

Transforming Museum Specimens into Genomic Resources*

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Abstract. Scientists have been studying ancient DNA for three decades, but next-generation sequencing (NGS) has made the process of sequencing ancient DNA much easier. This technological leap has huge potential for genomic analysis of the very museum specimens that already form the foundation of prior knowledge about biodiversity. We are still determining which methods of preparing ancient DNA for NGS work best for various questions, especially with regard to whether whole genomes need to be sequenced or whether a subset of the genome is more desirable and computationally tractable. In this chapter, we discuss different methods for preparing ancient DNA for NGS, and various analytical issues specific to NGS data output from ancient DNA sources. We focus mainly on birds and discuss several case studies where NGS has been applied to very old museum specimens, like subfossils, as well as younger specimens, like those from museum study skins collected in the last century. Although few recently published studies using NGS are about ancient DNA from bird museum specimens, the number is expected to grow rapidly. The case studies demonstrate that systematics and

taxonomy are important applications of NGS to museum specimens, and that plenty remains to be learned from specimens about population-level processes such as the genetics of changes in population size, some of which have led to extinction. Better methods of extracting ancient DNA from museum specimens are badly needed, as well as careful consideration of how the research community archives DNA extractions and the billions of DNA sequencing reads now being produced from museum specimens on NGS platforms. Methods for correcting errors that occur during NGS, as well as those introduced during the process of DNA degradation in the specimen itself (e.g., deamination), are in development, but easy-to-use pipelines are still lacking. In sum, although methods are still in development, the field of next-generation museum genomics is burgeoning, with high potential to extend the utility of museum specimens in bird systematics, historical demography, and conservation.

Key Words: birds, evolution, genomes, natural history collections, next-generation sequencing, phylogenetics.

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BACKGROUND: FIRST FORAYS INTO THE GENOMES OF MUSEUM SPECIMENS

The first museum specimen to reveal anything about its genomic content to the world was a salted pelt of a quagga (*Equus quagga quagga*), a handsome extinct subspecies of the plains zebra stored at the Natural History Museum in Mainz, Germany (Higuchi et al. 1984). Lacking a method to increase trace amounts of DNA to high concentration, the researchers of 1984 required large amounts of starting material, over 10 ng of DNA for a single reaction in the case of the quagga study, to clone into a bacterial vector and sequence with a relatively new method at the time, dideoxy sequencing, also called Sanger sequencing (Sanger et al. 1977). Recovered DNA sequences allowed the researchers to place an extinct animal in the molecular tree of life for the first time.

Progress in ancient DNA research has always been tightly linked to technological advancements. Not long after the quagga success, the polymerase chain reaction (PCR) was invented (Saiki et al. 1985), which allowed small amounts of DNA from ancient samples to be amplified hundreds of thousands of times to the levels needed for Sanger sequencing (Pääbo 1989). The subsequent discovery of highly conserved regions in mitochondrial DNA (mtDNA) provided convenient priming sites for PCR amplification of variable DNA across major branches of the tree of life (Kocher et al. 1989). With this plethora of new genomic sites to target, ancient DNA studies began to proliferate.

The first published use of ancient DNA from a bird museum specimen was an attempt to describe a new shrike by comparing its DNA to the DNA of other bird species, some of which was derived from study skins (Smith et al. 1991). A deeper time study of ancient avian DNA, from birds more than 3,000 years old, occurred when researchers extracted DNA from skin, bone, and muscle tissue of four specimens of an extinct order of birds, the moas of New Zealand (Cooper et al. 1992). The 390 DNA bases that Cooper and colleagues painstakingly stitched together from many smaller pieces, each targeted by individual primer pairs, suggested that moas were not the closest relative of another endemic New Zealand bird order, the kiwis. New Zealand, according to these data, had been colonized twice by a largely

flightless group of birds known as the paleognaths, which also includes birds like emus and ostriches. This study marked a major achievement for the use of DNA sequencing with ornithological museum specimens.

Every new technological advance comes with its problems, and this was also true for PCR and DNA sequencing in their application to museum samples, particularly at their inception. Amplifying tiny amounts of genetic material made DNA sequencing more accessible, but it also greatly increased the risk of amplifying contaminant DNA, either introduced to the specimen through years of handling or by exposure to laboratories that were now, thanks to the advent of PCR, awash in ultrahigh concentration DNA. Several early successes in ancient DNA were found to be the result of contaminating DNA (e.g., DeSalle et al. 1992). Researchers soon called for ultraclean laboratory conditions (Cooper et al. 2001, Ho and Gilbert 2010) and exacting standards for replication of results (Handt et al. 1994, Cooper and Poinar 2000).

Sequencing DNA from museum specimens, even when it was the right DNA, was not an easy process. DNA degrades and fragments over time through a variety of biochemical processes (Willerslev and Cooper 2005). A recent study using extracted DNA from a time series of museum specimens (McCormack et al. 2016) demonstrates this fragmentation process through time (Figure 9.1). Recent specimens have DNA quality similar to fresh tissue. Specimens up to 20 years old might still contain high molecular-weight DNA. But specimens 30 years old and older are increasingly fragmented, with most fragments eventually being less than 500 base pairs (i.e., low quality). Thus, a researcher starting from high-quality DNA—extracted, say, from frozen tissue—can use universal primers to target a long span of variable DNA (0.5 to 10 kilobases), but this is rarely possible with ancient DNA. Instead, the degraded fragments of ancient DNA require many sets of primers, often designed from scratch, that span variable sections of DNA; design of such primers is a time-consuming and challenging task. In addition to being difficult to develop, the resulting primers, because they are placed in regions of variable DNA specific to the organism under study, often lose their universality, which is one of their principal benefits.

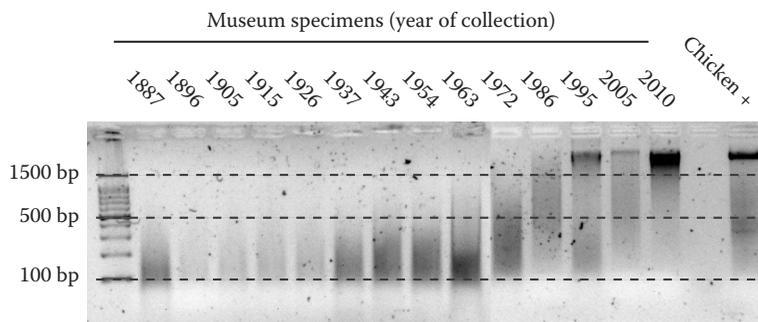


Figure 9.1. DNA extracted from a time series of museum specimens, as compared to DNA extracted from fresh, frozen tissue from a chicken.

THE GAME-CHANGER: NEXT-GENERATION SEQUENCING

Most of the truly remarkable feats in ancient DNA sequencing in recent years were made possible by what is colloquially referred to as “next-generation sequencing,” or NGS. Developed during the late 1990s, NGS was a radical departure from previous DNA sequencing methods (Shendure and Ji 2008). Although NGS platforms differ in their exact approaches and chemistries (Glenn 2011), all NGS methods clonally produce millions of DNA sequencing reads from a single run in “massively parallel” fashion compared to the achingly serial nature of Sanger sequencing.

NGS appeared just as ancient DNA studies were becoming more numerous. If an aura of exuberance surrounds the potential of NGS to transform our view of biocollections (Nachman 2013, Burrell et al. 2015, Wood and De Pietri 2015, Linderholm 2016), it is because NGS—at least in concept—appears to solve many of the problems that plagued prior ancient DNA studies (Knapp and Hofreiter 2010). First, the dramatically increased throughput of NGS, combined with the resulting decrease in the cost of sequencing each DNA base, turned the problem of contaminating DNA into less of an existential concern. The issue became less whether any target DNA would be sequenced at all, and more about how to separate target DNA from the inevitable contaminating DNA. Second, NGS platforms typically output short DNA reads, which seemed well suited to the degraded DNA input of ancient DNA studies. It is thus not surprising that many of the first applications of NGS involved museum specimens (Noonan et al. 2005, Poinar et al. 2006, Mason et al. 2011), with the first published study on

birds focusing on the development of microsatellite loci from “shotgun” NGS reads of the extinct moa genome (Allentoft et al. 2009).

CHOOSING A NEXT-GENERATION SEQUENCING TECHNIQUE

The term “next-generation sequencing” belies a uniformity of method that does not exist. The applications of NGS to studies of museum specimens are as varied as the specific protocols used to prepare the samples for sequencing and the specific platforms used for sequencing (e.g., see Buerki and Baker 2015 and references within). One of the early decisions a researcher must make is whether to target the whole genome of the study organism (Figure 9.2a) or to focus on a subsample of the genome (Figure 9.2b). This decision rests in large part on the specific research questions. Guidelines can be found in other reviews (Lerner and Fleischer 2010, Ekblom and Galindo 2011, McCormack et al. 2013b, Toews et al. 2015). For whole genome sequencing, the method is relatively straightforward. One of the first steps of standard genome sequencing is to shear genomic DNA into smaller fragments because most NGS platforms sequence short reads between 100 and 250 base pairs. Ancient DNA is already fragmented, so this step is often unnecessary although more recent samples often need to be sheared (McCormack et al. 2016). Degraded ancient DNA can often be input directly onto the sequencer after some preparation steps.

If whole genomes are not desired, then the researcher faces another series of choices (Figure 9.2c–f). Here, the specific attributes of ancient DNA may play a larger role in method selection than

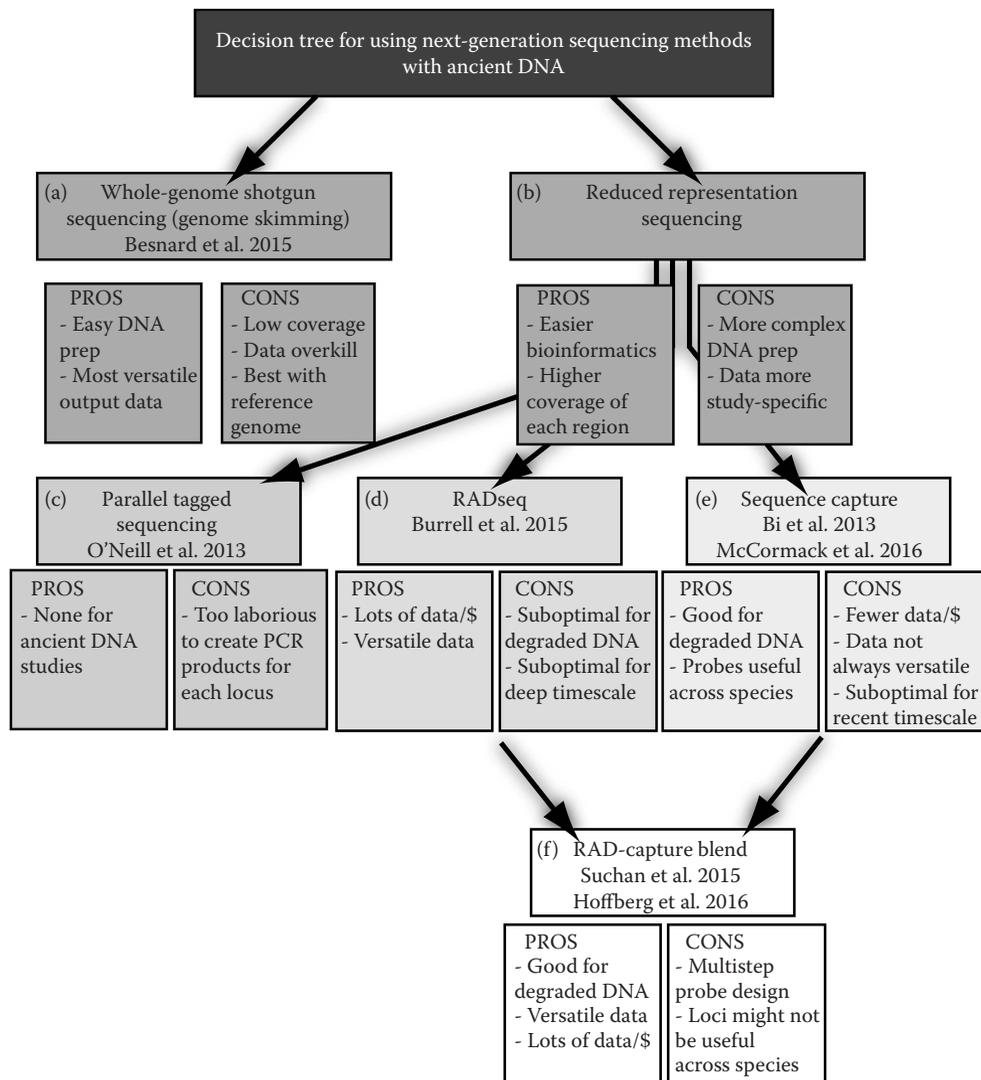


Figure 9.2. Decision tree for using NGS with ancient DNA from museum specimens. Methods denoted by letters are referenced in text.

the research question itself because certain ways of subsampling the genome might not be as effective, or even possible, when using degraded DNA. For example, parallel tagged sequencing (Figure 9.2c), a method of pooling PCR products for NGS (Meyer et al. 2008), suffers from many of the same inefficiencies of pre-NGS methods. Although the sequencing of NGS products occurs in parallel, which makes it suitable for medium-sized projects from cryopreserved tissue (O'Neill et al. 2013), the process is still time intensive when applied to ancient DNA because it requires primer design, PCR optimization, and serial generation of PCR products.

Restriction-site associated DNA sequencing (RADseq; Figure 9.2d) is a popular method that operates by creating a genomic subsample using restriction enzymes to cut up DNA in a systematic fashion, resulting in DNA fragments of similar sizes that are sequenced en masse on an NGS platform (Baird et al. 2008). When starting from a high-quality DNA source, RADseq produces many thousands of single nucleotide polymorphisms (SNPs). A major benefit of RADseq is that a reference genome is not required to identify variant sites, making it especially useful when applied to the many species found in biocollections that lack existing genomic resources.

The uses of RADseq data for studies of birds range from conservation genetics (Oyler-McCance et al. 2015) and phylogeography (Harvey and Brumfield 2015) to migratory connectivity (Ruegg et al. 2014b) and speciation genomics (Ruegg et al. 2014a). However, when applied to older or ancient samples present in biocollections, RADseq can be problematic. For instance,

as shown in Figure 9.3b, DNA degradation can produce very small DNA fragments, most of which lack the needed restriction sites (Burrell et al. 2015), especially when using double-digest RADseq (ddRADseq; Peterson et al. 2012) that requires two different restriction sites on the same fragment. Also, the lack of restriction sites on some fragments can produce null alleles, which

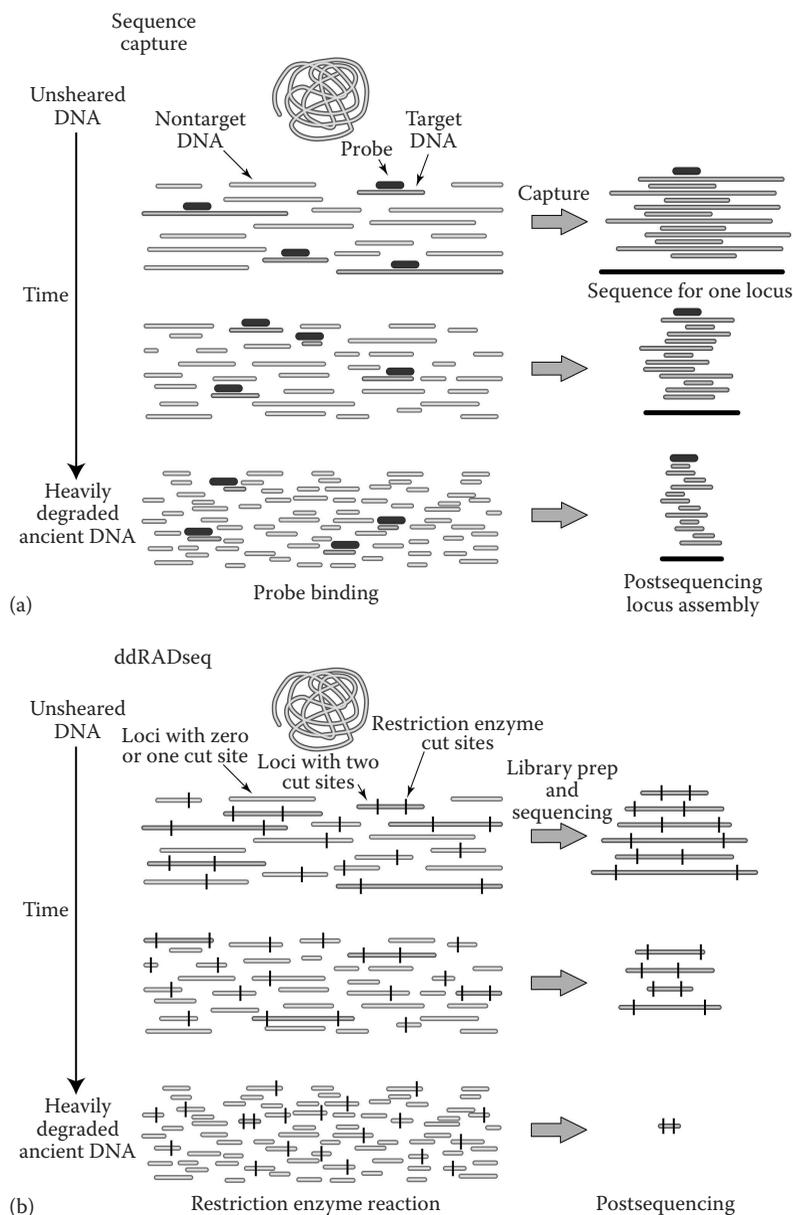


Figure 9.3. A comparison of how degraded DNA affects both (a) sequence capture and (b) ddRADseq methods. Sequence capture on ancient DNA leads to shorter assembled loci after sequencing. Ancient DNA in conjunction with ddRADseq leads to fewer loci sequenced because fewer fragments contain both restriction-digest cut sites.

may mislead downstream analyses (Graham et al. 2015). RADseq also generally requires a larger amount of high-quality starting DNA than other methods (especially ddRADseq; Puritz et al. 2014), which is not always accessible from ancient DNA.

Target enrichment—also called sequence capture, hybrid enrichment, or bait capture (Figure 9.2e)—offers another efficient alternative to whole genome sequencing that is well suited, at least in concept, to short, degraded DNA regions (Jones and Good 2016). Here, DNA or RNA probes that target a reduced subset of the genome are added to sequencing libraries prepared from extracted DNA (Gnirke et al. 2009). The probes are hybridized to their targets, and magnetic beads separate target and nontarget DNA (Mamanova et al. 2010). Following a round of PCR amplification, the targeted DNA is sequenced all at once using an NGS platform. One benefit of target enrichment is that it does not rely on systematic fragmentation of DNA inputs. Thus, in theory, the vagaries of how and where DNA degrades are less of a concern. After sequencing the captured products, the resulting DNA data for a given locus are assembled from the variously sized DNA sequences that overlap a given probe (Figure 9.3a). Although some preexisting genomic information is required to design the probe regions, once designed, probe sets are often broadly applicable across the tree of life, especially when they are designed from conserved genomic regions like exons (Bi et al. 2012, Ilves and López Fernández 2014, Prum et al. 2015) or ultraconserved elements (UCEs; Faircloth et al. 2012, Faircloth et al. 2013). Probes can even be created from PCR products (Peñalba et al. 2014).

A concern of using highly conserved genomic regions with ancient DNA is that assembled ancient DNA loci tend to be shorter than loci assembled from fresh tissue samples (Figure 9.3a; McCormack et al. 2016). If sequence capture probes use a highly conserved central core, then the worry of short loci is that insufficient variable DNA sites will be captured from the flanking regions. Another concern is that sequence capture usually targets a particular locus type (e.g., UCEs or exons), which can limit the versatility of the resulting data for addressing many types of questions. For example, DNA flanking UCEs is thought to be largely noncoding compared to the core UCE region, which is thought to be under strong stabilizing selection (Katzman et al. 2007). UCE flanking DNA, being largely noncoding, is

therefore likely not useful for making associations between genotype and phenotype. It is, however, quite useful for questions of phylogenetics and demographic history that prefer “neutrally evolving” DNA (Crawford et al. 2012, McCormack et al. 2013a, Smith et al. 2013). Meanwhile, RADseq data typically include both coding and noncoding sites, and are therefore more versatile in their application to different research questions, especially given the recent advent of phylogenetic methods that use SNP data (e.g., Bryant et al. 2012). One drawback of RADseq data, however, is that they appear to be better suited to questions at more recent timescales because increasing genetic divergence over time mutates the cut sites, leading to fewer and fewer homologous fragments among more distantly related species (Rubin et al. 2012).

New methods are now being developed that blend the best attributes of RADseq and sequence capture (Ali et al. 2016, Hoffberg et al. 2016, Suchan et al. 2016). In these approaches, sequence-capture probes are designed from RADseq markers originally detected from data generated from fresh tissue samples (Figure 9.2f). This minimizes the concern over where the RADseq digest cut sites will occur in degraded museum specimen DNA, while also allowing for the collection of a large number of genomic loci that are maximally versatile for addressing research questions involving both neutral and nonneutral processes. A remaining question is whether these approaches will be useful across species or whether new probes will need to be repeatedly designed as divergence increases among the targeted species.

PROBLEMS WITH SEQUENCE DATA SPECIFIC TO ANCIENT DNA

Thanks to NGS, DNA data production now outstrips, by a wide margin, our ability to render judgment on the quality and value of those data. To restate a prior point: the problem now is not whether we are going to sequence any of the target organism’s genome, but how we are going to assess the quality of the resulting sequence data. For instance, how will we separate the DNA of museum specimens from contaminant DNA? And once we have done that, what are the specific problems with ancient DNA that we must account for?

After an organism dies, there are relatively rapid biochemical processes that break down tissues

as well as slower-acting biochemical processes that degrade and damage DNA. Because storage conditions for museum specimens are not optimized for molecular stability, these degradation processes affect the DNA in museum specimens, which is one major factor making molecular work with older museum specimens difficult (Wandeler et al. 2007). The processes of DNA degradation are incompletely understood, but include both enzymatic and biochemical effects that alter DNA bases (mainly conversions from cytosine [C] to thymine [T]) through deamination (Dabney et al. 2013) and inhibit the effectiveness of DNA polymerase when synthesizing new copies of ancient DNA using PCR. Although each of these effects is troublesome, fragmentation of ancient DNA is perhaps the most problematic. Beyond a certain point, DNA sequences become too short to capture and sequence with current technology. Empirical studies showing an excess of purines (adenine and guanine bases) in the genomic positions directly adjacent to the ends of ancient DNA fragments suggest that depurination is the major cause of fragmentation (Briggs et al. 2007, Orlando et al. 2011).

The multifarious processes that are responsible for degrading DNA in museum specimens also differ in how they operate through time. Deamination events, primarily C to T transitions, appear to increase at a steady rate with specimen age (Sawyer et al. 2012), a pattern that is evident in DNA from mammal skins (Bi et al. 2013) and in plant herbarium tissue (Staats et al. 2013) collected during the last 100 years, as well as in DNA extracted from more ancient samples (Hofreiter et al. 2001, Briggs et al. 2009). As far as fragmentation is concerned, a study on specimens ranging in age from 18 to 60,000 years old did not find that DNA became more fragmented with age (Sawyer et al. 2012). However, this contrasts with a recent study that focused exclusively on DNA extracted from avian toe pads collected during the previous 120 years (McCormack et al. 2016). This study found that the length of assembled DNA loci was shorter when using DNA from older specimens, even when older specimens had large numbers of NGS reads associated with them. In this latter study, the rate of DNA fragmentation was faster during the first 30 years of specimen storage, after which time the DNA was heavily fragmented (also see Figure 9.1). It is possible that Sawyer et al. (2012) did not observe this trend because

their study examined a much longer window of time that included few specimens younger than 30 years. Supporting the relationship between fragmentation and age, another recent study looking at herbarium specimens also found shorter fragments in older specimens preserved over the last 300 years (Weiß et al. 2015).

While the processes affecting DNA degradation and timing of ancient DNA damage are still being investigated, it is clear that these issues are a concern for those working on samples thousands of years old. But it is important to realize that these same processes also affect DNA of historical specimens collected during the last 100 years. A simple way to identify deamination in samples is to plot the occurrence of all possible DNA base changes (e.g., A to G, C to T, etc.) against distance from the end of sequenced DNA fragments (Briggs et al. 2007, Bi et al. 2013). Because deamination is known to occur in greater frequency toward the ends of fragments, a signature of deamination is an elevated signal of C to T transitions close to the 5' end of the sequenced fragment. In fact, this pattern is so ubiquitous that it has been proposed as a way of validating that DNA is truly derived from an ancient source and not the result of more modern contamination (Dabney et al. 2013). Other, more sophisticated analytical methods for estimating deamination, like mapDamage2.0 (Jónsson et al. 2013), and contamination, like PMDtools (Skoglund et al. 2014), build on the models originally described in Briggs et al. (2007). At the moment, these techniques are better suited for human data where high-quality reference sequences are available, but they may become more suitable for nonhuman genetic studies, including those of birds, as additional avian reference sequences are developed (Zhang et al. 2014). In addition, kits for correcting deamination prior to sequencing are commercially available.

CASE STUDIES IN ORNITHOLOGY

Still relatively few studies apply NGS to bird museum specimens. Early studies, however, provide tantalizing hints of the potential for NGS to extend the mission of ornithological biocollections, especially collections that contain older or extremely rare specimens from which modern sources of cryopreserved DNA are lacking. Many smaller, regional collections at state and county natural history museums contain these

kinds of rare specimens. Genomic applications for museum specimens might therefore have their biggest impact on these smaller collections, which hold important specimens, but often struggle to obtain needed space and funding from administrators and funding bodies (Snow 2005).

The study of extinct or highly endangered species is an obvious and important application of NGS to museum specimens because no high-quality DNA will likely ever be available for many of these species. In fact, most published studies to date using NGS with museum specimens focus on extinct or endangered species. The Passenger Pigeon (*Ectopistes migratorius*) is a recent favorite subject for genomic study as a result of the 100-year anniversary of its demise (Greenberg 2014) and ongoing efforts to revive the species through de-extinction approaches (Seddon et al. 2014). Recent NGS studies of Passenger Pigeons sequenced two complete mtDNA genomes (Hung et al. 2013) and roughly half the nuclear genome of four specimens from different parts of their geographic range (Hung et al. 2014). By combining DNA extracted from museum specimens, broad sampling of the genome, and new analytical methods for assessing effective population size from limited population sampling, the nuclear genomic study revealed novel conclusions about Passenger Pigeons that could not have been reached in any other way. In particular, these analyses suggest that Passenger Pigeons frequently went through dramatic population fluctuations, which left them vulnerable to extinction, and were in a decline phase that was exacerbated by human exploitation (Hung et al. 2014).

Another use of whole genome shotgun sequencing using NGS, where many random parts of the genome are targeted, is a recent study of crowned pigeons in the genus *Goura*. The three living members of this genus are threatened, which makes the acquisition of fresh tissue impossible (Besnard et al. 2015). Here, the authors used “genome skimming” on DNA extracted from museum specimens. Genome skimming involves filtering through millions of shotgun NGS sequence reads to find the few reads associated with particular DNA regions of interest. While perhaps not the most efficient use of sequencing effort, this method allowed the authors to assemble a small phylogenetic dataset consisting of both mtDNA and nuclear genes that placed crowned pigeons with high confidence into the existing pigeon

tree of life, closely related to several extinct or highly endangered island species like the Dodo (*Raphus cucullatus*). The Dodo itself could not have been included in this genetic analysis if DNA had not been sequenced previously from the cortical bone of a museum specimen (Shapiro et al. 2002). Perhaps more than anything else, this study demonstrates the kind of opportunistic genome skimming studies that will arise in greater frequency as more and more NGS data are produced for species across the bird tree of life, provided such data are archived (see later).

Other studies used target enrichment to subsample genomic DNA from museum specimens. One such study used an mtDNA probe set to assemble whole mtDNA genomes from subfossils of elephant birds (family Aepyornithidae), showing that they are most closely related to the New Zealand Kiwi. This surprising result implicates dispersal, not vicariance, as a major diversifying force in ratite birds (Mitchell et al. 2014). Another study enriched thousands of UCE loci from a time series of California Scrub-Jay (*Aphelocoma californica*) and Woodhouse’s Scrub-Jay (*Aphelocoma woodhouseii*) study skins and identified variable loci and SNPs that allowed both phylogeographic and population genetic analyses (McCormack et al. 2016). This study demonstrated that sequence capture of conserved regions could produce phylogenetic data from degraded nuclear DNA in museum specimens. But along with this success, it also showed that age and starting DNA quality and quantity mattered for data matrix completeness and locus lengths, both important mileposts for producing high-quality phylogenetic datasets. Compared to whole genome shotgun sequencing, the targeted capture approach allowed for many individuals to be queried at thousands of loci, with high efficiency and little missing data.

FUTURE DIRECTIONS

Returning to Fundamentals: DNA Extraction

Underlying the previous discussion is the pre-eminent importance of starting DNA quality. It is ironic, given the pace of technological advancements in DNA sequencing, that researchers are more frequently returning to retool antiquated protocols for retrieving DNA from museum specimens. Unlike the case with DNA sequencing, we are still awaiting paradigm-shifting advances in

DNA extraction, and they are badly needed. A few studies have tested different ancient DNA extraction protocols (e.g., Rohland and Hofreiter 2007), without a clear consensus emerging on universal best practices. At least one study claims that the low yields of DNA from ancient material is more a product of inefficient extraction methods than low DNA content of the samples themselves (Barta et al. 2014), which serves to highlight the need for improved protocols.

New studies should not only test different protocols, but different sources of starting material from bird study skins. For example, toe pads are currently the most commonly used source material (Mundy et al. 1997), but other options include skin punches from featherless skin tracts or from the feathers themselves (Sefc et al. 2003, Rawlence et al. 2009). Ancient DNA studies on mammals suggest that finely ground bone produces the highest DNA yields (Pruvost et al. 2007, Hawkins et al. 2016). It would be interesting to test whether the same is true in birds, perhaps by arthroscopic retrieval of small bone pieces from inside bird study skins, which would have the added benefit of leaving no trace of the sampling on the outside of the specimen. Of course, differences in bone structure between mammals and birds might have important implications for the retrieval of DNA between these two groups. Until new DNA extraction methods are developed, we must work with what we have.

Archiving Data: One Researcher's Trash Is Another's Treasure

Subsampling the genome with NGS approaches like sequence capture and RADseq appears to be taking hold in phylogeography and phylogenetics as methods of reducing the overall complexity of datasets that still contain a large and representative genomic sample (McCormack et al. 2013b). However, this is not universally true, and many high-profile studies have featured whole-genome sequencing, with subsets of the genome later being extracted *in silico* and analyzed independently in a manner similar to genome skimming (e.g., Jarvis et al. 2014). The utopian situation time and money are maximized by sequencing whole genomes, with different teams later tackling different questions with different parts of the genome and different analyses, is becoming a reality for living organisms with the rapidly

advancing genomes initiatives for multiple animal groups (Haussler et al. 2009, i5K Consortium 2013). Making sure these initiatives sequence genomes linked to museum vouchers whenever possible is one important step toward increasing the scientific value of individual specimens by linking a genotype to a vouchered phenotype.

By the same token, independent researchers conducting *ad hoc* low-coverage, shotgun sequencing of museum specimens should make all their data open to the broader scientific community, using, for example, the National Center for Biotechnology Information's Short Read Archive (<http://www.ncbi.nlm.nih.gov/sra>). It is probably also worth archiving all sequencing reads generated from genomic subsampling approaches like sequence capture, because these methods produce millions of off-target reads from the nuclear and organellar genomes, including off-target mtDNA reads generated during sequence capture, that might be of interest to other researchers (e.g., off-target mtDNA reads generated during sequence capture; Meiklejohn et al. 2014, do Amaral et al. 2015).

Analytical Programs for Detecting DNA Damage and Contamination

A number of analytical issues that are specific to ancient DNA exist for which automated pipelines are currently lacking. For instance, analysis to detect sources of contamination should be carried out as a matter of course for ancient DNA studies, but few tools are available for automating this process. One could, for instance, imagine a program that keeps a database of all DNA sequences that have passed through a lab. When a new study is carried out, the resulting sequence data are screened against the database as the most likely source of contamination. Deamination is another issue particular to ancient DNA studies whose detection and correction would benefit from more study and automated pipelines to detect deamination events. Similar to other analytical issues associated with NGS, methods of analysis and data processing pipelines lag behind our ability to generate incredible amounts of data.

CONCLUSIONS

Although currently few studies use NGS on bird museum specimens, this number is expected to grow rapidly in the coming years, as protocols are

developed and computational pipelines become more user friendly. This will undoubtedly mirror the growth in use of NGS on nondegraded samples, which multiplied rapidly in the last 5 years as researchers started to speculate about possible applications (Lerner and Fleischer 2010) and later tested various methods and described those that seemed most successful (Ekblom and Galindo 2011, McCormack et al. 2013b). Similarly, as NGS methods using degraded DNA from museum specimens become more established, we will undoubtedly see a shift from studies that focus almost exclusively on systematics and phylogenetics toward those that require deeper sampling from populations. This will open the door to large-scale study of genetic change through time (Holmes et al. 2016), both natural and human-mediated, already hinted at in the existing case study of the Passenger Pigeon (Hung et al. 2014). The field of paleornithology is poised to benefit greatly from NGS methods (Wood and De Pietri 2015), as ancient DNA has been successfully extracted from eggshells (Oskam et al. 2010), ancient feathers (Rawlence et al. 2009), and even coprolites (Wood et al. 2013) and sedimentary deposits (Willerslev et al. 2003). As those in the museum community know and have long advocated, one of the truly unique features of biocollections is that they offer a snapshot of biodiversity at a particular moment in time. Accessing the genomes of the organisms captured in each successive snapshot will add to the extended specimen and will be the work of future generations.

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