Genetic load in Evolutionarily Significant Units (ESUs): conservation and management implications

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Abstract

The Conservation Genetics Specialist Group of the International Union for Conservation of Nature (IUCN) proposes introducing Evolutionarily Significant Units (ESUs) as an additional new assessment unit in the IUCN Red List and Green Status. This proposal is made because ESUs possess unique evolutionary trajectories present within species and harbour genetic diversity that requires safeguarding. Given that genetic diversity is not formally incorporated in the Red List or Green Status assessment, the inclusion of ESUs would help improve the protection of valuable intraspecific biodiversity. In this framework, ESUs can be identified by non-genetic and/or genetic data, including data on karyotype differences, distinct adaptations, and deep evolutionary distinctiveness. Genetic load is purposefully not included in ESU delineation, even though its important role in species conservation and management is increasingly recognised. Here, we report on the discussion that led to this decision, explaining the rationale and challenges that led us to exclude the genetic load from the ESU-defining framework. We also discuss recent research on genetic load, and how this could help advance conservation science and improve species conservation and recovery programs.

Background

The International Union for Conservation of Nature (IUCN) has assessed the extinction risk of over 163,000 species in the Red List of Threatened Species. Their risk of extinction was assessed based on the best available data on occupation of the species' past and present range, census size and trends, habitat extent and quality, and level of fragmentation (IUCN, 2024). Extinction risks have historically been assessed at three levels: entire species, subspecies, and subpopulations (IUCN, 2024). Genetic or genomic data are not explicitly considered in these assessments, although there are increasing calls that they must be (Willoughby et al. 2015; Garner et al. 2020; Norderhaug et al. 2024). Furthermore, the Red List assesses the extinction risk over 3 generations or 10 years (whichever is longest). Such a short time frame is insufficient to assess the long-term threat to biodiversity caused by genomic erosion (Jackson et al. 2022; Pinto et al. 2024). The IUCN recently established the Green Status of Species, and although it too does not include genetic diversity in its assessment framework, its assessment is over a 100-year timespan (IUCN, 2021). The Green Status of Species assessment provides an appropriate platform to include genetic data and evolutionary genetic concepts relevant to population recovery, in particular when combined with computer-modelling based population viability forecasts. The Green Status of Species framework can help assess the contribution that management of evolutionary genetic health can make to population trajectories (Jeon et al. 2024; Norderhaug et al. 2024).

The absence of genetic and genomic data in the IUCN's assessments has been a subject of considerable debate encompassing reasons why inclusion of these data would beneficial, and how do so (Garner et al. 2020; Schmidt et al. 2023; Jeon et al. 2024; Norderhaug et al. 2024). The Conservation Genetics Specialist Group (CGSG) was established as part of the Species Survival Committee (SSC) of the IUCN with the aim to promote research, collaboration, and awareness of genetic and evolutionary principles in conserving biodiversity. Growing concern about the lack of recognition of evolutionary and genetic principles motivated the CGSG to propose Evolutionarily Significant Units (ESUs) as an additional new unit of conservation in the IUCN Red List and Green Status assessments (Geue et al. 2025). ESUs are defined based on their unique evolutionary trajectories, and inclusion of ESUs in IUCN's assessments would help protect intraspecific genetic diversity and enhance conservation management (Geue et al. 2025).

The importance of ESUs

The first mention of the term ESU was by Ryder (1986). In his paper, he discussed the need to conserve unique genetic attributes within a species that are important for present and future generations. Ryder (1986) furthermore suggested a combination of genetic data, geographic distribution data, life history information and morphometrics to delineate ESUs within species. Considerable research on ESUs has since followed, briefly summarised in Geue et al. (2025). The application of the term ESU has suffered long-standing issues resulting from a lack of standardisation, partly due to the multidisciplinary nature of conservation science. Moreover, scientists from different disciplines have defined and used ESUs with different approaches and types of data. Geue et al. (2025) proposes a framework intended to consolidate the best elements of ESU approaches into one feasible, stepwise approach, incorporating both genetic data as well as data that do not require genetic analysis.

The new ESU assessment framework proposed by Geue et al. (2025) comprises two steps: (1) identifying distinct subpopulations and, (2) assessing whether these subpopulations meet the criteria to be all or part of an ESUs or flagged as possible ESUs (pESUs) in case of data limitations. Three types of data are used in this delineation framework: *genetic* evidence, *recorded biological* evidence and *inferred* evidence. *Genetic* evidence derives from genetic or genomic data (e.g., single nucleotide polymorphisms, microsatellites, mitochondrial haplotypes, structural variants) or from quantitative genetic (breeding) studies. *Recorded biological* evidence comprises observed biogeographic patterns, phenotypic or behavioural variation. *Inferred* evidence is not observed directly in the focal units but inferred from biogeographic patterns based on modelling techniques, related species, and traditional knowledge (Geue et al. 2025).

From a genetics point-of-view, ESUs can be identified based on their deep evolutionary distinctiveness, as evidenced from long-term restricted gene flow, reciprocal monophyly, or genetic estimates of divergence times. In some cases, the distinction between ESU and species can be blurry. Even in the absence of genomic evidence for local adaptation, “drift speciation” can occur if ESUs are completely isolated and population sizes are sufficiently small (Black et al. 2024). Such cases are likely to be rare, and often genetic data (e.g., karyotypes) can be used to identify heritable differences in chromosome numbers or ploidy, or provide strong evidence of no/rare/unfit hybrids. Furthermore, ESUs can be defined based on their adaptive uniqueness in the form of local adaptation driven by natural- or sexual selection. Although the genetic load of deleterious mutations is increasingly recognised as an important aspect in species conservation and restoration (van Oosterhout 2020), this aspect of genetic diversity was ultimately not included in the ESU- defining framework.

Rationale to exclude genetic load from the ESU-defining framework

Here we report the debate concerning the relevance and issues relating to the exclusion of genetic load in the ESU assessment framework. There are two fundamental arguments against the inclusion of genetic load to define ESUs. First, these units are defined based on their evolutionary uniqueness that needs to be conserved, whereas contemporary maladaptive variation is by definition not useful and worthy of protection. Second, the framework focuses on ESU delimitation, whereas ESU assessment and management—for which genetic load is more relevant—is a separate process of goal-setting and decision-making. The practical arguments against incorporating genetic load in ESU delineation include that identifying and quantifying the impact of deleterious mutations remains challenging. We also explain why, despite the exclusion of the genetic load from the ESU-defining framework, the assessment of harmful mutations can be critical for the management of ESUs and how genetic load is currently being analysed.

Analysis of genetic load

Genetic load can be considered a fitness concept, in which case it is estimated as a decrease in the average fitness of a population relative to that of the optimal genotype (Bertorelle et al. 2022). Genetic load can now also be estimated based on genomic data, but the link between the fitness concept and the molecular genetic concept of the genetic load remains largely untested in wildlife (Grueber and Sunnucks 2022).

Studying genetic load tends to involve advanced bioinformatic approaches to assess the impact of genetic variants, which are then ranked or categorised according to the level of assumed severity (Bertorelle et al. 2022). Popular variant-prediction scores include GERP (Genomic Evolutionary Rate Profiling), CADD (Combined Annotation-Dependent Depletion), and SIFT (Sorting Tolerant From Intolerant), and many others (reviewed in Bertorelle et al. 2022). Recent papers have proposed frameworks to examine the dynamics of genetic load in threatened and recovering populations, and these approaches are increasingly being applied in conservation genomics (Grossen et al. 2020; Mathur & DeWoody, 2021; Dussex et al. 2023;

Smeds & Ellegren 2023; Kyriazis et al. 2023; Kleinman-Ruiz et al. 2022; Femerling et al. 2023; Cavill et al. 2024; Hogg 2024; Hasselgren et al. 2024).

Unfortunately, the rank scores of variants do not directly translate into selection (*s*) coefficients. Consequently, unlike *s* coefficients, rank scores (e.g., CADD scores) cannot simply be summed to estimate the genetic load. An additional complication is that the fitness impact of genetic variants is often context-dependent. For example, the sickle cell mutation at the beta-globin gene can be beneficial or deleterious depending on whether an individual carries one or two copies, and the environmental conditions (notably whether malaria is endemic). More broadly, many variants of polygenic traits can be deleterious or beneficial depending on their genetic background and environment (van Oosterhout et al. 2022).

Nevertheless, some genetic load consists of variants that are unconditionally deleterious in (nearly) all individuals and environments. For example, genetic variants that are shared by highly diverged species are likely to be conserved by strong purifying selection. The evolutionary conservation of such a variant suggests that its substitution is likely to be harmful to fitness under nearly all conditions and genetic backgrounds. Ultra-Conserved Elements (UCEs) consist of such highly invariable sites (Speak et al. 2024). Although it might not be possible to determine the exact deleteriousness of a given variant at a conserved site in every individual, we can be confident that the long-term *average* effect of a mutation at such a site is harmful when considered across multiple individuals. Moreover, we can rank the impact of these mutations using their variant-prediction score and compare the distribution of these scores between individuals or populations. This enables researchers to estimate what proportion of harmful mutations is expressed as realised load, and how much remains hidden from selection as masked load (Speak et al. 2024).

Fitness-impact of genetic load in wildlife

Linking these variant-prediction scores to individual fitness remains a big challenge. Studies addressing the issue are beginning to emerge. In *Arabidopsis lyrata*, an indicator of genetic load (i.e., weighted mean frequency of derived alleles, $P_n f_n / P_s f_s$) correlated with a fitness proxy, population growth (Willi et al. 2018). More recently, arctic fox (*Vulpes lagopus*) individuals with more homozygous loss-of-function genotypes (LoFs) were shown to have lower lifetime reproductive success and reduced lifespans compared with individuals with lower proportions of LoFs (Hasselgren et al. 2024). Similarly, LoF variants at specific loci associated with reproductive health reduced male reproductive success in the northern elephant seal (*Mirounga angustirostris*) (Hoelzel et al. 2024). Moreover, in five loci associated with transcriptional regulation during hypoxia, frequencies of minor alleles (e.g., putatively deleterious alleles) were higher in individuals with lower dive performance. Importantly, genome-wide heterozygosity was not a good predictor of the fitness components (Hoelzel et al. 2024), implying that the genetic load at particular loci, rather than genome-wide diversity, was a better predictor of the chosen fitness indicators in this species.

These are the first studies showing that it is possible to link the effects of putative harmful mutations to fitness of individuals in wild populations of non-model organisms. There is an urgent need for more studies that link individual fitness to genetic load and other genomic data. We also need to critically test the potential added value of assessing and managing deleterious mutations in biodiversity conservation (van Oosterhout 2020; Grueber and Sunnucks 2022; Jackson et al. 2022). Genomics data have immense value for biodiversity conservation, but this potential will be fully realised only when we go beyond categorising or ranking genetic variants in relation to their relative severity (or benevolence). We need to be able to estimate the fitness effects of genetic variants and express them into selection coefficients, which would enable us to engage the powerful quantitative– and population genetic frameworks. That would be a step-change in the use of genomics data in the studies of ecology, evolution, and conservation.

Genetic load in ESUs

Genetic load plays a pivotal role in biodiversity conservation and management. However, the framework proposed by the CGSG is designed to delineate ESUs for consideration in conservation management, not to prescribe particular conservation interventions. Conservation management of ESUs can benefit from a better understanding of genetic load. If individuals from one ESU have become fixed for deleterious variants at different loci than those in another ESU, gene flow between these ESUs could improve fitness. Conservation management that promotes gene flow can help to mask realised load by making loci heterozygous, thereby improving fitness and population viability. Future conservation management of threatened ESUs subject to genetic rescue may benefit from balancing the harm of introducing deleterious variants versus the benefits of adding beneficial alleles and replenishing lost adaptive variation (Mathur et al. 2023). Knowledge derived from genomics-informed conservation about genetic load is valuable in conservation management, but at present, these analyses are prohibitively complex for applied conservation of most threatened species (Ralls et al. 2020; Speak et al. 2024). Important progress can be made by combining population genomics with quantitative genetics and linking detailed observational fitness and experimental data to genomics-based estimates of genetic load (Willi et al. 2018; Bertorelle et al. 2022; Grueber & Sunnucks 2022; Hasselgren et al. 2024; Hoelzel et al. 2024). Improving and validating fitness inferences from genetic variant predictions would be a major advance in conservation science, enabling better use of genomics data for the conservation and recovery of ESUs and threatened species.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception. The first draft of the manuscript was written by Cock van Oosterhout and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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