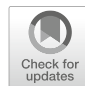
Advances in Using Non-invasive, Archival, and Environmental Samples for Population Genomic Studies



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Abstract Recent advances in DNA sequencing and genotyping technologies are rapidly building our capacity to address ecological, evolutionary, and conservation questions for wildlife species. However, a large portion of wildlife genetic research relies on samples containing low quantities and quality of DNA, such as non-invasive, archival, and environmental DNA (eDNA) samples. These samples present unique methodological challenges that are largely responsible for a lag in the adoption of new genetic technologies for many areas of wildlife research. Nonetheless, steady progress is being made as researchers test and refine laboratory protocols and bioinformatic methods tailored to low-quality samples. Here we provide an overview of the progress toward low-quality sample applications for amplicon sequencing, single nucleotide polymorphism (SNP) genotyping, DNA capture, mitogenome sequencing, restriction site-associated DNA sequencing (RADseq), and whole-genome sequencing. We also review methods for generating DNA sequence data from samples comprised of multiple individuals and species, such as eDNA or fecal samples, including metagenome sequencing, metabarcoding, metagenome skimming, and metatranscriptomics. The implementation of these

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approaches has provided insight into a wide range of questions such as modern and historic population genetic structure and diversity, adaptation, inbreeding, ancient hybridization, occupancy, diet composition, microbiome composition, and many more. As the development of methods tailored for low-quality DNA sources continues to advance over the coming years, we expect these samples to provide unprecedented insight into the ecology, evolution, and conservation of wildlife species.

Keywords Ancient DNA · Historical DNA · Metagenomics · Museum samples · Next-generation sequencing

1 Introduction

The development of “next-generation” DNA sequencing (NGS) and new genotyping technologies over the last decade has dramatically increased our ability to obtain genetic data for wildlife species. This growing capacity to mine the genomes of non-model organisms has both expanded and refined questions that can be addressed in wildlife ecology, evolution, and conservation. For example, population genomic data provide new opportunities for detecting natural selection and quantifying adaptive genetic variation in natural populations (Schoville et al. 2012) while simultaneously increasing the accuracy and precision of estimates of genome-wide diversity (Väli et al. 2008), population structure, and demographic parameters (Luikart et al. 2003). The application of genomic information has further driven the development of novel approaches for delineating conservation units (Funk et al. 2012), improving wildlife management strategies (Hoffmann et al. 2015), and increasing phylogenetic resolution (Jarvis et al. 2014; Wagner et al. 2013). Furthermore, these new technologies also facilitate the characterization of entire species assemblages from environmental samples (Andersen et al. 2012; Jørgensen et al. 2012).

Despite the increasing availability of new techniques for generating genomic information, the ability to obtain a source of DNA to generate these data can be limited for many wildlife species due to logistical and ethical constraints on tissue sampling. These challenges have been addressed over the past 25 years by optimizing laboratory and data analysis techniques for generating genetic data from non-invasively collected materials, such as feces, hair, and feathers, as well as archival (e.g., museum specimens and subfossils) and environmental samples (Goldberg et al. 2015; Orlando et al. 2015; Waits and Paetkau 2005). However, all of these types of samples often contain DNA of low quantity and poor quality (Pääbo 1989; Shapiro and Hofreiter 2012), as well as DNA from non-target species, all of which can pose challenges for generating genetic and genomic data.

Here, we briefly review the history of non-invasively collected, archival, and environmental sources of DNA for addressing questions in wildlife ecology, evolution, and conservation and highlight the challenges involved in adapting these sampling strategies in the genomics era. We then discuss a variety of NGS-enabled approaches to studying wildlife genetics and genomics with a specific focus on their application to non-invasively collected, archival, and environmental sources of DNA. Throughout, we highlight important considerations for employing the various approaches and explore novel opportunities in light of current and future advances.

2 Non-invasive, Archival, and Environmental Sources of DNA for Wildlife Genetics

The field of genetic non-invasive sampling (gNIS) has revolutionized wildlife ecology and management. This field was launched in 1992 when researchers demonstrated that it was possible to obtain and amplify mitochondrial DNA (mtDNA) from hair and fecal samples of brown bears (Höss et al. 1992; Taberlet and Bouvet 1992) and hair samples of chimpanzees (Morin and Woodruff 1992). Over time, researchers expanded the application of gNIS to include feathers (Morin et al. 1994a; Taberlet and Bouvet 1991), egg shells (Pearce et al. 1997), urine (Nota and Takenaka 1999; Valiere and Taberlet 2000), saliva (Wheat et al. 2016; Williams et al. 2003), sloughed skin (Bricker 1996; Valsecchi et al. 1998), insect exuviae (Feinstein 2004), owl pellets (Taberlet and Fumagalli 1996), and other regurgitates (Sugiyama et al. 1993; Valiere et al. 2003). More recently, researchers demonstrated it was possible to obtain bullfrog DNA from water samples (Ficetola et al. 2008). This work launched the new field of environmental DNA (eDNA) analysis which uses samples such as water, soil, and air to target DNA fragments that have been shed by organisms into the surrounding environment (Goldberg et al. 2015; Pedersen et al. 2015; Thomsen and Willerslev 2015). Both gNIS and eDNA analyses have become the genetic sampling and monitoring method of choice for many species because they provide valuable genetic information without catching, handling, or, in some cases, even observing animals (Box 1; Beja-Pereira et al. 2009; Bohmann et al. 2014; Rees et al. 2014; Schwartz et al. 2007; Waits and Paetkau 2005). Also, multiple studies have demonstrated that gNIS and eDNA analyses are more cost-effective than traditional sampling methods (De Barba et al. 2010; Jerde et al. 2011; Solberg et al. 2006; Stenglein et al. 2010).

Box 1 Genetic non-invasive samples (gNIS), archival samples, and eDNA samples have been used to address a wide range of research questions, including those described below

Detection of taxa

An important application for eDNA samples and gNIS is the detection of target taxa (Waits and Paetkau 2005; Bohmann et al. 2014). These samples can be used to detect the presence of rare, elusive, or invasive species; for example, eDNA approaches have been effective for early detection of invasive species such as Asian carp (Jerde et al. 2011), American bullfrog (Dejean et al. 2012), New Zealand mud snails (Goldberg et al. 2013), and Burmese python (Piaggio et al. 2014). eDNA and gNIS can also be used to conduct biodiversity surveys for both contemporary and ancient environments (Pedersen et al. 2015; Thomsen and Willerslev 2015), detect disease (Kohn and Wayne 1997; Kolby et al. 2015), identify factors influencing occupancy and distribution patterns (Lonsinger et al. 2017; Pansu et al. 2015), and characterize species composition of dietary samples (De Barba et al. 2014; Höss et al. 1992; Taberlet and Fumagalli 1996) and microbial communities (Amato et al. 2013).

Detection of individuals

gNIS can be used to identify the presence of individuals across space and time, providing valuable insights into demographic parameters such as individual-level movement patterns (Dixon et al. 2006; Fabbri et al. 2007; Proctor et al. 2005), population size and density (Arandjelovic et al. 2010; Davidson et al. 2014; Kendall et al. 2009; Woods et al. 1999), survival (Rudnick et al. 2005; Woodruff et al. 2016), and sex ratios (Rudnick et al. 2005; Woods et al. 1999). For example, DeMay et al. (2017) used DNA extracted from fecal samples collected from a Columbia Basin pygmy rabbit reintroduction site to estimate dispersal distance and survival rates of reintroduced individuals. Individual identification from gNIS can also be used to identify individual predators responsible for killing domestic animals (Blejwas et al. 2006; Caniglia et al. 2014; Williams et al. 2003) or wildlife species of conservation concern (Ernest et al. 2002; Mumma et al. 2014; Wheat et al. 2016).

Population genetic analysis

Population genetic analyses using gNIS and archival samples have been used to characterize modern and historic population structure, diversity, and gene flow (Bi et al. 2013; Epps et al. 2005; Miller and Waits 2003; Quemere et al. 2010; Wultsch et al. 2016). For example, genetic analysis of modern and museum Tasmanian devil specimens indicated genetic diversity has been low for at least 100 years and has not declined as a result of recent disease-related population bottlenecks (Miller et al. 2011). gNIS and archival samples have also been used to identify environmental variables driving population structure

(continued)

Box 1 (continued)

(Braunisch et al. 2010; Castillo et al. 2014; Cushman et al. 2006; Martinez-Cruz et al. 2007; Wasserman et al. 2010), estimate effective population size (Eggert et al. 2003; Gonzalez et al. 2016; Miller and Kapuscinski 1997), assess recent hybridization and introgression (Adams et al. 2003; Lawson et al. 2017; Steyer et al. 2016), and identify related individuals (Constable et al. 2001; Ford et al. 2011; Morin et al. 1994b; Rudnick et al. 2005). For example, genetic parentage analysis with non-invasive hair and fecal samples collected over 7 years from a reintroduced brown bear population revealed that although population size increased rapidly after reintroduction, reproduction was dominated by a single male, leading to inbreeding and diversity declines (De Barba et al. 2010).

Phylogenetic analysis
Phylogenetic analysis using gNIS and archival samples has been used to characterize phylogenetic relationships (Guschanski et al. 2013; Willerslev et al. 2009), assess ancient hybridization (Cahill et al. 2013; Miller et al. 2012; Prufer et al. 2014), and understand transmission pathways of disease (Bos et al. 2011; Schuenemann et al. 2011). For example, phylogenetic analysis of modern, historical, and ancient polar bear and brown bear samples revealed ancient hybridization between these two species (Cahill et al. 2013; Miller et al. 2012). Phylogenetic analysis is also used for eDNA analysis to identify taxa (e.g., Fonseca et al. 2017; Klymus et al. 2017).

Archival specimens represent another important DNA source that does not require traditional sampling and can provide an invaluable resource for reconstructing patterns and processes of evolution across time and space (Wandeler et al. 2007). Such specimens can take the form of preserved hard or soft tissues collected within the last ~200 years for natural history collections (Wandeler et al. 2007), yielding what has been generally termed “historical DNA” (Bouzat et al. 1998). A variety of tissue types have been used as sources of historical DNA including bones (Russello et al. 2005), teeth (Wandeler et al. 2003), skins (Mundy et al. 1997), claw pulp (Casas-Marce et al. 2010), baleen (Rosenbaum et al. 1997), feathers (Ellegren 1991), and fish scales (Nielsen et al. 1999). The quality and quantity of historical DNA can vary by tissue type, preservation method, and storage conditions (Martínkova and Searle 2006; Morin et al. 2007). On a deeper time frame, subfossils, permafrost-preserved specimens, and coprolites can yield “ancient DNA” that is typically <100,000 years old (Lindahl 1993) but can extend up to 700,000 years depending upon state of preservation (Orlando et al. 2013). In the pre-NGS era, population-level studies using archival DNA largely relied on conventional markers, such as fragments of the mitochondrial genome and nuclear microsatellite loci, and, later, targeted single nucleotide polymorphisms (SNPs, Morin et al. 2004), to investigate a range of questions in wildlife ecology, evolution, and conservation (Box 1).

The main challenges associated with obtaining genetic data from non-invasively collected, archival, and environmental sources are the low quantity and quality of target DNA present, as well as contamination by "exogenous" DNA, either from non-target species or, in the case of archival specimens, modern DNA. In terms of quality and quantity of recovered DNA, degradation from these sources typically results in fragmentation to about 100–500 bp, with the majority of strand breakage occurring shortly after sampling, shedding, or death as a result of enzymatic and nonenzymatic processes (Hofreiter et al. 2001; Pääbo 1989; Pääbo et al. 2004). The extent of degradation can be further influenced by the environmental conditions at the time of collection (e.g., temperature, pH, humidity, exposure to UV radiation; Barnes et al. 2014; Poinar et al. 1996) and preservation method (Frantzen et al. 1998; Rees et al. 2014). For archival specimens, postmortem DNA damage can be particularly significant, leading to strand breaks, DNA cross-links, and oxidative and hydrolytic lesions that can all impact DNA quality (Pääbo et al. 2004). Co-purification of elements (e.g., aluminum, copper, chromium) or other compounds not thoroughly removed during the DNA extraction process can also bind to active sites and inhibit PCR, the magnitude of which can be influenced by age of specimen and preservation method (Hall et al. 1997). Contamination is another significant concern when working with non-invasively collected, archival, and environmental DNA samples. Ancient samples and contemporary fecal samples are often comprised of more than 95% microbial DNA (Carpenter et al. 2013; Chiou and Bergey 2018; Perry et al. 2010; Snyder-Mackler et al. 2016).

3 New Sequencing and SNP Genotyping Technologies

The last decade has seen dramatic advances in DNA sequencing technologies. Whereas Sanger sequencing was the dominant technology for three decades starting in 1977 (Sanger et al. 1977), a diverse array of new technologies started appearing in 2005, each capable of generating orders of magnitude more data than Sanger sequencing. These new technologies have been broadly described as "massively parallel sequencing," "high-throughput sequencing," and "next-generation sequencing;" hereafter we adopt the widely used term "next-generation sequencing" (NGS). Illumina currently dominates the market with short-read "sequencing by synthesis" technology that produces reads up to 300 bp (Metzker 2010; Shendure and Ji 2008), whereas long-read technologies can sequence up to 60 kb with Pacific Biosciences single-molecule real-time sequencing (Eid et al. 2009) and 200 kb with Oxford Nanopore technologies (Goodwin et al. 2015). These technologies have opened up a wide range of new applications for exploring the genomes of both model and non-model organisms (Fig. 1).

A variety of SNP genotyping platforms have also been developed over the last decade. Rather than producing DNA sequence reads, these platforms output genotypes from a preselected set of SNPs for large numbers of samples, with lower cost, effort, and genotyping error rate than NGS (Fig. 2). Single-tube, single-locus SNP

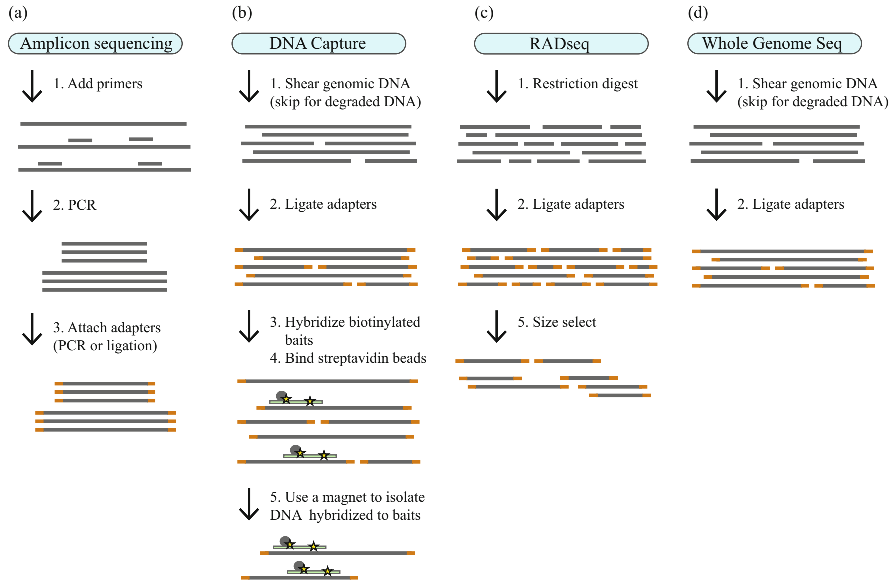

Fig. 1 Basic steps of four approaches for producing DNA libraries ready for next-generation sequencing: (**a**) Amplicon sequencing, (**b**) DNA capture, (**c**) RADseq, and (**d**) whole-genome sequencing. All methods start with extracted genomic DNA. Orange indicates sequencing adapters. For DNA capture, stars represent biotinylation of baits, and gray circles represent streptavidin-coated magnetic beads bound to biotinylated baits. One RADseq method is illustrated, but many types of RADseq methods have been developed (reviewed in Andrews et al. 2016)

genotyping assays such as TaqMan™ (Higuchi et al. 1993; Holland et al. 1991) and Amplifluor® (Nazarenko et al. 1997) have been used for more than two decades. More recent technologies interrogate hundreds or thousands of loci and individuals simultaneously using a wide range of techniques (Garvin et al. 2010; Ragoussis 2009). SNP genotyping platforms require prior genomic knowledge to design primers and probes, which can be a disadvantage for wildlife species with limited genomic resources available. However, NGS is providing greater access to genomic information in non-model species, thus indirectly contributing to increased use of SNP genotyping platforms in wildlife research.

Non-invasive, archival, and environmental samples present limitations to all methods for generating genetic data but in particular for approaches generating non-targeted data from large numbers of loci. Therefore low-quality samples have limited use under standard protocols for many of the new DNA sequencing and genotyping technologies. However, a number of approaches have been developed to circumvent the limitations of low-quality sequence data for these new technologies, as we describe in the following sections. We start by describing techniques for generating sequence and genotype data for individual target organisms, beginning with the most feasible techniques for low-quality samples. We then describe techniques for generating data from samples comprised of communities of individuals, such as eDNA samples.

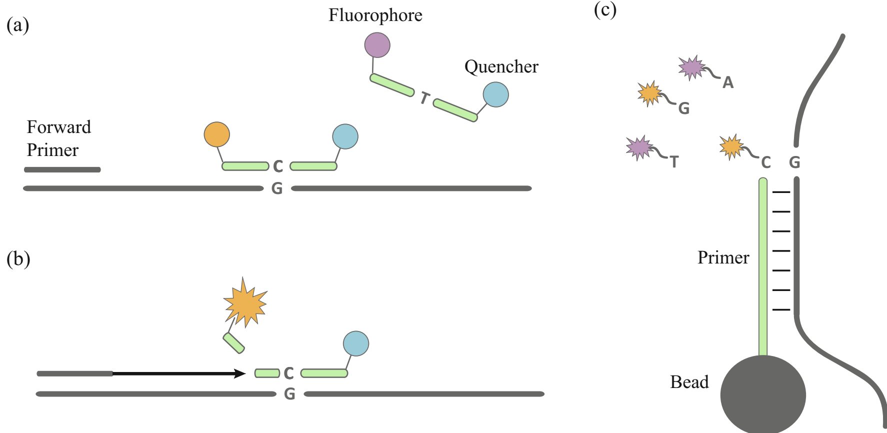

Fig. 2 Two examples of the many methods employed by SNP genotyping assays. In contrast to next-generation sequencing methods, these assays do not produce sequence reads but instead produce genotypes at a preselected set of SNPs. (**a**, **b**) TaqMan™ assays can be used on Fluidigm Dynamic Arrays. Each assay uses two probes that are complementary to one of the two possible nucleotides at the target SNP. Each probe has a fluorophore and a quencher molecule attached; the quencher inhibits fluorescent signal when in close proximity with the fluorophore. The complementary probe anneals to the sample DNA (**a**). During PCR, Taq polymerase extends the primer and degrades the annealed probe, breaking the association between the fluorophore and the quencher and resulting in a fluorescent signal that is used for genotyping (**b**). (**c**) The primer extension method (also called single-base extension method) is used by iPlex® assays on the MassARRAY and by Illumina GoldenGate and Infinium assays. An oligonucleotide primer is attached to a bead; this primer is complementary to the target site and terminates immediately prior to the SNP site of interest. The primer is enzymatically extended by one fluorescently labeled nucleotide base, and the fluorescent signal of the incorporated base is used for genotyping

4 Amplicon Sequencing

Amplicon sequencing (Fig. 1a) is a powerful tool for generating large quantities of genetic data from low-quality samples because it relies on the generation of PCR products using template-specific primers, a method with a well-established success rate for these sample types. However, instead of using traditional Sanger sequencing of PCR products, amplicon sequencing uses NGS to simultaneously sequence millions of DNA fragments, thus generating orders of magnitude more sequence data than prior technologies at a fraction of the cost. Amplicon sequencing is a cost-effective and efficient method for generating sequence data from a relatively small number of loci but for a very large numbers of samples. These methods have the added advantage of being highly flexible in the numbers of loci targeted.

The laboratory methods for amplicon sequencing differ in several ways from those of traditional Sanger sequencing of PCR products. For PCR products to be sequenced on an NGS platform, specific double-stranded oligonucleotides called “sequencing adapters” must be attached to both ends of the PCR products to create a

"DNA library." In addition, PCR products for each individual sample must have a unique barcode identifier so that amplicons from multiple individuals can be sequenced simultaneously, thereby substantially reducing sequencing cost. These requirements are typically accomplished through an initial PCR with target-specific primers, followed by addition of sequencing adapters through a second PCR or a ligation reaction. The barcodes can be incorporated into the primers used for either the first or second PCR. PCRs can be conducted either in singleplex (one primer pair per reaction) or multiplex (multiple primer pairs per reaction). Although singleplex reactions necessarily require many more individual PCRs than do multiplex reactions, Fluidigm Corp (San Francisco, USA) has improved the efficiency of singleplex PCRs by developing chips on which thousands of independent PCRs can be conducted simultaneously in microfluidic chambers. One challenge of multiplex PCR is that amplification performance may be inconsistent across loci within a single reaction. However, Campbell et al. (2015) used a multiplex PCR approach to sequence 192 loci for 2,068 steelhead trout fin tissue samples in a single Illumina HiSeq lane and found that 187 loci were genotyped in >90% of samples, with only three loci genotyped in <70% of samples (Table 1). This study also found 99.9% concordance in genotypes previously generated for the same loci using TaqMan assays.

Amplicon sequencing of microsatellites has been tested for a number of wildlife species and found to have many advantages over traditional microsatellite fragment length analysis including increased accuracy, efficiency, and consistency of genotyping (Table 1; Darby et al. 2016; De Barba et al. 2017; Farrell et al. 2016; Suez et al. 2016; Vartia et al. 2016; Zhan et al. 2017). Specific benefits afforded by amplicon sequencing of microsatellites include unambiguous allele identification, increased information regarding sequence variation not detectable with fragment length analysis, and direct genotype comparability among platforms and laboratories due to automation of the genotyping process. Thus far, one study has used NGS of microsatellite amplicons for non-invasively collected samples and reported significant improvements in genotyping success and error rates from brown bear hair and scats (De Barba et al. 2017).

A very different kind of amplicon sequencing called "Nextera-tagmented, reductively amplified DNA" (NextRAD) genotyping has been tested on low-quality samples (Russello et al. 2015). This method sequences all regions of the genome containing a preselected 9 bp sequence by fragmentation of genomic DNA, ligation of Illumina sequencing adapters, and PCR amplification with a primer containing the 9 bp sequence and the adapter sequence. This method was first used for 96 American pika hair samples and produced data from 3,803 SNPs (Fig. 3a, Table 1; Russello et al. 2015). NextRAD differs from other amplicon sequencing methods in that it indiscriminately generates sequence data for both target and non-target species and therefore will be inefficient for samples containing large quantities of non-target DNA, such as fecal samples, without incorporating a target DNA capture step (see below). NextRAD also targets many more loci than most other amplicon sequencing methods.

Table 1 Examples of studies using high-throughput DNA sequencing and SNP genotyping for low-quality DNA samples

Method	Example system	Source material	Number of loci	Application	Citation
Amplicon sequencing					
Microsatellites	Brown bear (*Ursus arctos*)	Feces	13 microsatellites, 1 sex marker	Proof of method, individual identification, parentage	De Barba et al. (2017)
NextRAD	American pika (*Ochotona princeps*)	Hair	3,803 SNPs	Proof of method, divergent selection and diversity across habitats	Russello et al. (2015)
SNP genotyping platforms					
Fluidigm	European gray wolf (*Canis lupus*)	Feces, saliva, hair, urine	96 SNPs	Proof of method, individual identification	Kraus et al. (2015)
MassARRAY	Puma (*Puma concolor*)	Feces	25 SNPs	Proof of method, individual identification	Fitak et al. (2016)
Illumina GoldenGate BeadXpress	Coyote (*Canis latrans*)	Feces	63 SNPs	Hybridization assessment	Monzón et al. (2014)
DNA capture					
Exons	Alpine chipmunk (*Tamias alpinus*)	Museum toe pads	~11,000 exons	Temporal shifts in genetic structure and diversity	Bi et al. (2013)
Ultraconserved elements (UCEs)	Western scrub jay (*Aphelocoma californica*)	Museum toe pads	4,460 SNPs	Phylogeny	McCormack et al. (2016)
Microsatellites	Lemur (*Propithecus diadema*)	Feces	5,000 microsatellites	Proof of method, parentage	Kistler et al. (2017)
Mitogenome sequencing					
Amplicon sequencing	East African white-eyes (*Zosterops* spp.)	Feathers	13,523–13,596 bp	Phylogeography	Meimberg et al. (2016)
Genome skimming	Tasmanian devil (*Sarcophilus harrisii*)	Museum hair shafts	16,940 bp	Temporal shifts in genetic diversity	Miller et al. (2011)
Capture	Western chimpanzee (*Pan troglodytes verus*)	Feces	16,554 bp	Proof of method	Perry et al. (2010)

RADseq					
ddRAD	Lake whitefish (*Coregonus clupeaformis*)	Degraded muscle	Not reported	Proof of method	Graham et al. (2015)
Ribo-tailing	Ants (three species)	Museum whole specimen	16–1,275 SNPs	Proof of method	Tin et al. (2014)
Capture	Common ragweed (*Ambrosia artemisiifolia*)	Museum leaf	22,813 SNPs	Proof of method; historic population structure	Barreiro et al. (2017)
Whole-genome sequencing					
Shotgun sequencing	Polar bear (*Ursus maritimus*)	Bone	2.53 Gb	Evolutionary history	Miller et al. (2012)
Capture	Przewalski's horse (*Equus ferus* ssp. *przewalskii*)	Bone	2.40 Gb	Evolutionary history	Der Sarkissian et al. (2015a, b)
Capture	Baboon (*Papio papio*)	Feces	127,654 SNPs	Parentage, relatedness	Snyder-Mackler et al. (2016)
Metagenomics/metabarcoding/metagenome skimming					
Metagenomics	Giant panda (*Ailuropoda melanoleuca*)	Feces	37 Mbp	Taxonomic and functional composition of microbiome	Zhu et al. (2011)
Metabarcoding	Large herbivores (seven species)	Feces	1 barcode locus	Dietary niche partitioning	Kartzinel et al. (2015)
Metagenome skimming	Banded leaf monkey (*Presbytis femoralis*)	Feces	3 barcode loci	Diet, parasites, population genetics	Srivathsan et al. (2016)

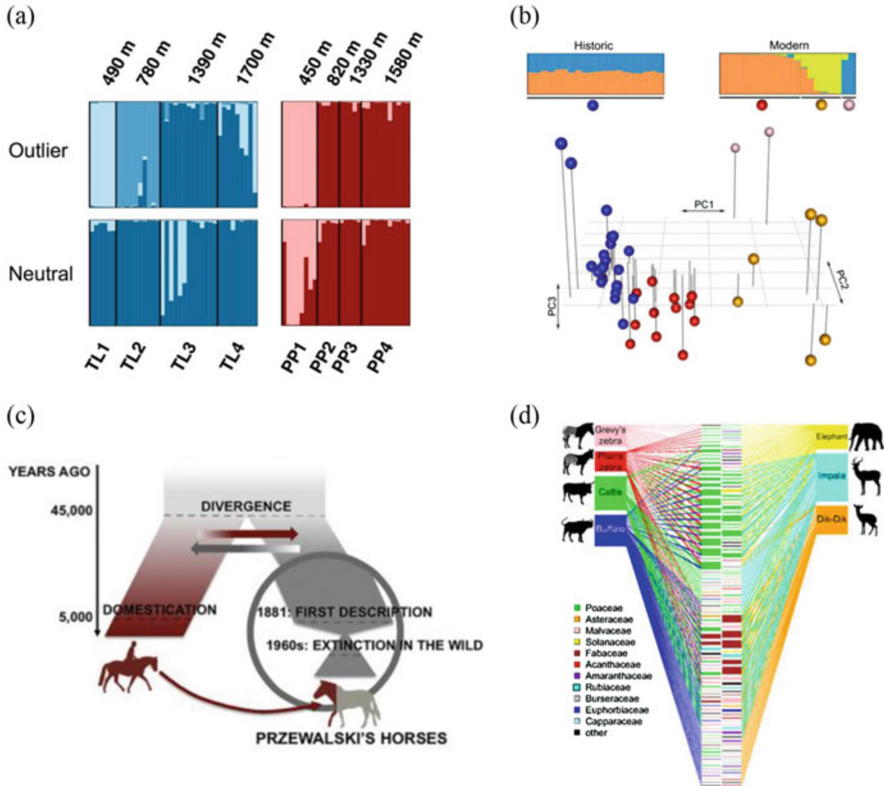

Fig. 3 Examples of studies using next-generation sequencing of low-quality samples. (**a**) Russello et al. (2015) used NextRAD to evaluate 3,803 SNPs for non-invasively collected hair samples from American pika across two elevational gradients (TL and PP). Outlier analysis and Bayesian clustering analysis revealed 55 loci that are candidates for divergent selection at different elevations. (**b**) Bi et al. (2013) used DNA capture with ~11,000 exons for both contemporary alpine chipmunk samples and museum samples collected in 1915. Bayesian clustering analysis and principal components analysis revealed greater genetic structure for contemporary (non-blue dots) than historic (blue dots) samples. (**c**) Der Sarkissian et al. (2015b) sequenced the genomes of 11 contemporary captive and five historic Przewalski's horses dating 1878–1929. Comparative genomics indicated divergence from domestic horses around 45,000 years ago with ongoing gene flow, variable introgression of domestic alleles, and inbreeding resulting from captivity. (**d**) Kartzinel et al. (2015) conducted fecal sample metabarcoding for seven large mammalian herbivores and discovered unexpectedly strong diet partitioning across taxonomic groups

With the exception of NextRAD, amplicon sequencing requires template-specific primers and therefore requires prior genomic knowledge, which may be unavailable for many wildlife species. However, genomic information is becoming increasingly available for non-model organisms, thus increasing the feasibility of this approach.

5 SNP Genotyping Platforms

Numerous SNP genotyping platforms are currently available for analyzing a preselected set of loci (reviewed in Garvin et al. 2010; Ogden 2011; Ragoussis 2009; Slate et al. 2009). These platforms do not use NGS techniques but instead use probes and primers, a wide range of hybridization and enzymatic reactions, and genotype resolution through fluorescent dye or molecular weight detection (Fig. 2). The data output of these platforms is genotype calls rather than sequence reads and therefore requires less bioinformatic processing. Furthermore, the data is generated at a much lower cost and effort than NGS data and usually with a lower genotyping error rate. However, these platforms also require custom-designed taxon-specific probes and primers, which must be developed from prior genomic data. In addition, most platforms require costly, specialized equipment.

The numbers of loci and individuals analyzed at a time, and the level of flexibility in those numbers, vary widely across genotyping platforms. Small-scale platforms analyze tens of individuals and loci at a time and include the Fluidigm Dynamic Array (Fluidigm Corp, San Francisco, USA), MassARRAY (Agena Biosciences, San Digeo, USA), and SNPlex (Applied Biosystems, Foster City, USA). In contrast, platforms developed by Illumina and Affymetrix can genotype thousands of individuals and/or loci simultaneously; for example, the Illumina GoldenGate platform can assay between 96 and 3,072 loci for up to 1,536 samples, and the Illumina Infinium HD iSelect BeadChip arrays can genotype between 3,000 and one million SNPs for up to 24 samples.

The use of SNP genotyping assays is growing in wildlife research, largely due to an increase in accessibility of genomic resources that can be used to design probes and primers (Table 1). Some wildlife studies have taken advantage of genotyping assays developed from closely related domestic species. For example, domestic dog assays have been used for wild canids (von Holdt et al. 2011), cattle assays have been used for deer (Haynes and Latch 2012), and domestic sheep assays have been used for bighorn sheep (Miller et al. 2014). However, the use of SNP assays designed for a different species often results in high proportions of monomorphic loci due to ascertainment bias (Clark et al. 2005; Lachance and Tishkoff 2013). Other wildlife studies have designed species- or population-specific SNP assays using genomic data obtained from transcriptome sequences (Cullingham et al. 2013; Fitak et al. 2016; Hoffman et al. 2012), whole-genome sequences (Nguyen et al. 2014), or other sources of DNA sequence (Holman et al. 2017; Veale and Russello 2016).

Most SNP genotyping assays are not amenable to non-invasive samples due to requirements for high-quantity and/or high-quality input DNA. However, several studies have found success with non-invasive samples when using SNP genotyping assays that target relatively small numbers of loci. For example, a study using 96×96 Fluidigm Dynamic Array chips, which genotype 96 loci and 96 samples at a time, found low missing data rates (<10%) and low genotyping error rates (~1%) for non-invasive European gray wolf samples including tissue, blood, scat, saliva, hair,

and urine (Kraus et al. 2015). Another study using the same type of assay found even lower rates of missing data and genotyping error (<1%) for fecal samples collected from brown bears in Sweden (Norman and Spong 2015). Thus far, success rates have been lower for studies using MassARRAY for low-quality samples. For example, Fitak et al. (2016) found a relatively high missing data rate (40.2%) for a 25-locus MassARRAY assay for puma fecal samples; however, this was lower than the missing data rate for microsatellites for the same samples (60.1%), indicating sample quality may have been a driving factor.

6 DNA Capture

DNA capture is increasingly being used for NGS approaches with low-quality samples (Fig. 1b, Table 1; Gnirke et al. 2009; Jones and Good 2016; Mamanova et al. 2010). This method first involves the creation of genomic DNA libraries for sequencing on a NGS platform. These libraries must contain DNA fragments of the appropriate size for sequencing, which is about 500 bp for the widely used Illumina sequencing platform. For high-quality DNA samples, genomic DNA is usually reduced to the appropriate fragment size range through mechanical or enzymatic shearing, but low-quality samples may already have DNA fragments this size or smaller. Genomic DNA libraries must also have sequencing adapters ligated onto the ends of the DNA fragments. These DNA libraries could be sequenced directly, but for many applications this would generate data from many more loci than are needed. Furthermore, this approach would indiscriminately sequence all DNA present in the sample, which in the case of low-quality samples would often include high proportions of non-target DNA, such as bacteria in many archival samples. DNA capture circumvents these problems by targeting selected loci through hybridization of biotinylated oligonucleotide probes or "baits" to the library and then isolating the hybridized DNA for sequencing. Baits must be designed using prior genomic knowledge, such as a reference genome, transcriptome, restriction site-associated DNA sequencing (RADseq) data (described below), or PCR products. Any number of baits can be synthesized, or baits can be created by directly biotinylating PCR products, RADseq libraries, RNA-transcribed PCR products, or RNA-transcribed DNA libraries. If prior genomic information is not available for the target species, several studies have shown that capture efficiency can be high for baits designed using genomic resources from a moderately divergent species (Bi et al. 2012; Enk et al. 2014; Hedtke et al. 2013; Jin et al. 2012; Vallender 2011).

A number of studies have used DNA capture to target hundreds or thousands of loci for population genetic or phylogenetic analyses with low-quality samples. For example, DNA capture was used to investigate temporal shifts in genetic structure and diversity for alpine chipmunks by sequencing ~11,000 exons from 20 museum samples collected in 1915 and 20 contemporary samples (Fig. 3b, Bi et al. 2013). Similarly, two studies employed DNA capture targeting 9,000 SNPs and the full mitogenome, respectively, to directly investigate the genetic consequences of rapid

population decline and recovery in Pinzon Galapagos giant tortoises sampled pre-bottleneck in 1906 ($n = 78$) and post-bottleneck in 2014 ($n = 150$) (Jensen et al. 2018a, b). Another study used sequence capture of 5,060 ultraconserved elements (UCEs) for phylogenetic analyses of 27 western scrub jay museum specimens up to 120 years old (McCormack et al. 2016). Kistler et al. (2017) described a bioinformatic pipeline for designing probes for a large panel of microsatellite markers for non-model species without a reference genome and developed a capture-based approach for enriching genomic DNA libraries for thousands of these loci. They tested this method on tissue and fecal samples from an endangered lemur species and reported high efficiency in recovering targeted loci, as well as high genotyping accuracy.

DNA capture has also been used to sequence whole mitogenomes, subsets of RADseq loci, and even whole genomes. These approaches are described in more detail in later sections.

7 Mitogenome Sequencing

Mitochondrial DNA has been used frequently in analyses of ancient, historical, non-invasive, and forensic samples, because it is present in high copy number in cells relative to nuclear DNA (Table 1). This disparity in copy number helps facilitate recovery of mtDNA sequences through PCR amplification and other approaches (Alacs et al. 2010; Ho and Gilbert 2010; Höss et al. 1992; Paijmans et al. 2013). Mitochondrial markers are linked and maternally inherited and therefore do not provide as much information as multiple nuclear loci. Nonetheless, whole mitogenome sequences can provide greater resolution than single mtDNA markers for phylogenetic and population genetic analysis (Duchêne et al. 2011; Meiklejohn et al. 2014; Paijmans et al. 2013; Rohland et al. 2007) and can enhance the discriminatory power of forensic analysis (Chaitanya et al. 2015).

Before the development of NGS technologies, mitogenomes could be sequenced by PCR amplification and Sanger sequencing of overlapping fragments covering the entire mitogenome, provided sufficient prior knowledge was available for primer design. This approach has been used successfully with a variety of low-quality sample types, including non-invasively collected feces (Bjork et al. 2011; Finch et al. 2014; Matsui et al. 2007) and ancient (Bon et al. 2008; Rogaev et al. 2006) specimens. This process can also be accomplished using two-step multiplex PCRs, which reduce the amount of source biological material required compared to a singleplex PCR approach, and does not require cloning prior to Sanger sequencing (Edwards et al. 2010; Krause et al. 2006; Römpler et al. 2006). However, low-quality samples present a challenge for PCR-based techniques, because DNA degradation can limit the size of fragments available for amplification.

NGS technologies have overcome many of the challenges of Sanger sequencing-based approaches to mitogenome sequencing for low-quality samples. NGS can be used to sequence mtDNA singleplex or multiplex PCR products more efficiently and

inexpensively than Sanger sequencing. Alternatively, mtDNA sequences can be recovered from NGS sequence data generated from entire genomic DNA libraries and assembled into whole mitogenomes, thus eliminating the need for prior knowledge for PCR primer design. This can be accomplished using relatively low quantities of sequence data from genomic DNA libraries, because the high copy number of mtDNA in cells results in a high proportion of the data being of mtDNA origin. The technique of using low quantities of sequence data from genomic DNA libraries to assemble mitogenomes has been called "genome skimming" and can also be used to assemble other high-copy loci like nuclear ribosomal genes, histone genes, and plastomes (Straub et al. 2012). This approach has become widely used for mitogenome sequencing with archival samples, with examples including an ancient polar bear bone sample dating 110,000–130,000 years ago (Lindqvist et al. 2010), a woolly mammoth sample dating 14,000–60,000 years ago (Gilbert et al. 2007), an ice-age woolly rhinoceros sample (Willerslev et al. 2009), and Tasmanian devil museum samples from the early 1900s (Miller et al. 2011).

Mitogenomes can also be sequenced using DNA capture from genomic DNA libraries, and this technique has been used in a number of studies with low-quality samples. For example, this approach was used to sequence mitogenomes for guenon specimens as old as 117 years (Guschanski et al. 2013), extinct sloth species from bone and coprolite samples (13,000–20,000 years old; Slater et al. 2016), and contemporary fecal samples for chimpanzees (Perry et al. 2010). This approach requires prior mitogenome sequence knowledge for bait design, but some studies have found success using baits designed from species 10–20% divergent from the target species (Hawkins et al. 2016; Mason et al. 2011).

8 RADseq

Restriction site-associated DNA sequencing is a widely used NGS-based method for sequencing a subset of the genome for non-model organisms (Fig. 1c, Table 1; reviewed in Andrews et al. 2016; Davey et al. 2011). This method sequences regions adjacent to restriction cut sites, which occur across the genome in both coding and noncoding regions. RADseq requires no prior genomic knowledge, uses relatively small quantities of genomic DNA (usually around 100 ng), and is highly flexible in the numbers of loci that can be surveyed. Numerous methods have been developed to generate RADseq data (reviewed in Andrews et al. 2016), but all share some common features: digesting genomic DNA with one or more restriction enzymes, ligating adapters required for sequencing, and sequencing with a high-throughput platform. Most RADseq protocols ligate adapters with unique, sample-specific barcodes early in the library prep; this decreases the time and cost involved in library prep by allowing many samples to be multiplexed early in the protocol and also decreases sequencing costs by allowing many individuals to be sequenced together. Although this approach typically generates data from several thousands to tens of thousands of loci, smaller numbers of loci can be assayed by adding a DNA capture

step to the end of the RADseq library prep using baits designed directly from RADseq data for a selected subset of loci ("Rapture," Ali et al. 2016; "RADcap," Hoffberg et al. 2016).

Few studies have tested the performance of RADseq on low-quality samples. RADseq may be more negatively impacted by degraded DNA than other methods, because digesting degraded DNA with restriction enzymes may result in fragments too small to generate sufficient sequence information. Graham et al. (2015) systematically evaluated the performance of one RADseq method on low-quality samples by allowing lake whitefish muscle samples to degrade by incubation at room temperature for up to 96 h before DNA extraction and library prep. This study found little reduction in RADseq data quantity and quality for moderately degraded DNA but a substantial reduction for severely degraded DNA. Another study used a modified RADseq protocol for six ant specimens collected between 1910 and 1953 and found that a large proportion of sequence reads were not useable because they were too small for genome mapping or were PCR duplicates, although several protocol modifications were proposed that could increase the success of this technique in future studies (Tin et al. 2014). The performance of RADseq for archival and non-invasive samples is likely to vary across the wide range of laboratory protocols that have been developed and also across the variety of sample types, due to the associated variation in quantity and quality of DNA.

Another approach for generating RADseq data from low-quality samples is to use DNA capture of genomic DNA libraries with RADseq locus-specific baits. These approaches differ from Rapture and RADcap (described above), which may perform more poorly for low-quality samples because loci are captured from RADseq libraries rather than genomic DNA libraries. For example, Barreiro et al. (2017) used RADseq data from 190 modern, high-quality common ragweed samples from 37 populations to design and synthesize capture baits for 20,000 RADseq loci. These baits were then used for DNA capture of genomic libraries generated using 38 herbarium samples dating 1835–1913. Although the numbers of sequence reads varied substantially across targeted loci, this approach led to the discovery of 22,813 SNPs in the herbarium samples. Suchan et al. (2016) used a similar approach for museum samples of *Lycaena helle* butterfly but, instead of designing and synthesizing baits, created baits by directly biotinylating RADseq libraries generated from high-quality DNA samples.

9 Whole-Genome Sequencing

NGS can also be used to sequence entire genomes (Fig. 1d, Table 1; reviewed in Ekblom and Wolf 2014; Ellegren 2014). Currently, most WGS projects with high-quality samples rely on direct sequencing of genomic DNA libraries ("shotgun sequencing") using the Illumina HiSeq platform. However, the Illumina HiSeq generates sequence reads that are a maximum of 150 bp long, and the short lengths

of these fragments can present a limitation when assembling a genome de novo. Thus, many whole-genome sequencing projects with high-quality samples use a combination of both Illumina sequencing and long-read sequencing technologies such as those developed by Pacific Biosciences or Oxford Nanopore. Even when combining short- and long-read technologies, however, the process of de novo genome assembly from raw sequence data requires a large quantity of sequence data and complex computational algorithms (Ekblom and Wolf 2014; Ellegren 2014). Once a genome has been assembled for a species, "genome resequencing" of additional samples from the same species can be conducted with less sequencing and computational effort, because the raw sequence data can be aligned to the reference genome (Ellegren 2014; Fuentes-Pardo and Ruzzante 2017). Furthermore, a reference genome can provide a valuable resource for sequencing and genotyping approaches that target a subset of the genome, such as those described above.

WGS and genome resequencing are challenging for low-quality samples due to low quantities of DNA, degradation of DNA, and high proportions of non-target DNA which is sequenced indiscriminately with standard WGS protocols. However, the high-throughput sequencing capability of NGS, along with the ability of NGS to sequence short DNA fragments, dramatically increases the feasibility of WGS for low-quality samples when compared to Sanger sequencing (Hofreiter et al. 2015; Rizzi et al. 2012). A growing number of studies are using NGS to sequence whole genomes from archival samples, including samples from extinct and endangered species (Table 1; reviewed in Der Sarkissian et al. 2015a; Leonardi et al. 2017). For example, Mikheyev et al. (2015) sequenced and compared the genomes of honeybees collected from 32 colonies in 1977 and 2010, and Der Sarkissian et al. (2015b) sequenced the genomes of five historic Przewalski's horses dating to 1878–1929 (Fig. 3c). A number of genomes have been sequenced from ancient bone, tissue, and hair specimens including human, Neanderthal, mammoth, polar bear, horse, common rat, and pigeon (e.g., Cahill et al. 2013; Green et al. 2006; Hung et al. 2014; Miller et al. 2008, 2012; Orlando et al. 2013; Poinar et al. 2006; Rowe et al. 2011).

Although NGS has dramatically increased the feasibility of WGS with low-quality samples, this approach is still more costly than WGS of high-quality samples and requires special protocols for DNA extraction, library prep, and bioinformatic analyses to accommodate degraded DNA and the high prevalence of non-target DNA in the samples (reviewed in Orlando et al. 2015). One of the primary laboratory methods used for WGS of low-quality samples is DNA capture with probes designed from genome sequence data generated using high-quality modern samples, and this approach was used for most of the examples listed above. Some recent DNA capture studies have reduced the time and cost involved in bait development by generating a genomic DNA library using a high-quality sample from the study species and then transcribing the library into biotinylated RNA fragments which can be directly used as baits. For example, Carpenter et al. (2013) used this approach for genome-wide enrichment of ancient human teeth, bone, and hair samples, with baits generated by transcribing a DNA library created using a blood sample from a contemporary human. For this study, DNA libraries sequenced without the capture step yielded an average of 1.2% reads that mapped to

the human genome, but libraries sequenced with the capture step yielded up to 59% mapped reads. Notably, this technique requires a large quantity of genomic DNA from a high-quality sample, which can be a limitation for many wildlife studies.

The use of WGS for contemporary non-invasive samples has lagged behind that for ancient samples and has focused on primate fecal samples with a DNA capture approach. Perry et al. (2010) demonstrated the feasibility of WGS from fecal samples by sequencing more than 1.5 Mb of the genome (including the whole mitogenome and parts of two chromosomes) from six western chimpanzee fecal samples, using 55,000 120 bp capture baits designed from a chimpanzee reference genome. Snyder-Mackler et al. (2016) used a capture approach similar to that of Carpenter et al. (2013) described above for genome-wide enrichment of 62 wild baboon fecal samples, with baits generated by RNA transcription of a genomic library from a high-quality blood sample from the same species (Table 1). This study found up to 40-fold target enrichment as a result of the capture step.

Chiou and Bergey (2018) introduced another method called "FecalSeq" for enriching whole genomic DNA from low-quality vertebrate samples that requires no prior genomic knowledge or high-quality samples. Instead of using baits, this method uses methyl-CpG-binding domain (MBD) proteins to selectively bind DNA having high levels of methylation, taking advantage of the fact that vertebrate DNA has a higher frequency of methylation than does bacterial DNA. This approach resulted in a 195-fold increase in the amount of target DNA for baboon fecal samples that started with a mean of 0.34% host DNA (Chiou and Bergey 2018).

10 Metagenome Sequencing, Metabarcoding, and Metatranscriptomics

Whereas the techniques we have described thus far generate sequence and genotype data for single target organisms, other techniques called metagenome sequencing, metabarcoding, and metatranscriptomics generate sequence data for samples comprised of multiple individuals and species, such as eDNA samples, insect traps, plankton tows, fecal samples, oral swabs, and many other sample types (reviewed in Aguiar-Pulido et al. 2016; Mendoza et al. 2015; Taberlet et al. 2018; Wooley et al. 2010). "Metagenomics" refers to studies generating data from a large portion of the genomes present in these samples (the "metagenome"); these studies typically use a shotgun sequencing approach and focus on both taxonomic-informative and functional genes present in species that have relatively small genomes, such as microbial species. For example, Zhu et al. (2011) used wild giant panda fecal samples for metagenomic analysis of the gut microbiome and identified putative bacterial genes coding for cellulose-digesting enzymes, thus clarifying how giant pandas can partially digest bamboo despite the absence of genes coding for cellulose-degrading enzymes within the panda genome (Table 1). Similarly, fecal metagenomics revealed functional genes related to metabolism in the microbiome of Asian

elephants (Ilmberger et al. 2014), snub-nosed monkeys (Xu et al. 2015), and Galapagos iguanas (Hong et al. 2015).

"Metabarcoding" differs from metagenomics because it does not focus on a large portion of the genome but instead typically uses amplicon sequencing of PCR products for a small number of taxonomic-informative loci to delineate the species composition of a sample (reviewed in Taberlet et al. 2018). Metabarcoding is similar to "DNA barcoding," but starts with samples comprised of communities of individuals rather than single individuals, and simultaneously generates sequence data from these different individuals and species. As in DNA barcoding, metabarcoding usually focuses on a certain taxonomic group, such as bacteria, plants, or plankton, and usually uses high-copy genetic markers such as mtDNA and chloroplast loci. However, shorter fragments are amplified for non-invasive and eDNA samples to accommodate DNA degradation, and highly conserved and versatile primers are preferred for robust and efficient amplification of many taxa (Deagle et al. 2014; Valentini et al. 2009). The development of NGS technologies has led to a dramatic rise in metabarcoding studies because these technologies eliminate the need for a time-consuming cloning step that would be required for Sanger sequencing of samples containing amplicons from multiple individuals and species.

Metabarcoding is becoming widely used for wildlife studies with non-invasive and eDNA samples. For example, fecal sample metabarcoding has been used to characterize diet in herbivores, carnivores, insectivores, and omnivores (e.g., Fig. 3d, Table 1; Bohmann et al. 2011; De Barba et al. 2014; Deagle et al. 2009; Kartzinel et al. 2015; Shehzad et al. 2012; Valentini et al. 2009). Fecal metabarcoding has also been used to characterize microbial communities in a number of wildlife species, primarily using 16S ribosomal RNA markers (reviewed in Escobar-Zepeda et al. 2015). For example, Amato et al. (2013) used fecal metabarcoding to characterize microbial communities of howler monkeys across different habitats and found lower microbiome diversity in suboptimal habitats. Metabarcoding of eDNA water samples has been used to characterize fish, amphibian, and invertebrate communities, detect invasive species, and even infer population structure and diversity (e.g., Klymus et al. 2017; Sigsgaard et al. 2016; Thomsen et al. 2012). This approach has also been used to characterize ancient and contemporary species assemblages of flora and fauna using eDNA extracted from soil samples (reviewed in Pedersen et al. 2015).

Another approach for generating sequence data from barcode loci is "metagenome skimming" (Crampton-Platt et al. 2016; Papadopoulou et al. 2015). This method is conceptually similar to genome skimming (described above) and starts with sequencing of metagenomic DNA libraries, followed by bioinformatic identification of the portion of the sequence data that is the barcode loci. This technique is feasible even with relatively low quantities of metagenomic sequence data, because barcode loci are typically high copy number in cells, and therefore a large proportion of the sequence data generated by metagenome sequencing will be from the barcode loci. This technique is more expensive than metabarcoding, but overcomes several of its limitations, including PCR bias, lack of taxonomic resolution from using small numbers of barcode loci or short barcode loci, and detection of species for which

primers anneal poorly. Furthermore, metagenome skimming can also be used to assemble mitogenomes and identify non-target taxa. For example, Srivathsan et al. (2016) used both metagenome skimming and metabarcoding of fecal samples to assess the species composition of banded leaf monkey diet and found that metagenome skimming had higher taxonomic resolution (Table 1). In addition, the fecal sample metagenome sequence data confirmed the presence of parasites and allowed assembly of whole mitogenomes of the monkeys. Metagenome skimming has been described using a variety of terms in the literature, including "metagenomics," "targeted metagenomics," "metabarcoding," and "PCR-free single/multiple loci metabarcoding," potentially leading to confusion (Mendoza et al. 2015).

Metatranscriptomics involves sequencing RNA extracted from a sample comprised of a community of individuals and species (Aguiar-Pulido et al. 2016; Carvalhais et al. 2012; Moran 2009). This method provides information regarding the genes expressed by the community and has primarily been used to investigate microbial communities. For example, metatranscriptomics may help identify bacterial genes with functions making them effective probiotics for the fungus responsible for chytridiomycosis in amphibians (Rebollar et al. 2016). However, metatranscriptomics has a number of limitations, including the instability of RNA, the requirement for reference data regarding gene sequences and functions, and others (Moran 2009).

11 Choosing a Method

The optimal choice of sequencing or genotyping method for a project involving low-quality samples will depend on the research question and budget, as well as the quantity and quality of DNA available. Different research questions vary in the required numbers and specificity of targeted loci, and the numbers of loci generated by different methods vary substantially (Fig. 4). Currently, DNA capture is the most widely used technique for generating sequence data from low-quality samples because it targets specific loci of interest and has high flexibility in the number of loci targeted. Amplicon sequencing and SNP genotyping also target loci of interest and are less time-consuming and expensive than DNA capture for large sample sizes; these methods are likely to become widely used for research projects with low-quality samples requiring fewer than several hundred loci. For studies targeting the mitogenome, the choice between amplicon sequencing, genome skimming, and DNA capture will largely rest on project budget and the availability of reference sequences for designing primers or baits.

In contrast to DNA capture and amplicon sequencing, non-targeted approaches like RADseq and WGS are advantageous because they require no prior genomic information (Fig. 4), thus eliminating the time, cost, and bioinformatic expertise required to design primers and probes. Furthermore, non-targeted approaches are minimally affected by the many disadvantages associated with ascertainment bias

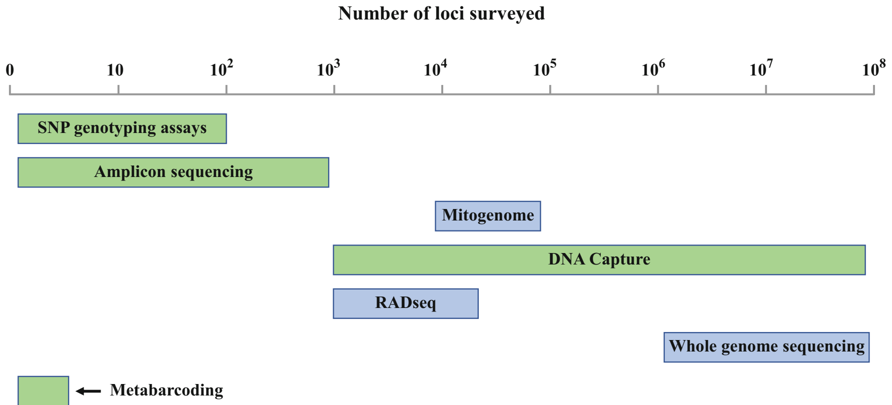

Fig. 4 Numbers of loci typically interrogated for next-generation sequencing and SNP genotyping approaches with wildlife species. Green indicates methods that require prior genomic knowledge to design primers and probes, and blue indicates methods that require no prior genomic knowledge. Note the scale is logarithmic

(Clark et al. 2005; Lachance and Tishkoff 2013). However, generating RADseq and WGS data is more technically challenging for non-invasive samples than targeted approaches, due to greater sensitivity to DNA quantity and quality. In addition, these methods non-discriminately sequence all DNA present in the sample and therefore will waste sequencing effort for samples containing large quantities of exogenous DNA, unless a DNA capture step is incorporated. In addition, RADseq and WGS generate data from much larger numbers of loci than are required for many research questions and thus will not be time- and cost-efficient for many studies. However, WGS could be highly valuable for species or populations for which few samples are available, such as endangered or extinct species, because a number of ecological and evolutionary insights can be gained from whole-genome sequences from even a single individual. In addition, both RADseq and WGS data can be used as a resource for designing primers and probes for targeted approaches.

For studies of environmental samples, metabarcoding is currently the most efficient, affordable, and widely used approach for taxonomic composition analysis. Metagenome skimming can also be used for this type of analysis and can overcome some of the limitations of metabarcoding but requires more sequencing and bioinformatic effort. Metagenomic and metatranscriptomic approaches can provide insight into the functional genomic composition of communities but require even greater sequencing and bioinformatic effort; currently, these approaches are typically restricted to species with relatively small genomes, such as microbes, and are often limited by the availability of functional genomic information in reference databases.

In summary, recent advances in DNA sequencing and genotyping technologies have dramatically increased the amount of genetic data available for low-quality samples, thereby expanding the types of questions we can address using non-invasively collected, archival, and environmental samples. These technologies

are rapidly evolving to become cheaper, faster, and easier and produce longer and more accurate sequence reads. Although applications of these technologies for low-quality samples will continue to lag behind those for high-quality samples, nonetheless we expect steady advances in techniques tailored for these sample types in the coming years. Continued efforts should also be made to maximize initial amount and quality of DNA for low-quality samples by optimizing sample collection, preservation, and DNA extraction to minimize DNA degradation and loss, and improve recovery of endogenous DNA. Furthermore, development should continue for effective bioinformatic tools and standards tailored for data generated from low-quality samples to ensure accurate data collection. In the coming decade, we expect these advances to provide unprecedented insights into wildlife ecology, evolution, and conservation.

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Glossary

Amplicon sequencing High-throughput sequencing of PCR products from multiple individuals simultaneously

Archival sample Historic (collected within the last ~200 years) or ancient (usually up to ~100,000 years old) tissue sample

Ascertainment bias Inference bias resulting from the process by which genetic loci were selected

Bait Biotinylated oligonucleotide probe used to isolate or "capture" target DNA for sequencing

DNA capture Method relying on baits to isolate or "capture" target DNA prior to high-throughput sequencing

DNA library DNA that has been prepared for high-throughput sequencing, with DNA fragments the appropriate length, and with sequencing adapters ligated to ends of fragments

Environmental DNA (eDNA) DNA present in environmental samples such as water, soil, and air

Genetic non-invasive sample (gNIS) DNA sample collected without handling the study organism, e.g., shed hair or fecal sample

Genome skimming Using low quantities of sequence data from genomic DNA libraries to study high-copy loci like mitogenomes, nuclear ribosomal genes, histone genes, and plastomes

Metabarcoding High-throughput sequencing of PCR products generated from taxonomic-informative markers for an environmental sample

Metagenome skimming Using shotgun sequencing data from samples comprised of multiple individuals and/or species (e.g., environmental samples) to study high-copy loci like mitogenomes, nuclear ribosomal genes, histone genes, and plastomes. Often focuses on taxonomic-informative markers to identify community composition

Metatranscriptomics Sequencing RNA extracted from an environmental sample

Next-generation sequencing A variety of high-throughput DNA sequencing technologies developed over the last decade

Nextera-tagmented, reductively amplified DNA (NextRAD) High-throughput sequencing of PCR products from genomic regions containing a preselected 9 bp sequence

Restriction site-associated DNA sequencing (RADseq) High-throughput sequencing of genomic regions adjacent to restriction cut sites

Sanger sequencing "Traditional" low-throughput DNA sequencing technology developed in 1977

Sequencing by synthesis High-throughput sequencing technology used by Illumina that detects the incorporation of single bases into replicating DNA strands

Shotgun sequencing Direct sequencing of genomic DNA libraries

Single nucleotide polymorphism (SNP) Variant at a single nucleotide site

SNP genotyping platforms A variety of technologies that generate SNP genotype data rather than sequence reads

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