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Opinion

Where to now with the evolutionarily significant unit?

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The designation of units for conservation has been a necessary but challenging objective since conservation efforts began. Most species are divided, typically by environment, into populations with independent evolutionary trajectories. There are practical conservation objectives for defining these boundaries. Separate genetic clusters provide future evolutionary potential as environments change, and individuals in isolated populations may lose fitness when population size is reduced. The history of the effort to define units is briefly reviewed here, but I focus on finding a process that may facilitate uniform and effective application at a time when conservation urgency is great. I propose a refinement of the designated unit concept, distinguishing between conservation units (CUs) and evolutionarily sustaining conservation units (ESCUs).

Highlights

The history of the evolutionarily significant unit has deep roots, back to our appreciation that what we defined as species were often themselves divided into separate units with some degree of isolation.

There are many approaches for what is generally recognized as a common objective, the identification of intraspecific groupings that require separate designation for effective conservation.

Some definitions of the evolutionarily significant unit have received greater recognition than others, and interpretation can be varied leading to varied outcomes.

I propose a process that first identifies conservation units broadly, and then recognizes those that are divergent beyond defined thresholds based on quantitative assessment. The process can be facilitated in a four-step decision tree.

History of the evolutionarily significant unit

At a meeting of the American Association of Zoological Parks and Aquariums in Philadelphia 38 years ago, the delegates discussed the thorny issue of subspecies, and more generally variation within named species [1]. Linnaeus recognized varieties within species in the 18th century, although he did not classify them [2]. Darwin [3] also recognized varieties, and thought of variation as a continuum, predicting more variety within species when there were more species in a lineage [4]. The term subspecies was introduced in 1872 [5]. The wider context of the meeting was the establishment of species survival programs. For example, at the time there were seven named subspecies of black rhino (*Diceros bicornis*) – which ones should they conserve? Parks and aquaria have limited space and resources, and so how should decisions about prioritization be made? Another part of the discussion was the lack of precision and comparability for subspecies designation, even including a story about how littermates may have once been erroneously identified as separate subspecies [1]!. Ultimately, the conference members agreed to introduce a new term, the evolutionarily significant unit (ESU). The objective was to better characterize the range of diversity found among conspecific populations, based on various metrics including natural history, morphometrics, distribution range and genetics. The idea was evidently inspiring, because many different approaches have been proposed for the identification of an ESU (see examples in Box 1).

Discussion of ESU designation and legislation

The foundations that made an ESU especially influential were laid when the Endangered Species Act (ESA) was passed in the USA (1973). The ESA (as amended in 1978) states that '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.' (16 U.S. Code § 1532 – Definitions). Similar legislation in Australia (Endangered Species Protection Act 1992; replaced by Environment Protection and Biodiversity Conservation Act in 1999) and Canada (Species at Risk Act, 2002) followed. In Canada a designatable unit represents a named subspecies or variety identified in accordance with the Committee on

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Box 1. Evolving definitions of the ESU

Ever since the term was proposed there have been attempts to interpret, clarify, or extend its definition, and consider its application. Here are listed proposed characteristics, and the dates when the ideas were first published.

1986: Ryder [1]: concordant evidence (such as morphology plus genetics).

1991: Waples [6,7,43]: substantial reproductive isolation that represents an important component in the evolutionary history of the species. Emphasizes importance of both isolation and adaptation.

1992: Dizon *et al.* [47]: discontinuous, significant genetic divergence, and geographic isolation.

1994: Moritz [8,9]: binary approach based on reciprocal monophyly at mtDNA markers, and significant differentiation at nuclear markers. The later paper contrasts his interpretation with that of Crandall *et al.* [11] and emphasizes the need to incorporate the idiosyncratic features and needs of individuals species.

1994: Vogler and DeSalles [48]: ESU should have a wholly unique heritable trait.

1994: Avise [49]: populations derived from congruent gene phylogenies at multiple markers.

1998: Bowen [50]: populations that have been in isolation for a long time.

1999: Paetkau [51]: emphasizes the importance of drawing from a range of factors, both genetic and non-genetic.

2000: Crandall *et al.* [11]: ecological and genetic exchangeability together with isolation for a sufficient period of time. Defined categories based on genetic or ecological, recent or historical isolation.

2001: Fraser and Benachez [10]: adaptive evolutionary conservation – incorporating the ideas from past definitions as they best fit the focal species.

2007: De Guia and Saitoh [45]: full and partial ESU, with a full ESU requiring differentiation for both neutral and adaptive variation.

2012: Funk *et al.* [15,16]: emphasized the importance of incorporating emerging genomic methodologies.

2013: Casacci *et al.* [52]: importance of dynamic biodiversity parameters and the incorporation of both molecular and nonmolecular data.

2018: Coates *et al.* [46]: emphasizes the role of hybridization and interlineage gene flow (and the contribution of genomic methods).

2019: Quiroga *et al.* [53]: molecular evidence from phylogenetically independent taxa delimiting biogeographically significant units.

2023: Molinari [54]: a matrix integration of molecular and morphological characters.

the Status of Endangered Wildlife in Canada (COSEWIC). Under the Wildlife Protection Act in India (1972) subspecies are sometimes included, which is also the case for the EU Habitat Directive, which also discusses the protection of populations [see https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=PI_COM:C(2021)7301]. Other countries have focused on lists of protected named species (e.g., legislation in China in 1989, and South Africa in 2005), typically without listing subspecies or incorporating the ESU concept.

For the ESA (USA), the link between distinct population segments (DPSs) and an ESU was established in [6,7], which stated that ‘A population (or group of populations) will be considered “distinct” (and hence a “species”) for purposes of the ESA if it represents an evolutionarily significant unit (ESU) of the biological species.’ Waples [6,7] further stated that an ESU must be ‘substantially reproductively isolated from other conspecific population units’ and ‘represent an important component in the evolutionary legacy of the species’. He emphasized the importance of establishing

Glossary

Admixture: mixture of the alleles from one population in the gene pool of another population.
Assignment: clustering of individuals into populations based on certain criteria (such as compliance with equilibrium expectations).
Bayesian posterior probability distribution: distribution of likelihood outcomes in the context of the Bayesian analysis.
Census population size (Nc): number of individuals of the target species in the population.
Common garden experiments: quantifying the genetic component of phenotypic variation by raising organisms in a shared environment.
Effective population size (Ne): size of an ideal (no evolutionary forces impacting allele frequency) population that would show the same rate of loss in diversity as the observed population.
Genetic drift: random changes in allele frequencies over time due to stochastic processes.
Island model: Sewall Wright’s model that assumes equal subpopulation size and equal rates of migration among all subpopulations and bases evolutionary inference on those assumptions.
Ordination: representation of individuals in Euclidean space based on their genotypes.
Phenotypic plasticity: potential for a given genotype to produce different phenotypes under different environmental conditions. Can be driven by epigenetic modification of gene expression.
Positive selection: directional natural selection increasing the frequency of the relevant allele.
SNPs: single base-pair variation among individuals in a population.
Structural variation: genetic variation in the genome affecting multiple base pairs caused by translocation, transposition, inversion, expansion, or contraction of repetitive elements.

reproductive isolation using all available lines of evidence, and in the context of the species as a whole. From that point on, the ESU became an essential part of the process of endangered species conservation in the USA, and to a large extent, elsewhere in the world. However, the definition of an ESU often uses language like 'substantial' and 'important' which makes consistent designation challenging. A few years after the ESU was introduced, Moritz [8,9] proposed an approach that was essentially binary (Box 1), based on reciprocal monophyly of mitochondrial (mt)DNA, although others raised issues with the dependence on mtDNA [7,10,11]. The method also required significant differentiation at nuclear genetic markers, although interpretation would depend on resolution (number of markers and level of diversity). An important advantage was that decisions could be made based on an established set of rules.

However, Crandall *et al.* [11] were concerned about forcing a dichotomy (ESU or not) in a system that is continuous by nature, the use of phylogenetics to the exclusion of other metrics, and the possibility that high resolution genetic markers may lead to ESUs being defined among functionally equivalent groups. Their main concern was the focus on isolation and **genetic drift** (see Glossary), without an explicit consideration of adaptive differences. They suggested instead a focus on ecological and genetic exchangeability (Box 1), although fitting populations to the different proposed categories requires further tests, assessment, and judgement (including binary decisions). By the start of the 21st century, many embraced a process that integrated both genetic and phenotypic data, considered the whole species context (all extant populations), and then assigned ESU status if that seemed appropriate or needed. The practical application was often guided by the process initially described in [6].

A proposal for conservation unit designation

Rationale

Essential to the utility of an ESU metric is a clear understanding of the objective. Proximately, this is the identification of populations or groups of populations that are evolving independently to manage as independent units for effective conservation. Ultimately, the objective is to preserve evolutionary potential and promote the survival of populations and species. An ESU concept should remain separate from taxonomy and focused on conservation. The process of identifying conservation units needs to be as efficient as possible and widely agreed. As Michael Soulé noted in 1985, conservation biology is driven by crisis [12]. The identification of the conservation unit is an integral part of the broader process of assessing conservation priority. Genetics can help with questions about diversity, effective size, demographic trajectory, and connectivity. Those data can then be integrated with information about present and future risks, ecology and ecosystem services.

Proposal

Therefore, I propose a revision of the ESU concept that takes on board the following features. First, the decisions made to identify conservation units are binary and based on quantitative measures. Although it is true that natural systems are more likely to be continuous [11], the decision itself is binary (to conserve or not), sequential binary choices can usefully partition a continuous pattern, and binary systems provide clear inference for conservation managers and policy makers. Second, data on genetic differentiation should be assessed sequentially, first on the basis of neutral, and then on adaptive variation [13,14], and including genomic methods whenever appropriate [15–17]. Neutral variation is considered first because neutral differentiation implies isolation (after which phenotypic differences likely follow). Proxies for adaptation may often remain necessary (e.g., when phenotype is determined by many small-effect loci making their identification difficult), but should be defined quantitatively and differences tested for significance. As technologies develop, the incorporation of **structural variation** in the genome should be considered when this determines local adaptation [18]. For example, a large chromosomal inversion has led to local adaptive differentiation in the common qual (*Coturnix coturnix*) [19]. Third, aspects of the broader conservation considerations should be integrated into the decision tree (such as origins and demography). Fourth, the initial identification of CUs should be separate from more stringent designation (e.g., ESU). Instead of ESU, I propose adopting the term evolutionarily sustaining conservation unit (ESCU,) in part to reflect the two-step process. I would change ‘significant’ to ‘sustaining’ because the objective is to conserve established demographic units on independent evolutionary trajectories. A CU is similar to a management unit (MU) [8], while an ESCU is similar to an ESU. Both, however, are based on a broader set of characteristics, as defined in Box 2.

Decision tree

The proposed decision tree (Boxes 2 and 3) begins with an assessment of origins (Step 1). Some populations are known to be invasive or unintentionally introduced, and in those cases management may more likely be about eradication. Some may be mixed populations or unknown introductions, in which case further analysis (e.g., testing for **admixture** or **assignment** to determine origin) could be required and usefully use genomics. Step 2 is an assessment of the **effective population size (Ne)**, trajectory, and the relationship between Ne and the **census population size (Nc)**. Ne assessment benefits from nuclear genomic data sufficient for robust resolution (~1000 or more **SNPs**). Various methods are then possible (e.g., using demographic modeling appropriate to the timeframe of interest) [20–23]. Most genetic methods assume panmixia (a single population), so putative populations are treated independently. The important conservation context is threefold: (i) populations with smaller Ne lose diversity more quickly; (ii) a larger Ne means selection is stronger and beneficial alleles are less likely to be lost to drift; and (iii) some populations may have a small Ne/Nc ratio [24], and therefore, census counts can give false security about the conservation health of those populations. A meta-analysis [25] suggested that the median Ne/Nc ratio is about 0.1, and hence the threshold proposed in the decision tree (Box 2). I suggest that a large, stable population would usually not be worth conservation investment, but this depends on the Ne/Nc ratio, and smaller populations or those in decline should be considered.

A CU or ESCU would then be designated when appropriate thresholds could be demonstrably passed. It could be argued that under the precautionary principle, independent CU management is generally appropriate when diversity is differentiated among populations at any level (F_{ST} significantly greater than zero, separate **ordination** or assignment clusters), since local diversity may become important as environments change, and connectivity can be lost. Management needs can be judged on the extent of differentiation, noting that patterns of connectivity can change over time and units become more or less differentiated. At the same time, high power can resolve very small differences. Therefore, marker number should be limited to avoid supporting very small F_{ST} values that are not biologically meaningful (Box 2). This method is based on rejecting panmixia, which has been commonly applied. An alternative of assessing demographic independence [26] would be facilitated by using genetics to track past demography [21] or F_{ST} threshold estimates based on the **island model** [26], however independent demography can sometimes be sustained with migration rates too high for genetic differentiation [27].

For loci under selection, outlier loci that are divergent from neutral expectations and suggest **positive selection** should be identified. This can be done using various methods [28–32]. Loci may be outliers because they are under positive selection or strong drift. Comparing the pattern of population structure seen for outlier loci with that found at neutral loci (e.g., using ordination methods) may show the same pattern, less or no structure due to reduced power (fewer outlier than neutral loci), a stronger version of the same pattern (possibly due to strong drift or selection

Box 2. Decision tree for conservation unit designation

The main point of the decision tree (Figure I) is to provide a sequence of choices that permits logical progression, inclusive of the considerations to be applied for conservation designation. Depending on the strength of the differences, the final classification can be either a CU or a more stringent ESCU. The process follows four steps (Figure I).

Step 1: consider origins

If the species is a non-native invasive, and not endangered or extirpated in its native range (in which case translocation may be appropriate), then it need not be a conservation priority. Proceed through the decision tree if it is native or re-introduced.

Step 2: population demography

Large populations need not be a conservation concern unless they have historically been greatly reduced in number or their diversity is reduced by some other process. Therefore, there are separate parts to this assessment. It is ideally based on estimates of both Ne and Nc. Proceed to the next step if Ne is <1000 (see [55]), which would be Nc = 10 000 if Ne/Nc = 0.1 [25]. If Ne/Nc is <0.1 then Nc could be larger and the population should still be conserved. Populations clearly in decline should also be conserved.

Step 3: differentiation by drift

This refers to differentiation that is seen across multiple, presumably neutral genetic markers, and is the first step that can distinguish CU from ESCU. For a CU, the threshold could be any significant differentiation (e.g., $F_{ST} \neq 0$) when there are ≤5000 nuclear markers (see main text). However, the most appropriate threshold may depend on Ne in each population, with high Ne leading to lower F_{ST} values. When Ne is small, many fewer SNPs may be sufficient to show clear differentiation. Ordination can help identify separate clusters. For an ESCU, an estimate of the number of migrants per generation (Nm) should be <1, or the confidence intervals for the estimate should overlap with one (see Box 3 in the main text).

Step 4: differentiation by selection

For a CU it is appropriate to use proxies for selection, such as consistent phenotypic differences or genetic inference (e.g., see Box 3 in the main text). When possible, the extent to which phenotypic differences are heritable or environmentally driven (plastic) differences should be assessed, for example, by using common garden experiments [35,56] or genome association studies (but, see [35]). For an ESCU, in the absence of differentiation by drift, there should be a fixed genomic difference clearly associated with adaptive differentiation or a strong phenotypic difference that can be shown to not be wholly due to phenotypic plasticity.

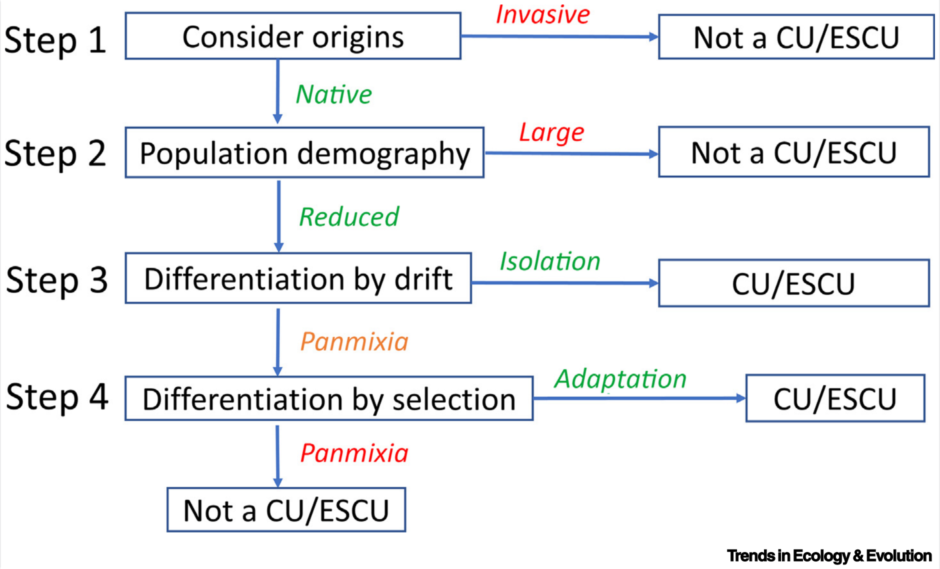

Figure I. Decision tree for determining conservation unit (CU) and evolutionarily sustaining conservation unit (ESCU) designation.

Box 3. Case studies of CU and ESCU designation

(i) Killer whales (*Orcinus orca*) in the Northeast Pacific (focusing on the southern resident population) (Figure I). Regional populations in the North Pacific are divided into two main ecotypes, feeding on fish or marine mammals [57]. This is not an invasive species in this habitat (Step 1), so proceed to the next step. Estimates of Ne (Step 2) suggest a small population size of Ne = ~100 [58]. Estimates of divergence at neutral markers (Step 3) were high and significant against all six population comparisons in the North Pacific (F_{ST} = 0.067–0.162) [58]. Therefore, the CU threshold is passed. Using the isolation with migration model, it was found that the number of migrants per generation overlapped with Nm = 1 for all comparisons [58] (Figure I), so designation as an ESCU would be appropriate, and the process can stop at this step. Note that this population has been designated as a Discrete Population Segment under the US Endangered Species Act (so equivalent to an ESU), and later studies using genomics [59–61] support the earlier assessment and add inference about local adaptation. Posterior distributions from the Bayesian analysis in the isolation with migration model [38] are shown [58]. Strong population structure is seen among other Delphinid (dolphin) species as well, comparable with that seen in some terrestrial mammals [62].

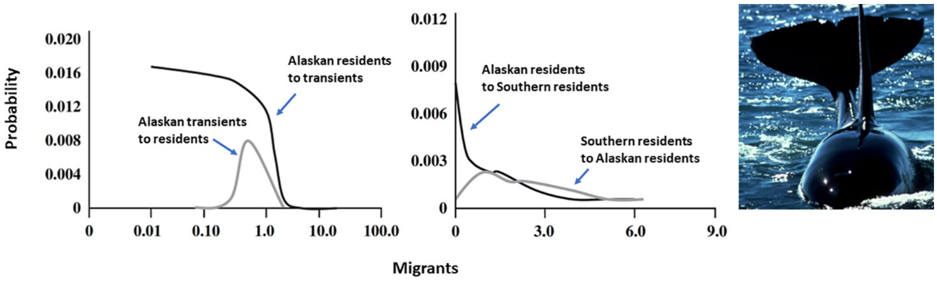

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Figure I. Posterior distributions for Bayesian estimations of directional migrants per generation between resident (fish eating) or transient (marine mammal eating) killer whale populations [58]. Photo of killer whale is by A.R. Hoelzel.

(ii) Orange roughy (*Hoplostethus atlanticus*) in the North Atlantic. This deep water fish species is historically taken in large numbers, breeding over sea mounts throughout its global distribution. The example assesses the population at Faraday Seamount, North Atlantic. This is a native species (Step 1) with a small estimated Ne/Nc ratio (Step 2) [33]. Based on 3756 neutral SNP loci, all F_{ST} values are nonsignificant (Step 3). Outlier loci are identified, putatively under selection, and ordination tests show greater divergence at Faraday for these loci than for putatively neutral loci (Figure II) [33], although the precise functional locus or loci are not identified. Therefore, at Step 4 it is possible to identify the population at Faraday Seamount as a CU, and further research may establish it as an ESCU.

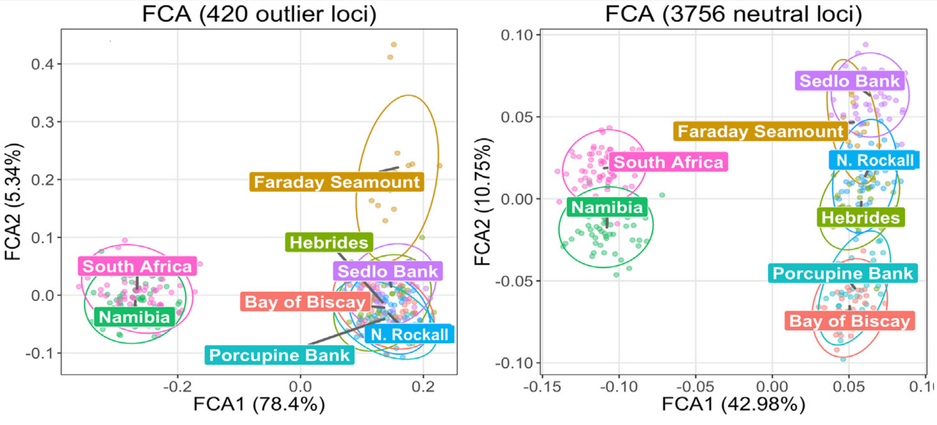

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Figure II. Ordination of samples from Atlantic locations based on loci putatively under selection (outliers) or neutral loci (after [33]). Abbreviation: FCA, factorial correspondence analysis.

at some or all outlier loci), or a distinctly different pattern (e.g., see [33] (Box 3). The latter is less likely to be associated with genetic drift and should be sufficient to identify a population that needs to be managed as a CU due to differential selection. In some cases, it may be possible to identify relevant loci, but without knowing the function [34], although using whole genomes can facilitate this. If differentiated phenotypic distributions are used to define CUs, a heritable genetic component should be established (e.g., through **common garden experiments** when feasible – ruling out differentiation solely due to **phenotypic plasticity**) and relevant loci or structural genomic differences identified when possible. The challenges of confirming heritability and the continuing utility of common garden experiments in the genomic age are described in [35].

The next step would be to identify populations that qualify as an ESCU. This more stringent level is equivalent, for example, to a DPS in the USA (under the ESA) or a designatable unit in Canada (under COSEWIC). For neutral differentiation, the key metrics are Ne and gene flow (m). Based on the theoretical construct of an island model [36], panmixia should exist when Nm (number of migrants) >1, and equilibrium diversity [4Nm/(1+4Nm)] should be equivalent in all populations. However, real populations may behave differently (many published examples where differentiated populations show estimated Nm >1). Furthermore, the estimation of Ne can be imprecise and potentially violate assumptions (e.g., that F_{ST} is at equilibrium; [37]). It would also be best to consider gene flow in a temporal context, since the continuous regular transfer of genes at a low level could look similar to the recent cessation of gene flow, but the conservation inference may be different. Alternatively, recent fragmentation and isolation may not yet have resulted in strong genetic differentiation. For these questions one could apply isolation with migration models, for which 100–300 SNP loci would be sufficient [38,39] (Box 3). Based on this method I define an ESCU for neutral variation as either a population with no gene flow [estimated **Bayesian posterior probability distribution** (PD) for the time of separation does not overlap with zero, and PD for migrations does overlap with zero], or where the PD for Nm <1 or overlaps with 1 (even if the peak for PD is >1).

If the evidence of differentiation due to genetic drift is weak or absent, then the evidence based on adaptive variation would need to be strong to support an ESCU (Box 2). Fixed genetic differences where the relationship between genotype and phenotype is known would likely suffice, but this information is still rare. This raises the question of selection at one or a few loci, like dark and light pigmentation in oldfield mice (*Peromyscus polionotus*) driven by changes at the MC1R and Agouti loci [40] and industrial melanism in peppered moths (*Biston betularia*; a transposon insertion in the *Cortex* gene) [41]. Another example is Müllarian mimicry in the red postman butterfly (*Heliconius erato*) where wing pigment patterns, based on large-effect loci, vary among populations [42]. Each of those examples are ultimately driven by predator avoidance, but there are also other drivers [43]. In the cases I have listed, the adaptive value is relatively easy to identify (i.e., protection from predation), but this is harder in many cases [43]. For ecotypes of the spinner dolphin (*Stenella longirostris*) off southern California, there is extensive gene flow based on neutral markers, but also strong phenotypic differences and fixed genomic differences associated with social behavior [44].

Concluding remarks

The essential idea is a framework based on a decision tree and the utility of genomic methods [45,46]. Whether the system fits a CU or/and ESCU can be assessed quantitatively, although as many have pointed out in the past, all systems are to some degree idiosyncratic. The decision tree can provide a framework and a basis for discussing exceptions (e.g., when the importance of plasticity versus genetic differentiation is uncertain (see Outstanding questions). At the same time, there is an increasing urgency and limited resources. We need to ensure that the funding and

Outstanding questions

As technologies develop and more species are sequenced for high quality genomes, resequencing becomes more practical, and information on the structural variation of genomes becomes accessible, how do we ensure that current methods for the identification of units in need of conservation management continue to be effective?

What role may environmental DNA play in the identification of the range limits of endangered species and populations, and understanding how their distributions have changed over time?

The identification of differentiation due to adaptation is challenging, especially since phenotypic variation may be based in part or wholly on plastic changes (such as those driven by epistatic changes in gene expression in response to environmental conditions). How do we ensure that conservation resources are used to protect diversity that is not entirely plastic?

How do we deal with adaptive changes based on many loci each of small effect? If we develop the means to effectively identify the genomics of these traits, should we default to that instead of accepting distinct CU or ESCU designations based on phenotype?

I suggest that we should exclude introduced species that are or have been invasive, but what if the introduction was long ago and the species in question is now an integral part of the local ecosystem? Should we reconsider if the loss or lack of effective management of those species will be damaging to ecosystem function?

How can we best unify strategy and interpretation so that the mechanism for identifying units to conserve is uniformly applied and accepted globally, and resilient to challenges (legal or otherwise)?

effort put into protecting species is robust (facilitated by available methods), effective, and conserves biodiversity required for evolution into the future.

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Declaration of interests

No interests are declared.

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