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PHENOTYPE WITHOUT GENOTYPE: LIMITS OF DE-EXTINCTION

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Bioinformatics, De-extinction, CRISPR, Somatic Cell Nuclear Transfer, Genome Editing, Conservation Biology, Functional Proxies

ABSTRACT

This research evaluates the scientific boundaries of de-extinction, distinguishing between phenotypic proxies and genotypic resurrection. Through a comparative analysis of various case studies such as the Quagga Project, Bucardo, and Dire wolf, we will understand the process like selective atavism, cell cloning, and CRISPR gene editing, respectively, including their impacts, both positive and negative. The study identifies critical limitations in mitochondrial compatibility, epigenetic reprogramming, and the Behavioural Void. The paper concludes that methods like back-breeding, cloning, and CRISPR editing can increase the genomic fidelity but cannot currently achieve true species resurrection. The most pressing part is not technical but ecological, as there is a lack of a validated framework on how one can introduce these function proxies in modern ecosystems. The study concludes that the success of these projects must be measured by their contribution to modern ecosystem integrity rather than genomic fidelity alone.

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INTRODUCTION

Popular representation of de-extinction is dominantly shaped by media from movies like Jurassic Park, often portraying de-extinction as the direct resurrection of extinct animals through the recovery of ancient DNA. The International Union for Conservation of Nature (IUCN) has defined de-extinction as the artificial creation of functional proxies that can replicate the ecological roleplay which used to be played by the respective extinct animal [1]. However, these portrayals are unrealistic in the scientific community. DNA degrades over time, with its half-age being 521 years; hence, it is not possible to clone DNA from long-extinct animals like the dinosaurs, which are extinct 66 million years ago [2].

De-extinction is often confused with complete resurrection. However, this interpretation is scientifically inaccurate. Even in the case of cloning, complete resurrection is not possible due to differences in mitochondrial DNA, epigenetic modifications, and developmental environments. Cloning an animal successfully requires intact living cells, which are not available for most of the extinct creatures. As a result, de-extinction today is primarily centred around genome editing. While this method can introduce targeted genetic modifications to approximate the traits of the extinct animal, it cannot produce an exact taxonomical replica. This highlights the fundamental distinction between being phenotypically similar (observable morphological and behavioural traits) and being genotypically similar (genetic equivalence). [3]

De-extinction has transitioned from being an experiment or scientific curiosity to an environmental necessity. Current extinction rates, often described as “The Sixth Mass Extinction”, reflect an accelerated loss of species driven by anthropogenic factors. [4] These engineered proxies can be reintroduced into their former habitats, which increases the range

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of habitat, and may contribute to ecosystem restoration, which was disrupted due to the extinction of that particular species.

Replicating the animal's external looks (phenotype) does not equate to recreating the original long extinct animal (genotype). For example, an elephant edited to have a thick coat of fur and smaller ears may give it a physical appearance similar to the long gone wooly mammoth, but at the end of the day it still internally remains a modified Asian elephant (*Elephas maximus*). A lab by the name of Colossal Biosciences, based in Dallas, Texas, is one of the first labs to successfully work on functional de-extinction. Functional de-extinction is the process of developing an organism that has both an appearance and a genetic code similar to that of its extinct relative. In this process, the resistance is engineered, and adaptability is enhanced in such a way so that it thrives in today's environmental conditions and ecosystems. [5] Once, de-extinction was seen as an experiment or scientific curiosity, but now it is seen as an environmental necessity.

A keystone species is defined as an organism that exerts a disproportionately large influence on its environment relative to its numerical abundance, fundamentally maintaining the structural integrity of its ecosystem. [5] A perfect example of the importance of a keystone species would be that of the Woolly Mammoth. Its extinction led to the collapse of "Mammoth Steppe," which was once a highly productive grassland covering the Northern Hemisphere. [6]

After the extinction of the Woolly Mammoth, the once nutrient-rich Mammoth Steppe had been converted into a moss-dominated tundra. Without the Mammoth's constant trembling, the winter snow created an insulating layer over the soil, preventing the Arctic cold from reaching the permafrost. [7] Restoring this functional proxy is no longer viewed as a scientific experiment; it is looked at as a strategic intervention to prevent the Arctic from being a "Carbon Bomb". [8]

Section 1 of the paper examines the definition and process of selective atavism, covering the case study of The Quagga Project. Section 2 of the paper examines the definition and the process of gene cloning, covering the case study of the resurrection of Bucardo. Section 3 of the paper examines the definition, and the process of CRISPR gene editing, covering vast case studies of the successful dire wolf project (2025), and the upcoming Wooly Mammoth project.

This paper argues that while back-breeding, SCNT, and gene CRISPR editing increase the genomic fidelity, none currently achieve true species resurrection, and that the most pressing unresolved challenge is not technical but ecological: the absence of a validated framework for accessing functional integration of de-extinct proxies in today's modern ecosystems.

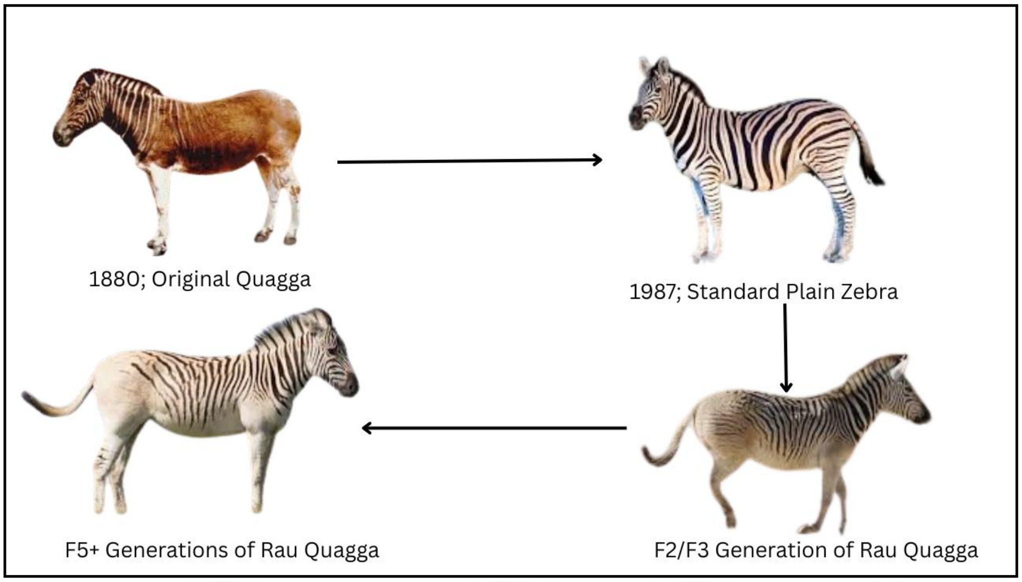
BACK BREEDING

Back breeding (selective atavism) is a type of artificial selection done by scientists to recreate the phenotype of the extinct species. This is done by selectively breeding the living relatives of the extinct species, containing diluted traits, which concentrates the traits in the new progeny, leading to the proper development of the phenotype. [10] It relies on the fact that when a particular subspecies goes extinct, it distributes its alleles in various other individuals. Naturally, the alleles are repressive and dilute, but when bred repeatedly with one another, the traits get more and more dominant and concentrated. [11, 12]

CASE STUDY: The Quagga Project

The Quagga was a unique subspecies of a plain zebra, native to the areas of Karoo and the southern Free-State of Africa. The prominent difference between Quaggas and Zebras was the progressive fading of the stripes from the front to the rear part of the body. At the bottom end, they had no strips and had a solid creamy-brown hide. [13] In the 19th century, the European settlers arrived in South Africa. Extensive anthropogenic pressure, primarily in the form of trophy hunting and livestock competition for grazing land, led to a rapid population decline during the 19th century.. They couldn't scientifically differentiate between the quagga and the zebra, and hence considered all striped equines as "Quagga." The last wild Quagga was killed around the late 1870s. The species officially went extinct when the last known Quagga died in the Natura Artis Magistra zoo in Amsterdam. [14]

The Quagga Project was initially started in 1987 by a group of dedicated scientists and conservationists, led by Reinhold Rau in South Africa. [15] Rau's discovery through mitochondrial DNA analysis of the skins of Quagga led him to conclude that Quagga was a southern subspecies of Plain Zebra (*Equus quagga*). The project's main aim was to reverse-engineer the extinct phenotype of Quagga. [16] The goal is to breed the Zebra's having less stripes in order to concentrate the



trait in the upcoming progeny. Thereby producing a population of 'Rau Quaggas 'which are morphologically identical to their extinct relatives, and also restores the African ecological heritage. [17,18]

Fig. 1: Progressive Phenotypic Reversion in the Quagga Project. The schematic illustrates the transition from the extinct Original Quagga (1880) to the initiation of the project using the Standard Plains Zebra (1987) as a founder population. Through successive generations of selective atavism, the F2/F3 generations show a marked reduction in posterior striping, culminating in the F5+ Rau Quagga, which serves as a functional morphological proxy for the extinct subspecies. (Source: [10, 17, 21]

The restoration of the Quagga Project relies on the following scientific mechanisms:

1. The project initiated with finding the "founder" individuals amongst the species of Plain Zebra, meaning the individuals with reduced distal striping and light brown hue on the posterior side of the body. These individuals were selected on the basis that the Quagga phenotype was a result of clinical variation. [19]
2. The scientist paired the most "Quagga-like" zebras; they aimed on increasing the homozygosity of the trait in the progeny. This is a form of artificial selection that mimics the process of natural evolution but in an accelerated manner. [20]
3. The project tracks the progression of the similarity in physical appearance in successive generations. While F_1 generation might show significantly less similarity, the F_4 & F_5 generations (currently termed as "Rau Quagga") show higher phenotypic similarity and are starting to show a closer appearance to the specimen skins of the original quagga kept in the museums. [21]
4. Beyond the mere physical similarity, the project evaluates the biological fitness of the "Rau Quaggas" according to the Karoo environment. This ensures that the Rau Quaggas not only remain and phenotypic replica but also play the important role in the ecological niche which the original quaggas had played for so many years in the past. [22]

While the quagga project had succeeded in morphological restoration, the methodology has certain downsides to it, as follows:

1. The most significant failure is the inability to prove genetic identity. Selective breeding only manipulates visual alleles or traits, not genetic ones. It does not account for any physiological adaptation, such as unique metabolism or immune responses that may have existed in the original quaggas. [23] Critics are saying that Rau Quagga is merely a thin-striped Zebra rather than a resurrected species. [24]

2. The project's reliance on restricted "founder" group individuals creates a high risk of inbreeding depression. In successive generations, a lack of genetic variety often arises from breeding animals with similar traits. This may lead to reduced reproductive fitness and a compromised immune system. The new Rau Quaggas become vulnerable to the diseases which the original quaggas were vulnerable to. [25]
3. Back breeding is fundamentally a subtractive process, meaning it can successfully suppress the stripes, but it cannot reintroduce the lost genetic sequences. For example, if there was a specific enzyme, protein or behavioural trait exclusive to the quaggas, it is now completely purged out from even the existing generation of zebras due to repetitive back-breeding. [26]

CELL CLONING
Cell cloning is a laboratory process for creating a viable embryo from a somatic cell and an egg cell. It involves transferring the nucleus of the donor adult cell into the enucleated oocyte to generate identical copies. Due to this nucleus transfer, the process is also known as Somatic Cell Nuclear Transfer (SCNT). [27]

Molecular cloning is used to copy DNA segments, and SCNT is used to copy/duplicate the entire genome to make a living duplicate. In de-extinction, the goal is to use the preserved cells of the extinct animal to reanimate it with the exact same genetic code, in the womb of the closest living relative surrogate.

Core Requirements:
- The Donor: An intact, non-degraded nucleus is needed from the extinct species.
- The Recipient: A healthy egg cell is needed from the proxy recipient
- The Surrogate: A living animal capable of carrying the inter-species embryo to term.

Case Study: Bucardo
Bucardo is a subspecies of Spanish ibex native to the Pyrenees. By 1999, only one female named Celia was left. In 1999, the scientists captured her to take fibroblast from her ear for cryopreservation in liquid nitrogen at a temperature of -196 degrees Celsius. On January 6 2000, Celia was found dead. Her skull was crushed by a fallen tree. At that moment, the subspecies was declared extinct. [28, 29]
Under the leadership of **Dr. Jose Folch**, a team from the Centre for Research and Food Technology of Aragon (CITA) attempted to clone Celia using the preserved fibroblasts.

Researchers used 439 reconstructed embryos. These were created by inserting Celia's nuclei into enucleated oocytes from domestic goats (*Capra hircus*). 57 embryos were implanted into various recipients, including domestic goats and hybrid ibex-goat crosses. Only one surrogate carried the pregnancy to term. In July 2003, a female Bucardo was born via cesarean section [29, 30].

Many embryos were transferred to about 50 surrogates. Most ended in early loss, but one hybrid carrier went full term. In mid 2003, a surgery delivered a seemingly healthy female bucardo goat, becoming the only extinct species to be cloned successfully. The newborn faced breathing issues due to the malformations in the development of the lungs, and it passed away within minutes. [29]

Case study: Dire wolf

The Dire Wolf scientifically known as *Aenocyon dirus,* was an apex predator in North America. The Dire Wolf was estimated to go extinct around 10,000-12,000 years ago in the late Pleistocene period. Dire wolves are known to have disappeared during the terminal Pleistocene megafaunal extinction. This coincides with a massive prey-loss and climate change. [31]

Early morphological analysis using skeletal comparisons shows that dire wolves are related to grey wolves. But, nuclear genome studies indicate that the dire wolves diverged from the ancestors of grey wolves and coyotes ~5.7 million years ago. There is no evidence of a relation between any modern canids and dire wolves. [32]

Currently, the de-extinction of dire wolf by Colossal Biosciences is focused on replicating its ecological function, i.e. making sure about the similarity in phenotype and not complete genomic resurrection. For extinct species like dire wolves, certain fragmented ancient DNA is available which makes the process of gene cloning impossible, as it requires intact living cells. Even advanced gene editing, like the CRISPR methods, cannot recreate an extinct species ʾfull genetic identity. According to the current scientific achievements in human history, no current methods can restore an extinct genome in full, only functional proxies or trait approximations. [33]

Comparative Genomic and Evolutionary Features of *Aenocyon dirus* and *Canis lupus*

Feature	**Dire Wolves (*Aenocyon dirus)***	**Gray Wolves (*Canis lupus*)**
Last Known Existence	~13,000 years ago	Extant
Evolutionary Divergence	~5.7 million years ago	Recent divergence with domestic dog
Gene Flow with Other Canids	None detected	Gene flow with coyotes & dogs
Ecological Role	Apex predator	Generalist predator
Full Genome Availability	Fragmented ancient DNA	Many modern genomes
De- extinction Suitability	Low (no close surrogate)	Higher (genomic data available)

Source: Adapted from [32] & [33]

Reproductive biological waste refers to the wastage of biological materials like embryos, fetuses, neonates etc. and the physiological toll on the surrogate mothers to create a single functional proxy. [29, 34]

The development of de-extinction proxies in canids is a prominent example/case study which demonstrates significant biological wastage in creating a single proxy organism. Studies in cloning and somatic cell nuclear transfer (SCNT) show that a very large proportion of reconstructed embryos fail to reach term, with only a small fraction resulting in live births, and an even smaller fraction surviving postnatally. [29, 34, 35]

There are three total reasons:

1. Cloning and editing of somatic cells make them act like embryo cell. If the DNA methylation reset is incomplete, the embryo may develop a large offspring syndrome (LOS), leading to a difference in the rate of growth of different organs. This developmental instability is a well-documented outcome of incomplete epigenetic reprogramming in cloned embryos. [33]
2. In interspecies nuclear transfer, the mitochondria belong to a different species than the nucleus. This metabolic mismatch disrupts cellular respiration and energy regulation, often leading to embryonic failure during development. [34]
3. Making multiple genomic edits simultaneously can lead to DNA damage, including double-strand breaks. Even if the cell survives, the resulting genomic instability can cause chromosomal abnormalities, reducing embryo viability. [35]

Behavioural Void

Colossal made sure that the outside of the proxy looked and felt like the real dire wolf through editing targeted morphological traits; however, such modifications are limited to physical characteristics and do not extend to complex neurological or behavioural traits. [35]

The proxy being born through a surrogate and being raised outside of its natural ecological context can never attain the highly specialised behavioural adaptations which were evident in the original species. Behaviour in mammals, especially in social predators, is influenced by both genetic and environmental factors, including learning and social interaction. [35]

The result of such a proxy organism is that it may resemble the extinct predator morphologically but lack the ecological and behavioural functionality required to perform the same role in the ecosystem. [35]

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Conclusion

The paper shows us that even though modern biotechnology has moved from being science-fiction to functionally restoring ecosystems by addition of proxies, yet there is a significant gap between phenotypic de-extinction and genomic resurrection. Be it the Quagga project using selective atavism, Bucardo de-extinction using cell cloning or dire wolf de-extinction using CRSPR gene editing, the result is consistently a functional proxy rather than a taxonomical equivalent.

While functional de-extinction causes a reasonable amount of technical hurdles like epigenetic reprogramming, mitochondrial mismatches, and biological waste. A proxy born to a surrogate and raised in modern environments cannot replicate the behavioural traits which were once observed in the original species. Therefore, the future of de-extinction must shift its focus from the laboratory to the landscape. The success of these proxies should not be measured by their genomic fidelity alone, but by their ability to successfully integrate into and restore the ecological integrity of modern ecosystems. Overall, de-extinction shall not be viewed as an undo button rather it shall be viewed as a way to safeguard the future biodiversity.

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