



Application of conservation genetics in conservation planning, with a special focus on the impacts of hybridization on birds and their conservation

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ABSTRACT

Biodiversity consists of all different live forms found in Earth. It is important to sustain it as it has several important aspects, such as maintaining ecosystems, helping with their proper functioning and it also plays a big part in providing ecosystem services. Conservation biology studies the loss of biodiversity on earth and its patterns, followed by conservation actions that aim to prevent extinction by implementing management plans. Large numbers of bird species are threatened or endangered, which makes conservation planning a more common topic nowadays to help protect the species from extinction. Additionally, as a consequence of decreased population size, species will begin to suffer from reduced genetic diversity and inbreeding, which is when conservation genetics is involved. Conservation genetics have been an important discipline for a long time, and its inclusion has been discussed throughout the years. It deals with genetic factors that are causing rarity or extinction of a specie, and uses genetic markers in order to resolve taxonomic uncertainties and to help in reconstructing phylogenetic trees. Genetic diversity is important in terms of conservation, as it provides species with adaptive potential that increases their chance of survival when ecosystems go through changes. The field of conservation genetics addresses several aspects that are important for conservation planning such as (i) the extinction risk of small populations, (ii) the delimitation of conservation units and (iii) the effects of hybridization., which will be the main focus of this thesis. Smaller populations are vulnerable to inbreeding, which can lead to inbreeding depression, this phenomenon might lead to the extinction of the species, as more deleterious alleles will be fixated and the population size keeps on decreasing. Conservation genetics also helps in delineating conservation units such as Evolutionary Significant Units (ESUs) and Management Units (MUs). As for hybridization, species might interbreed after expanding their geographical range, or after mating with invasive species, which may result in negative effects on fitness. This is where hybridization between species may lead to genetic swamping, fitness decrease and loss of important alleles. Hybridization has several effects on the affected species, and can have positive or negative effects, that ought to be understood for conservation planning. The focus in this thesis will be on conservation genetics in regards to hybridization in bird species interacting and interbreeding together. The goal is to

understand the negative and positive consequences of hybridization on species' survival and adaptation, which may vary in different cases and the importance of including genetic data in conservation planning and how conservation genetics helps with hybridization. After the literature review, it is clear that hybridization is affecting populations differently, in some cases the hybrids are viable, in others they are not, deleterious genetic processes are as a result contributing to the loss of rare alleles. Following the results of studies that have used conservation genetics, it is recommended that conservation planners take into consideration genetic data when implementing plans as it might reveal more important threats on the molecular level.

Vanessa Hajjar

DEDICATION

This work is dedicated to my family and friends that have continuously supported me throughout this journey. I am forever grateful for my loving parents, Elias and Gretta, whose words of encouragement were my strength and source of motivation. I am beyond thankful for my sister, Lea, who was always there for me when I was at my lowest. This work goes out as well to my beloved people, my angels, who mean the world to me. My grandfather Zaki, who always lit a candle to wish me good luck and I know he is doing so in heaven. My dear friend Mario, who was very helpful throughout my academic years back in Lebanon. I present this thesis with both of you in mind.

I hope my work will make you proud.

I love you all, beyond words.

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SEMINAR ANNOUNCEMENT



University of Cyprus
Department of Biological
Sciences

*BIO 681 Scientific Methodology in Biodiversity and
Ecology*

Student Presentation

Wednesday, 01 June 2022 at 10:00
Building XOΔ01, Room 003, Panepistimioupoli Campus

This seminar is open to the public

Vanessa Hajjar

Thesis Supervisor: Assoc. Prof. Alexander Kirschel

“Application of conservation genetics in conservation planning, with a focus on the impacts of hybridization on birds and their conservation”

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risk of small populations, (ii) the delimitation of conservation units and (iii) the effects of hybridization., which will be the main focus of this thesis. Smaller populations are vulnerable to inbreeding, which can lead to inbreeding depression, this phenomenon might lead to the extinction of the species, as more deleterious alleles will be fixated and the population size keeps on decreasing. Conservation genetics also helps in delineating conservation units such as Evolutionary Significant Units (ESUs) and Management Units (MUs). As for hybridization, species might interbreed after expanding their geographical range, or after mating with invasive species, which may result in negative effects on fitness. This is where hybridization between species may lead to genetic swamping, fitness decrease and loss of important alleles. Hybridization has several effects on the affected species, and can have positive or negative effects, that ought to be understood for conservation planning. The focus in this thesis will be on conservation genetics in regards to hybridization in bird species interacting and interbreeding together. The goal is to understand the negative and positive consequences of hybridization on species' survival and adaptation, which may vary in different cases and the importance of including genetic data in conservation planning and how conservation genetics helps with hybridization. After the literature review, it is clear that hybridization is affecting populations differently, in some cases the hybrids are viable, in others they are not, deleterious genetic processes are as a result contributing to the loss of rare alleles. Following the results of studies that have used conservation genetics, it is recommended that conservation planners take into consideration genetic data when implementing plans as it might reveal more important threats on the molecular level.

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INTRODUCTION

Conservation genetics understands and reduces the risk of population and species extinction by applying genetics (Frankham 2019). It deals with genetic factors such as inbreeding and loss of genetic diversity which can cause rarity, extinction and endangerment (Frankham 2019). It consists of genetic management to reduce these impacts (Frankham 2019). Conservation genetics uses genetic markers to aid in resolving taxonomic uncertainties in threatened species and understand their biology (Frankham 2019). Preventing biodiversity loss and understanding both its drivers and consequences are of high importance to humanity (Bull and Maron 2016). However, with a sixth mass extinction coming, mostly driven from human activities and their impacts, preventing biodiversity loss and ecosystem degradation has become a complicated task. Discussions about a sixth mass extinction have been common for several years, which is translated by the earth's loss of about 75% of its species (Briggs 2017). The sixth mass extinction was first mentioned by Leakey and Lewin's (1995) book where it was estimated that annual extinction rates will range in between 17.000 and 100.000 species (Briggs 2017). It is important to conserve species as species diversity is decreasing rapidly due to human actions, directly or indirectly (Frankham 2019). This led to the extinction of many species, and others to have severely reduced population sizes, putting them at risk of extinction (Frankham 2019). The International Union for Conservation of Nature (IUCN) recognizes three main levels as to why it is important to conserve biodiversity, (a) genetic diversity, (b) species diversity and (c) ecosystem diversity (Frankham 2019). This mass extinction is mostly driven and caused by human activities, which are using natural resources uncontrollably and disregarding the importance of balance in nature (Briggs 2017). Ecosystem degradation has a long-term impact on an ecosystem's structure, function and capacity to provide ecosystem services (Delgado and Marín 2020) which fulfill and sustain human life by offering processes provided by species and their natural ecosystems (Daily 1997). The UN Millennium Ecosystem Assessment recognized four types of ecosystem services: provisioning, regulating, cultural and supporting services (Whelan et al. 2008). Loss of biodiversity is the most serious aspect of the environmental crisis, because the extinction rate nowadays is far above the older rates in the previous five mass extinctions (Ceballos et al. 2015). This massive extinction is

affecting human beings as biodiversity loss is interfering with ecosystem services which are a must for the environment to stay healthy, and for humans to benefit from (Ceballos et al. 2015).

The focus in my thesis will be on bird species, their hybridization and how is conservation genetics applied to help understand its effects. Birdlife lists 1.250 bird species out of 10.000 under the category of risk of extinction, 182 species are seriously threatened as they fall under the “critically endangered category”, of which many doubtfully still exist (Pimm et al. 2006). About 13% of bird species are categorized as threatened by the UCN (Frankham 2019). The main reason why so many species are threatened by extinction is mostly habitat destruction which includes deforestation, desertification, eutrophication, urbanization, water and oil extraction (Yadav et al. 2019), as most of nature’s habitats have been impacted, in a way, by humans (replaced or fragmented) (Pimm et al. 2006). A more recent study maps bird diversity worldwide, and the percentage of threatened species, (Figure 1, Tilman et al. 2017). Tropical South America, Southeast Asia, India, China and sub-Saharan Africa have a total of 5.200 species, and these are the most impacted areas with the highest percentages of bird species (Figure 1.b). The most affected habitats are forests, and particularly tropical forests, which are known for being a hotspot of bird diversity (Pimm et al. 2006). Habitat fragmentation does not simply lead to extinction, but it might also result in hybridization between previously isolated species’ populations, which might lead to the extinction of one of the parental lineages (Yadav et al. 2019).

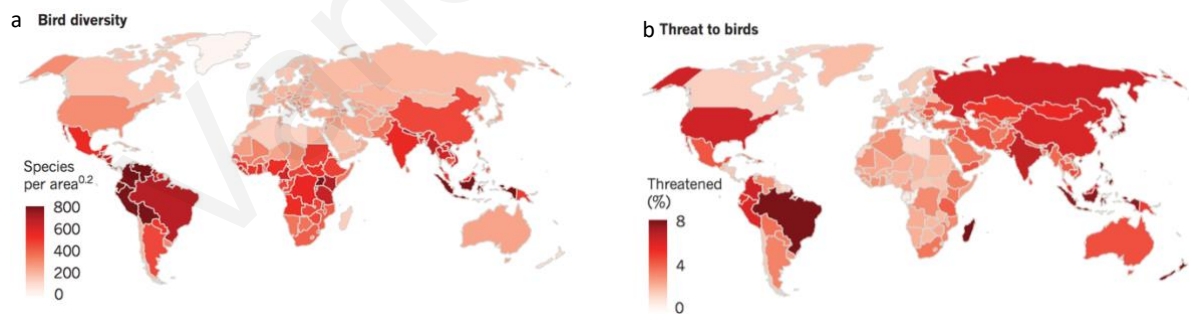


Figure 1. Diversity of birds and the extent of threats worldwide. (a) Species per area (100.000km²) in each country. (b) Percentage of threatened bird species in each country (Tilman et al. 2017).

Hybridization, one aspect of conservation genetics

Conservation genetics and genomics approaches allow the understanding of the genetic basis of inbreeding depression and adaptation, these insights can be applied in order to understand the effects of hybridization and predict outbreeding depression and evolutionary responses to climate change (Allendorf et al. 2010). Genomics can assess introgression rates across different genomic regions following hybridization between species (Allendorf et al. 2010). Hybridization is the interbreeding between two different species that will produce an offspring, regardless of the state of their offspring in terms of their fitness (Yadav et al. 2019). It can occur naturally or as a result of anthropogenic activities (Yadav et al. 2019). Natural hybridization, is the natural reproduction between two distinct species and anthropogenic hybridization on the other hand, occurs due to habitat fragmentation or introduction of species (Yadav et al. 2019, consequently, the offspring produces will have a mixed genotype between parental species, that could result in an intermediate phenotype. Hybridization can also lead to introgression which is the incorporation of alleles from one species into the gene pool of a second one after repeated backcrossing of hybrids with one of its parental species (Harrison and Larson 2014). Species are mostly vulnerable to hybridization in 4 scenarios (a) if they represent a small population size, this is a possible cause explaining lesser mate availability after their habitat is altered, (b) following habitat destruction, species will migrate, evade or introduce newer surroundings (c) artificial hybridization by breeders and (d) interferences, could be visual, chemical or acoustic, which are important factors in breeding, as they can lead to reduction in the ability of species to distinguish between the same and opposite species, this has been only seen in aquatic species (Yadav et al. 2019). Haldane's rule, which states that heterogametic sex hybrids have lower fitness, e.g. are sterile or inviable (Rosser et al. 2021) has been shown to also apply in birds (Ottenburghs 2022). Bird female hybrids (and those of any other organism with a ZW sex chromosome system) tend to have lower fitness than male hybrids (Ottenburghs 2022). Nature follows a 1:1 sex ratio, meaning that both sex chromosomes should be presented equally a species' population, but this tendency is not true as sex chromosomes are not present in both sexes in equal proportions (Johnson and Lachance 2012). Even though hybridization often results in sterile offspring, lowered fitness and has several disadvantages, it can also benefit hybrids as it can provide advantages as well (Yadav et al. 2019). Some outcomes from hybridization could be the gain of adaptive traits on several levels, either biochemical, morphological or behavioral (Hughes 1999).

It is expected that in the next 50 years threats to biodiversity will further increase, with the growth of human population (Tilman et al. 2017). Which is why conservation planning is becoming a very important topic and scientists are finding methods to save the threatened species before it is too late. Birds are very important actors in ecosystems, as they take part in all ecosystem services from provision, to regulation, to cultural services and supporting services (Garcia et al. 2010). They are able to connect ecosystems through migration and via seed (Garcia et al. 2010). Which is one of the many reasons why it is important to protect those species and prevent their extinction. Conservation planning is based on demographic analysis of species, by estimating their population, their range, and their habitat preferences (Greenwood 2007). When all this data is gathered, it becomes easier to understand which species are of more importance in terms of conservation urgency (Greenwood 2007). When planning on implementing a conservation strategy for a certain species, the first step is to have a strong knowledge of the species' distribution (Marini and Garcia 2005). Following that, is the identification of threatened species (Marini and Garcia 2005), then finding out which threats are mostly affecting them (Tilman et al. 2017) and finally starting with conservation initiatives. Several biodiversity actions are being implemented, by completely avoiding human activity impacts and retaining biodiversity, if impossible, minimizing impacts as much as possible, restoring and renewing biodiversity (Milner-Gullan et al. 2021). Applications of such actions are being performed by national governments (Milner-Gullan et al. 2021), however it is not only governments and NGOs that are working on conservation, these plans always require the help of the citizens and volunteers in order to record data and provide them to the entity in charge (Greenwood 2007). Even though all the aforementioned is crucial for conservation planning, one factor that has less often been taken into consideration is genetic information and data (Frankham 1995). Conservation genetics is helpful in conservation biology as it uses genetic information of populations and species to help with their conservation (Frankham et al. 2002). It relies on molecular analysis in order to document the extent and pattern of genetic variation (Hedrick and Miller 1992). Since conservation biology is aiming to preserve diversity, this should include conservation of genetic diversity as well (Taft et al. 2020). Several factors are affecting genetic diversity and variation, and should be identified in order to proceed with conservation genetics *e.g.* habitat alteration, fragmentation (Nakahama 2020). Genetic data is mostly helpful when working on species with little knowledge on their status and biology, it is capable of providing further information (Muniz et al. 2019). Moreover, historic genetic data has been

disregarded at times, even though molecular studies have shown that with the help of museum data, they can discover how a species evolved and what genetic processes did it undergo (Nakahama 2020). Several genetic processes tend to affect a species population and its genetic diversity, such as inbreeding depression, deleterious mutations, decrease of genetic variation (Frankham 1995). Genetic studies of endangered species have become more widespread recently (Hedrick 2001). Several methods are used in conservation genetics, and they are more relevant for long-term conservation plans (Moritz 1994). Conservation genetics uses neutral, detrimental and adaptive variants for conservation application (Hedrick 2001). Moreover, genetic studies play an important role in phylogenetic findings and relationship between species, by using DNA fingerprinting for example (Degnan 1993). Various methods can be used in conservation genetics to document the extent and pattern of genetic variation in endangered species (Hedrick 1992). Four common techniques, that are outdated now, were used in conservation genetics in the past (a) Protein electrophoresis to help in species identification and tracking evolutionary paths, by analyzing sizes of different bands and comparing them (Ladizinsky and Hymowitz 1979) (b) Mitochondrial DNA (mtDNA) analysis, used to determine genetic variation and geographical range of species (Hedrick 1992) and (c) DNA fingerprinting, by using unique tandem repeats can identify species and subspecies and their genetic variations (Lynch 1990). Nowadays, with the development of genetic techniques, Next Generation sequencing is being used, which is able to sequence bigger DNA samples than the older methods, up to a whole genome. Genetic rescue is also a technique used which consists of breeding a bottlenecked population with richer ones (in terms of genetic diversity) in order to increase genetic diversity and populations' fitness. Although incorporation of molecular and genetic information into conservation biology have been found to be a positive addition to conservation biology (DeSalle and Amato 2009), it is still rare to find studies including them. Several reasons lead to this absence of genetic data in conservation planning, (a) genetic concepts are complex, complicated and hard to handle, (b) there's lack of communication between scientists and conservation managers and (c) because genetic manipulations and analysis techniques require high costs, it is hard to find adequate funding (Taft et al. 2020). However conservation managers are aware of the importance of incorporating genetic information in their studies, but due to the previously mentioned inconveniences, it is a bit challenging to do so.

The emphasis in the following paragraphs is on bird species and more precisely on conservation genetics of bird species. Interbreeding between species is leading to several outcomes, some of which are positive and some of which are negative for the offspring and the following generations. Conservation planning, as surprising as it is, does not always occur while relying on genetic data. However it is widely known that hybridization may result in lower hybrid fitness, loss of alleles and extinction. After giving an overview of conservation planning and management in general, the questions addressed and subjects of interest are the following. (a) What is hybridization, and how does it affect the species in different ways? (b) What is conservation genetics and how is it relevant in conservation planning? Techniques of conservation genetics are mentioned, including the goal of each one and why it is used. Several reasons explain the lack of genetic data and information used in conservation planning. The few studies reporting the inclusion of genetic information in conservation planning demonstrate that it leads to more accurate results, and will give a clearer idea on which species should be prioritized before it is too late. Finally I give some recommendations as to how to make genetics a part of conservation biology planning, and improve conservation planning outcomes.

OVERVIEW

Bird conservation planning and management

Conservation biology addresses problems that species are facing, helps to understand their biology, their communities, and habitats that are being perturbed directly or indirectly by human activities (Soulé 1985). Successful conservation planning depends on detailed demographic analysis which relies on recorded data such as migration patterns, species distribution, population fluctuations, habitat requirements etc. (Greenwood 2007). A lot of work and effort is required in order to reverse endangered species' declines by implementing protected areas, coming up with conservation initiatives and direct management of threatened species (Develey 2021). The availability of genetic data will allow conservation officers to prioritize species that need urgent help (Greenwood 2007). It is important to mention that not all the threatened species get the same attention (Brooks et al. 2008). A study on bird conservation done by Brooks et al. (2008), on

threatened species, showed that 50% (over 600 species of birds) of the threatened bird species in the analysis do not have any publication dedicated to them. However, BirdLife International and Birdlife partner organizations are willing to provide necessary data and advice on how to help the species lacking attention, especially in poor countries as they have lower rate of conservation studies but host most of the threatened bird species (Brooks et al. 2008).

Brazil is known for hosting a high diversity of bird species in North and South America. However it is top on the list for the number of threatened bird species, with 166 species in 2021 (Develey 2021). The following paragraphs regarding the steps taken in bird conservation are mostly focused on examples from Brazil as it is the leader country in this field.

1. Strong knowledge of birds distribution and composition:

First of all, strong knowledge of the bird populations and status (endemism, migration...) in the area of interest is required, including the species' richness and abundance and the types of habitats they seek to live in (Marini and Garcia 2005).

2. Number of threatened species and their distribution:

Relying on international relevant data such as the International Union for Conservation of Nature (IUCN) Red List of globally threatened species and, if available, the national list of threatened species (Marini and Garcia 2005). After defining the number of threatened species in the studied area, other information should follow *e.g.* depict the number of species that are endemic to the area of study (Marini and Garcia 2005). Additionally, it should be checked whether the threatened species are found in Endemic Bird Areas (EBA), which makes endemic threatened species belonging within these areas, of high priority for conservation (Marini and Garcia 2005). Table 1, shows the different categories of importance when assessing threatened species, a study on bird conservation in Brazil. The highest bird diversity is found in the Amazon and the Atlantic Forest, the latter however, has the highest number of threatened taxa which might be due to very high rates of human activities such as deforestation and urbanization.

Table 1. Number of bird species in Brazil, endemic ones and threatened taxa, including their biomes (Marini and Garcia 2005)

Biome	Species	Endemic species	Threatened taxa ^a		
			total	endemic to biome	endemic to Brazil
Amazon	1,300 ^b	263 ^b	20	6	10
Atlantic Forest	1,020 ^c	188 ^c	112	54	90
Cerrado	837 ^d	36 ^e	48	14	14
Caatinga	510 ^f	15 ^g	25	7	15
Southern Grasslands	476 ^c	2 ^c	20	3	0
Pantanal	463 ^b	0	13	0	1
Coastal and marine	>130 ^f	0	23	0	0
Total Brazil	1,696-1,731 ^f	504	193	84	119

^aIBAMA 2003; IUCN 2004.

^bMittermeier et al. 2003.

^cMMA 2000.

^dSilva 1995.

^eSilva 1995; Cavalcanti 1999; Silva & Bates 2002; Lopes 2004.

^fSilva et al. 2003.

^gUniversidade Federal de Pernambuco et al. 2002.

^hTubelis & Tomas 2003.

ⁱVooren & Brusque 1999.

^jCBRO 2003; IUCN 2004; NatureServe 2004.

3. Depicting major present and future threats:

Numerous species are threatened with extinction due to anthropogenic activities, as future population growth of humans is estimated of imposing further threats on more species (Tilman et al. 2017). A lot of threats could be held accountable for resulting in species extinction or vulnerability such as habitat loss, fragmentation, invasive species, pollution, natural disasters, human disturbances (hunting, settlement), trade and persecution (Marini and Garcia 2005). Tourism is also affecting protected areas, as they are becoming a popular destination to explore by nature enthusiasts (Balmford et al. 2009). Studies of the effects of forest fragmentation in Brazil were initiated by Willis (1979), and thereafter followed a long term study monitoring bird populations before and after habitat fragmentation, and this study is still ongoing as more studies are coming out on the Amazon fragmentation (Marini and Garcia 2005). In addition to habitat destruction, another threat is bird trading, in Northern Brazil for example, 92 wild bird species were found to being killed, kept as pets, used for medical purposes or even for food (Ferreira et al. 2012). The Glaucous Macaw and Spix's Macaw have been victims of illegal trading which has led to their probable extinction – at least in the wild (Marini and Garcia 2005).

4. Conservation initiatives:

When it comes to conservation initiative, it is important to include members of citizen and make them involved (Greenwood 2007). Birdwatchers for example contribute to generate information and fill some gaps. University programs raise awareness for the public and teach them about their country's biodiversity and the threats it is facing, and additionally provide necessary guidance for

the public to participate in data collection (Develey 2021). Usually the public is welcome to help and volunteer in some tasks that do not require a scientific background, as long as they are interested in the environment and want to make a change (Greenwood 2007). The Ministry of Environment in each country should also be involved in conservation programs, have projects and programs that will help in conservation of birds and also fund projects (Marini and Garcia 2005). In extremely critical cases, assigning protected areas is not enough, if the population has already reached very low levels, direct management takes place in many aspects, predator control or management of key sources for feeding and breeding (Develey 2021).

After all what's been mentioned above, not once, has it been stated that conservation planning takes into consideration genetic information of threatened species. After checking what are the most addressed questions when writing an article, the results showed that the majority of papers are concentrated on species' status when considering conservation planning, and very little do they look into genetic and taxonomic matters (Table 2, Brooks et al. 2008). Which means that if a population seems to be stable, conservationists are indirectly assuming that the species is safe, and not threatened in anyway. However, as much as it might seem to be maintaining a good fitness, things might be radically different on a molecular scale. In other words, phenotypic characteristics do not always reflect what is happening on a genotypic scale, the more hybridization occurs, the more pure genotypes and important genetic information might be lost. It is more common and frequent for hybridization to result in loss of alleles, genetic drift and decrease in genetic diversity in the offspring, than the gain of adaptive traits. The following paragraphs will highlight the effects of hybridization and the importance of including genetic data in conservation planning, complemented by examples of bird species proving its significance.

Table 2. Most focused-on issues among 114 papers published in Bird Conservation International (2004 – 2008) (Brooks et al. 2008)

Issue	Number of papers
Species status – includes population estimates, declines	62 (43%)
Ecology – includes breeding	29 (20%)
Threats – papers dealing with a specific threat	17 (12%)
Habitat associations	16 (11%)
Taxonomy	6 (4%)
Methodological	4 (3%)
Captive breeding	3 (2%)
Genetics	3 (2%)
Results of conservation actions	3 (2%)
Socio-economic studies	1 (1%)

Importance of conservation genetics

Among the large variety of goals conservation aims to achieve, preserving genetic diversity is an essential one (Taft et al. 2020). Genetic diversity is crucial in the conservation biology field because it helps to further understand the temporal, spatial and evolutionary processes (Flores-Manazanero et al. 2021). Genetic diversity can be affected by habitat fragmentation and changes in a habitat's quality either from management methods or from natural disturbances (Nakahama 2020). For example, oceanic islands are known for their high endemism rates, unique biota and genetic differentiation of populations after their establishment from a small sized population of mainland migrants (Martínez et al 2019). Their populations are often facing damaging genetic processes such as drift and bottlenecks (Flores-Manazanero et al. 2021). A study on the effects of inbreeding depression on natal dispersal in Red-Cockaded Woodpeckers in 2000, showed that as a result of close inbreeding, offspring showed lowered fitness, lower hatching rates, natal dispersal of females was not sufficient to avoid mating with close males (Daniels and Walter 2000). Hence, genetic disturbance results in the loss of original spatial genetic structure of a certain species leading to different behaviors (Nakahama 2020). Genetic disturbance also tends to threaten outbreeding depression, meaning a reduced fitness as a consequence of hybridization between genetically divergent individuals (Nakahama 2020). The genetic information is important for conservation planning of endangered species as it helps preventing inbreeding leading to decline in fitness in some cases, hence avoiding genetic disturbance and genetic diversity decrease (Nakahama 2020). Conservation genetics relies on tools and concepts of genetics in order to apply them when facing problems in conservation (Hedrick and Miller 1992), it uses species' genetic information to preserve them (Frankham, et al 2002). It provides insights into the extinction risk of small populations, defines conservation units and helps to understand the effects of hybridization (Frankham, et al 2002). Conservation genetics relies on molecular genetic techniques which started with mitochondrial DNA (mtDNA) and protein electrophoresis, that are very old methods and haven't been used since the emergence of new more developed techniques. However they have been fundamental in documenting the extent and pattern of genetic variation in endangered species (Hedrick and Miller 1992). New genomics techniques in Novel Generation Sequencing techniques (NGS) are Restriction-site Associated DNA sequencing (RADseq), exon capture and whole genome sequencing (WGS).

Conservation genetics has accomplished several achievements, (a) Massive applications of markers generated a huge data-base on the effects of small population size on the extent and distribution of neutral genetic variation (Ouborg et al. 2010). (b) It designed efficient methods to translate neutral marker patterns in space into inferences about demography, gene flow, effective population size, metapopulation structure and phylogeography (Ouborg et al. 2010). (c) Important insights into processes that are associated with conservation genetics paradigm (Ouborg et al. 2010).

Including genetic information when preparing conservation plans is an essential need that helps preventing inbreeding and outbreeding depression as a result of genetic disruption (Nakahama 2020). Even though it has become well recognized, conservation management and policy are more often than not made without including genetic data (Taft et al. 2020). There are some examples however, that show the importance of genetic data, such as the study by Muniz, et al. (2019), on *Dimorphandra exalata* combined both geographical and genetic data to assess the extent of threat this species is facing. The geospatial results classified the species as endangered, additionally the genetic data showed that the species has low adaptive potential. The overall results showed that the species is even more threatened when the genetic information was added to the geospatial analyses (Muniz et al. 2019). Additionally, Taylor and Kearns (2021) stressed on the importance of genetic factors in species' long-term persistence by showing the opportunities that it provides to conservation planning. Another reason why including genetic information into conservation plans is important, is the challenging task of prioritizing sites containing endangered species that are broadly distributed and have high genetic diversity (Sork et al. 2009). In a report by Sork et al. (2009), on California Valley Oak conservation, their results show that in order to protect genetic diversity of the species efficiently, sites of high allelic diversity should be considered. In fact, ignoring genetic diversity in conservation planning of threatened and endangered species, tends to end up in management outcomes that are lower than the expectations and in some cases could lead to disastrous decisions (Frankham et al. 2002).

Molecular conservation techniques:

Molecular conservation genetic techniques are useful in conservation as they resolve taxonomic uncertainties and provide new information on little known species (Frankham et al. 2002). Genetic surveys can include genes that might affect a population's fitness (Wayne and Morin 2004). They

are used as an aid to help conservation biologists to implement programs aiming to preserve genetic diversity in captive and natural populations (Hedrick and Miller 1992). A large range of genomic resources is being used to categorize diversity at multiple evolutionary levels, ranging from close relationship of an individual to the relationship of populations (Wayne and Morin 2004). The following briefly explains the aspects that conservation genetics covers, the very first techniques used, and the newest ones, and how they help in conservation genetics.

Small populations:

Endangered species, by definition, have a small or declining population and are sensitive to environmental changes (Freeland 2005). Small isolated populations are vulnerable to genetic drift which is translated by a random fluctuation of allele frequencies over time leading to the random loss of alleles, and inbreeding (Ouborg et al. 2010) due to higher chance of mating between relatives, leading to an increase in homozygosity (Freeland 2005). Genetic drift is common and quick in small populations (Freeland 2005). There are three consequences to the small population size, (i) Increased homozygosity and frequency of deleterious alleles leading to inbreeding depression (Ouborg et al. 2010). (ii) Loss of genetic variants which will reduce the long-term viability of a population especially in changing environments (Ouborg et al. 2010). Since levels of genetic diversity are low, it results in dangers on the small population size, because chances of adapting to a changing environment will get lower (Freeland 2005). As more deleterious alleles will be reach fixation, their accumulation consequently will lead to a reduction fitness of the population (Freeland 2005). (iii) Increase of genetic divergence in small isolated populations as a consequence of genetic drift in the population (Ouborg et al. 2010).

Conservation Units (CUs):

Conservation units are population units identified and used to help in conservation management. They are the first step in conservation in order to help in assessing a population's status and aim to increase population growth rates (Funk et al. 2012). When using genomic data to define CUs, it is important to take into consideration, that genomic data allows quantification of adaptive variation, microsatellite data can be used to identify ESUs and MUs, species with significant adaptive variation such as environmental variation among habitat patches, large effective population size and low migration rates (Funk et al. 2012). In order to overcome some of the

taxonomic problems, conservation biologists delineate conservation units of which (a) Management Units (MUs) and Evolutionary Significant Units (ESUs) (Freeland 2005).

ESU is a population or group of populations that warrant different management and priority for conservation due to the high genetic and ecological differences (Funk et al. 2012). Their maintenance will maximize the evolutionary potential in the face of environmental change.

MUs are populations that do not depend on demography, and their growth rate depends on local birth and death rather than migration (Funk et al. 2012). Their maintenance is useful for short-term management and ensures a long-term persistence of the species.

Hybridization

Conservation genetics helps to understand the effects of hybridization. Many studies using microsatellites were conducted to estimate hybridization events (Ouborg et al. 2010). Genome-wide markers are being developed and used for studies of hybridization events (Ouborg et al. 2010). More details on hybridization figure in the next part.

Protein electrophoresis

Protein electrophoresis was one of the most common techniques used to analyze genetic variation. It was a very useful approach, that helped in species identification and was able to track back evolutionary path of the species in question (Ladizinsky and Hymowitz 1979). It was also used to estimate the purity of captive populations.

Mitochondrial DNA analysis

As mtDNA is maternally inherited, it allows depicting the maternal lineage of the studied species (Hedrick 1992). Second, being a highly polymorphic DNA, the mtDNA can help in studying the geographical range of populations or examine the differentiation between closely related species (Hedrick 1992). This analysis gives important information for phylogenetic studies and is relevant for long-term conservation planning (Moritz 1994). This technique could be used in three ways, (a) to measure genetic variation within populations, (b) to identify evolutionary divergence of populations, and (c) from an evolutionary or phylogenetic point of view, to assess conservation value of populations (Moritz 1994).

DNA fingerprinting

DNA fingerprinting was used to reveal restriction fragment length polymorphisms at hypervariable microsatellite loci scattered in the genome (Degnan 1993). The variable number of tandem repeats (VNTR) also known as microsatellites, are mostly used for DNA fingerprinting (Hedrick 1992). These loci consist of short head-to-tail tandems, which are repeated units of 10 – 60 nucleotide base pairs (Degnan 1993). DNA fingerprinting is used to understand levels of genetic variations in natural and captive populations (Lynch 1990). Unrelated individuals tend to have different and unique genetic “fingerprint” (Hedrick 1999). They allow scientists to differentiate between populations of animals (Hedrick 1992). It has been proved to be very useful when studying relationships among animal species as each individual has its own unique fingerprint (Degnan 1993).

Genomics and Next Generation Sequencing (NGS)

Sequencing technology in which collections of DNA molecules can be sequenced simultaneously (Barbosa et al. 2020). This technology keeps on providing new platforms for sequencing and increase the feasibility and speed of generating reference genome assemblies for wildlife species (Barbosa et al. 2020). NGS tools can provide a large amount of data (Hohenlohe et al. 2021).

- RADseq:

Population genomics techniques can target thousands of loci across a genome, they can either be pre-selected or anonymously distributed across the genome, as a result of RADseq which uses restriction enzymes. Data from RADseq, are usually used as SNP genotypes. In case of wanting to develop a smaller panel of markers for genotyping large numbers of samples overtime, RADseq is used (Barbosa et al. 2020). When studying wildlife species, this technique does not require any prior genomic knowledge for the species (Hohenlohe et al. 2021). This application has seen progress in application to low-quality samples (Hohenlohe et al. 2021). RADseq can be used to estimate the effective size of a population and its declines overtime, which have been proved to be very effective (Hohenlohe et al. 2021).

- Whole-genome sequencing (WGS)

Produces data from every part of the genome and is increasingly feasible for most taxa (Hohenlohe et al. 2021). It does not require prior genomic knowledge of the species of concern as in the

RADseq (Hohenlohe et al. 2021). Whole-genome Sequencing data from a relatively small number of individuals can provide information across a range of timescales from demographic history and phylogenetic relationships among widely separated populations over the last two million years, to inbreeding (Hohenlohe et al. 2021). It has progressed in application to low-quality samples (Hohenlohe et al. 2021). It is important in small populations as it helps gathering the maximum amount of genetic information (Hohenlohe and Rajora 2012).

- Exon capture

DNA capture is becoming increasingly used for NGS approaches with low-quality samples (Andrews et al. 2018). This method involves creation of genomic DNA libraries for sequencing on a NGS platform (Andrews et al. 2018). Exon capture can target areas under selection more directly and provide more accurate results regarding population-specific adaptive responses (Barbosa et al. 2018).

Genetic rescue

Other conservation solutions are emerging as well such as the genetic rescue (Hogg et al. 2021). This solution is capable of increasing a small population's fitness by introducing new alleles (Whiteley et al. 2015), it is part of the novel solutions (Hogg et al. 2021). Genetic rescue techniques are highly effective as they can reverse the deleterious effects of inbreeding (Heber et al. 2012). However, this is not always a reliable solution for critically endangered species where only one viable population remains, in that case, options of introduction of new alleles are limited (Hogg et al. 2021). Genetic rescue has been used on 2 bottlenecked South Island robin (*Petroica australis*) populations, by using translocations of inbred donors (Heber et al. 2012). Reciprocal translocation increased genetic diversity which was accompanied by the increase of juvenile survival rate, increase of sperm quality and immunocompetence of hybrid individuals, the results compared the offspring result of crosses between the donor populations and the bottlenecked populations, to the inbred control individuals which are crosses within each population (Heber et al. 2012).

Hybridization

Hybridization is a very common phenomenon in bird species and specially among waterbirds (Muñoz-Fuentes et al. 2007). Several cases of hybridization between bird species due

to human activities have been reported. After the introduction of mallards within the Hawaiian duck (*Anas wyvilliana*) and New Zealand grey duck (*Anas superciliosa superciliosa*) populations, in both cases there has been extensive hybridization with the introduced species (Muñoz-Fuentes et al. 2007). This hybridization led to the near disappearance of Hawaiian Duck alleles (Browne et al. 1993). Hybridization is the occurrence of species interbreeding with each other (Grant and Grant 1992), and has important evolutionary consequences (Rutherford et al. 2019). It can occur naturally which is mostly common in plant species (Zheng et al 2021), it is also possible to find it in animal species, one of the most popular species affected by natural hybridization are fish, where it has been found that sympatric species of *Astyanax paranae* and *A. fasciatus* are hybridizing in the natural environment in a waterfall area in the Grande River, Southeastern Brazil (Pinheiro, et al 2019). Natural hybridization often occurs in hybrid zones where several species live in sympatry (Neri et al 2017). However, hybridization does not always occur naturally and is often human driven, indirectly (Todesco et al. 2016). Human actions have been affecting natural environments, by altering habitats, introducing invasive species, climate change, deforestation and habitat fragmentation (Brennan et al. 2014). These anthropogenic activities are consequently affecting biodiversity in several ways, one of them being hybridization (Brennan et al. 2014), as they resulted in the expansion of some species' geographical ranges (Muñoz-Fuentes et al. 2007) and inducing interactions between species that have been previously isolated, and have never been in contact (Todesco et al. 2016).

Case study of Prince Ruspoli's Turaco and White Cheeked Turaco

A recent example is found in Ethiopia, where two endemic species of turaco are found in the Ethiopian plateau, the White-cheeked turaco, *Tauraco leucotis* and Prince Ruspoli's turaco, *Tauraco ruspalii*. The latter are restricted in a small range on the southern part of the plateau (Borghesio et al. 2014). White-cheeked turaco are almost exclusively found in *Podocarpus* forests and select a smaller range of habitat when co-occurring with Prince Ruspoli's turaco (Borghesio and Massa 2000). Prince Ruspoli's Turaco on the other hand, inhabits transition formation and wet woodland and are better adapted in dry habitats than the white-cheeked Turaco (Borghesio and Massa 2000). These species' ranges share a common boundary (Borghesio et al. 2014). The White-cheeked turaco's population is still well maintained, however the Prince Ruspoli's is listed on the

Red List of threatened species (Borghesio et al. 2014). Due to habitat fragmentation White-cheeked Turaco seems to have expanded its region between 1995 and 2003 when most forested areas have been cultivated, overlapping with the Prince Ruspoli's Turaco (Borghesio, 2014). In 1995 Ruspoli's were still reasonably common, then occurred in the same region as White-cheeked turacos but with different habitat choices separating them and no evidence of hybridization was obtained (Borghesio et al. 2014). In 2002, the first hybridization between the two species was reported which might have been caused due to habitat fragmentation (Borghesio et al. 2014). In 2007 – 2008, several hybrids were found indicating that hybridization was becoming more widespread. Habitat degradation might be favoring this hybridization (Borghesio et al. 2014). This hybridization event might be of serious threat to Prince Ruspoli's turaco (Borghesio, 2000). Other major threats Ruspoli's turaco are facing are also human driven, such as egg collection used for lung diseases and habitat alteration as human presence keeps on expanding (Borghesio and Massa 2000). Occurrence of hybrids is widespread, however all the studies carried out on the hybrids have not yet obtained enough data to generalize the hybrids' status and have a clear view on the effects of this hybridization (Borghesio, 2014).

An important thing to keep in mind, is that hybridization between two species that were totally isolated from each other is radically different and has a dissimilar outcome than mating individuals of the same species being genetically different (Stebbins, 1959). Hybridization can have several effects on species' evolution (Todesco et al. 2016). When species interbreed, gene flow generates new genetic recombinations (Rutherford et al. 2019). The consequences of hybridization range between two extremes, the first being stability by procuring new adaptive solutions to different environments (Rutherford et al. 2019), or even increasing the diversity, which may occur in stable hybrid zones, the second, however, being the opposite and leading to a decrease in diversity by causing breakdown of reproductive barriers (Todesco et al. 2016). The white-headed ducks, for example, suffered from hybridization with the introduction of ruddy ducks in Spain, after being added to a zoological collection in England and have expanded to other western countries (Muñoz-Fuentes et al. 2007). Effects of hybridization were studied by using molecular genetic markers to assess the degree of genetic introgression between the two species in Spain (Muñoz-Fuentes et al. 2007). The latter study inferred that there might be introgression occurring at a low pace, as few to no alleles of Ruddy ducks were detected in White-headed ducks. The fact that no ruddy-duck alleles have been found in the white-headed duck, shows that introgression is happening at a very

low rate, in case it is happening, which means it is not too late and there is time to prevent the extinction of the White-headed ducks (Muñoz-Fuentes et al. 2007).

Effects of hybridization:

Disadvantages

As already mentioned, hybridization has several impacts on species, it can be beneficial to the hybrids as it might introduce adaptive alleles or disadvantageous when hybrids lose alleles of high importance. One of the most common negative effects of hybridization is that it might result in extinction (Todesco et al. 2016). Two mechanisms are capable of doing so, genetic swamping and demographic swamping (Figure 2, Todesco et al. 2016). Genetic swamping also known as introgressive hybridization, results in hybrids being partially fertile and viable but replace rare pure parental genes which may result in extinction of pure parental genotypes (Gibson et al. 2019). Hybrid sterility, which is a complex phenotype, results from the breakdown of spermatogenesis (Larson et al 2022). This high change of having sterile or inviable hybrids is caused by post-zygotic incompatibilities between species (Rosser et al. 2021). Genetic swamping is known for being powerful as it strongly impacts patterns of evolution. It has affected speciation and diversification in several species (Bougie et al. 2021). A case of conservation concern is when this happens between native and non-native species, resulting in extinction of the native species along with its rare alleles (Gibson et al. 2019). In 1998, a study on Greater Prairie Chicken (*Tympanuchus cupido*), compared genetic variation between four populations with different demographic histories by using Polymerase Chain Reaction to estimate the allelic diversity, geographic differentiation and levels of heterozygosity (Bouzat et al. 1998). The four populations were from: Illinois (which had previously encountered a severe demographic bottleneck), Kansas, Nebraska, and Minnesota (Bouzat et al. 1998). The findings showed that the Illinois population shares more than 95% of its alleles with all the other populations (Bouzat et al. 1998). This explains the loss of genetic diversity of the Illinois population due to introgression leading to the decrease of its population size and suggests that the populations might have had higher levels of genetic diversity that were lost through hybridization (Bouzat et al. 1998). This genetic loss, might lead to the extinction of the Illinois Greater Prairie Chicken population. Figure 2 (B) shows how genetic

swamping works, some of the hybrids are fertile and the more they reproduce with the parent species, the more the parental genotypes are being lost.

Demographic swamping, on the other hand, is when hybrid individuals are not viable, they are entirely removed and consequently with them goes all the rare lineage alleles, leading to the population or lineage's extinction (Todesco et al. 2016). In other words, demographic swamping results in sterile hybrids, or hybrids showing lowered fitness, therefore population growth of the hybrid individuals may be lower than the growth required for replacement of one or both parental taxa (Prentis et al. 2007). E.g, as a result of hybridization between the pied flycatcher (*Ficedula hypoleuca*) and the white-collared flycatcher (*F. albicollis*) in an area of sympatry, hybrid females are sterile, and males are not, however they are less successful when forming breeding pairs (Rubtsov, 2015). Demographic swamping is less frequent than genetic swamping (Todesco et al. 2016). Figure 2 (A), explains better how each mechanism works. When demographic swamping occurs, rare parental genotypes (red flowers) are going extinct, due to unviability or sterility of hybrids. On the right-side of each figure, percentage of rare genes keep on decreasing with each generation, thus leading to extinction.

Advantages

Despite these disadvantages, hybridization can lead to transfer of genes that will offer advantages in novel environments. Procuring adaptive traits, is one of the most important traits hybridization can give to the hybrids, it can cover many levels as it could be biochemical (e.g., produce enzymes), morphological (e.g., beak shape in birds) or behavioral (e.g., fleeing when in danger) (Hughes 1999). Adaptive traits allow the individual to perform a function that will be beneficial to its fitness (Hughes 1999). For example, the Italian sparrow, a hybrid species, which has resulted from hybridization between the house sparrow and the Spanish sparrow, showed significant introgression with both parents, the study showed that mosaic parental inheritance and new divergence in the hybrid lineage facilitated the maintenance of the hybrid species, Italian sparrow (Elgvin et al. 2017). The Italian sparrow is a good example that shows the benefits that hybridization brings to hybrids by showing how it contributes to diversity (Elgvin et al. 2017).

In plants, as well, there have been cases where hybridization was beneficial to the hybrids, which is the case of *Helianthus*, where hybrids have showcased a more rapidly evolving higher fitness than non-hybrids in an eight-year study (Mitchell et al 2019).

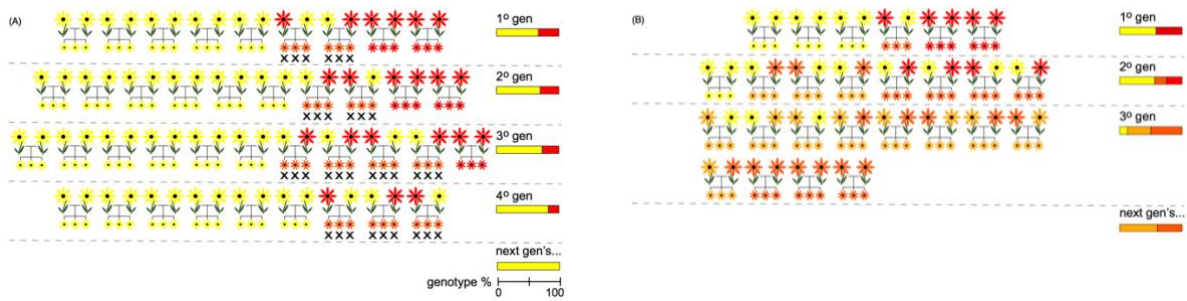


Figure 2. Hybridization results; (A): Demographic swamping where unfit hybrid individuals (designated by light and dark orange flowers) are completely removed, this results in the extinction of the whole population or lineage. (B): Genetic swamping, hybrids are partially fertile and viable, they replace their pure parental genotype (extinction of parental genotype). Red: rare; yellow: common. On the right side of each figure: percentage of rare, common and hybrid genotypes are indicated by color-coding (Todesco et al. 2016)

In addition, interpopulation hybridization has the ability to increase the viability of small populations undergoing inbreeding and genetic drift. After inbreeding, heterosis can take place, which is an increase in fitness of the hybrid offspring generation, this is due to the masking of recessive deleterious alleles in heterozygous individuals (Pekkala et al. 2012). Other positive fitness effects can result from positive interactions between loci, or from perturbation of negative interactions that may have been fixed in small populations through genetic drift (Pekkala et al. 2012).

Haldane's rule:

A great fitness reduction, translated as lower viability or sterility (Johnson and Lachance 2012), is common in hybrids and evolves first in the heterogametic sex, this pattern is known as Haldane's rule (Rosser et al. 2021). Usually two factors lead to this result, the faster X effect and the large X effect (Presgraves 2008). The faster X effect is simply explained by the fact that genes belonging to the X chromosome have a faster evolution than autosomes, as they reveal more interspecies divergence in transcript expression levels than autosomal genes (Yoshida et al. 2014). The large

X effect, is the disproportionately large role of the X chromosome in reducing hybrid fitness (Presgraves 2018). Birds, are known for having a ZW system to determine their sex, according to Haldane's rule, female hybrids (heterogametic: ZW) will have lowered fitness compared to male hybrids (ZZ) (Ottenburghs 2022). Haldane's rule has been found to affect birds in general and some specific bird groups such as ducks (Ottenburghs 2022). A study on the secondary contact zone between the Herring gull (*Larus argentatus*) and Caspian gull (*L. cachinnans*) was done to estimate the probability of survival rate of hybrids to further show how Haldane's rule also applies on bird species, found that hybrid males did not show a lower survival rate than nonhybrid ones, however hybrid females turned out to have the lowest survival rate (Neubauer et al. 2014). The first recorded female hybrid between the Common Nightingale (*Luscinia megarhynchos*) and the Thrush Nightingale (*Luscinia luscinia*) in nature was found during the breeding season, but did not show any signs of reproductive activity even though there were males calling for mating and it was the peak time of breeding, which suggests the sterility of the individual (Reifová et al. 2011). A more recent study conducted in 2018 identified 136 regions of elevated differentiation of genomic islands in the hybrid nightingale females, one of the pathways affected was the oocyte meiosis pathway, and had high differentiation in genomic islands, which explains the sterility of the female hybrid (Morkovsky et al. 2018). In order to explain this faster X effect and large X effect which both apply to the Z chromosome as well, following the 1:1 sex ratio, which was first described by Fischer (1930), each autosome should be equally represented in both sexes (half the time in males and half the time in females) (Johnson and Lachance 2012). However, when it comes to sex chromosomes, there is no equal representation. In the XY system, the Y chromosome will be exclusively found in males, while the X chromosome will be found 2/3 of the time in females and 1/3 of the time in males (following the 1:1 ratio) (Johnson and Lachance 2012). In the ZW system, the W chromosome will be exclusively found in females, and the Z chromosome will be found in males 2/3 of the time and 1/3 of the time females (Figure 4, Johnson and Lachance 2012). This unequal distribution of sex chromosomes between the sexes, will have the sex chromosomes undergo different effects of evolution (Johnson and Lachance 2012).

On a molecular level, Haldane's rule has several effects, more specifically with introgression rates of different loci (Ottenburghs 2022). First, as female hybrids (ZW) tend to be less fit than males, all of the maternally inherited loci will have low levels of introgression (Ottenburghs 2022).

Second, the faster evolution of Z-linked loci has the ability of speeding up the accumulation of incompatible alleles on this chromosome (Ottenburghs 2022).

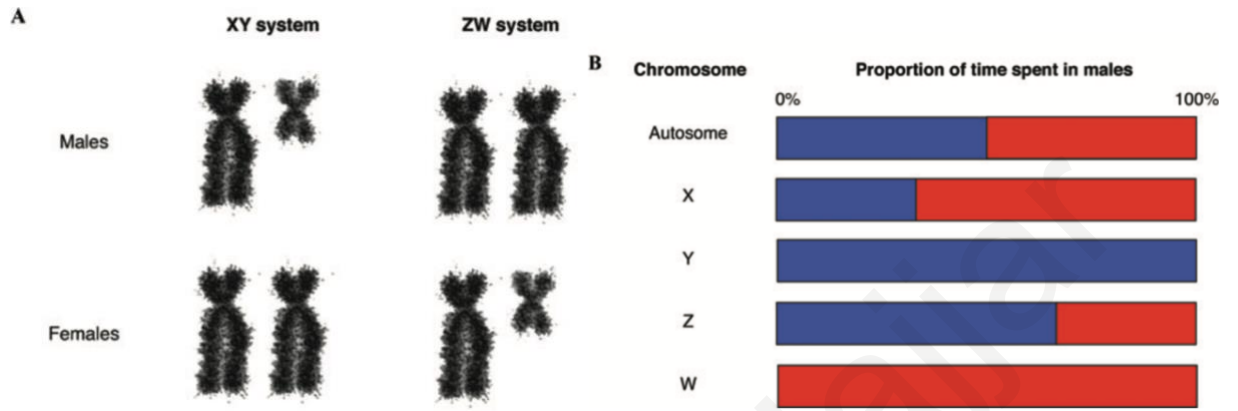


Figure 3. (A) Representation of sex chromosomes in XY and ZW systems, where males and females are heterogametic respectively. (B) Representation of the distribution of each sex chromosome in males, red indicating the females proportions and blue indicating the the male proportion (Johnson and Lachance 2012).

Why is biodiversity conservation important?

Most of the people's concern about biodiversity conservation relates to instrumental and utilitarian reasons, to satisfy their needs (Randall 1991). Instrumental services provided by nature, such as food, water and chemicals for pharmaceutical reasons, are important, however, it is not the only purpose of biodiversity (Randall 1991). Biodiversity, on all its levels, including animals, plants and micro-organisms, is fundamental in many forms, as it maintains the integrity of the environment through several processes (Mutia 2009). It maintains CO₂/O₂ balance, it regulates biochemical cycles such as hydrological cycles, it is in charge of waste decomposition by consuming it, determines and regulates natural world climate (through influencing temperature, precipitation and air turbulence) and acting as bioindicators (Mutia 2009). The environment procures ecosystem services that benefit humans, and are maintained with the help of biodiversity (Sekercioglu et al. 2004). For example, ecosystems such as wetlands have an important role in

purifying water, forests assimilate greenhouse gases and contribute in maintaining the oxygen balance (Randall 1991). Additionally, if one species is lost, it will affect the whole chain, as everything in the environment is interconnected (Randall 1991). Yellowstone Park, a very famous example highlighting the importance and influence of each species in its ecosystem. Wolves have been removed from Yellowstone Park for 7 years, during which the trophic level collapsed and allowing elk to take over and affect the ecosystem (Ripple and Beschata 2012). Elk being primary prey of wolves, took advantage of their absence and their populations increased, leading to overgrazing, and may other species population density have changed and led to negative effects (Smith et al. 2003). After the reintroduction of wolves in the park, all the aspects of the park have changed, as they induced a more balanced ecosystem, most importantly by preying again on the elk, thus leading to an improvement of the collapsed trophic chain (Ripple and Beschata 2012).

Birds contribute to all four types of ecosystem services (a) provisioning, (b) regulating, (c) cultural and (d) supporting services (Whelan et al. 2008). As most bird species fly, migratory ones link ecosystem processes that are very far apart in time and distance (Whelan et al. 2008). They play an important role in decomposition, pollination, seed dispersal, pest control and many others (Sekercioglu et al. 2004). They modify the environment in ways to benefit other species as well (Whelan et al. 2015). Here are some details regarding the birds' contribution in ecosystem services. (a) Provisioning services comprise mostly marketable services such as food, feathers, skin and blood for medicinal values and nutrient fertilizers such as Guano-seabirds (Mahendiran and Azeez 2018). (b) Regulating services; pest control through birds, does not only mean their consumption, but more importantly affecting the pests' population resulting in a positive impact (Whelan et al. 2008). Scavenging *e.g.* vultures, which are scavengers consume most of the carcasses available, thus contributing to waste removal and disease regulation (Whelan et al. 2008). Nutrient transfer, where birds move nutrients from rich to poorer areas which is mostly seen from aquatic to terrestrial areas, *e.g.* tons of nutrients are transferred annually via bird Guano (Mahendiran and Azeez 2018). (c) Cultural services, which range from religious to entertainment and photography (Mahendiran and Azeez 2018). Finally the supporting services (Table 3, Mahendiran and Azeez 2018). As seen in table 3, birds play a role in pollination, *e.g.* 58 Indian bird species are involved in pollinating 93 flowering plant species (Subramanya and Radhamani 1993). Over 920 species worldwide are pollinators of plants, including Hummingbirds, Flowerpeckers, Honeyeaters and others (Whelan et al. 2015), with the help of their body size and beak morphology that varies a lot

between nectarivore species (Subramanya and Radhamani 1993). They are involved in seed dispersal as well, which is among the most important ecosystem services provided by birds (Whelan et al. 2008). Fruit-eating birds for example, defecate seeds after digesting them, which is known as accidental dispersal (Howe 1986).

Table 3. Supporting services of birds (Mahendiran and Azeez 2019)

Non-market values*		Categories of Non-Market Services						
		Species dispersal (Pheasants, Ducks, Passerines), Pollination (Humming birds, Sunbirds, Flowerpecker, White eyes, Honey eaters, Lories, Hawaiian Honeycreepers, Warblers), Big seeded tree disperser (Bellbirds, Hornbills)	Role of disease control (Vultures), Obligate Scavengers (old world & new world Vultures), Facultative Scavengers (Heron, Rails, Skuas, Willet, Turnstone, Gulls, Plovers, Raptors, Woodpeckers, Crows, Tits, Starlings)	Energy Transfer Top level predators / Fish eating birds, Nutrient Transfer (Colonial Waterbirds, Seabirds), Bioturbation (Swans, Flamingoes)	Colonization & Regeneration of forest lands (Hornbills, Jays, Bellbirds, Cormorants), Frugivores effect on forest biome (Toucans, Manakins, Birds of paradise, Waxwings, Bulbuls, Thrushes, Tanagers & Bellbirds)	Rodent control (Owls & Hawks), Insect control (Sparrows, Cuckoos, Indigo buntings, Babblers, Warblers, Flycatchers)	Maintenance of vegetation profile in waterbodies (Grebes, Diving, Dabbling ducks), Shoreline biomass (Charadriiformes: Waders)	Human bird interactions (Honeyguidesetc)
Supporting / Habitat Services	Intrinsic Value ²	Seed disperser spatially & temporally (Waterbirds, Shorebirds, Waders); Plant, Animal dispersal -endozoochory, epizoochory (Anseriformes)	Primary cavity excavators (Woodpeckers, Trogons), Cavity Drillers (Sapsuckers), Seed Catching (Jays & Nutcrackers)	Bioperturbation (Burrowing seabirds) Soil burrowers, soil regeneration & enrichment (Penguins, Seabirds, Parrots, Owls, Kingfishers, Song birds, Bee-eaters)	Leaf litter gleaners (Antbirds), Beaters & Followers (Waders, Kites, Kingfishers; Woodpecker, Grackles, Drangos) Mixed flock foragers (Drangos, Flycatchers, Bluebirds, Treepies, Bulbuls, etc)	Feeding opportunities (Antbird follows army ants which was followed by Lepidopteranfemalebutterfly) Ship following birds (Albatross, Gulls & Skuas), Tractor following bird in Agricultural field (Cattle Egrets, Mynas, Crows)	Exclusive bird pollinating large seed plant taxas (Burseraceae, Sapotaceae, Lauraceae)	
	Intrinsic Value, Non Use ³	Taxonomic diversity	Ecological diversity	Genetic diversity	Natural History knowledge related to cultural identity, traditions etc	Knowledge value		
Bequest/vicarious values ⁴	Existence Value ⁵	Bird Sanctuary	National Park, Forests	Important Bird Areas	Heronries, Rookeries	Wetlands, Ramsar sites	Migratory Bird flyways	

Birds are ecosystem engineers, because they are constantly modulating resource availability for other species directly and indirectly (Mahendiran and Azeez 2018). Ecosystem engineers are organisms that create, modify or maintain habitats for other species to use, beavers (*Castor canadensis*) for example are a famous and ideal example, as they build dams that will trap nutrient-rich sediments, which after being abandoned, will result in meadows forming and persisting for more than 50 years (Wright et al. 2002). The European bee-eater for example, has the ability to dig burrows in sandy cliffs in order to breed there, these burrows would be later on used by numerous organisms for several purposes (nesting, completing stages of their lifecycle, reproduction, resting) (Casas-Crivillé and Valera 2005). Indeed, 19 vertebrates used the old bee-eaters' burrows, for mostly breeding, roosting or seeking shelter (Casas-Crivillé and Valera 2005). Additionally these burrows also play a role in basic ecological processes such as erosion, because

by digging these burrows they redistribute resources (Casas-Crivillé and Valera 2005). Some of the birds' diets have contradictory effects on the environment, scavenger birds for example which help in disease control can also spread diseases, e.g. American crows, *Corvus brachyrhynchos*, might facilitate proliferation and dispersal of prion disease, if the scrapie prions can survive the digestive system of the birds (Whelan et al. 2008). Another example of negative impacts of birds is eutrophication, after the colonization of southwestern Finland by the Pochard, *Aythya ferina*, the Reed Bunting, *Emberiza schoeniclus* and the Reed Warbler, *Acrocephalus scirpaceus*, dense vegetation growth appeared around shores, which is known as a consequence of eutrophication (Von Haartman 1983). This is explained by their nutrient cycling which leads to high levels of nitrogen and phosphorous ending up in aquatic system, where aquatic birds such as ducks and geese excrete (Whelan et al. 2008).

Researchers have predicted how the loss of pollinating birds might affect the plants that depend on them. Bird number reductions could stop or decrease ecosystems services, such as pollination (Anderson et al. 2011). This study on the North Island of New Zealand, show that seed production per flower will be reduced by 84% and fewer juvenile plants. Another study also supported these expectations, by studying the long-term demographic effects of pollination failure following pollinating birds' extinction (Sekercioglu 2011).

DISCUSSION

Conservation is very common nowadays, as we are witnessing the sixth mass extinction, which englobes 75% of earth's species. It is crucial to sustain the environment as one species' extinction can lead to catastrophes, just as what happened in Yellowstone Park, so what could happen if 75% of the species went extinct? It is dreaded that such a big number of species would be lost, which is why scientists, NGOs and Governments are constantly working on conservation plans to implement and execute as soon as possible, and always aiming for the best results they can achieve. However conservation planning requires a lot of work and challenges to face, one of the fundamentals is to have a very strong knowledge on all the species and their geographical range, next is to assess and infer through the data collected, which species are mostly in critical cases and on the verge of extinction. It is also important to make sure which ones are endemic and what range do they cover. Following that, it is important to depict the threats that are affecting the endangered species, how long have they been affected, what has been the outcome of each threat. Then depicting future threats that would also have negative impacts on the species in question. After collecting all this information, conservation planning starts accordingly, as different methods and techniques can be used in different cases. In some cases it is possible to avoid the threat, in others it is not so the second option is to minimize the threats as much as possible. Conservation planning requires scientists, NGOs and governments, however, in order to get a grip on all the data mentioned, it is quite challenging and this requires a lot of efforts. This is why, it is very important for the people to be more involved in such studies. Some tasks are given to them, which do not require a scientific background, mostly, these plans get help from birdwatchers, wildlife photographers or nature enthusiasts. This public's involvement accentuates the importance for awareness that should be spread among the people. Awareness programs, fieldwork and workshops should become more common. These activities will teach citizens about birds, about their biology, they will also teach them how to identify species by their songs and their morphology. After putting the people in contact with nature, they will become more interested and more devoted in helping. As most of the people like birds just for what they look like and sound like. However, this is only the tip of the iceberg, as birds are fascinating creatures that play very

important roles in the environment and ecosystem services. After learning all the above, more interaction will be observed from society willing to help and take part in such important events. With the help of these programs, people will be more interested and willing to help and they will learn not to hurt and overconsume nature's gifts.

As already stated, conservation planning rarely includes genetic information, however, with all the changes in birds' habitats and their fragmentation, hybridization is a very common process resulting from all the threats. Ignoring such events, may not lead to good long-term sustainable conservation plans. These interspecies relations will lead to hybridization, which is not always a concrete thing that can be seen, as not all hybrids need to show different morphologies or abnormalities, most of the time hybridization makes changes on molecular levels and alters the whole genome of the species. Sometimes hybridization might lead to positive effects on fitness such as adaptive behaviors and beneficial alleles. Hybrids have genetic variation that can lead to the extinction of one of the parental lineages in the case of genetic swamping, in other words, it can lead to extinctions. In heterozygous species (following ZW or XY systems), heterogametic hybrids tend to develop lower fitness than homogametic hybrids. This applies to bird species, as hybrid females show lower fitness signs, either by their low viability rate or their sterility. As hybridization results in different and various outcomes, it is important to clearly understand what is really going on, on a molecular level, and see how genotypes are being affected after interspecies interactions, than relying on their phenotypes. Since hybridization might lead to extinction, on the long-run, it is recommended to include genetic information in conservation planning as it can bring clearer views on what the species are going through. This will be possible with the application of Next-generation sequencing methods, as when sequencing a genome, or specific loci, with the right analysis, it is possible to see how hybridization is affecting the hybrids and what alleles are being lost throughout the course of this hybridization, and what alleles are still available in the populations. Genetic diversity is crucial for the long-term sustainability of a species, as some alleles that are considered neutral, and are found in the species' genotype, could be of high adaptive importance when environmental conditions change. It is also crucial to save species, not just for our benefit when it comes to food for example. However following what have been aforementioned, every species has a very important role to play in their ecosystems. And birds are one important factor, because they contribute to four ecosystem services. Additionally, hybridization has played an important role in evolution, and it is with the help of genetic analysis

and molecular techniques that researchers were able to depict the path species followed to end up looking and acting like what they do nowadays. More light should be shed on hybridization and molecular studies in regards to genetic variation between interbreeding species. As several examples have shown that hybridization led to different plumage coloration or different beak morphology, it is important to understand what is causing these changes, and what will result on the long-run from all the changes species are facing. Hybridization should not be disregarded and should be considered when working on conservation plans as it can help depict important knowledge that could change the way conservation planning is working.

Hybridization is one main reason to combine conservation genetics in conservation biology, as it brings a lot of knowledge on species' genetic variation. Nevertheless genetic data can contribute to lots of benefits for conservation planning. It helps in resolving problems that conservation faces and fails to depict, by improving a species' population status, by managing small populations, figuring out taxonomic uncertainties and even understanding a species' biology and behavior. Genetic studies have always been used to understand relationships between species, the pattern of their genetic variation, and how they have evolved, by using molecular techniques. The older, and the first conservation genetics techniques relied on mtDNA, which can help in depicting the maternal lineage it is issued from, as mtDNA is maternally inherited. Or used protein electrophoresis to identify genetic variants. DNA fingerprinting, to understand whether 2 species are related or not, because each species has its own unique DNA fingerprint. If it weren't for genetic data studies, none of the phylogenetic trees, taxonomic classification and relationships between species known to date, would have ever been discovered. These techniques weren't able to sequence bigger DNA samples, however with the development of these techniques, it is possible now to sequence the whole genome of a species.

One important example that supports the importance conservation genetics is the study that have been carried out by Muniz et al (2019) on angiosperm. As they have followed two strategies of conservation, one relied on the usual factors that conservation biology uses (demography, without including genetics) and led to concluding that the species classifies as endangered. The other relied on genetic data and showed that the species has very low adaptive potential and may not survive rapid long-term environmental changes. Both results combined made it clear that it is urgent to conserve the species, because genetic data showed that its adaptiveness is not of high importance

and the plant won't be resilient. Since genotypes have been found to be more stable than phenotypes, their analysis can bring to light very useful information on the species in question and they can solve problems that conservation biology fails to solve without their use, it is enough to show how important conservation genetics is, and how far it has come. Genetics played a big role in classification of species and relationships between them. Their combination with conservation biology would be a big advantage for the field and will make more, positive, powerful and sustainable outcomes in terms of conservation priority.

After all the literature that I have read, and the findings that conservation genetics has shown. I personally, support the combination of conservation genetics in conservation biology. So many techniques are used to answer different questions and problems tackled by conservation managers. It has been shown to be of high efficiency in all the studies that mentioned working with conservation genetics. Even though some techniques are expensive and require higher, stronger capacities and skills to conduct, it is going to end up in the favor of conservation planning and have promising results. Additionally, it was harder in the past to include conservation genetics in such studies due to the lack of data. However now, scientists and researchers have come a long way and know a lot more about many species. More importantly, 10.000 bird species are known to humans and data about the majority, if not all of them is collected. This should be a push for scientists to start including genetic information and data when planning on conserving bird species, specially that they are facing a lot of threats and a good percentage of them is facing extinction. Moreover, birds have contributed in conservation planning, as they are the most studied species in this field. Due to their study, conservation biology have gained so much improvement.. Conservation genetics should not only be included when conserving endangered bird species, however studies should cover as many species as possible, because when researchers study genetics of all the species, they will find a lot of undiscovered and unknown threats on the molecular level. Further studies should be conducted on all the extremely critical cases, and focus should be on newly hybridizing species, as it would be much easier to repair early problems, than when a lot of genetic variations pile up and end up being impossible to reverse what has happened. Even in such cases, it would still be possible to protect parental lineages, before they go extinct. It is also important to bring more attention to poor countries, as they host the majority of endangered species, and little work is achieved in order to prevent the species' extinction. This could be done by funding of richer countries and work of international researchers with the help of national

researchers on the species in question. More attention should be brought to the less “famous” species or “charismatic” species, as most of the publications are centered on the same famous species that will get the public’s attention, which is wrong, as all species have equal importance and should all be saved and protected without discrimination.

In conclusion, conservation planning is doing a great part in species survival and protection. It has come a long way and has shown a lot of positive results. However, how long can these results last if hybridization keeps on intervening? This question is a major problem in conservation biology, and the only component that could answer it is genetic data. For improvement in conservation planning results, it is important to include genetic data and information. This could be achieved after good communications between scientists and conservation managers. Moreover, providing appropriate funding for such analyses and having more people involved in such tasks. Even though these techniques require high skills and high costs, they will be rewarding. As much as it seems that conservation genetics is going to improve conservation planning, there will still be gaps to be filled, such as lack of genetic information of certain species. More scientists should focus on improving their skills in order to be able to work with conservation managers on problems they are facing. And with the little DNA information, researchers are able to assemble from fossils and historic museum, this task is also more challenging.

Finally, more improvement should take place in collecting species’ genetic information, because the more we know about a species the more we can understand what techniques will have a better result , for example being more successful in estimating the effects of hybridization on the affected species. And what should be urgently done to get the best efficiency in terms of conservation plans results. Researchers and conservation managers should come to a compromise to further show that this combination would be a step forward in biodiversity conservation.

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