Journal of Heredity, 2023, **114**, 300–311
https://doi.org/10.1093/jhered/esad011
Advance access publication 23 February 2023
Invited Reviews and Perspectives

American Genetic Association

OXFORD

Invited Reviews and Perspectives

Where to draw the line? Expanding the delineation of conservation units to highly mobile taxa

Sheela P. Turbek, W. Chris Funk, Kristen C. Ruegg

Department of Biology, Colorado State University, Fort Collins, CO, United States

Address correspondence to S.P. Turbek at the address above, or e-mail: sturbek@colostate.edu.

Corresponding Editor: William Murphy

Abstract

Conservation units (CUs) are an essential tool for maximizing evolutionary potential and prioritizing areas across a species' range for protection when implementing conservation and management measures. However, current workflows for identifying CUs on the basis of neutral and adaptive genomic variation largely ignore information contained in patterns of isolation by distance (IBD), frequently the primary signal of population structure in highly mobile taxa, such as birds, bats, and marine organisms with pelagic larval stages. While individuals located on either end of a species' distribution may exhibit clear genetic, phenotypic, and ecological differences, IBD produces subtle changes in allele frequencies across space, making it difficult to draw clear boundaries for conservation purposes in the absence of discrete population structure. Here, we highlight potential pitfalls that arise when applying common methods for delineating CUs to continuously distributed organisms and review existing methods for detecting subtle breakpoints in patterns of IBD that can indicate barriers to gene flow in highly mobile taxa. In addition, we propose a new framework for identifying CUs in all organisms, including those characterized by continuous genomic differentiation, and suggest several possible ways to harness the information contained in patterns of IBD to guide conservation and management decisions.

Key words: adaptive divergence, conservation units, evolutionary significant units, genomics, isolation by distance

What are conservation units?

Effective protection and management of biological diversity often requires specification of conservation units (CUs), or intraspecific groupings used to guide conservation and management decisions (Allendorf et al. 2022). CUs are delineated with the goal of maximizing a species' evolutionary potential and can be used to pinpoint areas across a species' range that should be prioritized for protection given limited resources for conservation (Fraser and Bernatchez 2001). The goal of this paper is not to provide a comprehensive review of the literature on CUs, as this has already been done elsewhere (e.g. Fraser and Bernatchez 2001); instead, we offer a brief overview of the types of CUs that exist to provide context for the rest of our discussion.

While there are numerous ways to designate CUs, a major distinction can be drawn between CUs aimed at categorizing biological differences among populations and those designated by federal agencies to implement policy. For the purposes of this paper, we focus on biological entities, rather than their analogous policy units, when discussing how to split taxa into discrete CUs. The designation of biologically meaningful CUs is an essential step in recovery planning for threatened species (Allendorf et al. 2022), as it facilitates the application of laws aimed at conserving the genetic variation needed for local adaptation (Waples 1991) and population persistence in the face of environmental change (Davis et al. 2005; Parmesan 2006).

Evolutionary significant units (ESUs), generally defined as populations or groups of populations characterized by high genetic and ecological distinctiveness (Allendorf and Luikart 2007; Funk et al. 2012), are one of the most recognized CUs. While exact definitions vary (Ryder 1986; Waples 1991; Moritz 1994; Crandall et al. 2000), ESUs represent an important component of the evolutionary history of a species and are biological entities that can be used to focus conservation efforts below the species level. Distinct population segments and designatable units, policy units analogous to ESUs, are designated by federal agencies and granted legal protection under the U.S. Endangered Species Act and Canadian Species at Risk Act, respectively (Green 2005; Coates et al. 2018).

Adaptive units (AUs), or groups characterized by differentiation at adaptive loci, are sometimes designated within ESUs and can provide additional resolution when identifying sources of unique genetic diversity (Funk et al. 2012; Barbosa et al. 2018). Finally, management units (MUs) refer to demographically independent populations whose population dynamics primarily depend on local birth and death rates as opposed to immigration (Palsbøll et al. 2007). While ESUs are used to inform larger scale range-wide conservation planning, MUs guide smaller scale management decisions, such as setting harvesting quotas, monitoring populations, and designating hunting and fishing areas (Funk et al. 2012). ESUs are typically the largest CU, AUs are often intermediate in size, and MUs are generally smaller than both ESUs and AUs,

Received April 27, 2022; Accepted February 21, 2023

such that a single ESU can encompass multiple AUs and MUs (Funk et al. 2012).

High-throughput genomic sequencing has revolutionized our ability to accurately delineate CUs by providing data at thousands of loci across the genome, allowing researchers to detect and characterize neutral and adaptive genetic variation with unprecedented precision. However, the flip side of this increased resolution is that we now have the ability to detect fine-scale patterns of population structure and signatures of isolation by distance (IBD), making it more difficult to identify biologically meaningful divisions across a species' range for conservation purposes. To leverage this growing body of genomic data for conservation planning, several workflows have been proposed to facilitate the identification of CUs on the basis of neutral and adaptive genomic variation (Funk et al. 2012; Barbosa et al. 2018; Xuereb et al. 2021). For example, Funk et al. (2012) recommend that researchers delineate ESUs using both neutral and adaptive single nucleotide polymorphisms (SNPs), identify MUs with neutral loci, and quantify adaptive differences among MUs with putatively adaptive loci identified from F_{ST} outlier tests. However, none of these existing workflows explicitly discuss how to conserve genetic variation in species characterized by strong patterns of IBD. Here, we build on previous work by proposing a workflow for researchers charged with designating CUs in highly mobile organisms.

The problem of IBD

While the framework detailed above is useful for species characterized by limited dispersal distances and clear population structure, many highly mobile species, such as birds, bats, and marine organisms with pelagic larval stages, are continuously distributed, lack geographic barriers to migration, and exhibit subtle genomic differentiation across their range (Palumbi 1994; Veith et al. 2004; Kekkonen et al. 2011), making it difficult to clearly define discrete units using existing guidelines. This clinal genomic variation arises from IBD, a pattern of decreasing genetic similarity with increasing geographic distance that occurs due to constraints on dispersal. First described by Wright (1943), IBD has been documented in a wide range of species (Perez et al. 2018) and is now readily detected with the growing availability of high-throughput genomic sequencing data.

Genetic differentiation arising from IBD can lead to significant behavioral, ecological, and phenotypic differentiation that, in ring species and other extreme cases, is known to result in reproductive isolation and speciation (Irwin et al. 2005; Devitt et al. 2011). However, because IBD does not create distinct breaks, instead generating subtle changes in allele frequencies across geographic space, it can be difficult to draw clear boundaries for conservation purposes. Prior to the advent of high-throughput genomic sequencing, researchers primarily used phylogenetic approaches (e.g. neighbor-joining and unweighted pair-group method with arithmetic averages; Salemi and Vandamme 2003), Bayesian clustering algorithms (e.g. STRUCTURE), and multivariate statistical methods (e.g. principal component analysis [PCA]) to delineate CUs on the basis of either a single marker (e.g. mitochondrial DNA) or a handful of loci (e.g. microsatellites) (Funk et al. 2012). However, these methods can yield spurious results when applied to organisms that are continuously distributed and/or strongly structured by IBD, regardless of the marker type used (Pritchard et al. 2000; Frantz et al. 2009; Meirmans 2012; DeGiorgio and Rosenberg 2013). In particular, uneven geographic sampling combined with a strong pattern of IBD can generate inflated estimates of genomic differentiation, thereby resulting in the erroneous designation of CUs when using genomic data.

The primary goal of this paper is to present a more inclusive workflow for identifying ESUs, AUs, and MUs in all taxa, including highly mobile species showing signatures of IBD, as well as organisms that exhibit discrete population structure. This framework builds on existing workflows that guide the delineation of CUs (Funk et al. 2012; Barbosa et al. 2018; Xuereb et al. 2021), but includes steps explicitly aimed at detecting subtle barriers to gene flow in organisms subject to IBD. First, we review common methods for delineating CUs and the problems that arise when these methods are applied to taxa subject to IBD. Next, we discuss existing statistical programs for detecting subtle breakpoints in patterns of IBD that can be used to detect barriers to gene flow. Finally, in cases where IBD is the only detected population structure, we suggest several possible ways forward for identifying priority conservation areas across a species' range. Throughout the paper, we highlight several case studies that illustrate the challenges associated with delineating CUs in continuously distributed organisms and the ways in which our workflow can provide additional resolution. Ultimately, the delineation of CUs is not an end goal in itself, but rather a useful tool for conserving genetic, phenotypic, and ecological distinctiveness in a species. While some organisms may lack clear boundaries that can be used to divide a species' range into discrete CUs, information contained in patterns of IBD can still be used to inform conservation and management decisions.

Potential pitfalls when identifying CUs

Tree-based approaches are useful for detecting deep phylogenetic splits and can be an appropriate first step when assessing population structure in clearly divergent organisms. However, these methods are generally uninformative in highly mobile taxa, which often lack discrete units exhibiting hierarchical population structure (Diniz-Filho and De Campos Telles 2002). We therefore focus on Bayesian clustering algorithms and multivariate statistical methods in the following discussion.

As documented elsewhere (Schwartz and McKelvey 2009; Oyler-McCance et al. 2013; Tucker et al. 2014; Balkenhol et al. 2015), appropriate sampling design is essential for the accurate detection of population structure. This is especially true for organisms subject to IBD, as the uneven sampling of taxa that exhibit continuous genomic differentiation across space can lead to the detection of artificial clusters, particularly when using hierarchical clustering approaches (Serre and Pääbo 2004; Meirmans 2012). For example, STRUCTURE, the most popular clustering algorithm, does not take the geographic location of samples into account (Pritchard et al. 2000), and can therefore falsely identify multiple clusters if continuously distributed populations are structured solely by IBD (Frantz et al. 2009). While a recent meta-analysis found that signals of IBD are ubiquitous in population genomic datasets, many studies do not adequately account for spatial autocorrelation in allele

frequencies when using Bayesian clustering methods (Perez et al. 2018).

In contrast to clustering algorithms, multivariate analyses, such as PCA, can perform better with continuous data (Price et al. 2006; Petkova et al. 2016). However, PCA is likewise sensitive to uneven sample sizes and poor geographic sampling (McVean 2009; DeGiorgio and Rosenberg 2013), potentially inflating signals of population structure when used to delineate CUs in highly mobile organisms. Thus, researchers should keep these potential caveats in mind when using Bayesian clustering and multivariate statistical approaches and sample individuals as evenly as possible across the landscape, rather than from predefined populations, to reduce the likelihood of detecting artificial clusters.

Even when individuals are sampled evenly across the landscape, the best supported number of genetic clusters (i.e. *K*) inferred with Bayesian clustering methods may mask important genetic variation that should be protected to maintain adaptive potential (Lamichhaney et al. 2012). For example, a study on migratory connectivity in the yellow warbler (*Setophaga petechia*) found that $K = 2$, the optimal number of populations as inferred using STRUCTURE, provides lower resolution for visualizing important connections between breeding and wintering populations than higher values of *K* (Fig. 1.1; Bay et al. 2021). It is therefore critical to consider biological context and the objectives of the study when choosing the most appropriate value of *K* with hierarchical clustering approaches (Pritchard et al. 2000).

More recently, researchers have begun to take advantage of outlier detection tests and landscape genomic methods (e.g. genotype–environment associations [GEAs]) to identify putatively adaptive loci and incorporate this information into the designation of CUs (Funk et al. 2012; Guo et al. 2016; Peters et al. 2016; Barbosa et al. 2018; Xuereb et al. 2021). Outlier detection methods screen the genome for loci showing unexpectedly high levels of genomic differentiation and therefore assumed to be under divergent selection (Nosil et al. 2009), while GEA analyses test for correlations between genomic variation and environmental variables thought to be involved in local adaptation (Forester et al. 2018). F_{ST} outlier tests can yield high rates of false positives and negatives and often rely on strict assumptions about the demographic history and evolutionary independence of samples, making them less useful for the analysis of continuous data that exhibit IBD (Meirmans 2012; Lotterhos and Whitlock 2014). Nonetheless, several studies on adaptive divergence in continuously distributed species have successfully used F_{ST} outlier tests along environmental gradients to identify putatively adaptive SNPs that show marked signatures of divergent selection despite clinal patterns of neutral genomic differentiation (Fig. 1.2; Milano et al. 2014; Wilder et al. 2020).

GEA approaches can be powerful for detecting local adaptation in taxa showing strong signatures of IBD; however,

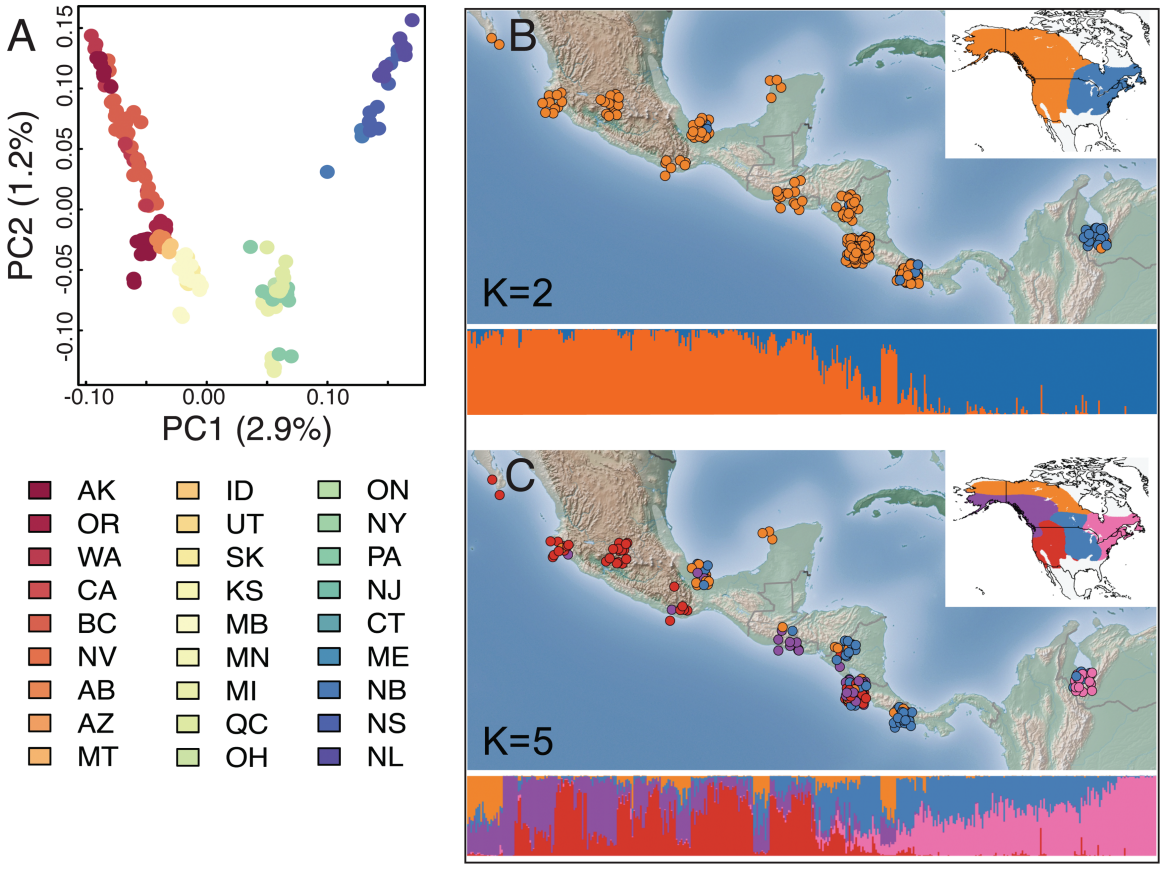

Fig. 1.1. A) PCA of breeding yellow warblers using 104,711 SNPs derived from RAD-Seq data. Points are colored by breeding state and ordered by mean longitude. Patterns of migratory connectivity under B) $K = 2$ and C) $K = 5$ as inferred from STRUCTURE. Points are colored by breeding group assignments indicated by STRUCTURE (shown in the inset) and the plots beneath each map show STRUCTURE results for 419 breeding yellow warbler samples genotyped at 157 SNPs, with individuals ordered by longitude. Figure modified from Bay et al. (2021).

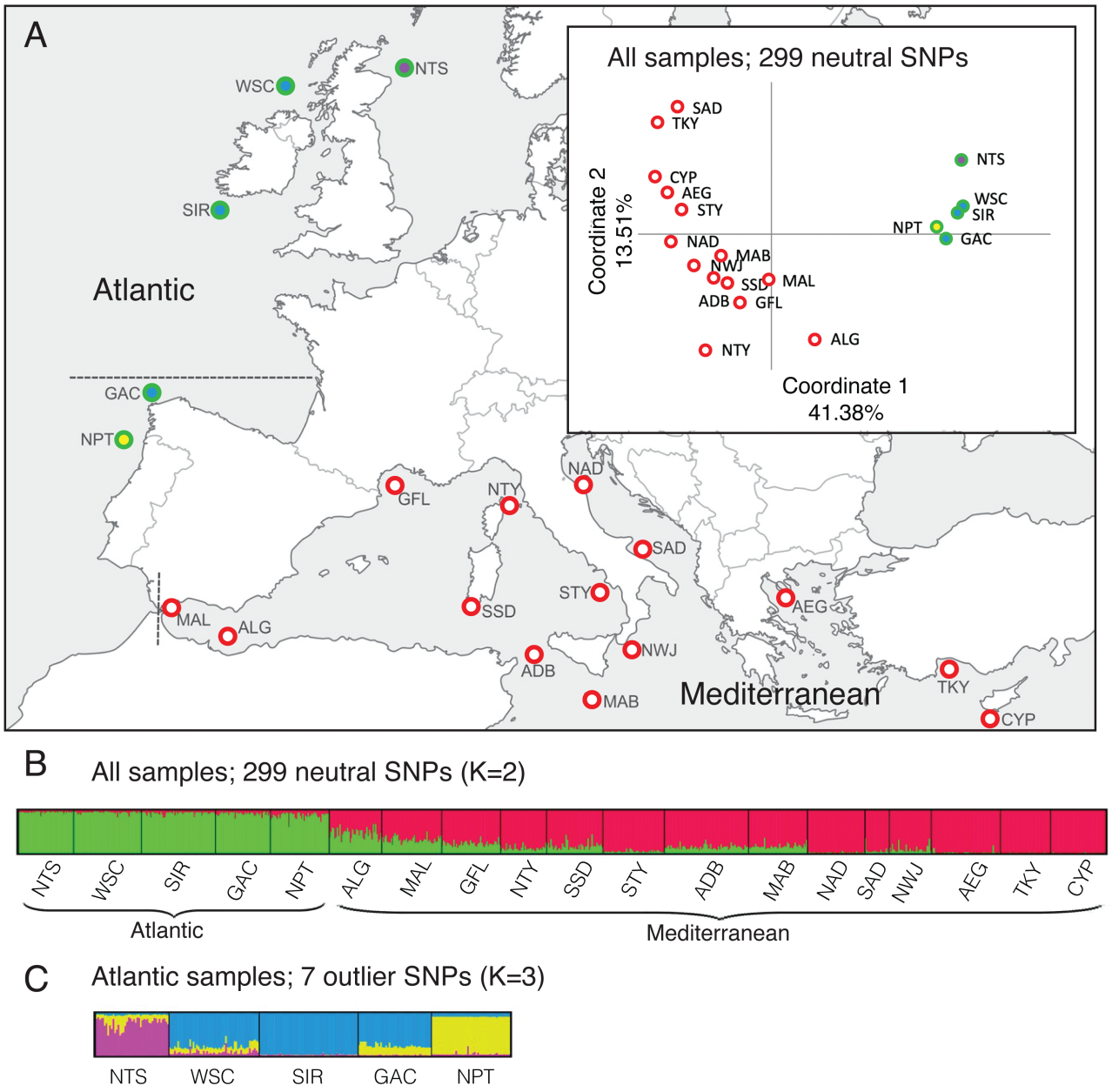

Fig. 1.2. A) Sampling locations across the European hake distribution. Dashed lines indicate boundaries between northern and southern stocks in the Atlantic, and between Atlantic and Mediterranean stocks. The inset shows a principal coordinate analysis based on Edwards' genetic distance calculated using 299 neutral SNPs. The outside ring of each point is colored according to the STRUCTURE results based on neutral loci, while the inside color represents the STRUCTURE results based on outlier loci (for the Atlantic). STRUCTURE results for B) 299 neutral SNPs across the hake distribution (K = 2) and C) 7 outlier SNPs within the Atlantic basin (K = 3). While STRUCTURE only distinguished between Atlantic and Mediterranean populations on the basis of neutral genetic variation, 3 distinct genetic clusters were detected within the Atlantic using outlier SNPs. Figure modified from Milano et al. (2014).

their results must be interpreted with caution if spatial population structure is not taken into account. For example, environmental gradients are often correlated with neutral population structure in organisms that exhibit clinal genomic variation across their geographic range, potentially resulting in false positives when putatively adaptive loci are identified with GEAs (De Mita et al. 2013; Lotterhos and Whitlock 2015). As a result, sampling should ideally maximize environmental variation while minimizing the collinearity between environmental gradients and neutral population structure to reduce the potential for false positives (Nadeau et al. 2016).

Using all genomic loci to delineate ESUs

Given the risk of detecting artificial clusters and false positives when IBD is not properly accounted for, researchers should test for signatures of IBD if initial explorations of genomic divergence reveal clinal patterns of genomic differentiation or a lack of discrete population structure (Fig. 2, Steps 1 and 2). The simple Mantel test, which examines the correlation between matrices of pairwise genetic and geographic distances, is one of the most common approaches for detecting IBD (Guillot and Rousset 2013). Nonetheless, simple Mantel tests can yield unreliable results in taxa that do possess geographically distinct population clusters (e.g.

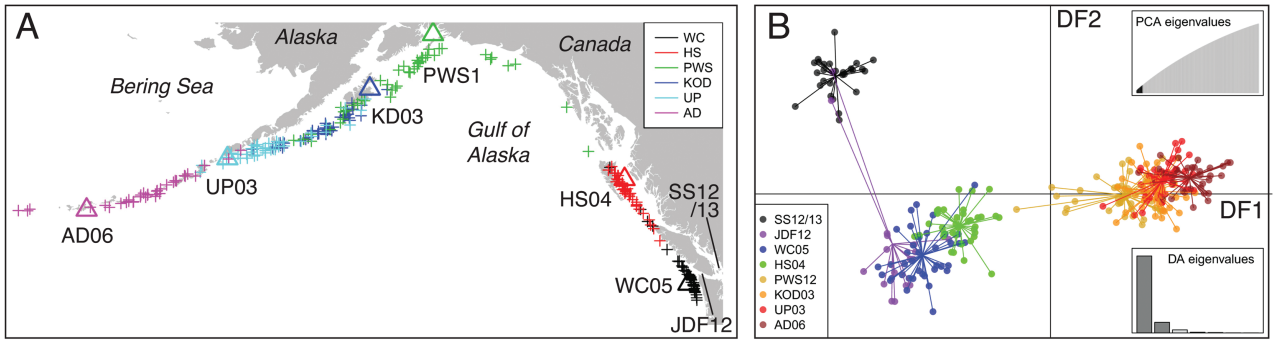

Fig. 1.3. A) Individual assignment locations for coastal populations of Pacific cod using SCAT to assign geographic locations of origin based on patterns of IBD. Plus signs indicate assignment locations and triangles represent sampling locations. Despite a strong pattern of IBD, genomic loci successfully discriminated spawning populations of Pacific cod and accurately assigned individuals to populations of origin. B) DAPC for sampling locations shown as triangles in (A), as well as 2 additional sampling locations in the Salish Sea (SS12/13) and Strait of Juan de Fuca (JDF12). While samples from the Salish Sea (SS12/13) were differentiated from the coastal populations along discriminant function 2 (DF2, *y* axis), discriminant function 1 (DF1, *x* axis) reflected a pattern of range-wide IBD. The authors attribute the clustering observed along DF1 to the lack of samples from southeast Alaska rather than true genetic differentiation among isolated populations. Figure modified from Drinan et al. (2018).

populations that have undergone postglacial expansion from multiple glacial refugia), as they are unable to distinguish between patterns arising from hierarchical clustering and those produced by IBD (Meirmans 2012). In taxa that likely show signatures of both hierarchical population structure and IBD, stratified Mantel tests, which permute the locations of populations within clusters, or redundancy analysis (RDA), which combines PCA and multiple regression to evaluate the influence of a matrix of geographic coordinates on a matrix of allele frequencies, should be used to distinguish between these 2 processes (Fig. 2, Step 3; Meirmans 2015; Perez et al. 2018).

In the event that IBD is the overwhelming signal of population structure, a number of statistical approaches can be used to examine genomic variation across space and identify breakpoints in patterns of IBD, or areas where genetic distance is greater than expected under IBD (Fig. 2, Step 4). Several studies using nonspatial Bayesian methods to identify genetic clusters have investigated the potential for artificial clustering by plotting genetic distance (i.e. pairwise F_{ST}) against geographic distance and coloring the points according to cluster membership (Rosenberg et al. 2005; Fontaine et al. 2007; Guillot et al. 2009). If genetic variation is indeed explained by factors other than geographic distance, genetic distance should be much larger for pairs of sites belonging to distinct genetic clusters than those belonging to the same cluster for a given class of spatial distances. In addition, Bayesian clustering algorithms have been developed that incorporate spatial information by placing spatial priors on cluster membership, such that the probability that 2 individuals belong to the same genetic cluster decreases with increasing geographic distance between them (e.g. GENELAND [Guillot et al. 2005], TESS [Chen et al. 2007], and BAPS [Cheng et al. 2013]). However, like nonspatial clustering approaches, these methods assume constant allele frequencies within clusters across a species' range and are therefore similarly susceptible to the effects of IBD (Frantz et al. 2009; Guillot and Santos 2009).

In contrast, the clustering method conStruct simultaneously models continuous and discrete patterns of population structure by explicitly incorporating a model of IBD (Bradburd et al. 2018). Although the program can be computationally demanding to run on many samples, conStruct avoids some of the overfitting issues observed with nonspatial model-based clustering algorithms and more accurately captures spatial structure in continuously distributed taxa (Bradburd et al. 2018). Finally, several recent methods for detecting barriers to gene flow between populations can be leveraged to identify breakpoints in patterns of IBD and inform the identification of CUs. For example, EEMS (Estimated Effective Migration Surfaces), and its faster counterpart FEEMS (Fast Estimation of Effective Migration Surfaces), allow researchers to visualize spatial patterns of population structure in organisms that exhibit IBD and identify regions where gene flow is either higher or lower than average (Petkova et al. 2016; Marcus et al. 2021). The program DResD (i.e. distribution of residual dissimilarity) can also be used to identify geographic regions where genetic distance between individuals is significantly higher than expected from the effect of IBD alone, indicating possible barriers to gene flow (Hindrikson et al. 2013; Keis et al. 2013; Fedy et al. 2017). While multivariate statistical analyses (e.g. PCA) and Bayesian clustering methods (e.g. STRUCTURE) are routinely used in CU delineation, we advocate the use of the lesser-known approaches listed above when identifying ESUs in organisms subject to IBD. These programs will aid the interpretation and visualization of Bayesian clustering results and ensure that important genetic variation necessary to maintain evolutionary potential is captured when designating ESUs (Fig. 2, Step 5).

Case study: IBD masks important biological information in the yellow warbler

The yellow warbler case study (Box 1A) illustrates how relying on the best supported value of *K* revealed by Bayesian clustering approaches can cause researchers to miss critical biological information relevant to the designation of CUs. Bay et al. (2018) use restriction site-associated DNA (RAD) sequencing of 229 individuals sampled from 21 locations across the breeding range of the yellow warbler to identify 104,711 SNPs (Fig. 1.1A). Using a simple Mantel test to correlate geographic distance with pairwise genetic distance between sampling locations, they document a strong signal of IBD (Mantel's $r = 0.85$, $P = 1 \times 10^{-5}$). In addition, they conduct a multiple regression analysis with geographic and

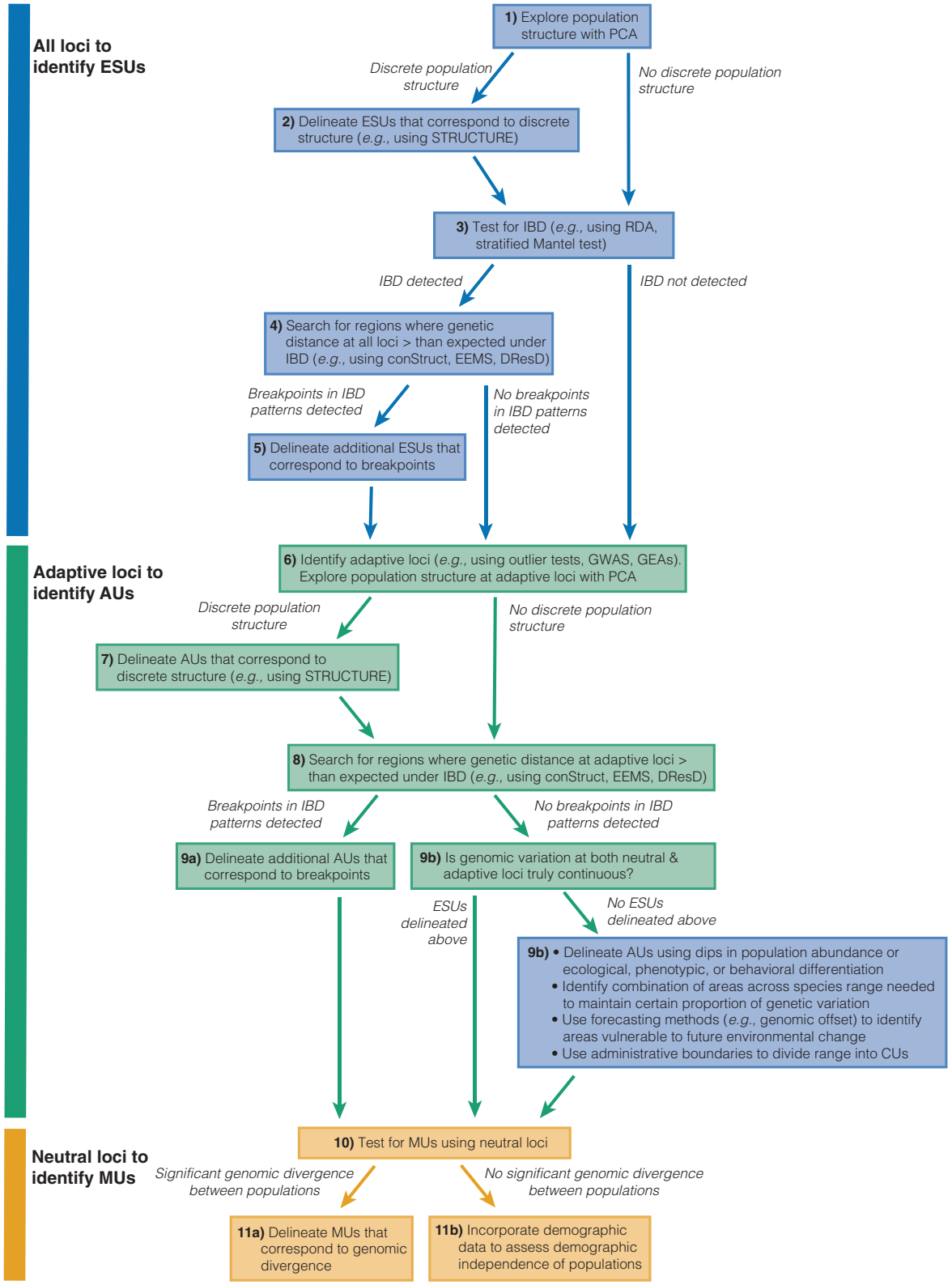

Fig. 2. Decision tree for identifying ESUs, AUs, and MUs in taxa exhibiting discrete population structure and/or continuous genomic differentiation. Analyses using all loci (adaptive and neutral) are indicated in blue, analyses using putatively adaptive loci are depicted in green, and analyses using neutral loci are shown in orange.

Box 1. Case studies illustrating the challenge of designating CUs in continuously distributed taxa

A. Yellow warbler (*Setophaga petechia*)

The yellow warbler is a migratory songbird that breeds from Canada to Mexico. While common across its breeding range, the species has suffered local population declines and is considered a species of conservation concern in certain areas (Shuford and Gardali 2008). According to microsatellite loci and SNPs derived from RAD data, genetic variation in the yellow warbler is strongly structured by IBD (Gibbs et al. 2000; Bay et al. 2018, 2021). The top principal components in a PCA were strongly correlated with both longitude and latitude (Bay et al. 2021), and a Bayesian clustering analysis using the program STRUCTURE found support for $K = 2$ as the optimal number of populations. Nonetheless, as revealed by gradient forest analysis of RAD-Seq data, the yellow warbler exhibits standing genetic variation for adaptation to different climate regimes across its breeding distribution, with populations in the Rocky Mountains revealing the greatest mismatch between current and predicted future genotype–environment relationships (i.e. genomic vulnerability) (Bay et al. 2018). In addition, patterns of migratory connectivity as interpreted under $K = 2$ obscure important connections between breeding and wintering populations that can help inform conservation efforts. These findings suggest that 1) yellow warblers possess important genetic variation across their breeding range (beyond the 2 genetic clusters revealed by STRUCTURE) that should be conserved to maximize evolutionary potential, 2) populations in the Rocky Mountains may be especially vulnerable to future climate change, and 3) $K = 5$ provides more information that can be used to pinpoint geographic areas across the annual cycle that should be prioritized for protection than $K = 2$. Therefore, traditional methods of delineating CUs (e.g. Bayesian clustering algorithms and PCAs), which find support for 2 genetic clusters, do not adequately capture genetic and ecological distinctiveness across the breeding distribution of the yellow warbler.

B. European hake (*Merluccius merluccius*)

The European hake is a widely distributed fish of commercial interest that resides in environmentally variable regions from the North Sea and Atlantic Ocean to the Mediterranean Sea and suffers from overfishing. Hake populations in the Atlantic and Mediterranean are separately managed and exhibit clear differences in demographic and life history traits, such as growth rate, size at maturity, and spawning season (Milano et al. 2014). Despite a lack of evidence for population structure, a northern and southern stock within the Atlantic Ocean have been delineated on either side of the Cap Breton Canyon, a supposed barrier to fish movement. Using SNP genotyping, Milano et al. (2014) sequenced 850 individuals from across the entire hake distribution and used outlier detection tests to investigate large- and fine-scale population structure on the basis of neutral and putatively adaptive SNPs. The panel of 299 putatively neutral markers confirmed the genetic break between Atlantic and Mediterranean basins, but revealed weak genetic differentiation and a strong pattern of IBD within basins, indicating high connectivity between the previously delineated northern and southern stocks within the Atlantic (Milano et al. 2014). STRUCTURE analyses of outlier loci within basins, however, revealed additional fine-scale population structure, identifying 3 genetic clusters in the Atlantic that correspond to the North Sea, Northern Portugal, and the remaining Atlantic locations (Fig. 1.2C), and genetic structure within the Mediterranean (not shown) (Milano et al. 2014). Thus, while neutral markers show a clinal pattern of population differentiation within the Atlantic and Mediterranean basins, failing to provide clear breakpoints in patterns of IBD that can be used to identify CUs, putative adaptive variation provides additional resolution that should be incorporated to revise existing CUs and more effectively conserve adaptive potential.

C. Pacific cod (*Gadus macrocephalus*)

The Pacific cod is a commercially harvested species of groundfish that inhabits coastal regions in the North Pacific Ocean. According to microsatellite markers, Pacific cod exhibit a tight pattern of IBD along the western coast of North America, complicating the identification of clear MUs (Cunningham et al. 2009; Spies 2012). Nonetheless, the species is currently managed as 4 separate stocks: Bering Sea, Aleutian Islands, Gulf of Alaska, and Pacific coast. Using restriction site-associated DNA (RAD) sequencing, Drinan et al. (2018) identified 65 outlier loci showing evidence for selection among coastal spawning populations and documented a strong signal of IBD along the Washington coast, British Columbia, Gulf of Alaska, and Aleutian Islands. Genomic loci were sufficiently differentiated among coastal populations to allow successful assignment to populations of origin using a continuous assignment test called SCAT, which interpolates allele frequencies in an IBD relationship, and a leave-one-out approach. The authors suggest that established MUs may not accurately reflect stock structure in Pacific cod and highlight population structure over small spatial scales (e.g. Prince William Sound: PSW1 vs. Kodiak Island: KD03) that may be relevant to management decisions.

environmental distance matrices; only geographic distance was significant (MRM: $R^2 = 0.73$; geography $P = 1 \times 10^{-5}$; environment $P = 0.12$), indicating that IBD is the primary force structuring genome-wide variation in the yellow warbler.

To investigate patterns of discrete population structure across the breeding range, Bay et al. (2021) run STRUCTURE with the same breeding samples to estimate individual ancestry proportions. They find support for $K = 2$ as the optimal number of genetic clusters, suggesting that the yellow warbler is comprised of at least 2 ESUs (Fig. 1.1B). However, additional analyses (e.g. gradient forest and patterns of migratory connectivity) suggest that yellow warblers possess additional genetic variation across the breeding range (beyond the 2 genetic clusters revealed by STRUCTURE) that may be important to conserve in order to maintain adaptive potential (Fig. 1.1C). While this study did not focus on the delineation of CUs, a logical next step in order to achieve that goal would be to use all identified genomic loci to search for geographic regions where genetic distance is greater than expected under IBD within each genomic cluster (e.g. using conStruct, EEMS, or DResD; Fig. 2, Step 4). The detection of additional breakpoints using these programs could indicate that the yellow warbler should be split into further ESUs.

Using adaptive loci to delineate AUs

In addition to clustering algorithms and programs designed to identify barriers to gene flow in continuously distributed taxa, statistical methods aimed at searching for signatures of local adaptation can also be integrated to delineate AUs (Fig. 2, Step 6). Certain GEA methods (e.g. Bayenv2, LFMM, and RDA) are capable of controlling for neutral population structure while identifying adaptive variation, thereby reducing the risk of detecting false positives (Rellstab et al. 2015). A simulation study comparing several GEA methods found that RDA, a multivariate ordination technique that can analyze numerous genomic loci and environmental predictors simultaneously, outperforms random forest and several univariate methods when detecting adaptive variation across various strengths of selection (Forester et al. 2018).

Additionally, genome-wide association studies (GWAS) can be conducted to identify genomic loci that underlie adaptive traits and examine how these loci are distributed among populations. However, phenotypic data are lacking for many species of conservation concern and the phenotypic traits involved in local adaptation are often unknown, limiting the utility of GWAS for the delineation of CUs. Outlier detection methods can also be used to identify loci that exhibit high genetic differentiation relative to overall population structure and are likely involved in divergent selection. Unlike GEAs, F_{ST} outlier tests do not require knowledge of the environmental factors underlying local adaptation, but are less useful when applied to continuously distributed organisms that show signatures of IBD and lack clear neutral population structure given their high type I and II error rates (Lotterhos and Whitlock 2014). Nonetheless, outlier tests that estimate and account for evolutionary nonindependence among samples (e.g. FLK, Bayenv2, and OutFLANK) show some promise for accurately identifying loci under divergent selection in nonequilibrium scenarios with IBD (Lotterhos and Whitlock 2014; Whitlock and Lotterhos 2015). After identifying candidate SNPs using these approaches, researchers can search for evidence of discrete population structure (e.g. using PCA or STRUCTURE; Fig. 2, Steps 6 and 7), as more commonly done, or subtle breakpoints in patterns of IBD (e.g. using conStruct, EEMS, or DresD; Fig. 2, Step 8), less commonly done, using putatively adaptive loci to delineate AUs (Fig. 2, Step 9a). Despite their shortcomings, GEA, GWAS, and outlier detection methods can provide additional resolution of adaptive genomic differences across a species' distribution.

Case study: Adaptive loci reveal additional population structure in the European hake (*Merluccius merluccius*)

The case study in European hake (Box 1B) demonstrates how adaptive loci can provide additional resolution needed to split continuously distributed organisms subject to IBD into discrete CUs. Using SNP genotyping, Milano et al. (2014) sequence 850 individuals from 19 locations across the distribution of European hake (Fig. 1.2A). The authors detect outliers using Arlequin and Bayescan to define a panel of 299 putatively neutral SNPs and a panel of basin-specific outlier SNPs within the Atlantic (n = 7) and Mediterranean (n = 19). To test for IBD, the authors correlate geographic distance with linearized pairwise F_{ST} based on neutral SNPs and document a significant pattern of IBD within both the Atlantic (r = 0.84; P = 0.014) and Mediterranean (r = 0.48; P = 0.003) basins. They use the GEA method Bayenv to test for associations between genetic variation at outlier loci and 2 environmental variables (seawater salinity and surface temperature). Finally, they run STRUCTURE to infer genetic clusters based on 1) neutral SNPs, 2) outlier SNPs within the Atlantic, and 3) outlier SNPs within the Mediterranean. While K = 2 was the best supported number of genetic clusters based on neutral markers (Fig. 1.2B), the putatively adaptive outlier loci revealed additional population structure within each basin (e.g. 3 genetic clusters within the Atlantic; Fig. 1.2C). Several of the identified outlier SNPs within the Atlantic basin were strongly correlated with environmental variables, providing support for the designation of multiple AUs in European hake. While the authors stop here, running conStruct, EEMS, or DResD with putatively adaptive SNPs (Fig. 2, Step 8) could reveal additional breakpoints in patterns of IBD and provide further resolution when splitting European hake in the Atlantic and Mediterranean basins into AUs.

What if genomic variation is truly continuous?

In cases in which neutral and adaptive genomic variation are clinally distributed and no clear breakpoints in patterns of IBD can be detected across a species' range, it may be impossible to draw boundaries corresponding to discrete ESUs or AUs on the basis of genomic variation alone. We propose several possible ways forward for identifying portions of a species' range that should be protected to ensure the maintenance of evolutionary potential in the absence of discrete population structure (Fig. 2, Step 9b). First, when available, information on life history traits, ecological, phenotypic, and behavioral differences among populations, and population abundance can be incorporated to help inform the delineation of AUs (Allendorf et al. 2022). For example, adaptive phenotypic traits that are acquired primarily through social learning (e.g. bird song) or controlled by few genomic loci (e.g. migratory timing in fish or avian plumage coloration) may show clear differentiation across a species' range despite a lack of genome-wide genetic divergence (Prince et al. 2017; Semenov et al. 2018; Turbek et al. 2021). Thus, these traits can be useful for dividing continuously distributed species into discrete AUs when high resolution genomic data cannot be obtained or breakpoints in patterns of IBD are not detected (Vredenburg et al. 2007; Delarue et al. 2009; Mahoney et al. 2021). However, defining AUs on the basis of single-gene differences that encode adaptive traits is generally not advisable unless these traits are of fundamental ecological and evolutionary importance and directly underlie reproductive isolation, such that they warrant special consideration for defining CUs (Waples and Lindley 2018). Additionally, variation in spatially explicit estimates of population abundance (e.g. based on Wright's neighborhood size [Shirk and Cushman 2014]) can indicate regions across a species' distribution that potentially represent barriers to gene flow that are not yet reflected at the genomic level. In the absence of additional data, dips in population abundance can therefore be used to help draw the lines necessary to split highly mobile species into discrete CUs.

Alternatively, genomic information contained in patterns of IBD can be used to identify particular areas across a species' range that should be prioritized for protection in order to maximize the likelihood of population persistence. For example, if the objective was to maintain 90% of allelic richness or heterozygosity within a species, one could examine spatial patterns of genomic variation across the distribution and identify key areas that must be protected in order to maintain that proportion of neutral and/or putatively adaptive genetic diversity. Another option to guide decisions regarding the allocation of limited conservation resources could be to use forecasting methods, such as genomic offset modeling, to predict the vulnerability of populations to future environmental change. Genomic offset models assess relationships between patterns of adaptive genomic variation and environmental variables in the present and project these relationships into the future to estimate the degree of genomic vulnerability (i.e. the amount that allele frequencies must shift in order for populations to keep pace with environmental change) (Fitzpatrick and Keller 2015; Bay et al. 2018; Capblancq et al. 2020). These models generate continuous predictions of allelic turnover across a species' range and can be used to pinpoint portions of the distribution that are most vulnerable to future environmental change and should therefore be prioritized for protection. Finally, in the absence of additional ecological, phenotypic, or population abundance data that can be leveraged to inform the delineation of CUs, one could simply use existing administrative boundaries to divide a species' range into separate units that can guide conservation and population management decisions. While these administrative boundaries may be somewhat arbitrary with respect to an organism's biology, their adoption for conservation purposes would enable conservation practitioners to implement different conservation measures to protect populations located on either end of a species' distribution, which often exhibit clear genetic, ecological, and behavioral differences that should be conserved to maintain evolutionary potential.

Using neutral loci to delineate MUs

Even when clear boundaries cannot be drawn across the distribution to divide a species into either ESUs or AUs, patterns at neutral loci can be used to delineate discrete MUs that represent demographically independent populations (Fig. 2, Step 10). According to Palsbøll et al. (2007), the delineation of MUs should be based on the observed estimate of population genomic divergence, as this estimate is a function of the dispersal rate among populations. While the threshold level of divergence that corresponds to demographic independence will depend on the conservation context, effective population size, and various aspects of the target species, Palsbøll et al. (2007) provide guidelines for setting this threshold and suggest several simulation programs that can be used to estimate the expected level of genomic divergence under different dispersal rates. In addition, population assignments tests, which use genomic data to identify the geographic origin of sampled individuals, can provide insight into the demographic independence of populations (Benestan et al. 2015; Drinan et al. 2018). In particular, high population assignment success for individuals sampled in different geographic regions may provide support for the designation of discrete MUs (Fig. 1.3). In cases in which genomic data alone fail to resolve MU status, additional demographic data (e.g. from mark-recapture and long-term monitoring studies) can also be incorporated to quantify spatial variation in population dynamics and identify demographically independent populations (Fig. 2, Step 11; Rushing et al. 2016; Forester et al. 2022). The delineation of MUs in species strongly structured by IBD will allow conservation practitioners to apply different management strategies to populations that potentially exhibit genetic, phenotypic, and ecological differences despite clinal genomic variation across a species' range. In addition, dividing species subject to IBD into distinct MUs can increase sustainable yield for harvested taxa and avoid overexploitation and population collapse, especially when highly mobile organisms are faced with spatially disproportionate fishing and/or poaching pressure (Spies et al. 2015).

Case study: Population assignment tests provide insight into stock boundaries in Pacific cod (*Gadus macrocephalus*)

The Pacific cod case study (Box 1C) illustrates how population assignment tests can be leveraged to aid the designation of MUs in highly mobile taxa structured by IBD. To inform stock boundaries in the Pacific cod, Drinan et al. (2018) use RAD sequencing to identify 6,425 SNPs from 276 individuals across the species range in the eastern Pacific Ocean (Fig. 1.3A). The authors use a simple Mantel test to estimate the magnitude of IBD, documenting a strong pattern of IBD among coastal samples (R^2 = 0.81). In addition, they carry out a discriminant analysis of principal components (DAPC) to visualize genomic relationships among spawning locations. The DAPC indicated that the samples formed 3 genomic clusters, one comprised of individuals from the Salish Sea and 2 other clusters along the coast (Fig. 1.3B). However, given the strong pattern of IBD, clustering among the coastal samples was likely due to uneven geographic sampling rather than true genetic differentiation among isolated populations. These results thus highlight the importance of appropriate sampling design when examining population structure in organisms subject to IBD.

The authors then use BayeScan and OutFLANK, 2 outlier detection methods, to identify candidate loci under selection. Sixty-five SNPs were identified as putatively under selection across the sampled coastal range using both outlier detection approaches. Finally, the authors use a number of different assignment methods (GeneClass2, Assigner, and SCAT) to assess the power of all loci to accurately assign individuals to their sample of origin. SCAT (Smoothing and Continuous AssignmenTs), leverages information in patterns of IBD to assign individuals to a geographic location of origin, rather than a discrete population. The authors point to successful population assignment rates despite the strong pattern of IBD exhibited by coastal populations as evidence that established MUs in Pacific cod may not reflect true stock structure (Fig. 1.3A). However, carrying out this population assignment analysis separately with putatively neutral and outlier SNPs would allow the authors to distinguish between AUs and MUs.

Conclusions

The accurate delineation of CUs is essential to maximize adaptive potential and ensure the effective protection and

management of biological diversity. Current guidelines for designating CUs largely neglect information found in patterns of IBD; however, clinal genomic variation in continuously distributed species often contains important information that can be used to aid conservation efforts and identify particular areas across a species' range that should be prioritized for protection. We explore the ways in which researchers can extract information relevant to conservation from patterns of IBD and highlight additional data that can be incorporated to ensure that conservation efforts capture unique genetic variation necessary to maintain evolutionary potential. While emphasis is often placed on drawing clear lines to divide populations into discrete CUs that can be separately managed, some organisms may be characterized by subtle patterns of genomic variation and lack obvious boundaries across their geographic range. CUs are merely a tool to achieve the ultimate goal of protecting the distribution of genomic, phenotypic, and ecological variation in a species. Therefore, all genomic information, whether useful for drawing clear boundaries across a species' range or not, should be used to inform conservation and management decisions.

Acknowledgments

We thank Eric Anderson and Robin Waples for their valuable input during initial brainstorming sessions and members of the Ruegg and Funk labs, as well as 2 anonymous reviewers, for their thoughtful feedback on the manuscript.

Funding

This work was supported by the National Science Foundation (Postdoctoral Fellowship in Biology 2208881 to SPT; DEB 1942313 to KCR; DEB 1838282 to WCF; DEB 1754821 to WCF) and the US Fish and Wildlife Service (F21AC02312 to WCF).

References

Allendorf FW, Funk C, Aitken SN, Byrne M, Luikart G. *Conservation and the genomics of populations*. 3rd ed. Oxford (UK): Oxford University Press; 2022.

Allendorf FW, Luikart G. *Conservation and the genetics of populations*. Oxford (UK): Blackwell Publishing; 2007.

Balkenhol N, Cushman S, Storfer A, Waits L. *Landscape genetics: concepts, methods, applications*. Hoboken (NJ): John Wiley & Sons; 2015.

Barbosa S, Mestre F, White TA, Paupério J, Alves PC, Searle JB. Integrative approaches to guide conservation decisions: using genomics to define conservation units and functional corridors. *Mol Ecol*. 2018;27(17):3452–3465.

Bay RA, Harrigan RJ, Le Underwood V, Gibbs HL, Smith TB, Ruegg K. Genomic signals of selection predict climate-driven population declines in a migratory bird. *Science*. 2018;359(6371):83–86.

Bay RA, Karp DS, Saracco JF, Anderegg WRL, Frishkoff LO, Wiedenfeld D, Smith TB, Ruegg K. Genetic variation reveals individual-level climate tracking across the annual cycle of a migratory bird. *Ecol Lett*. 2021;24(4):819–828.

Benestan L, Gosselin T, Perrier C, Sainte-Marie B, Rochette R, Bernatchez L. RAD genotyping reveals fine-scale genetic structuring and provides powerful population assignment in a widely distributed marine species, the American Lobster (*Homarus americanus*). *Mol Ecol*. 2015;24(13):3299–3315.

Bradburd GS, Coop GM, Ralph PL. Inferring continuous and discrete population genetic structure across space. *Genetics*. 2018;210(1):33–52.

Capblancq T, Fitzpatrick MC, Bay RA, Exposito-Alonso M, Keller SR. Genomic prediction of (Mal)adaptation across current and future climatic landscapes. *Annu Rev Ecol Evol Syst*. 2020;51(1):245–269.

Chen C, Durand E, Forbes F, François O. Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. *Mol Ecol Notes*. 2007;7(5):747–756.

Cheng L, Connor TR, Sirén J, Aanensen DM, Corander J. Hierarchical and spatially explicit clustering of DNA sequences with BAPS software. *Mol Biol Evol*. 2013;30(5):1224–1228.

Coates DJ, Byrne M, Moritz C. Genetic diversity and conservation units: dealing with the species-population continuum in the age of genomics. *Front Ecol Evol*. 2018;6(165):1–13.

Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK. Considering evolutionary processes in conservation biology. *Trends Ecol Evol*. 2000;15(7):290–295.

Cunningham KM, Canino MF, Spies IB, Hauser L. Genetic isolation by distance and localized fjord population structure in Pacific cod (*Gadus macrocephalus*): limited effective dispersal in the northeastern Pacific Ocean. *Can J Fish Aquat Sci*. 2009;66(1):153–166.

Davis MB, Shaw RG, Etterson JR. Evolutionary responses to changing climate. *Ecology*. 2005;86(7):1704–1714.

De Mita S, Thuillet A-C, Gay L, Ahmadi N, Manel S, Ronfort J, Vigouroux Y. Detecting selection along environmental gradients: analysis of eight methods and their effectiveness for outbreeding and selfing populations. *Mol Ecol*. 2013;22(5):1383–1399.

DeGiorgio M, Rosenberg NA. Geographic sampling scheme as a determinant of the major axis of genetic variation in principal components analysis. *Mol Biol Evol*. 2013;30(2):480–488.

Delarue J, Todd SK, Van Parijs SM, Di Iorio L. Geographic variation in Northwest Atlantic fin whale (*Balaenoptera physalus*) song: implications for stock structure assessment. *J Acoust Soc Am*. 2009;125(3):1774–1782.

Devitt TJ, Baird SJE, Moritz C. Asymmetric reproductive isolation between terminal forms of the salamander ring species *Ensatina eschscholtzii* revealed by fine-scale genetic analysis of a hybrid zone. *BMC Evol Biol*. 2011;11(245):1–15.

Diniz-Filho JAF, De Campos Telles MP. Spatial autocorrelation analysis and the identification of operational units for conservation in continuous populations. *Conserv Biol*. 2002;16(4):924–935.

Drinan DP, Gruenthal KM, Canino MF, Lowry D, Fisher MC, Hauser L. Population assignment and local adaptation along an isolation-by-distance gradient in Pacific cod (*Gadus macrocephalus*). *Evol Appl*. 2018;11(8):1448–1464.

Fedy BC, Row JR, Oyler-McCance SJ. Integration of genetic and demographic data to assess population risk in a continuously distributed species. *Conserv Genet*. 2017;18(1):89–104.

Fitzpatrick MC, Keller SR. Ecological genomics meets community-level modelling of biodiversity: mapping the genomic landscape of current and future environmental adaptation. *Ecol Lett*. 2015;18(1):1–16.

Fontaine MC, Baird SJE, Piry S, Ray N, Tolley KA, Duke S, Birkun A, Ferreira M, Jauniaux T, Llavona A, et al. Rise of oceanographic barriers in continuous populations of a cetacean: the genetic structure of harbour porpoises in Old World waters. *BMC Biol*. 2007;5(30):1–16.

Forester BR, Lasky JR, Wagner HH, Urban DL. Comparing methods for detecting multilocus adaptation with multivariate genotype–environment associations. *Mol Ecol*. 2018;27(9):2215–2233.

Forester BR, Murphy M, Mellison C, Petersen J, Pilliod DS, van Horne R, Harvey J, Funk WC. Genomics-informed delineation of conservation units in a desert amphibian. *Mol Ecol*. 2022;31(20):5249–5269.

Frantz AC, Cellina S, Krier A, Schley L, Burke T. Using spatial Bayesian methods to determine the genetic structure of a continuously distributed population: clusters or isolation by distance? *J Appl Ecol*. 2009;46(2):493–505.

Fraser DJ, Bernatchez L. Adaptive evolutionary conservation: towards a unified concept for defining conservation units. *Mol Ecol*. 2001;10(12):2741–2752.

Funk WC, McKay JK, Hohenlohe PA, Allendorf FW. Harnessing genomics for delineating conservation units. *Trends Ecol Evol*. 2012;27(9):489–496.

Gibbs HL, Dawson RJG, Hobson KA. Limited differentiation in microsatellite DNA variation among northern populations of the yellow warbler: evidence for male-biased gene flow? *Mol Ecol*. 2000;9(12):2137–2147.

Green DM. Designatable units for status assessment of endangered species. *Conserv Biol*. 2005;19(6):1813–1820.

Guillot G, Leblois R, Coulon A, Frantz AC. Statistical methods in spatial genetics. *Mol Ecol*. 2009;18(23):4734–4756.

Guillot G, Mortier F, Estoup A. Geneland: a computer package for landscape genetics. *Mol Ecol Notes*. 2005;5(3):712–715.

Guillot G, Rousset F. Dismantling the Mantel tests. *Methods Ecol Evol*. 2013;4(4):336–344.

Guillot G, Santos F. A computer program to simulate multilocus genotype data with spatially autocorrelated allele frequencies. *Mol Ecol Resour*. 2009;9(4):1112–1120.

Guo B, Li Z, Merilä J. Population genomic evidence for adaptive differentiation in the Baltic Sea herring. *Mol Ecol*. 2016;25(12):2833–2852.

Hindrikson M, Remm J, Männil P, Ozolins J, Tammeleht E, Saarma U. Spatial genetic analyses reveal cryptic population structure and migration patterns in a continuously harvested grey wolf (*Canis lupus*) population in north-eastern Europe. *PLoS One*. 2013;8(9):e75765.

Irwin DE, Staffan B, Irwin JH, Price TD. Speciation by distance in a ring species. *Science*. 2005;307(5708):414–416.

Keis M, Remm J, Ho SYW, Davison J, Tammeleht E, Tumanov IL, Saveljev AP, Männil P, Kojola I, Abramov AV, et al. Complete mitochondrial genomes and a novel spatial genetic method reveal cryptic phylogeographical structure and migration patterns among brown bears in north-western Eurasia. *J Biogeogr*. 2013;40(5):915–927.

Kekkonen J, Seppä P, Hanski IK, Jensen H, Väisänen RA, Brommer JE. Low genetic differentiation in a sedentary bird: house sparrow population genetics in a contiguous landscape. *Heredity*. 2011;106(1):183–190.

Lamichhaney S, Barrio AM, Rafati N, Sundström G, Rubin C-J, Gilbert ER, Berglund J, Wetterbom A, Laikre L, Webster MT, et al. Population-scale sequencing reveals genetic differentiation due to local adaptation in Atlantic herring. *Proc Natl Acad Sci USA*. 2012;109(47):19345–19350.

Lotterhos KE, Whitlock MC. Evaluation of demographic history and neutral parameterization on the performance of F_{ST} outlier tests. *Mol Ecol*. 2014;23(9):2178–2192.

Lotterhos KE, Whitlock MC. The relative power of genome scans to detect local adaptation depends on sampling design and statistical method. *Mol Ecol*. 2015;24(5):1031–1046.

Mahoney SM, Pasch B, Theimer TC. Subspecies discrimination based on song structure by Willow Flycatchers. *J Field Ornithol*. 2021;92(2):173–183.

Marcus J, Ha W, Barber RF, Novembre J. Fast and flexible estimation of effective migration surfaces. *eLife*. 2021;10:e61927.

McVean G. A genealogical interpretation of principal components analysis. *PLoS Genet*. 2009;5(10):e1000686.

Meirmans PG. The trouble with isolation by distance. *Mol Ecol*. 2012;21(12):2839–2846.

Meirmans PG. Seven common mistakes in population genetics and how to avoid them. *Mol Ecol*. 2015;24(13):3223–3231.

Milano I, Babbucci M, Cariani A, Atanassova M, Bekkevold D, Carvalho GR, Espiñeira M, Fiorentino F, Garofalo G, Geffen AJ, et al. Outlier SNP markers reveal fine-scale genetic structuring across European hake populations (*Merluccius merluccius*). *Mol Ecol*. 2014;23(1):118–135.

Moritz C. Defining 'evolutionarily significant units' for conservation. *Trends Ecol Evol*. 1994;9(10):373–375.

Nadeau S, Meirmans PG, Aitken SN, Ritland K, Isabel N. The challenge of separating signatures of local adaptation from those of isolation by distance and colonization history: the case of two white pines. *Ecol Evol*. 2016;6(24):8649–8664.

Nosil P, Funk DJ, Ortiz-Barrientos D. Divergent selection and heterogeneous genomic divergence. *Mol Ecol*. 2009;18(3):375–402.

Oyler-McCance SJ, Fedy BC, Landguth EL. Sample design effects in landscape genetics. *Conserv Genet*. 2013;14(2):275–285.

Palsbøll PJ, Bérubé M, Allendorf FW. Identification of management units using population genetic data. *Trends Ecol Evol*. 2007;22(1):11–16.

Palumbi SR. Genetic divergence, reproductive isolation, and marine speciation. *Annu Rev Ecol Evol Syst*. 1994;25(1):547–572.

Parmesan C. Ecological and evolutionary responses to recent climate change. *Annu Rev Ecol Evol Syst*. 2006;37:637–669.

Perez MF, Franco FF, Bombonato JR, Bonatelli IAS, Khan G, Romeiro-Brito M, Fegies AC, Ribeiro PM, Silva GAR, Moraes EM. Assessing population structure in the face of isolation by distance: are we neglecting the problem? *Divers Distrib*. 2018;24(12):1883–1889.

Peters JL, Lavretsky P, DaCosta JM, Bielefeld RR, Feddersen JC, Sorenson MD. Population genomic data delineate conservation units in mottled ducks (*Anas fulvigula*). *Biol Conserv*. 2016;203:272–281.

Petkova D, Novembre J, Stephens M. Visualizing spatial population structure with estimated effective migration surfaces. *Nat Genet*. 2016;48(1):94–100.

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet*. 2006;38(8):904–909.

Prince DJ, O'Rourke SM, Thompson TQ, Ali OA, Lyman HS, Saglam IK, Hotaling TJ, Spidle AP, Miller MR. The evolutionary basis of premature migration in Pacific salmon highlights the utility of genomics for informing conservation. *Sci Adv*. 2017;3(8):e1603198.

Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics*. 2000;155(2):945–959.

Rellstab C, Gugerli F, Eckert AJ, Hancock AM, Holderegger R. A practical guide to environmental association analysis in landscape genomics. *Mol Ecol*. 2015;24(17):4348–4370.

Rosenberg NA, Mahajan S, Ramachandran S, Zhao C, Pritchard JK, Feldman MW. Clines, clusters, and the effect of study design on the inference of human population structure. *PLoS Genet*. 2005;1(6):e70.

Rushing CS, Ryder TB, Scarpignato AL, Saracco JF, Marra PP. Using demographic attributes from long-term monitoring data to delineate natural population structure. *J Appl Ecol*. 2016;53(2):491–500.

Ryder OA. Species conservation and systematics: the dilemma of subspecies. *Trends Ecol Evol*. 1986;1(1):9–10.

Salemi M, Vandamme A-M. *The phylogenetic handbook: a practical approach to DNA and protein phylogeny*. Cambridge (UK): Cambridge University Press; 2003.

Schwartz MK, McKelvey KS. Why sampling scheme matters: the effect of sampling scheme on landscape genetic results. *Conserv Genet*. 2009;10(2):441–452.

Semenov GA, Koblik EA, Red'kin YA, Badyaev AV. Extensive phenotypic diversification coexists with little genetic divergence and a lack of population structure in the White Wagtail subspecies complex (*Motacilla alba*). *J Evol Biol*. 2018;31(8):1093–1108.

Serre D, Pääbo S. Evidence for gradients of human genetic diversity within and among continents. *Genome Res*. 2004;14(9):1679–1685.

Shirk AJ, Cushman SA. Spatially-explicit estimation of Wright's neighborhood size in continuous populations. *Front Ecol Evol*. 2014;2(62):1–12.

Shuford WD, Gardali T. California bird species of special concern: a ranked assessment of species, subspecies, and distinct populations of birds of immediate conservation concern in California. *Stud West Birds*. 2008;1:332–339.

Spies I. Landscape genetics reveals population subdivision in Bering Sea and Aleutian Islands Pacific cod. *Trans Am Fish Soc*. 2012;141(6):1557–1573.

Spies I, Spencer PD, Punt AE. Where do we draw the line? A simulation approach for evaluating management of marine fish stocks

with isolation-by-distance stock structure. *Can J Fish Aquat Sci*. 2015;72(7):968–982.

Tucker JM, Schwartz MK, Truex RL, Wisely SM, Allendorf FW. Sampling affects the detection of genetic subdivision and conservation implications for fisher in the Sierra Nevada. *Conserv Genet*. 2014;15(1):123–136.

Turbek SP, Browne M, Di Giacomo AS, Kopuchian C, Hochachka WM, Estalles C, Lijtmaer DA, Tubaro PL, Silveira LF, Lovette IJ, et al. Rapid speciation via the evolution of pre-mating isolation in the Iberá Seedeater. *Science*. 2021;371(6536):eabc0256.

Veith M, Beer N, Kiefer A, Johannesen J, Seitz A. The role of swarming sites for maintaining gene flow in the brown long-eared bat (*Plecotus auritus*). *Heredity*. 2004;93(4):342–349.

Vredenburg VT, Bingham R, Knapp R, Morgan JAT, Moritz C, Wake D. Concordant molecular and phenotypic data delineate new taxonomy and conservation priorities for the endangered mountain yellow-legged frog. *J Zool*. 2007;271(4):361–374.

Waples RS. Pacific salmon, *Oncorhynchus* spp., and the definition of "species" under the Endangered Species Act. *Mar Fish Rev*. 1991;53(3):11–22.

Waples RS, Lindley ST. Genomics and conservation units: the genetic basis of adult migration timing in Pacific salmonids. *Evol Appl*. 2018;11(9):1518–1526.

Whitlock MC, Lotterhos KE. Reliable detection of loci responsible for local adaptation: inference of a null model through trimming the distribution of F_{ST}. *Am Nat*. 2015;186(S1):S24–S36.

Wilder AP, Palumbi SR, Conover DO, Therkildsen NO. Footprints of local adaptation span hundreds of linked genes in the Atlantic silverside genome. *Evol Lett*. 2020;4(5):430–443.

Wright S. Isolation by distance. *Genetics*. 1943;28(2):114–138.

Xuereb A, D'Aloia CC, Andrello M, Bernatchez L, Fortin M-J. Incorporating putatively neutral and adaptive genomic data into marine conservation planning. *Conserv Biol*. 2021;35(3):909–920.