.</p> <p>On moving to University of Glasgow in 1996, Griffiths significantly improved the original PCR-based test. This new Glasgow test, published in 1998,<sup>1</sup> achieves a diagnostic result in a single PCR step, eliminating the additional laboratory processing and optimisation steps and thereby simplifying the test in terms of time required and cost. Furthermore, the Glasgow test also has greater specificity (thus a clearer result) than the earlier test or indeed other available DNA tests, a fact widely supported by extensive citation of the 1998 paper (1323, Scopus – excluding self-citations) representing the wide use by independent users.</p> <p>The Glasgow test amplifies a different region of CHD1, one that differs in length between CHD1-Z (which occurs in both sexes) and the female-specific CHD1-W. DNA from a male bird, therefore, yields a single PCR fragment (from the two Z chromosomes present in males), whereas the same PCR on DNA from female birds amplifies two products that are different sizes (one from the Z and one from the W chromosome). The additional 'W'-based product in the female DNA allows the</p>

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sexes to be clearly and easily distinguished.

**Key University of Glasgow researchers:** Dr Richard Griffiths (BBSRC Research Fellow 1996–2000; Lecturer 2001–2002); Dr R.J.G. Dawson (Postdoctoral Research Assistant, 1996–1997); Mrs Kate Griffiths (née Orr) (Research Technician, 1990–present).

**Key external collaborator:** Dr Mike Double (Postdoctoral Research Associate, Division of Botany and Zoology, Australian National University – assisted with manuscript preparation, providing samples and testing of the technique).

**3. References to the research**

Griffiths, R., Double, M., Orr, K. and Dawson, R. (1998) [A DNA test to sex most birds](#). *Mol Ecol.* **7**, 1071–1076. doi: 10.1046/j.1365-294x.1998.00389.x.

**4. Details of the impact**

The Glasgow avian sex test is a simple, yet effective, means to determine the sex of many bird species with minimal optimisation. The technology is also freely available, without requirement for license by users. These advantages have led to the test being adopted by commercial companies in the UK and USA, and by zoo-based research units worldwide, which have made the test internationally available to a broad range of users, including those involved in the management of conservation captive breeding programmes worldwide for some of the world's rarest bird species.

**Commercial use**

In 2000, the Glasgow avian sex test was adopted by Avian Biotech International, one of the largest providers of bird sex-determination tests in the USA. Avian Biotech International performs approximately 50,000 sex tests per year, 95% of which use the Glasgow avian sex test developed by Griffiths.<sup>a</sup> The test has been instrumental to the development of the company, and at an average cost of £13 per test, generates revenues of approximately £618,000 per year.<sup>a</sup> Avian Biotech International provides services to a broad user base including zoos, conservation organisations, commercial bird breeders and private bird owners across North America and Europe, as well as in Brazil, China, Japan and the Philippines. The company continues to use the Glasgow test because it represents '*...a simple, reliable, thus far unsurpassed molecular method to identify the sex of most of the approximately 9000 species of birds*' - Founder and Research Director of Avian Biotech International.<sup>a</sup>

Avian Biotech also provided services to a number of commercial bird breeding companies. Examples include Hurricane Aviaries in Florida, USA; Preferred Birds in Oklahoma, USA; Birds International in the Philippines; and Exotic Fauna NV in Surinam. Avian Biotech also works with industries such as Bayer AG, who sex a large number of quail for use in their veterinary medicines development work.<sup>a</sup>

The Glasgow test is also used by Biobest Laboratories Ltd., a large UK specialist in veterinary virology, serology and DNA diagnostics which undertakes avian DNA sex determination. Biobest Laboratories adopted the Glasgow test in 2002, and since 2008 has tested approximately 2,000 individual birds across a range of species for bird owners, collections, breeders, and zoos.<sup>b</sup>

**Use in avian conservation programmes**

Breeding programmes must maintain large, self-sustaining, genetically diverse captive populations; however, a single zoo might only have two or three individual birds. Zoos and other conservation organisations, therefore, collaborate to exchange birds in coordinated breeding programmes. In Europe, these are managed through the European Association of Zoos and Aquaria (EAZA), within European Endangered Species Programmes (EEPs) and the European Studbook system. Studbooks (also a component of EEPs) record the genetic history and movement of every bird within a given species in the programmes. Knowing the sex of each bird is a basic requirement for both EEP and studbook management.

### *Use in successful captive breeding*

Edinburgh Zoo uses the commercial services of Biobest Laboratories for sex determination. Since 2008, the zoo has sexed 352 individual birds from 39 bird species, nine of which are managed through EEPs. The majority of these tests (202 individual birds) are performed on Gentoo penguin chicks, an iconic colony for which the zoo is well known. This ensures that the colony maintains a stable 1:1 sex ratio, which is important because the birds breed as monogamous pairs. All bird species tested are listed on the International Union for Conservation of Nature (IUCN) 'Red List' of threatened species, which include four categorised as 'critically endangered' and three as 'endangered'. One species, the Socorro dove, is extinct in the wild; the fewer than 100 purebred birds that exist in captivity (which include four at Edinburgh Zoo) are vital for the species' survival.<sup>c</sup>

San Diego Zoo (SDZ) Institute for Conservation Research has used the Glasgow test for avian sex determination since 2000. While the majority of bird sex determination in the zoo is now performed by Avian Biotech, the test is also performed in-house as part of the zoo's Hawaii Endangered Bird Conservation Programme.<sup>d</sup> Since 2008, the zoo has used the Glasgow test on Hawaiian bird species, including the 'alalā (crow family), palila (a type of finch) and various species of Malkoha (members of the cuckoo family). The Hawaiian 'alalā is extinct in the wild, and the entire population of 110 individuals exists within two conservation programmes at SDZ. To date, 25 individual crow chicks have been sexed using the test, supporting the zoo's successful captive breeding efforts ahead of their reintroduction to the wild in early 2014. The palila is critically endangered, and since 2012 eight birds have been sexed. With the Malkoha species, despite trying a number of different DNA-based sex determination approaches, only the Glasgow test gave clear results, which has enabled five males and 18 females to be sexed.<sup>d</sup>

The Royal Zoological Society of Antwerp Centre for Research and Conservation has used DNA-based sex determination since 2003. Since January 2008 it has used the Glasgow test to determine the sex of 370 individuals across 55 bird species held at Antwerp zoo, 11 species of which are classified as threatened on the IUCN 'Red List'. One species, the Bali myna, is critically endangered, and seven hatchlings have been sexed since 2008, contributing to the strictly regulated captive-bred population of around 1000 birds worldwide.<sup>e</sup>

### *Use in successful reintroductions*

The Ara Project, a zoological park and conservancy in Costa Rica, is a customer of Avian Biotech International. Ara works with the Costa Rican Ministry of Environment and Energy to reintroduce and stabilise the populations of Scarlet and Great Green macaws, the two native and endangered macaw species in Costa Rica. The project uses the Glasgow avian sex test to aid their captive breeding programme, and determine appropriate sex ratios at release sites. Since 2008, 69 scarlet macaws and 34 great green macaws have been tested, all of which were subsequently released into the wild.<sup>f</sup>

The Seychelles warbler is a threatened bird species that originates on the tiny Cousin island in the Seychelles. In the 1970s the warbler nearly became extinct and was rescued by a conservation programme, which used Cousin as a source to establish populations on neighbouring islands. As part of continuous monitoring of the population, each of the roughly 60 birds hatched per year on Cousin are routinely collected from the wild, tagged and have their sex determined using the Glasgow test (performed at University of Groningen, Netherlands).<sup>g</sup> In the latest conservation programme in 2011, led by Nature Seychelles, a successful new population was established on the neighbouring private island of Frégate. The new population comprised 59 birds captured from Cousin, and because the sex of each of the Cousin birds is known, this enabled the translocation of a balanced sex ratio of birds.<sup>g</sup>

## **5. Sources to corroborate the impact**

- a. Statement provided by Founder and Research Manager, [AvianBiotech International](#), Florida; available on request.
- b. Data from [Biobest Laboratories](#) Ltd., Penicuik; available on request.

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- c. Data from Edinburgh Zoo/Royal Zoological Society of Scotland; available on request.
- d. Data from Genetic department, San Diego Zoo; available on request.
- e. Data from KMDA (Royal Zoological Society of Antwerp); available on request.
- f. Data from [Ara project](#). [Alternative [website](#)]; available on request.
- g. Data from the Seychelles Warbler Research Project, University of Sheffield; available on request.