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DNA from keratinous tissue Part II: Feather

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SUMMARY

Although good quality DNA can be recovered from the base of the calamus of freshly sampled feathers, as from other fully keratinized tissues such as nail or hair shaft, the quality and quantity of DNA in the majority of feather structures is much poorer. Little research has been performed to characterize the quality of this DNA is, and thus what a researcher might be able to achieve when using feathers as a source of DNA. In this review, we expand on our companion article detailing the quality of DNA in nail and hair, by synthesizing published, and new preliminary genetic data obtained from feathers. As with nail and hair, we demonstrate that although DNA can, in general, be recovered from all parts of the feather, the quality of such DNA varies. As such, although one can expect *a priori* that genetic analyses are possible on the feather, for PCR based analyses, it is extremely difficult to predict the size of amplicon that can be used in such analyses. However, PCR-free genetic analyses that can exploit much smaller DNA fragments may promise to be a powerful tool for future exploitation.

1. Introduction

Stemming from germinal cells at the bottom of an invagination in the skin (Prum and Williamson, 2001), a common feature of both hair, nail and feather is that during development, the cells that ultimately form the bulk of these tissues undergo keratinization, a process in which they fill themselves with keratin. During the cell death that accompanies this process, the organelles and DNA within the cells is degraded, making these tissues a challenging source of DNA for genetic study (e.g. McNevin et al., 2005). Despite this, as with other predominantly keratinized tissues such as hair, horn and nail (see Bengtsson et al., 2011), as well as reptile and fish scales (e.g. Fetzner, 1999; Yue and Orban, 2001; Feldman and Spicer, 2002), feathers have been successfully exploited as a source of material for genetic studies. In 1984, Marsden and May reported that protein, of suitable quality for electrophoretic study, could be 'non-destructively' recovered from within the shaft of freshly plucked feather (Marsden and May, 1984). In 1991, following the growing availability of polymerase chain reaction, Taberlet and Bouve demonstrated that a freshly sampled feather also yielded PCR amplifiable DNA (Taberlet and Bouve, 1991), and Ellegren reported similar success using feathers from museum specimens. While Taberlet and Bouve suggested that the source of their DNA may have been the presence of pulp cells attached to the keratinous parts of the feather, as opposed to within the keratin of the feather itself (Taberlet and Bouve, 1991), Ellegren (1991) argued that, given the age of his samples (over 100 years), it was unlikely that pulp cells had survived in the material, thus demonstrating that DNA could be recovered even in the absence of the pulp (although see our comments later). Subsequent to these initial studies, feathers have been used in a range of genetic analyses. From modern samples these include analyses of mitochondrial DNA (mtDNA) (e.g. Taberlet and Bouve, 1991; Mundy et al., 1997b; Morin et al., 1994; Srikwan and Woodruff, 1998; Haddrath and Baker, 2001; Petersen et al., 2003; Segelbacker, 2002; Horváth et al., 2005) and nuclear DNA (nuDNA) – in particular for molecular sexing of birds (e.g. Grant, 2001; Malagó et al., 2002; Jensen et al., 2003; Horváth et al., 2005; Harvey et al., 2006; Wang et al., 2006; Constantini et al., 2008; Ong and Vellayan, 2008) but also to recover microsatellites (e.g. Mundy et al., 1997a; Segelbacker, 2002; Horváth et al., 2005; Höglund et al., 2007; Gebhardt et al., 2009), and even viral pathogens of the birds (e.g. Borenshtain and Davidson, 2002; Davidson and Borenshtain, 2002; Sung et al., 2002; Zavala et al., 2002; Davidson and Borenshtain, 2003; Renz et al., 2006; Davidson et al., 2009). Historic (that is, from museum and archival collections), and even ancient samples have also been used as a source for both mtDNA (e.g. Ellegren, 1991; Robinson and Matthee, 1999; Payne and Sorensen, 2002; Rawlence

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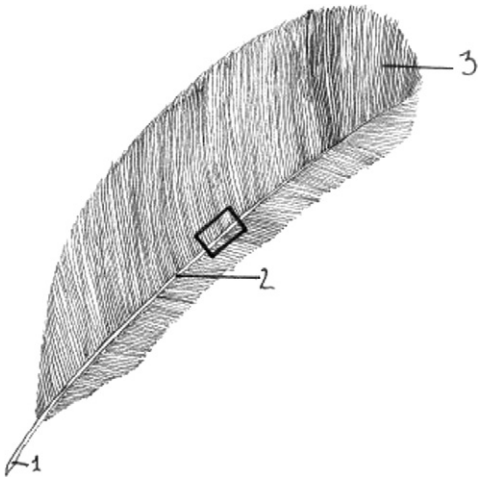

Fig. 1. Schematic representation of a pennaceous contour feather. (1) Calamus. (2) Rachis. (3) Barbs. For close up of barbs see Fig 2.

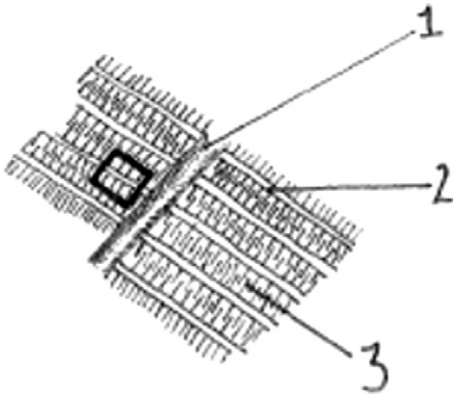

Fig. 2. Close up detail of rachis, ramus and barbules. (1) Rachis. (2) Ramus. (3) Distal and proximal barbules. Fig. 3 is a close up of the region enclosed by a square.

et al., 2009) and nuDNA (e.g, Sefc et al., 2003; Horváth et al., 2005).

Despite their use as a source of DNA for almost 20 years, however, much fundamental information is lacking regarding the quality and quantity of DNA in feathers. The feather is made up of several parts (henceforth referred to as feather 'structures': the calamus (sometimes referred to as root), the rachis (stalk) and the barbs (formed by the ramus and proximal/distal barbules) (Prum and Williamson, 2001) (Figs. 1–3), and to date there have been few systematic studies as to how the DNA quality varies throughout these structures. Although, as detailed above, studies have reported the recovery of many kinds and qualities of DNA from feather, in many cases it is difficult to infer general trends directly from the

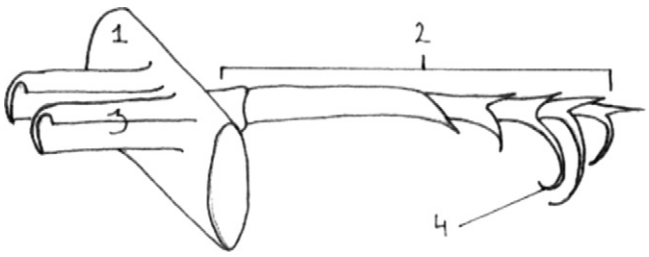

Fig. 3. Schematic representation of the ramus and barbules. (1) Proximal barbule. (2) Distal barbule. (3) Proximal barbules are hooked on the upper edge, and (4) Affix to distal barbules on the next ramus, to confer rigidity on the feather.

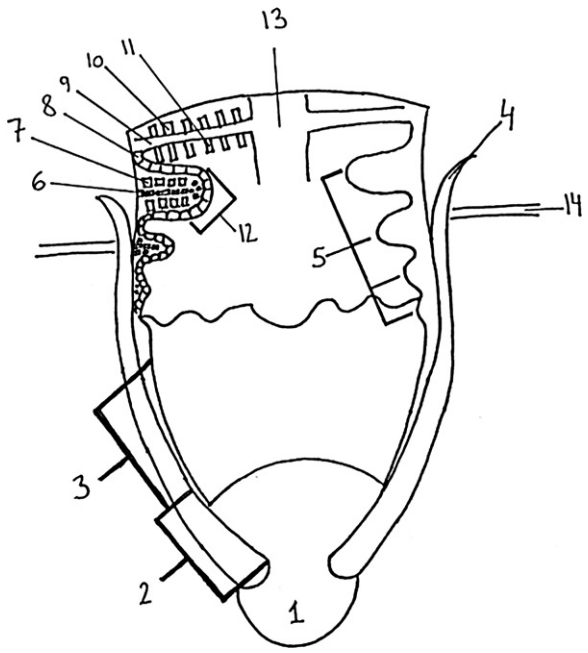

Fig. 4. Schematic reconstruction of the feather bulb with different stages of feather growth. (1) Dermal papilla. (2) Proliferation zone. (3) Ramogenic zone. (4) Feather sheath. (5) Barb ridges. (6) Axial plate. (7) Barbules plate. (8) Marginal plate. (9) Ramus. (10) Distal barbules. (11) Proximal barbules. (12) Growth zone of the barb ridge containing undifferentiated cells. (13) Rachis. (14) Dermis.
Modified from Prum (1999).

results, as the feathers studied may, or may not have included remnants of the pulp or other cellular remnants around the pulp, both derived from non-keratinized tissues that would contain high quality DNA. Furthermore, unless explicitly treated with DNA degrading solutions such as dilute bleach, it is possible that results may derive from DNA present on the outside of the feather, such as shed skin cells or saliva. The question of where and how the DNA survives is not simply of academic interest, but has practical benefits, as in many situations researchers may not have access to whole, freshly sampled feathers, and thus should the quality of DNA vary within a feather, this would shape their research strategy. The purpose of this review is to outline the current state of understanding in light of feather structure.

2. Feather structure and its implications for DNA studies

A typical vaned feather is structured around a hollow shaft (Fig. 1). The base of the shaft is the calamus, and the very end of this is referred to in some previous DNA-studies as the 'basal tip' or 'root'. The calamus extends into the rachis, from where the barbs radiate in a straight line on each side (Figs. 1 and 2). Each barb consists of a shaft, called the ramus, each of which has a number of barbules on both the proximal and distal side (Fig. 3). The proximal barbules point towards the tip of the feather, and each has little hooklets on the tip. The distal barbules point towards the base of the feather. A distal barbule is flattened and slightly curved dorsally, creating a shallow groove called the distal flange. The hooklets of the proximal barbules extend over and between the distal ones, and their hooklets fasten in the flanges of the distal barbules in a zipper-like manner. Down and plumulaceous feathers lack the hooked tips (Prum, 1999; Prum and Williamson, 2001). Some feathers also have an after-feather, which is a small feather structure placed at the bottom of the rachis (Prum, 1999; Prum and Williamson, 2001).

The growth zone of the feather is situated at the base of a follicle on the skin of the bird (Fig. 4). Here, the dermal pulp from