THE EXPANSION OF CONSERVATION GENETICS

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Abstract | The 'crisis discipline' of conservation biology has voraciously incorporated many technologies to speed up and increase the accuracy of conservation decision-making. Genetic approaches to characterizing endangered species or areas that contain endangered species are prime examples of this. Technical advances in areas such as high-throughput sequencing, microsatellite analysis and non-invasive DNA sampling have led to a much-expanded role for genetics in conservation. Such expansion will allow for more precise conservation decisions to be made and, more importantly, will allow conservation genetics to contribute to area- and landscape-based decision-making processes.

SYSTEMATICS
The field of biology concerned with the diversity of life on Earth. Systematics is usually viewed as having two components — phylogenetics and taxonomy.

Conservation biology has been accurately described as a 'crisis discipline'. Much like the human-disease crisis disciplines of human immunodeficiency virus biology and cancer biology, we urgently need to understand the patterns and processes that conservation biology aims to describe. The magnitude of the crises that are the subject of conservation biology is manifest in the large number of species that are facing imminent extinction. In fact, the past two centuries of human activity might be one of the most severe periods of mass extinction of all time[1–3]. In addition, because conservation biologists have to make rapid decisions based on currently available data, there is a heightened sense of crisis in the discipline.

Crisis disciplines often see periods of expansion of the tools that are used to solve the problems that these crises pose. Many of these tools help researchers to quickly and efficiently collect data relating to problems that need rapid and even immediate attention. Conservation biology is no exception: the increasing integration of geographical positioning system (GPS) technology, mathematical advances and genetics into this field are prime examples. In particular, the proliferation of relevant technologies in genomics, SYSTEMATICS and population biology over the past decade has been a key factor in promoting the integration of genetics into conservation biology.

This review attempts to articulate the current structure and scope of conservation genetics, as well as to demonstrate the usefulness of this structure in modern conservation biology. The expansion of the use of genomic technologies in data acquisition, storage and analysis has assisted the efficiency of the field in helping conservation decision-making. The improved precision and quantity of data that are relevant to endangered species that can now be used in conservation genetics allows us to examine the issues of breeding captive species, species-boundary problems and conservation forensics, three topics that are the primary focus of this review. Another emerging area of conservation genetics that we do not cover in detail comprises studies that combine genetic methodology with ecological and landscape approaches. Such studies can provide conservation biologists with a much more accurate picture of the complex systems that they work on.

Conservation biology is expanding to include many subdisciplines and approaches and is starting to incorporate genomics and high-throughput methods of data acquisition. This expansion enables the field to address more effectively the urgent problems that are involved in managing endangered species and critical areas. Nonetheless, significant challenges remain to be overcome. In particular, there needs to be a wider awareness that genetic information can place conservation decisions in context, and so ensure that the correct decision is made. Genetics can provide conservationists with unprecedented precision and can add greatly to their

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understanding of the genetic parameters, on the basis of which many decisions are made. Importantly, however, conservation genetics itself needs to be placed in the context of the difficulties of working across political boundaries, amidst economic challenges and in the face of the complexity of using science to inform management decisions.

RANDOMLY AMPLIFIED POLYMORPHIC DNA (RAPD). DNA fragments that are generated by PCR using one or two randomly selected oligonucleotides or primers and are polymorphic in size.

MINISATELLITES
Regions of DNA in which repeat units of 6–100 bp are arranged in tandem arrays that are 0.5–30 kb in length

RESTRICTION-FRAGMENT-LENGTH POLYMORPHISM (RFLP). A fragment-length variant of a DNA sequence that is generated through the gain or loss of a restriction site due to a DNA substitution.

ALLOZYMES
Co-dominant nuclear DNA markers that consist of enzymes that differ in their mobility on a charged gel.

The scope of conservation genetics

The introduction of high-throughput DNA sequencing and genotyping technology has expanded the scope of conservation genetics over the past decade. Conservation geneticists have followed the same trends in terms of technique usage as outlined by Schlotterer[4] for population genetics. Some techniques — such as those that use RANDOMLY AMPLIFIED POLYMORPHIC DNA (RAPD), MINISATELLITES, RESTRICTION-FRAGMENT-LENGTH POLYMORPHISMS (RFLPS) and ALLOZYMES — are either not used or are only sparingly used in population-level studies. However, four methods — the use of AMPLIFIED-FRAGMENT-LENGTH POLYMORPHISMS (AFLPS), DNA sequencing, SNP analysis and the use of MICROSATELLITES — are the main techniques that are used at this level to study animal conservation genetics [4]. By contrast, plant conservation genetics, as well as using AFLPs, has continued to use RAPDs and another DNA-variation technique that makes use of INTER-SIMPLE-SEQUENCE REPEATS (ISSRS)[5]. The development of techniques in systematics that incorporate high-throughput sequencing and SNP analysis has also helped in some areas of conservation genetics that focus on pattern description (TABLE 1). Taken as a whole, conservation geneticists have closely followed the development of genetic and genomic technology and have voraciously incorporated techniques that use SNPs[6–8] and microsatellite variation as the main tools of their trade. These researchers are continuously looking for more rapid and efficient screening techniques to detect genetic variability. Finally, some conservation geneticists have even contemplated using microarrays and quantitative-trait mapping in conservation biology[9,10].

The role of genetics in conservation biology is diverse and has been addressed in several publications[11–15] (TABLE 1). Here, we attempt to summarize the main approaches that are used in conservation genetics for understanding pattern and process. To do this, we first examine the early challenges to the introduction of genetics into conservation decision-making, and then discuss the present and future of pattern and process discovery in conservation genetics.

Early challenges. The expansion of the 'toolbox' of conservation biology in the late 1980s to include molecular-evolutionary genetic techniques and markers was immediately met with three challenges to the relative merit and priority of the use of genetics in the discipline. The first was Lande's cogent and direct challenge to the relevance of genetics to demographic issues in conservation biology[3]. In a landmark paper, Lande pointed out that demographic factors (the biology of population growth and life history) were much more important for understanding extinction than any of the genetic factors that could be incorporated into a theory of conservation biology. These demographic factors were viewed as outweighing most of the genetic factors that could cause extinction. Lande's challenge to conservation genetics was a healthy one: it opened the way for a better-defined role of the concepts of inbreeding and genetic variation in the discipline. It also cleared the path to the now well-accepted notion that the use of population genetics without demographics in conservation usually leads to less than useful recommendations[3]. However, careful integration of demographic and fine-detailed genetic approaches can often allow strong inferences to be made regarding conservation biology.

The second challenge concerned the tools used for pattern discovery; it came when Ryder challenged the definition of SUBSPECIES and the usefulness of these entities as conservation units[16]. The healthy debate that followed Ryder's original challenge embedded the term EVOLUTIONARILY SIGNIFICANT UNIT (ESU) in the conservation-genetics literature[17–19]. The problem of unit designation in conservation is often encountered at the interface of population genetics and systematics, because the goal is to discover species units (see also below). These debates, at both levels of resolution, resulted in a more applied focus in conservation genetics. Clearly articulating the goals of a specific conservation problem provided better guidance for the selection of techniques, tools and theory to be used.

Table 1 | **Classification of the diverse roles of conservation genetics**

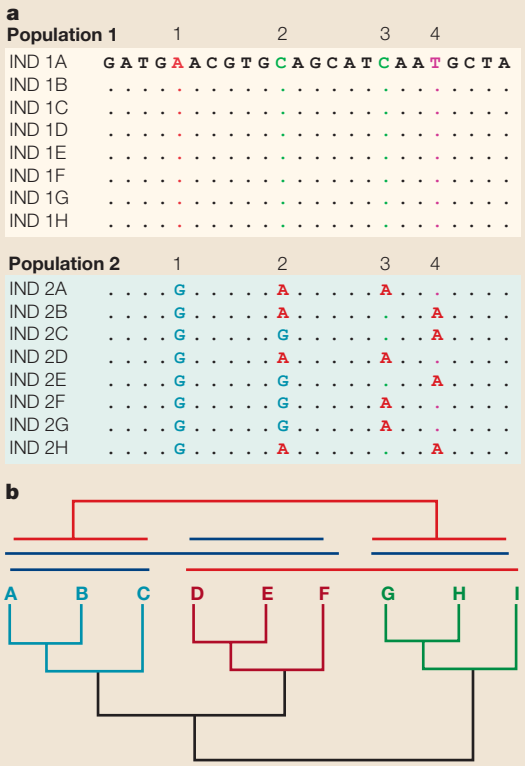
Role in conservation*	Pattern or process‡	Subdisciplines§
Minimizing inbreeding and loss of genetic variation	Process	Population genetics
Identifying populations of concern	Process/pattern	Population genetics
Resolving population structure	Pattern	Population genetics
Resolving taxonomic uncertainty	Pattern	Systematics
Defining management units within species	Pattern	Systematics
Detecting hybridization (genetic pollution)	Process	Population genetics/systematics
Detecting and defining invasive species	Process	Population genetics/systematics
Defining sites and genotypes for re-introduction	Process	Population genetics/systematics
Use in conservation forensics	Pattern	Systematics
Estimating population size and sex ratio	Process/pattern	Population genetics
Establishing parentage; pedigree analysis	Pattern	Population genetics
Understanding population connectivity	Pattern	Population genetics/systematics
Use in the management of captive populations	Process	Population genetics
Understanding relationships of focal groups of taxa	Pattern	Systematics
Implementing genotoxicity studies	Process	Population genetics
Increasing the reproductive capacity of organisms	Process	Population genetics

*The list of roles taken partly from REF. 22. ‡'Pattern' or 'process' indicates whether the role listed focuses on the genetic inference of processes or the description of patterns. §The subdiscipline of evolutionary biology that is the main source of the techniques used to fulfil the role.

Box 1 | **Approaches to diagnosing conservation units in nature**

The figure illustrates hypothetical examples of two important approaches to diagnosing conservation units. The example of the diagnostic character-based approach (**a**) shows hypothetical DNA sequences from samples taken from 14 individuals (INDs) from 2 populations. The top sequence is shown for IND 1A; dots in all sequences below indicate identity to this sequence. The DNA column numbered 1 indicates a DNA position in the sequence that unambiguously diagnoses population 1 (A in position 1) as being distinct from population 2 (G in position 1). The column numbered 2 indicates a position in the DNA sequence that, although polymorphic in population 2, still diagnoses population 1 (C in position 2) as being distinct from population 2 (either A or G in position 2). The DNA columns numbered 3 and 4 indicate positions in the sequence that are polymorphic and are not diagnostic on their own. However, in combination, the information in columns 3 and 4 does diagnose population 1 (C in position 3 and T in position 4 — CT) as being distinct from population 2 (A in either positions 3 or 4 — AT or CA). The approach described here is, of course, highly dependent on the sample size of the two populations.

The tree-based approach to conservation-unit diagnosis depends on assaying groups of populations that are reciprocally monophyletic. Part **b** in the figure, which shows a hypothetical phylogeny of three groups of populations (A–C in blue, D–F in red and G–I in green), can be used to illustrate the concept of reciprocal monophyly. Taken in isolation, each of the three groups is monophyletic (indicated by a blue line above the group) with respect to all other populations in the phylogeny; that is, each group includes the most recent common ancestor of that group and all its descendants. However, a larger group that includes both group G–I and either group A–C or group D–F is PARAPHYLETIC (indicated by a red line above the group) with respect to the remaining smaller group, because these groups do not contain some of the descendants of the most recent common ancestor of the populations that make up that group. Group G–I is, however, reciprocally monophyletic to the union of group A–C and group D–F.



PARAPHYLETIC
A term applied to a clade of organisms that includes the most recent common ancestor of all of its members, but not all of the descendants of that most recent common ancestor.

AMPLIFIED-FRAGMENT-LENGTH POLYMORPHISM (AFLP). A DNA marker generated by digestion of genomic DNA with two restriction enzymes to create many DNA fragments, followed by ligation of specific sequences of DNA (adaptors) to the ends of these fragments, amplification by PCR (using primers corresponding to the adaptors plus random combinations of three additional bases at the end) and visualization of the fragments by gel electrophoresis.

MICROSATELLITES
Co-dominant nuclear DNA markers that consist of sets of short, repeated nucleotide sequences.

The third challenge was issued in a posthumously published paper by Caughley who suggested that too much focus on technical approaches to conservation (including conservation genetics) had resulted in the neglect of more important issues, such as habitat threat and disease[20]. Subsequent expansion in the use of conservation genetics in ecology and applied wildlife management answered this criticism[21], as did the realization that genetics could aid landscape-ecology approaches.

Pattern and process — the targets of conservation genetics. The most significant result of debate on these three challenges was to define the roles of conservation genetics in understanding genetic and evolutionary processes and in delineating the patterns that are relevant to managing endangered populations. The most important applications of conservation genetics derive from its ability to help to create a more accurate picture of pattern and process in endangered species. Specifically, it allows a more precise description and understanding of the processes that gave rise to the current endangered state of a population or species. In particular, the quantification of INBREEDING DEPRESSION, BREEDING EFFECTIVE POPULATION SIZE, MINIMUM VIABLE POPULATION SIZE and levels of genetic variation and gene flow in natural populations[22–25] provides specific and comparable measurements of processes that affect endangered populations. Moreover, as exemplified below, such studies can indicate immediate, genetically based responses to the detrimental effects of these processes.

Ex situ genealogical and inbreeding analysis of populations[26–31] is a particularly important area from which such responses can be formulated. Several studies in which pedigrees derived from genetic analyses have informed 'matchmaking' (see below) exemplify the urgency of making the best possible genetic decisions in breeding programmes for endangered species[32–36]. In addition, pedigrees can be extremely important in examining life-history traits in endangered wild populations (for example, in whales; see below).

The more general study of population-level processes in endangered populations in the wild is perhaps less immediately useful for conservation decision-making than *ex situ* genetic 'matchmaking' but is equally important in the long term. Classic population-genetic measures, such as WRIGHT'S INBREEDING COEFFICIENTS (F statistics (F_{ST})), were initially used in such studies to characterize the levels of variation and genetic contact among the populations being studied, but more recently these classical measures have been criticized for being imprecise[6,10,37,38]. As much greater amounts of molecular data became routinely available, population-genetics methods such as refined F_{ST} approaches (analysis of molecular variance; AMOVA), COALESCENCE-THEORY-BASED ANALYSES and related approaches have become more important for the statistical analysis of population-level variation[39,40]. These methods have therefore become important in discovering process phenomena in conservation genetics. Another analytical approach that has become increasingly important at the population

INTER-SIMPLE-SEQUENCE REPEAT (ISSR). DNA fragments found between adjacent, oppositely oriented microsatellites. DNA is amplified by PCR, separated by gel electrophoresis and scored for the presence or absence of fragments.

SUBSPECIES
A physically distinct subunit of a species.

EVOLUTIONARILY SIGNIFICANT UNIT (ESU). A population of organisms that is reproductively isolated from other populations of the same species, and represents an important component in the evolutionary legacy of the species.

INBREEDING DEPRESSION
Reduction in fitness or vigour caused by one or more generations of inbreeding.

BREEDING EFFECTIVE POPULATION SIZE
The number of individuals that make up the breeding population in an idealized population.

MINIMUM VIABLE POPULATION SIZE
An estimate of the smallest number of individuals in a population that is capable of maintaining that population without significant manipulation.

WRIGHT'S INBREEDING COEFFICIENTS
Measures of inbreeding in a sub-population first devised by Sewall Wright to describe the amount of homozygosity in a population due to inbreeding. Measures of inbreeding at different hierarchical levels of comparison can be obtained using this approach.

COALESCENCE-THEORY-BASED ANALYSIS
A means of investigating the shared genealogical history of genes. A genealogy is constructed backwards in time, starting with the present-day sample. Lineages coalesce when they have a common ancestor.

POPULATION-VIABILITY ANALYSIS (PVA). The process of identifying threats faced by a species and incorporating these threats into an estimation of the likelihood of persistence of a species for a given time in the future.

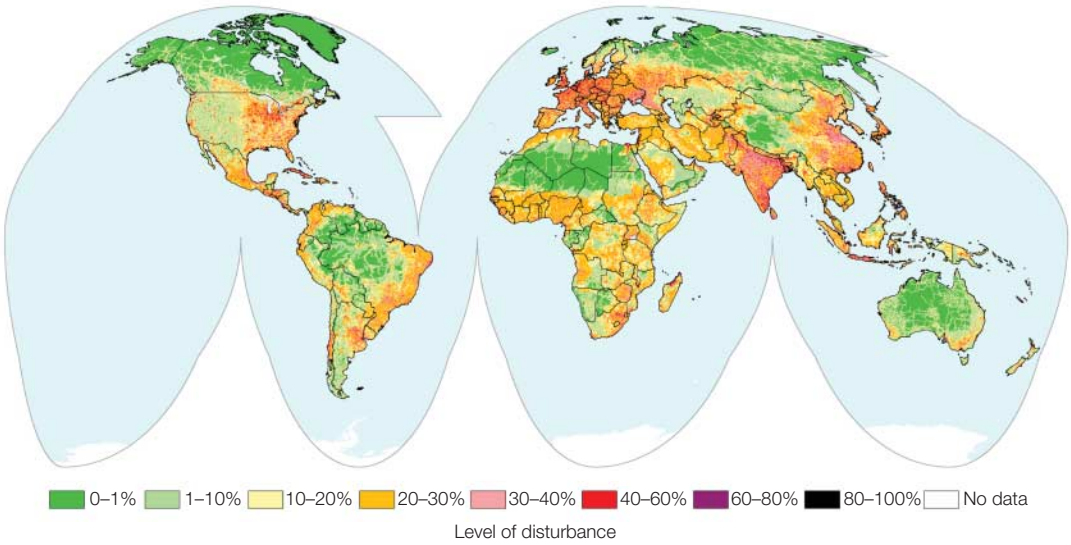


Figure 1 | **Map of conservation priority areas based on levels of human disturbance across all terrestrial landscapes.** Conservation genetics uses these landscape-prioritization exercises to indicate appropriate techniques and methods of analysis that are directed at threats specific to large protected areas, as well as highly fragmented habitats. For example, in largely undisturbed areas (0–1% and 1–10% disturbed), conservation genetics can have a significant role in identifying areas that contain significant biodiversity and high levels of endemism. In other areas that are highly disturbed (80—100%), conservation genetics focuses on restricted gene flow and metapopulation management. Modified with permission from REF. 116 © (2002) American Institute of Biological Sciences.

level is POPULATION-VIABILITY ANALYSIS (PVA). PVA uses models of population dynamics (sometimes incorporating genetic and pedigree information) to estimate minimal viable population sizes for threatened populations that are subject to a variety of conditions.

A second important area on which conservation genetics has had an influence is the delineation of appropriate units for conservation attention — an area that lies at the intersection of population genetics and systematics. Conservation decisions often rely on the determination of species boundaries, which is a contentious subject in evolutionary and systematic biology. The diversity of species definitions and the lack of agreement on how to objectively and operationally use data to delimit species boundaries based on a particular species concept or definition are the sources of these contentions[41]. However, several objectively based and operational approaches do exist.

Two general systematics approaches to delineation of unit boundaries have been taken — character-based methods (POPULATION-AGGREGATION ANALYSIS (PAA))[42] and tree-based methods[43–46] (BOX 1). All tree-based approaches (summarized in REF. 45) include the common step of producing a phylogenetic tree that indicates the position of a group of individuals in relation to other groups. In these approaches, regardless of the method of tree construction, the concept of reciprocal monophyly[45,47] is used to delimit the boundaries of entities in the analysis. The character-based approach results in the determination of diagnostic features from the attributes that are used to perform the PAA (BOX 1), and therefore provides easily applicable tools for identifying the units defined.

Pattern and process: the future. The most important applications of conservation genetics in the future will be those that incorporate both pattern and process into a cohesive approach to decision-making. There are three main areas in which this cohesion of pattern and process is being developed — nested clade analysis, CLADISTIC DIVERSITY measures and genetically informed demography-based approaches.

Nested clade analysis is an approach that has become particularly popular[48–51]. This approach uses a network constructed from genetic information that is nested according to a set of rules that results in increasingly inclusive nested groups, providing a detailed, pattern-based method for studying endangered organisms. The nested groups are then evaluated in the context of their geographical arrangement, such that the statistical significance of geographical associations is assessed. The last step is to link process to the nested patterns. A statistical approach is used in a step algorithm to designate aspects of restricted gene flow, fragmentation and range expansion, all of which are important processes that affect the genetic structure of endangered species[37;52].

The cladistic diversity method is more useful than nested clade analysis at higher taxonomic levels. The first step in this approach is the discovery of a phylogenetic pattern using genetic information and phylogenetic-tree-building methods. In this approach, taxa are given priorities based on their uniqueness in a phylogenetic tree. The patterns that are seen in a phylogenetic tree are interpreted as a reflection of the processes of extinction and speciation. Those taxa in the tree that are basal, with few close relatives, are considered to have more cladistic diversity, whereas those taxa with many

POPULATION-AGGREGATION ANALYSIS (PAA). Provides a straightforward criterion for demarcating a phylogenetic break between aggregates of individuals. An aggregate is said to be distinct when an attribute or combination of attributes in one aggregate is fixed and different from an attribute or combination of attributes in another aggregate.

CLADISTIC DIVERSITY A measurement that ranks areas for biodiversity-conservation priorities based on information in cladograms or phylogenetic trees. Also called phylogenetic diversity.

AREAS OF ENDEMISM Geographical areas where maximal numbers of endemic species exist.

CETACEAN An aquatic mammal of the order Cetacea, including whales, porpoises and dolphins.

MITOCHONDRIAL D-LOOP The highly variable, non-coding, portion of the mammalian mitochondrial genome. A D-loop is the configuration found during DNA replication of chloroplast and mitochondrial chromosomes wherein the origin of replication is different on the two strands. The first structure formed is a displacement loop or D-loop.

close relatives are considered to have less cladistic diversity. The cladistic diversity approach allows for an objective estimation of a cladistic diversity index, which some have suggested can be useful in making conservation decisions[53–57].

Although the genetic analysis of species and populations is a popular goal of conservation biologists, more recently there has been a growing realization that the field needs to focus on area-based recommendations (FIG. 1). Species-based approaches will address issues that are relevant to the immediate success of a species, but the goal of conservation biology should be the long-term health of areas in which endangered organisms exist. Consequently, steps to preserve ecological or geographical areas that address these more confined species problems represent the most efficient long-term approaches to conservation. Of most relevance to such area-based recommendations is the use of genetic data to inform demography-based approaches to landscape ecology and to defining AREAS OF ENDEMISM[46,58–63]. In particular, methods that discover genetic or genealogical patterns at these levels can assist in the assessment of connectivity in reserve formation and of the potential genetic impact of introductions of non-endemic populations and reintroductions of endemics. The planning of marine[59] and botanical reserves[58] provides good examples of the incorporation of genetic information into a multidisciplinary approach to conservation management. In particular, genetic information can be used to detail the spatial and often temporal continuity of the allelic composition of populations, and can help the selection of areas that are crucial for healthy reserve systems.

Two examples of the usefulness of combining demographics and genetics concern whales. Whaling has caused many CETACEAN species in the north Atlantic to undergo severe population reductions. Roman and Palumbi[64] used MITOCHONDRIAL D-LOOP sequences and coalescence theory to estimate current and pre-whaling population sizes of three north Atlantic whale species (FIG. 2a). Their study indicates that for each of these species — the humpback whale (*Megaptera novaeangliae*), the fin whale (*Balaenoptera physalus*) and the minke whale (*Balaenoptera acutorostrata*) — population sizes in the prehistorical past were ~300,000 individuals. By contrast, current population sizes are a fraction of pre-whaling population sizes. These results have prompted the recommendation that whaling of these species is further curtailed, even though inferences from historical whaling records would suggest that the current populations are doing well enough to sustain some harvesting. The second study attempted to understand the evolution of life-history traits in humpback whales in the north Atlantic[65] (FIG. 2b). Conducted over a 16-year period, this study demonstrated the potential of combining a genealogical approach with studies of life-history traits. A life-history study of the reproductive success of female humpback whales among two genealogically distinct maternal lineages indicated the correlation of life-history traits with maternal genealogy. Knowing that the trend in reproductive success differs dramatically between these distinct lineages might be crucial information for the effective conservation of this species.

Genetic threats to endangered species

The number of practical applications of genetics that help manage endangered species is increasing. Below, we focus on three areas of modern conservation genetics to illustrate the unique problems that face conservationists.

The conservation biologist as matchmaker. The use of pedigree analyses and genetic data has been extremely important in helping to direct efforts to breed endangered species in captivity[15,66]. Although managers of zoos and aquariums have struggled with the challenges of maintaining viable *ex situ* populations for a long time, only in the past 20 years have genetics-based

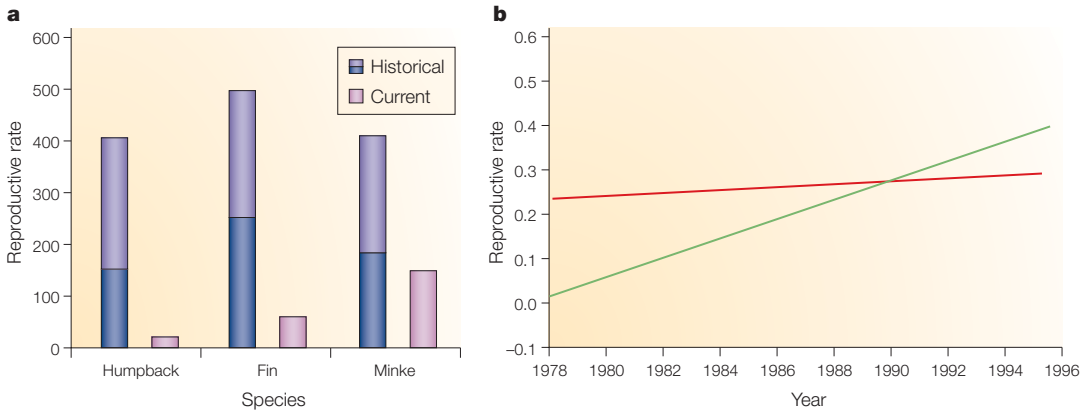
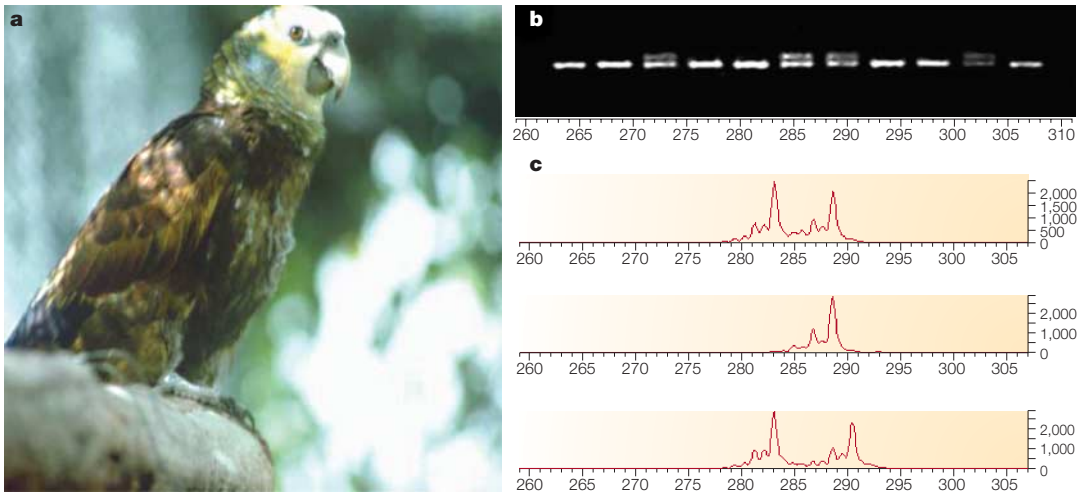


Figure 2 | **Cetacean conservation genetics. a** | The estimated number of north Atlantic humpback, fin, and minke whales in pre-whaling times is shown next to current census sizes for these species (confidence intervals are shown in purple). The current population sizes are a fraction of the pre-whaling population sizes. Modified with permission from REF. 64 © (2003) American Association for the Advancement of Science. **b** | Regression of reproductive success of female humpback whales, measured as the average fecundity among two genealogically distinct maternal lineages . The green line represents a clade of maternal haplotypes, termed the IJK lineage, and the red line represents a second lineage, BCD. Modified with permission from REF. 65 © (2002) American Genetic Association.

tools been incorporated into the current SPECIES-SURVIVAL PROGRAMS Intrapopulation and interpopulation assessment of genetic variation is particularly important for the management of single species and captive populations. Speke's gazelles provide a good example of the importance of considering genetic aspects of the biology of captive populations. The depleted genetic variability of this captive population highlighted the importance of genetic management and informed approaches to further breeding programmes for these animals[24].

The tools of population genetics have been used to assess levels of inbreeding[22–25], especially in *ex situ*



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	SVG080	SVG081	SVG093	SVG101	SVG108	SVG111	SVG116	SVG126	SVG127	SVG142	SVG151	SVG152
SVG052	0.3255	0.1967	–0.1170	–0.0891	–0.2330	0.3394	–0.2256	0.0538	0.1724	–0.1431	–0.3296	–0.1495
SVG054	0.0955	–0.1803	0.1220	–0.2551	–0.0198	–0.1597	0.1027	–0.1357	–0.1193	–0.0202	–0.1222	0.0995
SVG079	0.3174	–0.0602	0.0984	0.0558	–0.0820	0.4399	0.0826	0.2130	0.3098	0.0630	–0.0438	0.0774
SVG082	0.0087	–0.0631	–0.3314	0.3989	–0.2364	–0.1134	–0.3132	–0.2578	–0.3204	–0.0872	–0.1646	–0.3588
SVG084	–0.2546	–0.1454	–0.0658	–0.1324	0.2497	0.0846	0.1102	–0.1889	–0.0059	0.1827	0.2183	–0.0898
SVG087	0.4658	0.7122	–0.4084	–0.0413	–0.1770	0.2530	–0.3773	–0.2030	–0.2834	–0.3027	–0.3645	–0.4469
SVG097	–0.1979	–0.7000	0.6005	–0.1516	0.3467	–0.2819	0.6440	0.5538	0.6710	0.0244	0.4991	0.5880
SVG098	0.1400	–0.3506	0.4092	–0.2373	0.4082	–0.1285	0.2539	0.8992	0.7022	–0.3108	0.4514	0.5143
SVG103	–0.1527	0.0759	–0.2761	–0.0122	–0.0514	–0.2248	–0.2565	0.1389	–0.1646	–0.0554	0.0300	–0.0514
SVG112	–0.1692	–0.6774	0.3634	0.3024	–0.1712	–0.4547	0.4199	0.4485	0.4562	0.2092	0.2435	0.3477
SVG119	–0.1576	–0.6089	0.6537	–0.1208	0.4197	–0.1009	0.7913	0.4070	0.7080	0.1448	0.6408	0.5259
SVG135	–0.1490	–0.1311	–0.3365	0.2012	–0.1522	–0.2016	–0.3166	–0.2574	–0.3240	–0.2538	–0.1585	–0.3661
SVG139	0.2058	–0.2149	–0.2539	–0.3975	0.0674	0.0650	–0.2369	0.1516	–0.0434	–0.1539	0.2343	–0.1616
SVG141	–0.1377	–0.5502	0.0730	0.0640	0.1216	–0.3014	0.1531	–0.0749	0.0281	0.3269	0.3433	0.2617
SVG148	0.2493	–0.3651	0.0833	0.1569	–0.1311	–0.2862	0.0686	0.5565	0.2124	–0.1419	0.1864	0.0616
SVG149	0.2333	–0.1717	0.0826	0.0729	–0.1133	0.3277	0.2385	0.1096	0.2805	0.2235	0.0279	0.0630
SVG150	0.2584	0.1431	–0.1628	0.0604	–0.2652	–0.1095	–0.1557	0.3038	0.0205	–0.0763	–0.1741	–0.0749

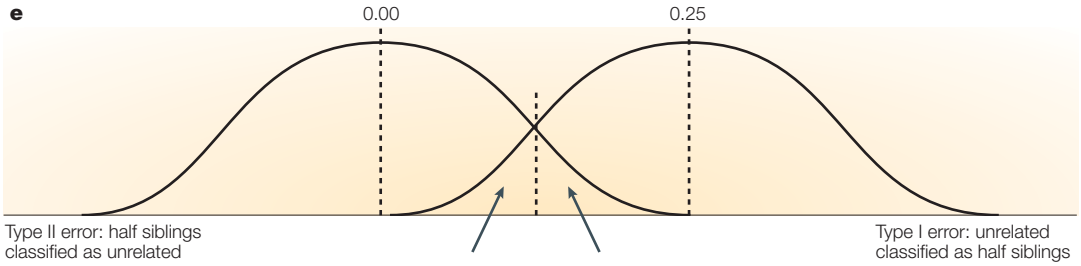

Figure 3 | ***Ex situ* conservation genetics: the Amazon parrot example.** A PCR-based sexing technique and relative relatedness measures calculated from microsatellite data allowed the design of a scientifically managed breeding programme for the *ex situ* population of *Amazona guildingii* (**a**), maintained by the Forestry Department on Saint Vincent. The first, most obvious, but previously difficult step in the process is to non-invasively identify the gender of all birds in the potential breeding population. A rapid and simple PCR test is available for this[35,36]: a single band indicates a male and a doublet indicates a female (**b**). In this figure, there are four females and seven males. The second step is to characterize variability using microsatellites at a number of loci[35] (an example of two heterozygotes (top and bottom) and a homozygote (middle) at one microsatellite locus is shown in part **c** of the figure. The third step (**d**) is to construct a pairwise genetic-relatedness table that indicates the degree of relatedness. White boxes highlight genetic relatedness values that indicate immediate kinship or half-sibship; light-grey boxes indicate an intermediate level of relatedness that is not discernable from half-sibship; dark-grey boxes indicate that individuals are 'unrelated'. The final graph (**e**) shows randomly generated distributions following two different models of calculating relative relatedness. The cutoff values indicated by the arrows are half way between the means of these two randomly generated distributions. In order to avoid errors in the classification of relationships, individuals from the areas indicated by the arrows were excluded. The data illustrated are from REF. 35.

SPECIES-SURVIVAL PROGRAMS Programmes established by the American Zoo and Aquarium Association to ensure the survival of selected wild or captive species through cooperative genetic and demographic management.

populations and in devising methods to avoid inbreeding depression. In addition, population geneticists who offer assistance to conservation biology pointed out that small population sizes (both captive and natural) tend to reduce genetic variation, and might therefore lead to a decreased ability of such populations to adapt to ecological challenges. It was later pointed out that outbreeding or the direct mixing of individuals from genetically distinct populations could also be deleterious to the genetic health of an endangered species[22,67]. Perhaps the most important outcomes of these approaches were the establishment of scientifically managed breeding programmes[68] and the use of population-genetics parameters to estimate minimum viable population sizes using PVA[68–71].

Recent work on Amazon parrots demonstrates many of the complications of captive-population matchmaking[35,36]. Several genetic analysis techniques were extended to develop a comprehensive breeding plan for this highly endangered species, which is restricted to fragmented oceanic tropical forest on the island of Saint Vincent in the eastern Caribbean. Among other threats, this species is vulnerable to stochastic events such as hurricanes. An *ex situ* population of approximately 100 birds was under the custodial care of the Saint Vincent forestry department as part of an amnesty programme on the island. Genetic relationships, and even gender, were unknown for most of the captive individuals. To optimize the breeding of these parrots, researchers determined the gender of all captive birds using a W-chromosome-specific PCR assay and genotyped microsatellite loci to determine the relatedness of all captive birds. Relative-relatedness values were established, allowing for a scientifically managed programme using empirical genetic data, rather than the assumed unrelatedness of the founders (FIG. 3).

Conservation matchmaking is also relevant to wild populations. Matchmaking in this context involves assessing the genetic health and integrity of an endangered population or species. Hybridization can sometimes occur between two populations or species that are conservation targets. Understanding whether hybridization is a natural phenomenon or is due to anthropogenic factors is important in developing conservation strategies. Two examples of hybridization events caused by humans that currently threaten species with extinction are the cases of Simien wolf/domestic dog hybrids in Ethiopia and Cuban crocodile/American crocodile hybrids in Cuba. Both cases involve rare, highly restricted endemic species. An example of natural hybridization that complicates conservation is the case of the red wolf (see also below). The red wolf was thought to be an endangered, unique taxon, and is the focus of a United States Fish and Wildlife Service species-recovery programme. It has now been shown that 'red wolves' are the descendents of a natural wolf/coyote hybrid zone that occurred in the south-eastern United States[72–74]. Now that coyotes have naturally recolonized the eastern part of the United States, they are interbreeding with the reintroduced red wolves, and there is no consensus in the conservation community as to what should be done.

Uninformed population genetics and 'bad systematics' can lead to species endangerment. A classic 'bad taxonomy' problem in conservation genetics concerns the tuatara, and the taxonomic grouping together of species of these unique New Zealand reptiles[75]. Tuataras look superficially like lizards, and are the sole surviving members of an ancient order of reptiles. For this reason, they are a high priority for conservation. However, before genetic analyses of these reptiles, tuataras had been mistakenly grouped into a single species. Some of the various demonstrable tuatara species units had small, geographically limited, populations so the failure to recognize their uniqueness and put effort into conserving them led to their endangerment and, in at least one case, extinction.

Another potential example of 'bad systematics' concerns the Russian sturgeon (*Acipenser gueldenstaedtii*). A DNA-based diagnostic test has been developed for the caviar that is produced by this fish (osetra caviar) and those produced by all of its close relatives[76,77]. In essence, these DNA-based diagnostics can serve as barcodes for the 24 species of fish in the family Acipenseridae. One of the criteria for including species in the CONVENTION ON IMPORTATION AND TRADE OF ENDANGERED SPECIES (CITES) 'red book' list of endangered species is that commercial products obtained from that species must be identified. As the caviar produced by the fish in this group can be identified as coming from a particular species using a molecular diagnostic approach, all species in this family were listed on the CITES red book list[78]. The other two commercial caviar-producing species in this group of sturgeons (*Acipenser stellatus* and *Huso huso*, which produce sevruga and beluga caviar, respectively) are easily diagnosed using DNA sequences, and the diagnosis of commercial products derived from these two species is not problematic. However, the recognized DNA-based diagnostic test is not totally reliable for *A. gueldenstaedtii* because a cryptic species might be present in the areas where caviar is harvested from this fish[79]. By examining diagnostic SNPs in commercial caviar, it was discovered that some commercial products from Russia that are labelled as osetra are being typed as a closely related lineage of the Siberian sturgeon (*Acipenser baerii*). Batches of osetra caviar that apparently originate from *A. baerii* are confiscated and the distributor is prosecuted for a violation of CITES regulations. The current DNA tests that are used to monitor the importation of commercial caviar might not be sensitive enough to detect the species origin of much of the osetra caviar that is exported from Russia. In this case, the Russian fishermen who rely on sturgeon fishing for a livelihood are at a disadvantage as a result of potential 'bad systematics'.

The delineation of conservation units is the main problem that underlies the two examples given above. Several unit definitions have been coined, the most prominent of which are the ESU and the MANAGEMENT UNIT (MU), both of which have been very useful in deciding on conservation priorities. Other units at or around this boundary are SEMISPECIES, INCIPIENT SPECIES and subspecies. The subspecies designation is often given

CONVENTION ON IMPORTATION AND TRADE OF ENDANGERED SPECIES OF WILD FLORA AND FAUNA
(CITES). A convention first started in 1973 and established amongst participating nations to restrict the international commerce of plant and animal species harmed by international trade.

MANAGEMENT UNIT
(MU). A population, stock or group of stocks of a species that are aggregated for the purposes of achieving a desired conservation objective.

SEMISPECIES
An emerging species in the early stages of speciation.

some formal taxonomic status. However, in conservation terms, we consider the determination of ESUs and MUs to be of paramount importance. To further complicate matters, hybrid populations or hybrid individuals have also been targets of conservation geneticists. Hybridization complicates the delineation and identification of distinct evolutionary units in conservation genetics. In addition, the ENDANGERED SPECIES ACT (ESA) does not protect hybrids between recognized endangered species. Good examples of these complications are provided by the cases of grey and red wolf hybrids[72,73,80]. The definition and study of different conservation units is also important so that the demography and genetics of source populations for reintroductions can be matched with those of the unit that was previously present in the reintroduction area. For example, the failure to consider how closely Texas panthers matched panthers that were previously present in Florida has led to the decreased fitness of a reintroduced population that was derived from this source. Another clearer and more extreme example of the impact of this approach comes from the lakeside daisy[81], for which a lack of diversity at a self-incompatibility locus has prevented sexual reproduction in the last remaining population of this plant in Illinois.

Forensics and 'dead' DNA. The final expanding area of conservation genetics that we discuss here concerns the ability to use unconventional sources of tissue for conservation work. Conservation biology, by definition, should be non-invasive. However, one of the main problems of carrying out conservation genetics at the level of DNA sequence is that non-invasiveness is hard to achieve when tissues (biopsies and necropsies of animals and plant tissues) are needed for genetic analysis. The use of PCR has opened doors to the analysis from a genetic perspective of many conservation problems that would have otherwise remained closed because of the requirement for conservation work to be non-invasive.

Currently, a wide range of unconventional sources of DNA are used in conservation work, including faeces, feathers, fur, sloughed-off skin and plants from herbarium sheets. Many studies use museum specimens and specimens of extinct organisms to detail the past genetics of endangered populations and species[82,83]. These studies range from using 100-year-old museum specimens (for example, specimens of whales[84], beetles[85], prairie chickens[86,87], muntjac[88], birds[89,90,91], fish[92], several endangered carnivores[93,94] and plants[95]) to using specimens of long-extinct organisms that are thousands of years old (for example, specimens of bears[96], moas[97] and sloths[98,99]).

Despite this increasingly ingenious plethora of sources of low- (but adequate) quality tissue samples, the collection of high-quality tissue samples from endangered species remains important. There are concerted efforts to store and archive high-quality samples from endangered species to preserve the genetic resources of these species, and possibly even to clone them at some future stage. For almost a century, botanists have stored plant seeds, and the continued attention to seed banks is also an important aspect of modern conservation. We now know that whole-organism cloning from extinct animal tissues is possible[100], so for this reason, high-quality freezing of tissues from endangered animals seems to be a worthwhile activity. Nonetheless, there are still significant problems with the viability of cloned animals. Moreover, the current endangered populations and the areas in which they live should be the foci of our conservation efforts. So, freezing gametes and tissues for the later regeneration of endangered species is certainly not the most important or efficient current approach to their management and recovery.

Another potential expansion of conservation genetics involves recent efforts to develop centralized DNA barcodes[101,102] and DNA registries. Barcodes in everyday life are 'unique identifiers' of commercial products. Commercial barcodes are placed on products, and when they are needed they are identified using barcode readers. Simply put, DNA barcoding makes use of specific DNA sequences as unique identifiers of species. The dynamics of DNA-sequence change has led some researchers to suggest that short DNA sequences can be used as a source of information to obtain unique identifiers (barcodes) for organisms. DNA barcodes are developed by sequencing specific genes — such as cytochrome oxidase 1 for animals and internal transcribed spacer 2 of the small subunit ribosomal RNA gene (*ITS2*) for plants — for a reasonable number of individuals from a species. There are several ways that barcodes can then be 'read' once these sequences have been obtained; multivariate statistical analysis[101], tree building[103] and population-aggregation analysis[42] are the most widely used so far.

Both excitement about[101,102,104] and criticism of[105–107] DNA barcoding have arisen in the scientific community. Criticism of the initiative has come mostly from classical taxonomists, with lively debate ensuing from both perspectives. We suggest that any source of character information that can be shown to be a unique identifier of a species should be considered as a potential barcode. Molecular techniques — such as those that use RFLPs, AFLPs, RAPDs, microsatellites, gene order, gene presence or absence and SNPs — would be appropriate methods for discovering unique identifiers for DNA barcoding. DNA barcoding can also be complemented and enriched using character information from other sources, such as morphological or allozyme approaches. Several initiatives towards barcoding specific groups have already been launched. Cetaceans[103] and bacteria (see the link to the Bacterial Nomenclature Up-to-Date web site in the online links box) are two prominent groups that are currently being studied using barcoding methods.

Individual barcoding in the form of DNA 'fingerprints'(obtained using sequences or microsatellite profiles) can also be used in populations of endangered species. Whales in particular have been an important focus of this approach[106]; samples are taken non-invasively from individuals in natural populations and a

INCIPIENT SPECIES
A newly formed species pair.

ENDANGERED SPECIES ACT (ESA). A US congressional act established in 1973 that articulates guidelines and rules for the protection of species on the brink of extinction.

suitable number of informative microsatellite probes is used to generate a genetic profile for these individuals. The genetic profile is then stored in a database for later reference and for matching to either living whales or to whale tissue that has been harvested or is being sold.

DNA barcodes and DNA registries are useful for two main purposes in conservation biology: for the identification of illegally imported animal or plant products (wildlife forensics)[108] and for the rapid assessment of biodiversity studies. DNA barcodes could speed up and make more precise the identification of specimens in biodiversity studies that are currently tedious and time-consuming.

The context-dependency of conservation genetics

The goals of conservation biology are highly context-dependent. Much thought concerning the specific goals of genetic and ecological analysis in conservation biology has been couched in terms of NATURALNESS and NATURAL IMPERATIVES[109]. These concepts are important in assessing what genetic, demographic and ecological information can offer to decision-making. An important consideration is that genetic and demographic information represents only a snapshot in time. Such a snapshot is only useful if a frame of reference is available. This frame of reference in conservation, placed in the context of naturalness, would result in establishing 'benchmarks'[110,111] for conditions at various times in the past as natural states for those periods. But which benchmark is the most appropriate to adopt in making conservation decisions — pre-human, pre-human-civilization, pre-Columbian (for North and South American initiatives), pre-industrialization or another benchmark? Establishing genetic benchmarks at these various stages is difficult, but not impossible, and has been accomplished in several cases[84,85,96]. An important role of genetics will be to establish current population genetics and systematics profiles for endangered species, with an eye on this information as a benchmark for conservation work in the future.

Future challenges

Many challenges still face conservation genetics. They include integrating genetic information with other biological and non-biological factors and, perhaps more importantly, using the results of conservation-genetics studies to implement successful conservation strategies. An understanding of landscape dynamics and area-based conservation will be an important part of the framework that is required for the successful use of genetic data to help make conservation decisions concerning areas, landscapes and species. We suggest that collections of organisms such as those in museums, herbaria, zoological parks, botanical gardens and the like will have an essential role in the future, in particular for the establishment of baselines for genetic comparisons. Such studies have already been extremely useful in deciphering the effects of land use, species protection and conservation efforts as well as the negative effects of the human use of biotic resources.

The next generation of collections that are relevant to conservation might be even more important than the museums and herbaria of today. We should seize the opportunity that we now have to implement the storage of high-quality genetic resources for a large number of endangered species[112], if only for the sole reason that this will enable us to monitor the effects of our current conservation efforts. In this context, it is important that we rethink current restrictions on the collection and storage of information regarding biodiversity, an issue that is complicated by national and international legal issues.

There is a great need to increase the ability of conservation geneticists to non-invasively collect, archive and use genetic resources for as broad a range of biodiversity as possible. This increased effort would entail a reconsideration of the current restrictions that conservation scientists and policy makers must conform to, such as CITES, ESA and CONVENTION OF THE PARTIES regulations. Because conservation biology is a crisis discipline, rapid assessment of biodiversity will become increasingly important in modern conservation decision-making. Methods for the rapid detection and assessment of biodiversity are essential. It seems to us that DNA-identification methods such as barcoding initiatives are an efficient and potentially effective way of assessing the biodiversity of complex communities. Genetic and genomic approaches, such as DNA barcoding and high-throughput population censuses, will enhance this aspect of modern conservation biology. We do not suggest that these high-throughput methods should replace classical taxonomy, but rather that the DNA-based methods will augment the rapid and automated identification and assessment of biodiversity. Another essential area for expansion is in the development of genetically focused analytical tools, such as quantitative genetic approaches[113] and Bayesian methods[114], for use in making important conservation decisions.

Most important is to recognize that conservation decisions depend on a number of factors that go beyond the scientific. So, one of the major challenges for modern-day conservation genetics is the more efficient and better-defined incorporation of genetics into conservation decision-making in the context of the complexities of social, cultural and political issues. Some conservationists have cogently argued that scientific issues should not be confused with social and legal issues[115] and that scientific terminology and inferences in conservation decisions (especially landscape management) should be explicit about the scientific content that is used in decision-making. The integration of scientific terminology and the scientific process into conservation decision-making should only be made when the implications of such use are clearly articulated and the impacts precisely assessed. The future of conservation biology will clearly need a broader theory for handling genetic information from population genetics and systematics, and the interface with ecology. Such a formalized theory would allow a more rationale and consistent approach to conservation decision-making at all levels.

NATURAL; NATURALNESS
A thing is natural if it is not made by humans. Naturalness is the degree to which something is natural.

NATURAL IMPERATIVES
An essential and urgent duty to restore some aspect of the environment to its 'natural' state.

CONVENTION OF THE PARTIES
Also known as the Conference of the Parties to the Convention on Biological Diversity. An international organization dedicated to fostering conservation, sustainable use and equitable benefit-sharing concerning biological diversity.

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Competing interests statement

The authors declare no competing financial interests.

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