Wildlife Population Genomics: Applications and Approaches

Soraia Barbosa, Sarah A. Hendricks, W. Chris Funk, Om P. Rajora, and Paul A. Hohenlohe

Abstract Population genomics provides a powerful and growing set of approaches for wildlife biology, revealing new insights into demographic history, population structure, adaptation, and the consequences of genetic diversity. Given the multiple threats faced by global biodiversity, it is critical for researchers to advance efforts to manage and conserve wildlife populations. In this chapter we provide an overview of the research questions that can be addressed in wildlife population genomics, applications to specific conservation and management issues, and the variety of technical methods at all stages from sampling to sequencing and data analysis. Wildlife species, here defined as vertebrate species of specific conservation or management concern, present unique challenges and opportunities. These include not only the necessity of using poor-quality samples from non-invasive or archival collections, but also the availability of genomic reference data from closely related domestic species. We highlight a number of case studies in particular taxa that illustrate recent progress in wildlife population genomics, including how population genomics approaches have been applied to date, and also how the field can continue to connect research to urgent conservation actions in wildlife populations. We also discuss prospects for applications of population epigenomics, transcriptomics, metagenomics, and eDNA approaches in wildlife.

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1 Introduction

Wildlife species face a number of threats, such as habitat loss and fragmentation, direct mortality from exploitation, invasive species, pollution, and climate change. The genomic revolution has democratized the field of population genomics, allowing high-throughput sequencing to be applied in nearly any organism, including natural populations of rare or difficult-to-study wildlife species (Luikart et al. 2019; Rajora 2019). Wildlife biology can benefit from population genomics in several ways: first, by improving our basic understanding of wildlife species and populations, including their evolutionary history and relationships, adaptation to local environments and ecological interactions, and population dynamics and viability. Second, this information can inform management and conservation efforts, such as delineating population units for conservation, maintaining genetic diversity in captive or wild populations, or predicting adaptive potential. The importance of genetic variation in conservation, including its role in setting conservation targets and monitoring the status of biodiversity, is increasingly recognized and can benefit from genomics tools (Hoban et al. 2020). Population genomics studies can provide efficient genetic approaches for monitoring and managing populations, and a number of technical improvements specifically make genomics more applicable to wildlife. These include methods for using non-invasive DNA samples or environmental DNA, and sequencing tools that can be used in the field.

Traditional wildlife population genetics has focused on mitochondrial, and less frequently, nuclear DNA sequences for population-level analyses, and microsatellites for individual-level analyses (Schwartz et al. 2007; Allendorf 2017). Mitochondrial DNA has been the most widely used molecular marker for population genetic diversity, phylogeography, conservation units, and species identification (DeYoung and Honeycutt 2005; Hajibabaei et al. 2007). Microsatellite analyses have focused mostly on identifying fine-scale genetic population structure and connectivity, population origin, estimating kinship, abundance and dispersal, as well as studying behavior, such as determining mating systems (Carroll et al. 2018). A great deal of wildlife conservation research has been dedicated to evaluate population connectivity and individual dispersal, improved by the ability of performing individual identification through non-invasive samples, for example in Cabrera voles (*Microtus cabrerae*; Ferreira et al. 2018) to tigers (*Panthera tigris*; Thatte et al. 2018). Here, integration with landscape ecology has enabled great insights into the identification of dispersal corridors and barriers to gene flow, which has shown the vulnerability of isolated small populations in many species of conservation concern (Proctor et al. 2005; Shah and McRae 2008; Waits et al.

2016). Very important were also the studies aimed at identifying links between variation at microsatellites associated with immune genes and selection and fitness, particularly using Major Histocompatibility Complex genes (Oliver et al. 2009; Palomares et al. 2012). Traditional markers like microsatellites continue to be extremely valuable for wildlife genetics studies, for instance for estimating relatedness in captive populations of Iberian lynx (*Lynx pardinus*; Kleinman-Ruiz et al. 2019) or fragmented wild populations of African leopards (*Panthera pardus pardus*; Naude et al. 2020).

Wildlife biology has much to gain from the transition from population genetics to population genomics methods, as over the past decade, the techniques of population genomics have been applied widely across the fields of biology (Rajora 2019). Applications to conservation have been slow to develop because of hurdles in translating research into concrete actions, due to limitations of costs, sample quality, or applicability of the methods. The power of emerging genomics methods to answer different questions is central to their application in wildlife. For instance, the use of RAD sequencing to detect adaptive variation has been under debate because the technique samples a subset of loci across the genome, and some important regions of the genome may be missed. This potentially limits inferences about the genetics of adaptation, but progress is also being made in how to quantify and assess trade-offs among methods (Catchen et al. 2017; Lowry et al. 2017a, b; McKinney et al. 2017; Hohenlohe et al. 2020). However, recent years have seen accelerating progress in translating genomics research into management (Allendorf et al. 2010; Steiner et al. 2013; Shafer et al. 2015; Breed et al. 2019; Walters and Schwartz 2020, this volume). For example in salmonid fish, multiple approaches, including SNP arrays, RAD sequencing, and whole-genome analysis, have been used to identify conservation units, quantify genetic diversity, detect local adaptation, and determine genotype–phenotype associations, all with consequences for the intensive management and conservation efforts in these fish (Waples and Lindley 2018; Waples et al. 2020).

A critical need in many wildlife studies is to gather genetic data from non-invasive samples, such as feces and hair. Mitochondrial DNA and microsatellites have been extensively applied in these situations, which promoted a rapid expansion of their use in wildlife conservation (Waits and Paetkau 2005; Beja-Pereira et al. 2009; Andrews et al. 2018, this volume). This was especially useful for threatened and elusive species, for which non-invasive genetic sampling provided more accurate estimates of species presence, density, kinship, and dispersal, often at a lower cost (Barbosa et al. 2013; Hedges et al. 2013; Ferreira et al. 2018). Due to issues of sample quality, the use of non-invasive samples in the genomic era has lagged and so have genetic monitoring studies that deal with threatened and elusive species (Carroll et al. 2018). Nonetheless, an expanding set of genomics tools can now be applied to non-invasive and low-quality DNA samples (Carroll et al. 2018; Andrews et al. 2018, this volume). For example, active research areas in genomic research for wildlife monitoring include the use of in situ sequence amplification, which has been used for bird sexing from blood and feather samples, DNA barcoding, and for single species detection from environmental DNA samples

(Centeno-Cuadros et al. 2017; Williams et al. 2019; Watsa et al. 2020). Environmental DNA samples may also have potential beyond species detection, for assessing population-level characteristics of genetic diversity or population structure (Goldberg and Parsley 2020, this volume).

In this chapter we provide an overview of the field of wildlife population genomics: the range of techniques and resources available for genomic studies, the biological questions that can be addressed, and applications of population genomics to wildlife management and conservation. We highlight a few key areas, such as whole-genome sequencing, that are emerging as central to the field. We also discuss approaches with future potential for applications to wildlife, such as population epigenomics, population transcriptomics, metagenomics, and eDNA for population genomics.

2 Addressing Research Questions in Wildlife Biology

2.1 Population Genetics Versus Genomics in Wildlife

Traditional population genetics has applied techniques like allozyme and microsatellite genotyping or sequencing of mitochondrial and chloroplast genes to provide a wealth of knowledge about natural populations (Allendorf 2017). However, these techniques provide data on a limited number of genetic markers across individuals, and a common assumption is that this sample of markers represents the action of neutral processes that affect the whole genome. Statistical power of these traditional genetics approaches is also limited by the sample size of loci or markers. Advances in next-generation sequencing technology have led to a proliferation of techniques for population genomics studies, all of which have the potential to provide fine-scale genetic data across the genome of multiple individuals. The central advance of next-generation sequencing is that heterogeneous pools of DNA fragments can be sequenced together, rather than requiring individual fragments to be isolated and amplified (Mardis 2008). This means that data can be gathered across thousands of loci, or even across the whole genome, in a single sequencing library. Critical for applications to wildlife, many approaches in population genomics are suitable even in taxa with little or no existing genomic resources.

Many basic questions in wildlife populations were addressed with traditional genetic tools, and these can be addressed with genomics techniques as well. An advantage of the number of loci that genomics approaches provide is much higher precision in estimating population genetic statistics or detecting patterns, such as genetic differentiation among populations or phylogenetic relationships among taxa (Hohenlohe et al. 2019). This use of high-throughput sequencing to address longstanding questions, but with greater precision or statistical power, has been called “broad-sense genomics” (Garner et al. 2016). For example, Zimmerman et al. (2020) compared microsatellite genotyping with reduced representation sequencing in Gunnison sage-grouse (*Centrocercus minimus*) and found finer-scale detection of

population structure with the genomics approach. Additionally, population genomics opens the door to address new questions in wildlife biology that were previously intractable with traditional genetic tools, what has been called "narrow-sense genomics" (Garner et al. 2016), particularly when genetic information can be arrayed along a map of the genome (Allendorf 2017). For instance, the scale of genomics tools can reveal features of neutral processes such as fine-scale historical reconstructions of inbreeding in small wildlife populations (e.g., Grossen et al. 2020). Narrow-sense population genomics enables the fundamental advance of being able to detect specific genomic regions or loci that are under natural selection or associated with ecologically important traits (Garner et al. 2016; Allendorf 2017).

Surveying examples of recent work in applying population genomics to wildlife reveal a few basic conclusions (Table 1). First, a wide range of scientific questions have been addressed, and these can be very roughly divided into those that affect the genome as a whole (e.g., demographic patterns, population relationships, and other "neutral" processes) and those that relate to a subset of the genome containing genetic variation related to adaptation, fitness, or important phenotypes. Second, these distinctions among types of research questions or genetics versus genomics techniques are often not clear. For example, many studies listed in Table 1 and described below address multiple questions at once, such as population structure and detection of adaptive variation, that span the "broad-sense" and "narrow-sense" aspects of genomics (e.g., Saremi et al. 2019; Oyler-McCance et al. 2020, this volume). Several studies also combine techniques, such as using next-generation sequencing tools to efficiently identify a set of marker loci that can be consistently genotyped over time, for instance in long-term monitoring of wildlife populations. The resulting marker panels may have relatively few loci and not constitute a "genomic" dataset in terms of representation across the genome. Nonetheless, such marker panels may target adaptive variation and represent a substantial advance in wildlife population genomics (Meek et al. 2016; Förster et al. 2018; Eriksson et al. 2020).

Studies in wildlife population genomics can occur across a wide range of taxonomic and spatial scales, and these factors drive the sampling design as well as choice of sequencing techniques and analysis tools (Fig. 1). At one extreme, questions about phylogenetic relationships or species presence in a community cut across related species, while requiring relatively few individual samples. At the opposite extreme, studies focused on individual relatedness or inbreeding can occur within a single population, sampling a large number of potentially related individuals. In the middle, studies of population structure or local adaptation gain statistical power by sampling individuals within a species across a broad range of populations, locations, or environmental factors. In all cases, the number of loci required varies widely among research questions, depending on whether a study needs a smaller representative sample of loci, or more comprehensive sampling to reveal factors like selection affecting individual genes.

Table 1 Examples of research goals that can be addressed in wildlife using population genomics, applications to conservation or management efforts, and recent illustrative studies

Research goal	Conservation or management application	Published examples
Demographic processes and population relationships		
Estimate phylogenetic relationships among taxa	Understand evolutionary relationships among threatened species	Wolves (*Canis* spp.); Sinding et al. (2018)
Estimate effective population size (N_e)	Assess ongoing loss of genetic diversity; identify conservation priorities	Gorillas (*Gorilla beringei* subspp.); van der Valk et al. (2019) Ibex (*Capra ibex*); Grossen et al. (2018)
Reconstruct historical trends in N_e	Understand historic influences on current genetic diversity	Tasmanian devil (*Sarcophilus harrisii*); Patton et al. (2019) Eurasian lynx (*Lynx lynx*); Lucena-Perez et al. (2020)
Identify geographic population structure	Identify population units for conservation	Pandas (*Ailuropoda melanoleuca*); Zhao et al. (2013) Yellow-legged frogs (*Rana boylii*); McCartney-Melstad et al. (2018) Pangolins (*Manis* spp.); Hu et al. (2020)
Quantify population distinctiveness	Establish whether populations meet criteria for conservation status listing	Rockfish (*Sebastes* spp.); Andrews et al. (2018)
Estimate population connectivity and levels of gene flow	Manage migration among populations to maintain genetic diversity	Polar bears (*Ursus maritimus*); Jensen et al. (2020)
Estimate levels of hybridization	Maintain locally adapted genotypes; characterize the spread of hybridizing invasive species	Westslope cutthroat trout (*Oncorhynchus clarki lewisi*); Muhlfeld et al. (2017)
Estimate current levels and historic trends of inbreeding	Identify priority populations for conservation action	Pumas (*Felis concolor*); Saremi et al. (2019)
Adaptive and functional variation		
Estimate heritability of phenotypic traits	Quantify the adaptive potential of populations to respond to selection	Hihi (*Notiomystis cincta*); de Villemereuil et al. (2019)
Test for inbreeding depression	Quantify population-level impacts of inbreeding; identify targets for genetic rescue	Red deer (*Cervus elaphus*); Huisman et al. (2016)
Assess the fitness impacts of deleterious mutations in small populations	Quantify the effects of genetic drift and purging on population fitness; identify targets for assisted gene flow	Island foxes (*Urocyon littoralis*); Robinson et al. (2018) Alpine ibex (*Capra ibex*); Grossen et al. (2020)

(continued)

Table 1 (continued)

Research goal	Conservation or management application	Published examples
Identify loci associated with adaptive differentiation, with either outlier or GEA approaches	Evaluate adaptive differences among populations; inform potential translocations or assisted gene flow	Thick-billed murres (*Uria lomvia*); Tigano et al. (2017) Pikas (*Ochotona princeps*); Waterhouse et al. (2018)
Test for contemporary genomic responses to selection	Identify populations currently adapting to environmental change	Chipmunks (*Tamias* spp.); Bi et al. (2019)
Identify loci associated with phenotypic traits (GWAS)	Manage populations for ecologically important phenotypes	Tasmanian devils (*Sarcophilus harrisii*); Margres et al. (2018)
Estimate adaptive potential	Assess the capacity of populations to adapt to environmental change without intervention	Willow flycatchers (*Empidonax traillii*); Ruegg et al. (2018)
Estimate genomic vulnerability	Identify populations that may be genetically maladapted to future environmental conditions and warrant management actions	Yellow warblers (*Setophaga petechia*); Bay et al. (2018)
Develop genetic marker panels for high-throughput genotyping	Genetic monitoring of natural populations, including tracking adaptive responses	Eurasian lynx (*Lynx lynx*); Förster et al. (2018)

2.2 Populations, Demography, and Neutral Processes

A central feature of wildlife biology is the size, distribution, and relationships of populations across a species' range. Population genomics tools provide an abundance of genetic data that can be used to understand wildlife populations (Hohenlohe et al. 2020). The size of a population strongly influences its viability, including its genetic capacity to adapt to environmental change, with implications for wildlife conservation and management actions. The genetic consequences of small population size are captured by N_e, the effective population size. N_e captures the rate of genetic drift in a population; formally, it is the size of an idealized population with the same rate of genetic drift as the population under study (Charlesworth 2009). N_e can be estimated with genetic data and multiple genomic data sources (Nunziata and Weisrock 2018; Grossen et al. 2018). Genetic and genomic data are also applied to delineate populations according to different criteria for conservation or management (Funk et al. 2012).

For example, Grossen et al. (2018) estimated N_e in several populations of Alpine ibex (*Capra ibex*) compared to the closely related Iberian ibex (*Capra pyrenaica*) and domestic goat (*Capra hircus*), using over 100,000 SNP loci identified through RAD sequencing. These data provide precise estimates of individual-level

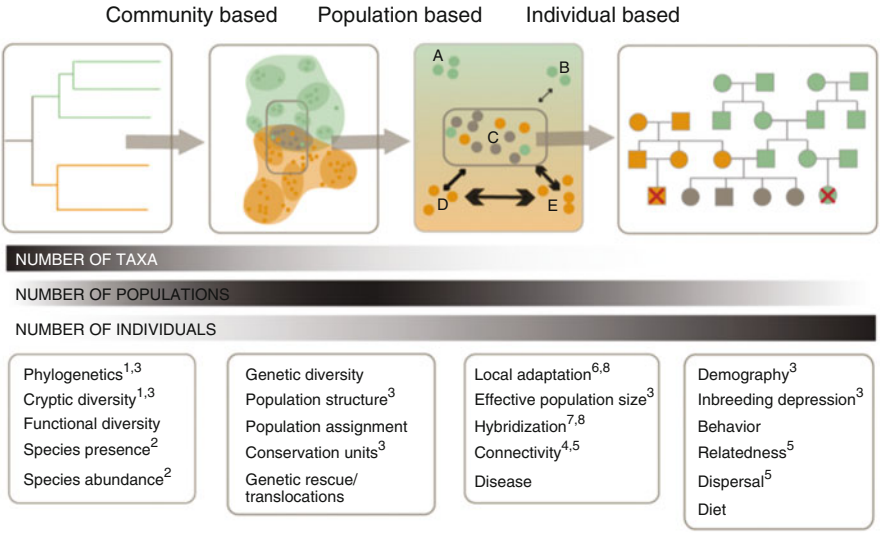

Fig. 1 Research questions in wildlife biology can be addressed with population genomics across a range of scales, from groups of related species (left) to populations within a species (middle), to individuals within a single population (right). At these different scales, the relative numbers of taxa, populations, or individuals that should be sampled for any particular study vary (darker gray represents relatively more sampling at this level). At each scale, different research questions may require different numbers of loci to adequately sample the genome, in order to provide statistical power for particular analyses or fine-grained genomic information. For all of these aspects, this figure is meant as a rough, non-quantitative guide. Case studies for research questions: (1) Barbosa et al. (2018); (2) Marshall and Stepien (2019); (3) Hu et al. (2020); (4) Eriksson et al. (2020); (5) Escoda et al. (2019); (6) Rellstab et al. (2019); (7) Peek et al. (2019); (8) Mills et al. (2018)

heterozygosity and standing genetic variation across the genome for each population (Fig. 2a). These were translated into estimates of N_e using linkage disequilibrium-based methods (Fig. 2b). Despite the large amount of genomic data, note that the estimates of N_e still have large confidence intervals, particularly on the upper limit. Nonetheless, these estimates of N_e in Alpine ibex populations, many of which were established by a series of reintroductions during the last century, provide useful information for understanding these populations. Further, genomic data can reveal the consequences of low N_e, such as inbreeding and accumulation of deleterious alleles, that provide a more detailed picture of the genetic status and viability of wildlife populations (Kardos et al. 2018; Robinson et al. 2019; Grossen et al. 2020).

Understanding the history of populations can be important for wildlife, including historical effects on genetic diversity. Even in the absence of historical samples, population genomics tools can provide a detailed picture of population demography, including current and historical trends in N_e (Fig. 3a; Salmona et al. 2019; Lucena-Perez et al. 2020). Genome-wide data can be used to estimate time scales of population bottlenecks and expansions as well as infer the severity of demographic changes, which can help explain current levels of genetic diversity. The combination

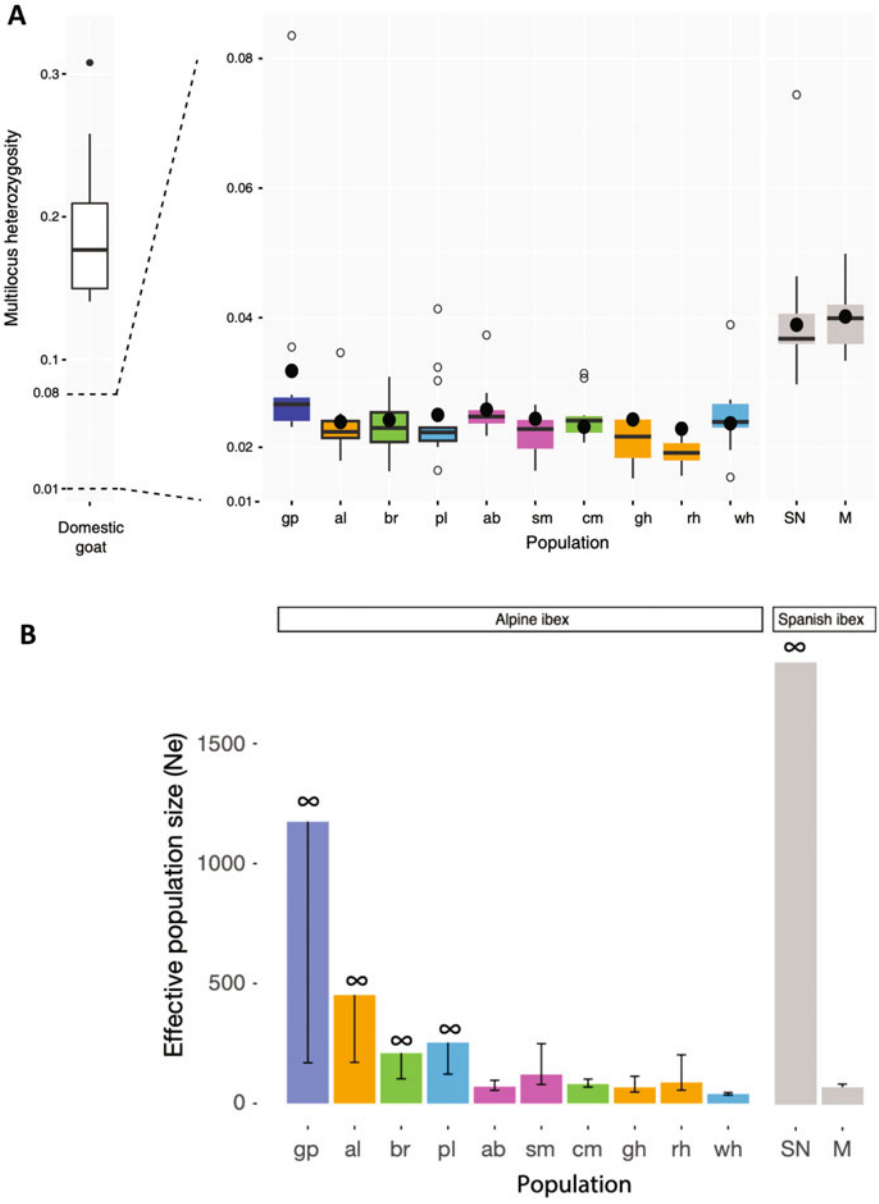

Fig. 2 Estimates of (**a**) genetic diversity and (**b**) effective population sizes across reintroduced populations of Alpine ibex (*Capra ibex*) and Iberian ibex (*C. pyrenaica*) based on RAD sequencing data. Reproduced from Grossen et al. (2018)

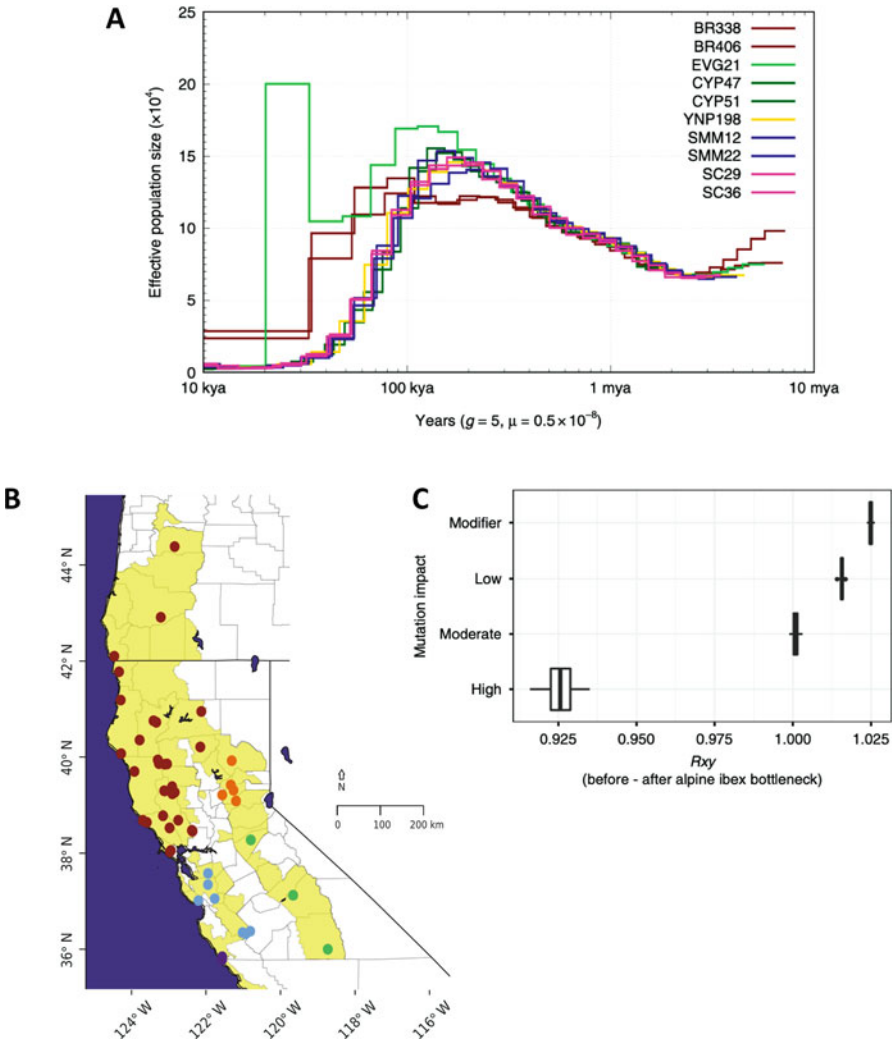

Fig. 3 Examples of population genomics to understand demographics and population structure in wildlife: (**a**) Reconstruction of historical effective population sizes using sequentially Markovian coalescent (SMC) analysis of whole-genome data from 10 individual pumas (*Puma concolor*) from populations across North and South America. Reproduced from Saremi et al. (2019). (**b**) Population structure of the foothill yellow-legged frog (*Rana boylii*) in the western USA, estimated by phylogenetic analysis of genomic data from a RAD sequencing approach, with colors indicating different population groups that could be managed as genetically distinct units. Reproduced from McCartney-Melstad et al. (2018). (**c**) The effects of population bottlenecks and inbreeding on deleterious mutations in Alpine ibex (*Capra ibex*), assessed by change in allele frequency of the derived allele (R_{xy}). $R_{xy} < 1$ indicates a decrease in frequency of these mutations after bottlenecks in Alpine ibex compared to Iberian ibex; $R_{xy} > 1$ indicates an increase in frequency after bottlenecks. The excess of modifier and low-impact mutations shows the influence of genetic drift, while the reduced frequency of high-impact mutations indicates purging of these mutations during population bottlenecks. Reproduced from Grossen et al. (2020)

of demographic methods to estimate historical population size as well as diversity metric estimation can further reveal the relationship between historical and current levels of genetic diversity in light of demographic history. In the case of African wild dogs (*Lycaon pictus*), whole-genome analysis indicated that despite historically low effective population sizes, heterozygosity remains high in the current population (Armstrong et al. 2019). A study using WGS of both museum and contemporary samples by van der Valk et al. (2019) showed that over the past century the mountain gorilla (*Gorilla beringei beringei*) population has remained small, but genetically stable, while the Graur's gorilla (*Gorilla beringei graueri*) underwent population declines which led to increased inbreeding and loss of genetic diversity.

2.3 Population Structure and Connectivity

The distribution of genetic variation, population structure, and connectivity can be estimated using population genomics tools. These analyses can be critical for addressing key questions in wildlife conservation and management, as described below. For instance, population structure analysis of the foothill yellow-legged frog (*Rana boylii*) in the western USA, estimated by phylogenetic analysis of genomic data from a RAD sequencing approach, indicated that different population groups could be managed as genetically distinct units (Fig. 3b; McCartney-Melstad et al. 2018). Because of greater statistical power and sensitivity, genomic data can often reveal population structure that is not apparent with mitochondrial sequencing or fewer microsatellite loci, as observed, for example, in Gunnison sage-grouse (*Centrocercus minimus*; Zimmerman et al. 2020). In species with specific environmental threats, like polar bears (*Ursus maritimus*) facing climate change, assessment of population structure is a necessary first step to understand how population might respond (Jensen et al. 2020).

Combined with environmental data, demographic studies can assess how geography and climatic history influence geographic ranges, population sizes, gene flow, divergence, and speciation (Salmona et al. 2019). Ancient demography is important for understanding the driving factors, such as environmental changes, behind past population fluctuations and factors involved in historical connections or barriers to connectivity among populations. For example, analyses of demographic history of Malayan pangolins (*Manis javanica*) showed the effects of long-term environmental changes, including climate (as measured in surface temperature) and sea-level oscillations, revealing multiple population size changes in their evolutionary history (Hu et al. 2020). In another example, Zhao et al. (2013) analyzed WGS data of wild giant pandas (*Ailuropoda melanoleuca*) finding the occurrence of multiple demographic events such as population expansion, bottlenecks, and population divergence. They inferred that the decline of pandas in the last 3,000 years is likely due to anthropogenic disturbances. Timing of historical splits between populations can also be identified by divergence in population size estimates, as observed between European and Asian populations of Eurasian lynx (*Lynx lynx*; Lucena-Perez et al. 2020). Historical demographic reconstruction has the power to assess population

changes in light of the past environmental and anthropogenic shifts and may be able to inform the effect of ongoing changes on spatial distribution of genetic diversity (Prates et al. 2016).

Many wildlife species have reduced and fragmented populations, leading to loss of genetic diversity and inbreeding, and the potential for reduced fitness from inbreeding depression. Overall levels of genetic diversity can be estimated with genetic tools, and genomic data provide precise estimates from densely sampling the genome. Population genomics tools can precisely estimate individual inbreeding coefficients and pairwise genetic relatedness – and the relationship between them – to test for inbreeding depression (Huisman et al. 2016). They can also be used to map loci associated with individual inbreeding or reduced fitness, similar to approaches for mapping adaptive variation as discussed below.

2.4 Hybridization

Biological aspects of some wildlife systems present particular challenges for management that can be addressed with population genomics; for instance, Toews et al. (2018, this volume) document how hybridization among bird species has been an important source of variation and possibly led to the formation of new species. In mallard ducks and their relatives (*Anas* spp.), hybridization has occurred between feral mallards introduced widely by humans and native *Anas* species across the globe (Lavretsky 2020, this volume). This creates challenges for management of native biodiversity in this group. Genomics methods for identifying hybrid individuals and monitoring the extent of hybridization across a landscape can answer basic questions that may inform management decisions. Additionally, identifying genomic regions or genes associated with admixture – the flow of allelic variation into a hybridized population – can reveal how selection operates in these populations. Alleles from an invasive species that spread rapidly into a native population may be considered "invasive alleles." For instance, some alleles from introduced rainbow trout (*Oncorhynchus mykiss*) move preferentially into native westslope cutthroat trout (*O. clarkii lewisi*), although selection predominantly acts against introduced alleles across most of the genome (Kovach et al. 2016). The spread of hybridization across the native species range in this system depends on a large number of factors, including water temperature and precipitation as well as proximity to sources (stocking locations) for the invasive rainbow trout (Muhlfeld et al. 2017). Climate change will continue to impact the spread of hybridization in this and other systems (Muhlfeld et al. 2017).

In addition to more accurately quantifying hybridization across individuals and populations, and identifying loci that are responding to selection in hybridized populations, population genomics can also reveal the history of hybridization. For instance, North American canids have a complex pattern of hybridization among taxa, both over evolutionary history and more recently in response to anthropogenic factors (vonHoldt et al. 2016; Sinding et al. 2018). Red wolves (*Canis rufus*), native

to the southeastern United States, have been subject to extensive hybridization with coyotes, which expanded their range eastward from the western US following European settlement. However, they also show evidence of historical hybridization with wolf and/or coyote lineages earlier in their evolutionary history (vonHoldt et al. 2016; Hohenlohe et al. 2017). Understanding not only the extent of hybrid ancestry but also the time scale over which hybridization occurred in red wolves is important for management decisions (Waples et al. 2018). Conversely, conservation policy can be informed by our growing understanding of the role of hybridization in wildlife taxa (vonHoldt et al. 2018; Heppenheimer et al. 2018; Funk et al. 2019).

2.5 Adaptive Variation

A central feature of wildlife populations is their adaptation to local environmental and ecological conditions, the genetic variation that facilitates adaptive responses, and how these affect population size, growth rates, dispersal, and the long-term viability of wildlife populations. Population genomics tools provide multiple approaches for assessing adaptive genetic variation in wildlife populations. At the phenotypic level, genomics tools can be used to estimate individual relatedness and heritability of phenotypic traits, including traits linked to ecological functions or even fitness as a phenotype (Gienapp et al. 2017; de Villemereuil et al. 2019). This provides a quantitative genetic assessment of the ability of populations to adapt (Reed et al. 2011).

Increasingly, population genomics tools are being used to detect specific loci associated with fitness, adaptation, or ecological functions (Luikart et al. 2019). One approach is outlier tests that identify loci that are strongly differentiated among populations, indicating a signature of local adaptation (Beaumont and Nichols 1996). Outlier tests have the advantage of relying only on sampling individuals from different populations, without requiring other data on phenotypes or environmental variables. However, several other factors such as recombination rate heterogeneity and demographic fluctuations can have strong effects on errors in outlier analyses, including high rates of false positives (Lotterhos and Whitlock 2015; Hoban et al. 2016). If both genomic data and phenotypic measurements are available on a set of individuals, loci can be associated with phenotypic variation using genome-wide association studies (GWAS; Wellenreuther and Hansson 2016). However, the power of GWAS in wildlife systems is often limited. Even for model species and those with high levels of genome-wide heterozygosity, sample sizes need to be thousands of individuals for sufficient power to detect loci of small or moderate effect (Joshi et al. 2015). However, GWAS with limited sample sizes in wildlife can still reveal important features of the genetic basis of key traits, even if the specific effects of individual loci cannot be quantified with precision (Margres et al. 2018). In other cases, for abundant or commercially harvested species, sample sizes are sufficient to unravel the genetic basis of complex traits in wildlife using GWAS (Sinclair-Waters et al. 2020).

For many wildlife species, populations are distributed across a variable landscape, and combining genomic data with measurements of environmental variables reveals insights into adaptation and ecological factors affecting wildlife populations (Manel et al. 2003; Forester et al. 2018, this volume). This approach is called landscape genomics. The field can be divided into neutral landscape genomics, which focuses on understanding gene flow and connectivity, and adaptive landscape genomics, which focuses on characterizing the genetic basis of adaptation and how natural selection structures the distribution of adaptive genetic variation across the range of a species (Balkenhol et al. 2019). However, both neutral and adaptive information are available from most genomic datasets, so many studies can address both concurrently. The central approach of adaptive landscape genomics is genotype–environment association (GEA) analysis, which links allelic variation to environmental variables. Forester et al. (2018, this volume) provide guidance for applying GEA analysis, including design of sampling, genomic and environmental data production, and specific issues that can be addressed in wildlife populations.

Some case studies illustrate the complementary use of multiple techniques in applying population genomics to wildlife, such as combining whole-genome sequencing with genotyping at a small panel of markers. This approach can address multiple questions, such as identifying population structure and conservation units along with adaptive differentiation. For example, researchers working on sage-grouse (Oh et al. 2019; Zimmerman et al. 2020; Oyler-McCance et al. 2020, this volume) used WGS to determine genome-wide differentiation between two species (greater and Gunnison) of sage-grouse (*Centrocercus* spp.) and found intraspecific population structure consistent with genetic drift due to limited gene flow among populations. Further, they used a high-density marker panel to probe SNPs exhibiting extreme population differentiation. They found candidate genes associated with local dietary adaptations, which calls for conservation strategies that account for the specific chemistry of local sagebrush on sage-grouse.

2.6 Deleterious Variation and Inbreeding Depression

Many wildlife populations have reduced and fragmented populations, leading to loss of genetic diversity and inbreeding, and the potential for reduced fitness from inbreeding depression. Population genomics tools can precisely estimate individual inbreeding coefficients and pairwise genetic relatedness to test for inbreeding depression. In red deer (*Cervus elaphus*), inbreeding coefficients estimated using SNPs were compared to several different fitness metrics (Huisman et al. 2016). Strong evidence for inbreeding depression was found including associations between annual breeding success, offspring survival, and juvenile birthweight and survival. Robinson et al. (2019) found evidence of severe inbreeding depression in the gray wolves (*Canis lupus*) of Isle Royale. They used population genetic simulations, comparison of inbreeding coefficients estimated from runs of homozygosity (ROH) from wolves from a variety of demographic histories, and morphological

analysis to determine that this population of wolves has undergone an increase in homozygosity of strongly deleterious recessive mutations. The use of WGS data to estimate ROH is particularly useful to both quantify inbreeding coefficients and identify causal loci for inbreeding depression (Kardos et al. 2018; Hohenlohe et al. 2020).

Population genomics tools can also be used to map loci associated with individual inbreeding or reduced fitness, although this inevitably suffers from small sample size in small wildlife populations. However, as with GWAS studies for identifying adaptive loci, mapping of loci associated with inbreeding depression or loss of fitness in small, bottlenecked populations can be facilitated by other sources of information. For example, a more powerful approach includes functional data on mutations identified across the genome from WGS data compared with well-annotated reference data (Fig. 3c; Robinson et al. 2018; Grossen et al. 2020). The functional consequences of mutations can be predicted based on where they occur in well-annotated genomic sequences, which are often available either for focal wildlife species or for close relatives.

2.7 Specific Threats and Adaptive Potential

A major threat to viability and persistence of wildlife populations is their response to environmental change. Two related questions determine whether wildlife populations may be able to adapt and persist under environmental change: what is the mismatch between the genetic state of a population and future environmental conditions (genomic vulnerability; Bay et al. 2018), and how much genetic variation exists in a population to allow it to adapt to changing conditions (adaptive potential; Dawson et al. 2011; Nicotra et al. 2015; Funk et al. 2019). The ability to assess the adaptive genetic variation present in a wildlife population is a key goal of wildlife population genomics. An emerging, powerful approach combines data on adaptive genetic variation from approaches like GEA with environmental data and climate modeling (Fig. 4; Ruegg et al. 2018; Funk et al. 2019; Forester et al. 2018, this volume). This includes genetic responses to climate change, where future changes can be compared to current adaptive genetic variation across climate variables (Bay et al. 2018; Razgour et al. 2019). It could be applied in other cases where genetic responses have been observed to other human-caused environmental changes such as habitat modification, changes in the ecological community, population fragmentation, or effects on behavior (Benazzo et al. 2017). These applications can combine multiple techniques that focus on the genetic basis of particular phenotypes, in addition to fitness under environmental conditions, along with multiple sources of non-genomic information (Funk et al. 2019). This can help identify populations, regions, or protected areas that contain hotspots of adaptive genetic diversity for evolutionary response to environmental change (Mills et al. 2018).

Wildlife population can persist in the face of climate change through a combination of genetic adaptation, phenotypic plasticity, range shifts, and management

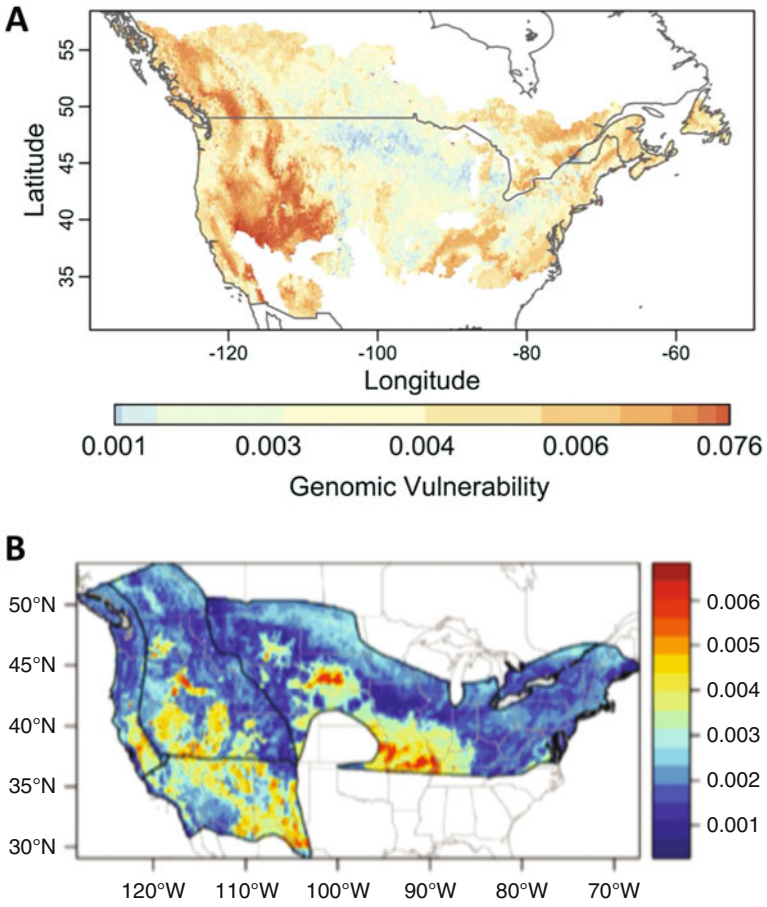

Fig. 4 Genomic vulnerability to climate change in two North American bird species, estimated by comparing current patterns of local adaptation to climate conditions with future predictions under climate change scenarios. (**a**) In yellow warblers (*Setophaga petechia*), areas of recent population declines corresponded with areas of highest genomic vulnerability to future climate change, shown here. Reproduced from Bay et al. (2018). (**b**) In willow flycatchers (*Empidonax traillii*), climate vulnerability is high in the endangered southwestern subspecies (*E. t. extimus*), shown on the map as the southwestern portion of the range bordered by dark lines. Reproduced from Ruegg et al. (2018)

intervention. Population genomic data can be combined with phenotypic and environmental information to understand a wide range of potential ecological and evolutionary responses (Waldvogel et al. 2020). By combining analysis of local adaptation with projections of future conditions, population genomics tools can also assess the vulnerability of wildlife populations to future change (Fig. 4; Bay et al. 2018; Ruegg et al. 2018). Climate change directly interacts with a number of key phenotypes, and population genomics tools can identify the genetic basis of this adaptive variation (Razgour et al. 2019; Höglund et al. 2019, this volume). For

example, climate change affects ecological interactions with invasive species, with consequences for competition or hybridization with native wildlife populations (Chown et al. 2015). Increasing water temperatures can increase the spread of invasive rainbow trout (*Oncorhynchus mykiss*) and hybridization with native westslope cutthroat trout (*O. clarki lewisii*), with genomic consequences for the native populations (Muhlfeld et al. 2017).

Another threat to many wildlife species is disease, which can be facilitated or exacerbated by other anthropogenic influences and potentially affects large swaths of biodiversity (for example, chytridiomycosis in amphibians; Scheele et al. 2019; Funk et al. 2018, this volume). Population genomics can assess the variation that may permit wildlife population to adapt to emerging diseases (Epstein et al. 2016; Gupta et al. 2020; Auteri and Knowles 2020; Storfer et al. 2020, this volume). Population genomics can be applied to wildlife disease in multiple ways, including pathogen detection, inferring disease transmission and predicting spread, as well as assessing genetic variation for resistance (Blanchong et al. 2016). Storfer et al. (2020, this volume) assess the applications of population genomics to disease in wildlife, focusing on four case studies: colony collapse in honeybees (*Apis mellifera*), chytridiomycosis in amphibians, whitenose syndrome in bats, and transmissible cancer in Tasmanian devils (*Sarcophilus harrisii*). All four of these diseases have arisen relatively recently, have spread widely across host species, represent major threats to population persistence, and include complex interactions among hosts, pathogens, and ecological communities that can be addressed with population genomics tools. A specific group of diseases, cancer, is poorly understood in wildlife species but may have widespread impacts as a result of genetic and environmental changes (Pesavento et al. 2018; Hendricks et al. 2020, this volume). The ability of wildlife populations to withstand cancer and other diseases is closely tied to their genetic diversity, demographic history, and inbreeding, factors that are tractable with population genomic data.

3 Applications in Genetic Management and Conservation of Wildlife

Population genomics approaches have multiple applications to wildlife conservation and management actions (Walters and Schwartz 2020, this volume). There has been criticism of the broader field of conservation genomics and its slow pace in achieving its potential for connecting research to direct conservation action (Shafer et al. 2015; Garner et al. 2016). But within the last several years, applications of population genomics tools to wildlife provide a rapidly growing set of examples illustrating the breadth of issues that can be addressed (Table 1). While many of the published examples in Table 1 still have not been implemented in management actions, they give wildlife managers and policymakers an overview of the types of information that can inform decisions. The issues addressed include basic features of wildlife

populations that have long been confronted with genetic data, such as microsatellite loci, as well as new issues focused on the genetic basis of adaptive or deleterious traits. Different strategies of sampling, data collection, and analysis are appropriate at different scales (Fig. 1).

Population genomics can be applied simply by providing basic information about wildlife populations without intervention: for example, estimating phylogenetic relationships, delineating population units, estimating population size and genetic diversity, determining whether populations meet criteria for conservation listing, or assessing population vulnerability to threats. Ongoing monitoring can estimate trends in these features over time. Additionally, population genomics tools can also inform decision-making for more intensive management actions, such as translocations of individuals or captive breeding, and monitor the consequences of these actions after they are carried out. In all of these cases, the power of population genomics to identify both genome-wide patterns and also identify and assess loci with adaptive significance can improve the utility of genetic data for conservation and management applications in wildlife.

3.1 Delineating Population Units for Management

Population genomics provides basic information on population sizes, distribution, and connectivity. This allows the delineation of conservation units for management and assessment of their size and distribution (Fig. 3b; Funk et al. 2012). It also facilitates prioritization of populations for conservation on the basis of genetic factors by quantifying the effects of current and historical population dynamics on genetic diversity, inbreeding, population fitness, and adaptive potential. For instance, some laws designed to protect endangered wildlife, such as the U.S. Endangered Species Act, take adaptive potential into consideration in endangered species listing and delisting decisions (Funk et al. 2019). As a result, different management goals rely on different criteria for delineating populations. For example, Evolutionarily Significant Units (ESUs) are defined by reproductive isolation and adaptive difference from other populations, so that an ESU represents a significant evolutionary or genetic component of the species (Funk et al. 2012). Management Units (MUs) are local populations that are demographically independent, so that management goals based on population size, such as regulation of harvest levels, may be designed at this level. Multiple MUs, defined by demographic connectivity, may be present within an ESU, defined by genetic connectivity. This can be understood in the context of basic population genetic models: demographic connectivity generally depends on the migration rate (m), the proportion of individuals that migrate among populations per generation, while genetic connectivity generally relies on the absolute number of individuals migrating (Nm, the product of population size and migration rate) (Lowe and Allendorf 2010). Specific adaptive differences may further lead to specific delineation of populations as adaptive units (Prince et al. 2017).

As an example, this framework was applied to the Iberian endemic and near-threatened Cabrera vole (*Microtus cabrerae*). This case study illustrates an important role of genomics to resolve gaps or inconsistencies from previous, smaller-scale genetic datasets. Early analysis of microsatellite and nuclear DNA sequencing data of Cabrera vole populations revealed little variation across the species distribution, contrary to mitochondrial DNA that showed a clear division in at least two genetic groups (Alasaad et al. 2013; Barbosa et al. 2017). Subsequent analysis of a subset of these samples with reduced representation genomic sequencing allowed for the identification of four ESUs, while the identification of neutral and outlier variation further led to the identification of six MUs and three adaptive units, respectively (Barbosa et al. 2018). Similar studies have also provided a better understanding of genetic population structure leading to changes in conservation listing and management, such as among rockfish species (*Sebastes* spp.; Andrews et al. 2018; Walters and Schwartz 2020, this volume).

3.2 Monitoring

Genetic monitoring of wildlife populations can address a number of basic issues, including abundance, effective population size, genetic diversity, vital rates, hybridization, as well as temporal trends in all of these factors (Carroll et al. 2018; Flanagan et al. 2018; Hoban et al. 2020). Genetic monitoring in wildlife populations has often used microsatellite markers, in part because a relatively small number of loci are typically sufficient to estimate individual identity, relatedness, dispersal, and metrics of genetic diversity and population differentiation (de Barba et al. 2010). Microsatellites can be genotyped with non-invasive and low-quality DNA samples, facilitating long-term monitoring of wildlife populations (Waits and Paetkau 2005; Selkoe and Toonen 2006; de Barba et al. 2016). Genomics techniques have overtaken microsatellites in many respects, with advantages in numbers of loci, cost per sample, consistency in genotyping, as well as advances in using genomics techniques for non-invasive samples in wildlife (Hunter et al. 2018). Nonetheless, microsatellites remain a key tool for genetic monitoring in wildlife populations, particularly where a panel of microsatellite loci has been used for long-term monitoring and maintaining a consistent dataset is important for understanding long-term trends.

Monitoring requires a set of genetic markers that can be consistently genotyped across many individual samples over time, using a standardized protocol that is rapid and cost-effective. Population genomics approaches can be most effective in providing a large set of loci from which to choose an optimal set of markers that can then be rapidly genotyped using another technique (Förster et al. 2018; von Thaden et al. 2020). For instance, Förster et al. (2018) started by designing targeted capture probes from the domestic cat (*Felis catus*) reference genome and using them to gather sequence data for 809 nuclear coding regions in Eurasian lynx (*Lynx lynx*). From these sequences, they optimized a marker panel of 96 SNP loci that could be

genotyped on a high-throughput Fluidigm platform. The 96-marker panel was able to identify individuals, assign individuals to source populations, and detect population structure. In contrast to panels of microsatellite loci that are often assumed to be neutral, marker panels developed from genomic datasets can specifically include loci that have adaptive or functional significance. This allows monitoring efforts to track genetic variation at specific adaptive loci, for instance to understand population responses to environmental stress or management actions, and to identify populations that lack adaptive variation (Flanagan et al. 2018; Leroy et al. 2018). Some powerful complementary approaches in wildlife population genomics would combine monitoring of genetic diversity at particular loci with an understanding of the consequences for population viability, both in terms of functional consequences of specific alleles and population-level consequences like inbreeding depression (Robinson et al. 2018; Leroy et al. 2018; Grossen et al. 2020).

3.3 Genetic Management of Wild Populations

Many wildlife populations are primarily managed by regulating harvest levels. This has genetic implications based on the resulting effective population size and potential loss of genetic variation through genetic drift in small populations (although the relationship between selective harvest and N_e is complex; Kuparinen et al. 2016). Genetic monitoring of N_e and levels of genetic variation can be informative, by tracking both average levels of variation across the genome and also maintenance of variation at adaptive loci. If adaptive loci are known for a harvested wildlife species, for instance through GEA tests, these should be included on genetic marker panels designed for monitoring. Still, panels should always also include a genome-wide set of loci to track average levels of genetic variation. This is because any genomic information on the genetic basis of adaptation will necessarily be incomplete, especially relative to future environmental change. Harvest levels may be set with a goal of maintaining target levels of variation, both in genome-wide average and at specific adaptive loci, to support future population persistence.

In many cases, selective harvest of wildlife populations leads to changes in particular phenotypes (Kvalnes et al. 2016). However, it is difficult to separate the effects of non-genetic factors, such as phenotypic plasticity, from genetic evolution in response to harvest (Kuparinen and Festa-Bianchet 2017). Genomic identification of loci associated with phenotypic variation, for instance with GWAS, and inclusion of these loci in monitoring panels could resolve this issue, by directly observing a response to selection at the genetic level. Some phenotypes, such as horn size (Miller et al. 2018; Sim and Coltman 2019), may be more tractable than others such as behavior (Leclerc et al. 2019). As above, harvest levels or regulations on harvest with respect to age, sex, or phenotype could be designed with the goal of maintaining genetic variation for particular phenotypes or to minimize genetic evolution in response to harvest. Alternatively, genomic monitoring of harvested populations

provides another means to identify the genetic basis of phenotypes subject to harvest-induced selection (e.g., Bowles et al. 2020).

In other cases, wildlife conservation efforts in natural populations are more intensive, involving movement of individuals among populations or reintroduction to unoccupied habitat. Individuals may be translocated into a population with the goal of genetic rescue, which is an increase in population fitness and decrease in extinction probability caused by the genetic variation added to the population. Fitzpatrick and Funk (2019, this volume) outline a variety of ways in which population genomics can help managers with decisions about genetic rescue. Genetic rescue may occur by reducing inbreeding depression via masking deleterious alleles expressed in the homozygous state, or by infusing additive genetic variation on which selection can act so that populations can adapt to changing environments (Bell et al. 2019). Genomics tools can help identify populations suffering from low genetic variation and inbreeding depression (Table 1). They can also help identify the best potential source populations that are not too adaptively divergent from the target recipient population, in order to avoid outbreeding depression, a loss of fitness caused by genetic mixing. Finally, if and when genetic rescue is implemented, genomic data can be used to monitor changes in genetic variation and the relative fitness of immigrants, residents, and hybrids to test whether gene flow is increasing fitness as desired (Miller et al. 2012; Flanagan et al. 2018; Fitzpatrick et al. 2020).

Ferchaud et al. (2018) provide a case study for using population genomics tools to quantify the genetic effects of population supplementation in lake trout (*Salvelinus namaycush*). The researchers used a reduced representation sequencing approach to genotype nearly 5,000 SNP markers in several stocked and unstocked populations. They found higher levels of neutral genetic diversity in stocked populations. They also used functional information from the related rainbow trout (*Oncorhynchus mykiss*) to identify deleterious alleles among the SNP loci that were genotyped, and found that deleterious alleles were more abundant in unstocked populations. These results suggest that supplementation not only adds genetic variation but may also improve the ability of selection to purge deleterious alleles in supplemented populations. However, the researchers also identified fixed deleterious alleles in a source population, emphasizing the role of genomic data in identifying suitable source populations for translocations.

3.4 Captive Breeding

Population genomics is also being incorporated into intensive management of captive wildlife populations (Russello and Jensen 2018, this volume). Captive breeding has typically relied on pedigree-based management, but population genomics tools can provide more accurate estimates of genetic relatedness (Kardos et al. 2015) to guide breeding decisions, as well as critical information on functional genetic diversity in captive populations (Brandies et al. 2019; Russello and Jensen

2018, this volume). One example is using genomics tools to monitor and minimize genetic adaptation to captivity. Genomic data can also help determine whether management goals are being met, such as maintaining overall genetic diversity or the integrity of different ancestral population groups, or maintaining variation are specific adaptive loci (Russello and Jensen 2018, this volume). Establishment of captive populations can also have genetic effects on small wild populations from which individuals are taken. For instance, Morrison et al. (2020) used reduced representation sequencing to genotype SNPs in wild and captive populations of the Australian orange-bellied parrot (*Neophema chrysogaster*) and found that removal of half of a juvenile cohort from the wild population to supplement the captive population nearly a decade ago still shows effects on genetic diversity in the wild population. Subsequent release of captive-reared individuals has restored the level of genetic diversity in the wild population (Morrison et al. 2020). Jensen et al. (2018) compared variation at >2,000 SNPs in Pinzón giant tortoise (*Chelonoidis duncanensis*) samples from a single island in the Galápagos Island from before and after a bottleneck that reduced their population size (N_e) to just 150–200 in the mid twentieth century. They found that the extent and distribution of genetic variation in the historical and contemporary samples was very similar, which they attributed to a successful ex situ head-start and release program.

With population genomics tools it is possible to identify loci associated with specific phenotypic traits, fitness, or inbreeding depression. It is increasingly possible to design management of captive populations around a specific set of functionally important loci, although there are substantial pitfalls in managing captive wildlife populations for a small number of loci (Kardos and Shafer 2018). However, the possibility of efficient genotyping of individuals with relatively large genetic marker panels means that genetic management of captive populations can target multiple goals at once – for instance, maintaining variation at specific loci or keeping phenotypically distinct populations separate, while still maintaining genome-wide diversity or minimizing genome-wide inbreeding. Another goal of captive population management may include maintaining genetic adaptive potential in the face of specific threats to wild populations, such as disease (Hohenlohe et al. 2019; Storfer et al. 2020, this volume). Genotyping approaches that can be applied across both captive and wild samples (e.g., including non-invasive samples) can help integrate management of captive and natural populations of the same species (Morrison et al. 2020).

3.5 *Improving Connections Between Research and Applications*

Despite the potential for wildlife population genomics to address a wide range of issues directly relevant to management actions, there remain gaps between research and application (Holderegger et al. 2019; Taft et al. 2020). It is important for

researchers and practitioners to establish professional connections and to communicate at all stages of wildlife population genomics research. In this case, collaborative partnerships benefit both sides (Taft et al. 2020). Before a research project begins, communication can guide the research toward key metrics or questions needed for management decisions and allow researchers to focus on the types of information and results that would be most informative for management decisions (Holderegger et al. 2019). Conservation practitioners can also learn what types of information are available from population genomics studies, and how to interpret them and apply them to decisions. Managers may be critical in facilitating research, for example by providing samples and providing biological knowledge about wildlife populations. When a study is complete, simply publishing in a scientific journal is often not sufficient for results to be useful for guiding management (Fabian et al. 2019); again, ongoing professional contacts and efforts to communicate results to broader audiences are critical for spreading information between research and practitioner groups.

Population genomics is a challenging science, with high bars to entry particularly given the complexity of laboratory methods, bioinformatics, and data analysis. Training opportunities are critical, and training workshops that involve a mix of researchers and practitioners are most effective at establishing professional connections as described above. However, it is not necessary for everyone involved in population genomics research or using the results to be directly proficient in lab or bioinformatics methods; instead, a major goal of training opportunities should be to teach concepts that allow people to understand what information population genomics studies can provide and to interpret the results in a broader context (Holderegger et al. 2019). Nonetheless, continued efforts to make bioinformatic analysis tools more user-friendly and accessible will facilitate applications of population genomics.

Many of these recommendations for improving connections between wildlife population genomics research and applications are being followed. For instance, Taft et al. (2020) identified a large number of partnerships between researchers and practitioners already established. In part the apparent gap in population genomics results that have actually influenced wildlife management decisions reflects an unavoidable time lag. Many of the case studies highlighted throughout this chapter have not led to direct changes in management of wildlife populations, but they may still contribute to future decisions as understanding of the potential for population genomics to inform wildlife management improves. More broadly, the growing body of population genomics research in wildlife species can contribute to general conclusions about management and conservation actions. For instance, examples of genetic rescue attempts have led to emerging conclusions about the efficacy of this strategy in improving population fitness (Ralls et al. 2018, 2020; Fitzpatrick et al. 2020), which can help provide general guidelines for management decisions. Genomics can contribute to this understanding, for instance by identifying the genetic basis of increased fitness in rescue.

4 Approaches and Resources

4.1 Options and Challenges for Wildlife

The wide range of population genomics techniques and approaches, research questions, and applications to wildlife conservation and management questions are illustrated by case studies in particular wildlife taxa (Table 1). These studies demonstrate how the diversity of population genomics techniques can be tailored to a particular study, depending on the resources available, the scientific or management question(s) being addressed, and limitations or challenges for the specific system (Matz 2018). Tools for population genomics are changing rapidly, and this includes advances at all steps in the process: from non-invasive sampling and extraction of DNA from archival or degraded samples, to library preparation protocols and sequencing platforms, to analysis pipelines and software (Luikart et al. 2019; Rajora 2019). At each step, researchers confronting the bewildering array of options should stay grounded in the scientific question(s) being asked and the suitability of any approach for the specific system, as well as how the conclusions might be used to inform a management or conservation action. The resulting choice of approaches may differ widely, and will also be constrained by the time and resources available. In addition to choices of sampling design, library preparation and sequencing, and analysis, there is a growing wealth of resources of genomic information that can be applied across species.

Planning a population genomics study is best done in an integrated way. For example, downstream analyses may require certain numbers of loci or numbers of individuals per population to increase their power to make useful inferences, and these considerations should drive sampling design. Alternatively, the availability of samples or a requirement to use non-invasive or archival samples may drive a study toward particular sequencing and analytical approaches. As an example of an increasingly useful approach, Box 1 discusses these considerations in presenting a general workflow for applying whole-genome sequencing (WGS) in wildlife population genomics studies.

Box 1 Whole-Genome Sequencing for Wildlife Genomics: A Practical Guide

The advances in sequencing technology and methods have made whole-genome sequencing (WGS) of multiple individuals a feasible approach for population genomics studies in wildlife. Here, we review a general workflow for WGS data, including library preparation, sequencing, and bioinformatic analysis. Further useful information on designing WGS studies and analysis pipelines is provided by Ekblom and Wolf (2014), Fuentes-Pardo and Ruzzante (2017), Pfeifer (2017), Wong et al. (2019), Bani Baker et al. (2020), and Pereira et al. (2020).

(continued)

Box 1 (continued)
Considerations for Library Preparation and Sequencing

1. *Sampling of individuals* for WGS is an important consideration because often a smaller number of individuals will be sequenced compared to other approaches. For instance, if the goal is to make inferences about a population, such as demographic reconstruction (Fig. 3a), the individuals chosen should be representative of the population in their ancestry. Similarly, inadvertent WGS of an inbred individual would lead to underestimates of population-level heterozygosity or genetic diversity.
2. *The quantity and quality of the DNA* may affect your choice of library preparation and sequencing platform. Most library preparations, which are proprietary for specific sequencing platforms, are optimized for a given range of DNA quantity and quality that are typically easy to achieve using fresh or recently frozen samples. However, often in wildlife studies, samples are degraded due to various factors, such as environmental field conditions or archival storage, making them more challenging to sequence. If sample quality or quantity is lower than specified for a library preparation protocol, it can lead to extensive troubleshooting and limit strong conclusions in downstream analysis. Recent methods have been developed specifically for the use of samples with limited quantity and/or low-quality DNA. For instance, Chiou and Bergey (2018) present a method for enriching target vertebrate DNA and reducing bacterial contamination from fecal samples.
3. *DNA template amplification* with PCR is often used when only low quantities of DNA are available. However, PCR can introduce biases such as potentially removing low-abundance variants from sequenced populations, producing uneven coverage across loci, or introducing mutations into clonally amplified DNA templates that subsequently appear as variants. There are several ways to address this: (1) choose the appropriate library preparation kit given the sample quality, as above, (2) adjust the PCR protocol by minimizing the number of PCR cycles (Aird et al. 2011), and (3) remove duplicates in silico using publicly available bioinformatic tools such as Picard (http://broadinstitute.github.io/picard). Note that removing duplicates reduces overall coverage, so accounting for this filtering step is important to determine how much total sequencing effort is required.
4. *Minimum coverage and insert size* are highly dependent upon the focus of the study and sampling design. The recommended coverage for whole-genome resequencing is >30×/individual when individual-level genotype data will be used (Sims et al. 2014). Recommended coverage for pooled sequencing and ultra-low coverage genome sequencing approaches may be much lower per individual, and inferences are made at the population level

(continued)

Box 1 (continued)

(Nielsen et al. 2011; Schlötterer et al. 2014; Wang et al. 2016). Further considerations are necessary when addressing questions using structural variants, such as insertion/deletion (indel) and inversion polymorphisms. Standard libraries with short reads (~350–550 bp insert size) are appropriate for detecting small structural variants, such as small indels and copy number variants (CNVs). The detection of large structural variants (>50 kb) such as inversions or translocations may require the use of long-read data (English et al. 2014; Chaisson et al. 2015; Sedlazeck et al. 2018; Mahmoud et al. 2019).

5. *The total sequencing effort* depends on the sequencing platform, accounting for the error rate, initial filtering, and the expected quantity of high-quality sequence data produced, in order to produce sequence data at the required coverage given the species' genome size. For instance, Illumina sequencing has relative low error rates and a multitude of options for models of sequencer, read length, number of reads per sequencing lane, number of lanes that can be run concurrently, and costs. It can be useful to distribute barcoded libraries across multiple lanes to reduce the effect of lane-to-lane variation that can occur with some sequencing platforms (Ross et al. 2013).

Bioinformatics Workflow for Whole Genomic Sequencing

1. *Quality filtering of raw sequence data* removes many of the errors produced during sequencing, and is facilitated by the standard *fastq* file format that contains quality scores for each nucleotide. Sequencing platforms differ widely in the error rate at individual nucleotide level, as well as other error types that may be specific to a particular technology. Regardless of the sequencing platform, some level of quality filtering of initial raw data is required (Laehnemann et al. 2016). A quality score is given to each base call by the sequencing platform using Phred scores, which is a logarithmic error probability (Ewing and Green 1998). For example, Q30 indicates that there is a 1 in 1,000 probability of calling an incorrect base (or 99.9% accuracy). Frequently there is an observable trend of decreasing quality with increasing base position, as the quality degrades after many cycles of sequencing (Kircher et al. 2009; Kircher and Kelso 2010), so trimming lower-quality ends of reads can be warranted. Additionally, residual adapter sequences, which are added during the library preparation to bind the DNA template to the sequencing platform, are removed from the ends of each read during initial filtering. Adapters and low-quality base pairs are trimmed using programs such as Trimmomatic (Bolger et al. 2014) and Cutadapt (Martin 2011). Although this trimming step reduces the total number and the length of reads, it raises the quality levels and alignment

(continued)

Box 1 (continued)

success to a reference which are crucial for the overall success of genomic data analysis.

2. *Read alignment and mapping* typically involves aligning the sequenced fragments to a reference genome or to a *de novo* assembly depending on whether a reference genome is available:

   (a) If a reference genome is available, it can be used to map high-quality reads based on sequence similarity. Burrows–Wheeler Aligner (BWA; Li and Durbin 2009) and Bowtie2 (Langmead and Salzberg 2012) are commonly used programs to perform alignments of short-read data against a reference. It is important to understand how to optimize parameters for each algorithm to minimize alignment artifacts that can arise due to factors such as divergence between the target reads and the reference genome and misalignments around indels. Multiple reviews of alignment and mapping provide further information regarding alignment algorithm and parameter choices (Fonseca et al. 2012; Hatem et al. 2013; Reinert et al. 2015; Ye et al. 2015; Kumar et al. 2019).

   (b) *De novo* assembly involves assembling a new genome without the help of external data. Several recent reviews provide information regarding achieving high-quality *de novo* genome assembly, particularly with non-model systems (Ekblom and Wolf 2014; Koepfli et al. 2015; Phillippy 2017; Liao et al. 2019).

3. *Mapping statistics*, obtained from data provided in the SAM/BAM files that are output from alignment programs, will provide information such as the fraction of reads mapped to the reference genome and mapping quality scores (Phred-scaled), indicating the confidence that the mapping position is likely to be correct based on a combination of sequence similarity to the reference and base quality. Programs such as Qualimap2 (Okonechnikov et al. 2016) and SAMtools (Li et al. 2009a) calculate these summary statistics to help evaluate mapping quality. Further, small targeted regions can be visually assessed for alignment quality using alignment viewers such as the Broad Institute's Integrative Genomics Viewer (IGV; Robinson et al. 2011).
4. *Post-alignment filtering* is recommended to detect and correct spurious alignments and improve the quality of downstream processes such as variant calling. Unpaired reads, reads mapped to multiple positions, and mapped reads with low-quality scores should be removed. Further, local realignment particularly around indels reduces the number of misidentified variants, although newer methods have incorporated this into variant calling algorithms.

(continued)

Box 1 (continued)

5. *Base quality score recalibration (BQSR)*, implemented in Genome Analysis Toolkit (GATK; McKenna et al. 2010; DePristo et al. 2011) helps to detect systematic errors made by the sequencer when it estimates the quality score of each base call. These non-random errors, caused by the physics or the chemistry of the sequencing reaction or manufacturing flaws of the equipment, can lead to over- or under-estimated base quality scores. These errors are modeled in BQSR by applying machine learning and then quality scores are adjusted accordingly.
6. *Variant calling* identifies sites where at least one individual differs from the reference sequence and estimate individual genotypes at all variant sites. Numerous variant caller methods have been developed, including but not limited to GATK (McKenna et al. 2010), SAMtools (Li et al. 2009a), VarScan (Koboldt et al. 2009), and SOAPsnp (Li et al. 2009b). Variant calling using GATK involves two major steps (Poplin et al. 2018). First, variant genotyping is completed per sample to create intermediate files. Second, another program, HaplotypeCaller, is run on all samples to simultaneously call SNPs and indels. This program reassembles the reads in areas showing signs of variation. HaplotypeCaller tends to be more accurate at calling variants in difficult regions such as regions that contain differing types of variants that are close to each other.
7. *Filtering of variants* with low-quality scores reduces false positives that should be removed from the dataset before downstream analyses. For systems with a large number of validated SNPs, filtering can be completed using variant quality score recalibration (VQSR; van der Auwera et al. 2013). However, often non-model systems do not have these variant databases readily available. In that case, hard filters are applied to remove false positives by detecting variants with characteristics outside their normal distributions. Appropriate choice of threshold values is a function of the data with low-quality scores, imbalanced strand specificity, and skewed allelic imbalance indicators of false positives. Hard filter thresholds can be implemented with programs such as GATK's VariantFiltration (McKenna et al. 2010; DePristo et al. 2011; van der Auwera et al. 2013) and VCFtools (Danecek et al. 2011). It is recommended to test the effects of a range of filtering thresholds particularly when applied to population genetic and demographic inferences, (Mastretta-Yanes et al. 2014; Shafer et al. 2017; Paris et al. 2017).
8. *Variant annotation*, implemented in programs such as Ensembl Variant Effect Predictor (VEP; McLaren et al. 2016) or SnpEff (Cingolani et al. 2012), is the assignment of sequence ontology terms and functional information to variants. This information can include estimates of sequence conservation, computational predictions of putative deleterious effects,

(continued)

Box 1 (continued)
and predictions about the effect of a variant on protein structure. Variants should be considered putative polymorphisms until validated by PCR amplification and Sanger sequencing or development of a marker panel for additional genotyping. This will ensure that variants discovered are not false positives.

4.2 Sampling

How many samples are required, and how they should be distributed among populations or across a landscape, varies widely depending on the goals of a study (Fig. 5). For instance, studies aiming to understand inbreeding within a population need to sample many individuals, while comparative studies across populations or taxa, such as phylogenetic analysis, may need only a single "representative" individual (Box 1). However, one advance of genomic data is that one or very few individuals can still provide a vast amount of information about a population to the extent those individuals are genetically representative of the population's history. Because each individual's genome derives from an expanding set of ancestors back in time, densely sequencing the whole genome leads to inferences about population history (Fig. 3a). This is particularly important in threatened wildlife species, where the availability of samples may be the greatest constraint on a population genomics study. However, the assumption that focal individuals are representative of a population is critical, and factors such as hidden population structure can strongly affect inferences about demographic history (Mazet et al. 2016; Gaughran et al. 2018).

Population genomics studies in wildlife often aim to use low-quality and/or low-quantity DNA samples, such as archival, environmental, and non-invasive samples collected from scat, hair, or feathers. These samples may have reduced total amounts of genetic material, DNA molecules that are fragmented or degraded, contamination from bacteria or other genetic material, or all of these issues. Andrews et al. (2018, this volume) describe the wide range of genetic and genomic techniques that can be applied in these cases. Many of the library preparation and sequencing approaches below can be optimized for low-quality samples, although others remain challenging. Environmental DNA (eDNA), which is DNA extracted from soil, water, or other environmental samples, has been used primarily for the detection of species presence, such as with species-diagnostic barcode sequences from mitochondrial DNA. Goldberg and Parsley (2020, this volume) describe the potential for eDNA approaches to be extended to population genomics studies in wildlife, in which allelic variation among individuals can be assayed from eDNA samples. This is challenging because eDNA fragments cannot be assigned to individuals, and eDNA samples may contain very few fragments of any particular locus. However, population genomics with eDNA will become more feasible as techniques improve for sequencing single DNA molecules.

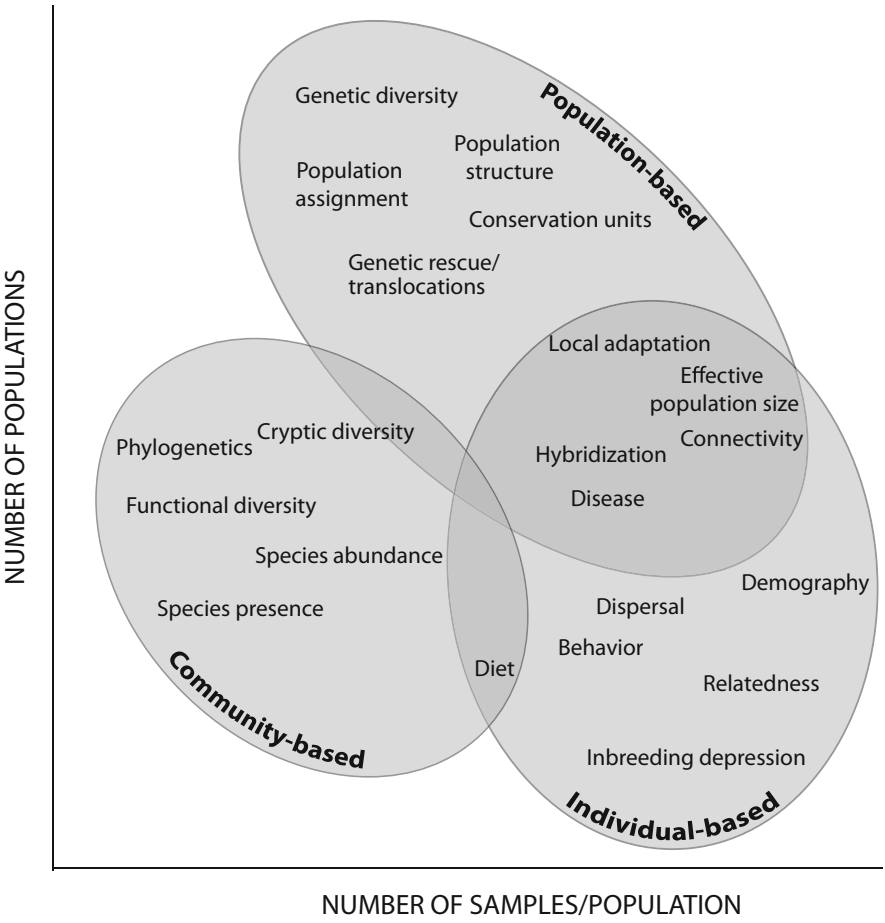

Fig. 5 Conceptual view of the range of sampling strategies that may be appropriate to address different questions in wildlife population genomics, at different scales as shown in Fig. 1. The number of populations sampled may range from a single focal population with inbreeding or demographic questions, to a large number of populations to address landscape-level questions. Similarly, the number of individuals sampled per population may range from just a single representative of each for comparative or phylogenetic questions, to a large number of individuals to address relatedness or demography within a focal population. Additionally, the total number of individuals sampled presents a trade-off with the amount of genetic information obtained for each individual, given constraints on total sequencing cost. Many population genomics studies in wildlife may be limited by the availability of samples, so that extracting more information per individual is appropriate (e.g., whole-genome sequencing; Box 1)

Many wildlife species are also represented in museum collections, and these samples can provide insights into temporal and spatial variation in many taxa. Often, historical museum samples may represent genetic variants, populations, or even species that are no longer extant in nature (Robinson et al. 2018; van der Valk et al. 2019; Sánchez-Barreiro et al. 2020). Application of genomic methods to

museum samples can reveal how genetic variation has changed in the past and inform understanding of adaptive genetic variation that may have been lost from current populations. For example, Bi et al. (2019) generated genomic sequence data from museum specimens of two species of chipmunk (*Tamias* spp.) spanning 100 years of collection history. They were able to reconstruct demography of the two populations and also identify signatures of positive selection based on rapid shifts in allele frequency.

4.3 Library Preparation and Sequencing

Population genomics in wildlife benefits from a bewildering and growing array of techniques for producing large amounts of genomic DNA sequence data (Fig. 6). Most of these are based on sequencing technologies in which heterogeneous

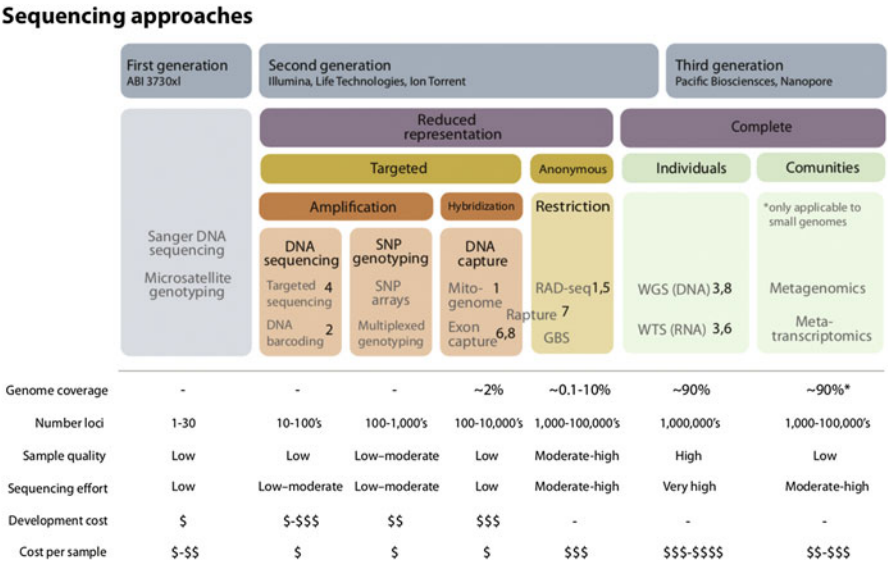

Fig. 6 Conceptual overview of sequencing approaches for population genomics in wildlife. The top row shows sequencing technologies progressing through methods based on Sanger sequencing (first generation), short-read parallel sequencing (second generation), and long-read sequencing (third generation) (Wong et al. 2019). Genomic sequencing can cover a subset of each genome (reduced representation) or the entire genome (complete). Reduced representation techniques can be either targeted at pre-identified loci, using either amplification with primers or hybridization with probes, or they can be anonymous, for instance using restriction enzymes to survey loci across the genome. Complete genome sequencing may cover individuals or include genomic sequence from multiple individuals or species in a community. Below these groupings are example techniques, with case studies as in Fig. 1: (1) Barbosa et al. (2018); (2) Marshall and Stepien (2019); (3) Hu et al. (2020); (4) Eriksson et al. (2020); (5) Escoda et al. (2019); (6) Rellstab et al. (2019); (7) Peek et al. (2019); (8) Mills et al. (2018). Note that some techniques combine approaches: for instance, Rapture (Ali et al. 2016) combines RADseq with targeted sequence capture. At the bottom are very rough estimates to quantify some features of these techniques, as they might be applied in a wildlife study such as those in Table 1

collections of DNA molecules can be sequenced simultaneously (often called next-generation sequencing, or second- and third-generation sequencing; Heather and Chain 2016; Wong et al. 2019). As a result of these technical advances in recent decades, population genomics techniques may target thousands of loci across the genome. These loci can be either pre-selected based on prior information using capture probes or primers or anonymously distributed across the genome as a result of protocols like RADseq that use restriction enzymes (Hohenlohe et al. 2019; Holliday et al. 2019; Luikart et al. 2019). Data from RADseq are typically used as SNP genotypes, but analyzing them as microhaplotypes can provide higher-resolution data (Baetscher et al. 2018). Alternatively, WGS across a sample of individuals is now feasible even in wildlife species and is particularly well-suited for reconstructing historical demography, estimating inbreeding with runs of homozygosity, or assessing the functional significance of deleterious mutations (Table 1; Box 1). The technology of sequencing continues to provide new platforms for sequencing, including the current transition to third-generation sequencing approaches that provide continuous sequence data for long DNA fragments (Fig. 6). These technologies continue to increase the feasibility and speed of generating reference genome assemblies for wildlife species, adding to the data resources for population genomic studies.

There are important considerations before choosing the most appropriate library preparation and sequencing technique, driven by the limitations of the study system and the scientific question (Benestan et al. 2016; Hohenlohe et al. 2019). One limiting factor may be DNA sample quality. Many genomics techniques require high quality and quantity DNA samples, especially for whole-genome and whole-transcriptome sequencing (Box 1), but also for some reduced representation techniques like some RADseq methods (Andrews et al. 2016). Other techniques, including targeted sequencing with amplification primers or hybridization probes, can be effective with lower-quality DNA samples (Carroll et al. 2018; Bi et al. 2019). Progress continues in optimizing techniques for low-quality samples, including WGS and modified versions of RADseq protocols, so that these approaches are increasingly accessible as well (Russello et al. 2015; Andrews et al. 2018, this volume). New methods for isolation of target DNA prior to library construction can help as well (Chiou and Bergey 2018).

Given a total amount of resources for building libraries and sequencing, the allocation of this budget among numbers of individuals, numbers of populations, and density of sequence data and loci across the genome depends on the scientific question being addressed (Fig. 1). These trade-offs drive the choice of sequencing approach, because sequencing approaches vary widely in cost and sequencing effort per sample (Fig. 6). For example, a study of genetic population structure across a landscape like the one illustrated in Fig. 3b can be applied to identify population units for conservation purposes. This scientific question is best addressed by sampling a relatively large number of individuals distributed geographically, but analyses of population structure require a moderate number of loci. Accordingly, McCartney-Melstad et al. (2018) sampled individuals across nearly the entire species range, and used RADseq to generate data on tens of thousands of SNP loci.

Similarly, Jensen et al. (2020) genotyped 13,488 SNP markers with a RADseq protocol across 358 individuals to identify genetic population clusters in polar bears (*Ursus maritimus*). Alternatively, demographic reconstruction of historical population trends and their consequences, especially in small populations, can be accomplished with high-density WGS on a small number of individuals (Box 1). For instance, this approach was used to identify fine-scale effects of inbreeding in pumas (*Felis concolor*; Saremi et al. 2019) and wolves (*Canis lupus*; Kardos et al. 2018). At the extreme, producing a reference genome assembly for even just a single individual can reveal deep insights into population history and functional genetic variation in wildlife species (Humble et al. 2020; Upadhyay et al. 2020).

Studies focused on adaptive variation may also span these trade-offs depending on the particular question. For example, tests of local adaptation to environmental variables using GEA analysis can benefit from a relatively large number of samples distributed geographically across a wide range of environmental variables, and can still be accomplished with reduced representation approaches (Catchen et al. 2017; Forester et al. 2018, this volume). Alternatively, studies seeking to comprehensively assess the adaptive or functional variation in a wildlife species' genome may require the complete sequence data of WGS, using analyses of gene content and functional inferences about the effects of polymorphisms, rather than analyses that rely on sampling across individuals (Robinson et al. 2018, 2019). For instance, Johnson et al. (2018) produced a high-quality reference genome assembly for koalas (*Phascolarctos cinereus*). They determined that koalas' decline is likely associated with human arrival to Australia, matching the decline of Australian megafauna, and detected decreased genetic diversity in translocated populations originating from a single source population. This study also found adaptations of koalas to the toxicity of eucalyptus foliage and to chlamydia, which has had large impacts on the koala populations over the past century (Polkinghorne et al. 2013).

As costs of sequencing continue to drop, WGS is feasible for larger numbers of samples in wildlife studies (e.g. Lucena-Perez et al. 2020 used WGS on 80 individuals in their study of lynx [*Lynx lynx*] population history), and an increasing number of wildlife population genomics studies apply this technique. However, WGS remains more costly than other techniques, both in the library preparation and sequencing and computational resources to handle WGS datasets, and WGS may not be necessary to answer many questions in wildlife (McMahon et al. 2014; Lewin et al. 2018). In some cases, wildlife taxa present specific challenges for applying population genomics and limit the choice of techniques that can be applied. For instance, some amphibian taxa have remarkably large and complex genomes that may preclude WGS (Funk et al. 2018, this volume), and Weisrock et al. (2018, this volume) provide detailed recommendations for calibrating other methods including RAD sequencing, sequence capture, and amplicon sequencing in this group. More generally, reduced representation approaches will continue to be effective in cases where the scientific question requires large samples of individuals without needing large numbers of loci, and where no prior panel of markers has been developed and an approach like RADseq can be used with no prior data (Andrews et al. 2016). Similarly, traditional genetic techniques like microsatellites will continue to play a

role in wildlife research (e.g., Naude et al. 2020), and even microsatellite genotyping can be accomplished with high-throughput genomic techniques (Bradbury et al. 2018; Tibihika et al. 2019).

In many wildlife applications, it can be useful to use a combination of genomic sequencing approaches. For example, multiple sequencing techniques are commonly combined to produce reference genome assemblies (Humble et al. 2020). Combining WGS of one or a few individuals (at higher depth of coverage), and shallower resequencing of a larger set of (geographically distinct) individuals can provide a greater understanding of the processes governing phylogenetics, population structure, demographics, inbreeding and adaptation, while reducing sequencing effort (Brandies et al. 2019). In many cases it is increasingly feasible for wildlife studies to generate a reference genome assembly concurrently with reduced representation sequencing across a large number of samples, gaining the benefits of a reference genome against which to align the population-level data (Ruegg et al. 2018; Liu et al. 2019). For instance, Oyler-McCance et al. (2020, this volume) describe this approach in sage-grouse (*Centrocercus* spp.), combining WGS to infer demographic history and reduced representation sequence data to detect adaptive differentiation among populations.

As described above for monitoring and other applications, it can be efficient to use an initial dense sequencing approach such as WGS, transcriptome sequencing, or RADseq to develop a smaller panel of markers for genotyping of large numbers of samples over time (Aykanat et al. 2016; Eriksson et al. 2020). These panels can be optimized from genomic data to include adaptive or functionally significant loci (von Thaden et al. 2020). This includes drawing functional genomic information from related species to contribute to wildlife studies, such as the annotated domestic dog (*Canis familiaris*) reference genome that has been used to assess the fitness consequences of mutations in wild canid taxa (Robinson et al. 2018). Genotyping panels can also be optimized for low-quality and non-invasive samples following the initial sequencing of a few higher-quality samples (Natesh et al. 2019; Schmidt et al. 2020). These marker panels can be used to detect species presence (Janecka et al. 2020), to perform individual identification and determine individual distribution (Bourgeois et al. 2019; Giangregorio et al. 2019), to detect and quantify hybridization (Tiesmeyer et al. 2020), or to infer kinship (Escoda et al. 2019).

4.4 Resources

Population genomics has developed a growing foundation of genomic data and resources that can facilitate studies in wildlife species. This includes reference genome assemblies for an increasing number of vertebrates, either wildlife species or their domestic relatives. Well-studied groups like ungulates (Martchenko et al. 2018, this volume) and birds (Toews et al. 2018, this volume) have large numbers of reference genome assemblies. In a more challenging group, Funk et al. (2018, this volume) provide recommendations for building a genome reference set across

amphibians, including at least one reference genome assembly in each amphibian family, and document progress toward this ambitious goal. Increasingly, technological advancements make it feasible to produce a high-quality reference genome assembly for nearly any wildlife species that is of interest for population genomics research (Gopalakrishnan et al. 2017; Armstrong et al. 2019; Rice and Green 2019).

Having a reference genome assembly in a population genomics study provides multiple benefits for all data types, including whole-genome resequencing across a population sample as well as any reduced representation approaches (Box 1; Rochette and Catchen 2017; Shafer et al. 2017). A reference genome allows positioning sequence reads and loci on a map, filtering of duplicate or problematic sequence, higher-confidence identification of loci, statistical analysis such as linkage disequilibrium and sliding-window analyses, identification of candidate genes near markers, and more. More broadly, the increasing number of species with genomic data allows for comparative genomics studies to better understand genomic evolutionary processes, such as changes in chromosome arrangement and recombination across birds (Toews et al. 2018, this volume), as well as phylogenetic analyses to reveal relationships among wildlife species and understand their evolutionary history (Lavretsky 2020, this volume; Ramstad and Dunning 2020, this volume).

Reference genome sequence data are maintained by several institutions that constitute the International Nucleotide Sequence Database Collaboration. They are publicly available online through GenBank of the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov/Traces/wgs/), the European Nucleotide Archive (ENA; https://www.ebi.ac.uk/ena/browse/genome-assembly-database), and the DNA DataBank of Japan (DDBJ; http://www.ddbj.nig.ac.jp/). GenBank (https://www.ncbi.nlm.nih.gov/bioproject/) and the Genomes Online Database (GOLD; https://gold.jgi.doe.gov/projects; Mukherjee et al. 2017) also provide a list of ongoing projects.

Many reference genome assemblies have also been annotated, meaning that putative genes and functional information have been inferred based on sequence similarity to known genes, transcriptomic data, gene prediction, and other analyses (Dominguez del Angel et al. 2018; Armstrong et al. 2019). As a result, a wildlife population genomics study that identifies loci that are differentiated among populations, subject to selection, or influenced by reduced diversity or hybridization can make functional inferences about these loci. For instance, Grossen et al. (2020) identified deleterious mutations in Alpine ibex (*Capra ibex*) and estimated their effects using genome annotation and functional data from related species, including gene models from the domestic goat (*C. aegagrus*). As a result, the researchers assessed the consequences of severe population bottlenecks and inbreeding on population-level fitness and genetic health of reintroduced ibex populations.

Genome annotation data is available from multiple databases, such as Ensembl (http://www.ensembl.org), RefSeq (http://www.ncbi.nlm.nih.gov/RefSeq; Pruitt et al. 2007), and the UCSC genome browser (http://genome.ucsc.edu). Functional information for gene families across species is also available from sources such as the Gene Ontology (GO) database (Ashburner et al. 2000; The Gene Ontology Consortium 2019), the Kyoto Encyclopedia of Genes and Genomes (KEGG;

Kanehisa and Goto 2000; Kanehisa et al. 2012), and the EggNOG database (Huerta-Cepas et al. 2019). Further functional information is available from protein databases such as UniProt Knowledgebase (https://www.uniprot.org; UniProt Consortium 2019) and Pfam (https://pfam.xfam.org). This type of detailed functional information is most useful in wildlife studies when population genomic data has identified a small number of candidate loci that may be important in adaptation, inbreeding depression, or population viability, and understanding the functional mechanisms is important (e.g. Waterhouse et al. 2018).

Reference genome assemblies, annotation, and functional information are also useful in wildlife studies for designing panels of markers that can be used for rapid genotyping, monitoring, or in-depth study of adaptive loci (Meek et al. 2016; Schweizer et al. 2018; Saint-Pé et al. 2019; von Thaden et al. 2020). Increasing publicly available data reduces the cost and investment needed to generate a marker panel for a wildlife species. For example, the large number of domestic ungulate species with genomic resources has translated into marker panel development for wild ungulate taxa (Martchenko et al. 2018, this volume), and genomic resources in dogs have facilitated research in wild canids (Schweizer et al. 2016). In Tasmanian devils, the Rapture approach (RADseq plus sequence capture; Ali et al. 2016) was used to design a panel of nearly 16,000 loci, most of which had putative association with devil facial tumor disease, either based on evidence of selection in response to disease (Epstein et al. 2016) or annotation to cancer or immune-related functions in the reference genome. This panel has been used to assess genetic variants associated with disease-related phenotypes (Margres et al. 2018), the genetic basis of local adaptation (Fraik et al. 2020), and selection in natural populations in response to disease (Stahlke et al. 2020) in a targeted way by genotyping thousands of individuals from natural populations.

4.5 Data Analysis

Population genomic datasets are large, and so bioinformatics and data analysis will be a significant portion of any population genomics study. The bioinformatics and analysis options for genomic data continue to grow. As with the choice of sampling and sequencing approach, the most appropriate analyses depend on the scientific question. The first steps in analyzing a large genomic sequence dataset are typically initial quality filtering, which tend to be fairly similar across data types. Filtering by quality scores, trimming adaptors and low-quality sequence, de-multiplexing samples, and other initial steps are critical (tools for conducting these steps with WGS shown in Box 1 are widely applicable across sequencing types). If a reference is available, sequence reads can be mapped to the reference, and several common software packages are designed for this task (Box 1). If not, *de novo* assembly can be done with multiple tools, depending on whether the data are transcriptome (e.g., Trinity; Grabherr et al. 2011), RADseq (e.g., Stacks; Catchen et al. 2013; PyRAD, Eaton 2014), WGS (see Box 1), or other types. Often the next step will be to identify

loci, such as SNPs, and/or to genotype these loci across a set of individuals. Several software tools, including GATK (McKenna et al. 2010) and SAMtools (Li et al. 2009a), are widely applicable across data types. Others are more specific, such as Stacks (Catchen et al. 2013) written specifically for RADseq data. In other cases, population-level allele frequencies or other statistics will be estimated rather than individual genotypes, using tools such as ANGSD (Korneliussen et al. 2014).

Typically, once a set of genotypes or population-level statistics are produced, further analyses depend on the scientific question and there is a multitude of possibilities. The case studies of wildlife population genomics in Table 1 provide examples of how different analyses are applied and combined. Many of these studies have sampled individuals across populations or a landscape, and a set of basic analyses to examine genetic population structure is common. These include principal components analysis and Bayesian clustering methods, such as STRUCTURE (Pritchard et al. 2000). Phylogenetic analyses are widely used, particularly with samples across divergent populations or species, but they can also be used to separate populations into clusters (e.g., the colors in Fig. 3b represent phylogenetic clusters identified in a maximum-likelihood analysis). Estimates of effective population size can be made in several ways, depending on the sampling (e.g., whether a single or multiple time points were sampled); Fig. 2b illustrates the results from a single time point, using the linkage disequilibrium method implemented in NeEstimator (Do et al. 2014). Historic demographic reconstruction often requires more genomic data; for instance, a few methods based on the sequentially Markovian coalescent (SMC) model are commonly applied to one or a few individuals with WGS data (Fig. 3a). Although these methods require continuous sequence data based on a genome assembly, results are somewhat robust to assembly quality. For instance, reference genomes for wildlife species that remain split into tens of thousands of scaffolds may still be sufficient for inferring demographic history (Patton et al. 2019). With a reduced representation of the genome, demographic inference is still possible with approaches such as approximate Bayesian computation (e.g., Bi et al. 2019).

Several analytical approaches address functional or adaptive variation in genomic data. Multiple software tools have been developed to identify adaptive loci from population genomic datasets, based either on outlier loci or genotype–environment association (Forester et al. 2018, this volume). For example, Tigano et al. (2017) applied outlier analysis to a RADseq dataset in thick-billed murre (*Uria lomvia*) populations, using the software package Bayescan (Foll and Gaggiotti 2008). They found evidence for adaptive divergence among populations, despite no evidence for genome-wide population differentiation. Genotype–environment association can be accomplished with tools such as LFMM (Frichot et al. 2013; see also Forester et al. 2018, this volume), as applied by Ruegg et al. (2018). In cases where samples are available across several time points, adaptive loci can be detected by testing whether shifts in allele frequency at particular loci are consistent with a neutral model of drift or other demographic scenarios. For example, Stahlke et al. (2020) identified signatures of ongoing selection in Tasmanian devils using tools designed for time-series data such as spatpg (Gompert 2016). Genome-wide association studies, for

which several analytical tools have been developed for model systems including humans, can also be applied in wildlife (e.g., Margres et al. 2018), using software such as GEMMA (Zhou and Stephens 2012). With WGS data, the genetics of inbreeding can be investigated by using runs of homozygosity (ROH) (Kardos et al. 2018; Robinson et al. 2019). This method identifies the genomic regions impacted by inbreeding within individuals and can also identify whether genetic rescue from other populations may be successful based on the complementarity of ROH (Saremi et al. 2019). Much can also be learned about adaptive or deleterious loci by inferring the functional consequences of specific alleles with a variety of methods that make use of genome annotations among related taxa (Robinson et al. 2018; Grossen et al. 2020).

The analytical tools described above are a small subset of those available for wildlife population genomics. Studies will often be most successful by combining multiple approaches, drawing multiple conclusions from a genomic dataset. However, specific analyses may not be appropriate in many cases, either because assumptions of the model are violated, the analysis is not designed for a particular data type, or because the amount of data is not sufficient for statistical power. With all steps of the analysis, a critical requirement is to test the effect of parameters and settings on the results (Paris et al. 2017; Shafer et al. 2017).

5 Future Prospects in Wildlife Population Genomics

5.1 Metagenomics and eDNA

The studies and techniques described above primarily focus on sequencing of samples from either a single individual or pool of individuals from the same population or species. As genomics tools continue to develop, wildlife population genomics may also make more use of metagenomic sequencing and metabarcoding. Metagenomic sequencing is defined as sequencing genetic material from multiple different taxa within a sample, while metabarcoding specifically refers to identifying the taxa present in a sample using sequence-based signatures, or barcodes (Taberlet et al. 2012; Luikart et al. 2019). These approaches can identify taxa in samples with low DNA quantity and quality like individual non-invasive samples (feces, hair, saliva), bulk samples (multiple individuals), or eDNA samples (Seah et al. 2020). One longstanding application of metagenomic sequencing is assessment of the microbiome – the community of microorganisms – associated with a sample. In wildlife, individual non-invasive samples have provided great insight into the role of the microbiome in adaptation and fitness, diet and diseases of wildlife populations, and even viral communities (Deagle et al. 2019; Hauffe and Barelli 2019; Roth et al. 2019; West et al. 2019; Bergner et al. 2020). Studies of eDNA have mostly focused on detecting species presence and abundance, for instance to detect cryptic or rare species or track invasive species such as Eurasian zebra and quagga mussels (Marshall and Stepien 2019). It is challenging to use eDNA to make population

genetic inferences that depend on data from multiple individuals at a set of loci, but still it has promise for population genomics applications in wildlife (Barnes and Turner 2016; Goldberg and Parsley 2020, this volume). For instance, Sigsgaard et al. (2017) produced estimates of genetic diversity in a whale shark (*Rhincodon typus*) aggregation by detecting mitochondrial DNA in seawater samples. Metagenomic sequencing of the microbial component of eDNA samples, while not directly sequencing wildlife species, can illuminate the environmental conditions in which wildlife populations exist by characterizing the functional genetic diversity of the microbiome (Seeleuthner et al. 2018).

5.2 Population Epigenomics

Population epigenomics is a fast-emerging area of research in population genomics (Rajora 2019; Luikart et al. 2019; Moler et al. 2019). It is now well established that epigenomic variation – alterations to genetic material that do not change DNA sequence – can contribute significantly to phenotypic plasticity, abiotic and biotic stress responses, disease conditions, and adaptation to a variety of habitat conditions (reviews in Richards et al. 2017; Moler et al. 2019). Because epigenomic variation may be inherited across generations, it could be of potential evolutionary significance. In wildlife populations, epigenomic variation may be important in the adaptive capacity of populations to respond to environmental pressures, such as climate change (Dawson et al. 2011; Nicotra et al. 2015). Recent advances in high-throughput sequencing technologies to assay genome-wide epigenetic marks, such as bisulfite DNA sequencing, have enabled the field to progress from studying individual epigenomes to investigating epigenomic variation across populations and species (Moler et al. 2019). In many wild animal populations, an abundance of epigenetic (DNA methylation) variation relative to genetic variation has been found (Hu and Barrett 2017).

The role of epigenomic variation in wildlife populations remains poorly understood, although there are some illustrative case studies. Riyahi et al. (2017) studied natural variation in DNA methylation within and among five subspecies of house sparrow (*Passer domesticus*) using the methylation-sensitive amplified polymorphism (MSAP) approach. DNA methylation was not found to be strictly subspecies-specific, but the European subspecies was differentiated from all other Middle East subspecies and the commensal subspecies was differentiated from the non-commensal species by differentially methylated regions. The methylation level was correlated with some morphological traits, such as standardized bill length. Liu et al. (2015) also applied the MSAP approach to three bat species (*Hipposideros armiger*, *Rhinolophus pusillus*, *Miniopterus fuliginosus*). The populations exhibited high epigenetic diversity and significant epigenetic structure within and among populations and individuals. The epigenetic diversity was higher than the corresponding genetic diversity. McNew et al. (2017) studied morphological, genetic, and epigenetic differences between adjacent "urban" and "rural"

populations of each of two species of Darwin's finches (*Geospiza fortis* and *G. fuliginosa*). They did not find differences in large-size copy number variation (CNV) but did find striking epigenetic (methylation) differences between the urban and rural populations of both species. Wenzel and Piertney (2014) examined epigenomic diversity and differentiation among 21 populations of red grouse (*Lagopus lagopus scotica*) in north-east Scotland and tested for association of gastrointestinal parasite load (caecal nematode *Trichostrongylus tenuis*) with hepatic genome-wide and locus-specific methylation states. The populations were found to be significantly epigenetically and genetically differentiated and displayed significant fine-scale epigenetic structure, and parasite load was associated with methylation patterns on a locus-specific, but not genome-wide level. The epigenetic differentiation observed among red grouse populations was considerably higher than genetic differentiation. This study provided an example for epigenetic mechanisms contributing to plasticity and adaptation in the context of host–parasite interactions in natural wildlife populations.

5.3 Population Transcriptomics

Population transcriptomics is another fast-emerging research area of population genomics (Rajora 2019; Luikart et al. 2019). Population transcriptomics uses transcriptome-wide data to study variation in gene expression within and among populations to understand mechanisms underlying acclimation and adaptation, phenotypic variation and plasticity, abiotic and biotic stress responses, adaptive evolutionary responses to new environments, and other evolutionary changes (Luikart et al. 2019). Addressing these issues in wildlife populations can be important for understanding population viability and adaptive potential in the face of environmental stressors. As discussed above, whole-transcriptome sequencing (RNASeq) can be applied to identify sequence variation at coding regions of the genome, but it can also be used to assess expression levels across genes, and it does not require prior information to target sequence effort. In animals, most of the population transcriptomics work has been conducted in fish and other aquatic organisms (Alvarez et al. 2015; Connon et al. 2018). Much of this work has addressed three questions: "(1) How much variation in gene expression is there in natural populations and how is it structured? (2) How do environmental stimuli affect gene expression? (3) How does variation in gene expression translate into phenotype?" (Alvarez et al. 2015).

Population transcriptomics research has been limited in terrestrial wildlife populations. In addition to quantifying the role of gene expression in adaptation and population differentiation, transcriptomic studies in wildlife can help understand response to disease. For instance, Campbell et al. (2018) used RNAseq to compare the gene expression profiles of frog (*Rana temporaria*) populations with a history of ranaviral disease and those without disease. They identified over four hundred transcripts that were differentially expressed between populations of different

ranaviral disease history. The differentially expressed transcripts included genes with functions related to immunity, development, protein transport, and olfactory reception. Population transcriptomics has been limited, including in wildlife, because transcriptome sequencing to estimate gene expression levels requires much more sequencing effort than that required to identify sequence differences, and also because RNAseq requires much higher sample quality than DNA sequencing approaches. However, as sequencing costs continue to drop, the feasibility of using RNAseq across individuals sampled in wildlife populations will increase. Technological developments are also likely to improve for preserving RNA from field-collected samples in wildlife.

6 Conclusions

The application of population genomics approaches to wildlife continues to expand. It is important for both population genomics researchers and wildlife conservation and management professionals to have an understanding of the range of approaches and questions that can be addressed in wildlife. An ongoing challenge is to improve the connections and communication among these groups. Efforts to provide venues for direct communication and interaction are critical, including cross-disciplinary training and workshops at all career levels. Research studies will benefit from coordination with wildlife professionals at all stages, from design of the questions and approach to interpretation and dissemination of results. New approaches will also emerge in the coming years, such as other "omics" techniques, the use of genetic engineering in wildlife, or approaches for multi-species or community-level genomics. Overall, population genomics provides a critical set of tools to address the biodiversity crisis in wildlife taxa. We hope this chapter provides an overview and framework to advance the field of wildlife population genomics and contribute to improving on-the-ground conservation efforts in urgent times for wildlife species.

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