Chapter 11: The role of genomics in the future of ESA decision-making

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INTRODUCTION

The U.S. Endangered Species Act (ESA) provides a framework for the protection and recovery of threatened and endangered species and their habitats in the face of anthropogenic threats, including habitat modification (e.g., loss, fragmentation, degradation, transformation), overexploitation, disease, and climate change (Leu et al., 2019; Naujokaitis-Lewis et al., 2021). Genetic information has played a role in decision-making under the ESA for decades, most commonly informing taxonomy and the designation of distinct population segments (DPSs; Avise, 1989) though detection of inbreeding also played an early role (O'Brien, 1994). The transition to genomic technologies has improved the precision and resolution of important population genetic metrics for at-risk species, such as genetic diversity and population structure (Gallego-García et al., 2021). For other parameters, such as inbreeding, genomic data have transformed our ability to precisely measure individual level variation, as well as quantify downstream impacts on population viability (Kardos et al., 2016). Genomic data have also democratized access to other parameters, such as estimates of evolutionary potential, that were once limited to model organisms and species amenable to experimental manipulation (Forester et al., 2022; Funk et al., 2019; Hoffmann et al., 2015). We use "genetics" to refer to small sets (e.g., tens) of neutral molecular markers. Common genetic markers referenced in this chapter include mitochondrial DNA (mtDNA) markers, derived from the maternally inherited, haploid DNA molecule found in the mitochondria of eukaryotes, and microsatellites, short sequences of repeated nuclear DNA. By contrast, we use "genomics" to refer to the genotyping of large sets of molecular markers (e.g., thousands to millions). The most commonly used genomic-scale marker is the single nucleotide polymorphism (SNP), which represents a single base pair difference within and among populations and/or species. In contrast to genetic data, genomic data can be used to investigate both neutral microevolutionary processes, such as gene flow and genetic drift, as well as adaptive processes that contribute to evolutionary potential and adaptive capacity (Allendorf et al., 2010; Hohenlohe et al., 2021).

In this chapter, we review how genetic data have informed decision-making under the ESA, and how the transition to genomics is improving the information that we can apply to both listing and recovery decisions. In some cases, genomic data are presenting new challenges to applied conservation under the ESA, providing an opportunity to evaluate and innovate existing practices. In all cases, falling costs and the increasing ease of genomic-scale data production in at-risk species are providing an unparalleled opportunity to improve applied conservation of threatened and endangered species and expand new frontiers for agency use of the "best available science" in ESA implementation.

THE ROLE OF GENETICS AND GENOMICS IN THE ESA: LISTING

The U.S. Fish and Wildlife Service (USFWS) and National Marine Fisheries Service (NMFS, collectively referred to as the Services) use the best scientific information available to determine if a species meets the definition of threatened or endangered under the ESA and, if so, whether it will be listed or placed on the candidate list. The first step in this process is identifying the conservation unit and determining whether or not it qualifies as a listable entity (i.e., species, subspecies, or DPS). Support for this determination of taxonomic and classification status has been one of the primary uses of genetic and, increasingly, genomic data in ESA listing decisions. However, these data can play important roles in the listing process beyond taxonomy, informing both the assessment of historical and current conditions of the species, as well as projecting the species' response to future conditions. Below, we review how these data are informing the listing process, providing examples of the effective use of genetic and genomic technologies in these efforts.

Taxonomy and classification status

The importance of appropriate taxonomic delineation in listing and recovery under the ESA cannot be understated: excessively broad designations can result in the under-protection of distinct lineages and potential outbreeding depression during conservation interventions (reduction in fitness from crossing diverged populations), while overly fine-scale taxonomic delineations can waste resources and limit the use of conservation actions such as genetic rescue (gene flow between populations to reverse inbreeding, recover genetic diversity, and improve fitness). However, delineating discrete units is challenging because speciation and species dissolution are dynamic processes that span a continuum of isolation, gene flow, and natural selection (De Queiroz, 2007). Processes such as hybridization and introgression complicate these dynamics because they can erode species identity while also facilitating adaptive radiations. Fortunately, genetic and genomic data can provide insight into these speciation processes, while also informing the delineation of intraspecific units such as subspecies and DPSs.

The ESA takes an inclusive approach to defining species: "The term 'species' includes any subspecies of fish or wildlife or plants, and any distinct population segment of any species of vertebrate fish or wildlife which interbreeds when mature" (U.S. Endangered Species Act of 1973, as Amended, 1973). This broad definition, while allowing for evaluation at both specific and subspecific levels, provides no guidance for interpreting the complexities of terms such as "distinct" (National Research Council, 1995; Waples, 1991). Beyond the ESA policy definition, species delineation can be contentious, given that there are over two dozen different sets of biological and evolutionary criteria for defining species. Generally, most species concepts focus on separately evolving lineages, but differ in how many defining properties are required to delimit a species (De Queiroz, 2007). The biological species concept (BSC) is one of the most widely used concepts and defines species as "…groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups" (Mayr, 1942). While this concept is relatively straightforward, it can be difficult to operationalize, and does not accommodate hybridization, sympatric speciation, asexual reproduction, or self-fertilization. A common alternative used by the conservation community is the phylogenetic species concept (PSC), which defines species as "…the smallest diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and descent" (Cracraft, 1983). The PSC can be applied across more taxonomic groups than the BSC, and can be easier to implement, given its reliance on molecular data. It has also been identified as a more inclusive definition in line with the policy-based definition provided in the ESA (National Research Council, 1995). However, a complication of the PSC is its tendency to identify many more species with small ranges and population sizes when compared to other species concepts, an issue with significant downstream biological, economic, and political consequences (Agapow et al., 2004).

The shift to genomics has heightened these concerns, since high resolution genomic data can be used to delineate fine-scale population structure, which could be interpreted as species-level divergence under some frameworks. Fortunately, genomic data also improve our ability to estimate demographic history and infer divergence, which can provide additional insight into the process of speciation and provide support for or against species-level divergence (Coates et al., 2018; Stanton et al., 2019). For example, genetic studies of the taxonomy of freshwater mussels in the genus *Cyprogenia* exhibited conflicting results based on a phylogenetic analysis using two mtDNA genes (Serb, 2006) and a study of population structure using one mtDNA gene and ten nuclear microsatellite markers (Chong et al., 2016). Resolving the taxonomy of this group is important, since it currently includes one ESA-listed endangered species (*C. stegaria*, Fig. 1) and a second species proposed as threatened (*C. aberti*). A recent study used a genomic approach to address these conflicting results, identifying ~10,000 SNPs distributed across the genomes of the two putative species (Kim & Roe, 2021). By integrating analyses of population structure, phylogenetic relationships, species tree inference, and demographic history, this study found support for three monophyletic groups (or "clades") distributed across biogeographic regions corresponding to *C. stegaria* and two clades within *C. aberti*. In addition to providing more resolution into relationships at the species level, this study identified clear intraspecific population structure that will be important for identifying management units and informing conservation actions in *C. aberti* (Kim & Roe, 2021).

Figure 1. Fanshell mussel (*Cyprogenia stegaria*) in the Ohio River near Williamstown, West Virginia, USA. Photo: Janet Butler, USFWS.

At the intraspecific level, delineating subspecies and DPSs can be even more complicated than species-level assessments. While subspecies are generally defined based on both geographic and phenotypic differentiation (Mayr, 1997), the parameters and thresholds used to delimit subspecies vary by taxonomic group. This is reflected in variable listing unit counts (i.e., numbers of species vs. subspecies) by taxon under the ESA (Haig et al., 2006). This variation in subspecies definitions across taxonomic groups has led to a history of contentious listing decisions for subspecies, with some of the most well-known cases involving differences in the interpretation of genetic data, such as the threatened Preble's jumping mouse (*Zapus hudsonius preblei*; King et al., 2006; Ramey et al., 2005) and California gnatcatcher (*Polioptila californica californica*; McCormack & Maley, 2015). As with species delineations, genomic data can inform the designation of intraspecific units through increased resolution into neutral processes and/or insights into adaptive differentiation. In the case of the endangered southwestern willow flycatcher (*Empidonax traillii extimus*), genomic data have provided additional lines of evidence to support the disputed subspecies delineation. Debate over the taxonomic status of this desert passerine has been based on geographic variation across the willow flycatcher complex in plumage, song, ecological settings, and a small mtDNA dataset (Mahoney et al., 2020; Theimer et al., 2016; Zink, 2015). A 2018 genomics study expanded both within-species sampling and genomic resolution, using 105,000 SNPs genotyped across four willow flycatcher subspecies (Ruegg et al., 2018). By analyzing SNPs distributed across the willow flycatcher nuclear genome, this study identified candidate markers related to adaptive differentiation across the species complex, including local adaptation of the southwestern subspecies to high breeding season temperatures. This genomic evidence of the ecological distinctiveness of the southwestern subspecies was further supported by follow-up ecological genomics work identifying the geographically restricted wintering grounds of the subspecies in Costa Rica and Nicaragua, as well as a highly constrained ecological niche relative to the other three willow flycatcher subspecies (Ruegg et al., 2021). Together these genomic studies have not only provided additional evidence of subspecies-level differentiation, but also identified the high level of niche specialization across the southwestern subspecies' breeding and wintering grounds and its vulnerability to future climate change, highlighting the need for restoration actions to enhance thermal refuges across the subspecies range.

The other intraspecific listable entity under the ESA is the policy based DPS (and its biological analog, the evolutionarily significant unit, or ESU, used by NMFS). A vertebrate population is considered a DPS if it meets the conditions of "discreteness" (substantial reproductive isolation) and "significance" (substantial contribution to evolutionary legacy and potential for persistence; USFWS & NMFS, 1996; Waples, 2006). The purpose of the DPS unit is to "…protect and conserve species and the ecosystems upon which they depend before large-scale decline occurs that would necessitate listing a species or subspecies throughout its entire range" (USFWS & NMFS, 1996). Because of insights provided by genomic data into both neutral and adaptive components of genetic variation, these data can inform both aspects of the DPS definition: discreteness and significance. For example, the USFWS recently proposed listing of four of six DPSs of the foothill yellow-legged frog (*Rana boylii*), two as threatened and two as endangered (USFWS, 2022a). The identification, evaluation, and status designation of these DPSs relied heavily on information from two population genomic studies (McCartney-Melstad et al., 2018; Peek, 2018). These studies built off of a previous genetic analysis using mtDNA and a single nuclear marker that was unable to clearly delineate population structure across the species range (Lind et al., 2011). By contrast, the genomic studies, using between ~25,000 and 45,000 SNPs, together identified the six distinct, biogeographic clades that formed the basis of the DPS discreteness analysis (USFWS, 2022a). These studies also provided evidence for significance due to marked differences in genetic variation across DPSs, contributing to overall adaptive capacity at the species level. Additionally, two DPSs were identified as unique in terms of adaptive potential due to a history of admixture (USFWS, 2022a), illustrating how genomic data can contribute to our understanding of spatial patterns of adaptive differentiation and evolutionary legacy.

In another example of the power of genomic data to inform and advance our understanding of adaptive differentiation, a series of recent genomic studies have uncovered an unexpectedly simple genetic basis for run timing in Chinook salmon (*Oncorhynchus tshawytscha*) and steelhead (*O. mykiss*), presenting an unprecedented challenge to how listable entities are delineated under the ESA (Waples & Lindley 2018; Waples et al., 2022). Run timing is an important phenotype in Pacific salmon that describes differences in freshwater entry times for adult migration from the ocean to spawning grounds, varying within and across species. Run timing has generally not been considered a feature that defines separate ESUs, although conserving diversity in run timing life history types within ESUs is a priority in Pacific salmonid conservation. This is due to previous genetic research that showed minimal genetic differentiation between early and late run life history types within basins (Waples et al., 2004; Kinziger et al., 2013; Arciniega et al., 2016) consistent with the quantitative genetic paradigm of many genes of small effect underlying the trait. This research had also suggested that early run timing evolved multiple times and was therefore a phenotype that could re-emerge from late run populations if it was lost, given the availability of appropriate spawning habitats. Beginning in 2016, a series of genomic-scale studies identified and confirmed the presence of a single genomic region associated with two genes, GREB1L and ROCK1, that are strongly associated with run timing in multiple populations of Chinook and steelhead (comprehensively reviewed in Waples et al., 2022). This research has established that the early run phenotype evolved only once in each species' evolutionary history and has spread through migration, rather than independent evolution (Prince et al., 2017; Thompson et al., 2019). This implies that the loss of the early run phenotype within a basin could result in extirpation of the variant conferring early run timing, which would likely be irreversible over ecological time scales without gene flow. This is important because early run populations of Pacific salmon have faced significantly higher levels of habitat loss, population declines, and extirpations compared to the fall run phenotype (Gustafson et al., 2007; Langin, 2018). These findings prompted multiple petitions to list early run populations of Chinook and steelhead under the ESA, arguing that these new genomic findings necessitated revision to the ESU delineation and/or listing status (reviewed in Waples et al., 2022). To date, three of the four petitions have been found not warranted, indicating that listing early run populations as separate ESUs is not appropriate due to shared genome-wide variation and lack of reproductive isolation between early and late run fish (NMFS, 2020a, 2021). These findings specifically reiterate, however, the importance of conserving and maintaining life history diversity within ESUs, including recovery actions dedicated to restoring and improving spawning habitat for the early run phenotype. This reflects part of a broader, long-standing approach to conserving intraspecific diversity and evolutionary legacy within and across Pacific salmonids (McElhany et al., 2000).

Finally, the transition from genetics to genomics will not always provide clear direction for taxonomic delineations when evolutionary histories are complex. For example, the red wolf (*Canis rufus*), was among the first species given federal protection under the precursor to the ESA, the Endangered Species Preservation Act of 1966. Since its listing as endangered in 1967, it has been the subject of many genetic and genomic studies using data ranging from mtDNA to the sequencing of multiple whole genomes, yet the taxonomy of the species remains a subject of contention (reviewed in Waples et al., 2018). A complicating factor is recent hybridization between red wolves and coyotes, which has impeded the evaluation of evolutionary history among North American canids in general and has led to multiple, conflicting hypotheses about the status of red wolves. A Congressionally mandated review of these taxonomic issues by an independent committee, initiated by the USFWS in 2018, upheld the validity of the species status for red wolves based on currently available data (Committee on Assessing the Taxonomic Status of the Red Wolf and the Mexican Gray Wolf, 2019). Interestingly, no matter which of the many hypotheses of red wolf evolutionary history is considered, it is likely that red wolves would remain a listable entity due to the ESA's broad definition of "species" and its flexibility in defining listable units at the species and subspecific levels (Waples et al., 2018).

These examples illustrate how genetic and genomic data will continue to play a critical role in informing the delineation of listable entities under the ESA, while not always providing the clear resolution needed to make decisions with complete certainty. In many cases, taxonomy and classification status under the ESA will be best addressed using an integrative taxonomic approach that includes analysis of genetic/genomic, morphological, behavioral, and ecological data (Coates et al., 2018; Smith & Carstens, 2022).

Assessing current conditions and relationships to past change

The next step in the listing process is to review the species' ecological needs and current status, including its current condition (i.e., the species' abundance, population trends, spatial structure, and diversity), and relationship to past and ongoing changes in abundance and distribution. The past provides a valuable reference point for establishing conditions under which the species was known to be viable (i.e., the "historical template" described by Waples et al., 2007). A historical range of variability for a species' distribution, abundance, and range of genetic, behavioral, phenotypic, and life-history traits provides a point of comparison for assessing a species' viability under current conditions (Waples et al., 2007). The likelihood of current viability becomes more uncertain under conditions that deviate significantly from the historical template, and more unlikely under conditions which have not historically supported self-sustaining, viable populations. Conversely, species may not be at significant near-term risk of extinction where current conditions roughly approximate historical conditions and fall within an expected historical range of variability (Waples et al., 2007). The historical template also provides context for evaluating potential changes in a species' adaptive capacity, the ability to accommodate, cope with, and respond to ongoing environmental change and novel stressors. Maintaining adaptive capacity requires conservation across the phenotypic, genetic, and environmental diversity of a species range, as well as maintenance of the evolutionary processes that drive adaptive change, such as gene flow and selection (Forester et al., 2022; Moritz, 2002). In evaluations under the ESA, this link between historical and current conditions has often been established based on patterns and changes in species distribution and abundance, though genetic and genomic analyses are increasingly playing a role.

For example, in the species status assessment of the threatened eastern massasauga rattlesnake (*Sistrurus catenatus*, EMR, Fig. 2), percent reduction in occurrence relative to historical conditions was central to describing the species' current resiliency to environmental and demographic stochasticity (USFWS, 2016b). The assessment also evaluated changes in adaptive capacity relative to historical conditions based on reduced occupancy across three representative units reflective of regional patterns of genetic diversity (defined using mtDNA; Ray et al., 2013). Adaptive capacity within units was evaluated based on fine-scale assessments of genetic diversity (using microsatellite data; Gibbs et al., 1997; Chiucchi & Gibbs 2010), and differences in behavior, life-history traits, and genetically-based variation in venom composition (USFWS, 2016b). These genetic studies were essential to deciphering the roles of isolation, small population sizes, low connectivity, and limited dispersal in shaping current patterns of population structure across the range. For many species, resiliency to environmental and demographic stochasticity requires connectivity among populations to facilitate gene flow and demographic rescue. However, the microsatellite-based study found evidence that limited dispersal, even among geographically close populations, is likely a long-standing biological characteristic of EMR, and that small population sizes are within the historical range of variability (Chiucchi & Gibbs, 2010). Unfortunately, a 38% reduction in the number of populations between historical and current time periods, in large part due to habitat loss, fragmentation, and modification, means that overall resiliency of these small populations had declined when compared to historical conditions (USFWS, 2016b). Additionally, differential losses across the three representative units were cause for concern, since two of the three units had large population declines (48% and 72%) implying that species-wide adaptive capacity was also at risk (USFWS, 2016b). Since the status assessment, a study using genome-wide SNPs in EMR identified recent demographic declines consistent with anthropogenic impacts that had not been detected in the previous genetic research (Sovic et al., 2019), providing additional evidence that ongoing stressors and threats are negatively affecting extant EMR populations.

Figure 2. Eastern massasauga rattlesnake (*Sistrurus catenatus*), Parry Sound District, Ontario, Canada. Photo: Nick Cairns.

Another recent genomic study in EMR illustrates the capacity for genomic data to not only provide greater resolution into historical and current demographic processes, but also transform how we understand and use important conservation genetic parameters such as inbreeding. Despite evidence for historically small population sizes and low connectivity (discussed above), previous genetic research had not identified signatures of inbreeding in EMR populations. A 2021 study tackled this apparent mystery by sequencing the entire genomes of 90 EMR individuals across nine populations and evaluating inbreeding using a genomic approach that identifies runs of homozygosity (ROH; Ochoa & Gibbs 2021). ROH are continuously homozygous regions of the genome which are identical by descent, meaning that both copies of the allele originated from a single copy in a common ancestor (Ceballos et al., 2018; Kardos et al., 2016; Keller et al., 2011). ROH occur when there is breeding among close relatives (inbreeding), which can lead to an increase in the frequency and expression of deleterious recessive alleles. This is particularly concerning in threatened and endangered species with small, isolated populations because it contributes to reduced fitness (inbreeding depression) and an increased probability of extinction. In the case of EMR, the genomic-scale analysis of ROH identified patterns consistent with increased inbreeding due to both historical and recent bottlenecks (Ochoa & Gibbs, 2021), illustrating the advantage of this approach over less accurate measures of inbreeding derived from population genetic data (Kardos et al., 2015). In addition to documenting the severity and magnitude of inbreeding in EMR, this analysis provided information on differential inbreeding levels across populations that will help ensure more successful conservation interventions, such as genetic rescue, in the future. Although uptake into ESA evaluations has been slow, genomic assessments of parameters such as population connectivity, demographic history, and inbreeding are becoming more common in the academic literature. Incorporation of these analyses in species assessments under the ESA are likely to follow as these tools are increasingly applied in species of conservation concern, such as the EMR.

Assessing response to future conditions

The Services are required to use the best available science to assess the risk that a species is in danger of extinction or likely to become so in the foreseeable future. Building off of the evaluation of a species' historical and current conditions, including the species' response to past and ongoing stressors, ESA assessments proceed with an evaluation of the future conditions of the species. These assessments estimate the viability of the species under a range of future scenarios that incorporate ongoing and future threats, stressors, and conservation actions, and use the species' response to past events as a template. When genetic and genomic data are available to evaluate historical and current conditions, simulations can be used to predict how genetic diversity will be impacted by ongoing threats, such as habitat loss and population isolation. This approach was used in a genomic study of EMR, where current populations were predicted to lose between 63% and 99% of their current neutral genetic variation over 100 years (Sovic et al., 2019). This suggests that historical and current trends of increasing habitat loss, population isolation, and population extirpation have imposed a genetic debt on EMR populations that will be realized in increased extinction risk in the absence of conservation intervention (Sovic et al., 2019).

While that study focused on neutral genetic variation, characterizing adaptive genetic variation and its potential to facilitate evolutionary responses to ongoing and future threats has become a topic of increasing attention in conservation genomics. In short, species will be better equipped to respond to current unprecedented rates of global environmental change if they retain the adaptive capacity to accommodate, cope with, and/or respond to changing conditions (Foden et al., 2019; Thurman et al., 2020). Rapid evolution in response to environmental change relies on the availability of standing genetic variation, or genetic differences among populations and among individuals within populations. For example, in an experimental study of a Mediterranean mussel (*Mytilus galloprovincialis*), standing genetic variation facilitated rapid adaptive responses to ocean acidification, highlighting the importance of maintaining genetic variation in wild populations (Bitter et al., 2019). Standing genetic variation provides the substrate of a species' evolutionary potential, defined as the capacity to evolve genetically based changes in traits that increase population-level fitness in response to novel or changing environmental conditions (Forester et al., 2022). Importantly, both neutral and adaptive genetic variation are essential to maintaining evolutionary potential since current adaptations may not always be adaptive under future conditions (Kardos et al., 2021; Kardos & Shafer, 2018; Moritz, 2002).

Evolutionary potential and the adaptive genetic variation underlying it are challenging to quantify, especially in species of conservation concern, necessitating the use of proxies (reviewed in Forester et al., 2022). ESA decision-making relies on many of these proxies to serve as indicators of evolutionary potential, such as phenotypic diversity, neutral genetic diversity, ecological diversity, and measures of candidate adaptive variation derived from genomic data (Forester et al., 2022). For example, the status assessment for the rusty patched bumblebee *(Bombus affinis)* addressed the species' adaptive capacity using ecological diversity as a proxy for evolutionary potential. The assessment described a decline in occupancy from historical to current conditions, with further declines projected under three plausible future risk scenarios (USFWS, 2016a). This deteriorating trend in occupancy and ecological representation was interpreted as a reduction in the bumblebee's evolutionary potential and informed the listing of the species as endangered (USFWS, 2017). Revisiting the EMR case, the evaluation of adaptive capacity across representative units relied in part on within-species variation in the molecular composition of venom. Among EMR populations, genes that control venom composition have evolved rapidly to effectively subdue locally available prey (Ochoa et al., 2020; USFWS, 2016b). Thus, the species assessment concluded that future evolutionary potential would be best conserved by ensuring the maintenance of multiple self-sustaining populations encompassing the full suite of phenotypic, genetic, and ecological diversity, including variation in venom composition.

Emergent wildlife diseases such as white-nose syndrome present a compelling case for the consideration of evolutionary potential when evaluating the future condition of at-risk species. White-nose syndrome is a disease caused by a fungal pathogen *(Pseudogymnoascus destructans)* that has rapidly reduced northern long-eared *(Myotis septentrionalis)*, little brown *(M. lucifugus)*, and tricolored *(Perimyotis subflavus)* bat populations by more than 90% over the last decade (Cheng et al., 2021). The tricolored bat and little brown bat are currently under review for ESA protection (as of March 2022) and an up-listing petition is under consideration for the threatened long-eared bat due to impacts of white-nose syndrome (USFWS, 2022b). Recently, two studies of white-nose syndrome in little brown bats have identified adaptive variation by comparing genomic data collected from survivors and non-survivors of the disease (Auteri & Knowles, 2020; Gignoux-Wolfsohn et al., 2021). Both studies, carried out in different parts of the species' range, identified adaptive genetic variants despite strong selective pressure imposed by the disease and the substantial impacts of population bottlenecks and resultant genetic drift. Adaptive variants were associated with genes related to hibernation, immunity, and fat metabolism that likely contribute to individual survival. Despite common pathways, candidate genes were unique to each study. This suggests that there may be within-species variation among evolutionary responses to white-nose syndrome and reemphasizes the importance of conserving genome-wide genetic variation to maximize evolutionary potential (Kardos et al., 2021).

Environmental stochasticity driven by global climate change will require a greater focus on evolutionary potential and the study of adaptive variation in forecasting species responses. However, given the aforementioned challenges in identifying adaptive variation, attempts to predict evolutionary responses to climate change for species evaluated under the ESA have been mostly qualitative in nature. For example, the recent species status assessment for southern white-tailed ptarmigan (*Lagopus leucura altipetens*, Fig. 3; USFWS, 2020) incorporated results from two genomic studies that spanned the white-tailed ptarmigan distribution, identifying signatures of adaptive differentiation both within southern white-tailed ptarmigan and across the species complex (Langin et al., 2018; Zimmerman et al., 2021). In the southern subspecies, two of the three southern white-tailed ptarmigan populations (southern Colorado and New Mexico) showed unique signatures of local adaptation to warmer summer temperatures and higher summer precipitation characteristic of climatic conditions in the southern part of the range. A future climate scenario reflecting very hot and dry conditions found that the New Mexico population was likely to be extirpated, reducing species-wide adaptive capacity to warming and drying conditions. Adaptive differentiation of the southern subspecies relative to the remainder of the species range was also identified, including potential dietary specialization, adaptation to high elevation and/or low latitude, and differences in seasonal plumage change (Zimmerman et al., 2021). These adaptive differences may both limit the capacity of southern populations to shift their range in response to climate change, as well as provide potential sources of adaptive genetic variation (i.e., heat tolerance) for northern populations (Zimmerman et al., 2021).

Figure 3. Southern white-tailed ptarmigan (*Lagopus leucura altipetens*) in summer plumage, Colorado, USA. Photo: Peter Plage / USFWS.

One path toward better integration of adaptive genomics into ESA listing is to facilitate full consideration of proxies for evolutionary potential into quantitative models of extinction risk (Forester et al., 2022). A recent study used a genetic simulation approach to forecast the role of evolutionary potential in mitigating extinction risk in the coral *Acropora hyacinthus* (Bay et al., 2017). This study integrated demographic parameters and adaptive genetic variation linked to thermal tolerance to forecast the extinction risk of a single *Acropora hyacinthus* coral population under a suite of climate change scenarios. Under a low-emissions scenario, the population possessed sufficient evolutionary potential to track changing conditions. However, under higher-emissions scenarios, the population was extirpated due to an insufficient evolutionary response. Translocating "pre-adapted" corals tolerant of warmer conditions promoted a quicker and more robust evolutionary response, mitigating the risk of extirpation under high-emissions scenarios (Bay et al., 2017). This case is an advanced application of ecological genomics, even by academic standards. However, it demonstrates the leading edge of what can be gained by querying adaptive variation across the genome. Assessments of extinction risk informed by evolutionary potential present an exciting frontier for molecular ecologists and a welcome improvement for managers and policymakers required to make categorical decisions about species' long-term viability. While challenges persist in the integration of genetic and genomic data into listing decision-making, the exceptional insights provided by these data into taxonomy and assessments of the historical, current, and future species condition will continue to motivate conservation genetics research and its practical applications.

THE ROLE OF GENETICS AND GENOMICS IN THE ESA: RECOVERY

Once a species is listed under the ESA, the Services work with partners to develop and implement a recovery plan with the goal of restoring and securing viable, self-sustaining wild populations. Recovery plans must include, at minimum, a description of site-specific actions necessary for recovery, objective, measurable recovery criteria, and estimates of the time and costs to meet the recovery goals (U.S. Endangered Species Act of 1973, as Amended, 1973). Genetic and genomic data can play a key role in this process by providing information to delineate recovery units, develop recovery criteria, identify recovery actions, and facilitate monitoring. Historically, the use of genetics in recovery planning has been limited. For example, a review of 181 recovery plans for listed animals spanning 1977-1998 found that genetic research was included in 41% of recovery plans, yet few specified how the collected data would be analyzed, or how they would inform the recovery plan or recovery actions (Moyle et al., 2003). However, technical innovations, reduced costs, and better integration of academic and manager collaboration (Taft et al., 2020) have facilitated the advancement of genetic and genomic methods in recovery planning. For example, a more recent evaluation of 100 recovery plans under the ESA dated 1997-2012 found that genetic factors were considered in risk assessments in 63% of plans, that 46% of plans already included some form of genetic information, and that 82% of plans included collection of (additional) genetic data to inform recovery goals (J. C. Pierson et al., 2016). Below, we review how genetic and genomic data are informing different aspects of recovery planning, providing examples of the effective use of these methods in the delineation of recovery units, development of recovery criteria, identification of recovery actions, and facilitation of monitoring.

Developing recovery units

Recovery units are an optional component of recovery planning under the ESA and are defined as population units that are identifiable (e.g., geographically and/or genetically) and are essential to the recovery of the listed entity (NMFS, 2020b). Recovery units are particularly useful for recovery planning in species with wide ranges, multiple populations, and/or a distribution that spans ecological settings, since these species will often require management of gene flow, metapopulation dynamics, and/or maintenance of adaptive diversity. In the same way that genetic and genomic data can inform intraspecific classification units (see *Determining the entity for assessment*), these data can be a critical component of identifying biologically relevant recovery units that improve recovery outcomes.

For example, the threatened Mojave Desert tortoise (*Gopherus agassizii*, Fig. 4) is a long-lived species occurring across the southwestern U.S. Its widespread distribution encompasses substantial intraspecific variation in ecological settings, genetic variation, behavior, and morphology. In the initial recovery plan, the USFWS delineated six recovery units spanning the entire species range in an effort to conserve "…important components of the evolutionary legacy of *Gopherus agassizii*" (USFWS, 1994). The recovery status of each unit is evaluated separately based on established recovery criteria and the effectiveness of recovery actions (USFWS, 2011). Genetic data have informed the delineation of recovery units since the initial recovery plan and subsequent revision (USFWS, 1994, 2011), including the use of mtDNA (Britten et al., 1997; Lamb et al., 1989; Rainboth et al., 1989) and microsatellites (Britten et al., 1997; Hagerty & Tracy, 2010; Murphy et al., 2007). These genetic studies, in addition to data on available habitat and environmental variation, supported a change in the number of recovery units in the 2011 recovery plan revision from six to five based on range-wide sampling. Interestingly, these studies differed in their recommendations for the largest recovery unit, with one finding no evidence of substructure (Hagerty & Tracy, 2010), while the other identified three within-unit groups (Murphy et al., 2007). A more recent genomic study using almost 7,000 SNPs supported this latter finding of substructure (Sánchez-Ramírez et al., 2018). Part of the reason for these discrepancies is the range wide pattern of isolation by distance in Mojave Desert tortoises, a spatial pattern where geographically close populations are more genetically similar than geographically distant populations. This continuous genetic differentiation can be difficult to partition into discrete groups, illustrating how genetic information can sometimes be inconclusive on its own. In the case of the Mojave Desert tortoise, the 2011 recovery plan used a holistic approach and retained larger recovery unit groupings based on genetic information, geographic barriers, available habitat, and environmental variability, while recognizing the importance of maintaining genetic variability within units (USFWS, 2011).

Figure 4. Mojave Desert tortoise (*Gopherus agassizii*) live in saltbush and Mojave Desert scrub habitats. Photo: Roy Averill-Murray, USFWS.

Identifying recovery criteria

All recovery plans for threatened and endangered species must identify objective, measurable recovery criteria that signify when recovery has been reached (NMFS, 2020b), a task that is challenging in general (Doak et al., 2015; Tear et al., 2005), even before considering the complexities of genetic-based indicators (Pierson et al., 2015). Effective population size is one of the more frequently used genetic indicators for recovery, likely because of the large literature on effective size thresholds for minimizing inbreeding and maximizing evolutionary potential (i.e., the 50/500 or 100/1000 "rules"; Frankham et al., 2014; Franklin, 1980; Mace & Lande, 1991). Effective population size is a genetically based measure of the size of an "ideal" population (e.g., random mating, no migration) that experiences the same amount of genetic drift as the focal population. It is typically smaller than the census, or observed, population size.

For example, the 2003 recovery plan for the threatened southern sea otter (*Enhydra lutris nereis*) bases delisting criteria on a census population size of 3,090 individuals (using a 3-year running average of census data), a threshold calculated to maintain an effective population size of 500 after a catastrophic event such as a major oil spill (Ralls et al., 1996; USFWS, 2003). Using a theoretically derived ratio of effective to census population size of 27% (Ralls et al., 1983) an effective size of 500 corresponds to a census population size of 1,850, with an additional 1,240 individuals included based on the expected mortality from a catastrophic spill (USFWS, 2003). Notably, the recovery plan specifically recommends reassessment of the effective size threshold if new data are made available. Illustrating the complexity of setting genetic thresholds in complex natural systems, a recent analysis combining 13 years of demographic and genetic data from over 1,000 southern sea otters found that genetic estimates of effective population size were much lower than demographic estimates, likely due to the impact of subtle population structure (isolation by distance) across the range in central and southern California (Gagne et al., 2018). Based on these results, including an inability to provide a working estimate of the ratio of effective to census population size, these authors recommend that the current delisting criteria be reevaluated, removing the reliance on effective size as the central criterion and expanding the analytical framework to incorporate both demographic and genetic factors (Gagne et al., 2018).

By contrast, the endangered Columbia Basin pygmy rabbit (*Brachylagus idahoensis*, Fig. 5) is a good candidate for the use of effective size recovery criteria, given its highly restricted geographic distribution and the discrete locations of recovery sites (i.e., minimal risk of cryptic population structure or isolation by distance). The current recovery plan uses multiple criteria for down-listing based on 5-year average effective sizes measured separately in different recovery units, with total sizes ranging from 500-750 individuals (USFWS, 2012). In another case, the recovery plan for the southern Oregon/northern California coast ESU of coho salmon (*Oncorhynchus kisutch*) combines effective size estimates as a lower bound for recovery in combination with other factors impacting species viability (NMFS, 2014; Williams, 2008). This integrative approach provides an illustration of how important genetic parameters reflecting population fitness and viability, such as effective population size, can be incorporated into recovery criteria even in species with complex spatial structure.

Figure 5. Columbia Basin pygmy rabbit (*Brachylagus idahoensis*). Photo: R. Dixon and H. Ulmschneider.

Identifying recovery actions

In addition to recovery criteria, all recovery plans for listed species must specify site-specific recovery actions that will alleviate threats and restore species viability (NMFS, 2020b). Some of the best known genetically based recovery actions for threatened and endangered species relate to captive breeding (Witzenberger & Hochkirch, 2011) and genetic rescue (Fitzpatrick & Funk, 2020; Whiteley et al., 2015). For example, a carefully planned captive breeding and intercross program in endangered Columbia Basin pygmy rabbits has played a major role in improving fitness while maintaining majority founder ancestry, despite highly consequential inbreeding depression in the species at the time of its emergency listing in 2001 (USFWS, 2012; WDFW, 2020). In the threatened endemic freshwater mussel Louisiana pearlshell (*Margaritifera hembeli*), recent genomic research has provided critical data to inform recovery actions related to captive production and reintroduction plans (Sikes, 2020; USFWS, 2019). Genomic analysis of captively reared Louisiana pearlshell offspring from a single gravid female recovered genetic diversity representative of the wild population, indicating multiple paternity, which was not previously known in this species (Garrison et al., 2021). This finding created an opportunity to produce genetically diverse captive bred cohorts for ongoing reintroduction efforts. The analysis also greatly improved resolution of population structure over two previous genetic studies, identifying a major river as a genetic break point and suggesting movement of host fish across minor drainages during flood events (Garrison et al., 2021). This improved understanding of wild population structure is being used to direct the reintroduction of captive bred cohorts, such as ensuring introduction sites are geographically proximate to where gravid females were sampled (Garrison et al., 2021; Sikes, 2020).

Perhaps the best-known example of genetically informed recovery action for an ESA listed species is the genetic rescue of the endangered Florida panther (*Puma concolor coryi*). Though listed under the ESA in 1967, Florida panthers continued to decline to an estimated low of 20-30 individuals exhibiting severe signs of inbreeding depression (Roelke et al., 1993). In 1995, eight female pumas (*P. c. stanleyana*) were translocated from Texas to Florida. Genetic, demographic, physiological, and morphological data indicate that the translocations were successful in reducing the negative effects of inbreeding and restoring fitness metrics, including positive population growth rates (Hostetler et al., 2013; Johnson et al., 2010). Because the Florida panther continues to be isolated from gene flow with other populations due to development and habitat fragmentation, the continued genetic health and persistence of the species will require additional human-mediated translocations to avoid subsequent declines. Recognizing this, the 2008 Florida panther recovery plan included among the recovery actions an integrative demographic and genetic model for use as a decision tool in genetic management of the species (USFWS, 2008). This model was published in 2019 and found that, without additional translocations, the probability of quasi-extinction of the Florida panther within 100 years was 17% when inbreeding and drift impacts were included in forecasts of population viability (van de Kerk et al., 2019). The authors were able to test the impact of alternative translocation scenarios, including varying the number of translocated animals and the interval between releases, and determined that translocating five females every 20 years would be sufficient to substantially reduce the extinction risk of the species (van de Kerk et al., 2019). In listed species where genetic and genomic data are available, it is likely that these types of integrative demographic and genetic simulation models will increasingly be used to plan recovery efforts that maximize species viability (Forester et al., 2022).

Finally, the transition from genetic to genomic data provides an opportunity to implement novel recovery actions to improve the adaptive capacity of threatened and endangered species. For example, the recovery plan for two threatened coral species, Elkhorn (*Acropora palmata*) and staghorn coral (*A. cervicornis*, Fig. 6), includes recovery actions related to increasing genotypic diversity in genetically depauperate populations and enhancing evolutionary potential in response to climate change related stressors, such as ocean warming and acidification (NMFS, 2015). These population enhancement actions include detailed guidelines for protecting coral health and genetic integrity, for the design of coral nurseries, coral collection, crossing, culturing, and out-planting, and best practices for monitoring and risk management (NMFS, 2016). Taking advantage of over a decade of visionary work by coral biologists and molecular ecologists (Baums 2008; van Oppen et al., 2015, 2017), recovery plans such as this are on the leading edge of genomic research to identify adaptive genetic variation (Devlin-Durante & Baums, 2017), map its distribution across seascapes (Selmoni et al., 2020), and apply it to restoration efforts (Quigley et al., 2020). These well-planned and carefully monitored efforts to improve the adaptive capacity of threatened and endangered species serve as outstanding examples that harness the power of genomics to inform recovery.

Figure 6. Staghorn coral (*Acropora cervicornis*) and chromis reef fish at Palmyra Atoll National Wildlife Refuge, equatorial Pacific. Photo: Amanda Meyer, USFWS.

Monitoring recovery

Well-planned monitoring is essential for recovery because it allows for the evaluation of recovery actions and for their modification, if needed, to meet recovery goals. The monitoring of genetic parameters, such as effective population size, genetic diversity, gene flow, and the distribution and frequency of adaptive alleles, is certain to become more common in recovery planning as genetic and genomic data increasingly inform listing and recovery decisions (Flanagan et al., 2018; Schwartz et al., 2007). One of many advantages of genetic monitoring is that it can often be accomplished using non-invasive methods. For example, monitoring of the endangered Columbia Basin pygmy rabbit through DNA extracted from fecal pellets has provided demographic and genetic information on the efficacy of reintroduction efforts including dispersal distances, survival, reproductive success, and genetic diversity of different cohorts (e.g., released vs. wild individuals; DeMay et al., 2017; USFWS, 2012). The ability of the pygmy rabbit monitoring program to collect large amounts of data over time without capturing or handling rabbits has been particularly valuable for evaluating reintroduction success and modifying future actions to meet recovery plan goals (DeMay et al., 2017). Another non-invasive genetic monitoring tool increasingly used in recovery monitoring of listed species is environmental DNA (eDNA), or the identification of DNA shed by organisms in the environment (on land or water) that is used as a proxy for the presence of the species (Bohmann et al., 2014). For example, eDNA methods have been developed to monitor diverse ESA-listed species including: eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*; Spear et al., 2015; Wineland et al., 2019) bog turtles (*Glyptemys muhlenbergii*; Kirtane et al., 2019) fairy shrimp (genus *Branchinecta*; Gold et al., 2020) and bull trout *(Salvelinus confluentus*; Dysthe et al., 2018). This method is also useful in monitoring for invasive species, such as brook and rainbow trout, whose presence threatens listed species (Wilcox et al., 2015; Sepulveda et al., 2019).

Recovery of threatened and endangered species is often a slow and iterative process in which progress is accomplished first by halting the species' decline through the removal or reduction of threats, then through stabilization of population trends, and finally by increasing the species' abundance and diversity with the ultimate goal of securing self-sustaining populations in the wild. Once this goal has been met, the species may be considered for delisting from the ESA in a process much like a status assessment undertaken by the Services for the initial listing. The ESA requires post-delisting monitoring of any delisted species for at least five years to ensure the species is able to remain viable and self-sustaining without ESA protections. As discussed above, genetic and genomic data can play an essential role in this process. However, because of the diversity of species considered under the ESA, there will never be a single, uniform approach to incorporating genetic and genomic data into listing and recovery decision-making. In many cases, genetic and genomic inferences will be most valuable when integrated with complementary demographic, morphological, behavioral, or ecological data. Most of all, increased collaboration and partnerships among molecular ecologists and conservation practitioners will be essential to realizing the potential of genetic and genomic data in informing species conservation.

FRONTIERS IN CONSERVATION GENOMICS & THE NEXT FIFTY YEARS

Advances in genome-sequencing technologies and the plummeting costs of employing them will continue to drive innovations in conservation genomics, granting new opportunities to sequence, screen, and even edit genetic material (Segelbacher et al., 2022). As a first step, efforts driven by scientific consortia such as the Vertebrate Genomes Project (Rhie et al., 2021) are producing reference genome assemblies for at-risk species across the taxonomic spectrum. A reference genome is a single, highly accurate and contiguous sequence of a species' genome, which serves as a shared public resource for conservation genomics research. The sequence and associated metadata (such as annotated gene regions) can then be used much like a map to identify areas of interest, query specific genes, and guide the analysis of large-scale population-level data from additional individuals. Access to a suitable reference genome (i.e., of the target organism or a closely related species) presents the first hurdle in applying some of the aforementioned conservation genomics analyses, such as runs of homozygosity, and is certainly a prerequisite for more experimental methods (e.g., genetic engineering). At present, reference genomes have been compiled for less than one percent of species listed as threatened by the International Union for Conservation of Nature (Brandies et al., 2019).

Ex situ conservation programs have historically served as a hub for conservation genetics innovation, as evidenced by the early publication of the giant panda *(Ailuropoda melanoleuca)* reference genome (Li et al., 2010), one of the first among non-model species. Albeit largely experimental, technological frontiers such as the use of biobanked genetic material and genetic engineering have principally been explored within *ex situ* programs. For example, cryopreserved gametes have been central to genetic rescue efforts in endangered black-footed ferrets (*Mustela nigripes*, Fig. 7), reducing inbreeding and restoring genome-wide variation (Howard et al., 2016). This approach has also been used to increase genetic diversity and improve thermal tolerance in threatened populations of Elkhorn coral (Hagedorn et al., 2021). Cloning, which uses a technology called somatic cell nuclear transfer coding, offers the possibility of introducing previously "lost" genetic variation into surviving populations of critically endangered species. Cloning was recently used in two *ex situ* conservation programs for ESA listed species, black-footed ferret and Przewalski's horse *(Equus ferus przewalskii*; Fritts, 2022; Sandler et al., 2021). These programs rely substantially, if not completely, on collections of captive or semi-captive individuals, gametes, tissues, and most recently, viable "living" cell cultures. The most longstanding program of viable cell culture banking, known as the Frozen Zoo®, was established in 1975 at the San Diego Zoo Institute for Conservation Research and includes viable cells for more than 10,000 vertebrate animals (Ryder & Onuma, 2018). While cellular technologies provide a measure of hope for species recovery, banking efforts must be undertaken prior to, rather than at the time of, extinction. Collaboration among geneticists and scientific consortia (e.g., Vertebrate Genomes Project) and federal, state, and non-profit partners in direct management of at-risk species will be necessary to secure the biological samples required for long-term biobanking and reference genome assembly (Dahn et al., 2021).

Figure 7. Black-footed ferret (*Mustela nigripes*), National Black-Footed Ferret Conservation Center, Carr, Colorado, USA. Photo: Ryan Hagerty, USFWS.

Although *ex situ* programs were early adopters of genomics, applications in other areas of conservation practice are steadily becoming more commonplace and uptake into ESA implementations is a readily achievable frontier. Increased familiarity with genomics will motivate practical applications for species across the conservation risk spectrum. Conservation practitioners interested in integrating genomic inferences into ESA listing and recovery decisions should first seek out neutral genomic assessments of diversity, gene flow, demographic history, and inbreeding. As applied to the threatened eastern massasauga rattlesnake, for example, inbreeding assessments such as ROH derived from genomics are more precise and offer greater resolution relative to traditional genetic assessments. The improved reliability of such inferences is especially important for critically endangered species and allows for a more accurate assessment of species' current conditions. A more distant, but certainly achievable frontier is the integration of adaptive genomic inferences into ESA listing and recovery decisions. At present, geneticists can characterize candidate adaptive variation throughout the genome and, in some cases, link it to traits which impact individual fitness. As illustrated by the coral simulation study referenced earlier (Bay et al., 2017), simulations that integrate demographic and genomic information can then be used to predict species responses to ongoing and future threats such as ocean acidification and global climate change. Incorporating such findings into quantitative models of extinction risk can more accurately forecast population viability for the purposes of listing and identify actions such as assisted gene flow which facilitate increases in evolutionary potential for the purposes of recovery.

The ESA is one of the most powerful and effective conservation laws in the world (Waples et al., 2013). However, the accelerating impact of synergistic threats including habitat loss and global climate change will test its effectiveness and require radical changes to how we conduct research and approach decision-making. In this chapter, we presented various cases demonstrating how information inferred from genetics and genomics can be integrated into ESA listing and recovery decisions. The next fifty years will be defined by a continued transition from conservation genetics to large-scale genomics (e.g., whole genome inferences). Therefore, it is our recommendation that status assessment teams intentionally include conservation geneticists who can seek out and interpret genomic findings and work collaboratively with practitioners to incorporate them into ESA implementation. However, the rapid advancement of the field has exacerbated an existing research-implementation gap, and uptake by conservation practitioners has been slow. The roots of this hesitancy are multifactor and include poor communication and low familiarity with genomic approaches (Kadykalo et al., 2020; Shafer et al., 2015; Taft et al., 2020; Taylor et al., 2017). Conservation genomics is inherently multi-disciplinary, requiring expertise in ecology, population genetics, molecular biology, and bioinformatics. The future advancement of the field requires greater interdependence between conservation geneticists and conservation practitioners, and a team-science approach which offers many advantages including resource-sharing, economies of scale, and cross-disciplinary collaboration. We urge conservation geneticists and practitioners alike to build collaborative relationships which facilitate the integration of the best available science into ESA decision-making.

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