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A Multi-disciplinary Approach to the De-extinction of the Pyrenean Ibex: Challenges and Opportunities

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ABSTRACT: Also known as "resurrection biology", de-extinction is reviving extinct species. Once known as science fiction, bringing species back to once-extinct life has gained much media attention with technological advances like genetics, selective breeding, and cloning. The Pyrenean ibex (aka bucardo in Spanish) is one of the subspecies of Iberian wild goat or Iberian ibex, which went extinct in 2000 when the last female Pyrenean ibex died. Recent genomics and nuclear transfer advancements have enabled reviving of extinct species.

This study will discuss the state of the art of various de-extinction technologies that can help revive extinct species like Pyrenean ibex and related challenges. This study will be based on secondary data collected from recent studies on de-extinction, Pyrenean ibex, and the revival of extinct species to fulfill this objective. Different processes are recommended for de-extinction, out of which cloning is widely proposed, even though selective breeding and genome editing have been used.

KEYWORDS: Cellular and Molecular Biology, Animal Behavior, Genetics, Pyrenean ibex, genomics, cloning.

Introduction

The revival of species that have been dead or extinct, or "de-extinction", has recently started getting a lot of scientific and media attention. Media reports constantly frame this topic in a positive limelight and convey that there is still a chance to bring fascinating beasts like mammoths back to life shortly.[1-6] Some of the most discussed species that should be brought to life are the thylacine (the well-known marsupial which looks like a dog in Australia), and the passenger pigeon (which was once found in billions in North America).[7]

People concerned about the environment are most concerned with their ethical duty for revival. This topic has sparked intense debate in the scientific community about how to best allocate funds for ecosystem restoration and conservation. While there are arguments that support de-extinction and ecosystem improvements, there are also ethical responsibilities to educate the public in sanctuaries or zoos and moral duties to protect life forms and nature.[4,8] There are some drawbacks to this type of project, such as equipment, time, resources, and the expertise needed to finalize a self-sustaining and fully functioning species, and financial costs will also be significant.[9]

One of the most echoing criticisms against de-extinction is the belief that the goals don't justify the resources at such a cost and urgent initiatives to maintain the environment and save extant species must be the priority before the revival of extinct species.[10,11] The attitude that de-extinction may be a billion-dollar business contradicts this view,[12,13] for the exchange of initial subsidy. Exotic animals are usually the major attractions in zoos and wildlife tourism and can develop awareness about sustainability in the common public to promote conservation.[14,15] Usually, de-extinction candidates should have something "big" for revival, such as claws, teeth, and horns. When showcased, these animals suggest raising a significant amount of money with ticket sales, i.e., probably enough to finance their own entertainment and promote further conservation.

1. Background:

Pyrenean Ibex was abundant in Southern France, the northern Pyrenees, and the Cantabrian Mountains. This species was most prevalent during the Upper Pleistocene and Holocene, during which their morphology was found to be larger than the "*Capra*" subspecies in "southwestern Europe" at the same time. The species went extinct with the death of the last Pyrenean ibex in January 2000. The Portuguese ibex had already been extinct due to overhunting, habitat loss, and competition from livestock. However, other subspecies survived, including the western Spanish ibex and the *Beceite ibex* or south-eastern Spanish ibex.[16]

Figure 1: A sketch of Pyrenean Ibex by Joseph Wolf. (Source – Lydekker (1898))

Since the extinction of the last Pyrenean ibex before the possibility of being analyzed by scientists, the taxonomy of this specific subspecies has been controversial. After several failed experiments to de-extinct the subspecies with the help of a cloning procedure, a live sample was finally born in July 2003.

Still, she couldn't survive after birth because of a lung defect.[5,17] The Pyrenean ibex was among the 4 "Iberian ibex subspecies." Portuguese ibex was the first Iberian ibex that went extinct in 1892.[18] The Second subspecies that went extinct was the Pyrenean ibex when a female "Celia" died in 2000. Pyrenean ibex were abundant in the Middle Ages in the Pyrenees region, but their population reduced significantly in the 19th and 20th centuries because of poaching. Only a few Pyrenean ibexes survived in the mid-20th century in the "Ordesa National Park, Spanish Central Pyrenees".[17]

Another major cause of extinction was their competition with wild and domestic ungulates. Most of its range contained competition from domestic goats, sheep, horses, and cattle, especially in high mountain meadows during summer. It caused overgrazing and interspecific competition, significantly affecting the species of ibex in dry seasons.[6,19] Celia, the last known Pyrenean ibex (female), died from a fallen tree. There is still a lack of evidence to justify the decline of this subspecies. Some theories suggest they could not compete with other herbivores regarding food, diseases, infection, and hunting. It was the first in the taxonomic group to be "unextinct" in July 2003.[5,18-21]

Literature Reviews

Siipi & Finkelman (2017) discussed the four alternatives to understanding de-extinction or resurrection biology results. They discussed the implications of each of the ways regarding the concepts of extinction and species – (1) Replication – It consists of animals created by de-extinction, replicas of original species. It supports the finality of extinction and specific ecological and biological species. (2) Non-extinction – It consists of animals from original species that have never gone extinct. It may be consistent with phylogenetic and phenetic species along with the finality of extinction. (3) Re-creation – It consists of animals that are members of earlier species but species that are extinct, irrespective of their existence. The view cannot be compatible with the concepts of all species presented. (4) Resurrection biology reverses the impact of extinction while creating new species members who were once extinct. This view presupposes non-finality and can support the concept of phenetic species as per literal resurrection.[1]

De-extinction is the promising concept of reviving extinct species and returning them to life one day. Even though a successful attempt to successfully revive the extinct species is yet to be seen, various technologies are there that might deliver a plausible solution one day. Hence, this concept is getting much attention from the common public and scientists. How far are we placed by this existing technology from the ultimate goal? Richmond *et al.* (2016) addressed several leading de-extinction techniques like cloning, back-breeding, genome editing, and synthetic genomics and discussed some significant challenges. They also discussed bigger challenges for de-extinction, including what might define "success and what might be required to take the recreated animal successfully and confer the ability to get it back to the wild".[22]

Different groups look forward to bringing back species that have been dead for a long time. Minteer (2014) argued that these efforts have the potential to undo some of the hard-learned lessons. Passenger pigeons once covered the North American skies. However, the sky was made clear of birds by the mid-19th century due to hunters. The last bird of the species, Martha, died in 1914 in the Cincinnati Zoo. There are several convincing arguments from the de-extinction lobby. The most powerful argument appeals to the feeling of justice, i.e., de-extinction, is how to get things right and compensate for moral failures. They argued that restored species would revive the lost environmental functions and improve ecological diversity,[23] meanwhile, de-extinction also has some concerns. Restored species could cause trouble in current environments and trouble native species that have evolved without them by competing with them for resources. There is also the risk of biological invasion and disease transmission with the arrival of a new species in the environment. There is also a fear that the environment won't support the de-extinct populations due to decades of human development and environmental change.[24]

1. Research Gap:

Most studies related to the de-extinction of species are based on either de-extinction processes and technologies or the de-extinction of certain species. Pyrenean ibex is still one of the topics that has been untouched by the research community. Hence, this study is based on the de-extinction of the Pyrenean ibex, which has been extinct twice. To the best of our knowledge, the novel attempts to understand the challenges and opportunities in the revival of Pyrenean ibex and fill the most important knowledge gap.

2. Research Question:

- What are the modern de-extinction techniques used to revive extinct species?
- What are the challenges and opportunities for the de-extinction of Pyrenean ibex?

3. Research Objectives:

- To understand the state-of-the-art de-extinction techniques to revive extinct species
- To explore the challenges and opportunities for the de-extinction of Pyrenean ibex

Research Methodology

To fulfill the above objectives, this study is based on secondary data collected from various online sources and websites like Google and Wikipedia using keywords like de-extinction, Pyrenean ibex, de-extinction techniques, and ethical implications of de-extinction. Data was also collected from studies conducted in the recent past related to Pyrenean ibex and the de-extinction of extinct species, which are published in peer-reviewed journals and news articles and reports published by reputed sources.

Analysis of Studies

1. Modern De-Extinction Techniques Used to Revive Extinct Species:

With the help of the SCNT technique, Somatic Cell Nuclear Transfer, which involves transferring the nucleus from a somatic cell into an enucleated egg cell, scientists almost achieved successful de-extinction in 2003 for the first time to revive the extinct Pyrenean ibex. They produced a clone from saved tissues, but the ibex couldn't survive after a few minutes of birth due to a severe lung defect. The near success sparked controversies about whether species must be revived, how it must be done, and whether species must be managed if brought back. There are several deserving species for de-extinction, and some of the high-profile examples are the passenger pigeon, the wooly mammoth, the gastric-brooding frog, and the thylacine. De-extinction is partly impossible for dinosaurs due to ancient specimens and extreme DNA degradation with age.[25] Here are some of the existing de-extinction techniques used to revive extinct species.

1.1. Back-breeding:

Before discussing the most technologically-heavy approaches, it is vital to examine whether such methods are warranted by de-extinction or "if, in some species, it could be achieved with natural crosses among extant organisms, partly where there are closely related and sufficient species. Those pools of genetic diversity can be either sister species or hybrids that can be the beginning of de-extinction by breeding back the version of any species that has been extinct. The first domesticated species was "European cattle (*Bos taurus*)" around 10,000 years ago from the aurochs.[26,27] The original species of auroch went extinct in 1627 in Poland after a decline with human pressure like overhunting and habitat loss due to agricultural expansion and deforestation over the centuries.[28]

The de-extinction of aurochs was attempted during the interwar era in Germany, making it the first and most popular example of back-breeding.[29,30] The ambition of initiators was to make the fusion of European cattle breeds selected per important morphological factors like body size, coat, and horns to revive wild aurochs' phenotype.[30] Although there are no specific resemblances between Heck cattle and aurochs, they retain clear differences in phenotypes,[31] (Figure 2), and several initiatives have constantly crossed Heck cattle with other breeds to improve phenotype.[32,33]

In Figure 2, some of the significant differences are illustrated between Heck cattle and aurochs. Leg and face are shorter in Heck cattle, and horns are elevated in Heck cattle. The body size was varied in aurochs; it is suggested that the height of aurochs bull is 170 to 180 cm at the shoulder, while the Heck cattle are considerably shorter by 20 cm.[31,33]

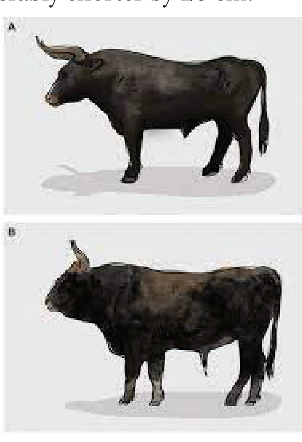

Figure 2: Phenotypic differences between (A) aurochs and (B) Heck cattle. (Source - Stokstad, 2015; Van Vuure, 2005).

1.2. Cloning:

Cloning is a reproductive technique that creates genetically identified new organisms from a "parent" cell. This principle applies to growing a completely viable multicellular diploid organism from an individual nucleus without meiosis. It transplants the nuclei from a target cell to the host cell vacant from its own nuclei, and the cell is manipulated to enter mitosis to develop a completely cloned animal.[34] Cloning has got much attention for de-Extinction as a way to generate authentic living organisms genotypically from the well-preserved subsamples of the cell, either taken from species before extinction deliberately or recovered from extinct species that have been naturally frozen.[35-37] Cloning is currently well regarded as the fastest route to de-extinction, and various attempts have been made that have successfully recreated Pyrenean ibex with frozen skin cells from the last individual who survived.[18]

1.3. Synthetic genomes:

These are created in a lab and involve constructing DNA sequences from basic chemical components to create entirely new or modified genetic material. This is done by combining small oligo strings and synthesis.[38] Khorana *et al*. (1972) were the first to synthesize a gene when the synthetic genomes constantly increased up to "ca. 1 million" base pairs by IV recombination.[39] The synthesis of "functional eukaryotic genomes" is much more complex because of larger sizes and added complexities associated with eukaryotic cells, like the presence of non-coding DNA, which requires more sophisticated methods and careful design to achieve desired biological functions.

1.4. Genome editing:

It is an alternative technique to recreate extinct species, which is more quickly viable in editing the genome of living species, closely related to a draft genome of extinct species. Based on the editing of Asian elephants, the woolly mammoth genome is used, but the principles are applied to other potential cases with ancient DNA. It has shown common barriers, such as differences in gestation and reproductive period when working with an elephant or large mammal as a surrogate. Most of the genes of mammoths are similar to Asian elephants,[40] instead of building a genome from the beginning. Forming a synthetic genome would be easier and much quicker than back-breeding.

2. Challenges and Opportunities for De-Extinction of Pyrenean Ibex:

Also known as bucardos, Pyrenean ibexes were magnificent creatures bigger than two Spanish ibex subspecies that have survived. They adapted well to their mountain habitat and challenged the worst winter by climbing the steepest cliffs. But their curving, large horns had much demand from hunters, and bucardo went extinct in 1913. However, a small population was found the same year in the Ordesa Valley in the center of the Pyrenees. The government banned hunting and announced the area as a national park. But it was too late, and all efforts failed to recover them.[41]

They had short hair that varied per season, i.e., long, thick, and harsh in winter. On their neck, the hair remained long all year round. One can differentiate between female and male

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ibex with fur, color, and horn. The color of males used to fade grayish brown in summer months and were adorned with black in various spots of the body like forelegs, forehead, and mane. The ibex could have been more vivid during winters. The transformation of the male was made from grayish brown to dull gray, and he became faded and dull where the spots were black. Since the coat of a female ibex was brown during summer, it could be confused with a deer. There was no black coloring on the female ibex. Young ibex was the same color as females for the first birth year.[42]

The "male had thick, large horns, curving back and outwards, then out and downwards, and inside and outside. The horn was ridged, and the ridges were used to develop with age. They were supposed to represent a year. The total number of ridges represents the age of the ibex. The female had cylindrical, short horns. Ibex lived on herbs and grasses.[42] The species was usually spotted in parts of Portugal, France, Andorra, and Spain but not enough in northern parts of the Iberian Peninsula. Pyrenean ibex went extinct for the first time in the northern part of the Iberian Peninsula in areas like France and Andorra on the mainland.[43]

2.1. Opportunities for De-extinction:

The researchers first explored the possibility of reviving extinct species at the beginning of the 20th century with an approach called "back breeding," For producing a breed that has the traits of a wild ancestor, back breeding relies on selective breeding used by humans for centuries to develop animals with specific characteristics. German zoologists Heinz and Lutz Heck crossbred various cattle in the 1920s and 1930s to revive the breed for the aurochs-like animal, a European wild ox species that was extinct and the ancestor of today's cattle.

In the late 20th century, several tools emerged to analyze DNA and isolate it from dead animals' hair, bones, and other tissues. Combined with advancements in "*in vitro* fertilization" and other reproductive technologies, researchers could identify cattle that are closely and genetically relative to aurochs. Further improvements have also increased the opportunities for the de-extinction of species. Genetic technologies helped rebuild the genetic sequences of extinct species crypto-preserved or preserved samples.

Cloning for de-extinction has been based especially on using SCNT, which refers to transferring the nucleus from the body cell of the animal for cloning into the cytoplasm of a donor egg from another animal without its nucleus. The egg cell is then lab-stimulated to start cell division, causing embryo formation. Then, the embryo is transplanted into the surrogate's uterus, which is closely associated with the animal that is being cloned. Researchers moved the nuclei from melted fibroblasts of skin samples into enucleated eggs of goats. The embryos were transformed into either a hybrid of goat and Spanish ibex or Spanish ibex females.

Stem cells can also be used to revive extinct species. It is possible to reprogram somatic cells while introducing particular genes, causing the "induced pluripotent stem (iPS)" cells. It is possible to stir those cells to differentiate

into various types of cells, such as eggs and sperm, which can possibly grow living organisms. Like other de-extinction techniques, the success of stem cell approaches relies widely on DNA quality in preserved samples.

2.2. Challenges related to de-extinction:

Manipulation of stem cells, cloning, genome editing, and genome reconstruction are effective technologies, but they also have ethical challenges regarding de-extinction. For example, the inefficiency and cost of SCNT have raised many concerns related to the realism of reviving extinct species.[44]

The first concern is the potential of such technologies, along with back breeding, to change the sequence of natural history. Humans can fix previous mistakes and harms they have posed on other species and expand the diversity of species with de-extinction. However, revived species could be endangered soon and need conservation due to a lack of resources. Their natural habitats may have changed significantly since their extinction, making finding suitable food, water, and shelter challenging. Additionally, they may face competition from other species for these resources.

It is also important to keep the disease angle in mind, which is associated with both the vulnerability of revived animals to modern parasites and diseases as it couldn't evolve during extinction,[45] and whether it could spread the disease to other species like livestock.[46] A serious example of vulnerability is the extinction of the "Christmas Island rat" due to a contagious disease.[47]

Result and Discussion

Though the de-extinction techniques discussed above may not be efficient enough to have proper "de- extinction," considering the rapid growth of molecular biology, it is worth expecting that more organisms will be recreated shortly with partial genomic qualities matching with extinct species. Meanwhile, several challenges cannot be overlooked, especially with interactions between the natural world and recreated species as they are now. The species may impact the ecosystem unexpectedly, such as altering food chains, competing with native species, and potentially introducing new diseases or parasites. Additionally, the existing environment may not be suited for the recreated species due to significant changes in climate, vegetation, or human development.

An organism's microbiome is a vital consideration, well-known as a second genome. Microbial interactions can significantly influence health, nutrition, and behavior from food in an animal, and microbiomes vary considerably across populations and species.[48,49] There is a lack of information on how to equip a de-extinct animal with a working microbiome to make its symbiotic potential meet the needs of the real world. Even though it is possible to recreate "ancient microbiomes", it is not guaranteed to work in the environment or climate they will face now.

Ultimately, it is worth considering that an animal is more than a living entity. It is a combination of behavioral details that are passed to another generation. The journey to their existing condition started billions of years ago for every living species on earth and has unfiltered and continued to this day. Behavioral transfer and evolution potentially range as far back as the evolution of learning capability in the lineage of species.

Conclusion

Today, there are so many approaches available that can make de-extinction easier and help understand what plays a vital role in the de-extinction of species. It is found that though each of those methods is promising, they also have some limitations. Ultimately, it is a matter of choosing the most suitable method for both the desired target for success and the species to be considered.

De-extinction efforts today focus on bringing back species such as the woolly mammoth, passenger pigeon, and thylacine (Tasmanian tiger) through advanced genetic techniques. One popular method is gene editing using CRISPR technology, which allows scientists to modify the DNA of existing animals to closely resemble extinct species. Another technique involves cloning, where scientists use preserved DNA samples to create embryos that can be carried by surrogate animals of closely related species. Both these techniques were used in the case of the Pyrenean ibex and aimed to restore ecological balance by reintroducing species that played crucial roles in their environments.

However, the ecological impacts of de-extinction are complex and uncertain. Just like in the case of the Pyrenean ibex, there is fear that introducing revived species could disrupt existing ecosystems, potentially harming current species or leading to unforeseen consequences. There is also concern that focusing on de-extinction might divert attention and resources from conserving endangered species that are currently alive.

The role of ethics is significant in de-extinction projects. Ethical considerations include the welfare of surrogate animals used in the process, the potential suffering of revived species in environments different from their original habitats, and the risks of creating animals with unknown or uncontrollable behaviors. Additionally, there are concerns about the long-term responsibilities of managing these populations and ensuring they do not become invasive species.

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