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The reintroduction process of the endangered species Acinonyx jubatus: a focus on the rewilding phase

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To all those who never stop believing, those who dare to dream big

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Summary

The cheetah (Acinonyx Jubatus), has been classified as vulnerable to extinction by the International Union for Conservation of Nature (IUCN). Habitat loss, human-wildlife conflict, and poaching have contributed to a significant decline in the global cheetah population, up to the point that an urgent intervention is strongly needed to prevent the complete loss of the species.

Cheetah population is expected to continuously decrease if conservation efforts remain superficial and no communication nor cooperation occur among the various bodies involved. Luckily, an increasing interest in the condition of this species has been shown in the last decades, and many efforts have been making to help cheetahs recovering from this situation: awareness on the importance of this species is increasing, livestock husbandry is being improved in order to reduce depredation by cheetah, a deeper control of captive breeding centres and of borders to avoid illegal trade is being applied, reintroduction and release programs have been approved...

This thesis provides general information on the species Acinonyx Jubatus, underlining the high extinction risk that it is running. It also treats the conservation of cheetahs through captive breeding and reintroduction, focusing mainly on a particular release method, the phased release. The nucleus of this approach is represented by the rewilding period, which consists in readying captive raised animals to live in the wild, permitting their release.

Introduction

Acinonyx Jubatus is one of Africa's most iconic species: everybody knows the cheetah, "the fastest land animal in the world". Unfortunately, not all of us are aware that the cheetah boasts another primate: it is the most endangered African felid.

The numerous challenges such as habitat loss, human-wildlife conflict, poaching, are decreasing its global population. Not by chance, the cheetah has been defined "vulnerable" by the International Union for Conservation of Nature (IUCN). Since cheetah's critical situation has been documented and recognized, conservation organizations and governmental agencies have been implementing different strategies for the safeguard of the animals and their habitats as well. Among them, the reintroduction of captive individuals in the wild is considered a promising and valid tool, as a matter of fact it is obtaining more and more attention. This method is beneficial both for aiding metapopulations and boosting genetic diversity. The reintroduction of captive individuals that own alleles not represented in the wild population, is one of the advantages of this approach (Magliolo et al., 2022). Important considerations have to be done about population size, since the smaller it gets, the more critical the issue of low genetic variability is. Inbreeding can lead to inbreeding depression, a massive threat that cheetahs' wild populations are likely to face.

This thesis explores generally the current situation of cheetahs and their distribution in Africa, it touches upon some conservation efforts employed for the protection of the species in the continent, going into detail on a particular conservation technique based on a period of rewilding.

In the past, captive cheetahs were subjected to a preparation programme of only two steps before being released. At first, they were held in a boma, secondly, they were directly released into free ranging conditions; the animal was required to hunt and feed by itself to survive. Due to the low success of this technique, because of insufficient hunting abilities and complications in monitoring in free ranging conditions, a different approach has been developed: the phased wilding. This method includes more phases between the captive facility and the final release, so that cheetahs can undergo a gentler evolution, improving their hunting skills and their wild behaviour in a safe environment, without competing predators. Furthermore, the passage from the captive to the wild condition, tends to be less stressful, due to the fact that it is not sudden. The animal is accompanied from the beginning until the end of the process, as a matter of fact the first phase involves the animal while still in captivity. This kind of release programme does not only adapt to the individual candidate, allowing the cheetah to progress at its own pace, but focuses also on the demographic

and genetic needs of the metapopulation, since individuals are evaluated from the genetic point of view to confirm they are suited to be released in all respects.

Despite the support provided by the implementation of a phased released involving a rewilding phase, the cooperation of different conservation strategies is necessary to enhance the chances of success in the protection of cheetahs. Furthermore, dearth of suitable habitats, the experimental character of this approach, the excessive costs and practical difficulties, make these projects not easily achievable (Kleiman, 1989). Last but not least, captive-born individuals might affect in a negative way the wild population due to their lack of fitness and natural behaviours (Buehler, 2018). The basic factors that need to be prioritized in order to increase the rate of success of such programs, are the presence of a strong self-sustaining captive population, the identification by research studies of appropriate habitats, valid transportation and release techniques, attentive post-release monitoring activity and a massive educational policy (Kleiman, 1989). Throughout the following pages, these different aspects will be touched and discussed, to provide a general depiction of the constantly developing strategies exploited to assist cheetah population recovery.

"Rewilding", considered from the ecological perspective, is quite a new and recent concept, this is why many studies to find the best application method in species conservation are still undergoing. Providing a sort of guideline for the implementation of this technique during the phased release, has been quite challenging. This essay relies on both scientific papers and on opinions and studies performed by some experts committed in this field in South Africa. A wilding protocol based on years of trial and error with the wilding of captive cheetahs prepared by Mr Van Der Merwe and Mr De Lange, in collaboration with Kuzuku Game Lodge has been exploited.

Chapter 1. Acinonyx jubatus: Ecological background

1.1 General information on biology, habitat, diet and social behaviours

Cheetah (*Acinonyx jubatus*) is a member of the family Felidae. Its reputation of fastest land mammal over short distances made it famous all around the world. The scientific genus name Acinonyx refers to its semi-retractile claws, while Jubatus (from the Latin "maned"), refers to the crest on the shoulders and the backs of juveniles (Nyhus et al., 2017).

Small head, large eyes close to each other to maximize the binocular vision, broad nostrils and extensive air-filled sinuses (Ewer, 1973), a thin and lightweight body with a musculature adapted to accelerate while allowing greater swing of the limbs (Hildebrand 1959, Hildebrand 1961, Neff 1983), semi-retractable nails for a powerful traction, highly flexible spine, long muscular tail, are only some of the physiological and morphological adaptations developed during years of evolution that enable this animal to touch 113 km/h in roughly 3 seconds. The explosion of energy needed to reach this incredible speed is facilitated by the presence of a powerful and enlarged heart, oversized liver, bronchi, lungs and strong arteries (Eaton 1974, O'Brien et al. 1983). The cheetah implies all its energetic reserves in the short-distance (300-400m), then it gets exhausted and vulnerable to the threats of other large predators.

The cheetah is the most specialized sprinter of all the land animals, as a matter of fact it has developed for speed over strength and aggression; this is why it mostly chooses a "flight" strategy rather than fighting. Furthermore, the cheetah was required to develop a hunting technique different from the other large cats (>25 kg), which can rely on strong teeth and jaws and muscular forelimbs: it puts its prey off balance during the chase (Londei, 2000), and once it falls on the ground the cheetah bites its throat suffocating it (Caro, 1994).

This animal can survive in areas relatively poor in prey density, thanks to their excellent hunting abilities, but its dietary requirements must be adequately satisfied to cover its energetic needs. The cheetah is an opportunistic predator, sure enough it can feed on a large variety of preys (Thomson's gazelle, impala, dik-dik, kudu, gerenuk, oribi, wildebeest, gemsbok, hare...), even though it prefers medium-sized wild ungulates.

Cheetahs' natural habitat is savanna, the most extended biome in southern Africa which covers almost one third of South Africa surface (Low & Rebelo 1998). It is characterized by a grassy ground layer and an upper one constituted of woody plants. This environment has been satisfactorily conserved through years thanks to the presence of the Kruger and the Kgalagadi National Parks (Low & Rebelo 1998). Nevertheless, they can readily adapt to a wide variety of different environments, including super-arid biomes.

Differently from the vast majority of Felidae family members that is nocturnal, cheetah is primarily diurnal, mostly because of the nocturnal behaviour of competitors.

Cheetahs are considered more social than the other felids, as a matter of fact large groups (up to 19 exemplars) were observed more than once, even though some differences between the two sexes have to be mentioned. Before reaching independence and maturity, all the siblings use to remain together in the den, then females and males split (more or less around six months after they left their mother). Adult females live solitarily except from when breeding or raising cubs, while males form lifelong groups of two to four members with their brothers ("coalitions") (Caro 1994; Marker et al. 2003).

Population grouping dynamics may be altered by factors such as food availability, loss of habitat, human and predators conflicts and stress (Caro 1994; Caro and Laurenson 1994). Cheetahs occupy large home ranges (Marker et al. 2007; Houser et al. 2009), which can be more extended when in competition with larger predators. Females use to occupy larger territories than males, who might either defend very small areas where females gather, or move around larger ranges. It is not rare that cheetahs' territories overlap, since they show tolerance.

Cheetahs are non-seasonal breeders, their gestation period lasts three months; they become sexually mature around two years, usually producing the first litter at three, and they are reproductively viable until twelve years of age approximately. They are induced ovulators, like most felids, this is the reason why, together with several uterine pathologies, artificial insemination is a challenging topic in this species. Cheetahs experience long periods of anestrous, independently from age and environment. Cheetahs do not show sexual dimorphism: males are slightly bigger than females (Caro 1994, Eaton 1974, Wrogemann 1975) and have larger heads. The average life expectancy is twelve years for both sexes.

1.2 Distribution, current status and challenges

The first records on the presence of cheetahs in Africa, show that they were widely distributed throughout all suitable sites, from the Cape of Good Hope to the Mediterranean (Marker 1998). Nowadays cheetahs are gathered in the northern part of South Africa, moving from the Northern Cape, North West province and Limpopo.



The 44.5% of the total land suitable for cheetah habitat (55,654 of 125,150 km2), belongs to formal conservation. This includes some of the largest parks such as the Kruger National Park, the Pilanesberg National Park, the Kgalagadi Transfrontier Park, etc. Some recent field studies performed by the De Wildt Wild Cheetah Project have been used to build an updated map on the distribution of naturally occurring and free roaming cheetahs (Boitani et al. 1999; Friedmann & Daly 2004).

The wild cheetah population underwent a significant decline over the last century and it was estimated at around 7100 individuals in 2017 (Buk & Marnewick, 2010; Durant et al., 2017), even if a precise information on this decrease is difficult to obtain because of their wide range and low density. The cheetah is part of the IUCN Red List as a "vulnerable" species in most of Africa and as "critically endangered" in North-West Africa and Iran (Durant et al., Reference Durant, Mitchell, Ipavec and Groom, 2015). It is an Appendix I species under the Convention in International Trade in Endangered Species (CITES) as well, gaining the title of Africa's most endangered felid.

The majority of the global cheetah population, at least 4500 adults, gathers in the southern African region, more precisely in Botswana, Zimbabwe, South Africa and Namibia, which retains more than 25% of the global population (Marker et al., 2018). Different areas result in different threats to the survival of this species, even though the one in common with all the range states is the conflict with wildlife ranchers and livestock. Cheetah population is threatened also by the decrease in number of wild preys, habitat loss, conflict and competition with other large predators, illegal trade in wild cheetahs due to unregulated captive breeding, captures and removal of cheetahs because considered as "pests", extremely low genetic variation because of bottlenecks (they have from 10 to 100 times less genetic diversity than the other felids) (Menotti-Raymond and O'Brien 1995). Additionally, cheetahs are extremely sensitive animals, sure enough they are very prone to develop gastritis and renal failure. Gastritis is a very common disease, which leads to high mortality risk in cheetahs. It can be triggered by several factors, such as diet, bacteriological infections, immunodepression, stress, lack of physical exercise, lack of genetic variability... These components can be distinguished between intrinsic, like the modified microbiome and bacteria leading to gastric infection, and extrinsic, such as those related with life in captivity. Even though both captive and wild individuals can suffer this condition, it is much more common among those living in captivity.

Chapter 2. Conservation efforts

2.1 Conservation works at different levels

Miscellaneous conservation methods have been used to help this species recovering from the current situation, by acting at different levels of intervention.

Increasing awareness and education for wildlife conservation is a good way to gain public support and to decrease human threats. The incremental empathy towards cheetahs' current situation, can effectively address conservation concerns (McPherson-Frantz and Mayer, 2014). Educational programs are categorized under formal and nonformal approaches. The kind of education that takes place in an institutional framework, like schools and universities, is defined "formal" (Eshach, 2007), while we refer to a nonformal program when people are provided with chances to learn out-ofschool, such as bush camps and site visits. Educational programs aim basically to the development of proactive behaviours, to the identification of measurable, achievable objectives in the long- and short-term (Hughes, 2012; Jacobson et al. 2015) and to have a positive impact on conservation outcomes.

One of the most valid ongoing conservation projects is The Cheetah Metapopulation Project (CMP). Established in 2011, it arose from the need of a new conservation tool that could remedy some complications provoked by a previous program. Between 1965 and 2009, free ranging cheetahs were translocated from Namibia and South Africa into fenced game reserves in South Africa, trying to reduce human-wildlife conflict (Marnewick *et al.*, 2009; Boast *et al.*, 2018; Buk *et al.*, 2018; van der Merwe, 2019). Unfortunately, the translocated individuals showed poor survival rates, this is why the initiative stopped at the end of 2009 (Boast *et al.*, 2018; Buk *et al.*, 2018; van der Merwe, 2019). A new program was necessary to preserve this steadily decreasing population; the CMP, aims to take care of the genetic and demographic integrity of cheetahs within the fenced areas (Boast *et al.*, 2018; Buk *et al.*, 2018; van der Merwe, 2019). Managed metapopulations adopt conservation tools like translocations. In ten years, the reserves associated increased by 23 properties, rising the natural habitat range available in protected areas (van der Merwe, 2019).

A conservation tool that Is gaining more and more valence, consist in moving individuals from one area to be released in another, also called "translocations" (Seddon et al., 2007). They can be run

for example to maintain gene flow during a reintroduction program or to reinforce an endangered population in its natural habitat. Thanks to their ability to adapt to different habitat types and to their wide prey species range, cheetahs are suited to translocation and reintroduction procedures (Caro, 1994). It has been observed that reconnecting fragmented populations and adding animals to small existing ones, can push genetic diversity locally (Johnson et al., 2010b), preventing inbreeding depression or other problems related to small populations. Reintroductions can also contribute in the connection between distant populations to recolonize large areas, since cheetahs' home ranges are quite wide (Marker, 2002). Generally speaking, reintroduction programs are designed to rise the current distribution of a population in areas originally occupied by that species; it is critical that the factors answerable for the removal of the original population in the area in question are investigated and uprooted before reintroducing new individuals (Hayward and Slotow, 2016).

Another crucial tool at the disposal of conservationists to fight against the risk of extinction are captive breeding programs. Captive breeding programs followed by reintroductions contribute effectively to the conservation and provide basis for *ex-situ* conservation ("the conservation of components of biological diversity outside their natural habitats") (Engels & Engelmann, 2002; Pritchard *et al.*, 2012; Braverman, 2014). They must be accompanied by valid conservation tools to provide a concrete contribution to the conservation of a species (Gusset & Dick, 2012).

Adequate knowledge on the biology and husbandry of the species and expert care are needed to breed specimens in captivity in the best way. An additional goal of captive breeding is the acquisition of the highest level of genetic diversity, so that individuals could adapt to a wider range of different environments once released in the wild; high levels of genetic diversity are usually correlated with higher fitness and lower percentages of inbreeding. Through the introduction of captive genetics, it is possible to diversify wild cheetah population: captive population helps with the management of free-ranging populations in the long term. Reaching an effective population size that could guarantee the maintenance of an adequate amount of genetic diversity is one of the critical purposes of captive breeding programs.

2.2 Bases of the reintroduction technique

Release of captive individuals can help mitigating pressures in vulnerable populations, although sometimes it is considered controversial due to the influence of captivity and the possible deficiency of natural behaviours. Lack of natural behaviours related to survival, foraging and reproductive fitness are the main causes for the failure of these projects.

There are two different types of release, the hard and the soft one. Hard release is about freeing the individuals soon after their arrival in the new environment, while the soft one consists in a period of acclimation in a "boma", a temporary holding facility, before the actual release (Hunter et al., 2007). In this way, captive individuals acquire wild behaviour, enabling their survival. Soft release aims at removing the impulse of going back to the capture site ("homing"), by creating a home range within the location of release (Hayward et al., 2007; Briers-Louw et al., 2019).

Once individuals are released, monitor protocol must be initiated through vary methods such as human observations, camera traps, GPS or VHF (Very High Frequency) collars, passive acoustic sensors, etc. Monitoring is essential to maximize the chances of survival and to help the animals coping with the new setting; in the case in which for example an individual has not made a successful kill yet, he is fed with a large meal, while his hunting behaviour is promoted continuously by reducing the frequency of meals. As soon as regular successful kills are recorded, supplemental feeding stops, otherwise a minimal feeding frequency is maintained to avoid starvation or loss of physical fitness. The point in which a cheetah no longer requires supplemental feeding, is when it can be defined "independent". Furthermore, once an animal mirrors constantly the normal behavioural routine of a wild cheetah (Marker et al., 2018; Wachter et al., 2018), monitor protocol is loosened, leading to individual control via GPS/VHF collars.

Reintroduction programs are made up of two phases, the pre- and the post-release periods. The pre-release includes everything concerning the selection and preparation of candidates, while the post-release covers the time from which animals are ready to be released, through the actual release, up until the post-release monitoring activity.

To guarantee a high success rate, a suited monitoring program during the periods anterior and posterior to the release is necessary. It includes the selection of appropriate candidates, the choice of adequate sites for the release, the monitoring of released individuals... rehabilitation of orphaned or trapped animals could be a valid tool for cheetah conservation.

In order to develop a reintroduction program in a correct way, it is necessary to follow carefully some steps:

selection of the releasing site \rightarrow selection of candidates \rightarrow pre-release protocols \rightarrow post-release protocols

The first aspect to be considered is the release site. A sufficient number of preys available, an adequate habitat, cheetahs' ecological needs and enough water points are the key characteristics that must be satisfied. The area should also give the opportunity to be left whenever necessary and provide a balanced intra- and interspecific competition. Because of the fact that cheetahs use to occur at low densities and have large home ranges, release sites must belong to a larger suitable landscape.

Secondly, candidates are selected in accordance to specific parameters: age at orphaning, level of habituation and social grouping. It is suggested to select individuals orphaned at an age not younger than six months, in order to reduce at minimum the level of habituation to humans during their captivity period, since younger orphans would require a much closer care. Furthermore, in nature, cubs remain in the den until two months old and go hunting together with their mother only at four months of age: younger individuals would not have ever witnessed kills and experienced hunting. A consideration has to be made on captive-reared cheetahs, which, differently from orphaned individuals, lack the experience and the learning from their mother: trial and error are necessary for survival skills to be totally acquired.

Habituation level	Behavioural criteria
Not-habituated	No tolerance for humans in any context: direct observation nearly impossible; extreme levels of stress when confined
Semi-habituated	Avoids human presence except in feeding contexts, immediately runs away to find cover after being fed; direct observation possible from suitable distance & in certain contexts;
Habituated	high levels of stress when confined Tolerates human presence in non-feeding contexts; does not exhibit normal cheetah flight response when approached; low stress levels when confined

Chart 2.1 Classification criteria for determining habituation level of release candidates. Adapted from (Weise et al., 2015)

The number of individuals to be released is another aspect to analyze, since it has been measured that coalitions have higher chances to survive. Moreover, living in coalitions is shown to improve hunting success and to facilitate defense. For what concern social grouping, creating artificial coalitions between release candidates can improve each member's chance of survival (Hunter, 1998; Gusset et al., 2008; Boast et al., 2018; Marneweck et al., 2019).

Next steps include protocols of pre- and post-release. Candidates' husbandry is a crucial aspect for the success of the release, because they need to maintain good physical and mental conditions. This aim might be reached through daily exercise, large off-exhibit enclosures and hunting training. It is important to accustom the animals to less frequent, larger amounts of food and to open and eat intact carcasses, by feeding them whole carcasses of natural preys. Providing the candidates with variable prey items is a good strategy not only to induce hunting behaviour but also to distinguish the different preys.

Their attitude towards the external environment influences their chances of survival too: braver individuals for example tend to be more exposed to risky situations, getting more vulnerable, while shier ones usually experience lower reproductive success.

A more general concept concerning candidates, common to all reintroduction programs, is their genetic value to both the captive and wild populations: during the early stages of the program, when success is still uncertain, it is suggested to select the least genetically valuable individuals, followed by specimens widely represented in the captive population but not in the wild and so on. In this way, reintroduced population is presenting an elevated gene diversity without weakening the genetic health of the captive one.

A release is considered successful when the released individual survives, when it is able to breed and its offspring reproduce and when this re-established population progresses; in other words, when recruitment exceeds adult death rate. Production and raise of offspring is a crucial point of any project linked to conservation, which can be successful only if released females survive for a minimum of two years post-release. Males, on the other hand, have a bigger conservation relevance in the short-term, since they can give their genetic contribution more quickly.

The period prior the release has a critical value, since it heavily influences the chances of survival. Candidates must be fully adapted to wild environments before being released and natural behaviours for mating, feeding and hunting must be exploited: they should be promoted while in

captivity, to assure they are displayed once in the wild. Captive animals, not only rapidly adapt to captivity from a behavioural point of view, but also from the physical and physiological: some animals showed changings in their bone conformation, that are likely to cause physical disadvantages in the wild.

Ethical and welfare problems can possibly arise during this time, like keeping animals in nearly suboptimal status or stress management.

Some problems might emerge during the post-release period, especially during the first 3-4 months, when cheetahs tend to explore the new environment and to move in the direction of home. Mortality rate is higher in this stage due to the numerous threats they are subjected to during these long and wide explorations. Human killings and other predators such as hyaenas and lions are the two central factors responsible for decreasing cheetahs' probability to survive, causing respectively the 50% and the 29% of deaths. Lions have already been recorded killing cheetahs, due to their highly territorial and opportunistic behaviour is their naivety towards predators. For the fact that captive cheetahs lose their natural anti-predatory behaviour by few generations, the failure in recognizing predators or responding to their cues must not be underestimated.

Nevertheless, once these initial months have been overcome, cheetahs use to settle into ranges and begin reproducing.

Fitness-related issues caused by variations in pathogen presence can be a further element contributing to mortality of captive individuals being reintroduced: disease control and pathogen exposure is a critical topic in both prior- and post-release phases. Newly exposed species are a threat for the disclosure of novel parasites and pathogens, even though transmissions from captive to wild individuals are common too. Isolation of reintroduction breeding populations from exotic species, might be a possible solution to deal with this risk in the prerelease period. Once released, screening for pathogens in the area is strictly necessary.

The serious low amount of genetic diversity of cheetahs, makes them much more susceptible to disease, reason why a deep control level is longer needed.

Giving that cheetahs are mostly threatened by interactions with humans, it is crucial to ensure protection also towards this peril, so that the efforts implied to carry out reintroduction programs were not fruitless. Different strategies have been practiced, like enforcing the protection of

endangered species, paid compensations for lost specimens, educating participants about the ecological importance of these animals and of reintroduction programs... It has been observed that conservation projects are most successful when they boast public support, this is why a substantial effort should be made to increase education and awareness. Nevertheless, sometimes compromises have to be found, since threats towards endangered species can be pushed by traditional or religious principles. Differently, other issues might be mitigated on a practical level in the short term; among these for example, the depredation on livestock and farmed game might be reduced using nonlethal techniques, such as guarding dogs or electric fences, rather than lethal ones, which are less cost-effective as well (McManus et al., 2014). Another appliable strategy consists in the addition of a compensation scheme: even if it is connected to a decrease in cheetah killing, it might turn to be advantageous for the reduction of killings of other carnivores (Maclennan et al., 2009). Anyway, the key to gain public support, relies on the idea that benefits of saving this species must outweigh the cost and the threats that it can cause to the local communities.

Chapter 3. Phased release: a focus on the wilding phase

3.1 An innovative releasing method

It is not easy to provide a definition of "rewilding", since definitions of many conservation terms vary. This word involves many concepts, such as reintroductions of endangered species, release of captive animals, restoration of Pleistocene megafauna etc. Generally speaking, it can be explained as a restoration strategy to increase the conservation of biodiversity, but in this essay, it is considered part of the process of releasing captive animals to wild regions (Jørgensen, 2014; Gammon, 2018).

Rewilding is the result of the commissure of advances in ecosystem and conservation science with innovations in management of natural areas. This term was coined in the 90s and the science of rewilding has been steadily implementing and expanding: now it is one of the tools used in conservation.

The rewilding period is supposed to teach captive animals to breed, to hunt and to feed on their own; it basically readies captive individuals for surviving in the wild.

The rewilding phase for the reintroduction of captive cheetahs can be considered part of a "phasedrelease" method together with the release phase. This type of release, thanks to its particular division in phases, permits a deep and individual evaluation of the candidates, so that only the ones ready can proceed in the programme (Ashia Management Plan, Unpublished).

Before starting the rewilding period, candidates spend some weeks in a boma, a livestock enclosure commonly used in Central and Southern Africa, to adapt to the new environment. Most of the times, cheetahs are ready to exit the boma when they do not show anymore stereotypic behaviours caused by stress, such as pacing. In general, the rewilding period begins in a fenced area without predators and stocked with preys. The wilding sections usually cover between 300 and 600 ha and aim to the development of hunting and behavioural abilities in a natural environment. Furthermore, individuals are constantly monitored and sure enough they get supplementary fed when required. Generally, when the cheetahs hunted at least twice a week successfully and do not longer need supplementary feeding, they pass to the release phase: they are released in pre-selected game reserves, following another period in a boma.

A scheme depicting this programme performed in 2019 by Ashia Cheetah Conservation (ACC; Paarl, Western Cape Province) together with Kuzuko Lodge (Eastern Cape Province), is provided as follows.



Figure 3.1 Flow chart depicting the Rewilding and Release programme that involves the use of a phased-release methodology (Ashia Managemet Plan, Unublished)

3.2 Stages of the wilding phase

Once the animal overcomes the period in the captive facility, it is going to be hosted in a boma for some weeks. These weeks are essential for the cheetah to: break its homing instinct, get used to the new environment and the unaccustomed bacteria and parasites, fortify its footpads, arouse its natural instinct by the presence of natural preys inhabiting the area. During these first two steps, the cheetah must be fed a natural diet for at least 3-6 months.

The boma is surrounded by a 250-600 ha area free of predators, called "pre-release section", where the cheetah is acceding after the boma stage. Inhere the animal has the opportunity to acuminate its hunting abilities, to increase fitness and to learn how to behave in the wild without being threat by competing predators. The animal is kept monitored via VHF or GPS satellite collars, in order to keep track of its improvements or declines and to supplementary feed if necessary. This third phase can last between 3 and 6 months, in accordance to how the cheetah reacts to the new stimuli and how fast it learns and adapts to the wild nature.

Finally, the ending phase can be the release whether into a 2000-5000 ha game reserve with a low number of predators or into a reserve with high predator densities ("hard" reserve). The latter requires a further step in cheetah training, since only fully ecologically functional animals can survive in such environment. They must develop vigilance and awareness of the presence of predators, for this reason they spend some time, which varies depending on the performance of the cheetah, in the former reserve. After some weeks in a holding boma within the new reserve, the cheetah can be released. It is recommended to monitor the released animals for at least six months after the release: daily, during the first month in the wild and then three times a week. This six-month range is sufficient to fully acquire ecological functionality (Van der Merwe et. al, Unpublished).

3.3 Fundamental aspects of rewilding

Diet

The diet, together with the hunting skills, have strong valence within the rewilding phase. Captive raised cheetahs eat differently from how they would do in the wild, both for the quality, the quantity and the timing of feeding. In captivity, most of the times, they know when they are supposed to be fed, their diet consists of dead prey items that, furthermore, differ from those they would catch in the wild. It is mainly due to costs since game meat is far more expensive than poultry, rabbits, smaller animals. On the contrary, it is impossible to predict feeding time in the wild, due to the variety of factors involved. Rewilding centres accustoms captive individuals to a natural diet through a step-by-step approach. They begin feeding them not only dead but also some alive preys, differing among poultry, wild preys and game meat, they introduce starvation days starting from once a week up to three or four in a row. In this way the animal learns to recognize hunger and to eat the whole meal, increasing the chances of hunting success in the wild.

The cheetah is supposed to be fed solely game meat, not only to encounter all the dietary requirements but also to get used to its taste and texture. It is essential to mirror as much as possible the life in the wild, this is why the animal needs to be provided consecutively with larger portions, up until it is able to eat a whole carcass, which is usually given every 3,5 days - carcasses are provided as a whole and not pieced because the wilding cheetah must learn how to open them. In the wild,

fluctuations in the feeding regime are commonplace, and the initial phases of rewilding programmes have been studied to make captive animals ready to cope with them. It must be fed a natural diet consisting on small-medium sized antelope and deer species, equids, lagomorphs, during the first two steps of the procedure for at least 3-6 months. Furthermore, wild pigs should not be fed, due to the fact that some species belonging to this family are cause of high injury and mortality rate: by providing this type of prey, cheetahs are encouraged to hunt them once released. An all-around diet can be obtained including skin, organs, fat and cartilage, while bones guarantee a sufficient calcium absorption and dental hygiene. It is recommended that the meat provided is indigenous to the range of cheetahs.

Cheetahs' daily energy requirements change according to many factors such as age, size of the social group and presence of cubs, maturity of the individual (Caro, 1994; Durant, 20000; Mills et al., 2004). By the way, awareness of the dietary requirements in terms of quantity and quality is crucial for the correct outcome of the wilding phase. Supplementary feeding in excess the animal negatively impacts release programs, by preventing the development of exploratory behaviour and opportunistic hunting, as individuals stay within the feeding area. It has been demonstrated that sufficient feeding interval: if a kill occurred by this time frame, the supplementary feeding should be delayed (Warmenhove et al., 2021). The application of a minimum supplementary feeding intervat feeding intervent the excessive and random supplementation, as the correct amount of food to meet individual needs without impairing exploratory and hunting behaviours is calculated.

Some indicators are helpful during the wilding period to monitor the progresses in the hunting activity of the rewilding cheetahs. Blood spread on face and body and bloated belly, show that a kill has been made and they fed within the past 24 hours; abdominal fullness can be quantified, especially when the stomach capacity has been reached, since the abdomen is obviously distended.

• Fitness and antipredator behaviour

Captive facilities have to treat not only the dietary aspect but also the physical fitness of the releasing candidate and the maintenance of a wild and natural behaviour. Behaviour results from interactions between genetic and environmental components, this is why the first step to achieve success and to avoid losing patterns of natural behaviour, is a captive environment as much faithful

of the wild as possible. A fitness training program should be included in their preparation as well, in order to develop muscle growth and health, speed, reflexes and natural behaviour. A helpful tool that can be used among captive individuals, is the running truck: it consists of a lure representing a prey item that is moved at high speed by a running track, inviting the cheetah to pursue it. It is suggested that the system bends the lure for a distance of 100-200 m and that it mimics preys sudden and fast movements. This method maintains the animals fit, trains their running abilities and preserves the hunting behaviour and natural instinct.

Individuals must be tested before the release for their predator-avoidance ability and it can be performed through auditory recordings that replicate lion's cue. These types of tests should be enriched with visual and olfactory stimuli, as well, so that a deeper insight into maladjusted behaviours can be obtained. Captive cheetahs are observed not to show any predator recognition, on the contrary they approach the acoustic cues, while semi-wild cheetahs demonstrate recognition at a certain extent; they approach the acoustic signal that replicates African bush crickets, but they flee from the one reproducing lion sounds. Finally, wild individuals flee from the cues. Sometimes, captive specimens do not approach with interest and curiosity the acoustic recording of the lion cue only, but they react in an aggressive way against it. This is an example of maladaptive behaviours that can be commonly observed in captive animals due to lack of encounters with predators. Prerelease training and the maintenance of some wild attitudes in captivity, are useful to avoid the expression of these behaviours once released. Also combining the predatory clue with an adverse outcome helps associating predators with a negative event.

Health

Before starting any rewilding programme, cheetahs must be vaccinated against some diseases such as the rhinotracheitis-, the calici- and the panleukopenia viruses and cured against Chlamydia Psittaci bacteria; further vaccinations and treatments are not endorsed once the specimens are released in the wild, since they must undergo natural evolutionary pressures. Moreover, only healthy individuals can be selected for releases, this is why they are subjected to DNA samples which are adopted in deeper genetic researches. Wilding projects must be aware that dangerous genetics accumulates in captive animals' pools, since they are not subjected to any evolutionary pressure. Anyway, it should get naturally uprooted once the individuals are released in the wild.

To ensure a well-rounded health monitoring, blood samples should be performed any time the cheetah is subjected to sedation for veterinary aims, for relocation and collaring, in this way it is possible to obtain an ample selection of tests to be performed in case of death. Prior to the final step of the procedure, the release, a health evaluation must be performed once again.

Habituation to human

The loss of interest in and the elusion of people is one of the most important achievements of the wilding phase: a cheetah standing in a condition in-between captive and wild is extremely dangerous, since it does not fear people and is still interested in them. Tourists and staff are usually threatened by habituated cheetahs, while it has never been recorded any damage to humans by wild born cheetahs in metapopulation reserves. Taking the human element off the equation is crucial for the correct development of the wilding period. The first positive behaviours that demonstrate improvements towards human presence are hissing, tail contracted between the legs and beating the ground with the fore paws. Whenever the animal shows an unresponsive attitude, it has lost most of its captive behaviours. To reduce this kind of issues, release candidates must not be touched while still in captivity and interactions must be limited at minimum; this is also why mother-reared cubs have a higher success in wilding programmes than hand-raised ones (Van der Merwe et. al, Unpublished).

• Reserve selection

Reserves are subjected to several controls and evaluations before being selected as releasing sites, as a matter of fact many requirements must be satisfied. They must guarantee the availability of proper preys, sufficient ecological carrying capacity for both cheetahs and their preys, high quality predator proof fencing, expert staff members and an adequate monitoring, financial stability in the long period, willingness to contribute to conservation and habitat suitability. Other important considerations to be made concern the density of competing predators in the area and, last but not least, the size of the reserve.

• Candidate selection

The first aim of the release of cheetahs is the contribution with not related genetics to the metapopulation, for this reason success is determined when offspring of released individuals reach the reproductive age and when they proceed adding different genetics to the metapopulation. In

order to maximize the chances of success, candidates must satisfy many requirements, which vary depending on the gender and on the age.

It is needed that females guarantee reproductive certainty, which can be compromised if they do not give birth within five years of age. It is known that in the wild cheetahs start breeding as soon as sexual maturity has been reached (24-40 months of age) and give birth again once the cubs become independent: females conceiving at this age and following this trend are able to reproduce until 10 years of age. On the contrary, if a female does not conceive by a certain period, she is going to produce smaller litter sizes, with the risk of not being able to conceive at all if 8 years of age are overtaken. This explains why females should get involved in a wilding process when they are 14 months old, so that they can be ecologically functional as soon as the age at which they can conceive (27 months) is reached (Van der Merwe et. al, Unpublished). Additionally, a statistical analysis has demonstrated that cub mortality increases sharply when the mother begins aging (more or less from seven years of age). Ideally, timing must be rigorously respected: the passage from the first two phases to the final ones, should take place by 18 months of age, so that the third and the fourth stages are completed between 24 and 27 months. It is also known that reproductive success is not stable and constant during the whole life of an animal, it tends to get more successful with age and experience. Promoting early reproduction and durable reproductive performance is a crucial aspect to increase the chances of success of a wilding project. Another aspect that should be taken into account is to reintroduce females prior to males, since established males might show aggressions against females and, potentially, kill them.

Talking about males, they should preferrable be reintroduced together with other conspecifics, maintaining the already established coalitions. Firstly, coalition cheetahs show higher chances of survival compared to singletons; secondly, the genetic profile of a member that is killed or lost, remains invested in its brothers and, moreover, it is not necessary to source another male. This is advantageous for reserves, since logistical pressures might be reduced. As coalition cheetahs cooperate in the hunting activity, they can enlarge their spectrum of preys, decreasing in this way predation pressure on preferred, small-medium sized species (Mills & Mills, 2017). An additional benefit is related to the acquisition and defense of a territory, contributing also to the increased access to females than a single male. This is why coalitions are helpful against competing males (Caro, 1994). Males' age has a much lower relevance in their reproductive success than in females': sperm quality and production increases with time, reaching maximal productivity peaks between 8

and 10 years of age (Durrant et al., 2001). Coalition structures advance to increase reproductive success as well, thanks to the territorial advantage and higher chance of survival they can guarantee. Despite these important assets, cheetahs taking part in large groups get eventually killed by other members or by members of other coalitions (Buk, 2020).

In the case in which some siblings are involved in this kind of reintroduction process, it is recommended that they face together the wilding phase, in order to increase their survival rate. If the group is composed by both sexes, it is even better; this is what happens in the wild for at least six months, depending on the hunting challenges, after leaving their mother (Durant et al., 2004; Mills & Mills 2017). Spending these crucial months hunting and perfecting killing techniques together, guarantees total ecological functionality. Furthermore, group vigilance during feeding time or while handling a prey, provides security during the initial release period. They should also be released onto the same reserve, giving them the possibility to choose when to separate and/or form a male coalition: this option would best fit their natural behaviour. When this is impossible, their relocation must be timed to correspond with their natural separation.

• Monitoring activity

Pre- and post-release monitoring make sure that the methods adopted are suited and guarantee the highest chances of success possible. The monitoring activity bases on observations of the released candidates. The IUCN had set some guidelines to be followed to monitor individuals, so that databases and protocols can be built up to record behaviour, spatial and foraging movements of captive cheetahs released (IUCN/SSC, 2013). Additionally, since post-release monitoring has a crucial value in documenting the progresses of the released subjects, it represents a useful tool to decrease the mortality rate and increase the possibilities of accomplishment.

The main factors taken into consideration, are the presence of coalition members or offspring, GPS location, peculiar behaviours, climate, Body Condition Score (BCS), belly index scores and the presence of a kill.

Prey consumption and body condition for example can be monitored monthly via feeding records and belly index observations (previously mentioned), comparing the results collected month by month to assess changes, exploiting the BCS index too. To improve the quality of the program, it is recommended to investigate the driving causes of these changes.

To evaluate the performance of behaviour, spatial and foraging ecology, it can be helpful to compare the registered data with literature on wild-born individuals.

Survival post-release could be assessed by checking cheetahs' physical conditions more or less twice a week. Furthermore, it is fundamental to check denning and breeding success as well, together with cubs' survival; because it is an extremely delicate moment during cheetahs' life, it is recommended to check the dens while the mother is hunting, in order to minimize disturbance. An important aspect to be taken into consideration is the high mortality rate during the first two months, since the youngs are lodged in a lair and the mother hunts from the dawn to the dusk, leaving the den unprotected. Roughly 5% of the newborns reach 14 months of age, while nearly 75% of the others are predated by lions (Kruuk and Turner, 1967).

Conclusions

Because of its title of fastest land animal, the cheetah is one of the most unique and iconic species in the world. Its history is characterized by a particular evolutionary background and by lively interactions with humans, that brought it on the verge of extinction.

This essay aims to explore general aspects of cheetah ecology, distribution and current situation, to discuss some possible strategies to conserve and support this species, with the focus on the rewilding period included in the phased release method of reintroduction. A whole chapter is dedicated to this innovative practice for individuals release, as it is showing to be an incredibly valid and promising tool. Nevertheless, many difficulties will arise in the process and the chances of success are not always guaranteed. The fact that a standard procedure has not been established yet, given the young age of this approach, should encourage experts to carry out studies and trials, instead of giving up on it. Additionally, some difficulties have been encountered when it came to provide an explanation of the methods and the techniques employed in the phased release. The essay bases on a protocol for rewilding cheetahs written by Mr Van Der Merwe and Mr De Lange. This document is the result of years of study, of animal observations, of trial-and-error approach, performed by experts committed in the field of cheetah conservation through reintroduction in South Africa.

The restoration of cheetah population in its original habitats, is not a project ending in itself, but is part of a way bigger and marvelous picture like the protection and sustainment of ecosystems and biodiversity. Even if in someone's opinion it might sound like a dream, rather than a realizable plan, cooperation and support are fundamental to reach such a demanding purpose. The decision of which species will survive, by sharing Earth resources, depends on people living today. As individuals, inhabiting the same planet and living in a perfect balance with nature, we must take action and do it now.

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