To pluck or not to pluck: the hidden ethical and scientific costs of relying on feathers as a primary source of DNA

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This article responds to the recent prominence of ornithological literature advocating the plucking or clipping of feathers to obtain DNA in avian studies. We argue that the practise of feather plucking or clipping should be strongly discouraged on both scientific and ethical grounds in the avian literature. Currently, despite claims to the contrary, it is not clear that feather sampling as a source of DNA has lower ethical impacts on birds than blood sampling. In addition, feather samples provide a smaller and less reliable biological resource, significantly jeopardising the short and long-term outcomes that can be gained by the sampling. In contrast, blood collection has been experimentally demonstrated to be relatively safe, subject to operators being skilled and following published guidelines, providing large yields of high quality DNA that facilitates archival storage of samples in a manner that the destructive sampling of feathers cannot.

In the past 20 years there have been major advances in molecular techniques, such that it is now possible to extract DNA from a number of avian sources other than the traditional blood sample (as defined by guidelines in Gaunt et al. 1999). For example, DNA has been effectively recovered from skin scrapings (Mundy et al. 1997) and even faecal material (Idaghdour et al. 2003), although by far the most commonly used alternative technique is extracting DNA from feathers (Taberlet and Bouvet 1991, Bello et al. 2001). Feathers sampled in this manner have either been plucked (the entire structure has been pulled from the live bird) or less commonly clipped (a portion of feather material is cut from a bird whilst leaving a portion of the rachis embedded in the skin). Our focus here is on obtaining DNA from feather material, and we shall refer to the invasive practises of either plucking or clipping as ‘feather sampling’ throughout to avoid confusion over the truly non-invasive practise of collecting freely moulted feathers.

Ornithologists have readily adopted feather sampling as a source of DNA material, with several articles recommending this as the preferred method of DNA collection, perhaps as the technique requires little training and storage of collected samples is relatively straight forward (Bello et al. 2001, Smith et al. 2003, Harvey et al. 2006). This has not escaped the attention of animal ethics committees at numerous institutions around the world; we have noted during conversations at recent ornithological conferences that these bodies are beginning to recommend feather sampling over blood collection, particularly to obtain genetic material. This view is supported by author’s recommending feather sampling as an ‘easier’ sampling method, relative to blood collection, to receive ethical permission for (Harvey et al. 2006). While we acknowledge that the ability to obtain DNA from small samples of feather material has opened up numerous research possibilities that might otherwise be logistically impossible to achieve (e.g., Griffiths and Tiwari 1995, Bush et al. 2005, Beja-Pereira et al. 2009), our problem lies with the fact that the procedure is advocated even for situations where a bird ‘in the hand’ could either be blood, or feather, sampled. In such situations, feather sampling is increasingly being advocated because it is suggested to inflict ‘minimal impact’ upon focal individuals (Bello et al. 2001, Smith et al. 2003, Harvey et al. 2006). This conclusion is routinely reiterated despite, to the best of our knowledge, no complete tests of the practise. On the contrary, there is a significant body of literature suggesting that the reverse may well be true; feather sampling is likely to have significant and far ranging impacts on individual fitness and even survival as we outline herein. Given this, we argue against feather sampling being the primary source of DNA material in avian studies until thorough and rigorous assessments of current known impacts have been undertaken and potential mitigation strategies developed, as should be required of any scientific technique (Gaunt et al. 1999). Note that we support the obtainment of feather material, after applying an appropriate degree of ethical rigour, when no other means exists for obtaining these data. However, readers should note that many of the issues raised herein apply to all eventual uses of data obtained via feather sampling when subject death is not
the end point (as we would hope is the case in the vast majority of studies).

Even in the event that feather sampling is without major health implications for sampled individuals, we believe that the most ethical approach is one that maximises the outcome from each time an animal is handled and sampled, not only for the current project but for unforeseen projects long into the future as advances in molecular technology open new opportunities. Many researchers may not be fully aware of the vast difference in quality of genetic data obtained from feathers in comparison to blood samples (e.g., Sacchi et al. 2004, Harvey et al. 2006, Maurer et al. 2010), a difference that may lead to the complete failure of projects to reach their initial aims. Therefore, we discuss two main issues: 1) that feathers yield low amounts of poor quality DNA that are potentially unlikely to meet the molecular requirements of a study, and 2) that the impact of feather sampling on focal individuals is largely unquantified, although several lines of evidence suggest that these effects may be pervasive.

**Feathers yield low levels of relatively poor quality DNA**

Whilst numerous studies have been able to extract DNA from feather material, the success of these studies typically falls below the complete dataset required when investigating brood sex ratios or mating strategies (e.g., Mc*N*Donald et al. 2005, Pryke et al. 2010). This is due to both the poor quantity and quality of feather-sourced genetic material. Feathers typically provide very low DNA yields in comparison to even a very small amount of avian blood/tissue, thanks to the nucleated nature of avian red blood cells. Moreover, even if sufficient DNA is obtained from the feather, it is typically of a much lower quality than blood-sourced material, partly because there is a reduced proportion of DNA relative to other potential PCR inhibitors, such as proteins like melanin and keratin, which can hinder extraction and amplification.

One problem that results from extracting DNA from low ratio samples, is that in these conditions it is necessary to further dilute the DNA to very low levels to reduce the effect of inhibitors such as proteins on the PCR. Coupled with the low amount of available DNA to start with, this can lead to a higher likelihood of contamination than when PCR is performed from a more concentrated DNA target (e.g., blood). Contamination occurs when the DNA from the target sample is so low that the PCR can amplify trace amounts of DNA from other sources such as other samples in the laboratory, any researcher that has collected the sample or even other organisms such as microorganisms.

In addition, the DNA present in the tip of a feather is not as well preserved as blood taken and stored directly into preservative such as Queens lysis solution or 95% ethanol. This generally manifests in the proportion of samples that fail to amplify once extracted, inevitably leading to resampling, lost opportunity and additional research costs. Higher costs stem from the low quantity of DNA extracted, as this frequently necessitates reliability sampling via splitting the sample across multiple tubes and reactions. This has a double effect of further reducing the amount of material available, whilst also increasing consumable and reagent costs. However, in many situations where feather-sourced material is used, the typically low yield may result in additional material not being available for both additional reliability tests, but also other researchers into the future as no material remains to be archived. This further highlights a long-term problem with feather-sourced DNA, in that by their conclusion most studies have little to no material and/or DNA remaining-one feather provides just one chance to extract DNA. Therefore, given the difficulties in extraction and amplification to assure a result from each individual, one would have to remove multiple feathers. This significantly reduces the long-term value of the research, as a) studies cannot be repeated if a subsequent discrepancy is noted, b) material is not available for further investigation by the same group or through collaboration with other groups to investigate temporal or regional differences, and c) there is no opportunity to revisit an archival source of individual or population samples for a later study that might present a valuable additional research opportunity.

A review of the various success of different projects is beyond the scope of this note, however several recent papers provide interesting examples in how these factors combine to negatively influence scientific outcomes. Harvey and colleagues (2006) provide a rare exception, as they extract sufficient DNA to correctly sex 102 black-capped chickadees *Poecile atricapillus* from a single rectrix feather from each individual. However this success required additional PCR cycles and slightly different protocols, as is often the case with feather-sourced DNA (Sacchi et al. 2004). Whilst in this study (Harvey et al. 2006) they may have been able to validate the outcome of the molecular sexing based on feathers it is often very difficult to do so. When a parentage analysis is conducted with microsatellite markers, a poor quality or dilute DNA sample sometimes produces a spurious allele or allelic dropout (e.g., Martinez and Burke 2003, Griffith et al. 2010). Typically, in the context of a parentage analysis this will provoke further examination because the genotype at one or more loci do not match or are implausible, and the sample will typically be subject to a process of re-extraction and amplification of DNA. One particular problem with sexing studies is that cross contamination from one sample to another, or allelic dropout, is virtually impossible to detect. For example if a sample is contaminated with a neighbouring sample in a study investigating molecular sex there is no obvious sign that anything is wrong (unlike in a parentage analysis in which multiple loci to a large extent validate one another). Whilst these problems can also occur with samples extracted from a blood sample they are more likely to occur when the target DNA is at low concentration in the initial sample (e.g., Arnold et al. 2003).

To an extent, even when a study has succeeded in amplifying DNA from all of the individuals sampled with a poor quality source of DNA, it is likely that these results are not as reliable as had the DNA been extracted from a more concentrated source of DNA. The extent to which different studies will succeed in correctly achieving good molecular outcomes will depend not only on the skills and care with which the sampling and laboratory work are conducted, but also on the type of genetic analysis conducted with some
marker sets more prone to spurious amplification than others. Success will also be driven by the length of sequence being investigated: the longer the length of sequence being amplified (either mitochondrial or nuclear), the lower amplification success tends to be (Broquet et al. 2007), a fact not limited to feather-sourced material.

For example, despite numerous precautions, Segelbacher (2002) was able to successfully analyse just 60% of plucked, and 50% of moulted feathers sampled. A recent review of the literature found similarly mixed success rates of amplification of DNA extracted from feathers, with success rates of up to 90% reported, though 50–60% was more common (see Beja-Pereira et al. 2009 for a review). The impact of these missing values is significant. This was highlighted in a study (Maurer et al. 2010) where 71% of feather samples were able to be successfully assessed, however this figure includes samples for which data on only 2–5 of the 8 loci tested were obtained. The principal reason for such low return using feather material was the low volume of extracted DNA, which prevented samples that did not amplify on the first run being re-tested (Maurer et al. 2010). Crucially, this resulted in the study being unable to determine if a maternal allele truly indicated an extra-pair mating, or was simply the result of confounds such as allelic dropout (which is more prevalent when the target DNA is very dilute or of poor quality). While methods have been proposed to minimise this problem (Knapp et al. 2009), these should only ever be seen as potential mitigators when no other option is available, rather than a justification of inferior basic data collection protocols. In contrast, when the same population was assessed using blood samples, all samples were successfully genotyped at all 8 loci (Maurer et al. 2010).

Two recent projects in our own institution (where the Animal Ethics Committee usually advocates feather sampling rather than blood sampling) further highlight the dangers inherent in relying upon feather-sourced DNA. The first involved captive fowl *Gallus gallus* sampled to assess paternity (Wilson et al. 2008). Initially, 6 rectrices were clipped for this purpose, with DNA extracted from these samples clearly visible on gels. However, when amplified at a set of 6 microsatellite loci using species-specific primers, these samples provided extremely inconsistent results across multiple runs of a given sample. A protracted period of troubleshooting failed to overcome this issue (D. Wilson pers. comm.). Subsequently, all 42 birds had to be recaptured and put through another collection protocol to facilitate blood collection; all samples were then successfully genotyped without further problems (Wilson et al. 2008). This double-handling involved considerable extra monetary and time costs, whilst also increasing the stress of focal birds to achieve the goals of the study. Luckily, as the birds were captive and long-lived they were all still available for this later blood sampling.

Additional sampling may not always be feasible or even possible, as the second case study from our institution demonstrates. The Australian brush-turkey *Alectura lathami* has one of the most extreme forms of parental care for any bird, in which eggs are incubated by the heat produced by composting vegetation in a mound built and tended by a male (Jones 1988). The offspring are completely independent on the day that they hatch and dig their way out of the mound, and are even apparently capable of flight within 24 hours (Jones 1988). As all of the eggs in a mound are laid over a period of months (by many different females) they will hatch over a very extended period. Therefore the only way to investigate hatching sex ratios or the maternity or paternity of the eggs in a mound is to excavate eggs and incubate them artificially. Unfortunately, due to their relative small size at hatching, it was also not possible to band or mark offspring for later identification. In this particular study, eggs were collected (under permit and ethical approval) for paternity analysis from a number of incubation mounds that had each been the focus of many months of detailed video analysis of male and female reproductive behaviour. When chicks hatched in the artificial incubator in the laboratory, a couple of feathers were plucked from the precocial young prior to release at their natal mound (D. Wells pers. com.). At the conclusion of the field component of this very labour intensive project, an attempt was made to extract and amplify DNA from these feathers. Unfortunately, the first attempt was not successful and, as sampling feathers is by nature a destructive process, no samples remained for any further attempts. This failure clearly impacted upon the outcomes of the project, and is presumably far more common than reported given null results are rarely published. More worryingly, a so-called ‘less invasive’ procedure has clearly resulted in great disturbance to the focal animals with little or no scientific gain in both examples, an issue to which we now turn our attention.

**Feather sampling is likely to significantly impact subject fitness and survival**

The primary argument for feather collection largely hinges on its ‘minimal impact’ (e.g., Grubb 1989; Smith et al. 2003; Harvey et al. 2006), despite these statements being based on no rigorous assessments. In contrast, a number of lines of evidence suggest that feather sampling is very likely to impart large costs upon focal individuals in a number of areas:

**Flight performance:** As more DNA can be extracted from larger feathers (e.g., Segelbacher 2002), many researchers advocate the sampling of flight feathers (Smith et al. 2003; Harvey et al. 2006). This is despite numerous studies investigating the impact of moult on flight performance as outlined below. Moult costs are typically experimentally simulated by feather plucking or clipping, allowing these experiments to also shed light on the impacts of feather sampling for DNA purposes. These studies show that even if wing shape and/or area is maintained following feather sampling, flight performance suffers markedly. For example, when two primaries on each wing of European starlings *Sturnus vulgaris* were either fully (clipped level with the skin) or partially clipped, flight performance fell relative to the level of asymmetry of wing shape and reductions in feather length (Swaddle et al. 1996). Worryingly, manoeuvrability and take-off trajectories were the most markedly affected traits, both of which are presumably critical to avoiding predators (Thompson et al. 2010). A similar study assessed ruby-throated hummingbird *Archilochus colubris* flight under controlled conditions after manipulating air
density during flight. Those with clipped or naturally moulting wings (including moult that influenced only secondary flight feathers) had the lowest capacity for hovering flight and the slowest air speed, producing comparably less mechanical power during flight at a much greater metabolic cost (Chai 1997, Chai and Dudley 1999). Remarkably, over time, the birds were able to increase their flight efficiency by reducing their body mass (Chai and Dudley 1999). This strategy may not always be possible in the field, where reduced resource availability during cold nights/migration would likely inflict significant costs. Nevertheless, a number of species are able to undergo moulting wings (including moult that influenced only secondary or retrix feather, a technique that leads to gross rather than subtle asymmetry and thus higher costs (Swaddle et al. 1996). These are the feathers favoured for DNA analysis, presumably given their size, however all would appear to carry significant costs on flight performance post-sampling.

**Physiological and metabolic costs**

Considerable evidence from the literature investigating moult suggests that generating replacement feathers incurs significant costs. This can be seen across a number of physiological measures, chiefly basal metabolic rate that can rise by as much as 10% in this period (Vézina et al. 2009). Rather than being a primary cost of feather production per se, these increases seem more closely linked to factors such as overcoming changing thermal conductance (Dietz et al. 1992, Lindström et al. 1993). These costs may be considerable, as demonstrated by Nilsson (1994) who plucked a single pair of retrices from European nuthatches *Sitta europaea* and monitored the length and mass of regrown feathers relative to food availability. Birds sampled during summer, a typical moulting period when food was plentiful, grew heavier feathers at a faster rate, whilst those plucked in winter had the slowest feather growth rates and produced the lightest and most asymmetrical feather pairs. Tellingly, those birds fed supplemental food during winter produced intermediate feathers, strongly implicating food restrictions as the source of this variation. Given this, sampling even small numbers of feathers during a critically stressful period of the life cycle may lead to significant costs in focal individuals, a possibility that requires further investigation.

**Alterations of individual status/attractiveness**

More subtle costs to feather replacement may also be incurred that cannot be measured by simple capture-recapture data. For example, male dark-eyed juncos *Junco hyemalis* have white patches on their rectrices, the size of which scales positively with both attractiveness to females and competitive ability against rival males (McGlothlin et al. 2007). Monitoring four regrown rectrices following plucking, a significant positive relationship between regrown tail patch size and the level of protein in a diet was found (McGlothlin et al. 2007). Thus feather sampling of this species during a protein-limited period would handicap the mating success and fitness of any male captured. Similar relationships are likely in other species, where feather colour is also influenced by diet (McGraw 2007), resource availability (Griggio et al. 2009), or season (Smith 1997).

**Confounding factors potentially exacerbating these effects**

At present there is insufficient data to predict the degree to which the above factors will influence individuals. Impacts are likely to covary with season and thus both the time and resources individuals can access to replace sampled feathers (Nilsson and Svensson 1996, De la Hera et al. 2010). Costs associated with poorly regrown feathers or feather gaps (if feathers are clipped) persist until they can be replaced during a typical moult period. These impacts are likely to be long-lasting, with poor quality feathers negatively impacting fitness even in subsequent breeding seasons (Nilsson and Svensson 1996). As poor quality feathers typically degrade faster than higher quality feathers (Dawson et al. 2000), any asymmetry or thermal conductance induced from feather sampling at inappropriate periods is likely to increase in scope over time. As the sexes may also follow different moulting strategies (Serra et al. 2010), the impacts may further differ within species. Considerable variation within populations is also likely, as feather clipping significantly alters foraging in the days immediately after sampling (Tsurim et al. 2010), presumably due to reduced flight performance and increased perceived predation threats (Thompson et al. 2010). Given this, birds in better condition at the time of sampling may be able to mitigate these costs more effectively. Together, these studies demonstrate many potential avenues for subtle and long-lasting impacts from feather sampling, none of which are apparent during the collection phase.

**The case for blood collection**

In contrast, several researchers have investigated the impacts of blood collection (reviewed in Gaunt et al. 1999, Sheldon et al. 2008). As long as collectors are suitably trained and guidelines followed, where a maximum of 2% of blood volume over any 14 d period or 1% at any given sampling period is obtained (Gaunt et al. 1999), blood collection has not altered fine-scale behavioural measures. We are aware of only one study where blood sampling has been claimed to increase mortality (Brown and Brown 2009). Indeed, much of the concern with blood collection appears to stem from anthropomorphic views on blood loss. However, as birds don't suffer from acidosis (Hoysak and Whitehead 1991), they can, in theory, tolerate far greater blood loss than equivalent sized mammals, although this should not be used...
as a justification for exceeding established sampling guidelines.

We are aware of only one study claiming decreased survival following blood sampling (Brown and Brown 2009) in the cliff swallow Petrochelidon pyrrhonota. However, these birds may have already been under considerable stress prior to sampling, as the population was heavily infested with ectoparasites and living in a highly arid environment. Under these conditions, the additional stress involved with handling and blood loss may have increased mortality from one year to the next (Brown and Brown 2009). However, there are a number of issues with this study (Voss et al. 2010), such as the inability to separate dispersal and mortality effects, and differential handling periods for bled and non-bled birds. Strangely, the taking of small (0.3–0.6%) over large volume blood samples (0.9–1.2% of average body weight) caused the greatest increases in mortality (Brown and Brown 2009), contrary to expectations under the author’s hypothesis that blood loss drives mortality post-sampling. Larger scope reviews of the process (Gaunt et al. 1999; Sheldon et al. 2008) have failed to find evidence of a systematic effect described by Brown and Brown (2009), indicating that at present there is little support for this result being systemic. Regardless, what these works do highlight is the need for researchers to examine the impacts of any sampling method on their focal system, make appropriate sampling adjustments to mitigate risks and, critically, publish their findings for dissemination to other ornithologists.

Thanks to the presence of nucleated blood cells, a relatively small blood sample (<50 µl) provides a greater opportunity for the initial extraction of DNA than a feather, and a template that will outperform feather-sourced material in the polymerase chain reaction that is the basis of most current molecular analysis (Harvey et al. 2006, Maurer et al. 2010).

An additional benefit of the amount of DNA readily available in an avian blood sample is that there is likely to be considerable DNA produced during the first extraction. Indeed the majority of a given blood sample typically remains following initial analyses, providing a long-term resource for other scientists/projects to utilise long into the future, reducing the need for researchers to collect additional samples. Archival sets of blood were extensively and very successfully re-visited for the determination of gender following the development of sex markers (Griffiths et al. 1998), enabling studies to investigate new questions about the evolution of maternal effects and sex ratio adjustment without having to subject additional animals to further invasive procedures (e.g., Hartley et al. 1999, Arnold et al. 2001, Thuman et al. 2003). In all of these cases, after this additional research was conducted, most of the blood from each individual remained because only a small proportion (<10%), was used in the chlex extraction of DNA (pers. obs. S. Griffith), leaving the blood sample archive largely intact for further study. An archive of appropriately stored blood will contain an almost unlimited resource for further research of the DNA of the individual bird, or even the parasites that lived in its blood (e.g., Bensch et al. 2000).

For several decades, a biologically inert stored avian blood sample has provided plenty of scope for analyses of nuclear DNA for studies of parentage, micro and macro population genetic structure, dispersal and phylogenetics (Sheldon et al. 2008). Furthermore, avian blood also contains mitochondrial DNA, albeit at a lower concentration than nuclear DNA. Mitochondrial DNA will provide an alternative perspective on an individual’s evolutionary history, though care must be taken to discriminate between mitochondrial DNA and paralogous copies of mitochondrial genes that have become incorporated into the nuclear genome (Sorenson and Quinn 1998, Bates et al. 2004). Avian blood samples also provide a target for future studies based on exciting, current developments. Telomere biology is an area of current intense research activity with recent studies suggesting that variation in telomere length and loss across individuals relates to an individuals survival prospects and life-history (Monaghan 2010). Whilst there are currently a variety of ways in which telomers are measured, and these are based on the type and quality of tissue available, it is certainly possible to get a biologically relevant assay from an archived blood sample (Monaghan 2010).

It seems likely that the investigation of telomeres in good archived blood samples from well-studied avian populations will make an important contribution to the future understanding of telomere biology, given that a number of important evolutionary questions concerning telomeres are focused on the type of life-history data that is often well represented by avian studies.

Another important avenue of future opportunities for those holding or generating archives of avian blood samples will be in genomics. In 2010 the zebra finch genome was published (Warren et al. 2010), providing a resource, along with the previously published chicken genome, for new lines of molecular inquiry that wish to explore the DNA that underpins all avian diversity at the species and individual level (Balarakrishnan et al. 2010).

In addition to the veritable treasure trove of DNA-based information contained within it, blood also conveniently facilitates assessment of other indicators, such as environmental pollutants. While feather plucking has been used to test for toxic chemicals in birds, feathers represent the concentration of pollutants during their formative stage only and may have significant contaminants on their outer surface. On the other hand, blood represents current circulating concentrations, and correlates much better with concentrations found in internal organs and tissues (Van den Steen et al. 2007, Summers et al. 2010). As both feather plucking and blood collection require a similar level of disturbance (i.e., capture), it seems prudent to simply take a blood sample in this and many other sampling scenarios given the additional scientific information likely to be obtained.

Conclusions

While molecular technology has advanced to the point where it is now possible to extract DNA from an ever-expanding array of sources, ornithologists should pause before adopting new techniques wholesale to determine if the scientific benefits outweigh the costs and inadequacies of these procedures. In our opinion, this is not the case with feather sampling from both an ethical and scientific viewpoint. Reliance upon feather sampling is likely to cause
significant distress to focal individuals that may have far-reaching impacts, whilst providing only poor quality DNA and thus fail to accomplish the goals of current and future research. Together, these factors argue strongly against feather plucking or clipping being used as the primary method of obtaining DNA from birds. While feather sampling has often been described as a ‘non-invasive technique’ (Bush et al. 2005, Beja-Pereira et al. 2009), we hope that we have clearly demonstrated herein that on current evidence this statement and assumption seems highly unlikely. Instead the term ‘non-invasive’ should be restricted to methods that do not require the capture and handling of the subject in any manner, such as collecting moulting feathers in areas and periods when the focal bird is not present. Further research on the impact of feather sampling across a range of species is clearly warranted given the widespread assumption that this practise has a limited impact, and stimulating this research is one of the goals of this manuscript. We acknowledge that sometimes blood collection is not possible and there may be no apparent alternative to feather sampling, however would recommend researchers strongly consider non-invasive techniques such as collecting moulting feathers in these scenarios (e.g., Beja-Pereira et al. 2009). It must be emphasised however that these techniques typically achieve marginal success rates (Segelbacher 2002, Bush et al. 2005, Gebhardt and Wait 2008). Until further data is gathered, we strongly recommend that feather plucking, as a greater provider of DNA material than clipping, be undertaken only when no reasonable alternative exists. Sampling should be restricted to relatively benign periods of a species life cycle, such as non-breeding periods when mass gains are high. Observer convenience is not an acceptable reason to sample outside of these periods, and wholesale calls for plucking flight feathers prior to assessment of the risks involved (e.g., Smith et al. 2003), particularly during critical periods such as migration (e.g., Harvey et al. 2006) are in our opinion irresponsible on current evidence. Until the true impact of feather plucking and clipping has been experimentally assessed and mitigated, we recommend that ornithologists, once suitably trained, use proven blood collection protocols (Gaunt et al. 1999) to obtain DNA samples, thereby ensuring both the best chance of project success, minimal disturbance to focal individuals, and the creation of an archive that will provide long-term potential for further research.

Acknowledgements – Michael Gillings, David Wells and David Wilson provided information on their recent experiences. Funding whilst this article was prepared was partially provided by a Macquarie Univ. Res. Fellowship to PM and an ARC QEII Fellowship to SG (ARC Grant DP0881019). Staffan Bensch and two anonymous referees provided helpful comments on the manuscript.

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