Lost in translation or deliberate falsification? Genetic analyses reveal erroneous museum data for historic penguin specimens

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Historic museum specimens are increasingly used to answer a wide variety of questions in scientific research. Nevertheless, the scientific value of these specimens depends on the authenticity of the data associated with them. Here we use individual-based genetic analyses to demonstrate erroneous locality information for archive specimens from the late nineteenth century. Specifically, using 10 microsatellite markers, we analysed 350 contemporary and 43 historic yellow-eyed penguin (\textit{Megadyptes antipodes}) specimens from New Zealand’s South Island and sub-Antarctic regions. Factorial correspondence analysis and an assignment test strongly suggest that eight of the historic specimens purportedly of sub-Antarctic origin were in fact collected from the South Island. Interestingly, all eight specimens were obtained by the same collector, and all are currently held in the same museum collection. Further inspection of the specimen labels and evaluation of sub-Antarctic voyages did not reveal whether the erroneous data are caused by incorrect labelling or whether deliberate falsification was at play. This study highlights a promising extension to the well-known applications of assignment tests in molecular ecology, which can complement methods that are currently being applied for error detection in specimen data. Our results also serve as a warning to all who use archive specimens to invest time in the verification of collection information.

**Keywords:** historic specimens; \textit{Megadyptes antipodes}; New Zealand; error; fraud; assignment test

1. INTRODUCTION

Museum collections, archived in natural history museums worldwide, provide invaluable resources of materials and knowledge that are of utmost importance to science and society (Suarez & Tsutsui 2004). These collections currently hold an estimated total of three billion specimens, of which 7–10 million are bird skins (Brooke 2000; Pennisi 2000). The crucial role of museum collections in defining species and their ranges started with the diligent efforts of nineteenth century collectors, and even today this wealth of information still plays a vital role in the documentation of species decline and conservation status assessment (Shaffer et al. 1998; Collar & Rudyanto 2003). During the last two decades, the use of museum specimens as sources of DNA samples has been facilitated by advances in molecular techniques, initiating a vast increase in the use of such archived specimens in population and evolutionary genetic studies (reviewed in Wandeler et al. 2007). Comparative studies now frequently compare levels of genetic diversity over time, thereby inferring changes in population size and population connectivity (e.g. Miller & Waits 2003; Johnson et al. 2004; Larsson et al. 2008; Taylor et al. 2008).

The potential problems arising from working with low-quality DNA from historic specimens are well known and can be addressed using clear laboratory guidelines (Sefc et al. 2003, 2007; Pääbo et al. 2004), but any additional pitfalls arising from errors in specimen data are potentially much more complex (reviewed in Rasmussen & Prýs-Jones 2003). Specimen data are recorded on attached labels (and collectors’ notes when available) and the scientific value of specimens ultimately depends on the accuracy of these data. The minimum information typically associated with a specimen includes the identity, location, collection date and the name of the collector, but one or more of these entries may be missing. Additionally, these labels are also the most prone to error in specimen collection (Winker 2000). Most errors are found in identity and location, which can lead to false representation of a species’ distribution (Graham et al. 2004). Causes of inaccuracy in museum specimen data vary from simple mistakes or carelessness during collection or post-collection to serious cases of fraud (Rasmussen & Prýs-Jones 2003). Neither inadvertent mistakes nor cases of deliberate fraud have received

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2. MATERIAL AND METHODS

(a) Sample collection, DNA extraction and genotyping

Yellow-eyed penguin blood samples were collected in 2005–2007 on the South Island \( (n = 249) \) and sub-Antarctic Auckland and Campbell Islands \( (n = 101) \) of New Zealand. DNA was extracted and purified using 40 μg proteinase K in 5 per cent Chelex (Biorad; Walsh et al. 1991). Historic toe pad samples were obtained from 55 specimens collected between 1840 and 1944 on the South Island \( (n = 35) \) and sub-Antarctic Auckland and Campbell Islands \( (n = 20) \). These specimens are held in 15 museum collections around the world (for specimen details see table S1 in the electronic supplementary material). Following rehydration, toe pad samples were finely chopped and DNA was extracted using the Chargeswitch Forensic DNA Purification Kit (Invitrogen) or the DNeasy Tissue Kit (Qiagen) following manufacturers’ instructions.

All samples were genotyped at 10 microsatellite loci previously developed for yellow-eyed penguins \( (\text{Man03, Man08, Man13, Man21, Man39, Man47, Man50, Man51, Man54, Man55}) \; \text{Boessenkool et al. 2008}) \). Microsatellite primer sequences and polymerase chain reaction \( (\text{PCR}) \) conditions for modern samples are described in Boessenkool et al. (2008). PCR reactions for historic samples were performed in 10 μl volumes containing 2 μl DNA, 0.5 μM of
In the model, allele frequencies were assumed to be
Second, the assignment test implemented in STRUCTURE
was performed using GENETIX v. 4.05.2 (Belkhir et al. 1996–2004).

Allele frequencies and unique alleles were evaluated using GENETIX v. 4.05.2 (Belkhir et al. 1996–2004). Markov chain parameters employed for the historic samples were left at default values. For the historic samples we applied the admixture model with a uniform prior for \( \alpha \) bounded by a maximum of 10, and we set ALPHAPROPSD to 0.025. The migration prior (\( \nu \)) for the assignment test was set to 0.01, but to account for uncertainty in \( \nu \) we ran replicate analyses using \( \nu = 0.05 \) and \( \nu = 0.1 \). The outcome of the analyses was unaffected by the migration prior and we only present the results from runs with \( \nu = 0.01 \). The Markov chain Monte Carlo simulation was performed with a burn-in of 100 000 followed by 500 000 iterations. We also performed a clustering analysis without specifying any \textit{a priori} geographical sampling information as implemented in STRUCTURE, using the same parameter settings as for the historic samples described above. This analysis revealed the same pattern as the analysis in which geographical information of contemporary samples was incorporated in the model (data not shown).

3. RESULTS
DNA was successfully extracted (i.e. resulting in positive amplification of \textit{M. antipodes} microsatellite markers) from 43 of the 55 historic samples. Twenty-seven of the successful extractions were from ‘South Island’ specimens, whereas 16 were from purportedly ‘sub-Antarctic’ specimens (Campbell and Auckland Islands; figure 1). Thirteen historic samples had missing genotypes at one (six samples), two (one sample), three (two samples), four (two samples), five (one sample) or six loci (one sample). The amplification of a false allele was encountered in one out of a total of 1066 successful PCRs. Allelic dropout was detected in 36 out of 420 PCR amplifications of confirmed heterozygous historic samples. In 26 out of the 36 cases dropout occurred in multiple replicate amplifications of samples at specific loci.

All 350 modern samples amplified at all microsatellite loci with the exception of six samples from the South Island, which had missing genotypes for one (three samples), three (two samples) or five loci (one sample), respectively. The dataset revealed no evidence for linkage disequilibrium between any pairs of loci and no significant departure from Hardy–Weinberg proportions in the modern \textit{M. antipodes} South Island and sub-Antarctic populations (see table S2 in the electronic supplementary material for diversity indices per locus per population).

the 27 historic samples with South Island origin, 20 have high $Q$ (i.e. $Q > 0.80$) to the South Island population. The other seven samples do not assign strongly to either of the populations; their $Q$-values lie between 0.20–0.80 indicating that they potentially have admixed ancestry (Lecis et al. 2006; Bergl & Vigilant 2007). Note that of these individuals with admixed ancestry, two have missing data at four loci. Of the historic samples with purported sub-Antarctic origins, eight have high $Q$ to the sub-Antarctic population ($Q > 0.80$), whereas seven show strong membership to the South Island population ($Q > 0.9$ for four samples and $0.80 < Q > 0.90$ for three samples, respectively). One sample has weak evidence for mixed ancestry ($Q = 0.744$ to the South Island population), but this individual lacked genotypic data at five of ten loci. The eight sub-Antarctic samples that have strong membership to the South Island population are the same eight samples that were placed among the modern South Island samples in the FCA. Four of these eight specimens were reportedly collected on the Auckland Islands (three in 1893, one in 1894) and the other four were reportedly collected on Campbell Island (two in 1893, two in 1894). Interestingly, these eight specimens were all collected by the same collector, namely Henry Hamersley Travers (1844–1928), and are currently held in the same museum collection (American Museum of Natural History).

The modern sub-Antarctic population has 18 unique alleles, whereas only one unique allele was detected in the modern South Island population (see also Boessenkool et al. 2009a). It is particularly noteworthy, therefore, that the eight historic samples supposedly collected from the sub-Antarctic, but genetically categorized as South Island specimens (figures 2 and 3), do not possess any of the alleles unique to the sub-Antarctic. By contrast, five of the eight historic samples with
confirmed sub-Antarctic origin possess a total of nine unique sub-Antarctic alleles. The probability of recovering zero unique alleles (by chance) in our sample of eight suspect individuals would appear to be very low. We investigated this probability further by calculating the probability distribution of sampling unique alleles (figure 4), assuming these eight suspect individuals were randomly sampled from the sub-Antarctic population. We simulated random resampling of the genotypes (100 million iterations and allowing for the missing data present in the original sample) of the suspect eight individuals using probabilities based on the allele-frequency vectors of the modern sub-Antarctic population and recorded the number of unique alleles in our sets of resampled genotypes. Our simulation confirmed that the probability of having zero unique alleles is extremely low (figure 4) and over 99.99 per cent of our random draws resulted in genotype sets with one or more unique alleles.

4. DISCUSSION

(a) Erroneous origins of historic Megadyptes specimens?

Individual-based genetic analyses and evaluation of unique alleles for 10 microsatellite loci indicate that eight historic *M. antipodes* museum specimens have incorrect specimen data with respect to geographical collection location. We show that these eight specimens were not collected on the sub-Antarctic Auckland and Campbell Islands, as is stated on their specimen labels, but were in fact collected on the South Island of New Zealand. We have previously analysed these specimens for mitochondrial DNA (mtDNA) variation (Boessenkool et al. 2009a,b), and these results do not conflict with our microsatellite data. However, *M. antipodes* mtDNA lacks the phylogeographic structure required to reliably discriminate between sub-Antarctic versus South Island (Boessenkool et al. 2009b).

The historic expansion of *M. antipodes* (Boessenkool et al. 2009a) and the current pattern of migration (Boessenkool et al. 2009b) both indicate an asymmetric pattern of rare dispersal in this species, involving migration northwards from the sub-Antarctic to the South Island. It is not surprising, therefore, that a few South Island individuals show admixed genetic ancestry (figure 3; see also Boessenkool et al. 2009b). On the other hand, the single batch of eight purportedly ‘sub-Antarctic’ birds that genetically have South Island origins, clearly conflicts with observed dispersal patterns for *M. antipodes*. Perhaps most intriguingly, all eight of these birds are attributed to the same collector who purportedly obtained them within two years of one another.

The eight incorrectly labelled specimens were obtained by the American Museum of Natural History (AMNH) from Lionel Walter Rothschild the second Baron Rothschild (in 1932; Rothschild 1983), who in turn seems to have obtained the specimens from the collector Henry Hamersley Travers. The specimens have Rothschild collection labels as well as labels from H. H. Travers, which state the collection location (i.e. Auckland or Campbell Islands) and date (i.e. 1893 or 1894; M. LeCroy 2009, personal communication). An assessment of the sub-Antarctic voyages documented from the 1890s (Anonymous 1895; Headland 1989) revealed that it is unlikely that Travers or any other collector visited the Campbell and/or Auckland Islands in 1893. It therefore seems that, in addition to incorrect collection localities, the collection dates for at least five of the eight incorrectly labelled specimens are also incorrect.

Whether the erroneous collection data for the eight penguin specimens is attributable to incorrect labelling or to deliberate fraudulence remains unclear. Monetary gain is considered the most evident motivation for the deliberate falsification of specimen information and cases within ornithology provide some well-known examples of major specimen fraud (Rasmussen & Pyys-Jones 2003). While *M. antipodes* is more abundant on the sub-Antarctic islands than on the South Island, specimens from geographically remote locations, such as the sub-Antarctic islands would nonetheless have been more valuable than readily available specimens from the South Island. The path from initial suspicion of specimen fraud to conclusive proof is, however, a long one (Rasmussen & Pyys-Jones 2003). H. H. Travers was a well-known professional collector and taxidermist in nineteenth century New Zealand (Encyclopedia Company Limited 1897) and although some have questioned the veracity of some of his collection data (i.e. R.A. Falla in Murphy & Pennoyer 1952; Scofield 2005), no substantive proof has been found of any incorrect labelling until now. Alternatively, it is possible that Travers may have bought the penguin specimens from someone else: indeed, he apparently had a standing agreement with crew members of the New Zealand government steamer to collect specimens on sub-Antarctic islands for him (Warham & Bell 1979). It is therefore currently not possible to trace the exact origin or cause of the erroneous specimen labels. Nevertheless, further investigation of Travers’ specimens as well as others that have been collected on sub-Antarctic New Zealand Islands in the late twentieth century may shed more light on the possibility of dishonesty.

(b) Genetic analysis as a means to detect errors in specimen locality data

The detection of errors in archive museum specimen information can be an arduous and time-consuming
process that often relies on the availability of historical data such as collectors’ field notes. The development of molecular techniques has now facilitated the use of DNA in this process. Genetic data have successfully been used to detect sexing errors in museum bird skins (Lee & Griffiths 2003; Bantock et al. 2008) and to verify the identity of species (e.g. Hennache et al. 2003; Olson et al. 2005) or even eggs (Lee & Prýs-Jones 2008). In this study we present an extension of this approach by using individual-based population genetic analysis to detect errors in the locality data of archive museum specimens.

The use of individual-based genetic analyses, including assignment tests, to identify an organism’s geographical origin is well known (Waser & Strobeck 1998; Manel et al. 2005). The extension of these approaches to the verification of locality data of archive specimens is a promising new direction in museum science. This method, however, is not without its limitations. First, the reliability of the method depends on the availability of specimens with confirmed geographical origin that can be used to calculate population allele frequencies. Often such historic samples are not available, so one relies on the assumption that allele frequencies of modern samples are representative of the allele frequencies of the historic populations. In our study we fortunately had good knowledge of the study system, including the colonization history and dispersal patterns of M. antipodes, and we can therefore be confident that this temporal assumption was not violated. Nevertheless, when populations are known to have suffered severe bottlenecks, for example, verifying the validity of this assumption will be challenging. Second, successful assignment of specimens is contingent on the existence of sufficient genetic structuring among populations and the statistical power of the applied markers to detect such a pattern. If limited levels of dispersal exist, the method can still be applied to detect errors in a sample of specimens that were collected by the same collector, or for example on the same voyage, but the detection of single misinformed specimens will probably be unreliable. If researchers aim to verify the origin of a single specimen, the guidelines already developed for the use of assignment tests in the detection of wildlife poaching should be applicable (Manel et al. 2002). Importantly, high thresholds (e.g. a probability of 0.999 that an individual belongs to a specific population) would have to be satisfied before a specimen can be confidently assigned to a specific population (Manel et al. 2002). Furthermore, care has to be taken when choosing an assignment method, as some techniques require sampling of all potential source populations (Pritchard et al. 2000; Manel et al. 2002; Piry et al. 2004). The present study underlines the value of combining distinct genetic approaches to improve the reliability of error detection.

Using individual-based genetic analyses to detect erroneous specimen information should be regarded as complementary to the methods that are currently being applied for error detection in specimen data (see Knox 1993; Rasmussen & Collar 1999; Rasmussen & Prýs-Jones 2003; Peterson et al. 2004; Olson 2008). The strength of the genetic method, however, is that potential errors can be detected without a priori suspicion. With the continuously increasing number of population and evolutionary genetic studies that use archive specimens (Wandeler et al. 2007), there is considerable scope to apply these methods.

(c) Consequences of erroneous specimen data

Historical material is a limited resource and studies employing historical specimens are typically constrained by low sample sizes. The detection of errors in specimen data consequently imposes a dilemma: should specimens with erroneous information be retained in the study or should they be removed from the dataset? If samples are retained in cases where errors were detected in locality data, then all subsequent analyses could be performed with samples re-allocated to their true geographical origin. Although this appears to be a valid solution, such an approach can result in some form of circularity, as the genetic data used to verify specimen location will probably be the same data used for subsequent analysis. Alternatively, if specimens found to be incorrectly labelled are completely removed from a dataset, this may severely limit sample sizes for subsequent analyses. In the case of yellow-eyed penguins, for example, we removed the eight specimens with erroneous specimen data from calculations of temporal effective population size (\(N_e\) see Boessenkool et al. in press). As a result, the sample size of the sub-Antarctic population was too low to estimate the population’s temporal \(N_e\) but we still had sufficient data to estimate the temporal \(N_e\) for the South Island population (Boessenkool et al. in press). Whether samples should be retained or removed from the dataset clearly has to be decided on a case-by-case basis, as determined by the availability of additional samples, the specific analysis to be undertaken, and the type of error encountered.

The results of the present study serve as a warning to all those who use museum specimens in population genetic studies. Especially when working on endangered species or populations, the reliability of specimen locality data is essential. Mistakes in locality data can potentially confound inferences of historical population connectivity, identification of conservation units, effective population size estimates and associated management strategies. Although it was recently argued that physical specimens provide the most reliable evidence for assessing species ranges (McKelvey et al. 2008), our analysis shows that specimen data are only as reliable as the associated collection details. Researchers are encouraged to invest in the verification of specimen data to ensure that archive specimens remain a valuable resource for many years to come.

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