A widespread gap in U.S. Endangered Species Act implementation: Risk of genetic erosion within populations

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Impact statement: Many populations of species assessed under the Endangered Species Act are too small to maintain genetic diversity, compromising recovery.

Keywords: conservation genetics, effective population size, endangered species, extinction risk, genetic indicators

Abstract:

Despite its importance to fitness and population viability, genetic diversity is rarely incorporated into biodiversity assessments. The recent adoption of indicators of intraspecific genetic diversity by the Convention on Biological Diversity has highlighted the importance of evaluating genetic diversity in wild species. Genetic indicators are useful even in the absence of genetic data because they reflect two major drivers of genetic diversity: population size and the maintenance of populations across a species' range. Here, we apply these indicators to 161 species assessed for classification under the U.S. Endangered Species Act (ESA), one of the strongest conservation laws in the world. We found that while most populations are being maintained across species' ranges, many of those populations are likely at risk of genetic erosion, compromising their ability to maintain genetic diversity and evolutionary potential. Additionally, ESA classification status was not correlated with the genetic indicators, suggesting that genetic health is either not being incorporated into species assessments, or is not being given full consideration by decision-makers during determinations of classification status. Fortunately, we found that data were widely available to calculate the genetic indicators. We suggest that the genetic indicators are a useful tool for evaluating and communicating genetic health in ESA assessments in a transparent and standardized manner. Inclusion of genetic indicators could support more timely and better-targeted protection of at-risk species prior to irreversible losses of genetic diversity. This, in turn, would increase the success of recovery actions while reducing time to recovery and overall costs, better ensuring the ESA goal of safeguarding biodiversity.

Introduction

Genetic processes within species impact individual fitness and population viability, yet genetic health is rarely integrated into extinction risk assessments due to limited data availability and the perception that genetic data are too complex to include (Pierson et al. 2016). Here, we define genetic health as maintenance of evolutionary processes that confer population and species-level viability. These processes include high within- and among-population genetic diversity, large effective population sizes, the maintenance of historical patterns of gene flow, and maintaining the distribution of populations across a species' historical range (Kardos et al. 2021). Threats to genetic health include erosion of genetic variation due to genetic drift in small populations, mating among close relatives causing reduced fitness (i.e., inbreeding depression), disrupted connectivity, and extirpation of locally adapted populations. These evolutionary processes have significant implications for conservation and management because low genetic diversity, small population sizes, and population extirpations negatively affect the successful recovery of declining species (Wilcove et al. 1993; Neel et al. 2012; Eberhard et al. 2022).

Inclusion of these genetic concerns in biodiversity assessments was recently improved with the December 2022 adoption of indicators of intraspecific genetic diversity into the Convention on Biological Diversity (CBD) Global Biodiversity Framework – the world's roadmap to halting biodiversity loss by 2030 (Hoban et al. 2023). This landmark achievement reflects a growing acknowledgement that evaluation of genetic health in wild species is critical to biodiversity monitoring and management, even though genetic data are lacking for the majority of species (Torres-Florez et al. 2018; Lawrence et al. 2019). Genetic indicators are useful even in the absence of genetic data because they reflect two major drivers of genetic health: population size, which governs the extent of genetic drift and inbreeding depression, and the maintenance of populations across a species' range, which conserves species-wide genetic diversity and evolutionary potential. Because the evaluation of these processes does not necessarily require DNA-based assessments, genetic indicators can be evaluated rapidly and affordably in many species (Mastretta-Yanes et al. 2024a).

The "proportion of populations with effective population size (N_e) greater than 500" indicator (hereafter the "Ne500 indicator") assesses if populations are large enough to maintain genetic diversity and evolutionary potential over the long term. It is based on the well-accepted principle that genetic erosion within populations accelerates when the population's N_e is less than 500 (Jamieson & Allendorf 2012; Hoban et al. 2020a; Willi et al. 2022). The Ne500 indicator can be measured using an estimate of contemporary N_e (using genetic or demographic methods) or using the population's census size (N_c) and an N_e/N_c ratio to convert N_c to N_e. The second genetic indicator, the "proportion of populations maintained" (hereafter the "PM indicator"), is based on the principle that environmental heterogeneity and distance drive adaptive divergence among populations, such that genetic composition varies spatially (Wang & Bradburd 2014; Forester et al. 2022). Thus, extirpation of populations results in a loss of evolutionary potential that cannot be easily recovered. The PM indicator can be measured using estimates of species' historical distributions and pragmatic approaches for delineating populations (reviewed in Mastretta-Yanes et al. 2024b).

Genetic indicators are useful not only for monitoring efforts under the CBD, but also in national biodiversity conservation programs and legislation, such as the U.S. Endangered Species Act (ESA). The ESA, signed into law in 1973, is one of the strongest and most effective conservation laws in the world (Greenwald et al. 2019; Fischman et al. 2023), though there remains room for improvement in its implementation (Puckett et al. 2016; Evans et al. 2016; Malcom & Carter 2021; Eberhard et al. 2022). In 2016, the U.S. Fish and Wildlife Service (FWS) adopted the Species Status Assessment (SSA) framework to improve the scientific rigor, efficiency, consistency, and transparency of extinction risk assessments, while keeping science distinct from the application of policy, which includes the determination of a species' classification status (i.e., threatened, endangered, or not warranted for listing; Smith et al. 2018). SSAs evaluate the capacity of species to withstand environmental and demographic stochasticity and disturbance, catastrophic events, and changes in the biological and physical environment. These assessments consider the best available science on population size, abundance, trend, and distribution as well as genetic, environmental, and phenotypic diversity. Unfortunately, many species assessed under the ESA are data limited (i.e., 36% of species in the 2024-2028 FWS National Listing Workplan; U.S. Fish and Wildlife Service 2016), challenging extinction risk assessment and recovery planning. The inclusion of genetic indicators may help fill some of this data availability gap.

Prior to the SSA framework, deficiencies had been identified in the evaluation and protection of within-species diversity under the ESA. In particular, it has been repeatedly noted that species typically do not receive ESA protections until their populations are very small and the number of extant populations is highly reduced relative to historical conditions, which exacerbates loss of genetic diversity and makes recovery more challenging (Wilcove et al. 1993; Abbitt & Scott 2001; Neel et al. 2012; Eberhard et al. 2022). Although the SSA framework calls for the evaluation of abundance, population trends, population distributions, genetic diversity, and adaptive capacity (including evolutionary potential), it is not clear whether the related aspects of genetic health (i.e., maintenance of populations with large effective sizes across species' ranges) are being incorporated into scientific assessments in SSAs and whether they are considered by decision-makers during determinations of classification status.

Here we evaluate the Ne500 and PM indicators for 161 species assessed under the ESA using the SSA framework, more than half of the species with SSAs available at the time of our analysis. Our goals were to (1) evaluate the genetic health of these species using the genetic indicators, (2) determine whether genetic indicator values are correlated with ESA classification status, taxonomic group, or geographic range size, (3) quantify data availability for calculating the indicators, while determining if data availability is influenced by the above covariates, and (4) evaluate the influence of uncertainty on indicator values. If indicators of genetic health are being effectively incorporated into SSAs and that information is being used by decision-makers in their determinations of classification status, we would expect a correlation between status (i.e., endangered, threatened, not warranted) and one or both of the genetic indicators (i.e., 'not warranted for listing' species should have higher genetic indicator values). Lack of a correlation would suggest that genetic health is not being given full consideration in extinction risk assessments, either during SSA production or during status determinations. We examined the additional covariates because taxonomic group may relate to both census and effective sizes, and range size may be positively correlated with larger population sizes (Ellegren & Galtier 2016). Finally, where data were available, we calculated the proportion of populations with N_e less than 50 to identify species with populations at risk of rapid genetic erosion and potential fitness impacts from inbreeding depression (Jamieson & Allendorf 2012).

Materials and methods

We collected data on genetic indicators for species or subspecies (hereafter "species") assessed under the ESA using the SSA framework. We limited our data collection for the indicators to SSAs for each assessed species, given that the SSA informs the classification status decision under the ESA (i.e., threatened, endangered, not listed, delisted) and we were interested in examining relationships between indicators and ESA status. We downloaded the list of species with SSAs from the FWS Environmental Conservation Online System on March 22, 2023 (https://ecos.fws.gov/ecp/report/ssa). We removed SSAs where most of the species' range occurred outside the US or was primarily pelagic, as well as those that assessed multiple species or focused on a distinct population segment. We then selected at random from the remaining SSAs.

To collect data on the genetic indicators, we used a previously developed and standardized KoboToolBox (https://www.kobotoolbox.org/) form (Mastretta-Yanes et al. 2024a, 2024b). Data collection using the Kobo form was calibrated on a set of six SSAs to ensure data collection was replicable (i.e., all individuals collecting data completed the Kobo form for the same six SSAs and results were compared and discussed). Then, smaller groups calibrated on another set of six SSAs before individuals were randomly assigned SSAs to evaluate. To further ensure data accuracy, every final Kobo form entry was reviewed and validated by at least one additional team member before the data were finalized. We downloaded this validated dataset as a .csv file and processed the file in R version 4.1.1 (R Core Team 2021). We used a modified version of R scripts created by Mastretta-Yanes et al. (2024a; available at the Dryad repository https://doi.org/10.5061/dryad.bk3j9kdkm) for quality control, data cleaning, and data formatting. Data and code for this analysis have been deposited to Dryad for peer review and will be made public upon publication.

We included covariates from data collected in the Kobo form in our analyses, including species range size, taxonomic group, and ESA classification status. Range size was categorized as either "restricted" or "wide-ranging"; categorization was based on extent of occurrence (< 20,000 km^2 for restricted range species), area of occurrence (<2,000 km^2 for restricted range species), or descriptions provided in the SSA (e.g., "narrow endemic"). Taxonomic group was analyzed at two scales when sufficient sample sizes were available: plants, invertebrates, and vertebrates; and bryophytes, gymnosperms, angiosperms, invertebrates, fish, amphibians, reptiles, birds, and mammals. ESA classification status was categorized as endangered, threatened, delisted, not listed, under review, and candidate. For statistical analysis and data visualizations, "proposed endangered" and "proposed threatened" species were set to "endangered" and "threatened", respectively; "delisted" was set to "not listed" since both categories mean the species does not meet the definition of threatened or endangered; and singleton categories (i.e., "candidate") were removed. Statistical analysis of relationships with ESA classification status were limited to endangered, threatened, and not listed categories, since there was an *a priori* expectation of differences in indicators among these three categories. We also determined what types of genetic studies were available for assessed species (i.e., phylogenetic, population genetic, both, or none) based on data reported in the SSA.

Some species were analyzed at multiple spatial scales in the SSAs (i.e., multiple approaches for defining populations or metapopulations) or included multiple assessments of population size or uncertainty about the extinct or extant status of populations. In these cases, multiple Kobo form submissions were completed reflecting these different approaches or uncertainties, called "multi assessments". Where species had multi assessments for one of the indicators, we averaged the indicator value for statistical analyses and data visualizations. We also assessed the impact of multi assessments on downstream indicator values.

The proportion of populations maintained (PM) indicator was calculated as: the number of extant populations / (the number of extant populations + the number of extinct populations). The baseline time period for defining populations was based on historical information available in the SSA and generally reflected human-induced changes in species' ranges from 5-200 years ago. The year or range of years used as a baseline was recorded for each species. Species with populations where the extant or extinct status was unknown were excluded from the PM calculation.

The proportion of populations large enough to retain genetic diversity (Ne500) indicator was calculated as: the number of populations with $N_e > 500$ / the number of populations with N_e data. Not all populations within each species had N_e or N_c data; only populations with N_e or N_c data were considered when calculating the Ne500 indicator, so not all populations are included for every species. For N_c data provided as a semi-qualitative measure or as a range, generalized N_c values were assigned to facilitate computation of N_e and the downstream Ne500 indicator. For example, if the N_c range was described as "more than 5,000 by much", a value of 10,000 was applied for the calculation. When the N_c range was described as "range includes 5,000", a value of 5,001 was applied. A default N_e/N_c conversion ratio of 0.1 was applied to approximate contemporary N_e from N_c. If a species had a reported N_e/N_c ratio, that ratio was used instead of the default value. We evaluated sensitivity of the Ne500 indicator to additional N_e/N_c ratios of 0.2 and 0.3. To calculate the proportion of populations with N_e less than 50, we used data from populations with N_e or N_c point estimates.

We evaluated relationships among indicator values and covariates using ordered beta regression (Kubinec 2023), which is designed for continuous data with upper and lower bounds (such as the indicator data), using the glmmTMB package version 1.1.8 (Brooks et al. 2017). We evaluated model fit using visual diagnostics and statistical tests (i.e., Kolmogorov–Smirnov test, dispersion test, and outlier test) with the DHARMa package version 0.4.6 (Hartig 2022). Deviations were addressed by fitting dispersion parameters in glmmTMB. For both the Ne500 and PM indicators, we evaluated the relationship between each indicator and range size and taxonomic groupings to determine if species with smaller ranges or specific taxonomic groups were at higher risk of genetic erosion or population extirpation. Similarly, for both indicators as well as Ne50, we evaluated the relationship between the indicator and ESA classification status to determine if species with elevated risk status (e.g., threatened or endangered) had lower (i.e., worse) indicator values.

Results

We assessed a total of 161 species, with angiosperms (n=48) and invertebrates (n=45) having the highest representation in the data set and gymnosperms and bryophytes the lowest (n=1 for both groups; Fig. S1). Most species had restricted ranges (n=118), with 43 categorized as wide ranging. For classification status, 59 species were endangered or proposed endangered, 41 were threatened or proposed threatened, 52 were not listed, eight were delisted, and one was a candidate for listing. Most species had data available to estimate the indicators, with 49% of species having sufficient data for the Ne500 indicator (Fig. S2), 74% for the PM indicator (Fig. S3), 39% for both, and 84% for at least one of the indicators. The baseline time period for defining the historical number of populations ranged from 1801 to 2021, with a mean of 1990, median of 2004, and standard deviation of 39 years. Of 161 species, 22% (36/161) had no genetic study available, 17% (27/161) had only a population genetic study, 22% (35/161) had only a phylogenetic study, and 39% (63/161) had both. Invertebrates were less likely to have a population genetic study compared to plants or vertebrates (pairwise Fisher exact test with Holm adjustment for invertebrates and plant comparison p = 0.008, estimate and 95% confidence intervals = 0.281 (0.108, 0.701); for invertebrates and vertebrates comparison p = 0.0006, estimate and 95% confidence intervals = 0.214 (0.086, 0.512)).

The distribution of the Ne500 indicator skewed low (i.e., Ne500 modal value = 0, Fig. S4) across all categories of ESA classification status and taxonomic groups (Fig. 1a,b), indicating that most populations within any given species may be too small to maintain genetic diversity over the long term. Of the 79 species with data, 47% (37/79) had no populations above the Ne500 threshold, while 18% (14/79) had all populations above. We did not detect a statistically significant relationship between ESA classification status and the Ne500 indicator (Fig. 1a, Table S1). Similarly, we did not detect statistically signficant differences in the Ne500 indicator among taxonomic groups (Fig. 1b) or between widespread versus range-restricted species (Tables S2-S4). We assessed the proportion of populations below the Ne50 threshold and found that 14% (8/59) of species with data had all populations below Ne50, 41% (24/59) had half or more populations below Ne50, and 31% (18/59) had all populations above Ne50. As with the Ne500 indicator, we did not detect a statistically significant relationship between ESA classification status and the number of populations with $N_e > 50$ (Table S5). Model residuals for Ne500 statistical tests showed some minor underdispersion; fitting dispersion parameters was helpful in some cases, though the deviations were small in all cases (full code, plots, and parameter estimates for these and all other statistical tests can be found at the Dryad peer review URL provided above under 1_data_analysis/4_analysis.html file). For the Ne500 models affected (i.e., Tables S2-S4, range size and taxonomic groupings), underdispersion could result in reduced power (Hartig 2022), though plots of the data (e.g., Fig. 1b) show that the Ne500 indicator spans the range of values for all taxonomic groups, pointing to a lack of relationship. However, the large confidence intervals for the invertebrate group (Table S2) reflects the small sample size for this category (Fig. 1b), likely reducing power. The Ne50 test had no misspecification issues.

Unlike the Ne500 indicator, the distribution of the PM indicator skewed high (i.e., PM modal value = 1, Fig. S5) across classification categories and taxonomic groups (Fig. 1c,d), indicating that populations are generally being maintained across species' ranges. Of the 119 species with data, 37% (44/119) had all populations maintained. As with the Ne500 indicator, we did not detect a statistically significant relationship between ESA classification status and the PM indicator (Fig. 1c; Table S6). Invertebrates had significantly lower values for the PM indicator when compared with plants (Fig. 1d; Table S7; p = 0.028, estimate and 95% confidence intervals = -0.531 (-1.003, -0.058)). We did not detect a statistically significant difference in the PM indicator for species classified as widespread vs. range-restricted (Table S8). Model residuals and diagnostics for the PM indicator statistical tests had no misspecification issues.

Uncertainty in assessing the indicators was relatively uncommon and did not have a large impact on downstream estimates. For the Ne500 indicator, 11% of species with data (9/79) required multiple assessments with different Ne500 inputs, but only four of those (5%) resulted in a change in the indicator value (average change of 0.25, Table S9). For the PM indicator, 22% of species with data (26/119) required multiple assessments with different PM inputs, with 19 of those (16%) changing the indicator value (average change of 0.15, Table S9). We also evaluated how the Ne500 indicator was impacted by the N_e/N_c ratio used in cases where a species-specific ratio was unavailable (default ratio = 0.1). As the ratio increased from 0.1 to 0.3, the overall distribution of the Ne500 indicator shifted towards one, since higher ratios translate to larger N_e values (Fig. S6). However, we did not detect statistically significant shifts in the value of the Ne500 indicator as a function of N_e/N_c ratio (Table S10), likely because 54% of species with data (32/59) had no change in their Ne500 indicator across ratios. Only three species had a reported N_e/N_c ratio: Luquillo Mountain babyboot orchid (*Lepanthes eltoroensis*; 0.14), San Francisco garter snake (*Thamnophis sirtalis tetrataenia*; average across population estimates = 0.47), and Gunnison sage-grouse (*Centrocercus minimus*; 0.2).

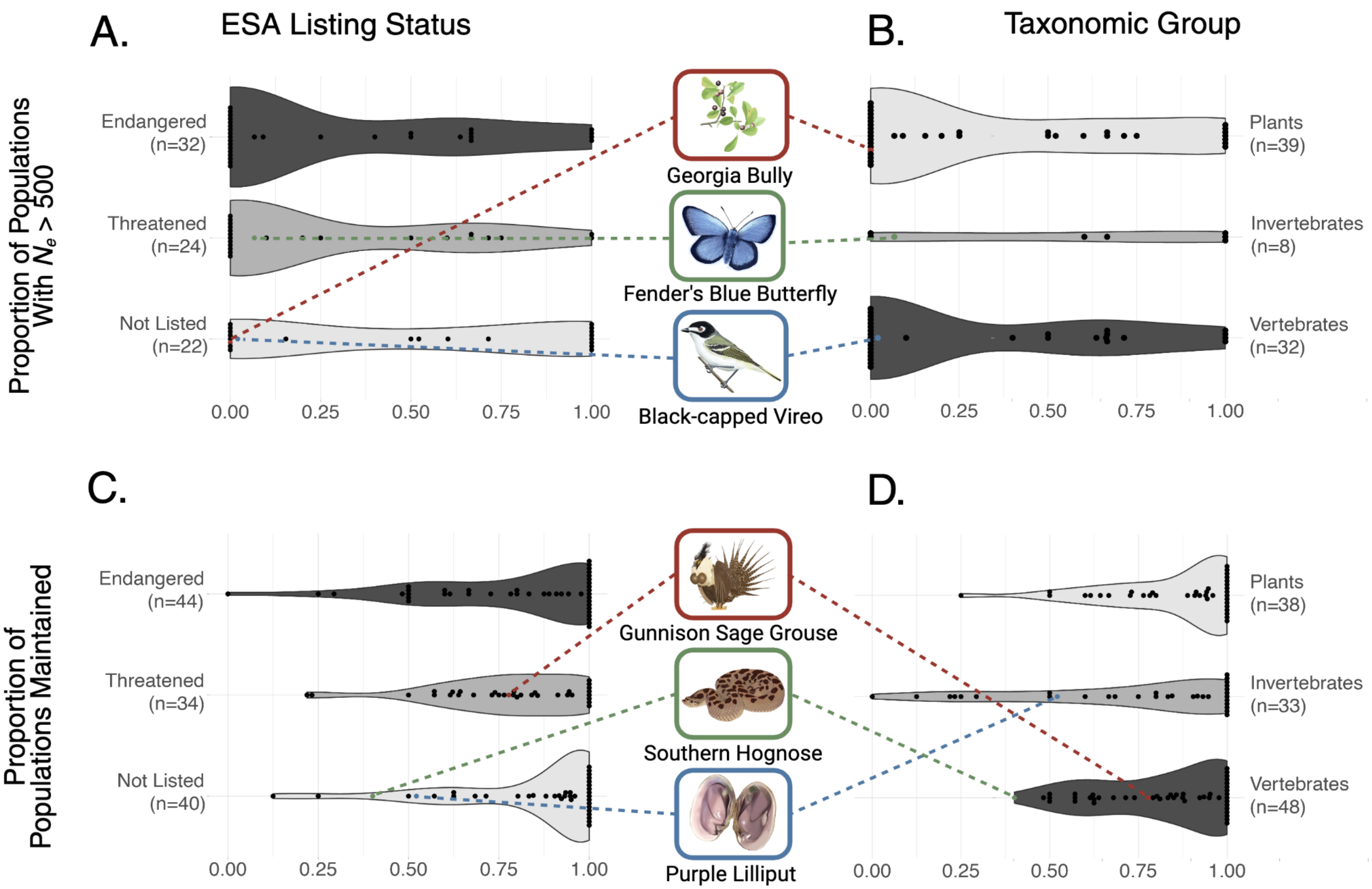


Fig. 1. Genetic indicators by ESA classification status and taxonomic group. Proportion of populations with $N_e > 500$ by (**A**) ESA classification status and (**B**) taxonomic group. A value of one means that all assessed populations have an $N_e > 500$, while a value of zero means that all assessed populations have an $N_e < 500$. Proportion of populations maintained within species by (**C**) ESA classification status and (**D**) taxonomic group. A value of one means that all populations have been maintained since the species' historical baseline, while a value of zero means that all populations have been extirpated since the historical baseline. Highlighted species shown at the center of the plots are referenced in the text.

Discussion

We found that most species recently assessed under the ESA are likely at risk of genetic erosion within their populations, compromising their ability to maintain genetic diversity and evolutionary potential over the long term. This is particularly concerning given that we did not detect a statistically significant relationship between ESA classification status and the Ne500 indicator, meaning that species that were not listed were just as likely to have Ne500 values of zero as listed species (Fig. 1a). For example, the Georgia bully (*Sideroxylon thornei*, Ne500 = 0, Fig. 1a,b), a flowering plant with a distribution restricted to the southeastern U.S., was classified as not warranted for listing despite 84% (38/45) of its populations having fewer than 50 individuals, and 51% of its populations (23/45) having fewer than 10 individuals (U.S. Fish and Wildlife Service 2021). Additionally, recruitment has been documented in only 15% (8/54) of element occurrences (a unit smaller than populations). These small population sizes and lack of recruitment point to reduced genetic and demographic viability, and are indicators of elevated extinction risk.

We also did not detect any statistically significant differences in the Ne500 indicator among taxonomic groups (Fig. 1b) or widespread vs. range-restricted species, indicating that reduced effective population sizes and the risk of genetic erosion are likely pervasive problems for at-risk species. One important implication is that wide-ranging species may still experience genetic erosion due to reduced population sizes despite having a larger distribution than range-restricted species. While the large distributions of wide-ranging species may offer the benefit of more gene flow due to more available source populations, this should be carefully evaluated since populations cannot be assumed to be in good genetic health just because they are distributed over larger areas. For example, the Purple lilliput (*Toxolasma lividum*), a freshwater mussel, is a wide-ranging species evaluated as not warranted for listing despite all populations falling below the Ne500 metric (Ne500 = 0, using either 64 or 146 populations; U.S. Fish and Wildlife Service 2020b).

Even more concerning are the 58-63% of populations with data available (representing 36% of species assessed) that had $N_e < 50$, pointing to the importance of this threshold in flagging populations that are at high risk of extirpation. For example, the threatened Fender's blue butterfly (*Icaricia icarioides fenderi*) has nine of its 15 populations falling below the Ne50 threshold (and only one population above Ne500; U.S. Fish and Wildlife Service 2020a, Fig. 1a,b), increasing the risk of extirpation due to genetic factors. Similarly, the delisted black-capped vireo (*Vireo atricapilla*) has only one of its 49 populations above Ne500, and only four above Ne50 (U.S. Fish and Wildlife Service 2018, Fig. 1a,b).

While population size may be reduced, we found that most species recently assessed under the ESA are generally maintaining populations across their ranges. Nonetheless, there is a noticeable tail in the distribution of the PM indicator, such that 49% of species have lost at least 1 in 10 populations (indicator value ≤ 0.9) and 32% of species have lost at least 1 in 4 of their populations (indicator value ≤ 0.75). Overall, this is a positive observation for the state of U.S. biodiversity in that some of the distinct elements of genetic diversity found among populations are likely maintained. However, we did not detect a statistically signficant relationship between the PM indicator and ESA classification status, suggesting that population losses resulting in reductions in intraspecific diversity may not be sufficiently considered when assessing species viability and conducting status determinations. For example, the southern hognose snake (*Heterodon simus*) was evaluated as not warranted for listing despite a documented range loss of

60% (i.e., PM = 0.4, Fig. 1c,d), including extirpation of populations across entire units representing unique elements of adaptive diversity (U.S. Fish and Wildlife Service 2019a).

Invertebrates had significantly lower values for the PM indicator when compared with plants, suggesting that this group has experienced higher levels of population extirpation. This may be driven by the well documented high levels of endangerment in freshwater species, including crayfish, mussels, and snails (Lydeard & Mayden 1995; Haag & Williams 2014; Reid et al. 2019; Elkins et al. 2019), which comprised over half of invertebrate species we evaluated. Examples include many freshwater mussels, including the purple lilliput discussed above (not listed, with average PM = 0.52, Fig. 1c,d), the round hickorynut (*Obovaria subrotunda*, threatened, with average PM = 0.24; U.S. Fish and Wildlife Service 2019c) and the sheepnose mussel (*Plethobasus cyphyus*, endangered, PM = 0.29; U.S. Fish and Wildlife Service 2022a). This result is in concordance with previous work that identified higher rates of population extirpation in invertebrates and plants with ESA recovery plans (Leidner & Neel 2011).

Furthermore, when combined with results from the Ne500 indicator, our analysis suggests that although populations of these at-risk species are often being maintained, most are likely too small to prevent genetic erosion and loss of intraspecific diversity that is critical to species viability in both the short and long terms. This points to potential population losses on the horizon for many at-risk species. For example, the Apache trout (*Oncorhynchus apache*) maintains a high PM value (i.e., PM = 0.90-0.95 depending on the scale of analysis), but has an Ne500 indicator value of zero at both the stream and sub-basin scales (U.S. Fish and Wildlife Service 2022b). Even more concerning are species whose population sizes fall below the Ne50 threshold such as the Gunnison sage grouse (*Centrocercus minimus*), a threatened, range-restricted species with a relatively high proportion of populations maintained (PM = 0.78, Fig. 1c,d), but with no populations greater than Ne500, and only one of its seven populations greater than Ne50 (U.S. Fish and Wildlife Service 2019b).

We note that there are cases where species have high values for the genetic indicators but are still listed under the ESA. For example, the Dixie Valley toad (*Anaxyrus williamsi*) is an endemic species with PM = 1, yet this species is listed as endangered due to imminent (non-genetic) threats that could result in extinction (U.S. Fish and Wildlife Service 2023b). Additionally, there may be species with highly restricted habitats that have persisted at population sizes less than Ne500 for long periods with minimal consequences to viability; however, these cases are expected to be infrequent, and monitoring of population sizes over time remains valuable even for these species (Hoban et al. 2024a). These cases clearly underscore the importance of integrating genetic indicators and/or direct measurements of genetic health with other aspects of species' viability and historical, ongoing, and future threats to best inform extinction risk assessments and classification status.

We found that the integration of genetic indicators into SSAs and downstream decision-making is feasible, given that most species have data available to evaluate one or both indicators. Overall lower data availability for the Ne500 (and Ne50) indicator compared to the PM indicator highlights the need for increased biodiversity inventory work, as well as the importance of estimating contemporary N_e in population genetic studies of at-risk species. Surprisingly, although 56% of species assessed had a population genetic study, only seven (4%) had any genetic estimate of contemporary N_e, suggesting that researchers should give more priority to ensuring that sampling designs and analyses allow for the estimation of this important conservation-relevant parameter (Waples 2024). We did identify some striking differences in data availability among taxonomic groups for the Ne500 indicator, with only 18% of invertebrates having sufficient data, compared with 78% of plants and 48% of vertebrates. This is likely due to the difficulty of approximating the census size of many invertebrates due to their small size and/or low detection probability. For example, there are 11 freshwater mussels in our dataset, and surveys often take the form of counting occurrences without an attempt to translate this into modelled N_c. However, estimates were made for some of these species, suggesting there is potential for getting at least rough estimates (and thus, sufficient data) for the Ne500 indicator for other invertebrates in the future. For species with low detection probabilities, genetic approaches for estimating N_e may actually be easier and more cost-effective than estimating N_c (e.g., Inoue et al. 2015; Smith et al. 2021; Gladstone et al. 2022). Additionally, because calculation of the Ne500 indicator requires only rough population size approximations (for instance, a description such as "population size is much less than 5000 individuals"), qualitative approaches to data production should be embraced in SSA data calls, such as data collection by local people and integration of indigenous knowledge (e.g., the use of traditional ecological knowledge in the Alexander Archipelago Wolf SSA; U.S. Fish and Wildlife Service 2023a).

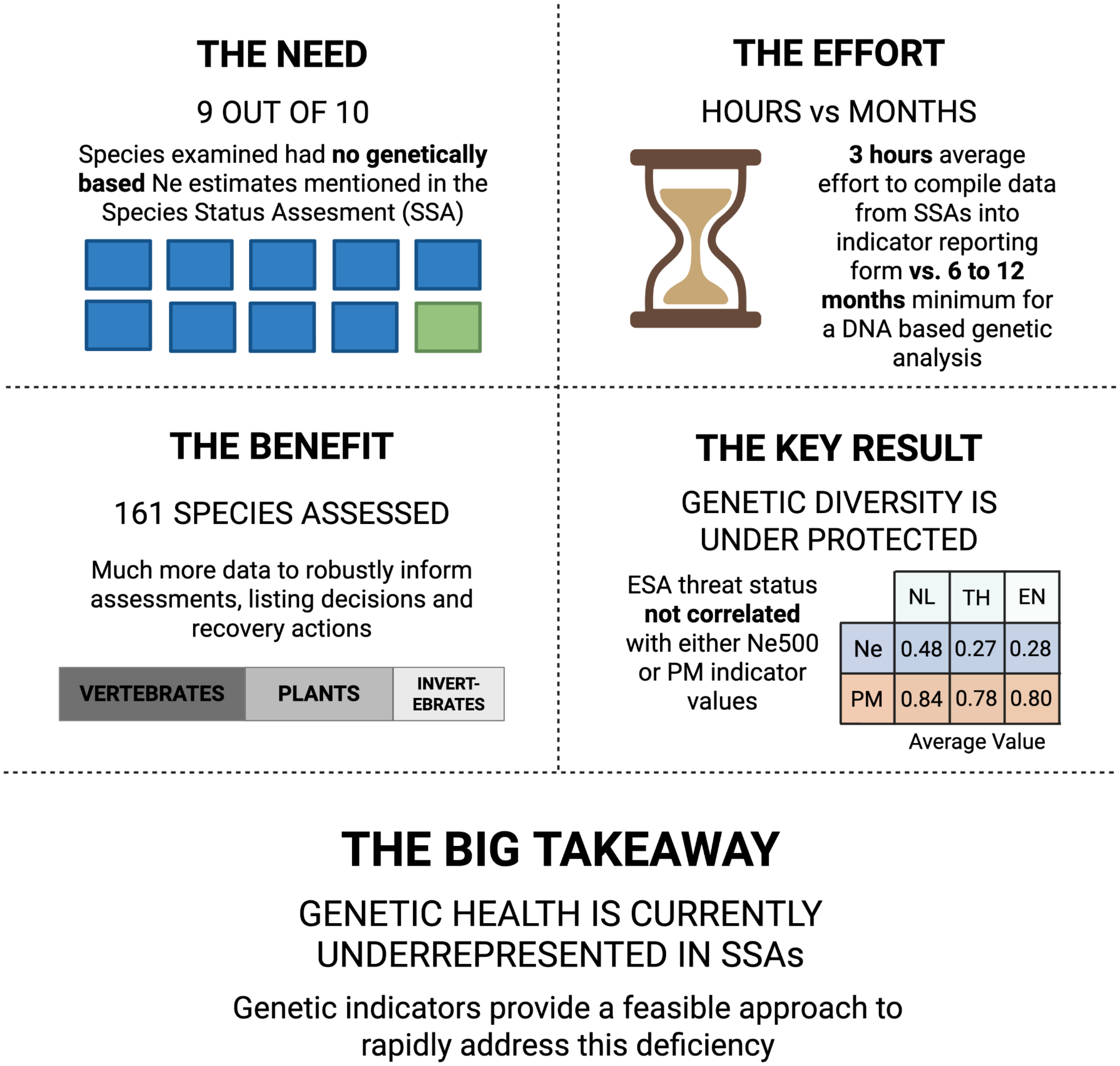
There are a few important considerations that should be addressed when integrating genetic indicators into SSAs. First, we recommend that, when data are available, the Ne50 indicator should be evaluated along with the Ne500 indicator to better inform status determinations (e.g., threatened vs. endangered), since populations with $N_e < 50$ are at elevated risk of extirpation and may contribute to rapid population losses rangewide. Second, the scale of gene flow should be carefully considered by SSA teams when assessing the indicators: the Ne500 and PM indicators should be evaluated at a metapopulation scale, since even small amounts of gene flow among populations can increase genetic diversity and help maintain evolutionary potential (Hoban et al. 2020b; Mastretta-Yanes et al. 2024b; Waples 2025). Application of the Ne50 indicator can be complex, though it generally is most appropriately applied to local, isolated populations (Waples 2025). Metapopulations that fall below $N_e < 50$ can clearly be identified as being at high risk of rapid genetic erosion and near-term inbreeding depression. Third, while the genetic indicators can be used to inform extinction risk assessments for any species, conversion of N_c to N_e using an N_e/N_c ratio should be avoided in cases where the census size is unlikely to reflect the genetic status of the population. This includes populations that are known to have undergone severe bottlenecks followed by population expansion, species with asexual (clonal) reproduction, and heavily managed populations, such as *ex situ* collections (Mastretta-Yanes et al. 2024b). Finally, the genetic indicators do not provide a comprehensive assessment of the genetic health of a species (Hoban et al. 2024b). However, when integrated with other data informing species viability, they can provide critical insight into population-level processes that impact extinction risk, in addition to helping guide recovery planning and providing transparent metrics for tracking recovery progress over time.

Conclusions

One of the major findings from this work is that there was no detectable correlation between classification status and indicators of genetic health in species recently assessed under the ESA (Figs. 1 and 2). In particular, species that were not listed or delisted were just as likely to have low genetic indicator values as listed species. This suggests that, at least so far, genetic health is either not being effectively incorporated into many SSAs along with other data informing species' viability, or that the viability impacts of genetic health included in SSAs are not being given full consideration by decision-makers during determinations of classification status. The ongoing lack of integration and/or consideration of genetic health in ESA decisions may have several causes, including the perception that genetic data are only relevant to taxonomic questions or delineating populations, the perception that genetic data are too difficult to incorporate into extinction risk assessments, lack of familiarity with genetic health metrics such as effective size, and difficulty communicating the relevance of genetic health to extinction risk with decision-makers.

Fortunately, data are available in most species to evaluate one or both of the Ne500 and PM indicators, helping to address this critical gap in assessments of extinction risk under the ESA. The time needed to calculate the indicators from SSA reports was minimal, at approximately three hours per species (Mastretta-Yanes et al. 2024a), which means that inclusion of the indicators is achievable for SSA teams, a critical point given the high FWS workload and chronically inadequate funding (Gerber 2016; Malcom & Li 2018; Eberhard et al. 2022). The indicators also provide a useful tool for communicating genetic health in a transparent and standardized manner, facilitating its consideration by decision-makers during classification determinations.

Our work demonstrates that the genetic indicators are a practical and worthwhile addition to the SSA framework, providing actionable information on genetic health that informs all aspects of ESA implementation, including recovery planning, permitting, and five-year classification status reviews. Consideration of genetic health in ESA decision-making would support more timely and better-targeted protection of at-risk species prior to irreversible losses of genetic diversity. This, in turn, would increase the success of recovery actions while reducing time to recovery and overall costs, better ensuring the ESA goal of safeguarding biodiversity in the U.S. and globally.



	NL	TH	EN
Ne	0.48	0.27	0.28
PM	0.84	0.78	0.80

Fig. 2. Summary of the need, effort, benefit, key results, and takeaway of evaluating the genetic indicators in at-risk species assessed under the ESA using the SSA framework. ESA threat status abbreviations: NL = not listing; TH = threatened; EN = endangered.

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Supporting Information for:

A widespread gap in U.S. Endangered Species Act implementation: Risk of genetic erosion within populations

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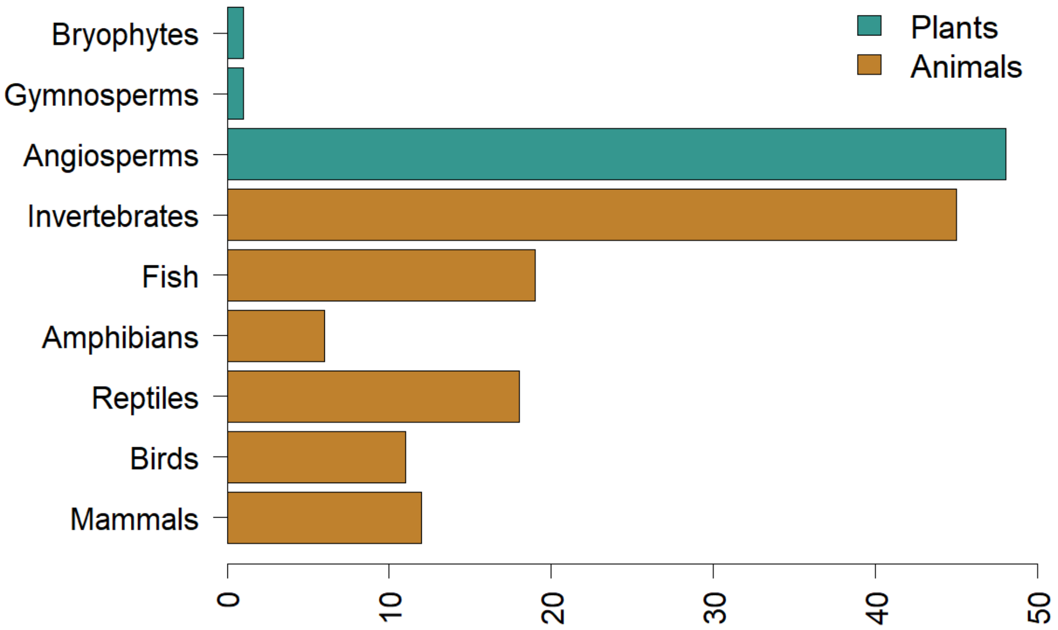

Fig. S1. Taxonomic distribution of 161 species status assessments analyzed.

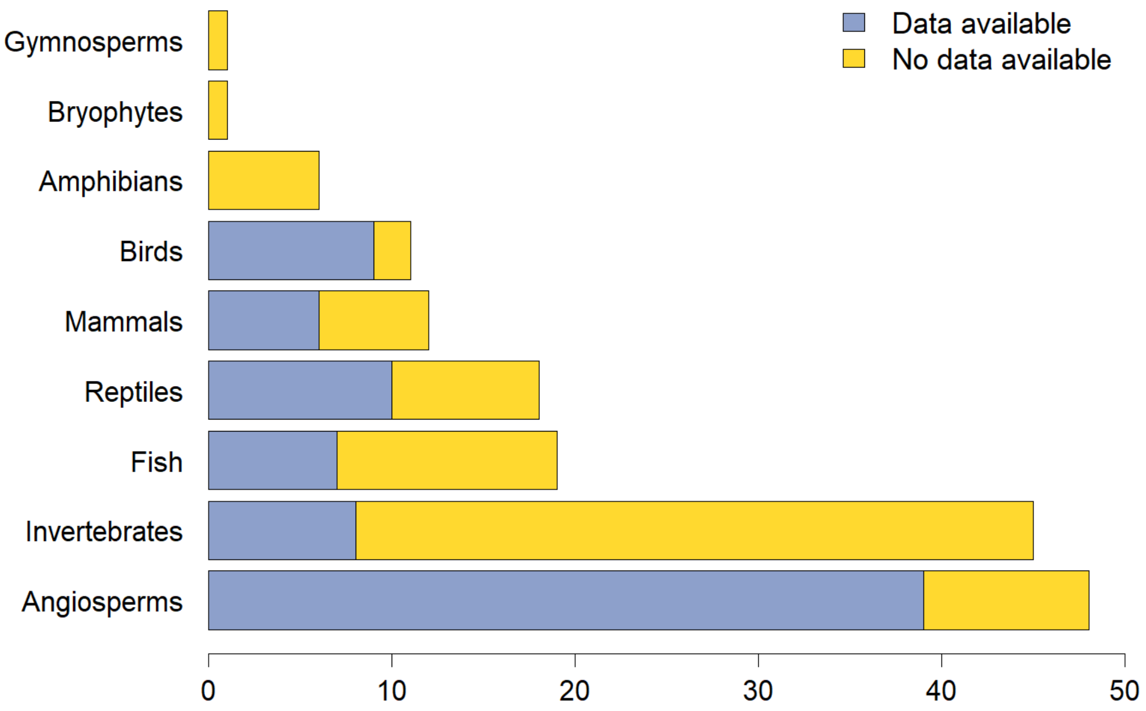

Fig. S2. Data availability for the Ne500 indicator for 161 species across taxonomic groups.

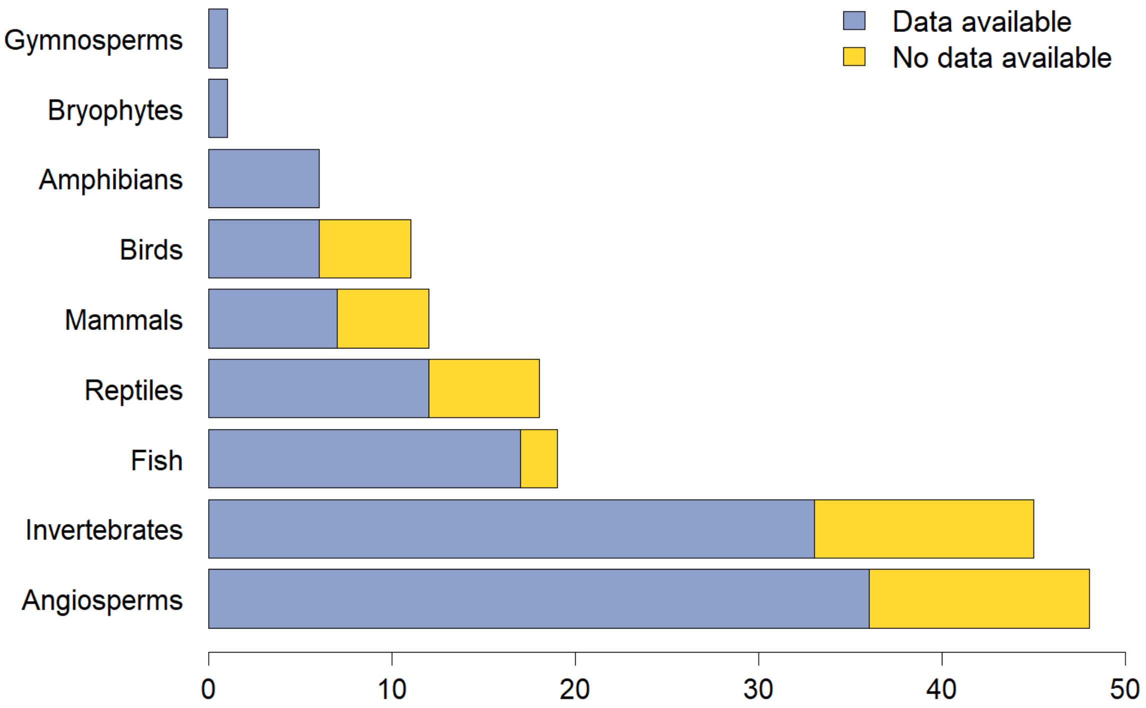

Fig. S3. Data availability for PM indicator for 161 species across taxonomic groups.

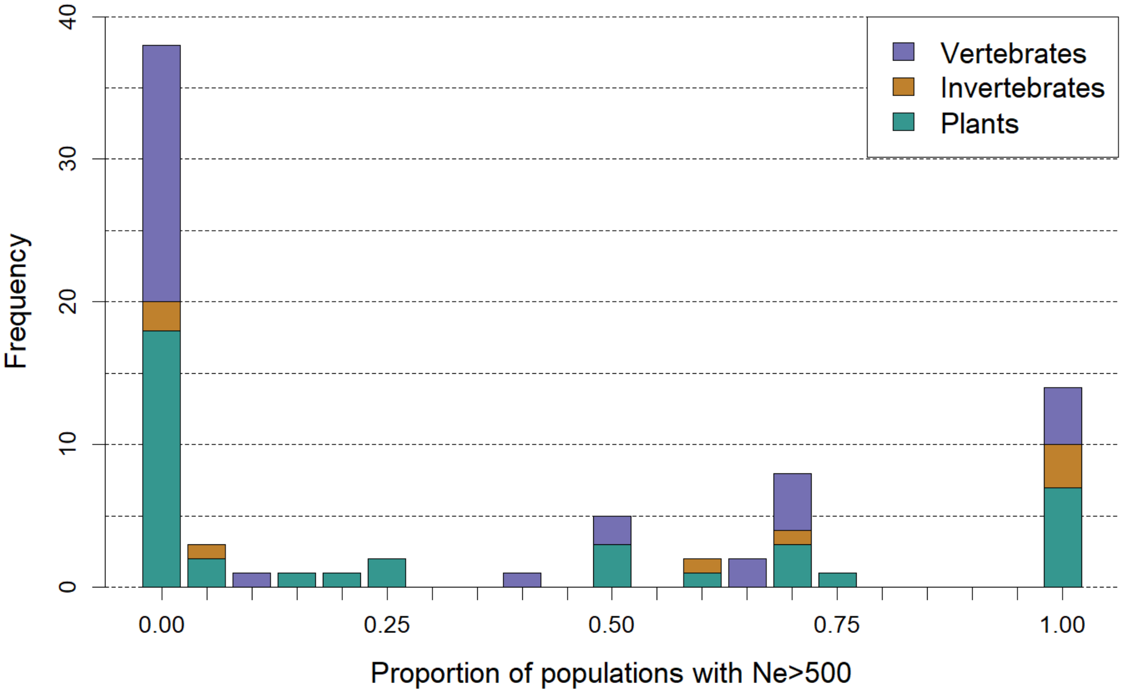

Fig. S4. Proportion of populations with Ne > 500 for 79 species. A value of one means that all assessed populations have an Ne > 500, while a value of zero means that all assessed populations have an Ne < 500.

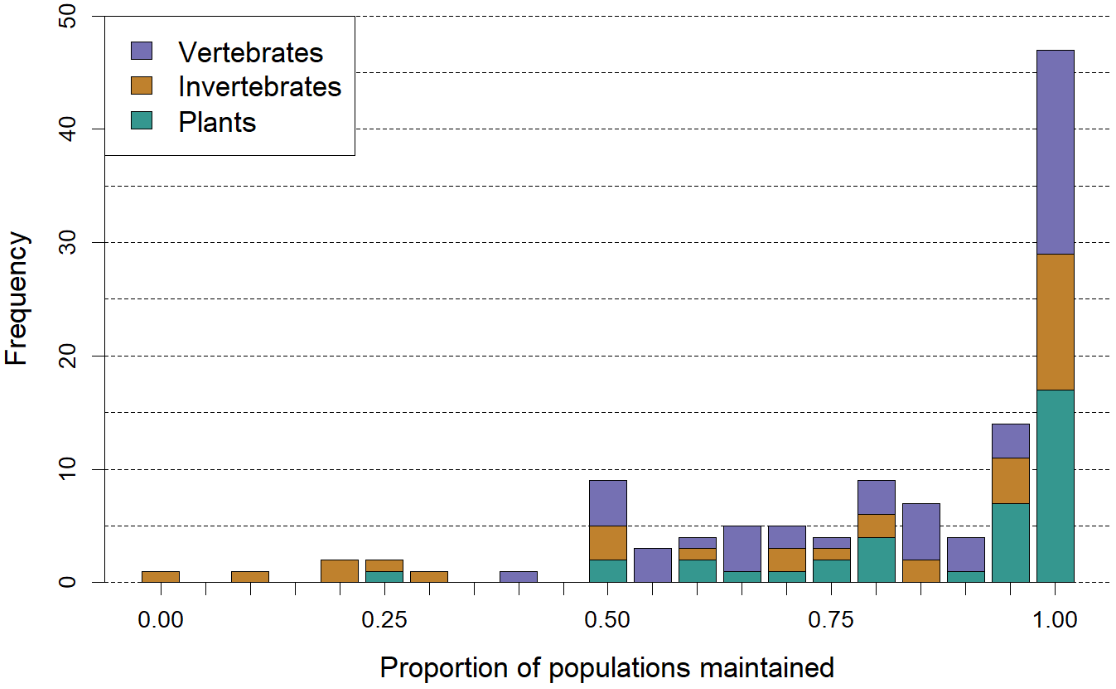

Fig. S5. Proportion of populations maintained for 119 species. A value of one means that all populations have been maintained since the species' historical baseline, while a value of zero means that all populations have been extirpated since the historical baseline.

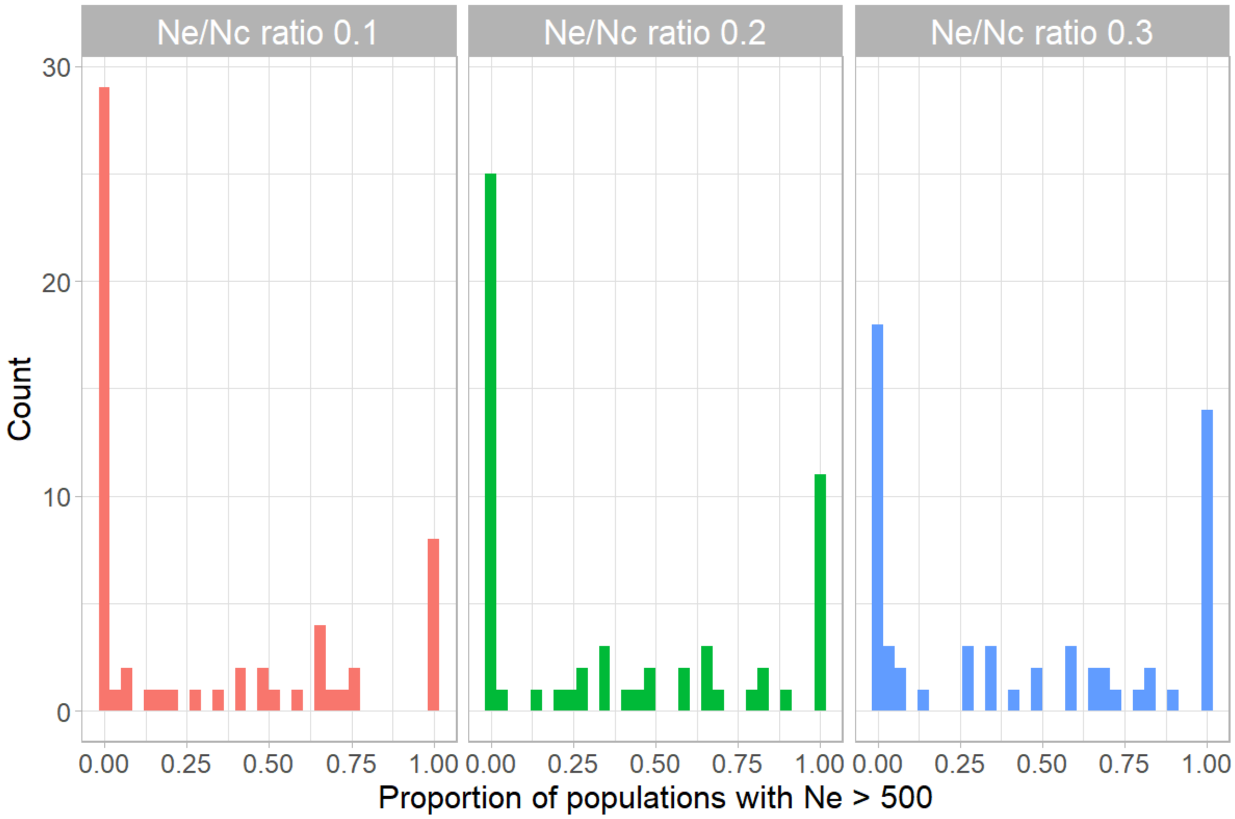

Fig. S6. Proportion of populations maintained for 59 species with Nc count data using different default Ne/Nc ratios of 0.1, 0.2, and 0.3. A value of one means that all assessed populations have an Ne > 500, while a value of zero means that all assessed populations have an Ne < 500.

Table S1. Ordered beta regression with ESA classification status evaluated as an ordered factor (levels = not listed, threatened, endangered): Does the number of populations with Ne>500 vary by ESA classification status? CI = confidence interval.

Variable	Estimate	Standard error	z value	Lower 95% CI	Upper 95% CI	Pr(>|z|)
Intercept	-0.256	0.193	-1.323	-0.634	0.123	0.186
ESA status (linear trend)	-0.211	0.260	-0.813	-0.721	0.298	0.416
ESA status (quadratic trend)	0.128	0.246	0.519	-0.354	0.609	0.603

Table S2. Ordered beta regression: Does the number of populations with Ne>500 vary by taxonomic group (3 groups represented)? CI = confidence interval; plants are the reference category.

Variable	Estimate	Standard error	z value	Lower 95% CI	Upper 95% CI	Pr(>|z|)
Intercept	-0.300	0.222	-1.351	-0.734	0.135	0.177
Invertebrates	0.491	0.529	0.928	-0.546	1.529	0.353
Vertebrates	-0.021	0.294	-0.070	-0.596	0.555	0.944

Table S3. Ordered beta regression: Does the number of populations with Ne>500 vary by taxonomic group (6 groups represented)? CI = confidence interval; mammals are the reference category.

Variable	Estimate	Standard error	z value	Lower 95% CI	Upper 95% CI	Pr(>|z|)
Intercept	-0.456	0.535	-0.853	-1.504	0.592	0.394
Birds	-0.283	0.648	-0.437	-1.553	0.987	0.662
Reptiles	-0.040	0.764	-0.052	-1.537	1.457	0.958
Fish	0.734	0.641	1.145	-0.522	1.990	0.252
Invertebrates	0.507	0.677	0.750	-0.819	1.834	0.453
Angiosperms	0.145	0.555	0.261	-0.944	1.233	0.794

Table S4. Ordered beta regression: Does the number of populations with Ne>500 vary by species range size? CI = confidence interval; restricted is the reference category.

Variable	Estimate	Standard error	z value	Lower 95% CI	Upper 95% CI	Pr(>|z|)
Intercept	-0.235	0.205	-1.145	-0.637	0.167	0.252
Wide ranging	-0.086	0.301	-0.286	-0.677	0.505	0.775

Table S5. Ordered beta regression with ESA classification status evaluated as an ordered factor (levels = not listed, threatened, endangered): Does the number of populations with Ne>50 vary by ESA classification status? CI = confidence interval.

Variable	Estimate	Standard error	z value	Lower 95% CI	Upper 95% CI	Pr(>|z|)
Intercept	-0.034	0.204	-0.168	-0.435	0.366	0.867
ESA status (linear trend)	0.024	0.293	0.083	-0.551	0.599	0.934
ESA status (quadratic trend)	0.022	0.277	0.081	-0.520	0.565	0.935

Table S6. Ordered beta regression with ESA classification status evaluated as an ordered factor (levels = not listed, threatened, endangered): Does the number of maintained populations vary by ESA classification status? CI = confidence interval.

Variable	Estimate	Standard error	z value	Lower 95% CI	Upper 95% CI	Pr(>|z|)
Intercept	0.855	0.111	7.715	0.638	1.072	<0.001
ESA status (linear trend)	-0.150	0.163	-0.921	-0.468	0.169	0.357
ESA status (quadratic trend)	0.160	0.158	1.010	-0.150	0.469	0.312

Table S7. Ordered beta regression: Does the number of maintained populations vary by taxonomic group (3 groups represented)? CI = confidence interval; plants are the reference category.

Variable	Estimate	Standard error	z value	Lower 95% CI	Upper 95% CI	Pr(>|z|)
Intercept	1.098	0.179	6.118	0.746	1.450	<0.001
Invertebrates	-0.531	0.241	-2.201	-1.003	-0.058	0.028
Vertebrates	-0.210	0.216	-0.976	-0.633	0.212	0.329

Table S8. Ordered beta regression: Does the number of maintained populations vary by species range size? CI = confidence interval; restricted is the reference category.

Variable	Estimate	Standard error	z value	Lower 95% CI	Upper 95% CI	Pr(>|z|)
Intercept	0.904	0.127	7.094	0.654	1.154	<0.001
Wide ranging	-0.151	0.200	-0.757	-0.543	0.240	0.449

Table S9. Species with multiple assessments (i.e., different values for calculating a given indicator: Ne500, PM, or both) and resulting indicator values. NA = no data (shaded in gray).

Species	Taxonomic group	# pops	# pops with Ne	# pops > 500	Ne500	# extant pops	# extinct pops	PM	Different inputs	Different value
Aphelocoma coerulescens	bird	10	10	1	0.10	10	0	1.00	Both	Both
		NA	NA	NA	NA	19	2	0.90		
Oncorhynchus clarkii virginalis	fish	122	122	40	0.33	122	6	0.95	Both	Both
		4	4	4	1.00	4	1	0.80		
Alasmidonta varicosa	invertebrate	241	171	35	0.20	241	NA	NA	Both	Ne500
		4	4	4	1.00	4	NA	NA		
Etheostoma chienense	fish	1	1	0	0.00	1	0	1.00	Both	Ne500
		2	1	1	1.00	2	0	1.00		
Mustela nigripes	mammal	14	14	0	0.00	14	15	0.48	Both	PM
		1	1	0	0.00	1	NA	NA		
Oncorhynchus apache	fish	30	30	0	0.00	30	14	0.68	Both	PM
		7	7	0	0.00	7	0	1.00		
Plestiodon egregius egregius	reptile	15	15	0	0.00	15	8	0.65	Both	PM
		19	15	0	0.00	19	4	0.83		
Toxolasma lividum	invertebrate	64	64	0	0.00	65	67	0.49	Both	PM
		146	146	0	0.00	146	119	0.55		
Pseudemys rubriventris	reptile	1	1	0	0.00	1	0	1.00	Both	No
		43	21	0	0.00	43	0	1.00		
Cambarus elkensis	invertebrate	NA	NA	NA	NA	1	0	1.00	PM	No
		NA	NA	NA	NA	6	0	1.00		
Necturus lewisi	amphibian	NA	NA	NA	NA	9	0	1.00	PM	No
		NA	NA	NA	NA	3	0	1.00		

Species	Taxonomic group	# pops	# pops with Ne	# pops > 500	Ne500	# extant pops	# extinct pops	PM	Different inputs	Different value
Notropis mekistocholas	fish	NA	NA	NA	NA	1	0	1.00	PM	No
		NA	NA	NA	NA	8	0	1.00		
Thoburnia atripinnis	fish	NA	NA	NA	NA	24	0	1.00	PM	No
		NA	NA	NA	NA	9	0	1.00		
Ambystoma cingulatum	amphibian	NA	NA	NA	NA	9	16	0.36	PM	PM
		NA	NA	NA	NA	4	0	1.00		
Bombus terricola	invertebrate	NA	NA	NA	NA	15	3	0.83	PM	PM
		NA	NA	NA	NA	47364	7908	0.86		
Cryptobranchus alleganiensis alleganiensis	amphibian	NA	NA	NA	NA	345	225	0.61	PM	PM
		NA	NA	NA	NA	4	0	1.00		
Erimystax harryi	fish	NA	NA	NA	NA	21	2	0.91	PM	PM
		NA	NA	NA	NA	22	1	0.96		
Etheostoma osburni	fish	NA	NA	NA	NA	18	17	0.51	PM	PM
		NA	NA	NA	NA	5	2	0.71		
Heterelmis stephani	invertebrate	NA	NA	NA	NA	0	2	0.00	PM	PM
		NA	NA	NA	NA	1	1	0.50		
Lavinia exilicauda chi	fish	NA	NA	NA	NA	2	2	0.50	PM	PM
		NA	NA	NA	NA	3	1	0.75		
Notophthalmus perstriatus	amphibian	NA	NA	NA	NA	116	74	0.61	PM	PM
		NA	NA	NA	NA	173	17	0.91		
		NA	NA	NA	NA	2	0	1.00		
Noturus munitus	fish	NA	NA	NA	NA	12	4	0.75	PM	PM
		NA	NA	NA	NA	5	1	0.83		

Species	Taxonomic group	# pops	# pops with Ne	# pops > 500	Ne500	# extant pops	# extinct pops	PM	Different inputs	Different value
Obovaria subrotunda	invertebrate	NA	NA	NA	NA	65	232	0.22	PM	PM
		NA	NA	NA	NA	34	104	0.25		
Pleurobema rubrum	invertebrate	NA	NA	NA	NA	28	108	0.21	PM	PM
		NA	NA	NA	NA	35	116	0.23		
Rhynchospora crinipes	angiosperm	39	19	0	0.00	35	4	0.90	PM	PM
		39	19	0	0.00	37	2	0.95		
Streptanthus bracteatus	angiosperm	16	16	0	0.00	16	2	0.89	PM	PM
		3	3	0	0.00	3	0	1.00		

Table S10. Ordered beta regression: Does the value of the Ne500 indicator significantly vary with the N_e/N_c ratio used? CI = confidence interval; reference group is N_e/N_c ratio = 0.1.

Variable	Estimate	Standard error	z value	Lower 95% CI	Upper 95% CI	Pr(>|z|)
Intercept	-0.431	0.176	-2.443	-0.776	-0.085	0.015
N_e/N_c ratio = 0.2	0.219	0.222	0.986	-0.216	0.654	0.324
N_e/N_c ratio = 0.3	0.329	0.223	1.472	-0.109	0.766	0.141