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Do species conservation assessments capture genetic diversity?

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ABSTRACT

The best known system for classifying threat status of species, the IUCN Red List, currently lacks explicit considerations of genetic diversity, and consequently may not account for potential adaptation of species to future environmental change. To address this gap, we integrate range-wide genetic analysis with IUCN Red List assessments.

We calculated the loss of genetic diversity under simulated range loss for species of *Delonix* (Leguminosae). Simulated range loss involved random loss of populations and was intended to model ongoing habitat destruction. We found a strong relationship between loss of genetic diversity and range. Moreover, we found correspondence between levels of genetic diversity and thresholds for 'non-threatened' versus 'threatened' IUCN Red List categories.

Our results support the view that current threat thresholds of the IUCN Red List criteria reflect genetic diversity, and hence evolutionary potential; although the genetic diversity distinction between threatened categories was less evident. Thus, by supplementing conventional conservation assessments with genetic data, new insights into the biological robustness of IUCN Red List assessments for targeted conservation initiatives can be achieved.

1. Introduction

Loss of genetic variation within a species may affect its ability to adapt to changing environmental conditions (Keller and Waller, 2002; Barrett and Schluter, 2008) and thus may increase risk of extinction. However, measures of genetic diversity have been largely absent from species conservation assessments (Mace and Purvis, 2008; Taberlet et al., 2012; Hoban et al., 2013) and are not explicitly considered in the IUCN Red List of Threatened Species, the most widely used system for assigning species' threat status (Laikre, 2010).

A striking dichotomy exists between assessors contributing to the IUCN Red List and practising conservation geneticists. A survey of papers published over the past decade (2004–2013) found 8897 papers on the topic "Red List", and 5505 on "conservation genetics"; however, only eighteen (<1%) of these papers included both these topics (Web of Science, consulted 26 January 2014). This suggests a lack of collaboration – that results in scientific publications – between the two scientific communities that needs to be addressed to ensure effective conservation measures for species.

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IUCN Red List criteria capture information on population decline and range loss (IUCN, 2001), implicitly assuming that this also captures loss of genetic variation; but the lack of an explicit genetic dimension means that IUCN assessments may not adequately reflect a species' potential for adapting to future environmental change. Genetic effects are implied in terms such as inbreeding, demographic stochasticity and effective population size. These properties of species would benefit from genetic analyses to achieve a better understanding of the biological and ecological impacts of changes in population size and ranges; however, there are no guidelines on how to incorporate such genetic data. Genetic analysis can also be used to test current thresholds used by the IUCN Red List. Specifically, the evidence that IUCN criteria, based on population size and dynamics, capture underlying genetic properties is limited. In order to remain as the leading system in assessing species' risk of extinction, the IUCN Red List needs to ensure that its underlying assumptions are based on a strong evidence base.

One of the biggest threats to biodiversity is habitat destruction, which directly impacts on species' ranges. Range loss can occur from the edge, or from within (fragmentation) and can have a negative impact on species survival. Conservation assessments undertaken for the IUCN Red List have two measures of range size: extent of occurrence (EOO) and area of occupancy (AOO). EOO measures the full spread of a species range whereas AOO measures the occupancy within that range. However, the genetic consequences of range loss in relation to the IUCN definitions of range are largely unexplored.

To create a stronger connection between range analysis (in particular in relation to IUCN Red List criteria) and conservation genetics, our aims were: (i) to determine the pattern of loss of genetic diversity in response to simulated range loss arising from habitat fragmentation; and (ii) to explore whether loss of genetic variation are encompassed indirectly in current IUCN Red List assessments. In other words, do IUCN Red List criteria based on range size dynamics reflect the evolutionary potential of threatened species? Our investigation centred on two tropical tree species in the genus *Delonix* (Leguminosae) occurring in Madagascar that are at ongoing risk of population loss due to land use conversion.

2. Methods

2.1. Study area and species information

Madagascar is one of the world's biodiversity hotspots with many species of conservation concern (Myers et al., 2000; Brummitt and Nic Lughadha, 2003; Mittermeier et al., 2005). Species endemism in Madagascar reaches over 80% among many animal groups (Goodman and Benstead, 2005) as well as several plant groups, such as legumes (Du Puy et al., 2002) and orchids (Hermans et al., 2007). However, Madagascar has a rapidly increasing human population and its unique biodiversity is under severe threat from habitat destruction and over-exploitation. A long-term vegetation mapping project showed that only 18% of primary vegetation still exists (Moat and Smith, 2007), and many species are in need of effective conservation action.

Leguminosae is the world's third largest angiosperm family and species in this family are important components of the Malagasy flora (Du Puy et al., 2002). Moreover, legume species are present in all major terrestrial ecosystems and as such this family is a useful proxy for evaluating global patterns of angiosperm diversity (Nic Lughadha et al., 2005). In order to conduct a comparative investigation of patterns of genetic variation at the species level, we chose two species of *Delonix* (Leguminosae) in Madagascar as the focal species for the present study – *Delonix decaryi* (R.Vig.) Capuron and *Delonix floribunda* (Baill.) Capuron – since the taxonomy and full species' ranges are well defined (Rivers, 2011).

The Malagasy species of *Delonix* are restricted to the dry forest and the spiny forest. Species of the genus are thought to be strongly outbreeding, although pollinators and seed dispersal agents have not been studied in detail (Du Puy et al., 1995). The floral characters of *Delonix decaryi* are indicative of moth pollination, including night-time opening, white petals with red, long-exerted stamens, and a narrowly tubular, nectariferous claw on the upper petal (Du Puy et al., 1995, Rivers pers. obs.). In contrast, the highly reduced petals in *Delonix floribunda* may be an adaptation to pollination by the Malagasy sunbird, *Nectarinia sovimanga* (Du Puy et al., 1995). The fruit pods in both species are large and woody, containing many seeds about 10–20 mm long, that often remain closed until after they have fallen (Du Puy et al., 1995). The genetic study of the genus (Rivers et al., 2011) reveals that the two species in question have similar levels of genetic diversity, although the genetic diversity of *D. decaryi* is related to spatial distance, whereas this is not the case for *D. floribunda*.

Delonix decaryi and *D. floribunda* were sampled (2007–2008) across their full geographic range in Madagascar (72 trees from 22 localities and 65 trees from 21 localities, respectively). These two tree species tend to occur in relatively small local populations; and all trees available at each locality were sampled. Leaf samples were placed in silica gel and stored at ambient temperature.

Genetic studies were based on AFLPs (Vos et al., 1995). We used this genetic marker because it was possible to include a large number of marker loci, ensuring broad genomic coverage. DNA extractions and AFLP analysis followed methodology previously outlined (Rivers et al., 2011). In brief, DNA extractions used a modified CTAB method (Doyle and Doyle, 1990). AFLP analysis followed standard protocol (Life Technologies, Core Reagent Kit cat no 10482-016, Starter Primer Kit cat no 10483-014). PCR fragments from three primer combinations (MseI-CAA/EcoRI-AAG, MseI-CAA/EcoRI-ACT and MseI-CAC/EcoRI-AGG) were analysed using a Beckman Coulter CEQ 8000 in order to separate fragments of different lengths. A mixture of 40 μ l of formamide, 0.5 μl PCR product and 0.5 μl of dye labelled size standard (60–420 bp) (Beckman Coulter-GenomeLab DNA Size Standard Kit 400 no 608098) was made and transferred to a single well on a 96 well PCR plate for analysis. The raw data were analysed using the fragment analysis module of the CEQ system software version 9.0. Signal peaks were filtered according to a number of criteria. Only signal peaks with an intensity of 5% or more of the

Table 1
The range thresholds for criteria A2-4, B and D2 in the IUCN Red List Categories and Criteria v. 3.1 (IUCN, 2001).

	Critically Endangered	Endangered	Vulnerable	Non-threatened
Criterion A2-4—EOO range reduction	>80%	>50%	>30%	<30%
Criterion B1 EOO* range size	<100 km^2	<5000 km^2	<20000 km^2	>20000 km^2
Criterion D2—AOO range size	n/a	n/a	<20 km^2 and $\leqslant 5$ locations	>20 km^2 and >5 locations

* Two out of the following three subcriteria also need to be fulfilled: (a) severely fragmented range/low number of locations; (b) extreme fluctuation in range, habitat or numbers of individuals and; (c) continuing decline in range, habitat or numbers of individuals.

maximum signal peak within a sample were included. Similarly, only peaks with a slope parameter >10% were retained. Fragment sizes for each peak were estimated based on an internal size standard. A binary table was constructed which recorded presence/absence of fragments ranging between 60 and 420 bp. Altogether, 1,006 polymorphic AFLP loci were scored. Robustness of AFLP scoring was tested by analysing replicated ALFP runs for thirty samples. The mean replication of PCR error rate was 4.1%, within accepted error rates for AFLP markers (Bonin et al., 2007).

2.2. Simulated range reduction and allelic richness estimation

We performed 1000 simulated range reductions for each species by dropping a random subset of localities for each simulation. Our random subsets were determined using the random number generation function in APLWIN version 3.2 (APL2000 Inc., www.apl2000.com). This exercise generated a dataset of 1,000 simulations covering a wide diversity of range loss, from near to the current range to almost complete loss of range. For each simulation, range size was calculated in terms of extent of occurrence (EOO) and area of occupancy (AOO) in accordance with recommendations from the IUCN Red List using the Royal Botanic Gardens, Kew, Conservation Assessment Tool (http://www.kew.org/gis/projects/cats/index.html) (Moat, 2007) in ArcView 3.2. EOO is calculated as the area of the minimum convex polygon; AOO is calculated as the area of all occupied grid cells. The minimum number for the subset of localities was three, as this is the smallest number for which EOO can be estimated. As there is still debate over the ideal size of grid cells for calculating AOO (Hernández and Navarro, 2007; Gaston and Fuller, 2009), EOO was the range measure used in our evaluations of loss of genetic diversity for criteria A and B (see below); for criterion D2, calculation of AOO was based the IUCN recommendation of a 2×2 km grid.

Allelic richness was used as the measure of genetic diversity, as this measure most effectively captured the full scope of our AFLP marker base. Allelic richness is the number of alleles found in the species across all localities. For AFLP data, with allelic identities of 0 or 1 at each locus, allelic richness reduces to the total number of polymorphic loci observed across all individuals sampled. In order to account for variation in sample size among localities (Leberg, 2002; Kalinowski, 2004; Szpiech et al., 2008), a multiple random subsampling procedure (Leberg, 2002) was used to estimate allelic richness. (Leberg, 2002). Subsampling was repeated 1,000 times within each locality, based on the minimum observed sample size among localities, again using APLWIN vers 3.2 (APL2000 Inc., www.apl2000.com). Allelic richness for each of the 1,000 simulated range reductions, was estimated as the average number of alleles observed over the 1,000 subsampling replicates.

2.3. Conservation assessments

IUCN Red List categories include two non-threatened (Least Concern [LC] and Near Threatened [NT]) and three threatened (Vulnerable [VU], Endangered [EN] and Critically Endangered [CR]) categories. For each simulation, we translated simulated range size and range loss into IUCN Red List categories using all applicable criteria: A (population decline), B (restricted geographic range, together with fragmentation, decline and/or fluctuations in range, habitat or population size) and D2 (restricted range-size and/or number of locations) (see Table 1). For criterion B, in addition to range size measures, individual trees sampled were assigned to locations/subpopulations based on spatial methods developed by Rivers et al. (2010); and continuing decline in habitat is seen in recent estimates of the deforestation rate of the dry spiny forest in Madagascar of circa 1% per annum (Harper et al., 2007; Moat and Smith, 2007; MEFT et al., 2009).

We tested the relationship between the IUCN Red List categories and remaining allelic diversity using the analysis of variance followed by Tukey's test for multiple comparisons (SPSS Statistics version 17.0).

3. Results

The relationship between the loss of range and the loss of genetic diversity is broadly linear for both *D. decaryi* and *D. floribunda* ($R^2 = 0.82$ and 0.61, $p < 0.001$, respectively) (Figs. 1A and 2A). The two species exhibited slightly different patterns of range loss. Specifically, a disjunction in the range of *D. floribunda* resulted in a gap in the simulated range loss estimates (Fig. 2A).

The relationship between IUCN Red List categories and range loss is more complex (Figs. 1B–D and 2B–D). For all three criteria (A, B and D2) there is a significant difference in genetic diversity between non-threatened (LC/NT) and threatened (VU/EN/CR) categories. For criterion A, levels of genetic diversity also differ significantly among the threatened categories

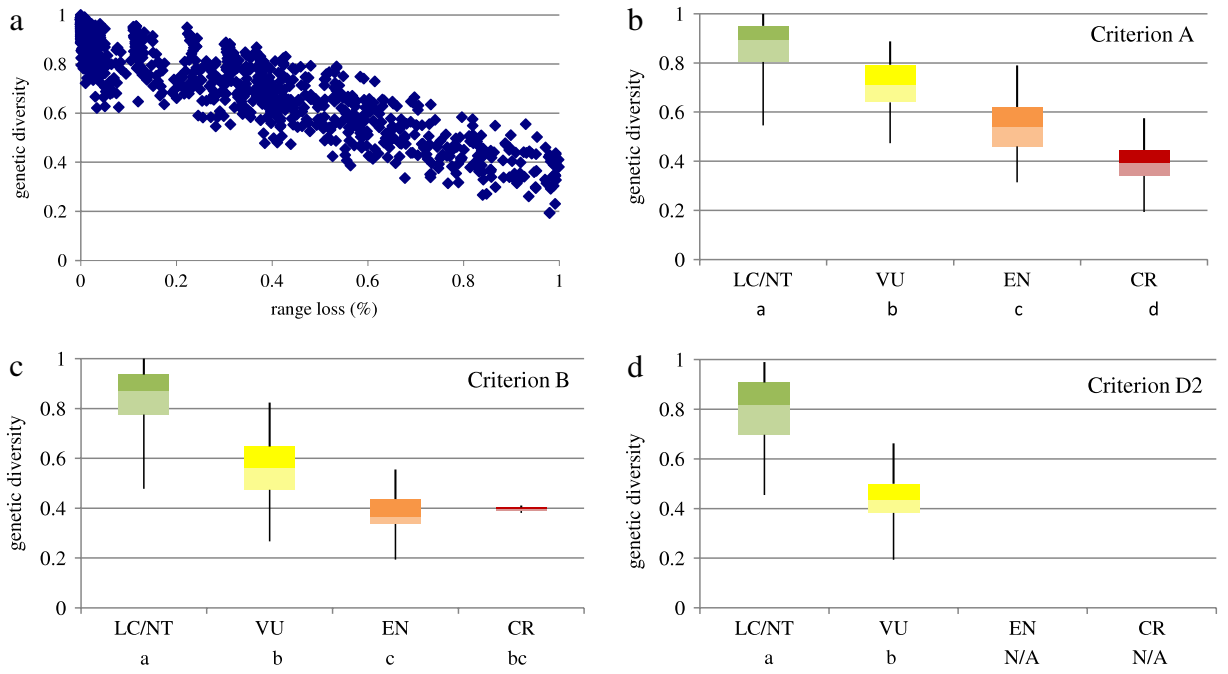

Fig. 1. (**A**) Genetic diversity of *Delonix decaryi* as a function of simulated range reduction measured as extent of occurrence (EOO); (**B–D**) Box-plots of percentage genetic diversity for the IUCN Red List categories under (**B**) criterion A, (**C**) criterion B, and (**D**) criterion D2. The lower-case letters (a–d) indicate significant differences between categories using ANOVA and Tukey's test for multiple comparisons.

(VU/EN/CR) (Figs. 1B and 2B). For criterion B, non-threatened and threatened categories differ significantly in genetic diversity, but there is limited discrimination among threatened categories (Figs. 1C and 2C). Criterion D is effective in distinguishing between non-threatened and threatened categories, but by definition criterion D2 is only applicable for the Vulnerable category so no comparison can be made between individual threatened categories (Figs 1D and 2D). The same patterns are found in both species: *D. decaryi* and *D. floribunda* (Figs. 1 and 2).

4. Discussion

Overall, our results for both *D. decaryi* and *D. floribunda* show that range loss is a good proxy for loss of genetic diversity. Both species have a linear relationship between the loss of genetic diversity and range loss with a similar rate of decline. Using neutral genetic diversity as a proxy of adaptive genetic diversity (see also Holderegger et al., 2006; Barrett and Schluter, 2008), we show that range loss measured in IUCN Red List conservation assessments can indeed capture the loss in species' genetic diversity.

It is often assumed that genetic diversity, or standing genetic variation, can be taken as a proxy for evolutionary potential (Keller and Waller, 2002; Barrett and Schluter, 2008). In order to invoke a relationship between genetic diversity and evolutionary potential, it is important to obtain a broadly based sample of the genome. By using AFLPs, we were able to include a large number of loci, and hence broad coverage across the genome. Thus, loss of range-wide genetic diversity would result in a corresponding loss in evolutionary potential. An alternative would be to identify genetic adaptation associated with specific genomic regions that are involved in response to specific environmental challenges. However, even closely related species can show very different genetic adaptations to the same environmental challenge (e.g., Zhang et al., 2013). For purposes of IUCN Red List classification or even validation, such a directed genetic investigation might be useful for case studies. On the other hand, the approach we have taken using standing genetic variation is more broadly applicable, perhaps even by making use of existing genetic data, and hence could be a basis for broader validation of the IUCN Red List criteria.

As expected, there are consistently higher levels of genetic diversity for non-threatened species than for threatened species. There is support for the thresholds set between non-threatened versus threatened IUCN Red List categories in all three range-based criteria examined (A, B and D2), where in each case levels of genetic diversity observed between threatened and non-threatened categories differ significantly. This means that the thresholds for assigning species to the Vulnerable category, which distinguishes non-threatened categories from threatened categories, are set at genetically meaningful levels. The amount of genetic diversity at a specific threat category, however, is not consistent across criteria, as different criteria are measuring different aspects of threat (range loss, absolute range and/or number of locations).

Our results also support the more precise thresholds between the threatened categories in one of the three criteria examined. Under criterion A, the levels of genetic diversity significantly differed among threat categories (Critically

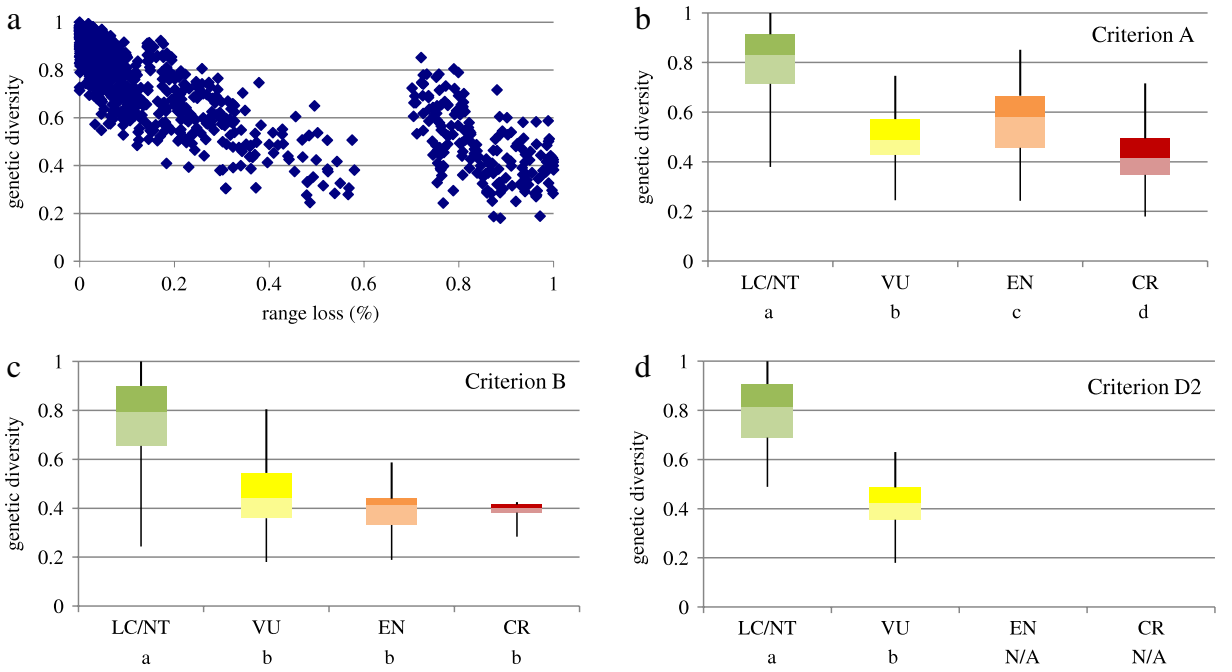

Fig. 2. (**A**) Genetic diversity of *Delonix floribunda* as a function of simulated range reduction measured as extent of occurrence (EOO); (**B–D**) Box-plots of percentage genetic diversity for the IUCN Red List categories under (**B**) criterion A, (**C**) criterion B, and (**D**) criterion D2. The lower-case letters (a–d) indicate significant differences between categories using ANOVA and Tukey's test for multiple comparisons. The gap in range reductions (**a**) is due to a North:South range disjunction.

Endangered, Endangered, Vulnerable), whereas this difference in genetic diversity between threatened categories was not detected under criterion B. The two thresholds within the threatened categories (i.e. between Vulnerable / Endangered and between Endangered / Critically Endangered) therefore appear to show greater genetic distinction under criterion A than under criterion B. This consideration does not apply to criterion D2, as only the Vulnerable category can be assigned here. Criterion D2 has previously been criticised for being too inclusive (Mace et al., 2008; Group, 2010); however, we demonstrate the opposite: the two species that qualify for Vulnerable under criterion D2 did so at higher levels of simulated range loss than those required to qualify them as Critically Endangered using criteria A and B. It is also worth mentioning that the genetic diversity is generally higher under criterion A than criterion B, for the same category of threat.

Our study models the loss of genetic diversity through loss of populations across the known range; it does not explicitly take into account other population genetic processes, such as inbreeding and genetic drift, which would accelerate loss of genetic variation as species' ranges contract. Although drift and inbreeding are critically important processes for the evolution and survival of species, these genetic processes are unlikely to have a major genetic impact over the 100 year time frame (as recommended by IUCN) over which this habitat loss is predicted. The generation times for *Delonix* are measured in decades and significant genetic turnover within populations over such a time scale is unlikely.

In our study, we combined the empirical data on genetic diversity with simulated loss of populations. Thus, the biological properties underlying the levels and distribution of genetic diversity in our data are embedded in the analysis. A potential further direction in which to take this work would be to construct simulated genetic data, building into the simulation underlying processes such as genetic drift and inbreeding, thus applying the power of a modelling approach to tease out the potential effects of such genetic processes on genetic decline (Hoban, 2014).

Our results differ from claims that tree species are slow to show loss of genetic variation under fragmentation because they are long-lived with long-distance gene flow (Dunphy and Hamrick, 2007; Meagher, 2010). Alsos et al. (2012) also used AFLPs to investigate the genetic consequences of anticipated range shifts, due to climate change, on a set of northern hemisphere plants. They showed that loss of genetic variation was nonlinear (in contrast to our findings for *Delonix*) and differed between species, partly due to genetic differentiation (Alsos et al., 2012) and probably also due to the directional nature of the range loss modelled under climate change scenarios. The genetic response to directional, climate change-driven range shifts has been modelled multiple times (see Pauls et al., 2013). In contrast, here we simulated the sequential loss of parts of the range at random, as for many areas, especially in the tropics, anthropogenic habitat destruction and fragmentation of species ranges are still the most urgent threats to biodiversity.

It is difficult to extrapolate the result found for these two tropical trees, although the demography and ecology of these species are not atypical. Moreover, the approach outlined in this paper could be applied to other species to enrich our understanding of the genetic implications of IUCN criteria. As more range-wide genetic studies across families and phyla become available, it will become possible to confirm whether the IUCN Red List truly represents evolutionary potential.

5. Conclusion

The IUCN Red List currently lacks an explicit genetic dimension. Although genetic analyses could lead to a better understanding of the biological and ecological impacts of changes in population size and ranges, there are currently no guidelines on how to incorporate such genetic data. For example, the distinction between species with small population sizes and restricted ranges, but with high genetic diversity, versus those that have undergone population bottlenecks, resulting in low genetic diversity, cannot currently be made under existing IUCN Red List criteria. This highlights the importance of incorporating genetic data to aid effective conservation strategies. As genetic data are becoming increasingly available (a trend that is likely to continue) it is essential to integrate such data with the IUCN categories and criteria in order to make the best informed conservation decisions.

Our novel approach shows that the current gap between IUCN Red List assessments and conservation genetics studies can be bridged: changes in genetic diversity can be incorporated and support current range-based criteria for the IUCN Red List. For the first time, by combining genetic data with full range analysis of conventional species conservation assessments we gain new and much needed insights into the robustness of the thresholds set in IUCN Red List. An integrated approach of this kind ensures that research outcomes in these two fields can be most effectively applied to biodiversity conservation. We call for further range-wide analyses of genetic diversity of species from different taxonomic groups, from different geographic areas, and with different life histories to validate this approach. Ensuring that the IUCN Red List captures genetic diversity is of critical importance for the survival of biodiversity in the face of ongoing environmental change.

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