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# Phylogenetics for Wildlife Conservation

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## Abstract

Recent extinctions and the continuing threats to the survival of rare species will make conservation biology crucial in the twenty-first century. Conservation genetics for wildlife is an emerging challenge for humanity because it is accepted that a number of species and its populations are under oppression by a huge human expansion. Conservation genetics is the science that aims to minimize the risk of extinction. The International Union for Conservation of Nature and Natural Resources (IUCN) recognizes three hierarchical levels to conserve biodiversity: genetic diversity (populations), species (taxon ascertainment), and ecosystems (living organisms and their interactions). In view of the world's imminent biodiversity crisis, the risk of extinction at several biotic levels is nowadays unavoidable and requires urgent action. One prime conservation goal is focusing on preserving the genetic variation. The main reasons are: (1) to preserve a representation of past evolution and (2) to maintain raw material for future evolution, favoring the balance of ecosystems. Having these aims in mind, a new approach utilizes different metrics, such as phylogenetic diversity, split distance, and heightened evolutionary distinctiveness, which are being considered for immediate practical use to manage threat species and stocks submitted to new policies for conservation.

**Keywords:** distinctiveness metrics, extinction risk, genetics wildlife management, phylogenetic and conservation, species diversity

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## 1. Introduction

*"In the face of inevitable future losses to biodiversity, ranking species by conservation priority seems more than prudent. Setting conservation priorities within species (i.e., at the population level) may be critical as species ranges become fragmented and connectivity declines."* [1]

Ever since the revolutionary ideas put forward by Darwin, the evolutionary perspective of wildlife has played a fundamental role and has aimed to the efficient protection and preservation of

biological diversity, which started off with an adequate, accurate or, at least, the best approached inventory of its current status. But the recent extinction or continuing threats to the disappearance of many species and populations has made conservation biology essential in the twenty-first century. The primary forces concerned with its long-term persistence of wildlife populations, may be ecological, political, economic, or other. Nowadays, these forces (or factors) use more objective genetics principles and related applications for conservation. In particular, the application of new molecular techniques, widely used in conservation research, has made genetics examination of endangered species feasible. Conservation genetics for wildlife is an emerging challenge for humanity because it is generally accepted that the extinction of present species, even some of its populations, were caused by the huge expansion of a sole species, the man (*Homo sapiens*). So, the number of studies based on genetic data aimed at understanding biological diversity patterns and processes has increased in recent years, partially, because biodiversity assessments made using species counts (e.g., total, endemic, threatened) may not be the most suitable metrics. In consequence, a more reliable approach has been proposed to improve the situation. On the one hand, using genetic data and phylogenetic analysis to adequately represent the processes that gave rise to the observed patterns of diversity and, on the other hand, allowing conservation efforts to apply not only to threatened species, but also to other particularly interesting populations. The metrics to be employed is yet under debate and an agreement needs to be reached.

As we said above, conservation genetics is the science that aims to minimize the risk of extinction from genetic factors [2]. Conservation genetics has flourished over the last 20 years and has shown that there are many ways genetic knowledge can help to conserve biodiversity, ranging from identifying the concerned populations to resolving taxonomic uncertainties, or understanding the biology of a focal taxon. The International Union for Conservation of Nature and Natural Resources (IUCN) is also focused on these ideas and recognizes three hierarchical levels to conserve biodiversity: genetic diversity (populations), species (taxon ascertainment), and ecosystems (living organisms and their interactions).

Although it is reasoned that endangered species have deserved a noteworthy attention on conservation research [3], less concerned species are also of research and sometimes conservation interest (e.g., European red deer [4, 5]). So, every species are important especially the distribution of their particular isolated populations when they are genetically distinct, although by not well known reasons. In this last case (including minor concern species), it reaches relevant importance to those inferior levels of taxonomic arrangement as subspecies, an historical nomination concept that is being replaced by evolutionary significant units (ESU), management units (MUs), and distinct population segments (DPS). In this way of thinking, the intra-specific diversity is officially recognized as one of three levels of biodiversity. This level of diversity, coupled with ecosystems and whole genetic diversity is worthy of protection [6] but often require more adequate information [7] about concerned species, ESUs, MU, or DPS [8].

In view of the world's imminent biodiversity crisis, referred to, by some people as the 'sixth mass extinction' but different from the five previous ones, "the next extinctions will be due to human impact", which are now unavoidable and need urgent actions to prevent it. Nowadays, optimistic scenarios predict significant changes in biodiversity around 2100, with most of the loss starting with isolated populations of whichever wild species.

A large portion of the conservation genetics is dealing with the genetic conclusions about the causes and consequences of isolated small populations characterized by its low effective populations size ( $N_e$ ), and simultaneously, the genetic drift effect because it causes a random change from generation to generation of gene pool. Whichever the case, they are both relevant issues associated to inbreeding under no random mating. The long-term effect of inbreeding leads to loss of genetic variability until reduced adaptability and ecosystem function, too [9].

Below the species level, it has been advocated the identification of populations that deserve long-term conservation or are derived from a recent rank fragmentation [10–12]. Although populations' relationships are being represented by bifurcating trees, it is known that bifurcating trees often fail to show everything and/or complex relationships, a major shortcoming if populations do need to be prioritized for conservation [1]. In this way of thinking, several studies have shown how measuring and maximizing phylogenetic diversity can be performed using phylogenetic networks and evolutionary isolation indices adapted for populations within species ([1, references therein]). The new approach utilizes different metrics, like phylogenetic diversity (PD), split distance (SD) [13], or Shapley metric (SH) [14], and heightened evolutionary distinctiveness (HED) (refined by [15]) to assess not only from the species level, but also to population differentiations within each other. These metric might be of immediate practical use to manage discrete populations within species with several degrees of threat and stocks submitted to new policies for conservation triage [16].

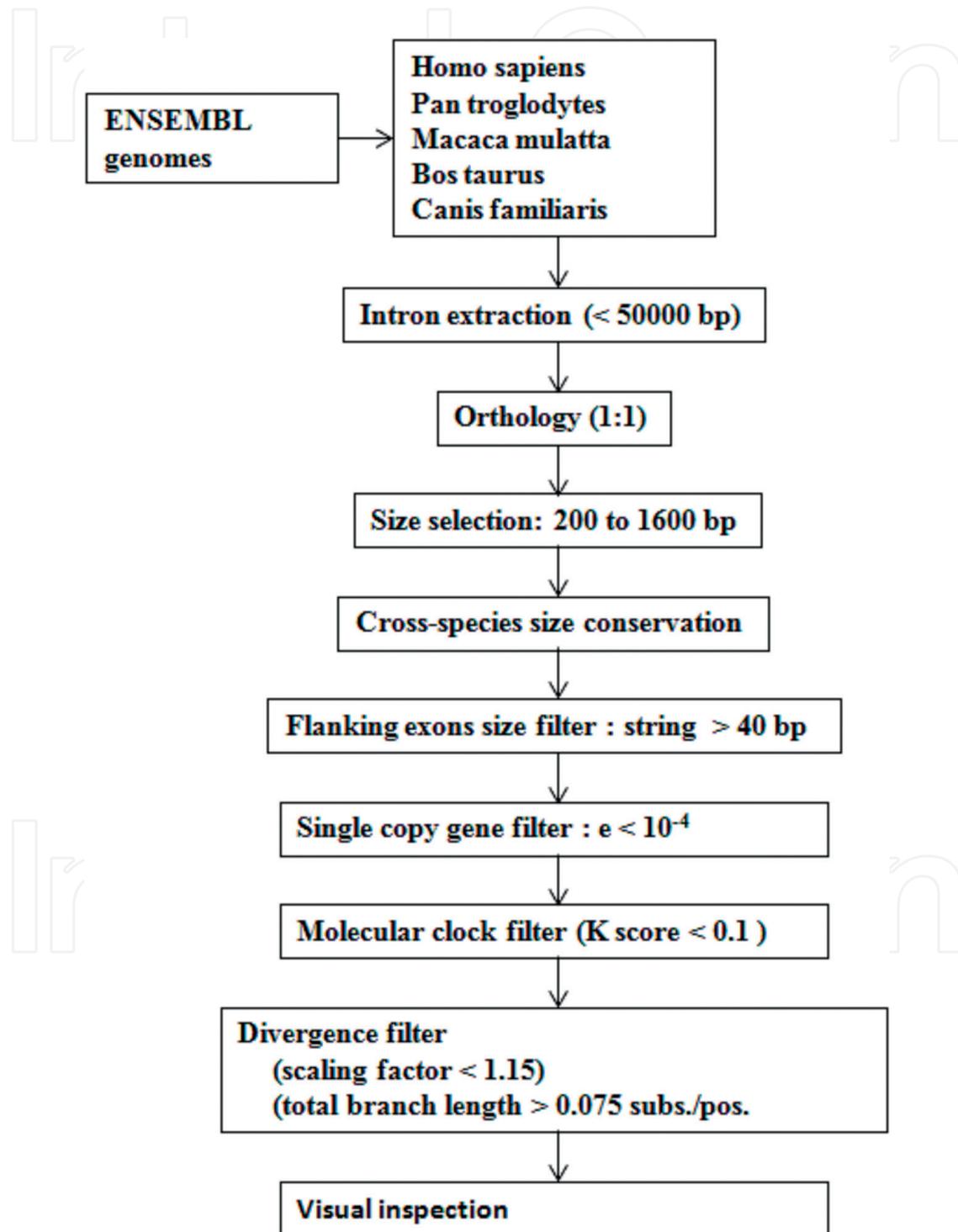
## 2. The phylogenetic context

The concepts of taxonomy are familiar for every biologist because they have spent a long time studying species names and retrospectively their order into genus, family, ..., kingdoms. Such a classification recalls a scenario like ancestor-descendent relationships among taxa (phylogeny), which result in a scheme describing an evolutionary relationship that could not be subject to critical analysis. Recently, modern phylogenetic science captures, as empirically as possible, the relatedness among similar taxa using the most orderly manner for mapping the path of evolution that leads to and represents the true ancestry relating the upstream organisms. The resultant classification must be reasonably and objectively assumed by worldwide biologists, undoubtedly. In this way, groups of species or its populations are essentially related by a set of both, morphological and molecular characteristics but, more importantly yet, these should be matched by properties such as its ecological abilities.

Firstly, phylogenetic studies have been proven to be of utility, of course, but in a research-oriented framework. In this way, a simple data research can provide guidelines to find gaps and strengthen interpretations to ensure management affirmations. So, multi-locus phylogenies can be used to infer the species tree whose nodes represent the actual separation between species, thus providing essential information about their evolutionary history or helping analyzes of species delimitation, gene flow, and genetic differentiation within species [17]. As an example, now adequate markers are available by extracting intron information from genomes of human, chimpanzee, macaque, cow, and dog (three mammalian orders) searching for the

ENSEMBL database. This analysis led to a final list of 224 intron markers randomly distributed along the genome for six mammal species, which can be useful to gather genetic markers with unambiguous phylogenetic signals (see [17] for details and design) (**Figure 1**).

Secondly, the use of phylogenetic diversity is of current interest in view of its objective metrics for conservation in evolution history (the past), genetic status of species (the present), and



**Figure 1.** Steps for intron extractions and filtering processes. Adapted from [17].

management for conservation in geographically split species (the future). The first two may be of general interest on research, but within a practical approach the last issue is of plentiful applicability to wildlife population management. The phylogenetic ramifications reflect more than simple systematic classifications. The molecular information and its association with other kinds of data can be an objective measure to identify species or population groups with different or similar vital aptitude such as habitat use among taxa or similar facts. A straightforward example has been pointed out in the case of strong associations between habitats and morphology in shorebirds, ducks, and other water bird species. However, supposedly described subspecies differentiation (e.g., the specimens of the whole geographic Iberian range was pooled as a single genetic population instead of delimiting them as lineage clusters) based on morphological information has been seen to fail, probably due to the mixing of genetic lineages. After a molecular survey of the Iberian desman (*Galemys pyrenaicus*), the data set suggested two main phylogenetic clusters delimited by mitochondrial DNA (**Figure 2**) in this emblematic species. Because of a strong geographic splitting in type localities of this species and the absence of clear morphological discrimination with nowadays data, its populations may easily be regrouped in two big clades that would correspond to two nominal subspecies *Galemys pyrenaicus rufulus* (clade A) and *Galemys pyrenaicus pyrenaicus* (clade B) [18]. Consequently, it has recently been suggested to treat these outstanding lineages as separated groups in the wildlife management contexts.

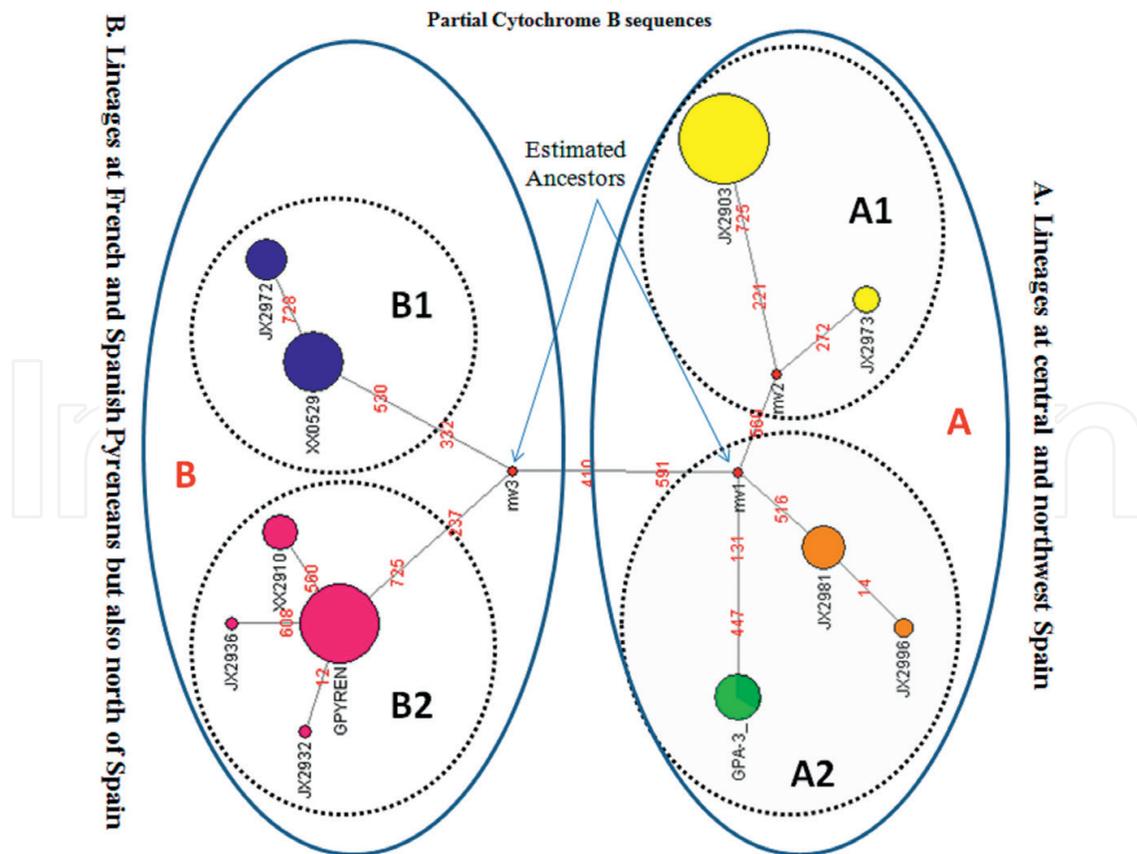
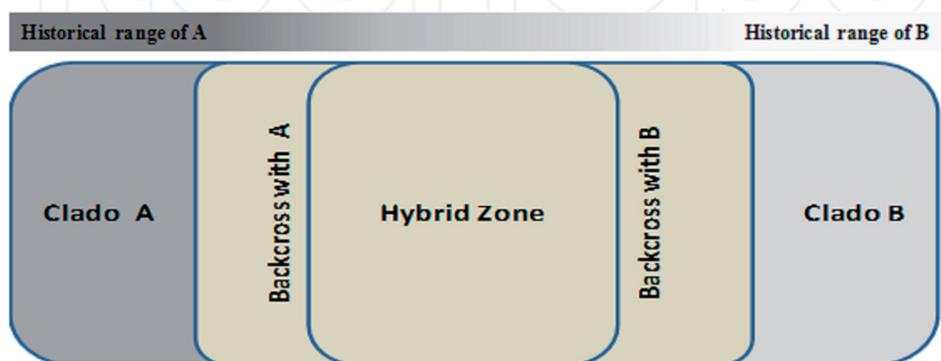


Figure 2. Main lineages in *Galemys pyrenaicus*. Adapted from [18].

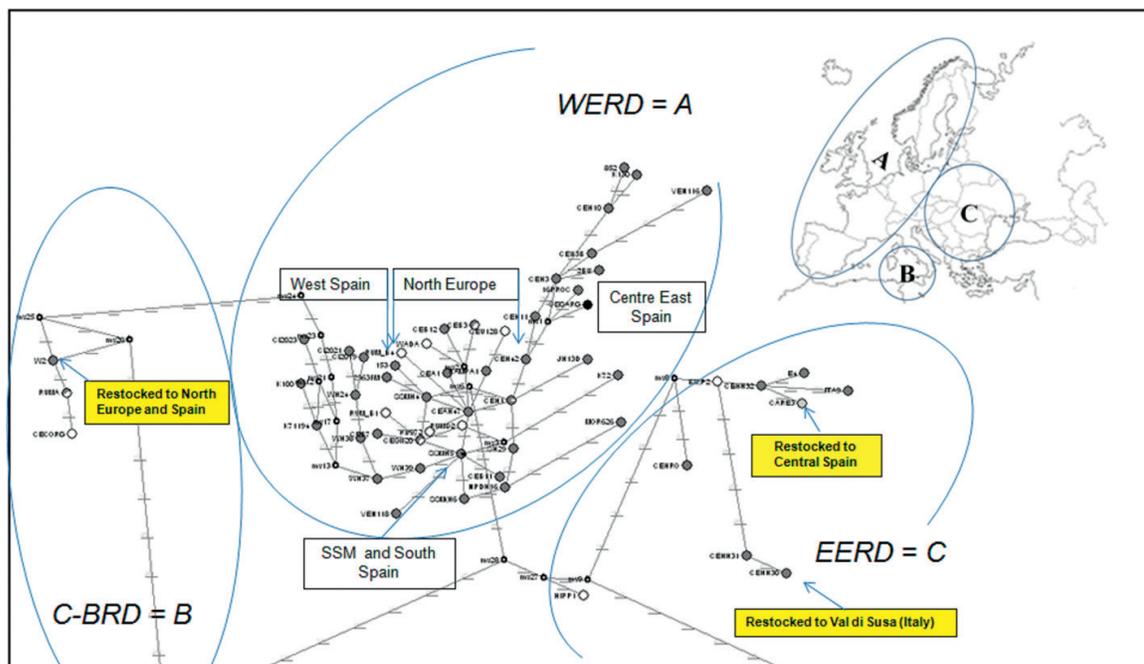
Thirdly, however, is the issue of hybridization: a cause for debate. Hybridizations have occurred for long and they are well known by managers and scientists around the world. The main question about hybridization is which, the species or its hybrid, should be prioritized and valued. The concept of hybridization understood to mean mating between different species has been extended to mating between two genetically distinct populations that produce offspring (F1 to several backcross; **Figure 3**), regardless of its fertility.

Two competing effects of such introgression are assumed but with different final results on species diversity: (1) a **negative view** is a feeling of concern when human activity is the main cause of the introgression [19] and (2) a **positive view** is when nature is the main responsible of admixture among populations but with a long-term component [20] because, at present, man intervention is in everything, so, consequently, the first view is the one that is considered of most concern.

One well-studied example about the negative effect of human impact on hybridization in wildlife in nonthreatened species is the European red deer. During the last century (past and currently also), there has been an extensive arbitrary trading of European red deer aimed at breeding improved trophies for hunting on extinct or nearly extinct autochthonous populations [21]. The direct consequence of the restocking and the action of introducing genetically-distinct populations has had various types of negative effects. On the one hand, hybridization with introduced animals has impaired the phylogenetic boundaries between former and natural populations, contributing to blurring true genetic history and confounding future researches. Worldwide allochthonous and indigenous red deer have been admixed (and are) through several Europe countries. It is believed that the scarce documentation about this fact is opposite to the true dimension of human impact, which should have been huge instead. Because of a generalized worldwide impact of anthropic action, a mixture of phylogenetic scenarios would probably be expected (**Figure 4**). Accordingly, though genetic variation is supposedly structured hierarchically, some exceptions occurred under hybridization associated to human activity. To overcome this drawback, an effective sampling strategy according to the specific problem should be design based on knowledge. In the European red deer example, due to the arbitrariness of admixture, these scenarios caused different effects. One of them may be the presence of mixture allochthonous lineages as in Val di Susa (Italy) being genetically similar to Bulgarian red deer. Although the origin of Val di Susa red deer was



**Figure 3.** The most probable distribution of hybrid and their backcross in a natural framework of admixture.



**Figure 4.** Phylogeography scenario of European red deer lineages. A = “Western European red deer” lineages, B = C-BRD “Corsican and Barbary red deer” lineages, and C = ERRD “Eastern European red deer” lineages. Map showing natural geography distribution of lineages. Network showed some restocked lineages into different areas of Spain and Europe (yellow quadrate).

Slovenia and Bulgaria; only Bulgarian blood survived, probably attributed to genetic drift. But in this case, the population could be easily qualified as allochthonous. On the other hand, outbreeding depression of the hybrid offspring due to lower reproductive success or survival of either parent has also been found. In our example, translocation of Wapitis and Asian red deer (today regarded as different species from European red deer) was unsuccessful by far, as a way to result in antler-size improvement. This failure was partially due to the **lack of adaptation** to local environmental factors or high susceptibility to local diseases. This example suggested outbreeding depression in hybrid populations [21]. Two mechanisms have been proposed for the outbreeding depression. An **intrinsic mechanism** upholds a reduced fitness of hybrids due to interactions between genes originating in different evolutionary taxa. Conversely, **extrinsic mechanisms** advocated for loss of adaptation to local environment with unsuccessful reproduction. Also, the interaction genotype-environment may be assessed.

Moreover, hybridized populations or species may consist on a hybrids swarm in which all individuals are to various degrees of admixture. In this respect, an important role in transferring or restocking species or populations to the wild is being played by enclosures (in zoos or collections), which serve as reservoirs of different populations and subspecies. Sometimes, these reservoirs have acted as the origin of feral populations of many different exotic species and subspecies contaminating autochthonous stocks. This was the case of the Woburn red deer from Bedfordshire [21] or the Mesopotamian fallow deer at the Opel Zoo [22]. In the latter case, phylogenetic studies can be used to assay the presence of hybridization in the Persian fallow deer from the Israeli Reintroduction Program started in 1996 and thus dispel all doubts.

The positive side of hybridization is more related to speciation. Hybridization occurs more frequently than previously recognized and is an important source of speciation. Hybridization leading to a new taxon, distinct from both parent species, is called (when homoploid) hybrid speciation or recombinational speciation [23]. Almost 50% of plant species originated from the hybridization of different species. For example, 10% of bird species are believed to hybridize with another species naturally. This sort of speciation promoted adaptive divergence and increased reproductive isolation. But introgressed genetic variation can also enhance the ability to coexist and promote invasiveness [24] enlarging the range of a hybrid populations. Moreover, a positive feedback between hybridization and speciation may exist [25]. So, hybridization may increase (1) the rate of speciation, (2) diversity of closely related species, and (3) adaptive radiation by incorporation into populations of selectively favored alleles or combinations of them; providing the basis for adaptive evolution and having important implications for the origin of new species.

The frequency of hybridization as a source of adaptive variation for speciation may be summarized as follows: firstly, hybridization among species occur about 10–30% of multicellular species regularly on a per-species basis but less frequently on a per-individual basis, the latter more frequently driven by humans (as the case of *Dama dama mesopotamica* described in [22]). Secondly, mutations are rare, around  $10^{-8}$  to  $10^{-9}$  per generation per base pair, that is, a considerable time for novel adaptations to appear but depending also on the population size. So, hybridization among species can act as a source of adaptive genetic variation rather than mutation [26–30]. For example, ‘New additive genetic variance introduced by hybridization in Darwin's finches, which has been estimated to be two to three orders of magnitude greater than that introduced by mutation’ [26], despite initial hybridization itself, which is unlikely to be adaptive because there is often evidence of selected against. Last but not least, adaptation is thought to be the most important process driving divergence during speciation [31–33] and divergence in ecology occurs almost exclusively under selection. Moreover, closely related species tend to hybridize more often. Species in rapidly diversifying adaptive radiations could especially be prone to hybridization [25, 34, 35].

### 3. Conservation genetics

Conservation genetics was born in the last third of the twentieth century integrating empirical and theoretical studies based on population genetic data, which were incorporated to the Conservation Biology doctrine giving rise to the discipline “Conservation Genetics” with a spectacular growth. The conceptual framework included all “genetics” issues that are phylogenetic, quantitative, evolutionary, ecological, and population genetics themes.

Nowadays, conservation genetics is being applied for practical conservation and wildlife management as a major paradigm. At first, the conservation of species was evaluated by indirect and phenotypic data but powerful advances on DNA technology resulted in a huge amount of genetic data more easily achieved, and also helped by an emerging sophisticated statistical procedures. Now, it is possible to gather the objective information coded long ago into genomes of every organism. Thereafter, the conservation genetic discipline raised its interest

when people became aware of the growing rate of human population and its unavoidable effect on planet biodiversity. The IUCN (World Conservation Union, formerly International Union for Conservation of Nature diversity either ecosystems or species) recognized three main levels worthy of protection and conservation: genetic diversity within species, species in themselves, and either local or global ecosystems. However, the first goal in the mind of conservation geneticist is the assessments of genetic variability in threatened and unthreatened organisms as a metric to trace the well-being of the planet.

### **3.1. Relevant items in conservation genetics: wildlife scenario from top to bottom**

#### *3.1.1. Kinship and genetic variation for within population conservation (population genetics)*

The loss of genetic variation due to inbreeding (as a result of mating among genetically related individuals) was (and is yet) the main issue regarding captive and natural populations of small size. Whichever the case, despite great scientific attention received by the deleterious effects arising from inbreeding depression; no less important are parentage, kinship, sex identification, and demographic history of population. Since a general scientific acknowledgement regarding inbreeding depression related to small captive populations and natural isolated populations as well, a preference position has been granted to those studies focused on inbreeding depression. The assessing of inbreeding depression has been the former issue in the design of conservation programs, formerly applied to domestic animals and plants, but today it has been extended to wildlife, both in captive breeding programs and in the management of natural isolated populations. An interesting case is the Pyrenean desman (*G. pyrenaicus*), which is annotated as vulnerable by the IUCN red list. However, the southernmost population in the Iberian peninsula (at the mountain place of the central system: green dashes in **Figure 2**) is listed as “endangered” with high extinction risk by the main Spanish government authority (MAGRAMA, that is, Ministry of Agriculture, Food and Environment) due to its almost null genetic variation (mtDNA studies suggested they carried a clonal lineage in several populations) and high level of anthropic threat but without possibility of implementing captive breeding programs [18].

Regarding population variations in wildlife, it is important to assess local kinship as offspring parentage, mating systems, sex determination, or lineages identification. The main field of study is the application of empirical data to be compared with theoretical assumptions as in the case of diploid lethal equivalents estimation to juvenile survival [36]. New DNA technologies are addressing molecular procedures to gather high informative loci as microsatellites and single nucleotide polymorphisms (SNPs) to finely estimate relatedness coefficient at several degrees of relatives, not only to parents-offspring pairs. An underestimate of the total impact of inbreeding has been declared and  $N_e/N$  bias between nonbreed and unmanaged wild population has been claimed after assuming statistical distribution of family size (Poisson distribution). The relative importance of the analysis of local kinships has several issues as follows: (i) isolated populations differ by drift and inbreeding but the first is more related to random sampling than specifically mating of relatives; (ii) balance among family sizes can be calculated by molecular procedures using as many genetic markers as possible in local or isolated populations; (iii) local populations exhibited correlations between diversity

and family sizes but unbalances in this last one may influence minimum viable populations size (MVPs) (number of individuals needed for long-term persistence of populations with high probability), which assist scientific and wildlife managers in population viability analysis (PVA). However, some discrepancies arose between theoretical and empirical studies comparison about the deleterious effect of inbreeding, suggesting a case-by-case analysis in wild species due to strong species specific conditionings: lifestyle, demographic history, genetics, and more. Other significant assessment related to conservation is heterozygosity. It may be useful to understand a species life history. This type of analysis allows us to give a retrospective look at the past to make current comparisons and to perform realistic predictions about the future.

### 3.1.2. Conservation genetic of geographic variation

Ecological and evolutionary sources of genetic variation, at the intra-specific level or higher, are also worth of being considered for conservation purposes. However, the two main areas of work in this broad field of study have its top representatives in phylogeography and genetics of populations. These two approaches are being used currently, one based on allelic frequencies (unordered polymorphism for population genetics but recently also phylogeography using e.g., network-net methods) and the other one based on mitochondrial DNA sequences (ordered polymorphism for phylogeography also using networks as in **Figure 4**). Both approaches are utilized because they can easily represent the pattern of spatial distribution of genetic variation for species and allocate the most genetically isolated populations and connectedness degree if any. Moreover, there is a current tendency to rejoin historical genealogical information plus contemporary forces modeling populations because it is believed to provide a larger resolution to illuminate the causes and consequences of such spatial pattern in nature. Moreover, practical biodiversity conservation is interested in conserving as many species or relevant populations within them allocated inside emblematic or unique places following the “species' genetic richness” concept.

At least, three competing concepts that connect researchers on conservation genetics and conservation managers, also needing to be delimited in the conservation biology context, are those that follow: evolutionary significant units (ESUs), managements units (MUs), and phylogenetic diversity (PD) of taxa as a way to estimate distinct population segments (DPS) [1]. Today, these three concepts are fully applicable for wildlife analysis and to take relevant decisions. The idea of MUs should be seen regardless of how recent the prior genetic history connections was, providing that exchange of individuals is so small as to be demographically independent units. By contrast, ESUs must imply a long historical separation of its populations. However, approaches based on demography and connectedness between populations can treat species or populations unequally. Consequently, a new appraisal introduces evaluations of phylogenetic trees connecting species (or populations within species) under a study approach called “Phylogenetic Diversity of taxa” (PDs). This approach has into account the edge length distances of the tree. Edge lengths depict the optimal number of features uniquely shared by all descending taxa below this edge and using a root. The set of taxa (populations) that maximizes the PD (normally less than the total populations considered) could be utilized in two types of projects. It has been employed to identify taxa and/or populations prone for

conservation purposes. On the other hand, it is also a way to identify important taxa or geographically isolated for sequencing projects.

### 3.1.3. Biodiversity of species

In this section, the basic idea is that unique evolutionary lineages may contribute largely to overall genetics diversity. Their extinction would constitute a far great loss of diversity than would the extinction of species that have extant close relative. Although under discussion, phylogenetic distinctiveness is dealing with resolution of taxonomic issues due to its recognized role as measurement of taxon worthy of investing conservation resources. It is generally admitted that the importance of research to delineate the influence of introgression and hybridization on species diversity. It is a topic that is reaching great relevance at the inter-specific level but also at the inter-subspecific level as a way of **silent extinction** due to human domestication of current wild species and random translocation of their products, overriding yet hidden evolutionary pathways unexpectedly by introgressive extinction (as for red deer subspecies, **Figure 4**).

In this state of things, the systematic evaluation focusing on elevating differentiated populations such as true species assessed by informative genetic loci of split populations. This is an important issue for wildlife conservation and for making management decisions. Several subspecies gathered the rank of species (e.g., historical nominal subspecies as Wapiti but today elevated to the species level: *Cervus canadensis* instead of *Cervus elaphus canadensis*) or at least will be considered from this point worth of deepest studies as Mesopotamian fallow deer (*Dama dama mesopotamica*) or Barbary red deer (*Cervus elaphus barbarus*), which are currently included in their respective conservation programs or even herdbooks. Hopefully, conservation programs and the creation of herdbooks to manage the most endangered species should be treated as a nonnegligible new Genetic discipline: "Domestic" wildlife issue. Nevertheless, it should not be obviate that the rate of speciation, diversity of closely related species and adaptive radiation by incorporation into populations of selectively favorable alleles or combinations of them may be increased by hybridization, providing thus, the basis for adaptive evolution and having important implications for the origin of species, as mentioned previously.

### 3.1.4. Wildlife forensic: the case of Pyrenean desman in ecological studies

Forensic identification by advanced DNA technology is also important for wildlife studies. But forensic analysis has several distinct fields of application. Firstly, free-ranging wildlife species, especially those endangered, where noninvasive methods are recommended to detect elusive or sensitive to human management species as sampling strategies (e.g., Pyrenean desman (*G. pyrenaicus*)). On the other hand, wildlife products from specimens under strict police management due to them are imperiled (e.g., rhinoceros horns).

Finally, the biology and ecology of species with elusive or with hidden activity, which are still poorly known. As an example, the nature of trophic interactions is a fundamental issue in ecology and has aroused the attention of biologists for decades. This knowledge is particularly important in endangered species such as the Pyrenean desman. Using DNA from feces of the Pyrenean desman, it is possible to identify 19 prey species by next generation sequencing

methods like the DNA minibarcode (133 bp) of the COI gene barcoding. This tool is able to simultaneously perform screening of species at large-scale because sometimes feces could be difficult to identify directly. Despite potential pitfalls in this methodology, it is based on one or a few genes at present state, each new genome incorporated into the data bank increases the validity of it. Consequently, more and more literature is arising in recent times.

#### **4. Biodiversity analysis by integrating phylogeny and conservation**

Quantification of biodiversity using phylogenetic analyzes has been proposed to provide a more objective framework to make conservation decisions. Three collaborative efforts among ecologists, evolutionary biologists, paleontologists, systematists, and conservation biologists from the USA, Canada, Australia, and England are driving these aims thorough the 'Tree of Life' project attempting to integrate phylogenetic and conservation biology. They are based on two complementary facts: (1) surprising amounts of phylogenetic diversity might remain even under high rates of extinction (random) and (2) it is feasible to detect current extinction events through missing phylogenetic diversity as it is mentioned in [37].

Three issues are being examined in the integrative framework as follow:

##### **4.1. Selectivity or random extinctions questions: species or upper taxonomic level**

The start viewpoint of New and May's was that simulated extinction occurred at random with respect to phylogeny [37]. However, phylogeny and conservation working groups ('phylogeny and conservation' working group sponsored by the National Center for Ecological Analysis and Synthesis (NCEAS) in Santa Barbara, CA, USA) reasoned that in this context randomness is not realistic due to extinctions and invasions tend to be strongly clumped for the most diverse taxonomic groups, for example, mammals or birds. After testing several statistics by simulation, the Moran's *I* index showed the high performance for detecting selectivity accurately, independent of tree size (i.e., number of species), tree shape (i.e., nodes with equal size groups in the tree), or prevalence of the desired trait (e.g., proportion of endangered or invasive species). As a result, it has been recognized that taxonomic selectivity is the main way to extinction and could be quantified, but hopefully selectivity varies across a wide variety of taxonomic groups, across geographical regions, between 'higher' and 'lower' taxonomic units, and extinction is related to selectivity for invasion within taxonomic groups.

##### **4.2. Levels below species**

A long-standing problem is how to designate conservation units below the species level: Subspecies, ESUs, MUs, and more. With the advent of molecular technologies, those historical concepts as "subspecies" fell in disuse. However, an overload of genetic information can lead to the designation of many small and isolated subunits hampering the standard delimitation of, for example, the ESU and MUs concepts. A survey of the recent literature revealed that most studies

follow the guidelines of Moritz, which advocate a purely genetic definition of ESUs. Nevertheless, a large fraction of conservation decisions require both genetic and ecological evidence.

The guidelines of Moritz admitted that ESUs should show significant divergence and reciprocal monophyly for mtDNA and significant divergence of allele frequencies at nuclear loci. This is straightforward because it requires to examine historical and recent restrictions to gene flow, that is, evidence for long-term divergence that continued in the mtDNA and nuclear loci (free from selection) where mutations accumulate relatively more slowly or very rapidly, respectively. Therefore, this molecular discrepancy is useful to evaluate restrictions to gene flow at different times or even detecting genetic distinctiveness but no adaptive potential. However, Moritz's definition no longer mentions the ecological distinctness because ecological divergence may or may not be necessarily associated to genetic divergence. Crandall et al. [8] proposed the “cross-hair analysis” to have into account the four important scenarios to decide whether ESUs or not is present (**Figure 5**).

Consequently, there is a worldwide agreement that decisions should be based on both genetic and ecological evidence but in the context of ecological and genetic exchangeability. Ecology together with an examination of recent and historical processes provide a more fine-grained, and therefore, more flexible categorization than the current system to be employed to diverse set “case studies” as red wolf (*Canis rufus*), dusky seaside sparrow (*Ammodramus maritimus nigrescens*), Florida panther (*Puma concolor coryi*), and Gila topminnow (*Poeciliopsis occidentalis occidentalis*) or Pyrenean desman (*G. pyrenaicus*).

#### **4.3. Areas with distinct population segments (DPS): hotspots places**

There are many criteria to determine the relative conservation value of different areas (e.g., species, threatened species or large numbers of species across different groups), but now below species level as areas containing DPS received attention since the late 1980s mainly for economic-important taxa. The problem is how we can quantify DPS value. Population-based management is being a necessary task for scientists and managers due to climate change and habitat degradation associated to growing human demands ensuring continued species-range fragmentation, which will be expected during this century. In order to address this work, phylogenetic diversity (PD) is being used as a measure of at least three stuffs to choose important areas to protect with accountability incorporating phylogenetic information.

Firstly, the exploration of the relations between PD and the spatial distribution of biodiversity would permit to get insight into the population structure complementary to the current statistical assessment of differentiation employed by MUs and DPS. Moreover, under this perspective, it is feasible, when constrained, to choose only a limited number of areas for conservation, to develop appropriate protocols to assess the complementarity predictions to preserve future biodiversity. Secondly, PD is being extended to simulations aimed to find taxonomically nonrandom extinction risk. Current threat scenarios are tested by comparing the spatial distribution of PD both before and after projected extinction. Finally, the predictions that suggest rapid environmental change leads to explore whether phylogenetic patterns of threat could predict the amount of ecological disturbance in a region.

+reject  $H_0$  of exchangeability, evidence exists for divergence.  
 -accept  $H_0$  of exchangeability, failure to detect divergence.

		Time Frame
+/-	+/-	Recent
+/-	+/-	Ancient
Genetic divergence	Ecological divergence	
$H_0$ Exchangeability		

Figure 5. Cross-hair analysis for management recommendations (adapted from [8]).

### 5. Prioritizing populations for conservation using phylogenetic distances from networks: split diversity (SD)

According to the “species richness” concept [38], practical biodiversity conservation has the aim to preserve as many species as possible. However, as previously said, such an approach has the hurdle of treating all species equally [39]. However, neither all species nor genetic lineages are equally important, with more isolated lineages providing more important contribution to total variation, that is, the base for identifying populations worthy of protection in law. Genetic variation is depicted perfectly onto a rooted phylogenetic tree, where the edge length represents the number of features uniquely shared by all descending taxa, say populations. Importantly, ESUs concept assumes that the relationships among populations can be represented by a bifurcating tree. However, these sort of phylogenetic trees often fail to capture complete genetic information among populations. Moreover, more complex interrelationships are expected for DPSs and MUs. So, it would seem a shortcoming could occur if populations do need to be prioritized for conservation on the basis of tree-based prioritization schemes. However, the prioritization approaches for trees can also be adapted for populations by using algorithms developed for network under the denomination “Neighbor-Net” procedures [1], where PD could be optimized via computing a circular split system. Optimal PD could be obtained by morphological and molecular data. Using PD, Faith [40] proposed a taxa selection once having a phylogenetic tree of  $n$  taxa by identifying the set of  $k$  taxa that maximizes the PD, where  $k < n$ . The optimal set is tested yet to determine taxa that are of interest for sequencing projects in wildlife [41]. Although mathematical formulations exceeded our aims, following [13] we summarize the example in the paper of these authors to show how it works.

In **Figure 6**, we show a network graph ordered in a circular format A to E taxon. Each split could be weighted according to edge distances from each bisecting taxon (arrows in **Figure 6**) to the rest of taxa. As an example, the procedure to get an optimal PD distance (PD is equivalent to SD in Ref. [13]) from circular taxon order of A–E will be constructed for an optimal three-set of split taxon as follows:

- (i) Formulae to be used ( $n^2$  in [13]).
- (ii) Compute the pairwise distance matrix  $d_{uv}$  (distance count).

	A	B	C	D	E
A	0	11	19	20	17
B	11	0	12	21	22
C	19	12	0	17	18
D	20	21	17	0	11
E	17	22	18	11	0

(iii) Index matrix to trace back the optimum. The 3-Path taxon maximizing DP (in blue an example: the ABC maximum 3-path DP; see **Figure 6**).

	A	B	C	D	E
$\alpha_{uv}^3 =$	A		B	C	C
	B			C	D
	C				D
	D				
	E				

(iv) Compute the longest ordered two-path using  $L^2 = d_{uv}$  (as in second line at formulae).

	A	B	C	D	E
$L^2 =$	A	11	19	20	17
	B		12	21	22
	C			17	18
	D				11
	E				

(v) Derived  $L^3$  from  $L^2$  (as in second line at formulae) but only three taxa.

Example A to C =  $(3+2+4+2) + (2+6+4) = 23$  (B features two times;)

Example B to E =  $(2+4+6+4+5) + (5+2+4) = 32$

	A	B	C	D	E
$L^3 =$	A		23	36	37
	B			29	32
	C				28
	D				
	E				

(vi) Calculate  $L^3 + L^2$  for the longest ordered three-path.

	A	B	C	D	E
A			42	56	54
B				50	54
$L^3 + L^2 =$	C				46
	D				
	E				

(vii) Determine maximal scores  $SD_{max}$  (three-circular tour) as  $(L^3 + L^2)_{max} = 56/2$ .

(viii) Determine the longest ordered three-path from A to D using  $\alpha_{iv}^3$ . As a result, the set ACD is an element  $SD_3$  with the highest scores for  $PD_3 = 28$ .

Several phylogenetic diversity measures have been adapted for nontree-like population genetic data. However, these methods could be conditioned to change when natural or artificial (human mediated) extinction alters the network structure. Given both the stochastic and/or selective nature of extinction, different metrics, like split diversity (SD; similar to PD) from [13] or Shapley metric (SH [14]), and heightened evolutionary distinctiveness (HED [15]) offer general ranking systems useful to wildlife managers rather than those based only on the present structure of a phylogenetic network trees. However, SH and HED rankings have been stated as able to allow lengthening or shortening the list of taxa to conserve in the event that resources become more or less available, which may give potential relevant frameworks or schemes for preserving future biodiversity [1].

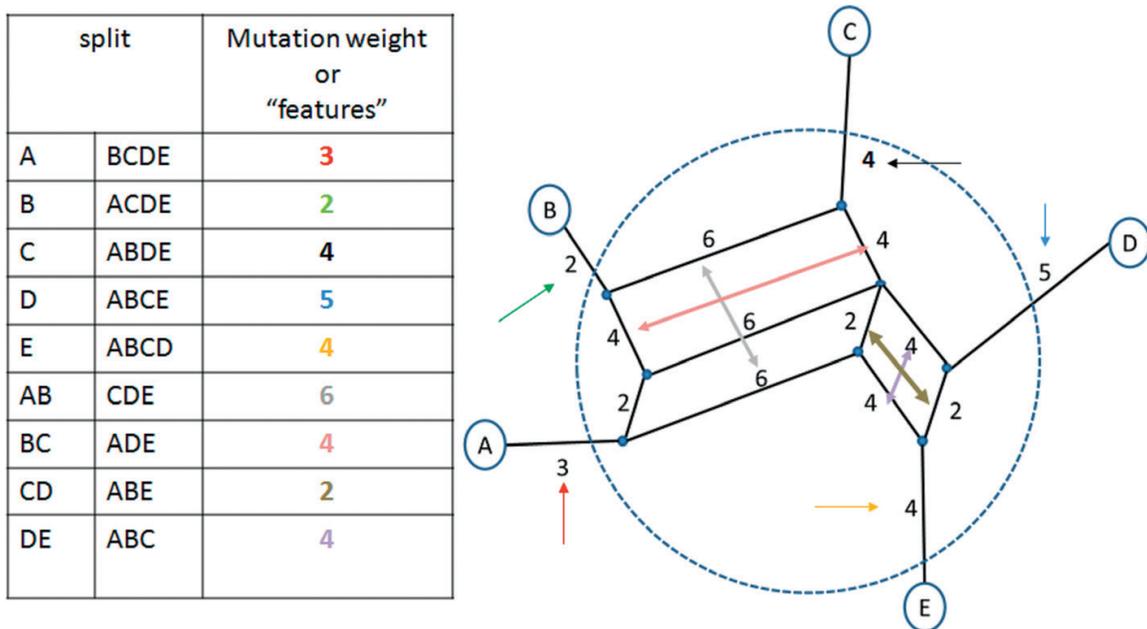


Figure 6. Split graph and its split systems (adapted from [13]).

Nowadays, the most recent, more inexpensive, and robust advances in molecular techniques make of the genetic sampling of populations a standard component of conservation planning. Moreover, there are views that value phylogenetic network approach because it offers insight into a species' population structure complementary to the current statistical assessments of differentiation employed by MUs and DPSs [11, 12]. Genotyping at multiple informative loci and networks will provide population genetic studies aimed at giving advice to conservation agencies, to do more informative and accurate estimates of population differentiation and of conservation-relevant processes, mainly those important onto genetic isolation and their effects on diversity [42].

## 6. Conclusion

Conservation genetics for wildlife is a recent challenge for humanity because biodiversity at several biotic levels need to be preserved to maintain desirable genetic variation for future generations. As a result, understanding biological diversity patterns and processes has increased the interest for phylogenetic analysis, remaining relevant all species. Nowadays, the imminent biodiversity crisis predicts significant new scenarios of biodiversity at the beginning of the twenty-second century for whichever wild species, which motivates to the geneticists in deal with preserve “all the gene pool”. However, two faced situations are clearly involved in the context of conservation decisions. On the one hand, the identification of small populations harbors any significant genetic relevance worthy of conservation. On the other hand, identification of natural hybridized populations or species, although do not lack detractors when artificially promoted, due to it is believed to be an important process causing divergence in speciation and enhances the ability for survive. So, practical biodiversity conservation has the aim to preserve as many species (populations) as possible, but the relative importance of species or its genetic lineages should be carefully studied for to be prioritized. Phylogenetic diversity measures have been adapted to offer potential relevant frameworks or schemes for preserving future biodiversity based on accurate estimates of population differentiation and conservation processes.

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