

# Selecting species and populations for monitoring of genetic diversity

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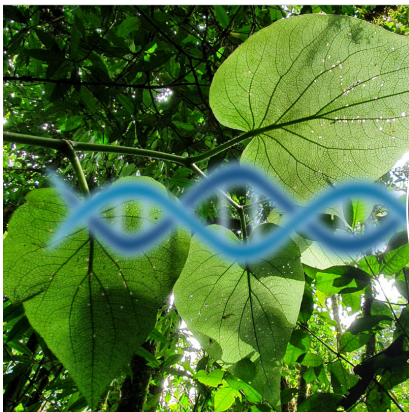
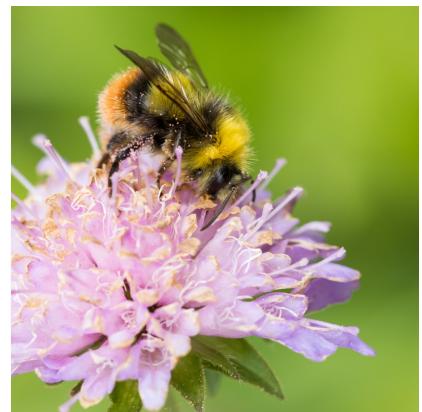
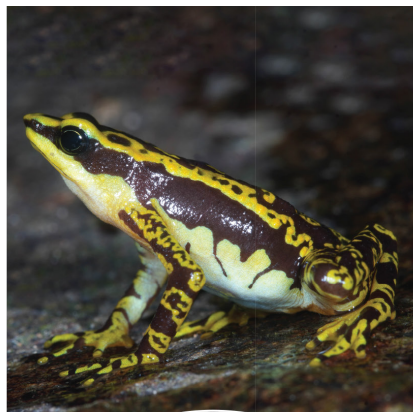
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C. Hvilsom, G. Segelbacher, R. Ekblom, M.C. Fischer, L. Laikre, K. Leus, D. O'Brien, R. Shaw and V. Sork



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# Contents

<b>Executive summary</b>	<b>iv</b>
<b>Drafting process and acknowledgements</b>	<b>iv</b>
<b>Scope</b>	<b>iv</b>
<b>Glossary of definitions</b>	<b>v</b>
<b>Section 1: Introduction</b>	<b>2</b>
What is genetic diversity?	3
Why is genetic diversity important and should be monitored?	4
To what degree has biodiversity conservation included genetic diversity?	8
What genetic diversity can tell us	8
<b>Section 2: The road to genetic monitoring</b>	<b>11</b>
How genetic diversity can be measured	13
What to consider when planning a genetic diversity monitoring project	16
Criteria for selecting species and populations	17
<b>Section 3: Cases</b>	<b>20</b>
Scotland – a scorecard approach	22
Libya – applying the Scottish scorecard approach	23
Sweden – a long term collaborative approach	26
California (USA) – biodiversity hotspot assessment	30
Switzerland – a genomic approach	34
Victoria (Australia) – a state initiative	38
South Africa – using the genetic indicators to ascertain the genetic status	40
<b>Synthesis</b>	<b>41</b>
<b>Online resources</b>	<b>42</b>
<b>References</b>	<b>43</b>



# Executive summary

One of the main challenges for conservation today is monitoring and understanding changes in biodiversity. Genetic diversity provides the foundation for biodiversity and is necessary for long-term survival, adaptation, and resilience not only for individuals, but also for populations, species, and entire ecosystems. If we want to preserve the whole entity of biodiversity, we need to know where the change in genetic diversity is occurring, why it is happening, and if conservation actions are having a positive impact. Monitoring genetic diversity across different time points is a first step to gain insight into the extent to which populations or species might be at risk, to guide conservation action and to provide evidence for solutions. However, putting genetic diversity monitoring into practice in the form of an effective, implemented project can be challenging.

Firstly, a suite of steps and considerations need to be taken into account when planning a genetic diversity monitoring project. Secondly, a list of criteria should be evaluated to select which species and populations will be monitored. Lastly, the monitoring of genetic diversity should be long-term, allowing repeated measures of genetic diversity over time and hence tracking the development of biodiversity and impacts of conservation efforts. This guidance document aims to be a resource that guides the reader through the decision and evaluation processes that take place when designing a genetic diversity monitoring programme and identifying the most appropriate set of species or populations to monitor.

## Drafting process and acknowledgements

A drafting team comprised of Christina Hvilsom (CGSG, CPSG Europe and Copenhagen Zoo), Gernot Segelbacher (Chair CGSG), Robert Ekblom (CGSG), Martin C. Fischer, Linda Laikre (CGSG), Kristin Leus (Convenor CPSG Europe, Copenhagen Zoo), David O'Brien, Robyn Shaw (CGSG) and Victoria Sork collectively drafted and co-authored the guidance document.

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## Scope

This guidance document is intended for practitioners (including governmental agencies, NGOs, but also locally responsible conservationists such as land managers) who need to monitor genetic diversity of species in the wild, whereby genetic monitoring is defined as monitoring genetic diversity of populations within species across (at least two) different time points. The focus of this guidance document is on selecting and prioritising which wild species or populations (from fungi, flora and fauna) to monitor and is aligned with international conventions and obligations, such as the Convention on Biological Diversity (CBD). Monitoring of genetic diversity in domesticated and cultivated species, and of ex situ populations (of whole living organisms as well as gametes, seeds, living cell lines etc.) is an essential part of their management and conservation, but is outside the scope of this guidance document. Because selecting which species or populations to monitor for genetic diversity can sometimes be challenging, this guidance document presents a practical tool and successful real-world examples under different resource frameworks to facilitate management projects.

# Glossary of definitions

## **50/500 rule**

Estimation of a minimum population size needed to prevent the loss of genetic variability (introduced in 1980 by Michael Soulé and Ian Franklin). A minimum population size of 50 is necessary to combat inbreeding and a minimum of 500 individuals needed to maintain evolutionary potential.

## **Adaptation**

Evolution via natural selection as environmental conditions change.

## **Admixture**

Two populations/genetic lineages of a species which have previously been isolated but now interbreed. Admixture introduces new genetic variation into a given population.

## **Allele(s)**

One, two, or more versions of a specific variant in DNA sequence on the chromosome. An individual inherits one allele from each of its parents, if both alleles are the same/different the individual is homozygous/heterozygous.

## **Barcoding**

DNA barcoding uses short sequences of DNA as a method of species identification.

## **Census population size ( $N_c$ )**

Usually, a count of the number of adult or mature individuals in a population.

## **Coding/non-coding**

Coding DNA is providing instructions for making proteins out of amino acids, whereas non-coding DNA does not code for any amino acids.

## **Effective population size ( $N_e$ )**

See Box 3

## **Environmental DNA (eDNA)**

DNA from environmental sources such as water, soil, sediment, or air. By sequencing specific areas of DNA from those samples, species identification and composition can be estimated.

## **Functional variation**

Genetic variation which is causing phenotypic variation. (e.g., MC1R for colour polymorphism)

## **Genetic diversity**

Heritable variation within species. Describes the variation at DNA level.

## **Genetic rescue**

Unrelated individuals from a genetically close population are introduced into a population with low fitness (Hedrick and Fredrickson 2010).

## **Homozygosity/heterozygosity**

An individual has inherited two identical/different alleles for a particular gene or genes from both parents. A measure of evenness; another common way to measure “genetic diversity”.

## **Inbreeding depression**

Loss of fitness (growth rate, lifespan, reproductive output) due to inbreeding and accumulation of genetic load, primarily recessive deleterious alleles.

**Introgression**

The transfer of genetic information from one species to another, as a result of hybridisation between them and repeated backcrossing.

**Neutral variation**

Genetic variation which is unaffected by natural selection.

**Non-invasive sampling**

Sampling of genetic material without the need to invasively collect a sample from an individual itself. Potential source material could be e.g., feathers, faeces, hair or seeds. On the other side invasive sampling will require direct contact with the individual to obtain tissue or blood from an animal or destructive sampling of plants.

**Population**

A group of interbreeding individuals in a defined area, genetically distinct from other such groups. Note this is not equivalent to “population” used by IUCN, who define “population size” as the total adult census of a taxon at the global level. Most species have multiple populations. Population genomic analyses or phenotypic/functional trait variation can be used to delineate populations (Funk et al., 2012).

**Single-Nucleotide Polymorphism (SNP)**

Different variants of a single nucleotide at a given position in the genome. Measures variability within populations or species.





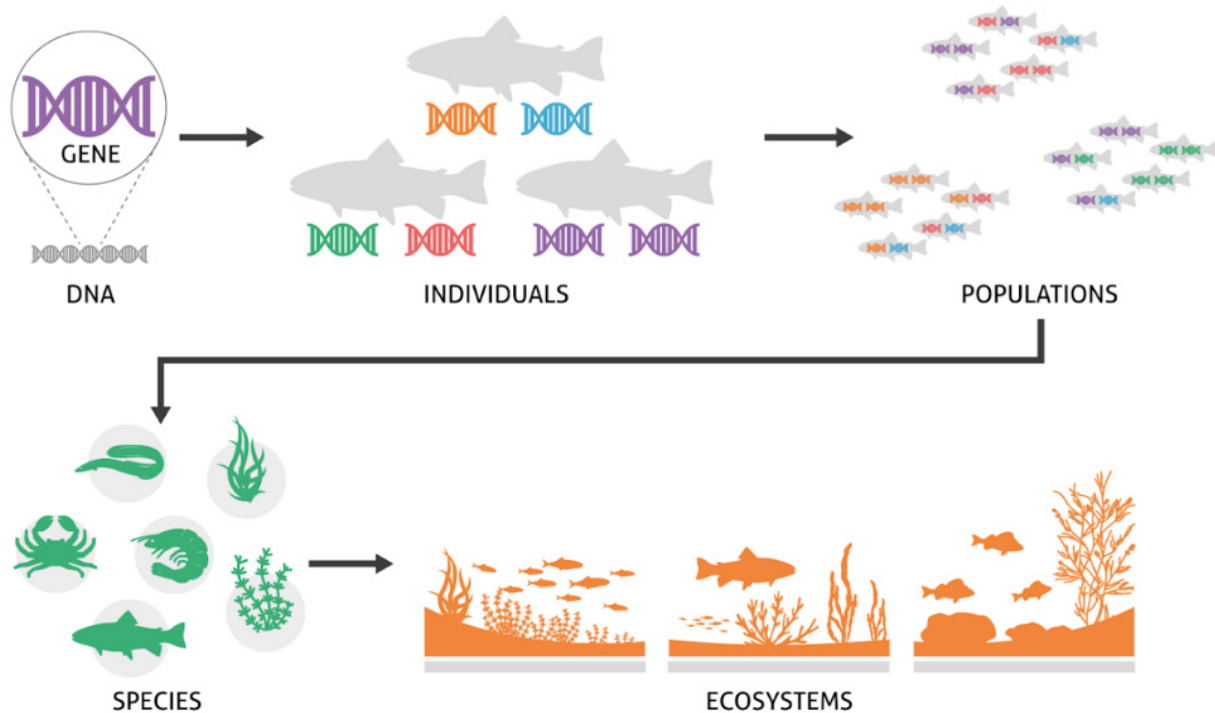
Natterjack toad (*Epidalea calamita*). Photo: © Mikkel Jezeguel



## Section 1: Introduction

Species all over the world face extinction. This current loss of species is estimated to be between 1000 and 10,000-fold higher than the natural extinction rate (Turvey & Crees, 2019). Globally, population sizes of monitored mammals, fish, birds, reptiles, and amphibians have declined an average of 68% between 1970 and 2016 (WWF, 2020). Figures for invertebrates (Eisenhauer et al., 2019), plants (Humphreys et al., 2019) and fungi (Lughadha et al., 2020) reflect greater uncertainty, but the available evidence suggests that population declines, and extinction rates are also high in these groups. In addition, habitats are being damaged, fragmented or altered at an unprecedented rate, leading to loss of the benefits ecosystems provide to people and nature.

Such a critical decline in biodiversity is a global threat to our own existence, as well as to our economies, societal equality, and the fight against climate change (Des Roches et al., 2021). To mitigate biodiversity loss, numerous initiatives and actions have been taken. Many national and international targets have been agreed on to halt biodiversity loss, but success to date has been very limited (Secretariat of the CBD, 2020). Three levels of biodiversity constitute the variation of life on our planet: Ecosystem diversity (variety of ecosystems such as e.g., forests, grasslands, swamps, or lakes), species diversity (number and distribution of species of plants, animals, fungi, and bacteria) and genetic diversity (amount and distribution of genetic variation within species or populations). Actions need to consider all three levels of biodiversity if they are to be successful for conservation. It is essential that biodiversity at all three levels is mapped, monitored, and used sustainably if we are to safeguard biodiversity and the benefits it brings.



**Figure 1:** Illustration of genetic diversity on different levels of an aquatic ecosystem. Variability within the DNA is called genetic diversity and can be detected among individuals and populations of the same species (intraspecific genetic diversity) which is the core of any genetic monitoring programme. The same gene may occur in different variants - alleles = genetic diversity. In most species individuals carry two copies of every gene, one from each parent. (Source: © Jerker Lokrantz/Azote).

## What is genetic diversity?

Genetic diversity is variation at DNA level, necessary for long-term survival, adaptation, and resilience not only for individuals, but also for populations, species and entire ecosystems. DNA, the raw material on which selection acts, is inherited from generation to generation. Thus, patterns of genetic variation reflect the demographic processes in a population, i.e., dispersal, reproduction, survival of individuals. The genetic differences within and among populations enable species to adapt to environmental changes, such as climate change or novel pests and diseases. The conservation of genetic diversity thus means safeguarding biodiversity (see also Figure 1).

## Why is genetic diversity important and should be monitored?

Increasing human pressure is leading to rapid environmental changes, often resulting in observable declines in population sizes or species distributions and declines in genetic diversity prior to extirpation (Spielman et al., 2004; Evans & Sheldon, 2008). But in some cases, population declines are less apparent (Faillace et al., 2021). Safeguarding the genetic diversity of all kingdoms of life provides the necessary basis for populations to adapt to changing conditions. Genetic diversity is a prerequisite for evolution and long-term survival of populations and species (Barrett & Schluter, 2008; Allendorf et al., 2013; Des Roches et al., 2021). It facilitates adaptation to environmental and climate change (Reid et al., 2016; Lai et al., 2019), new pests and diseases, and can augment species diversity by supporting ecosystem resilience (Reusch et al., 2005; Barbour et al., 2009). It provides resilience after extreme events and allows for species restoration after devastating declines (Hughes & Stachowicz, 2009; Morikawa & Palumbi, 2019). Genetic diversity supports productivity in many species that humans harvest (e.g., fisheries, forests and medicinal plants), and is used to guide animal and plant breeding to ensure a sustainable food system. In some species, genetic diversity supports ecosystem services like habitat formation and pollination (Des Roches et al., 2021; Hoban et al., 2021a; Stange et al., 2021). Ensuring genetic diversity in populations and species is thus essential for their long-term survival and human well-being in many ways (Figure 2). To safeguard genetic diversity, monitoring is critical to assess population trajectories so that preventative actions can be undertaken before reaching a critical point for survival (Schwartz et al., 2007; Laikre et al., 2008), and also to evaluate the outcomes of such interventions.

### Box 1 What can be found with genetic monitoring?

Seagrass (*Zoostera marina*) is an important habitat for many marine organisms (such as fish, molluscs, and invertebrates). It has been shown that under extreme climatic conditions (heatwaves with high water temperatures) those seagrass meadows with higher genetic diversity are the ones which recover quicker (Reusch et al., 2005). They also are the ones in which more species can be observed. Ecosystems such as seagrass meadows are thus a good example illustrating that high genetic diversity enables high species diversity and only when we protect high levels of genetic variability can these systems be resilient to strong environmental effects.

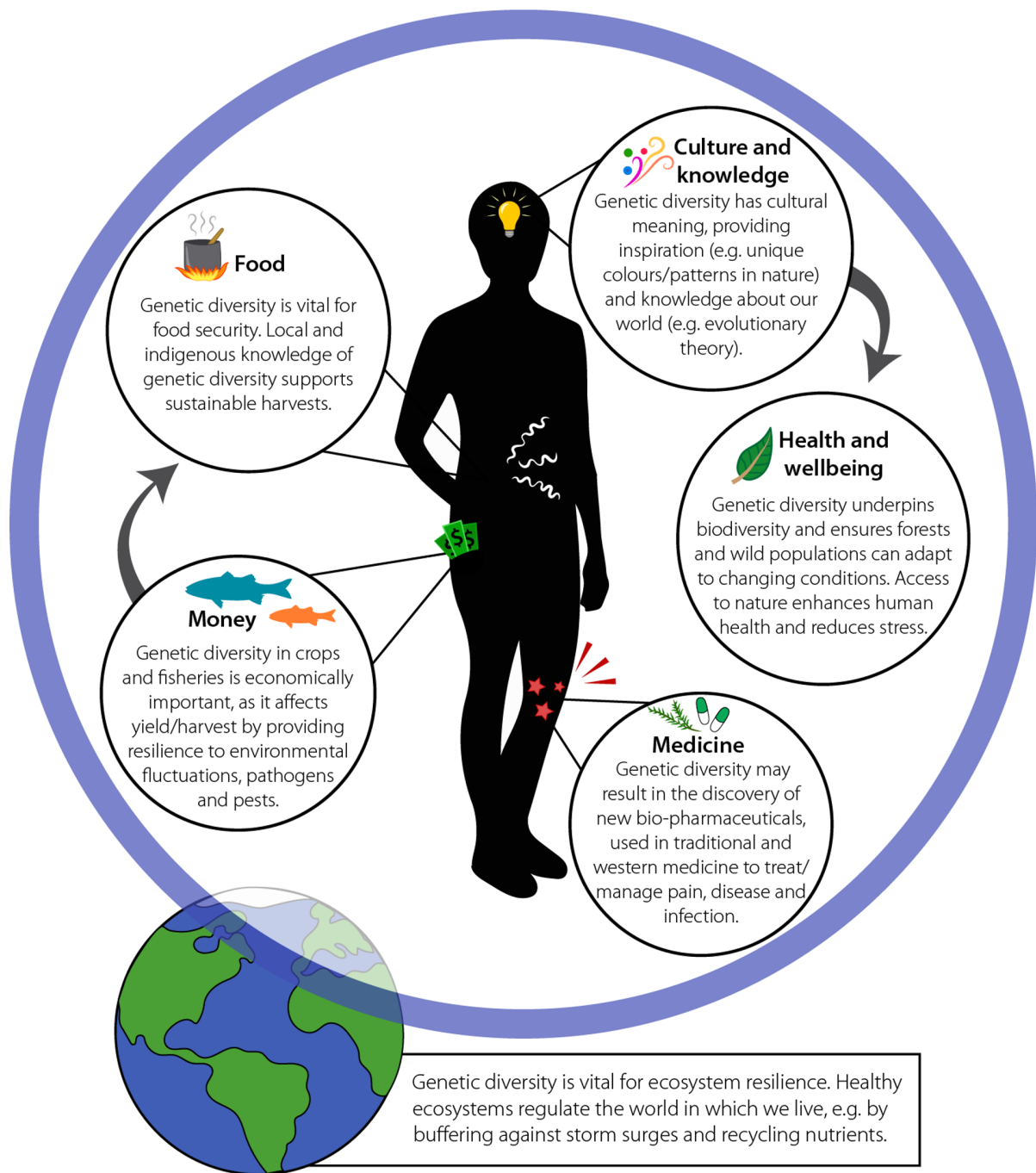
For more than two decades population demography, reproductive success, and functional genetic variation (see **Glossary**) was monitored in a small and isolated population of adders (*Vipera berus*) in Southern Sweden. Following a genetic rescue operation, whereby 20 genetically diverse males were introduced into the population, the researchers observed increased reproductive success and higher genetic diversity in the population.



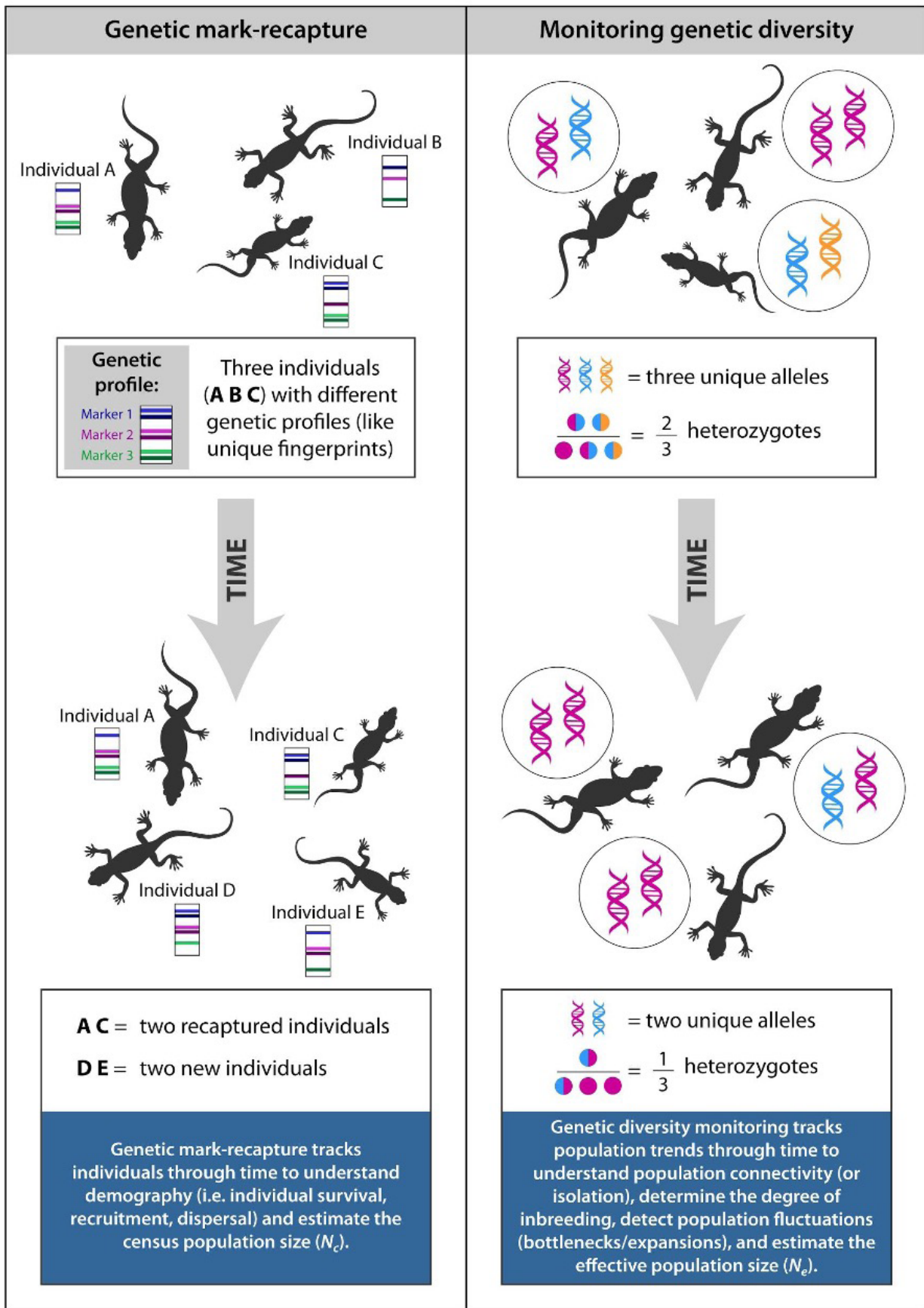
The term “genetic monitoring” is often applied to situations where molecular markers are used to monitor species via tracking of individuals using genetic methods or to identify species through barcoding, e.g., genetic mark-recapture or environmental DNA (eDNA) studies (see Box 2 and Figure 3). However, this type of genetic monitoring does usually not allow monitoring of population genetic parameters. We want to highlight that when we refer to monitoring genetic diversity in this guidance, this always means monitoring genetic diversity of populations across (at least two) different time points (category 2 monitoring in Schwartz et al., 2007). While genetic monitoring is common in sectors such as forestry and fisheries, it has been less frequently applied to other wild populations. Several national and regional efforts are currently embarking on or are already actively monitoring genetic diversity (see examples below). With growing awareness of the importance of genetic diversity in threatened species conservation, genetic implications are more frequently considered in recovery programmes for threatened species in the US and Australia than in Europe, (Pierson et al., 2016) though there has been important progress in Africa, Asia, and the Americas. However, beyond this, there is also an imperative to monitor genetic diversity in a wide range of species types, and not restrict the monitoring to those of economic importance or in imminent need of conservation action.

## **Box 2 What is not monitoring of genetic diversity**

Monitoring genetic diversity means monitoring genetic diversity of populations across (at least two) different time points. Therefore, although genetic methodologies can be used to investigate patterns and trends in species- and ecosystem diversity, not all genetic methods are currently fit for estimating genetic diversity. This could be for example methods such as barcoding or environmental DNA (eDNA) techniques, which can be applied to estimate parameters such as longevity, mating success and dispersal. Genetic data can also be used to track individuals and monitor populations through individual identification and sex assignment based on non-invasively collected samples. Recently, such data have also enabled population density estimations using spatial capture-recapture modelling (e.g., in large carnivores Bischof et al., 2020). These approaches are often phrased as genetic monitoring - as they “monitor” species using genetic tools (e.g., by tracking ID of individuals or by eDNA for identifying species). However, the objective of “monitoring of genetic diversity”, as used here, is to analyse temporal trends in genetic variation within populations and species, and data such as meta-barcoding is often not suited for this type of application. Anthropogenic threats, such as illegal wildlife trade, can be assessed and combatted using genetic tools that can help determine the geographical origin of confiscated individuals that are victims of the illegal trade, as well as pinpointing the illegal harvesting hotspots and trading routes.



**Figure 2:** Why genetic diversity is important for humans. Genetic diversity is important for human wellbeing in many ways. It is e.g. important to maintain the gene pools for crops as well as wild relatives for developing new varieties which will secure food availability, and by providing resilience it also delivers strong economic benefits. Genetic diversity also supports human culture and is essential for health and wellbeing through access to diverse nature and through medicine. (Source: Figure created by the authors of the guidelines).



**Figure 3:** The difference between monitoring using genetic techniques (left) and monitoring of genetic diversity (right). Depending on the number of genetic markers used to genotype individuals for genetic mark-recapture studies, it may be possible to also carry out genetic monitoring. (Source: Figure created by the authors of the guidelines).

## To what degree has biodiversity conservation included genetic diversity?

The need to monitor biodiversity at all three levels was globally recognised in international policy in 1993 when the CBD came into effect and in several subsequent programmes. However, monitoring of genetic diversity has not been given the necessary attention (Laikre, 2010; Laikre et al., 2016), particularly for wild species. Several parties to the CBD are looking to improve the recognition and reporting of genetic diversity, following concerns that early drafts did not give it sufficient prominence (Laikre et al., 2020; O'Brien et al., 2022). While most parties recognise the general importance of genetic diversity, in the past, reporting on genetic diversity has been inconsistent, superficial, or even overlooked in wild species completely (Hoban et al., 2021b). Encouragingly, there is increased emphasis on the importance of genetic variation for species' and ecosystems' resilience to climate change in documents such as the 2030 Biodiversity Strategy of the European Union ([https://ec.europa.eu/environment/strategy/biodiversity-strategy-2030\\_en](https://ec.europa.eu/environment/strategy/biodiversity-strategy-2030_en)) and there is a legal framework for the conservation of genetic diversity in the USA (through the Endangered Species Act) and Canada. The European strategy is linked to conservation efforts of genetic diversity in forest trees across the continent (de Vries et al., 2015) and there is a proposal for this approach to be extended to other taxa (Minter et al., 2021). While the conservation genetics gap (Taylor et al., 2017) still exists in policy and practice, such international developments reinforce that genetic diversity of wild species should be protected and maintained, including through in situ and ex situ conservation measures.





## What genetic diversity can tell us

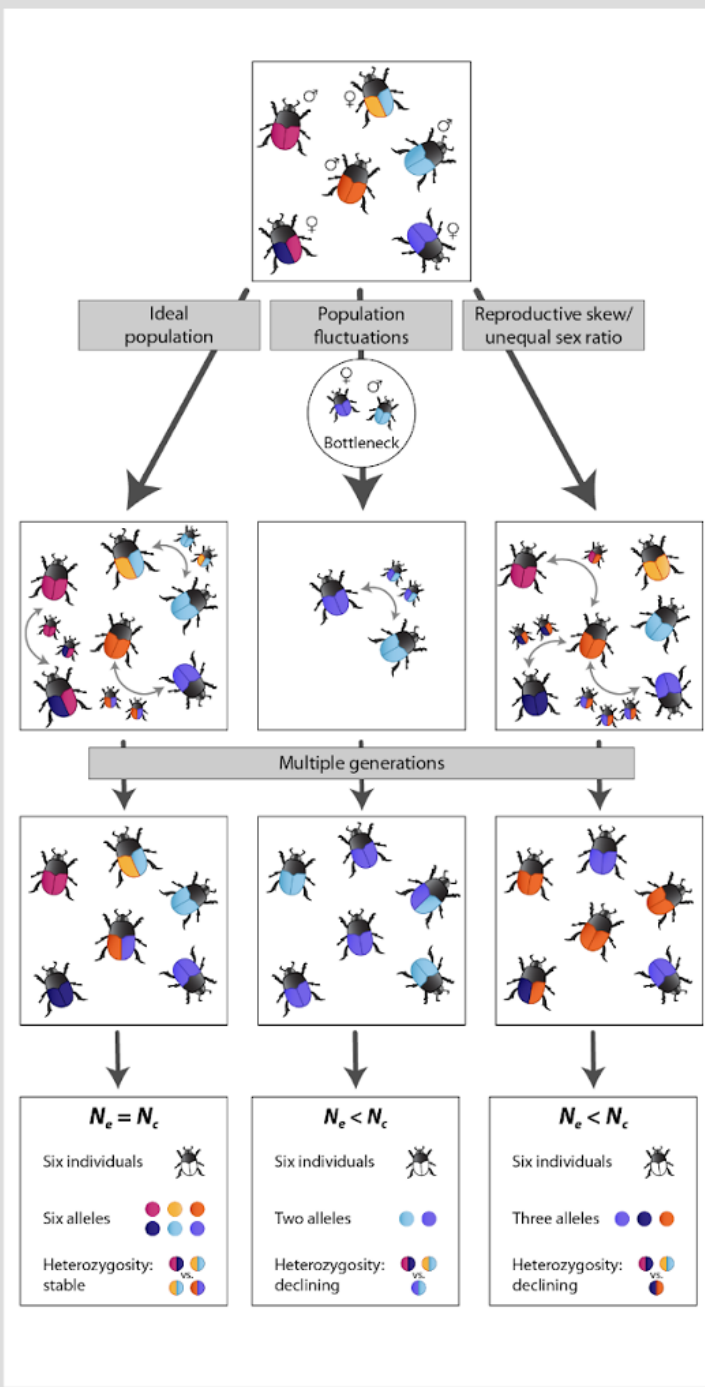
Genetic tools have become increasingly applied in conservation. There are different ways genetic tools can be used, but collectively genetic tools can provide managers with essential information on the status of species and populations to guide conservation efforts. Information on the number of individuals (census population size;  $N_c$ ) is one of the factors often requested, yet the census size does not always reflect the genetic diversity of the population. Rather, it is the so-called effective population size ( $N_e$ ) that determines the possibility for the population to maintain genetic diversity over time.  $N_e$  is one of the most important parameters in conservation biology (see Box 3) as it determines the rate at which genetic diversity is lost by stochastic processes. Within and among species or populations, genetic tools also enable evaluation of degrees of isolation, mixing or interbreeding of populations or species (commonly referred to as gene flow and hybridisation, respectively), and help unravel whether the cause is natural or unnatural.

However, to assess and monitor the viability of a species or population, its ability to survive and ultimately help achieve biodiversity goals, genetic diversity needs to be estimated over time to monitor changes. This could be over relatively short periods, such as a couple of weeks for species with a short generation time, but also span several centuries for other species. Monitoring genetic diversity can help quantify whether individuals are able to successfully disperse and reproduce, and how threats like habitat fragmentation might affect these processes. Genetic diversity can be lost in a population over time, even though the census size remains constant (e.g., if an environmental change is only affecting a certain genetic part of a population). There could be a massive loss of genetic diversity in underwater forests after a heatwave, however there may be no concurrent loss of forest cover (Gurgel et al., 2020).

### Box 3. Census size ( $N_c$ ) versus the effective population size ( $N_e$ )

The census size of a population ( $N_c$ ) does not in itself reflect how a population “behaves” genetically. Rather, the effective population size ( $N_e$ ) of a population determines the ability of the population to maintain genetic diversity over time.  $N_e$  is one of the most important parameters in conservation biology. It translates the census size of the population into the size of an idealised population showing the same rate of loss of genetic diversity as the real population of interest (Figure below).

In the left column of the figure, we have a hypothetical population with a census size ( $N_c$ ) of six individuals  an even sex ratio ♂:♀ and six unique alleles (DNA variants) . Individuals inherit two alleles (one from each parent). There are four homozygous individuals in the population (both copies of the allele are the same)  and two heterozygous individuals (two different alleles) 



In an ideal population, there is an equal sex ratio, non-overlapping generations and population size is stable. Alleles may be lost through random chance (genetic drift), and allele combinations (genotypes) may change from one generation to the next. However, all individuals have an equal chance of contributing genetic material to the next generation. Under such ideal situations  $N_e = N_c$ .

Real populations often deviate from the assumptions of an idealised population, so  $N_e$  is generally much lower than  $N_c$ . For example, population crashes (bottlenecks) can result in the loss of alleles (central column). Even if  $N_c$  increases over subsequent generations, the low  $N_e$  during the crash will affect  $N_e$  (measured over generations) for a long time.

$N_e$  is also reduced by unequal sex ratio, reproductive skew, individual fertility, and the survival of offspring (right column). If only a few individuals reproduce, and if the sex ratio among them is uneven, some individuals will contribute much more to the next generation than others. Alleles in non-mating individuals are lost, while other low frequency alleles may be lost over successive generations. Again, this results in a lower  $N_e$  compared to  $N_c$ .

Since  $N_e$  for most species is unknown, estimates are sometimes calculated using non-genetic data, based on known  $N_e/N_c$  ratios. Alternatively,  $N_e$  can be estimated using a general rule where  $N_e$  is approximately 10% of  $N_c$ . Whether assessed genetically or derived from census size, this indicator provides vital information about a population’s wellbeing. Although natural processes influence  $N_e$ , it can also reflect external pressure on a population, and is thus a useful indicator to monitor the effects of anthropogenic change.

(Source: Figure created by the authors of the guidelines).



## Section 2: The road to genetic diversity monitoring

Facing the loss of biodiversity, national and international policies have addressed the need for countries to halt and reverse its decline, and report back on the actions taken and progress achieved. Consequently, many countries have started monitoring programmes estimating species richness or population abundance for specific groups (such as pollinators, insects in general, selected plant species or carnivore populations) (O'Brien et al., 2022).





Hare's-tail cottongrass (*Eriophorum vaginatum*). Photo: © Martin C Fischer



Generally, biodiversity monitoring schemes mostly focus on species or habitats with few targeting intra-specific genetic diversity. This has led to missed opportunities in the past. As already noted, genetic diversity is essential for the resilience of biodiversity and to secure the benefits and opportunities nature gives to human health and well-being along with equitable development (Figure 2). The importance of genetic diversity is becoming increasingly recognised internationally, through bodies such as the United Nations and the European Union but relatively few countries report on them in their CBD National Reports (Hoban et al., 2021b).

## How genetic diversity can be measured

Genetic tools and techniques have become readily available to the scientific community and conservation practitioners alike. Genetic diversity can be monitored using a wide array of genetic markers and techniques but choosing the right markers appropriate for answering the study questions is crucial. A large number of DNA-based markers have been developed and established over recent decades and are now routinely applied (e.g., microsatellites, single nucleotide polymorphisms). Analytical workflows exist for some applied questions such as connectivity analysis (e.g., in Switzerland, Holderegger et al., 2020) enabling establishment of monitoring programmes to provide the required genetic information, often in collaboration with external laboratories. While technologies and analytical approaches develop rapidly (e.g., whole genome sequencing) conservation practitioners do not necessarily have to keep up with expert technological or statistical knowledge but can instead establish collaboration with qualified laboratories (Figure 4).

It is not always possible or desirable to collect invasive samples. In such cases, non-invasive samples, e.g., faeces, shed hair, feathers or seeds etc, can be used, minimising species disturbance. Non-invasive samples can to a great extent allow estimation of the same parameters as invasive samples, depending on sample quality and economical and technological resources available. Standardised workflows can help ensure genetic data are consistently collected spatially and through time and that appropriate statistics are presented to conservation practitioners to measure changes in genetic diversity. Such a workflow consists of different steps such as sampling of genetic and non-genetic data, laboratory analyses, statistical analyses and storing that information, to ultimately interpreting data (see Figure 4). If data are collected in a consistent and standardised manner, researchers can access, analyse, and share data, allowing genetic information to become more widely known to practitioners across the globe.

Establishing a genetic monitoring programme involves several key steps where the genetic analysis itself is only one out of many. We here present the typical workflow of a genetic monitoring programme. This includes the collection of many biotic and abiotic environmental variables, the sampling of the source materials for gaining DNA, the genetic analyses, subsequent bioinformatic procedures and then the synthesis of this information to inform policy and guide conservation action (Hoban et al., 2021a).

In many countries or regions, genetic analyses are still expensive and difficult to conduct due to the lack of qualified people or access to laboratories, as well as access to relevant scientific data and experience in analysing or interpreting e.g., genomic data. Furthermore, some countries have legislative barriers, where research permits are difficult to obtain and exporting samples to be analysed elsewhere through joint collaboration with external researchers is hindered. Consequently, assessing the genetic diversity of some threatened species, particularly those endemic or restricted to such countries is just not feasible due to national or international regulations (see e.g., Noreña et al., 2018). With very restricted resources and capacity additional approaches are needed to assess genetic diversity, even when genetic data are unavailable. Three new pragmatic indicators exist for measuring and reporting on the status and change of genetic diversity in species and populations. These indicators do not strictly require DNA based data and have been suggested as minimum requirements (Hoban et al., 2020); 1) the number of populations of a species with effective

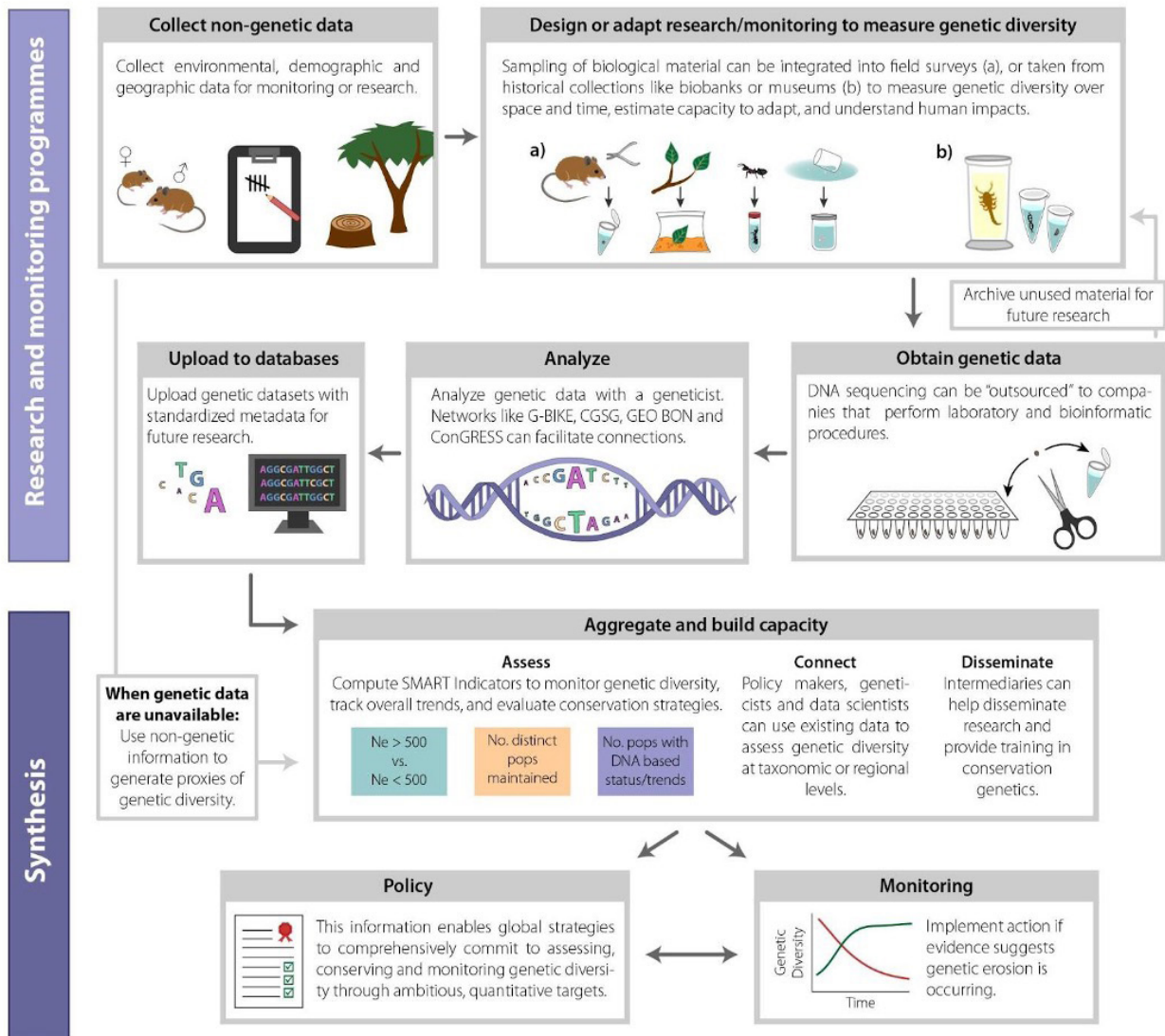
## Box 4 Sampling for a programme monitoring of genetic diversity

Selecting an appropriate sampling strategy is crucial for monitoring genetic diversity. Sampling biases can strongly influence genetic diversity estimates and reduce the power to detect changes in genetic diversity over time. The most appropriate sampling strategy might be a random or random stratified sampling (Lowe et al., 2004), where different biogeographical regions of a country are sampled to ensure a balanced sampling also within the entire climate-space of a species. In addition, the minimum distance between monitored populations must be defined in relation to the dispersal capacity of the species under study, so that sampling occurs at a geographically comprehensive scale and allows for inferring isolation and gene flow. Also worth considering is that genomic approaches (e.g., using a whole genome) require fewer individuals to be sampled to appropriately reflect a population, compared to traditional genetic approaches using only a few genetic markers (such as microsatellites or allozymes). Ten meticulously sampled individuals per population can provide enough power to accurately estimate the population genetic diversity (Nazareno et al., 2017).

Once samples have been collected, it is important to ensure their optimal long-term storage and registration (Jackson et al., 2012). Firstly, samples should be registered in a uniform and traceable manner, to enable future analysis. Secondly, storage of the samples should take place in centralised facilities, using standardised protocols. Thirdly, not only should the biological samples be documented and stored, but also the large amount of metadata and genetic data for genomic approaches (e.g. raw sequencing data, BAM files, SNP data in VCF files) as well as the results of any statistical analyses for the calculation of indicators. These data must be stored safely and reliably, with back-ups, and made freely available on public databases. Lastly, procedures to access samples and associated information should be defined with transparent requirements for anyone seeking to gain access. For an overview of the process see Figure 2.

population size ( $N_e$ ) above 500 compared to the number below 500 (See **Glossary**); 2) the proportion of populations maintained within a species; 3) the number of species and populations in a given country in which genetic diversity is monitored using DNA-based methods. For additional details see Hoban et al. (2020) and the Glossary. These indicators allow assessment of the impact of policy interventions (e.g., maintaining large and well-connected populations) and help guide decision making, even in the absence of DNA based data.

We here refer to populations as a discrete group of individuals living and interbreeding within a geographic area, relying on the same resources and environmental constraints. Populations often have limited migration to other areas and gene-flow will consequently be higher within than between populations. Often populations are defined a priori by geographic or ecological differences and then verified by genetic data. Subsequently, genetic data can be used to define and identify genetically distinct groups and populations based on observed genetic similarity and gene-flow.



**Figure 4:** Genetic monitoring steps (Source: Hoban et al., 2021a)

## What to consider when planning a genetic diversity monitoring project?

Designing a project to monitor, report on, and actively maintain genetic diversity requires incorporation of the following steps, considerations, and associated guiding questions:

First, define the purpose and scale of the monitoring:

- Determine the ultimate purpose of monitoring (e.g., to meet statutory goals, to inform on-the-ground conservation or to study the effect of certain threats).
- Determine the planned geographical scale, i.e., country wide, multiple countries or specific areas? And consider how that map onto the species spatial distribution.

Next, define the monitoring strategy to follow and its associated indicators. Two main approaches can be followed:

- Monitoring genetic diversity using molecular tools.
- Applying indicators that don't strictly require DNA based data (e.g., see Hoban et al., 2020).

If the planned genetic diversity monitoring project involves using molecular tools, the sampling and storage scheme should be designed:

- How is sampling going to be organised and what standards will be applied?
- Where are samples to be stored, genotyped and meta-data archived in the long term?
- Are there existing collections (museums, research institutes, biobanks etc) that can be explored for possible historical or extant samples?

With a sampling and storage strategy outlined, resources for both molecular and non-molecular approached need to be explored, assessed, and secured:

- Are there partners available for genetic studies, e.g., laboratory and data analysis?
- Have other countries already conducted genetic monitoring of the species of interest, i.e., are there methodologies that can be adopted, or possible collaborations explored?
- What data are already available, e.g., molecular datasets, holding and or abundance data? Or are new data and/or sampling attempts necessary?
- Is the planned monitoring feasible for all current and likely future partner countries (access to equipment or trained staff may differ)?
- Who is financing the different parts of the programme, what is the time scale of the financing and how can longevity be ensured?

Certain species-specific characteristics need to be considered in the project design as they can vary substantially across taxa and affect temporal comparisons. For example:

- What is the generation length of the target species and does information about mating patterns exist?

Analyses using novel genomic techniques and their application on species of conservation interest, depend on already published information of the species, related species, or non-model organisms.

- What is the genome size for the target species and is a reference genome available?

Depending on the focus of the monitoring project, conservation threats in specific regions may need to be taken into consideration when the taxonomic groups to monitor. E.g., one might choose different taxonomic groups for examining effects of overharvesting versus investigating the effects of long-term pollution.

- Have stakeholders been involved in the monitoring design (e.g., agriculturalists, foresters, local communities)?

Even if the monitoring is to take place using genetic indicators that do not strictly require DNA based data (see e.g., the indicators proposed by Hoban et al., 2020), many of the questions above would still apply, but different data sources on presence/occurrence/abundance need to be accessed.

## Criteria for selecting species and populations

For countries to monitor, report on, and take action to maintain genetic diversity, one of the key questions is to decide which species and populations to monitor. This selection of species or populations can be done based on criteria specified below.

Category A: basic criteria encompassing the most urgent conservation and management issues:

- Species or populations that are directly affected by human interventions resulting in the depletion of individuals, e.g., through substantial harvesting (hunting, fishing, trading, logging, medicinal use and use for other socio-economical values); or causing movement of individuals, including translocations and re-colonisations.
- Species of key ecological importance/ecosystem service delivery, including habitat-forming species, top predators and those which dominate habitats by virtue of coverage or numbers.
- National or regional populations that are genetically distinct from others over the distribution range; or species with their main distribution area confined to a given country, giving that country an international responsibility for that species (including endemics).
- Species already subjected to some other form of monitoring, enabling synergy with other national monitoring activities (such as projects evaluating the outcomes of any conservation action plans) and/or species complimentary to other (non-genetic) forms of biodiversity monitoring.

Category B: criteria which can be adjusted at a national level depending on the precise focus of the monitoring and the type and scale of resources available

- Populations likely to be strongly affected (increase or decrease) by climate change (species at the extreme of their thermal /physiological limits, e.g., in low or high elevation habitat, arid zones, or drought/fire sensitive species, species with low vagility, etc.).
- Species/ populations listed on national or regional directives/ under national or regional protection.
- Red listed species, including Near Threatened (NT)-classified species. Red Listing here indicates IUCN Red List Categories and Criteria applied at national level.
- Broad taxonomical spread across three kingdoms; animals (vertebrate and invertebrate), plants (vascular and nonvascular), fungi (Ascomycota and Basidiomycota).
- Emblematic and charismatic species/ culturally valuable species to ensure the conservation of these types of species of special importance and to increase public and policy-makers' acceptance and interest in monitoring genetic diversity. These species may be taken from surveys of species important to the public.

- Organisms that are particularly frequently used in nature conservation/restoration and/ or provide ecosystem services (e.g., pollinators, sentinel species, and pioneer species for plants of dry habitat establishment).
- Species/populations at risk of unwanted gene flow, such as hybridisation with non-native species following large scale releases or other human activities.
- Species that are key for ecosystem stability – may be rare, intermediate, or common.
- Species for which tissue collections are available (and where population genetic projects are already ongoing), providing the possibility for contemporary monitoring of genetic diversity.
- Representative species from main national habitat types (e.g., including biotopes)
- Indigenous wild relatives to domesticated livestock and crops, including fungi (c.f. Aichi Target 13).

**Table 1** showcases on the basis of which criteria a certain species was selected for genetic monitoring by respectively Scotland, Sweden, California (USA) and Switzerland.

	Basic criteria				Nationally prioritised criteria														
	Negatively affected by human interventions	Key ecological importance	National or regionally unique	Monitoring ongoing	Climate change affected	National or regionally listed or protected	Red - listed	Taxonomical spread	Culturally important	Frequently used in nature conservation	Risk of unwanted gene-flow	Ecosystem stability	Ecosystem level	Biomaterial collections available	Pollinators	National conservation priority	Representing national habitat	Indigenous wild relatives	Food / medicines
Papillose peatmoss ( <i>Sphagnum papillosum</i> ) Scotland	x	x			x					x		x					x		
Garden bumblebee ( <i>Bombus hortorum</i> ) Sweden	x	x	x*	x			x					x			x				
Valley Oak ( <i>Quercus lobata</i> ) California (USA)	x	x	x	x			x	x	x								x		
Natterjack toad ( <i>Epidalea calamita</i> ) Switzerland			x		x	x			x							x	x		

\* Historical samples available, present-day sampling ongoing in research project that includes genomics and a reference genome

(Source: Compiled by the authors).



Red-tailed bumblebee (*Bombus lapidaries*). Photo: © Ralph Martin



## Section 3: Cases

It is difficult to establish a standard monitoring programme and a similar suite of species suitable for all countries because challenges, importance, resources, and relevance will depend on the country or area. Hence approaches will be tailored to identify species for a specific genetic monitoring programme. We illustrate how Scotland, Libya, Sweden, California (USA), Switzerland, Victoria (Australia) and South Africa have established their respective monitoring programmes based on available resources as well as criteria and considerations relevant to them.





Peatmoss (*Sphagnum papillosum*). Photo: © Lorne Gill

## Scotland – a scorecard approach

The scorecard approach was designed to be applicable in any country or region, regardless of financial resources (Hollingsworth et al., 2020). While the scorecard approach is not genetic monitoring as defined in this document, the selection of species would be similar as if it was a genetic monitoring project. The project founders were concerned that reporting against CBD target 13, which had been agreed at the 2010 Aichi Conference, did not adequately reflect the importance of wild species. As a response the project was initiated in 2017, seeking to devise an easy to use method to assess genetic diversity of wild species. In order to build commitment to the project, a wide range of stakeholders were involved including botanic gardens, universities, research institutes, agricultural researchers, NGOs and government agencies. Lastly, people with a broad range of expertise in plants, animals and fungi, along with a government representative were brought in as a management team to spearhead the project.

The method devised used a set of criteria for defining terrestrial and freshwater species of socio-economic importance in Scotland and selected an initial list of 26 species. Aichi Target 13 method report. For each selection criterion, broad taxonomic diversity was pursued;

1. National conservation priority wild species. This was taken from a government-endorsed programme, the Species Action Framework. The steering group selected a subset with broad taxonomic coverage – two invertebrates (freshwater pearl mussel (*Margaritifera margaritifera*), great yellow bumblebee (*Bombus distinguendus*)), a fungus (hazelglove fungus (*Hypocreopsis rhododendri*)), and a vascular plant (woolly willow (*Salix lanata*)).
2. Species of national cultural importance. These species were taken from a national survey of culturally important species and included both animals and plants. Due to high levels of public concern over the decline of European ash (*Fraxinus excelsior*), this was added to this category.
3. Species providing key ecosystem services. Due to uncertainties over the relative contributions of different species, the steering group chose the three non-planted vascular plant species in terms of land cover, along with papillose peatmoss (*Sphagnum papillosum*) as a key species in carbon capture, and the common frog (*Rana temporaria*) as the most widely distributed vertebrate in mainland Scotland and an important regulator of invertebrate populations.
4. Species of importance for wild harvesting (food and medicine). Whilst wild foraged food is not an important dietary element in Scotland, it is culturally important, and the steering group also wanted the scorecard to be relevant to the needs of people in countries with less disturbed ecosystems. The species were selected from a survey of foragers.
5. Economically important game species. Hunting and fishing are economically important activities across much of Scotland and the species selected were two fish, two mammals and one bird. The selection was made on economic grounds but could alternatively have been made on dietary importance.

Each of the selected species was assessed by an authority on that species using the scorecard approach outlined below and then independently quality assured by a specialist, and finally by the editorial team to ensure consistency. The scorecard approach is not dependent on prior genetic knowledge, and instead uses expert opinion assessments of whether; 1) demographic declines are likely to lead to loss of genetic diversity (genetic erosion), 2) hybridisation is likely to lead to undesirable replacement of genetic diversity, 3) restrictions to regeneration/turnover are likely to impede evolutionary change, and 4) for plant species where seed-banking is a viable mechanism for holding genetic resources ex situ, users can also report on the representativeness of these ex situ collections.

Scotland published its first report in 2020 (see **Online Resources**) and the steering group plans to repeat the assessment every five years. While it is recognised that the ideal frequency of assessments will vary between species, the timeframe of five years was considered to be the minimum amount of time necessary, presenting a balance of enough time to measure meaningful changes of genetic diversity and reporting these results frequently to policy makers. The costs were limited to staff time as the approach used existing information and expert knowledge. One of the aims of the project was to design a method that could be applied in any country rather than relying on expensive equipment and extensive resources.

## Libya – applying the Scottish scorecard approach

The Scottish team are now working with the University of Benghazi to produce a scorecard for Libya and plan to use the knowledge gained to expand the approach to other countries and regions (O'Brien et al., 2022). The Libyan scorecard project includes endemics such as Cyrenaican wild artichoke (*Cynara cyrenaica*) and widespread but biologically and economically important species such as Aleppo pine (*Pinus halepensis*).



Hazelglove fungus (*Hypocreopsis rhododendri*). Photo: © David Genney



Aleppo pine (*Pinus halepensis*). Photo: © David O'Brien



European ash (*Fraxinus excelsior*). Photo: © Pixabay



Atlantic herring (*Clupea harengus*). Photo: @ Lars Hestbæk

## Sweden – a long term collaborative approach

Researchers within Sweden have long called for the need to map and monitor genetic diversity within populations (Ryman, 1981; Laikre et al., 2005; 2008) and several summaries on genetic diversity have been produced by the overarching authority for nature and wild species – the Swedish Environmental Protection Agency (SEPA) (Laikre & Ryman, 1997; Lönn et al., 1998; Lundquist et al., 2008; Posledovich et al., 2021a; 2021b), as well as by national researchers (Laikre et al., 2005, 2008; Wennerström et al., 2017). In 2005, Sweden's 16th national environmental objectives became effective stating that “Species are to live in long-term viable populations with sufficient genetic variation” and “species are spread within their natural habitats so that the genetic variation within and between populations is sufficient.” (pages 206-207 of 2005/06: MJU3). The government decision to reach the goals stipulated that mapping and monitoring of genetic diversity were to be initiated by 2015, albeit later set to 2020 (Swedish Government, 2017). Pilot programmes for monitoring of genetic diversity were therefore initiated during 2020 by SEPA and by the Swedish Agency for Marine and Water Management (SwAM) which is the national authority responsible for biological diversity of aquatic environments. In 2017, SwAM initiated a process to prepare a proposal for monitoring genetic diversity, including pilot work on a freshwater and a marine/brackish species as models, i.e., brown trout (*Salmo trutta*) and bladderwrack (*Fucus vesiculosus*). Two research teams were commissioned, working in close collaboration with SwAM managers. The work identified 12 priority species that were selected based on management questions and several indicators and limiting values to be applied by SwAM (Johannesson & Laikre, 2020; Andersson et al., 2021). Of these, four were selected for pilot monitoring during 2020, i.e., Atlantic herring (*Clupea harengus*), Atlantic salmon (*Salmo salar*), eelgrass (*Zostera marina*) and Atlantic cod (*Gadus morhua*). Concurrently, SEPA appointed three researchers to design a programme for initiating monitoring of genetic diversity. The work was carried out in close collaboration with SEPA managers (Posledovich et al., 2021a; 2021b). During this work, an extensive review was carried out focusing on scientific literature on genetic diversity of Swedish species (building from previous work referred above), which includes ongoing research/management including genetic monitoring (such as wolf monitoring where DNA is used to identify individuals), as well as ongoing management including collection of samples that could be used in monitoring of genetic diversity (such as fishes collected for monitoring of species occurrence), and existing tissue bank collections that could aid in providing time series DNA data, etc. Based on this review a collection of potentially suitable species was provided. To prioritise among these, the following criteria were considered particularly relevant:

1. Species/populations subjected to substantial harvest (hunting, fishing, collecting, logging, etc.).
2. Species/populations listed in the Annexes of the EU Habitats Directive and/or the EU Birds Directive.
3. Species/populations at risk of unwanted gene flow through, e.g., large scale releases or other anthropogenic activities.
4. Red listed species (including NT-classified species; Red listing here = IUCN Red List Categories and Criteria applied at national level).
5. Swedish populations that are genetically distinct from others over the distribution range.
6. Populations likely to be strongly affected by climate change (e.g., alpine and northern boreal species, Baltic Sea species with marine origin, low elevation species likely to not tolerate increasing temperatures).
7. Natural reference populations (presumed safe and non-exploited populations where “natural” and non-human induced rates of genetic change can be monitored and learnings on such rates obtained).

Other considerations given attention were:

- I. Species of key ecological importance, including habitat forming species and top predators.
- II. Pollinators (specifically asked for by SEPA to consider).
- III. Species for which tissue collections are available providing an immediate possibility for contemporary monitoring of genetic diversity.
- IV. Species already subjected to some form of genetic monitoring.
- V. Species subjected to other types of monitoring where individuals are sampled or handled during which samples for genetic analysis are possible to obtain.
- VI. Indigenous wild relatives to domesticated species (c.f. Aichi Target 13).

Following the suggested programme (Posledovich et al., 2021a; 2021b), during 2020 SEPA initiated a pilot monitoring of genetic diversity of five pollinator species: Apollo (*Parnassius apollo*), garden bumblebee (*Bombus hortorum*), common carder bee (*Bombus pascuorum*), red-tailed bumblebee (*Bombus lapidaries*), yellow-legged furrow bee (*Halictus rubicundus*) and moose (*Alces alces*). This work includes the use of present-day data as well as museum samples to provide historical genetic diversity data. Together with the four species selected by SwAM a total of 10 species are presently subjected to monitoring of genetic diversity in Sweden. Genetic markers used for separate species vary depending on availability and suitability and include both SNP arrays and whole genome resequencing. There is no long-term financial security for the programmes, rather, they depend on governmental instructions and funding. Consequently, the programmes will be evaluated by the two agencies on a need basis to decide on the next steps. Lastly, all the selected species are of direct importance for humans and or carry ecological roles. To date, the genetic diversity monitoring has helped the genetic rescue and comeback of the arctic fox in Sweden, assessed and ensured a genetically healthy population of the intensely hunted moose, evaluated the retention of genetic diversity of brown trout in protected areas, and helped evaluate the occurrence of over-harvesting of herring populations. In all cases, managers, agencies, and researchers are working in collaboration on genetic monitoring.



Eelgrass (*Zostera marina*). Photo: © Pekka Tuuri



Apollo (*Parnassius apollo*). Photo: © Ralph Martin



Moose (*Alces alces*). Photo: © Pixabay





Lace lichens (*Ramalina menziesi*). Photo: © Gernot Segelbacher

## California (USA) – biodiversity hotspot assessment

As one of the world's biodiversity hotspots (Myers et al., 2000), California has an extraordinary number of species in terrestrial and marine ecosystems, and most are under pressure from climate change, overexploitation, habitat loss, degradation, and, for many, ultimately species imperilment. In addition, the ecosystems they occupy are also in jeopardy. According to projections from California's Fourth Climate Change Assessment (Bedsworth et al., 2018), ~50% of the vegetation of California will be climatically stressed by 2100, and the areas destroyed by wildfire will increase annually due to hotter, drier conditions. All of these human disturbances necessitate both species and ecosystem management as well as environmental policies that will support better protections of species and improved land management.

The California Conservation Genomics Project (CCGP) (Schaffer et al., 2022), funded by the State of California, USA, is a collaborative effort to manage and help protect California's flora and fauna through landscape genomics-driven conservation research of threatened, endangered, commercially exploited, and ecologically significant species. The goal of the project is to provide a multi-species synthesis of state-wide diversity based on analysis of geographic and environmental trends in genotypes. The research design is built on the premise that genetics of organisms provide a unique geographic signature of historical evolutionary processes, such as gene flow, changes in population size, and natural selection, which will provide insight about regions where species or ecosystems could be particularly jeopardised by human disturbance. The resulting data can serve as a baseline of the genetic composition of species that could be compared with future sampling to assess the impact of climate change and other human-mediated landscape changes. Such information can guide policies and resource management strategies now and also serve as a basis for future monitoring projects of specific species of concern or regions that might be key for preservation.

The CCGP is being conducted as a joint effort by scientists from all ten campuses of the University of California, with input on choice of species and design from state legislators and scientists at state and federal agencies. The criteria and project design were agreed upon by a broad range of 50+ stakeholders and scientists during an initial planning meeting. The selection of species was based on whether they fell into one of these three categories: threatened and endangered, commercially exploited, or wide-ranging foundational species of great ecological significance. Species were also chosen from a broad set of investigator-initiated proposals to represent a taxonomically diverse set of vertebrates, invertebrates, and plants from marine, freshwater, and terrestrial ecosystems. Finally, species were selected to maximise geographic representation that encompassed the broadest possible range of California's 19 ecoregions.

At the planning meeting, several decisions were made to maximise the impact of the research for immediate and long-term conservation planning, resource-management, and policy relevant to threatened species and ecosystems. The overarching research approach is consistency. All studies generate whole genome sequence data rather than specific markers or reduced representation library sequences because these data should have the longest useful lifespan.

All projects use a landscape sampling approach by selecting approximately 100–150 individuals throughout the species' range, rather than a population approach of sampling clusters of individuals across fewer localities. This method was chosen because it maximises the statistical power to correlate individual genetic loci (e.g., single locus polymorphisms, SNPs) with environmental and geographic variables. All genera in the project will also have a high-quality chromosome-level reference genome, and almost all species will have reference genomes generated specifically for the CCGP. Genomic variants across taxa to be used in the landscape genetic analyses will be generated employing a uniform bioinformatic pipeline, including standard approaches and filters. CCGP will conduct synthetic conservation and landscape genetic analyses using the total dataset, while conducted using a uniform set of tools and pipelines individual and individual investigators

have access to their raw data for their own species-specific research needs. As a result of this research plan, CCGP has sampled 100–150 individuals from 246 species (144 genera) across ~15,000 localities, producing ~20,000 resequenced whole genomes.

In the short term, CCGP will utilise genomic information on gene flow, genetic diversity, adaptive genetic variation, and geographic patterns that is essential for endangered, threatened, and commercially exploited species to provide desperately needed evidence for management strategies. In addition, the landscape approach across diverse taxa will identify geographic regions of conservation importance. Over the long run, these data provide the foundations for genetic monitoring that might be implemented for specific species of concern or could be replicated in the future for most of these same species to assess whether climate change and other anthropogenic disturbances are jeopardising the ecological and evolutionary processes of natural populations. The utilisation of a landscape approach allows an assessment at the ecosystem level to identify geographical regions that should be prioritised for conservation and environmental policy protection.



Giant kelp (*Macrocystis pyrifera*). Photo: @ Claire Fackler



Feather millipede (*Brachybyge producta*). Photo: @ Jason Bond



Giant spiny sea star (*Pisaster brevispinus*). Photo: @ Jerry Kirkhart



False heath fritillary (*Melitaea diamina*). Photo: © Martin C Fischer

## Switzerland – a genomic approach

As a response to habitat loss and population decline, a national Biodiversity Strategy and Action plan was conceived using monitoring of genetic diversity as a tool to reach the established goals. Over decades, Switzerland has built knowledge of the diversity of national species and habitats, by means of e.g., federal inventories of biotopes of national importance and species diversity. Changes in species occurrence and abundance, as well as habitat quality and area, are recorded in various monitoring programmes, documenting that biodiversity in Switzerland is in a worrying state (Biodiversity Monitoring, Federal Office for the Environment (BAFU, 2017). Although the number of species have remained constant over the past 15 years, valuable habitats, such as dry meadows or raised bogs, have continued to lose quality and area, and common species continue to suffer population loss. In contrast to species and habitat diversity in Switzerland, little is known about the genetic diversity within species and even less about any changes in genetic diversity over time. Hence, the Swiss Federal Institute of Technology in Zürich (ETH Zurich) conducted a feasibility study on behalf of FOEN on how such monitoring could be implemented and what it would cost (Fischer et al., 2020). The feasibility study proposed to monitor the genetic diversity of 50 species every five years to allow similar cycles of reporting of already existing monitoring programmes. The study employs whole-genome resequencing, studying up to 60 populations and 150 to 600 individuals per species, dependent on their distribution range: in total about 17,000 individuals. To obtain more accurate estimates of changes of genetic diversity over time, it is proposed that genetic data dating back more than 100 years for ten of the species should be derived from collections and herbaria.

The following criteria were considered for the species selection for the Swiss monitoring of genetic diversity (Fischer et al., 2020):

1. The three major kingdoms – animals, plants and fungi are represented.
2. All main Swiss habitat types (TypoCH) are considered (BAFU, 2019).
3. Habitats of national importance are considered (FOEN, 2019).
4. All biogeographical regions of Switzerland are represented (Gonseth et al., 2001).
5. Rare, intermediate, and common (ecosystem relevant) species are studied.
6. National Red List species (Cordillot & Klaus, 2011) and national priority species (BAFU, 2019) are included.
7. Groups that are particularly frequently used in nature conservation are given special consideration.
8. Species likely to be affected by climate change (decrease or increase) are represented.
9. Emblematic species are included to increase public acceptance and interest in genetic diversity monitoring.
10. Possible synergies with other national monitoring efforts will be sought.
11. Genome size and availability of a reference genome are taken into consideration to reduce cost and enable a faster launch.

Starting directly with such large-scale monitoring would be overly ambitious, hence Switzerland initiated a first smaller scale pilot study in 2020 (see Online Resources), conducted by ETH Zurich on behalf of FOEN, supported by the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL). The three year pilot study aimed to i) test and standardise procedures for sample collection, laboratory work and data analyses, ii) establish cooperation with stakeholders – such as cantonal authorities, administrations, scientific collections and environmental offices, iii) establish reference values for the current genetic diversity of five selected species and document possible changes in genetic diversity over the past century for two of these species and, iv) test whether there is evidence that anthropogenic influences, such as habitat loss due to increased land use, have a measurable impact on the extent of genetic diversity in the studied species. The pilot study is set up to test the feasibility of monitoring genetic diversity for many species using a genomic approach. Of the 50 species identified for the full project, five species from different groups of conservation interest were selected for the pilot study: natterjack toad (*Epidalea calamita*),

yellowhammer (*Emberiza citronella*), false heath fritillary (*Melitaea diamina*), carthusian pink (*Dianthus carthusianorum*) and hare's-tail cottongrass (*Eriophorum vaginatum*). Between 150 and 300 individuals per species were collected in spring and summer 2021 using a random stratified proportional sampling scheme within Switzerland. The genomes of the five species were de novo assembled and for ~1200 individuals' whole genomes will be re-sequenced (WGS) to establish a reference value for the current genetic diversity of these individuals, populations, and species. Genetic indicators will be tested for their ability to allow conclusions to be drawn about genetic diversity, population structure, gene flow, inbreeding, hybridisation, genetic load, and adaptive potential. For two species, the hare's-tail cottongrass (*Eriophorum vaginatum*) and false heath fritillary (*Melitaea diamina*), 400 historical samples from scientific collections are being sequenced to provide a retrospective analysis of changes in genetic diversity over the past 100 or more years. Additionally, a survey among conservation stakeholders was conducted to ensure support and improve the foundation for a successful implementation of genetic diversity monitoring in Switzerland (Pärli et al., 2021). The monitoring is envisaged to be repeated every five years to have similar cycles of reporting to already existing monitoring programmes. This allows better comparability across the different biodiversity monitoring schemes and increases synergy effects.



False heath fritillary larve (*Melitaea diamina*). Photo: © Martin C Fischer



Carthusian pink (*Dianthus carthusianorum*). Photo: © Martin C Fischer



Yellowhammer (*Emberiza citronella*). Photo: © Fergus Gill





Greater glider (*Petauroides volans*). Photo: © Damien Esquerré

## Victoria (Australia) – a state initiative

To date, standardised inclusion of genetic data in conservation planning and government policy has been lacking at a federal level in Australia. However, States and Territories have proceeded with their own initiatives. In Victoria, CESAR Australia, Monash University, and the University of Melbourne have collaborated to develop a “Genetic Risk Index Tool” (Kriesner et al. 2019; see also **Online resources**), which the State Government (specifically the Department of Environment, Land, Water and Planning, or DELWP) now routinely considers in conservation planning. Victoria has the highest human population density, and the greatest historical proportion of land-clearing and habitat fragmentation of any state or territory in Australia, meaning early conservation intervention guided by genetic data may prevent species from reaching threatened status. This tool provides a way forward for incorporating genetic diversity into biodiversity conservation, even when genetic data are not available; grouping over 1,100 plant and animal species into low, medium, high, very high and uncertain risk categories.

To do this, information on genetic health was collated for as many Victorian plant and animal species as possible, using an automated Web of Science search, as well as a standard set of sources including websites (e.g., IUCN, Atlas of Living Australia), action/recovery plans, field guides, grey literature, and expert consultation. Where genetic data were not available, demographic data likely to be informative about genetic health were collected as proxies.

Using this approach, they were able to assess genetic risk for:

- All Victorian amphibian species (n=36).
- All birds that occur in Victoria for at least part of the year on a regular basis (n=349).
- All Victorian freshwater fish (n=53).
- Invertebrates listed under Victoria’s Flora and Fauna Guarantee (FFG) Act, as well as two crayfish species (n=62).
- All Victorian terrestrial mammals and one marine mammal (n=74).
- A selection of plants including species endemic to Victoria listed under the FFG Act (n=414).
- All Victorian reptiles (n=112).
- After the 2019–2020 bushfires the index was updated to include an additional 138 plants and animals of immediate concern identified in DELWP’s Victoria’s bushfire emergency.

These data were then evaluated for their potential to influence genetic risk, resulting in 17 metrics that were assigned weightings for each species. The overall genetic risk score for each species was generated by summing scores across each of the relevant genetic and demographic metrics. Finally, given the large amount of uncertainty around some metrics due to varying levels of data availability across taxon, uncertainty weightings were also included and summed to create a “doubt” score for each species. While the final risk indices and uncertainties need validation for their objectivity and accuracy for each species, the use of existing information and expert knowledge have provided a framework for incorporating genetic diversity into biodiversity conservation planning at low costs (limited to staff time). It has also highlighted research gaps and data deficiency for specific species.



Quiver tree (*Aloidendron dichotomum*). Photo: © Luke Verburgt

## **South Africa – using the genetic indicators to ascertain the genetic status**

South Africa is considered one of the most biologically diverse countries in the world due to its species diversity, rate of endemism and diverse ecosystems; and the National Biodiversity Assessment (NBA), led by the South African National Biodiversity Institute (SANBI), is the primary tool used to monitor and report on the state of this biodiversity. Until recently, the NBA has primarily focused on assessing biodiversity at the ecosystem and species levels; however, efforts are being made to include genetic level assessments. Building on the strong foundation of species assessments (i.e., all vertebrates and plants have been comprehensively assessed; invertebrate assessments are increasing), an ambitious project has been initiated to quantify the genetic status of all species based on two genetic indicators proposed by Hoban et al. (2020). This project will be carried out in three phases. The first being pre-screening, which will incorporate all known data for species (e.g., acquired from scientific literature, national assessments, Red List assessments) to obtain a preliminary genetic assessment. During the second phase, these assessments will be verified or revised by intensive expert consultation and a final genetic status for each species will be obtained. Following this, the third phase, species prioritisation guidelines for genetic monitoring, will be undertaken. This will likely involve critically examining guidelines set out by other nations and international organisations, applying them in a South African context, and revising the guidelines to better address the nation's unique and varied diversity. These prioritisation guidelines will feed into the National Biodiversity Monitoring Framework, currently under development by SANBI.

## Synthesis

Genetic diversity plays a fundamental role for nature and society (see e.g., Figure 2) and assessing, monitoring, and conserving genetic diversity in wild species is key for ensuring biodiversity.

Monitoring of genetic diversity is a tool to help guide and inform conservation actions, as well as reach policy goals, whether local, national, or international. Such monitoring programmes also generate samples and data, which are invaluable resources to safeguard for future use. For any programme to be developed, several key aspects need to be considered:

- Setting up a monitoring programme requires a sound stakeholder setup with a team consisting of practitioners, genetic experts (ensuring access to research facilities and analytical support and to assist with interpretation and management recommendations), links to ongoing monitoring programmes where existing and staff from the key agencies and governments, if possible. Ensuring top level stakeholder engagement could aid the financial aspect and help advocate for long term funding and ongoing reporting.
- Full archiving of modern samples, along with traceable origin information, should be secured in dedicated facilities such as biobanks or museums.
- A data management plan is needed, and an open data policy should be obligatory, ensuring that the data (along with metadata) are globally available and informative for future genetic diversity monitoring programmes and conservation efforts.
- Collaborations, whether new or existing, as well as overlapping initiatives should be explored for synergy; and partnerships and resources for the laboratory and bioinformatics work established where needed.
- Capacity building should be encouraged, to work towards building strong technical and analytical skills across all regions of the world, providing all countries with the necessary resources and skills to conduct genetic diversity monitoring of native species.
- As species and population challenges and questions may change over time, monitoring programmes must be and stay flexible to adjust as needed (standard metrics and methodology are needed to ensure comparability through time).
- Conducting a pilot study helps in gaining experience and establishing best practices and identifying main actors.
- Whatever monitoring design is chosen, project leaders must be transparent on why and how these choices have been made.

Setting up a monitoring programme needs the collaboration of many partners (such as scientists, practitioners, being embedded into ongoing monitoring programmes) and a long-term perspective. There will need to be nationally specific targets and methods. While this effort might be challenging for some countries, we argue that by linking with ongoing initiatives and establishing further networks, it is feasible for every country and can be adapted to their specific needs. Moreover, this approach provides opportunities for establishing a coordinated effort at national and international levels that can improve the impact and effectiveness of these monitoring programmes for the conservation of biodiversity.

## Online resources

<https://g-bikegenetics.eu/en>

### Existing monitoring programmes which could be linked to a genetic monitoring programme

<https://www.lter-europe.net/>

<https://www.tern.org.au/tern-observatory/tern-ecosystem-surveillance/>

<https://threatenedspeciesinitiative.com/>

[https://www.environment.vic.gov.au/\\_\\_data/assets/pdf\\_file/0029/518492/Genetic-Risk-Index-Report.pdf](https://www.environment.vic.gov.au/__data/assets/pdf_file/0029/518492/Genetic-Risk-Index-Report.pdf)

<https://geobon.org/>

<https://www.andis.org.au/working-with-data/fairdata>

### Cases highlighted in the guidance document

#### Scotland

<https://www.nature.scot/scotlands-biodiversity-progress-2020-aichi-targets-aichi-target-13-genetic-diversity-maintained>

#### Sweden

<https://www.naturvardsverket.se/globalassets/media/publikationer-pdf/6900/978-91-620-6959-9.pdf> (a proposal for species, methods and costs) and

<https://www.naturvardsverket.se/globalassets/media/publikationer-pdf/6900/978-91-620-6958-2.pdf> (pollinators)

#### Switzerland

<http://gendiv.ethz.ch/>

#### California (USA)

<https://www.ccgproject.org>

#### Victoria (Australia)

<https://www.environment.vic.gov.au/biodiversity/genetic-risk-index>

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