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Genetic diversity, population structure and conservation genetics approaches for the long-term protection of wildlife species

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Abstract

Genetic diversity underlies the adaptive potential, demographic resilience and long-term persistence of wildlife populations, yet it remains among the least monitored dimensions of biodiversity relative to species and ecosystem diversity. This review synthesizes current evidence on genetic diversity, population genetic structure and the conservation genetics and genomics approaches used to safeguard wildlife populations against extinction, drawing on peer-reviewed primary studies, invited reviews and international policy documents addressing effective population size thresholds, inbreeding depression, genetic rescue, landscape genetics, molecular-marker technologies and global genetic-diversity indicators. Small, fragmented populations are shown to be disproportionately vulnerable to genetic drift, inbreeding depression and reduced adaptive capacity. Historically influential thresholds such as the 50/500 rule remain widely cited but are now considered insufficient in isolation. Genetic rescue, illustrated by the Florida panther and Mexican wolf, together with the long-studied genetic depletion of the cheetah, demonstrates both the promise and the risk associated with restoring gene flow to isolated populations. The transition from microsatellite genotyping toward genome-wide single-nucleotide polymorphism (SNP) and whole-genome approaches has expanded analytical resolution without eliminating the practical value of earlier marker systems, and the 2022 Kunming-Montreal Global Biodiversity Framework has, for the first time, embedded measurable genetic-diversity indicators into binding international policy. Long-term protection of wildlife is found to require integrating genetic and genomic evidence into population viability assessment, landscape connectivity planning, captive-breeding management and international reporting frameworks, while addressing persistent gaps in monitoring capacity, data availability and equitable governance of genetic resources.

Keywords: Conservation genetics, genetic diversity, population structure, effective population size, genetic rescue, landscape genetics, conservation genomics

Introduction

The Convention on Biological Diversity recognizes biodiversity at three interdependent levels: ecosystem diversity, species diversity and genetic diversity, with genetic diversity forming the foundation upon which the other two levels ultimately depend (Tserendulam, Ulziisaikhan, & Delgerzul, 2023) ^[24]. Of the roughly 2.16 million species so far described, tens of thousands are currently classified as threatened with extinction on the IUCN Red List, and the great majority of extinction-risk assessments continue to rely almost exclusively on demographic and ecological criteria rather than genetic status (Tserendulam *et al.*, 2023) ^[24]. This asymmetry is significant because genetic diversity determines a population's capacity to resist disease, tolerate environmental change, and avoid the compounding fitness costs associated with inbreeding in small, isolated groups.

Conservation genetics emerged as a distinct discipline in the early 1980s, when early molecular studies revealed alarmingly low genetic variation in flagship species such as the cheetah (*Acinonyx jubatus*), prompting a re-evaluation of how genetic information should inform species management (O'Brien, Wildt, Goldman, Merrill, & Bush, 1983) ^[20]. Since then, the field has expanded from single-locus allozyme and microsatellite analyses toward genome-wide approaches capable of resolving population structure, effective population size, inbreeding load and adaptive variation with far greater precision (Hohenlohe, Funk, & Rajora, 2021) ^[11]. This shift, often termed the transition from conservation genetics to conservation genomics, has coincided with the adoption of genetic-diversity indicators within the 2022 Kunming-Montreal Global Biodiversity Framework, marking the first time that genetic status has been formally embedded in an international biodiversity policy instrument (Mastretta-Yanes *et al.*, 2024) ^[16, 17].

This review synthesizes evidence across four interconnected themes that are central to the long-term genetic management of wildlife: (i) the concept and significance of genetic diversity; (ii) population genetic structure and its drivers; (iii) the molecular and genomic tools used to characterize wildlife populations; and (iv) the applied conservation genetics approaches, including effective population size thresholds, genetic rescue, landscape genetics and genetic monitoring, that translate genetic evidence into management action. Case studies of the Florida panther, Mexican wolf and cheetah are used throughout to illustrate general principles with concrete, well-documented examples.

Genetic Diversity: Concepts and Significance

Genetic diversity refers to the heritable variation within and among individuals of a species, encompassing allelic richness, heterozygosity, and nucleotide-level polymorphism. It provides the raw material for natural selection and adaptive evolution, buffers populations against environmental and demographic stochasticity, and underlies resistance to emerging pathogens (Hohenlohe *et al.*, 2021)^[11]. Populations with depleted genetic diversity are, on average, less able to track environmental change, more susceptible to disease outbreaks, and more vulnerable to the cumulative fitness costs of inbreeding.

1. Commonly used measures

- Observed and expected heterozygosity (H_o , H_e), summarizing the proportion of heterozygous genotypes at sampled loci.
- Allelic richness, the number of alleles per locus, often standardized by rarefaction to allow comparison across unequal sample sizes.
- Nucleotide diversity (π), used with sequence-level genomic data to describe variation at the DNA base-pair level.
- The inbreeding coefficient (F), quantifying the probability that two alleles at a locus are identical by descent.

2. Sources and drivers of genetic erosion

- Genetic drift, the random change in allele frequencies that is proportionally stronger in small populations.
- Demographic bottlenecks, which sharply reduce the number of breeding individuals and, with them, the pool of segregating alleles.
- Inbreeding arising from restricted mate choice in small or isolated populations.
- Selective sweeps and background selection, which can locally reduce diversity around loci under strong selection.

The cheetah remains one of the most cited illustrations of extreme genetic depletion in a free-ranging mammal. Early biochemical and molecular studies found remarkably low variation across allozyme and mitochondrial markers, a pattern interpreted as the legacy of one or more severe population bottlenecks during the late Pleistocene (O'Brien *et al.*, 1983)^[20]. This low diversity has since been associated with poor semen quality, elevated juvenile mortality in captivity, and unusual tolerance of reciprocal skin grafts between unrelated individuals – a phenomenon normally observed only between close relatives or inbred laboratory strains. Later phylogeographic work using mitochondrial DNA and microsatellites nevertheless identified deeper-than-expected divergence among cheetah populations, supporting the genetic distinctiveness of the Northeast African and Asiatic lineages despite the species' overall low variability (Charruau *et al.*, 2011)^[3]. The cheetah case illustrates a key principle: overall genetic depauperation and meaningful population structure are not mutually exclusive, and both must be assessed before conservation units are defined.

Population Genetic Structure in Wildlife

Population genetic structure describes how genetic variation is distributed across space, typically as a result of restricted gene flow, historical biogeography, and local adaptation. Structure is most often quantified using F_{ST} and related differentiation statistics, Bayesian clustering methods such as STRUCTURE or ADMIXTURE, and multivariate ordination approaches such as principal component analysis and discriminant analysis of principal components.

1. Key drivers of genetic structure

- Dispersal limitation arising from species-specific life history and mobility.
- Physical and ecological barriers, including rivers, mountain ranges and vegetation transitions.
- Historical biogeographic events, such as Pleistocene range contractions and expansions.
- Anthropogenic habitat fragmentation, which increasingly overlays and intensifies natural patterns of structure.

2. Translating structure into management units

A central application of population structure analysis is the delineation of conservation units below the species level. The concept of the Evolutionarily Significant Unit (ESU) – a population or group of populations that is reciprocally monophyletic or otherwise historically isolated and represents a significant component of a species' evolutionary legacy – together with the more operational Management Unit (MU), defined by significant allele-frequency divergence among demographically independent populations, provides the conceptual basis on which many national and international conservation programmes allocate limited resources (Moritz, 1994)^[19]. In cheetahs, for example, the phylogeographic divergence documented between African and Asiatic lineages, and among African subspecies, has direct implications for whether individuals from different regions should be managed together or kept genetically separate in captive-breeding and reintroduction programmes (Charruau *et al.*, 2011)^[3].

- Correctly identifying structure prevents the artificial mixing of divergent lineages during translocation or captive breeding.
- It also prevents the opposite error – treating demographically independent but weakly divergent populations as a single unit and underestimating extinction risk at the local scale.

Conservation Genetics and Genomics Approaches: Molecular Tools

The toolkit available to conservation practitioners has expanded considerably since the discipline's origins in allozyme electrophoresis. Microsatellites (short tandem repeats) dominated the field for two decades because of their high per-locus polymorphism and relatively low cost, and they remain in routine use for parentage assignment, individual identification from non-invasive samples, and long-term monitoring programmes with established baseline datasets (Hauser, Athrey, & Leberg, 2021) ^[9]. The advent of next-generation sequencing has since enabled genome-wide genotyping through reduced-representation approaches such as restriction-site-associated DNA sequencing (RAD-seq) and genotyping-by-sequencing (GBS), SNP arrays, and, increasingly, low-coverage or high-coverage whole-genome resequencing (Davey & Blaxter, 2010; Tserendulam *et al.*, 2023) ^[4, 24].

1. Comparing marker systems

- SNPs typically offer greater resolution for detecting fine-scale population differentiation, migrant identification, and genome-wide estimates of heterozygosity, because thousands of loci can be surveyed simultaneously (Hauser *et al.*, 2021) ^[9].
- Microsatellites retain practical advantages for parentage and close-kinship analysis in species with reduced genetic variation, where their higher per-locus heterozygosity can outperform biallelic SNPs unless very large SNP panels are used (Hauser *et al.*, 2021) ^[9].
- Cost, sample quality (particularly for degraded non-invasive samples), and continuity with historical datasets often determine marker choice in ongoing monitoring programmes as much as raw statistical power does.

2. From population genomics to applied management

Population genomic approaches now allow simultaneous estimation of effective population size, historical demographic trajectories, inbreeding load at the level of individual genomes, and putative signatures of local adaptation, all from a single sequencing dataset (Hohenlohe *et al.*, 2021) ^[11]. Genomic tools have been applied to characterize evolutionary dynamics in small, fragmented populations such as the Brazilian merganser, and are increasingly central to case-study-based conservation programmes that must bridge the gap between academic genomic research and field-level decision-making (Garner *et al.*, 2016) ^[8]. Figure 3 summarizes the general workflow linking field sampling, marker and platform choice, genetic and genomic analysis, and downstream conservation applications.

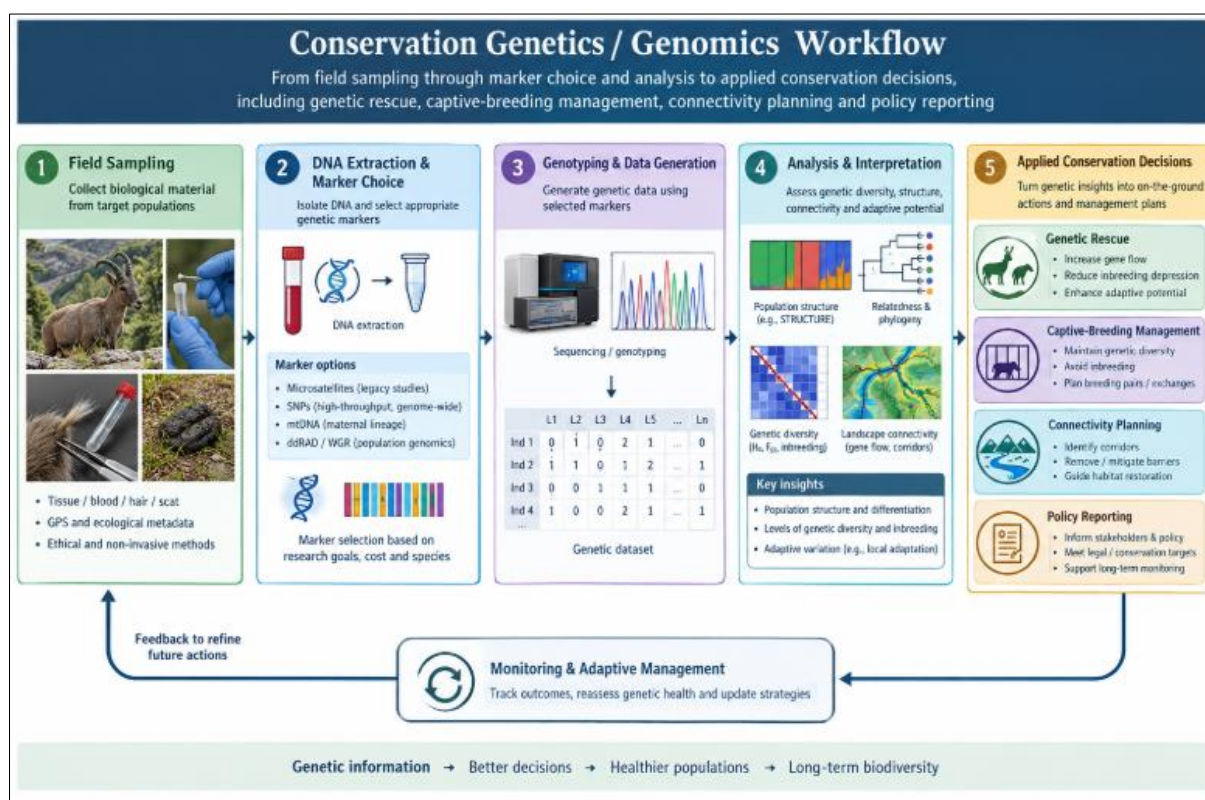


Fig 3: Conservation genetics / genomics workflow, from field sampling through marker choice and analysis to applied conservation decisions, including genetic rescue, captive-breeding management, connectivity planning and policy reporting.

Effective Population Size and Minimum Viable Population Thresholds

Effective population size (N_e) – the size of an idealized population that would experience the same rate of genetic drift or inbreeding as the real population under study – is widely regarded as a more meaningful genetic-risk metric than the census population size (N), because N_e accounts for unequal sex ratios, variance in reproductive success, fluctuating population size across generations, and overlapping generations. In most wild populations the N_e/N ratio is considerably below one, commonly falling in the range of roughly 0.1 to 0.2, meaning that genetically relevant population size is typically five to ten times smaller than the number of individuals counted in a census.

1. The 50/500 rule and its revision

The widely cited “50/500 rule”, originating with Franklin's (1980) ^[6] recommendation and later incorporated into IUCN Red List guidance, proposed that an effective population size of at least 50 is required to limit short-term inbreeding depression, while a larger threshold of at least 500 is required to retain long-term evolutionary potential (Jamieson & Allendorf, 2012) ^[13]. Subsequent critical reviews have argued that both thresholds are too low: evidence accumulated since 1980 indicates that N_e of 50 is inadequate to prevent meaningful fitness loss over five generations, that N_e of at least 100 is needed to limit total fitness loss to about 10 percent, and that retaining long-term evolutionary potential may require N_e in the order of 1,000 rather than 500 (Frankham, Bradshaw, & Brook, 2014) ^[5].

2. Genetic indicators in global policy

The revised $N_e \geq 500$ threshold has since been formally adopted as one of three genetic-diversity indicators under the monitoring framework for the 2022 Kunming-Montreal Global Biodiversity Framework, alongside an indicator tracking the proportion of genetically distinct populations maintained, and an indicator of the number of species monitored using DNA-based methods (Mastretta-Yanes *et al.*, 2024) ^[16, 17]. A multinational evaluation of these indicators across 919 taxa and 5,271 populations in nine countries found that although most species retained the majority of their historical populations, 58 percent of assessed species had populations too small to maintain genetic diversity under the $N_e \geq 500$ criterion, and that existing IUCN Red List assessments frequently failed to capture this genetic risk (Mastretta-Yanes *et al.*, 2024) ^[16, 17]. This finding underscores a persistent and largely underappreciated gap between demographic conservation status and underlying genetic vulnerability.

6. Inbreeding Depression and Genetic Rescue

Inbreeding depression – the reduction in fitness-related traits such as fertility, offspring survival and disease resistance that accompanies increased homozygosity – arises primarily through the increased expression of recessive deleterious alleles and the loss of heterozygote advantage at key loci. In small, isolated wildlife populations, inbreeding depression can interact with demographic decline in a self-reinforcing cycle: smaller populations experience greater drift and inbreeding, which further erodes fitness and survival, which in turn accelerates further decline (Figure 1).

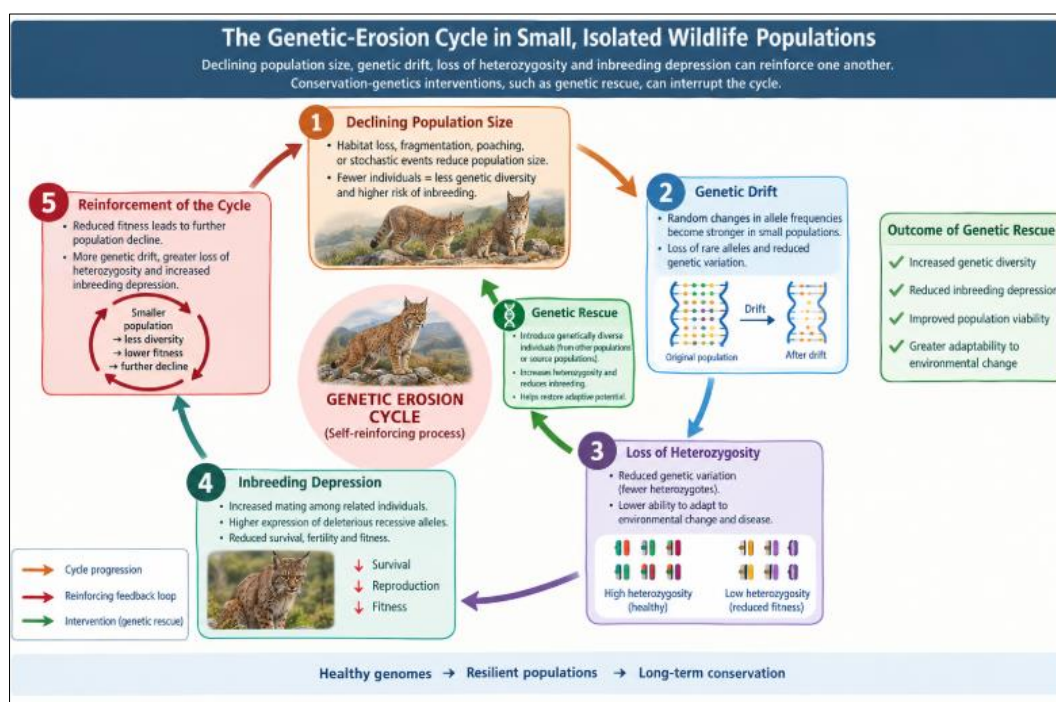


Fig 2: The genetic-erosion cycle in small, isolated wildlife populations, showing how declining population size, genetic drift, loss of heterozygosity and inbreeding depression can reinforce one another, and where conservation-genetics interventions such as genetic rescue can interrupt the cycle.

1. Genetic rescue as a management response

Genetic rescue – the deliberate introduction of unrelated individuals to restore gene flow and increase fitness in a small, inbred population – has been proposed as one of the most powerful tools available for conserving small and declining populations, yet it remains comparatively rarely applied in practice because of persistent concern about the risk of outbreeding depression, in which the introduction of poorly adapted or divergent genetic material can, in principle, reduce local fitness (Tallmon, Luikart, & Waples, 2004) ^[23].

2. Case study: the Florida panther

The Florida panther (*Puma concolor coryi*) is among the most thoroughly documented genetic rescue programmes in wildlife conservation. By the early 1990s the population had declined to fewer than 30 individuals confined to southern Florida, and exhibited a high frequency of phenotypic and congenital abnormalities associated with inbreeding, including kinked tails, a midline dorsal cowlick, cryptorchidism and atrial septal defects, alongside poor demographic performance. In 1995, wildlife

managers translocated eight female pumas of the related Texas subspecies (*P. c. stanleyana*) into southern Florida to restore genetic variation (Johnson *et al.*, 2010; Hedrick, 1995) ^[10, 15].

- Within roughly fifteen years, panther numbers had increased approximately threefold, genetic heterozygosity had approximately doubled, and survival and fitness measures had improved significantly, while morphological and biomedical correlates of inbreeding declined markedly (Johnson *et al.*, 2010) ^[15].
- A subsequent 40-year, nine-generation analysis of 1,192 individuals confirmed that these genetic, morphological and demographic benefits persisted after five generations of admixture, with population abundance increasing more than fivefold and effective population size increasing more than twentyfold relative to the pre-rescue population.
- Genome-wide analysis further showed that the beneficial effect of the translocation operated mainly through reduced homozygosity rather than the replacement of deleterious variants, and that original Florida panther ancestry was not genomically “swamped” by Texas ancestry, allaying a common concern about genetic rescue.

A broadly comparable pattern has been documented in Mexican wolves (*Canis lupus baileyi*), where the introduction of new genetic material into a highly inbred captive-origin population measurably ameliorated correlates of inbreeding depression (Fredrickson, Siminski, Woolf, & Hedrick, 2007) ^[17]. Taken together, these cases support genetic rescue as an evidence-based tool for small, severely inbred populations, while reinforcing the need for careful assessment of donor-population relatedness and local adaptation before translocation to minimize the risk of outbreeding depression.

Landscape Genetics, Habitat Fragmentation and Connectivity

Landscape genetics is an interdisciplinary field integrating population genetics with landscape ecology to relate patterns of gene flow and genetic differentiation to underlying landscape structure, including habitat configuration, land-cover composition and anthropogenic barriers (Holderegger & Wagner, 2008) ^[12]. As including habitat is converted and subdivided by agriculture, roads and urban development, populations that were once connected by regular dispersal become progressively isolated, altering the balance between gene flow, genetic drift and inbreeding (Figure 2).

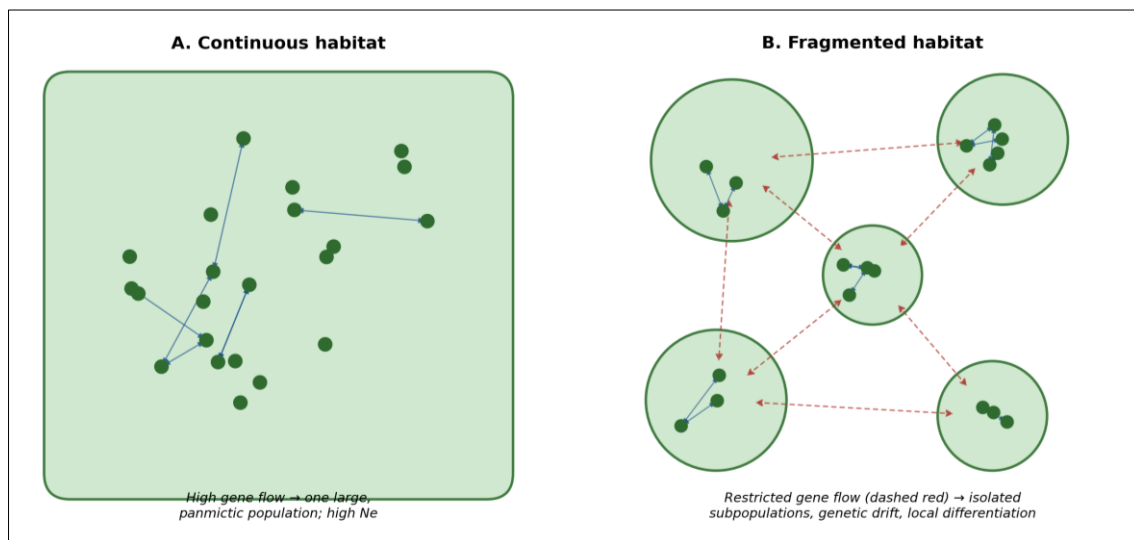


Fig 2: Effect of habitat fragmentation on population genetic structure and gene flow. In continuous habitat (A), frequent dispersal maintains a single, well-mixed population with a comparatively high effective population size. In fragmented habitat (B), restricted gene flow between remnant patches promotes genetic drift, local differentiation and elevated extinction risk within individual fragments.

1. Empirical evidence from fragmented landscapes

- In prairie riparian forest systems, even a highly mobile and widespread songbird showed measurable genetic structuring driven by small breaks in habitat continuity, demonstrating that fragmentation effects can be detected at surprisingly fine spatial scales (Adams & Burg, 2015) ^[1].
- Comparative studies of multiple woodland bird species have shown that the genetic consequences of habitat loss and fragmentation differ according to species-specific mobility and dispersal capacity, underscoring the need for species-tailored connectivity assessments rather than one-size-fits-all corridor design (Amos *et al.*, 2012) ^[2].
- Landscape genetic analyses are increasingly used to identify and prioritize dispersal corridors, evaluate the effectiveness of existing wildlife crossing structures, and predict the genetic consequences of proposed infrastructure or land-use change before it occurs.

2. Management implications

Landscape genetics provides an empirical basis for maintaining or restoring functional connectivity between remnant populations, through measures such as habitat corridors, stepping-stone reserves, and, where natural connectivity cannot be restored, assisted gene flow. Because the genetic consequences of fragmentation can take multiple generations to become fully apparent, landscape genetic monitoring is most effective when integrated into long-term, repeated sampling programmes rather than treated as a single baseline assessment.

Genetic Monitoring, Captive Breeding and Ex Situ Management

Captive-breeding and studbook-based management remain essential components of conservation strategy for many critically endangered species, particularly where wild population sizes are insufficient to guarantee short-term persistence. Because ex situ populations are typically founded from a limited number of individuals, they are especially susceptible to the loss of allelic richness and rare variants, even when overall heterozygosity is successfully maintained through careful pedigree management (Javarotti *et al.*, 2025)^[14].

- Studbook data, combined with quantitative and molecular genetic analysis, allow managers to reconstruct pedigrees, minimize mean kinship, and prioritize breeding pairs that retain the genetic contribution of founder lineages (Princée, 2016)^[21].
- Pedigree-based analyses of long-running captive programmes, such as that of Cuvier's gazelle, demonstrate how genealogical records spanning several decades can be used retrospectively to evaluate whether a chosen mating policy has succeeded in preserving founder genetic diversity, and to guide future management decisions (Moreno, Cervantes Navarro, Gutiérrez García, Fernández, & Goyache, 2024)^[18].
- Population viability analysis increasingly incorporates genetic parameters directly, using projected trends in heterozygosity and allelic richness over multiple generations to flag captive or reintroduced populations at risk of long-term genetic erosion, as demonstrated for the black lion tamarin (*Leontopithecus chrysopygus*), where ex situ and wild founder populations showed significant genetic differentiation and declining allelic richness despite stable heterozygosity (Javarotti *et al.*, 2025)^[14].

Integrating ex situ and in situ genetic data, rather than treating captive and wild populations as separate management problems, allows conservation programmes to make more informed decisions about founder representation, supplementation of captive stock from the wild, and eventual reintroduction strategy.

Policy Frameworks and Global Genetic-Diversity Indicators

For most of the history of international conservation policy, genetic diversity has been the most poorly operationalized of the Convention on Biological Diversity's three recognized levels of biodiversity, largely because standardized, broadly applicable genetic indicators were lacking. The adoption of the Kunming-Montreal Global Biodiversity Framework in 2022 changed this: 196 Parties committed to reporting the genetic status of wild and domesticated species, supported by three purpose-built indicators – the proportion of populations maintained above a genetically meaningful threshold, the $N_e \geq 500$ indicator described above, and an indicator tracking the number of species monitored using DNA-based methods (Mastretta-Yanes *et al.*, 2024)^[16, 17].

- A key practical advantage of these indicators is that they can, where necessary, be estimated using demographic or occurrence data even without direct DNA-based sampling, improving feasibility in resource-limited settings (Mastretta-Yanes *et al.*, 2024)^[16, 17].
- A multinational pilot assessment covering megadiverse and developing economies alongside high-income countries found that data were available to calculate at least one indicator for 83 percent of assessed taxa, demonstrating the broad feasibility of the framework (Mastretta-Yanes *et al.*, 2024)^[16, 17].
- The same assessment highlighted that conventional IUCN Red List status frequently does not align with genetic indicator status, meaning that demographically “secure” populations may nonetheless be genetically at risk, and vice versa (Mastretta-Yanes *et al.*, 2024)^[16, 17].

Detailed guideline documentation accompanying the monitoring framework has since been published to standardize data collection and indicator calculation across taxonomic groups and national reporting systems, supporting more consistent global implementation (Mastretta-Yanes *et al.*, 2024, Biodiversity Informatics)^[16, 17].

10. Challenges, Limitations and Ethical Considerations

Despite substantial technical progress, several structural challenges continue to constrain the application of conservation genetics in practice.

- Capacity and resource gaps: genomic sequencing infrastructure, bioinformatics expertise and long-term funding remain unevenly distributed, limiting genetic monitoring capacity in many biodiversity-rich but resource-constrained regions.
- The gap between data and application: population genomic datasets are increasingly large and statistically powerful, yet translating this information into field-level management decisions requires deliberate bridging between researchers and practitioners, and case-study-based approaches have been proposed specifically to close this gap (Garner *et al.*, 2016)^[8].
- Indigenous data sovereignty: genetic sampling of wildlife frequently occurs on or near Indigenous lands and territories, and the history of genetic data collection in other domains has, at times, involved practices that violated Indigenous sovereignty and eroded trust; equitable, collaborative approaches to genetic sampling and data governance are increasingly recognized as essential to the legitimacy and long-term success of conservation genetics programmes (Robbins, Hunt, Pelegri, & Gilbert, 2023)^[22].
- Balancing intervention risk: genetic rescue and assisted gene flow carry a well-documented risk of outbreeding depression if donor populations are poorly matched ecologically or evolutionarily to the recipient population, requiring case-by-case risk assessment rather than routine application (Tallmon *et al.*, 2004)^[23].
- Reporting and terminology discipline: conservation genetics claims should avoid overstated language such as unqualified novelty claims or causal statements unsupported by the underlying study design, particularly given that most wildlife genetic studies are observational rather than experimental.

Future Directions

Several emerging trends are likely to shape the next phase of conservation genetics and genomics practice.

- Continued cost reduction in whole-genome sequencing is expected to make chromosome-level reference genomes and population-scale resequencing routinely accessible for a much broader range of threatened, non-model species, extending the resolution currently available mainly to well-funded flagship programmes.
- Environmental DNA (eDNA) approaches offer the potential for non-invasive, landscape-scale monitoring of genetic diversity and population structure, particularly for cryptic, aquatic or otherwise difficult-to-sample species.
- Standardized implementation of the Kunming-Montreal genetic-diversity indicators across national biodiversity strategies and action plans will require continued methodological refinement and capacity-building, particularly in countries currently lacking DNA-based monitoring infrastructure (Mastretta-Yanes *et al.*, 2024)^[16, 17].
- Closer integration of genomic data with demographic population viability analysis, as already demonstrated in ex situ primate conservation programmes, is likely to become standard practice for both captive and wild populations of high conservation concern (Javarotti *et al.*, 2025)^[14].
- Continued methodological debate over genetic rescue, its risks, and the conditions under which it should be applied is likely to persist, and will benefit from additional long-term, multi-generational case studies comparable to the Florida panther programme.

Conclusion

Genetic diversity and population genetic structure are foundational, if historically under-monitored, dimensions of wildlife conservation. The evidence synthesized in this review indicates that small, fragmented populations face a genuine and often underestimated risk of long-term genetic erosion, that classical thresholds such as the 50/500 rule require upward revision in light of accumulated evidence, and that genetic rescue – while historically controversial – has produced well-documented, multi-generational benefits in cases such as the Florida panther and Mexican wolf when applied with appropriate caution regarding donor-population compatibility. The maturation of genomic tools, from microsatellites to genome-wide SNP and whole-genome sequencing approaches, has substantially improved the precision with which genetic diversity, structure and effective population size can be estimated, while landscape genetics has clarified how habitat fragmentation translates into measurable genetic consequences for wildlife populations. The formal adoption of genetic-diversity indicators under the Kunming-Montreal Global Biodiversity Framework represents a significant policy milestone, but its success will depend on sustained investment in genetic monitoring capacity, equitable and collaborative approaches to genetic sampling, and closer integration of genetic evidence into the routine demographic and ecological assessments that currently dominate conservation decision-making. Long-term protection of wildlife species will increasingly require treating genetic diversity not as a specialist add-on, but as a core, regularly monitored component of conservation practice.

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