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**Forum**

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# A practical framework for identifying genetic subpopulations and ESUs: Insights for IUCN assessments and broader management

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## Abstract

**Species conservation assessments evaluate extinction risk, and recovery potential, advancing species persistence through guiding resource prioritization and planning. Assessment frameworks, including the International Union for Conservation of Nature Red List and Green Status of Species, typically focus on species as a whole. Importantly, they do not routinely account for genetically distinct units or do not have standardized methods of unit delineation. This limits the representation of genetically distinct components, including adaptive genetic diversity that underpins long-term resilience and recovery. Incorporating standardized within-species units like subpopulations and Evolutionarily Significant Units (ESUs) into species assessments could help address this oversight. However, identifying and delineating such units remain challenging, particularly when molecular data are limited. Here, we propose a flexible framework that integrates molecular and non-molecular evidence to identify both subpopulations and ESUs across taxa, providing a practical tool to incorporate within-species diversity into conservation assessments.**


***Keywords*** **conservation policy, Evolutionarily Significant Unit, genetic diversity, IUCN species assessments, subpopulations**

**Box 1:** Key terms.

CBD, the United Nations Convention on Biological Diversity, is the foremost authority and key international treaty on biodiversity, which includes explicit commitments and agreements to conserve and monitor biodiversity (https://www.cbd.int/). The Kunming-Montreal Global Biodiversity Framework (KMGBF) is a recent agreement adopted in December 2022 with commitments from 196 signatory nations. The agreement provides a pathway for halting and reversing global biodiversity loss. Importantly, genetic diversity is explicitly recognized in this agreement as a core component of biodiversity that must be conserved, monitored, and reported.

Evolutionarily Significant Units (ESUs) are lineages demonstrating highly restricted gene flow from other such lineages within the higher organizational level of species (Fraser and Bernatchez 2001). Due to limited gene flow, these genetic units follow their own evolutionary trajectories and thus are likely to house unique adaptive genetic diversity (Funk et al. 2012; figure 1c).

A population within the IUCN Red List framework is defined as the total number of individuals of the taxon (i.e., species or subspecies) (IUCN 2001; figure 1a), which differs from its common biological usage (IUCN 2003). Due to the multiple definitions of this term, we have avoided it in the remainder of this manuscript.

Subpopulations in the IUCN Red List framework are geographically or otherwise distinct groups in a population between which there is little demographic or genetic exchange (typically one successful migrant individual or gamete per year or less (IUCN 2001; figure 1b).

## Why list subpopulations and Evolutionarily Significant Units in IUCN assessments?

Genetic diversity is the foundational level of biodiversity but remains rarely considered in global conservation programs (e.g., neglected in protected area design, Paz-Vinas et al. 2025; species recovery plans, Pierson et al. 2016; in IUCN assessments, Schmidt et al. 2023; and in recent policy like the European Union Nature Restoration Law, O'Brien et al. 2025). Though the recently adopted United Nations Convention on Biological Diversity (CBD; box 1) Kunming-Montreal Global Biodiversity Framework (also known as the KMGBF) includes explicit commitments with two key genetic indicators that aim to conserve and monitor genetic diversity (Target 4; CBD 2022, da Silva et al. 2026), a greater integration into other conservation initiatives could help to address the decline of global genetic diversity.

Irreversible allelic diversity and heterozygosity loss have been documented across hundreds of species (Leigh et al. 2019, Shaw et al. 2025). Human activities are frequently leading to the extirpation of entire subpopulation networks (Ceballos et al. 2017, Mastretta-Yanes et al. 2024), which likely harbored distinct alleles and possibly local adaptations, representing a loss of unique evolutionary trajectories. Genetic diversity underpins fitness, resilience, and adaptive potential (Reed and Frankham 2003, Swindell and Bouzat 2005, Hughes et al. 2008, Harrisson et al. 2014, Frankham 2015, DeWoody et al. 2021, Kardos et al. 2021, Meek et al. 2023, van Oosterhout et al. 2025), and its erosion is closely linked with species extinction risk through reduced capacity to respond to environmental change or disease threats (Frankham 2005, Evans and Sheldon 2008, Polishchuk et al. 2015). Importantly, demographic recovery of a population does not imply genetic recovery. Lost genetic variation may remain depleted for many generations, highlighting the need to maintain distinct subpopulations and networks of distinct subpopulations known as "Evolutionarily Significant Units" (ESUs; Nei et al. 1975, Frankham 2005, Allendorf et al. 2022). Thus, losses in genetic diversity represent a threat to all levels of biodiversity.

The International Union for Conservation of Nature (IUCN) and its Red List of Threatened Species (a catalogue of over 163,000 species) is the international standard in assessing the extinction risk of species based on the best available information. The Red List forms the basis for the A.3 headline indicator within the KMGBF, the Red List Index, which measures trends in the extinction risk of species over time to assess progress toward halting human-induced extinctions (CBD 2022). Extinction risk is calculated on the occupation of the focal species' historical range, census size and trends, habitat quality, and fragmentation level (IUCN 2001), but genetic diversity is not formally included in assessment criteria (Schmidt et al. 2023, van Oosterhout 2024). Although such frameworks provide scope to include genetic information, this aspect of biodiversity, which is critical for species' fitness, adaptive potential, and long-term extinction risk, remains underrepresented. As a result, Red List conservation status is not directly correlated with remaining levels of genetic diversity or magnitude of loss (Leigh et al. 2019, Mastretta-Yanes et al. 2024, Shaw et al. 2025). In the more recently established IUCN Green Status of Species (IUCN 2021), recovery of pre-impact census size is assessed as a measure of conser-

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vation success. Although genetic diversity is not explicitly assessed, it is sometimes discussed in Green Status assessments. In some cases, species with substantial, irreversible genetic diversity loss have received high Green Status recovery scores, despite persistent risk of inbreeding and limited adaptive capacity (e.g., Alpine ibex, *Capra ibex*; Brambilla et al. 2024). Though IUCN conservation status (i.e., extinction risk and recovery status) does not directly prescribe protection or species management, greater consideration of genetic diversity could help to identify important groups in need of protection that could help mitigate genetic erosion. In turn, incorporating genetically meaningful within-species units into IUCN assessments can help support the Red List and Green Status's main goals by ensuring a more accurate extinction risk and recovery is obtained, which is particularly pertinent for species with strong demographic independence across intraspecific units.

The two recently adopted genetic diversity indicators in the KMGBF provide access to genetic diversity assessment without necessarily requiring molecularly derived information (Mastretta-Yanes et al. 2024, Hoban et al. 2025). The "headline" indicator Ne500 reflects trends related to effective population size, and the "populations maintained" indicator reflects whether populations themselves are being retained across a species' range (CBD 2022). Both indicators track the maintenance of genetic diversity by assessing and monitoring key evolutionary processes, such as the persistence of subpopulations, connectivity between them, and the retention of adaptive variation across species. In practice, this means that even in the absence of genome-wide data, information from ecological surveys, demographic records, and well-documented populations can be used to infer whether genetic variation is being preserved and whether evolutionary processes are likely to continue. Adopting a similar approach within IUCN assessment criteria is a potential way to include genetic concerns without the need for scarce molecular data (less than 1.5% IUCN Red List assessed species have accessible molecular data, Paz-Vinas et al. 2025). Building on the KMGBF approach, the framework presented here offers a method to identify within-species units at scales relevant for evolutionary processes, which could inform assessments, within the IUCN and more broadly, to highlight units that contribute to overall genetic diversity.

Conservation-relevant evolutionary processes can be categorized into processes that distribute existing genetic diversity (gene flow and genetic drift) and processes that create or remove diversity (mutation and adaptation) (figure 1). Subpopulations, as defined under the IUCN, are akin to the common biological usage of "populations" (refer to key terms in box 1) and partially correspond to the units used for genetic indicators within the KMGBF (figure 1b; refer to Mastretta-Yanes et al. 2024). Gene flow and genetic drift shape the genetic structure of species over relatively few generations across subpopulations by redistributing genetic diversity. Processes like environmental adaptation (i.e., response to natural selection) and the accumulation of alleles through mutation involve the creation of new genetic diversity and act out over a greater number of generations to shape the evolutionary trajectory of a species across networks of several subpopulations, defined as ESUs (Allendorf et al. 2022; figure 1c). Due to the actions of adaptation, ESUs can follow their own evolutionary trajectory and may occupy unique or different ecological niches from other units within their species.

Identifying and considering ESUs can help maintain evolutionary processes beyond individual units, supporting broader ecosystem function. However, we explicitly do not consider human-mediated

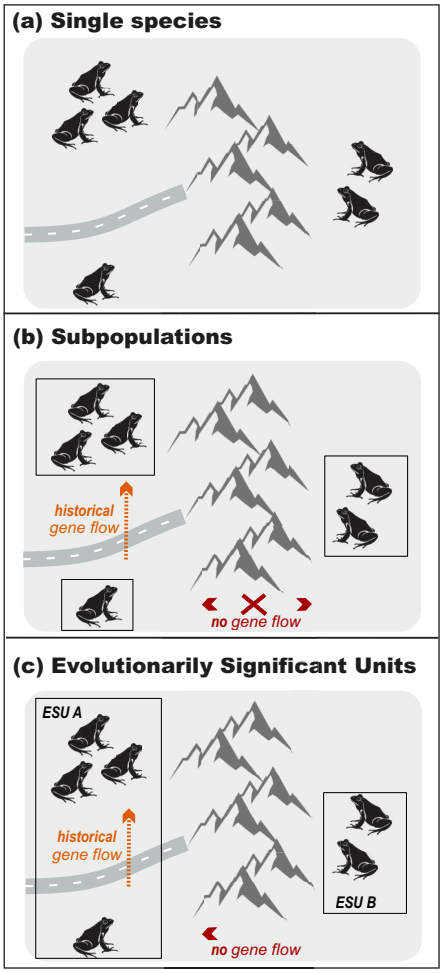



**Figure 1** Within the IUCN assessment guidelines, listing of distinct groups can occur at species (a), subspecies (not shown), or subpopulation (b) levels. We suggest the inclusion of Evolutionarily Significant Units (ESUs) (c). Note that one or several subpopulations together can form an ESU, and their grouping is determined by gene flow. In this schematic illustration, gene flow is depicted as being restricted between the hypothetical units [subpopulations (b) and ESUs (c)] by geographical barriers (mountains). The two subpopulations on the left side of the panel were historically linked by gene flow; although this was interrupted by a road, we do not consider them ESUs because the interruption of their gene flow is anthropogenic, and they have the same historical evolutionary trajectory thus they are one ESU. Alt text: Three-panel diagram showing conservation units. (a) A single species of frogs distributed on both sides of mountains. (b) Subpopulations separated by mountains, with no gene flow between left and right groups, but historical gene flow between two left-side groups despite a road barrier. (c) ESUs: left-side subpopulations grouped into one ESU due to shared history, and a separate ESU on the right, with no gene flow between them.

(e.g., selection for desirable phenotypes) or drift-driven change (e.g., due to human isolation) as valid evolutionary trajectories for ESUs.

The IUCN currently assesses species at three levels: species (figure 1a), subspecies, and subpopulations (figure 1b). Although ESUs (figure 1c) are not formally recognized as a separate unit of assessment within the Red List, they represent biologically meaningful within-species groups that are currently not reflected by these three existing levels (e.g., Cape parrot *Poicephalus robustus*, Coetzer et al. 2015 and leopard skink *Ctenotus pantherinus*, Prates et al. 2023). ESUs

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represent genetically distinct units, whereas subspecies often have little to no genetic basis and can reflect historical observations. Similarly, subpopulations delineate species at a finer scale than ESUs and do not reflect the important and valuable different evolutionary trajectories that can be present in a species. Subpopulations are a recognized unit, but fewer than 5% of species have them delineated on the Red List (Janet Scott, Programme Officer at IUCN, written communication, April 2024). This low uptake reflects the absence of a standardized methodology for defining subpopulations across taxa. Delineation is currently determined on a case-by-case basis by assessment teams and is often based on individual sites or locations rather than on demographically or evolutionarily independent units. As a result, subpopulation boundaries do not always have a clear biological basis. Importantly, although the IUCN Red List does not currently account for ESUs as a formal assessment unit (IUCN 2024), the IUCN Green Status of Species clearly recognizes ESUs as acceptable units for calculating recovery scores (IUCN 2021). Incorporating biologically meaningful subpopulations into both the Red List and Green Status frameworks, and ESUs into the Red List, as additional listing levels beyond those currently used in species assessments, could therefore provide insight into the structure and evolutionary potential of species, supporting more nuanced conservation prioritization and decision-making.

Conservation Units, including ESUs, have a longstanding history in scientific conservation assessments. The term ESU was first conceptualized by Ryder (1986), and subsequent studies have proposed varying criteria to define them as conservation-relevant units (overview in Fraser and Bernatchez 2001). However, challenges arise in standardizing ESU delineation due to the multidisciplinary nature of conservation science and differing approaches to genetic and ecological data (e.g., Moritz 1994, Crandall et al. 2000, Funk et al. 2012). For example, ecological factors such as habitat specialization or behavioral differences play a role in the divergence of ESUs and might be overlooked if genomic data alone are used to determine them. In turn, similar phenotypes that are underpinned by different genomic adaptations can also be overlooked if phenotypic data alone are used (Fenster and Dudash 1994). A holistic approach that integrates genetic and non-genetic metrics, including adaptive and ecological variation, has been recommended to improve comparability and relevance (Fraser and Bernatchez 2001, Robertson et al. 2014).

To support the consideration of both ESUs and subpopulations in IUCN and other conservation assessments, we present a comprehensive and flexible two-phase framework for improved subpopulation delineation (Phase 1) followed by standardized ESU identification (Phase 2). Our aim is to identify unique genetic units so that their passive loss can be mitigated. Enabling extinction risk assessments can help to reduce the loss of unique variation and prevent disruptions to evolutionary processes. Though we have developed our framework specifically to support IUCN assessments, we acknowledge that dividing species into subpopulations or ESUs could unintentionally affect downstream conservation management decisions. A recent meta-analysis found a strong historical tendency in conservation management to define units as genetically distinct through data misinterpretation and/or weak or no evidence (Liddell et al. 2021). These divisions have previously led to erroneously isolated units that unnecessarily increased extinction risk (e.g., the Eastern Barred Bandicoot *Perameles gunni*; Weeks and Rypalski 2021). We strongly stress that division of species into subpopulations or ESUs through any framework does not indicate a need to manage units in isolation (e.g., Senn et al. 2014) but rather could be used to help highlight unique units at risk of extinction and in need of protective actions (e.g., translocations; habitat restoration) to prevent irreversible genetic diversity loss or disruption of evolutionary processes. Risks and pitfalls concerning the designation of both subpopulations or ESUs are presented throughout the paper, with additional detail provided in the supplemental material Cost Benefits analysis, Section B.

## Subpopulation and ESU standardized framework

The framework we outline here for standardized identification of both subpopulations and ESUs has two phases: first, identifying genetically meaningful subpopulations and, second, grouping these into ESUs. Though we recognize the existence of several ESU frameworks (e.g., Fraser and Bernatchez 2001, Funk et al. 2012), a framework using multiple lines of evidence (both genetic and non-genetic) is necessary for objective and standardized identification of IUCN-relevant groups.

The null hypothesis for this framework is that all individuals within a species belong to a single genetic unit, that is, they form a single metapopulation, which in turn is one ESU. Deviations from this null hypothesis (manifested as genetic differentiation, biogeographic isolation, or adaptive divergence) indicate the presence of distinct subpopulations and/or ESUs. Accordingly, the null hypothesis serves as the baseline in each phase and must be explicitly tested in all assessments (the default first scenario).

In Phase 1 (identifying genetically meaningful subpopulations), subpopulations may reflect recent or human-mediated fragmentation, restricted gene flow, or local adaptation. Assessors must identify "candidate scenarios" representing different possible subpopulation groupings, with particular focus on those that are most likely or biologically relevant. Each scenario is evaluated using multiple lines of evidence, and in cases where several scenarios are plausible, each is scored separately, with the scenario achieving the highest total score representing the best-supported subpopulation delineation. Detailed guidance for applying these criteria is provided in the accompanying workflow document (https://github.com/iucn-CGSG/Subpop-ESU-Webtool). Once subpopulations have been identified, Phase 2 tests whether these subpopulations can be grouped into one or more ESUs (whereby the one ESU scenario is the null hypothesis). For Phase 2, new candidate scenarios can be evaluated independently of Phase 1, as the number of subpopulations does not necessarily correspond to the number of ESUs. As in Phase 1, the scenario with the highest score at the end of Phase 2 represents the most strongly supported ESU delineation.

Three types of data are used in this delineation framework: genetic, recorded biological, and inferred evidence (figure 2a). Genetic evidence derives from genetic or genomic markers (e.g., single nucleotide polymorphisms [SNPs], microsatellites, mitochondrial haplotypes) and is leveraged to assess differentiation and/or evolutionary distinctiveness, including adaptive divergences, of groups within a species. Recorded biological evidence does not require genetic data (e.g., observed biogeographic patterns, variation in transmitted traits) and comes directly from individuals in the assessed focal species. Inferred evidence also does not require genetic data and is not directly observed in the focal units, for example, it is deduced from biogeographic patterns based on modeling techniques. To guide assessors, we have comprehensively listed categories, specific analyses within each evidence type and data that can be used (table 1).

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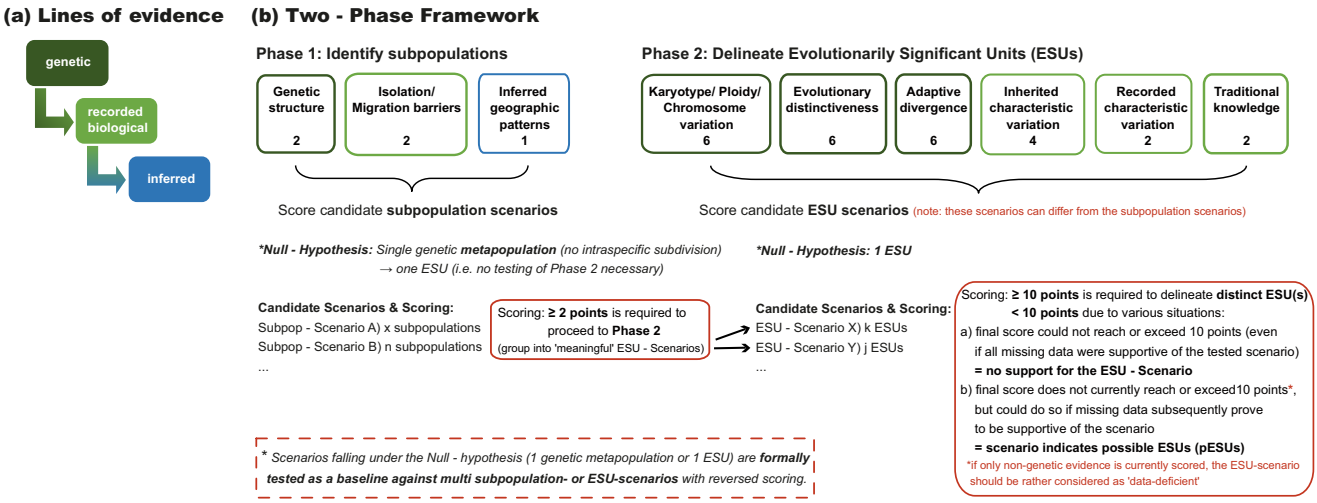



**Figure 2** (a) Lines of evidence and (b) the two-phase framework to assess the strength of evidence for subpopulations and Evolutionarily Significant Units (ESUs). Different lines of evidence are highlighted in dark green, green, or blue. Scores for each evidence category are indicated within the boxes. Higher scores indicate increased support. The scores quantify how informative each category of evidence is in assessing the likelihood that a subpopulation or ESU is distinct. The framework is followed using different "candidate scenarios" for various supported groupings of the species. In the case of conflicting evidence, assessors should divide the species into all different "scenarios" and score each scenario separately. If no data or unclear data are available, a score of 0 should be awarded, and the evidence category is marked as "missing." Achieving the Phase 2 minimum threshold of 10 points requires genetic evidence from at least one of the three described "genetic evidence categories." Alt text: Two-panel diagram showing (a) categories of evidence and (b) the two-phased framework used to evaluate whether groups qualify as subpopulations or Evolutionarily Significant Units (ESUs). Evidence types are color-coded within each of the phases and scores are displayed within boxes. Higher scores indicate stronger support. The scoring of each phase is also explained.

In the case of conflicting evidence, assessors can divide the species into all possible candidate scenarios and score each scenario separately then take the highest scoring. Comprehensive testing of scenarios based on existing knowledge, covering, for example, one to seven subpopulations or ESUs is, however, unnecessary, as many will be lacking sufficient data. The results of the scenario with the highest score (note this may be different for ESUs and subpopulations) could be used for listing assessment. When defining subpopulations, note that subpopulations can also be driven by human-mediated fragmentation or disruption of gene flow, whereas ESU divisions are likely to be more anciently derived.

To enable objective scenario evaluation, each of the three lines of evidence is given a score reflecting its strength (figure 2a). Scores range from 1 to 6. Values of 6 are for evidence types that offer the strongest support (i.e., genetic/genomic signals of prolonged reproductive isolation and/or local adaptations). A score of 6 is only possible in Phase 2 evaluation, as these differences occur between ESUs. Scores of 4 and 2 are given to strongly suggestive evidence categories that reflect genetic divergence or non-molecular signs of local adaptation. A score of 1 is given to data that are entirely inferred, such as information from projections (e.g., maps, species distribution models) or information inferred from closely related species. This is a common, but high-risk evidence type, and its low score reflects this associated risk. The relative weighting of evidence types reflects the IUCN's nature of evidence rule for assessment criteria (refer to IUCN 2024). A score of 0 is given if there are no data available, and categories are labeled "missing." The framework does not allow for fractions or partial scores, scores are in even increments to support quick summing. We have strived to keep the scoring system simple, but a degree of complexity is needed to capture all outcomes.

To identify distinct subpopulations, a total score of ≥2 is required in Phase 1. If multiple candidate scenarios are tested, the scenario with the highest score is used for assessments. If scores are tied between the tested scenarios, we recommend choosing the scenario that is based on the most robust data (e.g., higher resolution genetic marker or most comprehensive sampling of the range) as this will be the state-of-the-art. If two or more distinct subpopulations are delineated, the assessor then tests for ESUs in Phase 2. The scores from Phase 1 do not carry over to Phase 2, because in Phase 2, new candidate scenarios are tested as it is unlikely that the number of subpopulations and ESUs are identical. A minimum score of 10 is needed to delineate distinct ESUs. This can only be reached if genetic or genomic evidence is collected for the species (figure 2b and table 1). At least one genetic evidence category is needed as a "yes," coupled with either another genetic evidence category or with significant differences in inherited or acquired characteristics and/or traditional knowledge, to support a scenario with distinct ESUs. If fewer than 10 points are scored, the candidate scenario can either be regarded as (a) "not supported," because 10 points cannot be reached or exceeded even if all missing data were supportive of the tested scenario or (b) as a "possible ESU (pESU)" scenario, because the minimum threshold of 10 points could be reached or exceeded if missing data subsequently prove to be supportive of the tested scenario. However, if a candidate scenario currently only scores in recorded biological evidence types (so non-genetic), the scenario is regarded as "data-deficient" rather than as a pESU scenario. At the end of Phase 2, assessors should select the scenario with the highest score.

In cases where no candidate scenarios reach the minimum threshold for Phase 1 (2 points) or Phase 2 (10 points), even if additional data were collected, the null hypothesis would apply, and the species may be treated as a single genetic metapopulation (a set of spatially discrete

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**Table 1** Three categories of evidence used in the two-phase framework to delineate subpopulations and ESUs, each containing multiple lines of evidence.

| Types of evidence | Description | How to assess? | Example studies using evidence categories | Main costs of over- and under-splitting | Score |
|---|---|---|---|---|---|
| **Genetic evidence** | | | | | |
| Genetic structure<br><br>(Phase 1) | Substantial genetic structure and fragmentation mostly determined by limited gene flow between subpopulations | (a) Structure-like analyses such as structure, admixture plots, principal component analysis (PCA), and discriminant analysis of principal components (DAPC),<br><br>(b) High pairwise $F_{st}$ values (relative to the species wide pairwise levels),<br><br>(c) Abundance of private alleles, that indicate long-term limited gene flow between subpopulations | Abbott and Double (2003) | Over-splitting: cluster detection bias, over-estimation of genetic differentiation by visual inspection, over-interpreting private alleles<br><br>Under-splitting: generally low risk | 2 |
| Karyotype, ploidy, and chromosome structure variation<br><br>(Phase 2) | Documented heritable differences in chromosome number, ploidy, or chromosome structure between candidate ESUs | Using conventional cytogenetic methods (e.g., chromosome counting, karyotyping), flow-cytometry and/or molecular cytogenetics (with structural variant detection software) to detect:<br><br>(a) Karyotype variation,<br><br>(b) Differences in ploidy levels between units,<br><br>(c) Structural variation of chromosomes between units,<br><br>(d) Evidence of no/rare/unfit hybrids between proposed units | Ferreira et al. (2017), Ahrens et al. (2020), Hollenbeck et al. (2022) | Over-splitting: misinterpreting neutral karyotype variation as evidence for ESUs<br><br>Under-splitting: missing important karyotypic differences due to insufficient sampling or low technical resolution | 6 |
| Evolutionary distinctiveness<br><br>(Phase 2) | Genetic evidence of reproductive isolation, due to highly restricted or no gene flow between focal ESUs that may have fuelled evolutionary distinctiveness | (a) Reciprocal monophyly,<br><br>(b) Molecular estimates of divergence time<br><br>In complex cases, follow clades supported by ≥75% bootstrap or posterior probability values, derived from a statistically valid evolutionary model and tree-building method | Moritz (1994), Moritz and Faith (1998), Walsh et al. (2024) | Over-splitting: over-interpretation of reciprocal monophyly or long divergence times (dependent on methodology used and species context/history), assuming no current gene flow means no historical gene flow<br><br>Under-splitting: over-interpretation of lack of reciprocal monophyly, overlooking variation across the genome | 6 |



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**Table 1** Continued

| Types of evidence | Description | How to assess? | Example studies using evidence categories | Main costs of over- and under-splitting | Score |
|---|---|---|---|---|---|
| Adaptive divergence<br><br>(Phase 2) | Robust genetic evidence of candidate ESUs harboring unique local adaptation(s) driven by selection (e.g., environment, sexual selection) that are not shared with other ESUs | (a) Robust genomic signals of local adaptations supported by multiple selection detection methods and ideally in combination of non-genomic information (e.g., gene-environment associations using tools like Bayenv, RDA, RandomForest),<br><br>(b) Evidence of stable hybrid zones | Bonin et al. (2007), Rodríguez- Quilón et al. (2016) | Over-splitting: false positives in outlier tests, environment-based detection confounded by neutral structure, hybrid zones misinterpreted as ESU boundaries<br><br>Under-splitting: no outlier detection due to low statistical power, ignoring hybrid zone context | 6 |
| **Recorded biological evidence** | | | | | |
| Isolation and barriers to migration<br><br>(Phase 1) | Subpopulations show evidence of isolation (i.e., long-term signs of restricted gene flow due to geographic, environmental, or temporal differences) between them. Alternatively, subpopulations show evidence of recently restricted gene flow due to human-mediated change (e.g., habitat fragmentation, extirpation of connecting subpopulations, etc.) | (a) Occupation of different biogeographical zones (e.g., habitat maps, maps on environmental patterns),<br><br>(b) Occupation of discrete remnants of historical habitats with little to no chance of natural migration between habitat patches | Hewitt (2004), Lorenzen et al. (2012) | Over- and under-splitting: misinterpretation of observed patterns as long-term natural patterns, misidentification of species or environmental drivers for species | 2 |
| Inherited characteristic variation<br><br>(Phase 2) | Candidate ESUs show consistent heritable differences in life history or ecologically/species important traits (e.g., body size, color, breeding time, use of spawning grounds) | (a) Heritable differences in focal traits observed in common garden experiments or by other robust analytical tests (e.g., cross-fostering, trait measurements controlling for environmental variation) | Small et al. (1998), Wainwright et al. (2008) | Over-splitting: confusing plasticity with adaptation, overinterpretation non-functional traits, neglecting distorted variation in small subpopulations<br><br>Under-splitting: failing to recognize evolved differences in phenotypic plasticity | 4 |
| Recorded characteristic variation<br><br>(Phase 2) | Candidate ESUs show consistent differences in traits that are locally transmitted but not robustly shown to be heritable (acquired behavioral or phenotypic differences; environmentally modified traits) | (a) Cultural/learnt behavioral differences unique or specific between units,<br><br>(b) Acquired/transmitted traits (e.g., foraging techniques, alternative migration routes, regional birdsong "dialects", methylation differences, body size differences),<br><br>(c) General field-based morphological and functional trait assessments | Lundberg et al. (2017), Toews et al. (2019), Gu et al. (2021), Sanchez-Donoso et al. (2022) | Over-splitting: misinterpretation of acquired behaviors or phenotypic differences as barriers to gene flow<br><br>Under-splitting: study bias (lack of studies in general = data-deficiency, study selection bias) | 2 |

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**Table 1** Continued

| Types of evidence | Description | How to assess? | Example studies using evidence categories | Main costs of over- and under-splitting | Score |
|---|---|---|---|---|---|
| Traditional knowledge<br><br>(Phase 2) | Distinctiveness between candidate ESUs based on Indigenous, local, or traditional knowledge | (a) Recorded differences based on traditional and local knowledge (according to the IUCN Indigenous and Local Knowledge [ILK] framework, IUCN 2022). This encompasses information that is not yet statistically analysed, information that is analysed should be counted as inherited or recorded characteristics | a proposed tool to use ILK species assessments (Cross et al. (2017) | Over- and under-splitting: if not all relevant stakeholders are consulted, leading to a biased perspective | 2 |
| **Inferred evidence** | | | | | |
| Inferred geographic patterns<br><br>(Phase 1) | Subpopulation differentiation and fragmentation modeled from the focal species through, for example, species distribution modeling approaches, or measured/observed from closely related species | (a) Subpopulation disjunction from co-occurring species used as a proxy,<br><br>(b) Phylogeographic and/or biogeographic evidence from co-occurring species,<br><br>(c) Assumption of biogeographical data/refugia without direct evidence,<br><br>(d) Dispersal distance and buffer according to the genetic indicators accepted within the Kunming-Montreal Global Biodiversity Framework (KMGBF) | | Over-splitting: overinterpretation of detected patterns when they do not reflect genetic differentiation<br><br>Under-splitting: patterns in co-occurring species as proxies can obscure detected differences | 1 |

Note: For each line of evidence, a description of the methods and metrics used to assess it, examples of supporting studies, and the main risks of over- or under-splitting units are provided. Scores assigned to each line of evidence are also shown. Additional details on the risks of over- and under-splitting for each line of evidence and references are provided in supplemental material Costs Benefits analysis, Section B.



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or semi-discrete groups of individuals that are connected through sufficient gene flow to maintain shared genetic variation) and therefore a single ESU. Testing the null hypothesis of a single metapopulation or single ESU follows the same scoring framework, but in reverse. In this instance, points are awarded for evidence of genetic and biological cohesion rather than differentiation. In other words, scores are assigned based on evidence demonstrating, for example, lack of structure, high gene flow, or uniform traits across the species range and thereby supporting the conclusion that a species constitutes a single metapopulation and a single ESU.

This framework has been tested across multiple taxonomic groups (table 2), with detailed examples provided in box 2 and box 3 with the best supported candidate scenarios visualized in figures 3 and 4. The thresholds for delineating subpopulations and ESUs are derived from these tests, reflecting a range of data availability and species characteristics. We envision that through the continued application of this framework, more evidence types and exceptions will arise, allowing for refinement of the framework. Current efforts include testing species listed in the IUCN Green Status of Species framework (IUCN 2021).

## ESU and subpopulation division—risks and limitations

Choosing whether or not to split species into smaller units comes with an inherent risk of "under- or over-splitting," which is amplified when non-molecular data are used because genetic divisions may be cryptic (Frankham et al. 2019). Over-splitting a species into several ESUs could artificially inflate estimated extinction risks. Conversely, under-splitting a species could give a falsely optimistic picture of extinction risk or Green Status, or inhibit species management actions, in turn exacerbating extinction risk and fuelling genetic diversity loss (Frankham et al. 2019, Liddell et al. 2021). Careful interpretation is therefore critical, as ESU delineation influences threat assessments, management decisions, and recognition of adaptive potential in global conservation policy. ESU boundaries should be therefore treated as working hypotheses, subject to refinement as new evidence emerges.

An extensive cost–benefit analysis has been developed for the framework to help assessors and experts balance these challenges (supplemental material Section B and table 1). However, balancing the patterns identified with their uncertainty often requires genetic knowledge. Authors conducting molecular research can support managers and assessors by offering clear lay summaries of their results in publications and depositing data in open access repositories to support reuse (Leigh et al. 2024). In turn, assessors can seek advice from trusted sources during evaluations.

In the future, advances in genomics will provide us with deeper biological insights that could be relevant to management. For example, genetic load consists of deleterious alleles whose frequency and presence can vary across isolated genetic units. Undoubtedly, a diversity of genomic data types could become increasingly important for the effective management of ESUs (Dussex et al. 2023). Nevertheless, we have not yet included genetic load as a criterion in this framework, because it remains challenging to identify and is less relevant for ESUs delineation, which focuses on the evolutionary heritage that conservation managers aim to preserve (more details provided in the supplemental material, Section C and van Oosterhout et al. 2025). Though we have designed a framework that is not entirely reliant on molecular data, it is an important line of evidence where resolution will also improve in the future, and reassessments could be needed. Genetic or genomic data, often best capture long-term processes of adaptation (i.e., natural selection) and the accumulation of mutations that shape evolutionary trajectories (Allendorf et al. 2022). Data quality and resolution, such as uneven sampling, low marker density, or poor study design, can inflate or obscure signals of population genetic structure. The type and density of sequence data (e.g., microsatellites, SNP arrays, or reduced representation sequencing) strongly influence the resolution of ESU (and even subpopulation) delineation, with low-density markers or sparse sampling increasing the likelihood of misclassification.

## How subpopulations and ESUs could fit into Conservation Assessment Frameworks

The importance of delineating biologically meaningful units is underscored by recent cases where molecular evidence has reshaped IUCN Red List assessments. In some species, weakly supported subspecies designations were shown to be unfounded, leading to their collapse or consolidation (e.g., mountain lion *Puma concolor*: Culver et al. 2000, Nielsen et al. 2015; tiger *Panthera tigris*: Goodrich et al. 2015, Wilting et al. 2015). In other cases, genomic data revealed deep evolutionary divergences that had been obscured under traditional taxonomy, prompting recognition of distinct units with separate conservation assessments (e.g., giraffes, *Giraffa* spp.: Fennessy et al. 2016, Coimbra et al. 2021, Bertola et al. 2024; African elephants, *Loxodonta* spp.: Roca et al. 2001, Gobush et al. 2021). These examples illustrate how outdated or unfounded subspecies classifications may misdirect conservation resources, whereas delineating ESUs and subpopulations provides a more robust, evolutionarily grounded basis for assessments.

The framework proposed here provides a standardized approach for identifying and delineating both subpopulations and ESUs, which could potentially inform existing IUCN frameworks and assessments (Red List and Green Status of Species). Within the Red List, for example, delineated subpopulations and ESUs could be documented as additional levels under the taxonomy section or accompanying metadata to provide context on within-species genetic diversity. In some cases, this approach could serve as a more biologically grounded replacement, or supplement, to traditional subspecific assessments, particularly where subspecies designations are weakly supported or inconsistent across taxa. This information supports more nuanced assessments of extinction risk, particularly for subpopulations that are highly differentiated or potentially at risk due to fragmentation or isolation (Criterion B; IUCN 2024).

Within the Green Status of Species framework (IUCN 2021), delineating ESUs and subpopulations could directly inform assessments of representation (spatial units), through greater standardization and by clarifying whether historically distinct genetic units are retained across a species' indigenous range. In addition, once subpopulations or ESUs are delineated, genetic data or proxy measures could inform assessments of viability and ecological functionality by indicating whether spatial units (either those showing recovery or with recovery potential) are likely to maintain sufficient genetic diversity to support long-term persistence (IUCN 2024). Although viability within the Green Status framework is commonly assessed through extinc-

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**Table 2** List of species tested using the proposed framework under different candidate scenarios.

| Species or subspecies | Phase 1 | Phase 2 | Genetic Data and studies considered | IUCN Red List status | IUCN Green Status |
|---|---|---|---|---|---|
| Common eland (*Tragelaphus oryx*) | **Two subpopulations** (4/4 points) | **One ESU** (12/18 points) | Mitochondrial DNA (Gagnon and Chew 2000, Lorenzen et al. 2010, 2012) | Least concern | Not assessed |
| Mountain zebra (*Equus zebra*) | **Two subpopulations** (4/4 points) | **Two possible ESUs** (8/14 points) | Mitochondrial and microsatellite data (Moodley and Harley 2005, Kotzé et al. 2019) | Vulnerable | Not assessed |
| African buffalo (*Syncerus caffer*) | **Two subpopulations** (4/4 points) | **Two ESUs** (22/22 points) | Nuclear genomes, mitochondrial and microsatellite data (Van Hooft et al. 2002, Smitz et al. 2013, 2014, de Jager et al. 2020, 2021, 2025, Quinn et al. 2023, Colangelo et al. 2024, Talenti et al. 2024) | Near threatened | Not assessed |
| Rodrigues fruit bat (*Pteropus rodricensis*) | **One subpopulation** (4/4 points) | **One ESU** | Microsatellite data (O'Brien et al. 2007) | Endangered | Not assessed |
| Iberian lynx (*Lynx pardinus*) | **Two subpopulations** (2/4 points) | **One ESU** (10/16 points = human-mediated admixture due to captive breeding and translocations) | Microsatellite and whole-genome sequencing data (Rodríguez and Delibes 2003, Johnson et al. 2004, Pertoldi et al. 2006, Abascal et al. 2016, Casas-Marce et al. 2017, Kleinman-Ruiz et al. 2017) | Vulnerable | Largely depleted |
| African penguin (*Spheniscus demersus*) | **One subpopulation** (2/4 points) | **One ESU** | Microsatellite data (Labuschagne et al. 2016) | Critically endangered | Largely depleted |
| Western leopard toad (*Sclerophrys pantherina*) | **Four subpopulations** (2/4 points) | **Two possible ESUs** (6/12 points) | Mitochondrial and microsatellite data (Measey and Tolley 2011, da Silva et al. 2017, Stephens et al. 2022) | Endangered | Not assessed |
| Yellow-tufted honeyeater (*Lichenostomus melanops*) | **Two subpopulations** (4/4 points) | **Two possible ESUs** (8/14 points) | Microsatellite data, mitochondrial DNA and nuclear sequences (Pavlova et al. 2014, Harrisson et al. 2016) | Least concern | Not assessed |
| Sable antelope (*Hippotragus niger*) | **Five subpopulations** (4/4 points) | **Five possible ESUs** (6/16 points) | Mitochondrial genomes (Rocha et al. 2022) | Least concern | Not assessed |
| Black rhinoceros (*Diceros bicornis*) | **Seven subpopulations** (4/4 points) | **Seven possible ESUs** (6/16 points) | Mitochondrial DNA, microsatellite data, nuclear genomes (Moodley et al. 2017, Sánchez-Barreiro et al. 2023) | Critically endangered | Largely depleted |
| Rewarewa tree (*Knightia excelsa*) | **Two or four subpopulations** (two scenarios scored 4/4 points = assessment should be done by species experts) | **Two ESUs** (12/16 points) | Whole-genome sequencing data (McCartney et al. 2024) | Least concern | Not assessed |
| Hawaiian koa (*Acacia koa*) | **Seven subpopulations** (4/4 points) | **Four ESUs** (10/16 points) | Microsatellite data and genotyping-by-sequencing data (Shi 2003, Fredua-Agyeman et al. 2008, Baker et al. 2009, Gugger et al. 2018) | Vulnerable | Not assessed |
| Black wildebeest (*Connochaetes gnou*) | **One subpopulation** (4/4 points) | **One ESU** | Mitochondrial and microsatellite data (Corbet and Robinson 1991, Vrahimis et al. 2016, 2017, Grobler et al. 2018) | Least concern | Not assessed |

Note: All tested species here possess some form of genetic data, resulting in the "possible ESU" status for species where a score <10 was achieved. Only the highest scoring scenarios for Phase 1 (subpopulations) and Phase 2 (ESUs) are shown, as these best support the number of units tested. Scores are shown as points obtained out of the total points available. Relevant data sources supporting each scenario are provided as well as the IUCN Red List and Green Status.



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**Box 2:** Hawaiian koa (*Acacia koa*).

In this box, we present the results of all the tested candidate scenarios for the Hawaiian koa (*Acacia koa*). Background information and detailed testing output are provided in supplemental material, Section A and in the guidance document at https://github.com/iucn-CGSG/Subpop-ESU-Webtool. The best-supported candidate scenarios are visualized in figure 3.

**Phase 1: Four candidate scenarios are tested:**

Metapopulation scenario (=null hypothesis): No evidence was found to indicate the presence of a single metapopulation of the Hawaiian koa.

Subpopulation scenario 1: Genetic clustering and pairwise $F_{st}$ values based on microsatellite data suggest **two subpopulations**: one on Kaua'i and one spanning the Island of Hawai'i, Maui, and O'ahu (Fredua-Agyeman et al. 2008). No clear migration barriers were detected, and no indirect evidence of fragmentation was available. Thus, only genetic structure supported the subdivision.

Subpopulation scenario 2: Genetic clustering and pairwise $F_{st}$ values based on single nucleotide polymorphisms (SNP) data suggest **seven subpopulations** (Kaua'i, Maui, and O'ahu each with one, plus four on the Island of Hawai'i), though some overlapping clusters likely reflect human-mediated dispersal and are a side product of restoration programs (Gugger et al. 2018; figure 3 for visualization). No clear migration barriers were detected, and no indirect evidence of fragmentation was available. Thus, only genetic structure supported the subdivision.

Subpopulation scenario 3: Natural occurrence patterns across the four Hawaiian islands suggest **four subpopulations** (Kaua'i, Maui, O'ahu, and the Island of Hawai'i), aligned with distinct habitats (can be considered different biogeographical zones) and potential migration barriers (Baker et al. 2009). However, genetic/genomic studies did not confirm strong structure, and no indirect fragmentation evidence was available. This scenario is supported mainly by biogeographic isolation.

| | **Null hypothesis –a single metapopulation** | **Subpopulation scenarios** | | |
|---|---|---|---|---|
| | | **Two subpops** | **Seven subpops** | **Four subpops** |
| **Is there evidence of genetic structure?** (*2 points*) | Yes | Yes | Yes | No |
| **Is there evidence of natural isolation or subpopulation fragmentation (through migration barriers)?** (*2 points*) | Yes | No | No | Yes |
| **Is there inferred evidence of likely subpopulation fragmentation?** (*1 point*) | No data | No data | No data | No data |
| **Final scores** | **0 points** | **2 points** | **2 points** | **2 points** |

**Interpretation of Phase 1:** Three of the four tested candidate scenarios in Phase 1 scored two points and thereby reached the minimum threshold. The null hypothesis was rejected, and all three subpopulation candidate scenarios in Phase 1 are moved onto Phase 2.

When scores are tied as seen in this example (three subpopulation candidate scenarios scored two points), we recommend selecting the one based on the most robust data to maximize the conservation efforts of genetic diversity. In this example, we would consider subpopulation scenario 2 (seven subpopulations) as the one scenario based on the most robust data. Distinct subpopulation clusters were identified using high-resolution genomic markers (11,000 SNPs from >300 samples by genotyping-by-sequencing). Although microsatellites remain valuable in population genetics, subpopulation scenario 1 is unlikely to represent the true subpopulation structure. Instead, it might better reflect higher-level structuring at the ESU level, along with subpopulation scenario 3 (four island subpopulations). Therefore, the seven-subpopulation scenario better represents true subpopulation structure.

**Phase 2: The same four candidate scenarios are tested:**

A single ESU scenario (=null hypothesis): Karyotypic evidence shows Hawaiian koa is uniformly tetraploid across islands (Shi 2003). The remaining categories scored zero, based either on multiple genomic and provenance studies demonstrating island-specific genetic, adaptive, and inherited phenotypic divergence and contradicting the single-ESU null hypothesis or because of missing evidence. This scenario therefore scored 6 points.

ESUs scenario 1: Genetic data support two groups (Kaua'i versus the Island of Hawai'i–Maui–O'ahu), which are tested as **two distinct ESUs**, but no additional evidence (karyotype, adaptive divergence, or inherited characteristic variation) supports this split. Missing data were identified when scoring for evolutionary distinctiveness, recorded characteristic variation, and traditional knowledge. Provenance tests revealed phenotypic differences across all four islands, but these did not align with the two-ESU grouping. Overall, this scenario scored zero points.

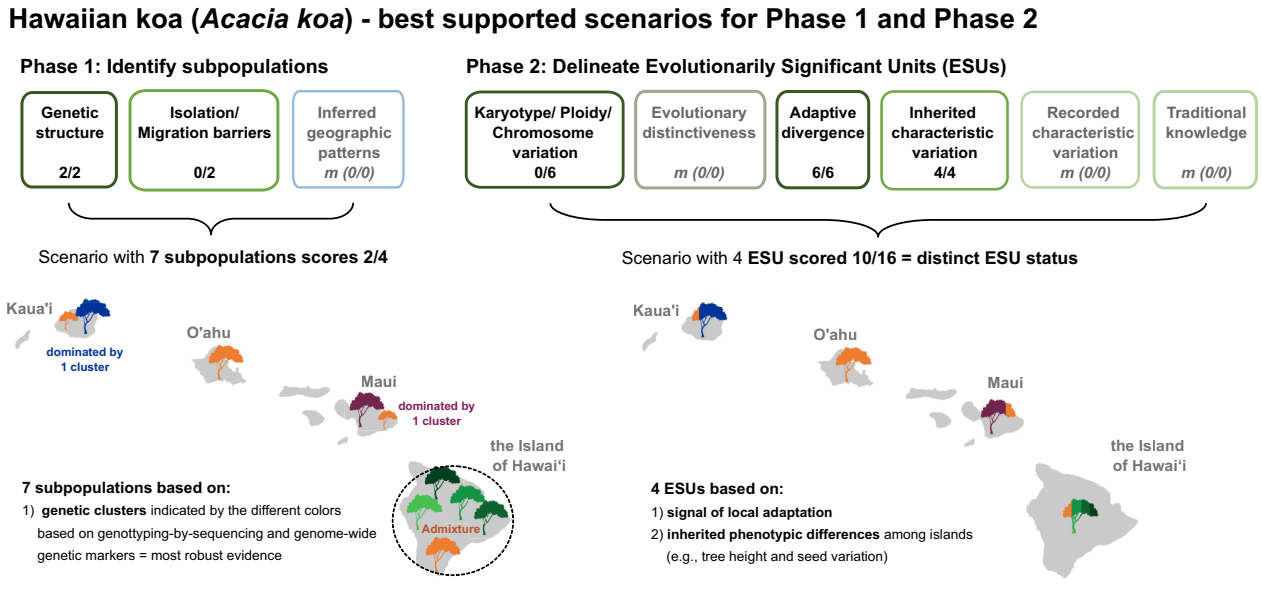
ESUs scenario 2: Genomic data identified seven subpopulations, which are tested as **seven distinct ESUs**, but admixture within the Island of Hawai'i contradicts the ESU definition of restricted gene flow. No evidence supported karyotype variation, evolutionary distinctiveness, or adaptive divergence. Provenance tests revealed phenotypic differences across all four islands, but these did not align with the seven-ESU grouping. Missing data were identified when scoring for recorded characteristic variation and traditional knowledge. Overall, this scenario scored zero points.

ESUs scenario 3: Natural occurrence patterns identified four subpopulations, which are tested as **four ESUs**. Adaptive divergence linked to precipitation supports four ESUs corresponding to the four islands (Gugger et al. 2018). These are reinforced by provenance tests showing inherited phenotypic differences matching the island divisions (Baker et al. 2009). No evidence supported karyotype variation, and missing data were identified when scoring for evolutionary distinctiveness, recorded characteristic variation, and traditional knowledge. Overall, with genomic and phenotypic evidence, this scenario scored 10/16 points.

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| | Null hypothesis | ESUs scenarios | | |
|---|---|---|---|---|
| | **- a single ESU** | **Two ESUs** | **Seven ESUs** | **Four ESUs** |
| **Is there inherited variation in chromosome numbers, ploidy level or chromosome structure?** (*6 points*) | No | No | No | No |
| **Is there evidence of long-term reproductive isolation?** (*6 points*) | No data | No data | No | No data |
| **Is there evidence of local adaptation or adaptive divergence?** (*6 points*) | Yes | No | No | Yes |
| **Is there evidence of inherited characteristic differences?** (*4 points*) | Yes | No | No | Yes |
| **Is there evidence supporting transmitted characteristic differences?** (*2 points*) | No data | No data | No data | No data |
| **Is there traditional knowledge suggesting distinctiveness between units?** (*2 points*) | No data | No data | No data | No data |
| **Final scores** | **6 points** | **0 points** | **0 points** | **10 points** |

**Interpretation of Phase 2:** ESU scenario 3 scored 10/16 points, providing the best-supported ESU scenario and highlighting that each Hawaiian islands (Kaua'i, Maui, O'ahu, and the Island of Hawai'i) can be considered a separate ESU.




[h]

**Figure 3** Visual summary of the best-supported scenarios for Phase 1 and Phase 2 of the Hawaiian koa tree (*Acacia koa*) case study. The top panel summarizes the evidence categories and scores (e.g., 2/2). Categories with insufficient data are assigned "m" for missing and are given 0 points. The lower panel presents a schematic illustrating the outcomes for each best-supported scenario. Each tree symbol represents a single genetic unit, with colors representing unique units. Further details are provided in supplemental material, Section A. Alt text: Figure summarizing two assessment phases for Hawaiian koa (*Acacia koa*). Phase 1 identifies seven subpopulations with low support (2/4), while Phase 2 identifies four Evolutionarily Significant Units (ESUs) with sufficient support (10/16). Evidence categories are scored, with missing data marked as zero. Maps show genetic groupings across Hawaiian islands.

tion risk (and therefore directly relates to the species' Red List status), genetic information can provide complementary insight into the capacity of populations to sustain evolutionary processes beyond demographic recovery.

Both phases of this framework can provide complementary information relevant to Red List assessments under Criterion B, which considers species with subpopulations that are severely fragmented (i.e., smaller than necessary to support a viable population; IUCN 2024). Severe fragmentation is currently inferred from habitat fragmentation and species traits. Information on subpopulations within the same ESU, particularly where high human-mediated genetic differentiation is observed, could provide complementary evidence to inform assessments on fragmentation. Furthermore, identifying ESUs could help distinguish whether observed subpopulation fragmentation is recent and harmful, or an ancient pattern that is unlikely to influence extinction risk.

Delineation of both subpopulations and ESUs can also assist with prioritizing site-based protection measures, from local to regional scales. Many conservation initiatives are focused on site-based protection (e.g., 30 × 30, Protected and Conserved Areas; CBD 2022),

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## **Box 3:** Black wildebeest (*Connochaetes gnou*).

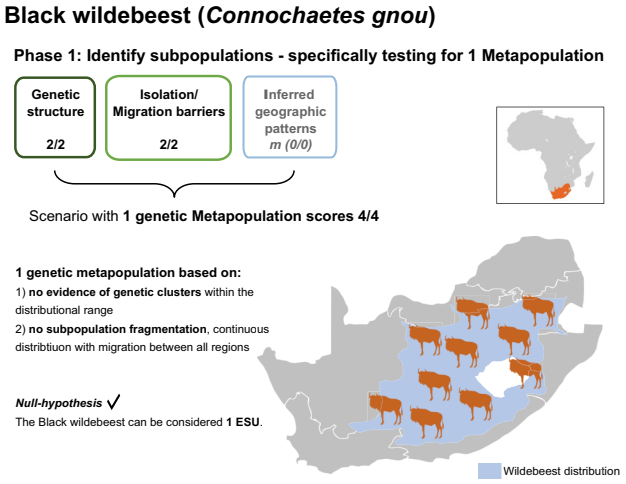
In this box, we present the results of all the tested candidate scenarios for the black wildebeest (*Connochaetes gnou*). Background information and detailed testing output are provided in the supplemental material, Section A. The best-supported candidate scenarios are visualized in figure 4.

**Phase 1**: Only the null hypothesis (a single metapopulation) is tested:

Metapopulation scenario (=null hypothesis): Genetic analyses using mitochondrial DNA (Corbet and Robinson 1991) and microsatellites (Grobler et al. 2018) showed no clustering, suggesting one single metapopulation, with ongoing migration and no barriers to gene flow (Vrahimis et al. 2016). No indirect evidence was available to indicate the absence of fragmentation. Thus, this scenario is supported mainly by a lack of genetic structure and a lack of biogeographic isolation.

| | **Null hypothesis** |
|---|---|
| | **- a single metapopulation** |
| **Is there evidence of genetic structure?** (*2 points*) | No |
| **Are there records of non-human-mediated isolation or subpopulation fragmentation (through migration barriers)?** (*2 points*) | No |
| **Is there indirect evidence of likely subpopulation fragmentation?** (*1 point*) | No data |
| **Final scores** | **4 points** |

**Interpretation of Phase 1:** Overall, the one metapopulation scenario scored 4/4, exceeding the threshold and supporting the null hypothesis that the black wildebeest represents a single genetic metapopulation and thus a single ESU, with no progression to Phase 2.




[h]

**Figure 4** Visual summary of the best-supported scenarios for Phase 1 and Phase 2 of the black wildebeest (*Connochaetes gnou*) case study. The top panel summarizes the evidence categories and scores. Categories with insufficient data are subdued in color and assigned m for missing and are given 0 points. The lower panel presents a schematic illustrating the outcomes for each best-supported scenario. The blue polygon represents the metapopulation distribution within South Africa. Further details are provided in supplemental material, Section A. Alt text: Figure showing assessment of Black wildebeest (Connochaetes gnou) identifying a single genetic metapopulation across South Africa. Evidence scores indicate strong support (4/4), with no genetic clustering or fragmentation. Map shows continuous distribution with individuals spread across one connected range.

where understanding the irreplaceability and vulnerability of individual sites is essential. The 30 × 30 target, adopted under the CBD's KMGBF, calls for the effective conservation and management of at least 30% of the world's terrestrial, inland water, and marine areas by 2030, emphasizing not only the extent but also the ecological representativeness, connectivity, and equitable governance of protected and conserved areas. Identifying which ESUs and subpopulations are present can help highlight differences in conservation value across a species' range, ensure that unique components of interspecific diversity are not overlooked, and provide an early indication of sites that may become conservation priorities in the future.

The primary IUCN tool recently developed to support site prioritization is the "Global Standard for the Identification of Key Biodiversity Areas" (also known as KBAs; IUCN 2016). Although ESUs often span multiple KBAs, standardized delineation of both subpopulations and ESUs could provide additional evidence for identifying such areas under several criteria. In particular, KBA criteria A1, B1, and B2 identify sites that support significant proportions of global populations of threatened species (A1), single geographically restricted species (B1), or multiple geographically restricted species (B2). Information on distinct subpopulations and ESUs can strengthen the assessments of KBAs by highlighting sites that support unique or irreplaceable genetic and evolutionary diversity. Such information may

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also contribute to assessments under criteria D and E, which consider the relative biodiversity value of sites supporting species that are not currently threatened or range-restricted, and can inform site-level management by helping to avoid actions that may erode locally adapted or evolutionarily distinct genetic units.

Importantly, delineating subpopulations and ESUs can help identify units experiencing different threats across a species' range. Recognizing these differences allows for unit-specific assessments of extinction risk or recovery, highlighting subpopulations that may require targeted conservation actions. This approach enhances the utility of the third level of IUCN assessments, ensuring that management priorities reflect both the evolutionary and ecological realities of within-species variation. Moreover, the units identified through this framework represent a first step toward linking ecological and evolutionary groupings to quantitative genetic indicators, such as the headline indicator accepted under the KMGBF—Ne500, the proportion of populations with an effective population size greater than 500 individuals. By defining biologically meaningful units in Phase 1, these groupings could subsequently serve as the basis for estimating effective population sizes and monitoring genetic diversity at scales relevant for conservation action.

In this paper, we outlined a two-phase framework to standardize the delineation of both subpopulations and ESUs by integrating genetic and non-genetic evidence. By providing a consistent approach to recognize biologically meaningful within-species units, the framework can complement existing IUCN assessments, offering additional context on species' genetic structure and evolutionary processes. Such information could inform conservation priorities, support more nuanced interpretation of recovery or extinction risk indicators, and contribute to efforts at maintaining and enhancing global genetic diversity.

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## Supplemental material

Supplemental data are available at *BIOSCI* online.

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The authors have no relevant financial or non-financial interests to disclose. There are no new data associated with this article.

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