Une histoire génétique de chats : comprendre le passé et le présent des populations de carnivores par des études comparatives
Amira Azizan

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DE L’UNIVERSITÉ DE MONTPELLIER

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“A Genetic Tale of Cats”
Understanding the Past and Present of Carnivoran Populations through Comparative Studies
- Une histoire génétique de chats
Comprendre le Passé et le Présent des Populations de Carnivores
par des Études Comparatives

Présentée par Amira AZIZAN
Le 15 décembre 2021
Sous la direction d’Emmanuel PARADIS

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Abstract

This PhD dissertation aims to shed light on the evolutionary history of mammalian carnivores through the analyses of their genetic diversity, and their interaction with the global expansion of human activities since the Quaternary. Conservation strategies focusing on this taxon have far-reaching societal and environmental impacts. However, the evolution of many carnivores remain poorly understood in human-dominated landscapes. To assess the state of knowledge of population genetic studies of carnivores in Southeast Asia (SEA), where the extinction risk of mammals is among the highest due to the disproportionate effect of habitat changes as well as illegal hunting for trade across other parts of the world, a general bibliometric review was conducted to address two questions in the first chapter: What is known about the genetic diversity of carnivorans in SEA? And: Do carnivorans in SEA have low genetic diversity due to recent human activities? In the second chapter I conducted a global analysis of the current pattern of within-population genetic diversity using the cat family (Felidae) as the study model given that it is one of the most studied carnivorous families and provides sufficient data for a large-scale study. In this context, a database of population genetic studies using microsatellite markers was built to ascertain how species traits, human population density, and geographical regions of the world can affect their levels of genetic diversity through a meta-analysis approach. The third chapter assesses the relationship between genetic diversity and population size within species, through the population density estimates from camera-trap surveys of Felidae. By combining our datasets, we showed that our framework can relate ecological indicators to the adaptive potential of species with small populations, which can be beneficial for extinction risk assessments and to test an important theoretical prediction in evolutionary biology and conservation genetics. In the chapter four, I examined the level of genomic diversity between wild and captive species and the genomic consequences of captivity. These analyses are based on the WGS data of cheetahs and Asiatic lions obtained from the Montpellier Zoo as a case study.
Résumé

Cette thèse de doctorat vise à mettre en lumière sur l'histoire évolutive des mammifères carnivores à travers l'analyse de leur diversité génétique, et leur interaction avec l'expansion globale des activités humaines depuis le Quaternaire. Les stratégies de conservation axées sur ce taxon ont un impact sociétal et environnemental considérable. Cependant, l'évolution de nombreux carnivores reste mal comprise dans les paysages dominés par l'homme. Afin d'évaluer l'état des connaissances sur les études de génétique des populations de carnivores en Asie du Sud-Est (ASE), où le risque d'extinction des mammifères est parmi les plus élevés en raison de l'effet disproportionné des changements d'habitat ainsi que de la chasse illégale pour le commerce dans d'autres parties du monde, une revue bibliométrique générale a été réalisée pour répondre à deux questions dans le premier chapitre : que sait-on de la diversité génétique des carnivores dans l'ASE ? Et : Les carnivores de l'ASE présentent-ils une faible diversité génétique en raison des activités humaines récentes ? Dans le deuxième chapitre, j'ai effectué une analyse globale du patron actuel de la diversité génétique au sein des populations en utilisant la famille des félins (Felidae) comme modèle d'étude étant donné qu'il s'agit de l'une des familles de carnivores les plus étudiées et qu'elle fournit suffisamment de données pour une étude à grande échelle. Dans ce contexte, une base de données d'études de génétique des populations utilisant des marqueurs microsatellites a été constituée afin de déterminer comment les caractéristiques des espèces, la densité de population humaine et les régions géographiques du monde peuvent affecter leurs niveaux de diversité génétique par l’approche de méta-analyse. Le troisième chapitre évalue la relation entre la diversité génétique et la taille de la population au sein d'une espèce, par le biais des estimations de la densité de la population provenant des estimations derivées de pièges photographiques sur les félidés. En combinant nos ensembles de données, nous avons montré que notre cadre peut relier les indicateurs écologiques au potentiel adaptatif des espèces à petites populations, ce qui peut être bénéfique pour les évaluations du risque d'extinction et pour tester une prédiction théorique importante en biologie évolutive et en génétique de la conservation. Dans le chapitre quatre, j'ai examiné le niveau de diversité génomique entre les espèces sauvages et captives et les conséquences génomiques de la captivité. Ces analyses sont basées sur les données WGS de guépards et de lions asiatiques obtenues au Zoo de Montpellier comme étude de cas.
# Table of Contents

ABSTRACT I  
RÉSUMÉ II  
ACKNOWLEDGMENTS IV  
LIST OF FIGURES V  
LIST OF TABLES VI  
LIST OF APPENDIX VII  
PROLOGUE 1  
GENERAL INTRODUCTION 3  
  Population Genetics And Genomics Of Threatened Species 4  
  Genetic Variation, A Keyhole To Past Population Size ? 11  
  Cats’ World Dominance 19  
AIMS OF THIS ThESIS 35  
CHAPTER I GENETIC EROSION IN CARNIVORES’ EVOLUTIONARY HOTSPOT ? 37  
  Introduction 39  
  Literature Search 42  
  What Is Known About The Genetic Diversity Of Carnivorans In SEA? 44  
  Do Carnivorans In SEA Have Low Genetic Diversity Caused By Human Activities? 54  
  Challenges In Sampling Genetic Data And Recent Progress 57  
  Conclusion And Future Recommendations 60  
CHAPTER II PATTERNS AND DRIVERS OF GENETIC DIVERSITY AMONG FELIDAE SPECIES 61  
CHAPTER III POPULATION DENSITY AND GENETIC DIVERSITY ARE POSITIVELY CORRELATED IN WILD FELIDS 89  
CHAPTER IV COMPARATIVE GENOMIC STUDIES AMONG CAPTIVE AND WILD INDIVIDUALS 106  
GENERAL DISCUSSION 138  
  Genetic Diversity In Tropical Regions Is Higher But Not Frequency Of Studies 139  
  The Unexpected Trend Of Life History Traits With Genetic Variation 140  
  Back To Basic: From Population Size To Population Density? 142  
  Stuck In A Captive Loop 143  
  The (small) Rise And (big) Fall Of Pleistocene Carnivores 144  
EPILOGUE 146  
RÉSUMÉ (VERSION LONGUE) 147  
  Introduction 147  
  Objectifs De Cette Thèse 156  
  Résumé De Chapitre I 158  
  Résumé De Chapitre II 159  
  Résumé De Chapitre III 160  
  Résumé De Chapitre IV 161  
  Discussion Et Conclusion Générale 162  
BIBLIOGRAPHY / RÉFÉRENCE BIBLIOGRAPHIQUE 170  
APPENDIX 201  

III
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IV
List of Figures

Figure 1. Genome-wide heterozygosity from autosomal scaffolds across different taxon obtained from published studies. 9

Figure 2. The extinction vortex 10

Figure 3 Schematic representation of the founder effect leading to a reduced genetic diversity (as depicted by the coloured DNA symbols) in captive population compared to those in the wild. 18

Figure 4. Dated phylogenetic tree of Felidae as inferred from nuclear DNA (Li et al. 2016), and their geographic distributions. 22

Figure I.1. Number of Carnivora species in each Southeast Asian country. 41

Figure I.2. Trend of population genetics study for Southeast Asian carnivorans since 1995. 43

Figure I.3. The relationship between the rate of publication per species and the percentage of species in each family / total carnivorans in SEA. 43

Figure III.1 Locations of the n = 399 records of population density and n = 156 records of population genetic from cluster data set across 15 Felid species. 95

Figure III.2 Positive relationship between within-population genetic diversity and population density from camera-trap surveys across wild felids using (a-c) cluster and (d-f) country data sets. 98

Figure III.3 Correlation coefficient between within-population genetic diversity (GD) and population density (PD) from camera-trapping surveys in some species with several records from the cluster datasets. 100

Figure IV.1 Phylogenetic relationship, based on biparental nuclear genome, between the cross-species reference genomes and the sampled species, cheetah (Acinonyx jubatus) and Asiatic lion (Panthera leo persica). 111

Figure IV.2 Summary statistics of the reads mapped on different reference genomes in cheetahs and lions. 121

Figure IV.3 Summary statistics of the filtered single nucleotide variant (SNV) called using ‘bcftools call’ and ‘freebayes’ based on different reference genomes in cheetahs and lions. 121

Figure IV.4 Distribution of heterozygosity from 18 autosomal chromosomes for each cheetah (red) and Asiatic lions (blue) according to various reference genomes: aciJub1, Felcat9 and PanLeo1. 123

Figure IV.5 Distribution of inbreeding coefficient based on runs of homozygosity (FROH) in cheetahs (red) and lions (blue). 125

Figure IV.6 Effective population size trajectory over time among captive (Ajabu, Aywa, Azrael, and Bappé) and wild cheetahs (Chewbaaka and Rico). 129

Figure IV.7 Effective population size trajectory over time among the Asiatic lion (Surina, Kiran and Atul) and African lion (Brooke). 130
List of Tables

Table I.1. Checklist of Carnivora species in Southeast Asian countries, with their IUCN Red List category and CITES status. 46
Table I.2. Sample source, molecular markers used and number of authors affiliated with research institutions in Southeast Asia in each study. 50
Table I.3. List of national biodiversity information online database in Southeast Asia. 59
Table III.1 Linear mixed model comparisons for predicting genetic diversity estimates using the cluster dataset. 102
Table III.2 Summary statistics and parameters from the best-fit linear mixed models in cluster (L) and country (C) data sets. 103
Table IV.1 A non-exhaustive compilation of studies investigating the differences of wild vs. captive genetic diversity in carnivores. 112
Table IV.2 Chromosome-level assembly of felid genomes. Genome assembly marked with an asterisk were used in this study for short-read alignment. 114
Table IV.3 Information on the nuclear genome sequences sampled and their coverage for this study. 119
Table IV.4 Mean and standard deviation (sd) genome-wide heterozygosity based on autosomal chromosomes of each species. 123
Table IV.5 Averaged inbreeding coefficients in cheetahs and Asiatic lions from Montpellier Zoo based on pedigree and genome-wide run of homozygosity (ROH) 126
Table IV.6 The mean effective population size, Ne near 10 kya averaged across 100 bootstraps inferred in PSMC and scaled to various mutation rate. 128

Appendix

ANNEXE I SOURCE OF THE GENOME-WIDE HETEROZYGOSITIES VALUES IN FIGURE 1.................................................................202
ANNEXE II DISTRIBUTION OF POPULATION GENETIC STUDIES IN FELIDAE........................................................................205
ANNEXE III SUPPLEMENTARY INFORMATION FOR CHAPTER 3........207
ANNEXE IV SUPPLEMENTARY INFORMATION FOR CHAPTER 4........213

List of Abbreviations and Symbols

AR Allelic richness
DNA Deoxyribonucleic acid
GD Genetic diversity within population
He Expected heterozygosity
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR</td>
<td>Allelic richness</td>
</tr>
<tr>
<td>Ho</td>
<td>Observed heterozygosity</td>
</tr>
<tr>
<td>IBD</td>
<td>Identity-by-descent</td>
</tr>
<tr>
<td>IUCN</td>
<td>The International Union for Conservation of Nature</td>
</tr>
<tr>
<td>mt</td>
<td>Mitochondrial</td>
</tr>
<tr>
<td>Ne</td>
<td>Effective population size</td>
</tr>
<tr>
<td>PD</td>
<td>Population density</td>
</tr>
<tr>
<td>PSMC</td>
<td>Pairwise sequentially Markovian coalescent</td>
</tr>
<tr>
<td>ROH</td>
<td>Runs of homozygosity</td>
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</table>
A Genetic Tale of Cats

Prologue

We are currently living in a new era of drastic climate change, the first Earth has ever seen - the Anthropocene. It began with our *Homo sapiens* ancestors who struggled to survive Mother Nature’s forces during the Pleistocene. From an initially small population in Africa, our species managed to successfully colonise all four corners of the world, leaving behind other hominin relatives. Then came our unbound population expansion extending to 7.9 billion people today, and all the while altering the composition of life in the seas, on land, and in the air, resulting in an unprecedented rate of climate change, loss of habitat, soil erosion, overexploitation, pollution, and increased carbon dioxide concentrations.

If Earth’s 4.5 billion year existence were to be compressed and scaled into one calendar year\(^1\), with the Big Bang being on January 1\(^{st}\) and today being midnight on December 31\(^{st}\), we have started to develop stone tools about 3 hours ago. Less than a second ago in the Cosmic Calendar which is equal to 200 years in real time, the temperature has risen by 1.2°C\(^2\). Carbon dioxide levels are increasing more than 250 times faster than it did after the last Ice Age, 18,000 years ago\(^3\). The rate of species loss have increased dramatically, unparalleled to past mass extinctions (Barnosky et al., 2011; Ceballos et al., 2015). The extinction of mammal species in the past 500 years is likely equal to the loss of 250 million years of evolutionary history (Gumbs et al., 2021). In this age of vast global changes, the scale of today’s ecological disasters such as more frequent transmissions of pathogens, proliferation of invasive species, the depletion of food supply, and the deterioration of genetic resources are jeopardising all benefits of human existence from nature.

Species extinction has always been a component of the Earth’s restless evolutionary history. This natural process commonly caused by multiple synergistic threats can provide new opportunities for surviving lineages best suited to survive the changing Earth and stimulate new branches in the tree of life. The fundamental fuel requires for evolutionary processes is in their raw genetic material and its diversity serves as a “capacity of ecosystems, habitats, species or genotypes to keep options

\(^{1}\) A la Carl Sagan’s Cosmic Calendar


A Genetic Tale of Cats

open in order to support a good quality of life” (Díaz et al., 2019; Hughes et al., 2008). The importance of maintaining genetic diversity at a healthy level has been widely acknowledged in conservation studies and policy since the 1970s (Díaz et al., 2019; Frankel, 1974; Frankham et al., 2002). The subject of this thesis contributes to the growing knowledge on solving the maintenance of genetic resources of carnivores whom are an intrinsic part of an ecosystem’s biodiversity and intimately tied to humanity. Understanding how the multitude disturbances have shaped the genetic diversity and the selection pressures that have altered the trajectory of species evolution since the rise of modern humans, is essential for deciphering the potential evolutionary mechanisms that will drive biodiversity in the future (Bull and Maron, 2016; Otto, 2018). As aptly stated by Ellis et al. (2021), “Depicting human use of nature largely as a recent and negative disturbance of an otherwise human-free natural world is not only incorrect but has profound implications for both science and policy”.
General Introduction

“It may be our evolutionary responsibility to keep evolutionary options open so far as we can without undue deprivations for those least able to bear them”

Sir Otto Frankel
(1974, Genetics Vol 78 Issue 1)
Population genetics and genomics of threatened species

All reproductive beings are subject to evolution driven by natural selection, from a whole organism to the cellular level, with genetic variation as its blueprint. Species that fit into the environment have the opportunity to survive and to reproduce. The best odds of continuing a viable legacy is to find potential mates in a large population with a unique set of genetic materials. Any gene pool, hereby defined as the total genetic variation present within an interbreeding population of a particular species, may fluctuate in size through the balance of mutation, migration and random processes referred to as ‘genetic drift’. The latter has significant consequences in a small population. The rate and direction at which speciation and extinction occur are governed by these mechanisms at the genomic level (Lynch and Walsh, 2007). The genetic variation measured within an individual or a population at a given time is thus a snapshot of the evolutionary forces at play throughout the history of the species.

The theory, experiments, and fieldwork in population genetic studies to describe the effect of evolutionary forces on the diverse patterns in nature have resulted in a unified theory of evolution since the foundation of Gregor Mendel’s model of inheritance of traits. Since then, a great diversity of statistical methods\(^4\) has been developed to perform parameter estimation and hypothesis testing based on the standing patterns and structure of genetic variation in wild populations. These have equally facilitated and improved research in conservation biology with the general aim to maintain genetic diversity as a species’ insurance for future vagaries of natural phenomena (Frankham et al., 2002). With the advent of the genomic era, measurements of genetic variation can be estimated with improved precision in each individual, and without necessitating a large sample size which is the case when studying species that are threatened, hard-to-find in the field or already extinct.

\(^4\) Computer programs for population genetic data analysis have been reviewed extensively in Excoffier et Heckel (2006) and Bourgeois (2016). The latter has made an updated list of these tools in this website http://methodspopgen.com/. Most of the programs are freely available. However an opensource software in R should be preferred as it allows great flexibility for any feature improvement and enhanced development in the codes to be realised without any restriction in any operating system (Windows, Linux, or Mac) - one would just need to pass over the language barrier. Publishing the code can be done easily and should be encouraged as the repeatability of the study can be examined by any users be it researchers or conservation agencies, and serve as a great educational tool for students.
“Small Population–Genetic Depauperate” Paradigm

When population genetic methods were at their early stage in conservation studies, the cheetah (*Acinonyx jubatus*), the fastest carnivore on land, was one of the first well-known genetic depauperate species, which highlights the important role of evolutionary history in species conservation (Frankham et al., 2002; O’Brien, 2003). The earliest work dealing with the genetic variation among cheetahs was undertaken by the prominent geneticist Prof. Stephen O’Brien and his research team. After an alarming call from the breeding centre in south Africa where cheetahs were pervasively difficult to reproduce in captivity, they examined the genetic variation in these individuals (O’Brien et al., 1986, 1983). They found no polymorphism detected based on allozyme variation and the proteins detected using two-dimensional gel electrophoresis revealed lower heterozygosity to those reported in other species at that time. Given the lower sperm counts in these cheetahs than other species, they suggested that the observed low genetic diversity has caused the low fitness in these individuals (O’Brien, 2003). Cheetahs in their natural habitat also appear to have similar structurally abnormal spermatozoa to these captive cheetahs (Wildt et al., 1987b). Additionally, cheetahs also exhibit highly uniform patterns in the major histocompatibility complex (MHC), a group of genes that are linked to the immune system, which has probably made them extremely vulnerable to the exposure and spread of feline infectious peritonitis caused by a coronavirus (O’Brien et al., 1985).

O’Brien et al. (1986) also compared the genetic variation of the cheetah to other African felid species such as leopards, lions, servals and caracals using additional allozyme loci. They found that cheetah’s genetic variation is consistently lower than other studied species, and even lower than the genetic variation in humans. The extreme scarcity of genetic variability and a high inbreeding coefficient in cheetahs compared to other felids and mammals were thought to be the reason why they were susceptible to disease and carried highly abnormal sperm, all of which contribute to the predicament of breeding in captivity. With more extensive sampling, the Asiatic lions from the Sakkarbaug Zoo in India showed a lack of polymorphism across all of the 50 allozyme loci tested and “produce ejaculates with more pleiomorphic sperm forms and fewer motile spermatozoa than even the reproductively compromised Crater lions” (Wildt et al., 1987a). The authors hypothesized that the lack of neutral genetic vari-
ation revealed in their study could be the result of ancient contraction in the natural population and not of captivity.

The analysis of the variation of mitochondrial DNA (mtDNA) and DNA fingerprints has allowed Menotti-Raymond and O’Brien’s (1993) to estimate the timing of this demographic contraction. Their estimation of the bottleneck event was about 10,000 years ago, which suggests the demographic contraction coincided with the Last Glacial Maximum during the Pleistocene. They argued that the extreme climatic changes, pushed cheetah populations into repeated genetic bottlenecks, resulting in being the only extant representative of the genus. These interpretations seem intuitive and have important considerations for conservation, but some found it rather controversial. For instance, Merola (1994) further assessed this hypothesis by comparing the patterns observed in cheetahs to other carnivores. She found that the low genetic variation is not unique to cheetahs - some carnivores have lower variation than cheetahs, and this group of terrestrial carnivores have even lower polymorphic levels when compared to other mammalian groups. O’Brien (1994) criticized this study, arguing that “the estimates of lower genetic variation in eight species of carnivores derive from early allozyme surveys that are almost certainly inaccurate because they deal with very few loci”.

Driscoll et al. (2002) revisited the genetic patterns of large felids using 90 microsatellite loci and a more robust approach to infer demographic history. They revealed that the levels of genetic variation from allozyme and microsatellite loci were similar, with the Asiatic lions showing the least genetic diversity. The most striking result in this study, however, is that depending on the markers and sample size, other species at population-level do appear to have lower diversity than the cheetah ‘model’. Although allozyme polymorphism was lower in cheetahs (2 – 4 %) than in the North American puma (5 – 10 %), the puma’s genetic diversity in microsatellites showed even lower values (42.9 – 75 % vs 81 – 84% in cheetahs) and was not significantly different than in Asiatic lions. They interpreted the results such that all three species had suffered genetic bottleneck at different periods and locations in the past. First the African cheetahs, then more recently the North American pumas, and finally the Asiatic lions likely caused by over-hunting by humans less than 100 years ago (Driscoll et al., 2002).
Carnivores have life-history traits such as long generation time and low reproductive rate making them more vulnerable to the drastic environmental changes during the Pleistocene and even more so by recent human activities (Jablonski et al., 2000; Johnson, 2002). From the maternal inheritance point of view, the order Carnivora seemingly does have the least nucleotide variation (Nabholz et al., 2008). Based on whole nuclear genome studies, other threatened species mostly carnivores have more depleted genome-wide levels of heterozygosity than cheetahs, such as the brown hyena (*Parahyaena brunnea*) (Westbury et al., 2018), narwhal (*Monodon monoceros*) (Westbury et al., 2019), island fox (*Urocyon littoralis*) (Westbury et al., 2018), Iberian lynx (*Lynx pardinus*) (Abascal et al., 2016; Casas-Marce et al., 2017), snow leopard (*Panthera uncia*) (Cho et al., 2013) and the orca (Westbury et al., 2018) (Figure 1). These examples support the hypothesis that genetic threats such as inbreeding depression, loss of adaptive variation, accumulation of mutations linked to deleterious recessive genes arising through long-term reduction of genetic diversity and recurring genetic bottlenecks are unavoidable in small and isolated populations of species with low density, as is the case with carnivores. Surprisingly, the domestic cat (*Felis catus*), one of the most famous pets in the world, has the least genomic diversity compared to other carnivores (Cho et al., 2013) and potentially lower than their closest wild relatives (Khan et al., 2017). This reflects back to Merola’s findings and her question raised in (1994): “Is it possible for a species to exist at what we have traditionally considered a perilous level of genetic uniformity?”, which remains an interesting scientific inquiry.

Although carnivores in general make up a small percentage of the animal kingdom, they occupy just about every array of ecological niches, from terrestrial to aquatic habitats, from the tropics to the poles. They have a very important place in the interaction webs of life specific to the ecosystem, and thus are integral for maintaining a balanced natural community. Furthermore, conservation effort focusing on carnivores with large area requirements could indirectly protect other threatened species (Roberge and Angelstam, 2004). Unfortunately, the effectiveness of carnivores as umbrella species is currently compromised by land use changes under human population pressures (Di Minin et al., 2016). Further decrease in size over time in these populations would undergo a process that drives the genetic variation and population size into a vortex towards extinction (Figure 2). Subsequently, the loss of these carnivores could
result in a catastrophic shift in the top-down trophic cascade leading to ecosystem changes that are abrupt and hard to undo, which carries significant implications for the maintenance of biodiversity (Estes et al., 2011; Schmitz, 2007).

There is clear evidence that the inexorable growth of recent anthropogenic activities driving for instance: habitat fragmentation and landscape changes, have severely jeopardized the gene pool in carnivores (e.g. Bull and Maron, 2016; Creel et al., 2019; DiBattista, 2008; Leigh et al., 2019; Lino et al., 2019; Miraldo et al., 2016; Rivera Ortíz et al., 2015; Schlæpfer et al., 2018). The diverse features of carnivores make them an interesting model-system, highly relevant for further investigation of the “Small population–genetic depauperate” paradigm, through an interdisciplinary framework of ecology and evolutionary genetic study. Uncovering the relationship between current and past patterns of genetic diversity can consequently lead to understanding the evolutionary history of carnivores, human-induced evolutionary changes in species and efficiently turn the spotlight on which carnivores and their locations are of conservation priority. However, population genetic studies on carnivores are still lacking in Southeast Asia, one of the hotspots for carnivores’ evolutionary history (Beheregaray, 2008; Sechrest et al., 2002) and also an area immensely impacted by human activities (Sodhi et al., 2004). In chapter I, I reviewed what is known about the anthropogenic impact on the genetic diversity of carnivores, addressed this gap, and presented some recommendations for future work.
### General Introduction

**Figure 1.** Genome-wide heterozygosity from autosomal scaffolds across different taxon obtained from published studies.

*Horizontal bars represent the standard deviation where multiple values exist. The food habit is indicated on the right, (O)mnivore, (C)arnivore and (H)erbivore. Numbers next to the taxon name refers to the references as listed in Annex 1. Figure is author’s own work.*
**Figure 2. The extinction vortex**

The concept was coined by Gilpin & Soulé in 1986 to describe the process that small and isolated population would experience greater stochastic process driving a negative feedback loop towards more reduced population size and thereby increased extinction risk. Consequently, where the population size or density falls below some critically threshold, a strong phenomenon called the Allee effect can alter the population dynamics and the functioning of social groups such as in carnivores, and accelerate the time-to-extinction until the last individual has disappeared (Courchamp et al., 2000, 1999; Sanderson et al., 2014). The figure above illustrate the genetic Allee effect (stronger as the thickness of the arrow increases) at the core of an extinction vortex (Luque et al., 2016). Figure is author’s own work.
Genetic Variation, A Keyhole To Past Population Size?

Traditionally, species monitoring using live-capture techniques or camera-traps are applied to survey the number of individuals and estimate the abundance within a population based on their unique marks (Boitani et al., 2012; Karanth et al., 2010). Comparing the abundance over time, across species, and in different locations, can provide explanation of the causal mechanisms in the population dynamics. However, carnivores are logistically more difficult to track than most other animals, as they are more secretive, nocturnal, live in remote areas inaccessible to researchers, and exist at low densities (Boitani et al., 2012). Gaining reliable and precise estimates of their population dynamic or fluctuation is a difficult task, as it would require long-term surveys over multiple years and in large areas with considerable logistical resources (Harmsen et al., 2017). Furthermore, there are neither reliable nor standardized data of species abundance from historic records to estimate past population sizes. Some scarce reports were mostly found in accounts of hunting expeditions by royalties and nobles or bounty hunters in the late 19th to early 20th centuries, and probably underestimate the actual abundance (Karanth et al., 2010).

Since each individual has its own genetic makeup, it is also possible to distinguish changes in population size over time based on the standing genetic diversity, using the effective population size, $N_e$, was introduced by Sewall Wright (1931) based on his observation on the genetic drift and in-breeding in real-world small populations. In a small population the genetic drift in that random fluctuation of alleles, leading to fixation from one generation to the next in a finite population, becomes important and can lead to great loss of genetic variation. Along with Ronald A. Fisher and J.B.S. Haldane, they mathematically generalized the $N_e$ leaning on the Mendelian mechanism of heredity and Darwinian evolution to account for a variety of convenient assumptions which are: the population is in constant size with random mating, with discrete generations, and without migration or gene flow, selection, or mutation. $N_e$ was thus defined as “the number of breeding individuals in an idealised population that would show the same amount of dispersion of allele frequencies under random genetic drift or the same amount of inbreeding as the population under consideration”. Empirical evidence in data from wild populations has also verified the validity that the level of genetic variation can be related to the variation in
Ne across species (e.g. Frankham, 1996; Hamrick and Godt, 1996; Gram and Sork, 1999). I further contributed to this knowledge by examining whether species traits (in Chapter II) and population density from camera-trap studies (in Chapter III) can be used to predict the level of genetic diversity within a population.

Considering that there are many parts of the genome that were not affected by fitness and can be assumed to evolve neutrally or non-adaptively, Motoo Kimura extended the Wright-Fisher model to only include neutral mutation and genetic drift, or a stochastic process in determining the genetic variation within a population (Kimura, 1964, 1983). From the neutral theory of molecular evolution’s point of view, Kimura defined Ne as the inverse rate of inbreeding effect, that is the smaller the population, a more rapid gain in homozygosity, thus loss of heterozygosity is expected without necessarily affecting fitness or evoking selection. The neutral theory can be used as a null hypothesis to determine the expected genetic variation and whether Ne in the past has contracted or expanded. For instance, in the event of bottleneck or founder effects in the past, where severe reduction of ancestral Ne and rapid loss of rare alleles within a population occurs, the average heterozygosity increases slower in case of population recovery, compared to a population with stable size over time (Maruyama and Fuerst, 1985; Nei et al., 1975). The usefulness of the Wright-Fisher model has served as an important theoretical foundation in evolutionary biology to describe the complex relationships between genetic diversity, population size, and phenotypic diversity (Ellegren and Galtier, 2016). This has also inspired the development of diverse approaches for demographic inference according to sample size, type of molecular data and the desired timescale (reviewed by Beichman et al., 2018).

Demographic Inference From High-throughput Sequences

The technological revolution in DNA sequencing since the breakthrough by Sanger et al. (1977) has offered powerful and more precise data to gain information of molecular variation, from a handful of co-dominant markers to high-throughput sequencing\(^5\) of a whole-genome. This development has stimulated research in other field in population genetics such as modelling complex demography of populations and DNA sequence evolution going backward in time. Based on the coalescent theory as mathematically formulated by John Kingman (1982), the variation among DNA se-

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\(^{5}\) Also sometimes referred to as next-generation sequencing (NGS)
quences expected under the neutral theory of molecular evolution can be used to estimate the probability that the two lineages coalesce in the past i.e. two sequences come from a single ancestral sequence or from the most recent ancestor (Emerson et al., 2001; Wakeley, 2008). In other words, the greater the variation within a locus, the smaller the probability of choosing random ancestors through a coalescent process in previous generations (1 / Ne), thus the branch length towards the most recent ancestor tends to increase with population size, whereas in a small population, their common ancestor will be recent (i.e. shorter branch lengths).

The coalescent-based approach provides a simple and useful model with many applications, which include assessing the time-varying population size that went through historical process, based on the contemporary variation of non-recombinant DNA sequences within a population, typically assumed at mutation-drift equilibrium. Thus sprung a new field in population genetics: phylogeographic study (Avise et al., 1987), which examined empirical data to infer how major geographical and climatic changes in the past inevitably shaped the population structure of species. Phylogeographic studies based on the comparison between intraspecific phylgroups and genetic distances, have the potential to elucidate carnivores recent speciation in the Pleistocene, since 72% of mammalian population divergence under a standard mtDNA-clock calibration, fall within the last 2 million years ago (Avise et al., 1998). However, mtDNA divergence only recovers the matrilineal history, in that neutrality-based predictions (i.e. random fixation of alleles varied by population size over selection) could be biased (Bazin et al., 2006; Nabholz et al., 2008), and the inferred time may be confounded by a wide margin of error (Lyrholm et al., 1996; Stiller et al., 2014). Nevertheless, the increasing study of phylogeography has provided ample raw mtDNA data in public repositories such as GenBank, which has paved the way for a global-scale comparative study correlating multi-species genetic variation with recent environmental predictors and human activities (e.g. Millette et al., 2020; Miraldo et al., 2016; Schmidt et al., 2020; Theodoridis et al., 2020). Similarly, such large-scale comparisons have also been conducted using neutral nuclear DNA such as allozymes and microsatellite markers, but these studies have been restricted to meta-analysis - by compiling genetic variability indices from published studies across multiple species (Almeida-Rocha et al., 2020; DiBattista, 2008; Leigh et al., 2019; Lino et al., 2019; Rivera Ortíz et al., 2015; Schlaepfer et al., 2018). In chapter II, I explore whether the
genetic variation within populations - derived from microsatellite markers within a
taxonomic family sharing similar life history traits, can be correlated to consequences
of the late Pleistocene and/or recent human population density.

Where sample size is restricted to few individuals for comparative studies,
which is the case when studying threatened species, the pairwise sequentially
Markovian coalescent (PSMC) approach developed by Li and Durbin (2011) would be
more appropriate, as it requires at least a single diploid individual. This method detects
the distribution of coalescent times by applying a hidden Markov model to contiguous
stretches of genomic segments to identify the local density of polymorphic sites and
the number of meiotic recombination events, thus high-throughput sequences as-
sembled to high contiguity would be ideal (Li and Durbin, 2011; Mather et al., 2020).
Previous studies have demonstrated the robustness of this method for comparative ana-
lysis of the variation in demographic history and population structure changes among
avian populations (Nadachowska-Brzyska et al., 2015), parasites and their host (Hecht
et al., 2018), marine and terrestrial snakes (Ludington and Sanders, 2021), forest tree
species (Patil et al., 2021) and rhinoceros species, including three extinct relatives (Liu
et al., 2021). One caveat is that changes in population size due to gene flow via migra-
tion between populations or types of bias in mate choice (e.g. mating system, group
structure), could create confounding signals of population contraction or expansion
over time. Hence the PSMC results require cautious interpretation and cross-examina-
tion of population structure from other analyses (Mather et al., 2020; Mazet et al.,

The PSMC approach works well for periods between 10,000 to 1 million years
ago, but tends to overestimate recent population sizes, and to spread sudden changes in
population size over several preceding tens of thousands of years (Li and Durbin,
2011; Mather et al., 2020). A more recent demographic history can be inferred based
on the individual inbreeding level from whole-genome data. Between a pair of gam-
etes, some regions of its genome can be a direct copy of DNA from their common an-
cestor. These identical-by-descent (IBD) regions were first identified in the sampled
reference families from Centre d’Etude du Polymorphisme Humain, to build a genetic
map of the human genome (Broman and Weber, 1999). It was later recognized that
these IBD regions are common and retain a high rate of homozygosity even in outbred
populations, and can therefore provide information about the demographic history of
human populations (Gibson et al., 2006; McQuillan et al., 2008). These long homozygous tracts in the IBD regions are also known as runs of homozygosity, ROH.

The length and distribution of ROH on mapped loci affected by recombination, natural selection, linkage disequilibrium and population structure are indicative of the magnitude and timing of decrease in $N_e$ (Gibson et al., 2006). Since its discovery the application has been manifold in domestic animal and managed captive population studies, of which the pedigree or parentage information across several generations is usually well constructed (reviewed in Peripolli et al., 2017). The inbreeding coefficient derived from pedigree is strongly correlated to this genomic inbreeding coefficient based on ROH and in principal, a high coefficient of both values indicates that their parents are more closely related on average (Kirin et al., 2010; McQuillan et al., 2008). According to Pemberton et al. (2012), the length of ROH in human populations can be divided into three classes, which can determine how recently they shared common ancestors. The first class being short ROH, measuring tens of kilobase pairs, that probably reflect homozygosity from historically shared haplotypes that contribute to the patterns of local linkage disequilibrium (i.e. non-random association of alleles at different loci). Secondly, intermediate ROH, measuring hundreds of kb to several Mb, that probably results from background relatedness due to limited population size. The third class with long ROH, measuring multiple Mb, that is probably resulting from recent parental relatedness less than 10 generations back. Additionally a comparative study across mammals based on this inbreeding measurement revealed that Red List status does not reflect the level of ROH burden (Brüniche-Olsen et al., 2018), which suggests that some species require more conservation attention than previously assumed.
**Data Source**

As previously mentioned, the choice of molecular markers is an important issue and must be considered carefully for comparative study. Recognizing the effect of population size changes on genetic diversity can be a difficult task, as the genetic variation with different inheritance mode can also be determined (or not) by an interplay of factors related to species biology, such as: geographic ranges and degree of isolation (island/mainland), temperate or tropical regions, body size, rate of chromosome evolution, and mutation rate in different taxonomic groups (vertebrates vs. invertebrates, animals vs. plants) (Nevo, 1978; Frankham, 1996; Doyle et al., 2015; Ellegren and Galtier, 2016; Nabholz et al., 2008). Allozyme, microsatellite and transcriptomic data do show concordance between genetic variation and species life-history traits, which reflects the population size variability (Flight, 2010; Romiguier et al., 2014; Vachon et al., 2018). At a taxonomic-family scale, the level of genetic diversity based on mtDNA in cetacean species (Order Artiodactyla) show strong association with social structure and species latitudinal range, whereas those based on nuclear DNA markers had a strong association with population size and habitat type (Vachon et al., 2018). Similarly in some Pinnipeds (Order Carnivora), nuclear genetic diversity shows strong signs of recent population decline and strong associations with the breeding habitat and mating system (Stoffel et al., 2018). Understanding which life-history traits can be a by-product of the within-species genetic variation is essential to understand how Ne varied with time among carnivores. In chapter II, I evaluate the role of species and ecological traits in determining the genetic variation derived from microsatellite markers across felids.

Tracking reliable changes in population size within a single population often requires a temporal reference point, or samples taken across many generations to establish a baseline level of genome-wide diversity before the population decline (Díez-del-Molino et al., 2018). In this context museum collections hold valuable resources for DNA sequences. Although the process of sampling from museum collections is destructive and the obtained ancient DNA is hampered by high contamination and low preservation rates (Wandeler et al., 2007), novel technical advances in DNA sequencing and bioinformatics are rapidly expanding to gather high quality of high-through-put sequences from ancient and historical specimens for robust inferences in evolu-
tionary biology (Der Sarkissian et al., 2015a). Comparing genetic structure among contemporary and historical specimens can not only quantify the genetic decline and differentiation, but concurrently help to ascertain misidentified or unlabelled specimens (Barnett et al., 2006a; Rodriguez-Varela et al., 2015), to identify unique alleles or haplotypes that are no longer present in the extant population (Dures et al., 2019; Janečka et al., 2014), to uncover the relationship of extinct populations or species with living close relatives (Charruau et al., 2011), to infer the timing of inter-specific introgression (Beaumont et al., 2001), or simply to increase the sample size in the study for more precise genetic diversity estimates (Cossios et al., 2012; Napolitano et al., 2014). Despite these advances in ancient DNA, fossil records - the primary means of understanding evolution in deep time are often under-represented in the tropics where about 70% of carnivores dwell6 (Burgin et al., 2018; Vilhena and Smith, 2013). According to the best available database of fossil occurrences, the Paleobiology Database (www.paleodb.org), there are 5,017 Quaternary fossil records, which represent 303 species from 13 families, but only 26% of these are located between 30°N and 30°S. In chapter I, I further discuss some challenges and perspectives of sampling from museum collections for population genetics in Southeast Asia.

To obtain high genomic data quality in terms of contiguity and completeness of threatened and elusive species, ample quantity of blood or tissue samples are required and these can be readily collected from animals held in captivity. Many critically endangered carnivores have been selected as important flagship species to increase awareness, raise support and generate funding for biodiversity conservation (Macdonald et al., 2015; Consorte-McCrea et al., 2019). Additionally, ex-situ research performed on the captive populations of carnivores can have large implications for both preserving wild populations and improving breeding management prior to reintroduction. Given that captive populations are initiated by several individuals taken from the wild, numerous concerns have been addressed on the genetic adaptation to captivity due to a phenomena known as the founder effect (Figure 3) – a reduced genetic variation that resulted when a few individuals from a large population in the wild were used to establish the captive population (e.g. Clubb and Mason, 2003; Frankham, 2008; Christie et al., 2012; Theodorou and Couvet, 2015; Grueber et al., 2017).

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6 The number of known carnivores could be as many as 304 living and 7 extinct species. They are classified into 16 families, with the estimated number of species occurred in the tropics (Afrotropic, IndoMalaya and Neotropic) is roughly 207 species (Burgin et al., 2018).
Using genomic data, Willoughby et al. (2017; 2019) demonstrated that the number of non-neutral SNPs have increased over time in captivity across multiple species with long-running captive-breeding programs. However, the level of genetic variation is tightly dependent on the management strategy (Willoughby et al., 2017; Humble et al., 2020). Some species have also exhibited changes in behavioural response due to captive conditions. For instance, the Tasmanian Devils show increased vulnerability to death by motor vehicles with increasing generations in captivity (Grueber et al., 2017). Another aspect of captive environment that we poorly understand is whether the inbreeding depression had negatively affected the genome structure and function in captive population. It seems clear that inbreeding depression can increase in stressful environments (Armbruster and Reed, 2005) and such interaction is less likely to occur in the wild probably due to a more effective genetic purging (Pemberton et al., 2017). Carnivores held in captivity offer interesting points of view on the baseline Ne and genomic variation imposed by the recent founder population, as well as the consequences of the recent environmental alteration on the estimated variation of the Ne over time. This is one of the objectives that I tackle in Chapter IV.

**Figure 3** Schematic representation of the founder effect leading to a reduced genetic diversity (as depicted by the coloured DNA symbols) in captive population compared to those in the wild.
**Cats’ World Dominance**

In this section I review some recent studies as to why Felidae is a particularly important mammalian family and a good proxy for a global-scale study of genetic variation and the demographic history of carnivores, including elucidating those patterns influenced by human interactions. Several reviews have covered the reproductive biology (Andrews et al., 2019), conservation and ecological research efforts (Tensen, 2018a; Zanin et al., 2015), relationships between felids and people based on archaeological and historical records (Faure and Kitchener, 2009), advances in the genomic study (O’Brien et al., 2008; O’Brien and Johnson, 2005) and one book in particular which covered the biology and conservation of felids extensively (Macdonald and Loveridge, 2010).

Felids with distinctive auditory bullae, retractable claws and blunt-nosed face are a rather successful group of hypercarnivores (i.e. species with 70% meat requirements in their diet). The Felidae is the second-largest family within Carnivora with 14 genera and 41 extant species of felids, including the domestic cat (Kitchener et al., 2017; Hunter and Barrett, 2019). Felids natural distribution encompasses habitats from forests to mountains and from wetlands to savannahs, in all continents except for Australia and Antarctica. They are highly diverse in tropical regions with the most number of sympatric species residing in Asia followed by South America, with 15 species spread in different types of habitat in the Indian subcontinent alone, which is one of the most human populated regions (Kitchener et al., 2017).

The entire cat family, including their extinct members, has been extensively studied compared to other Carnivora families, both globally and on each individual continent, providing unparalleled data suitable for large-scale studies across time and space (Pérez-Irineo and Santos-Moreno, 2013). Currently there are 11 extant felids with whole-genome sequences, the most representative within the order Carnivora according to wikipedia\(^7\). Other felids with sequenced genomes which have not yet been listed on this website are puma and jaguarundi from America (Ochoa et al., 2019; Tamazian et al., 2021), and the Chinese mountain cat (Yu et al., 2021). Additionally, the nuclear genomes of large cats that went extinct near the end of the Late Pleisto-

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cene, *Homotherium* (Barnett et al., 2020) and *Smilodon* (Westbury et al., 2021) have also been recently sequenced through ancient-DNA analyses.

The presence of felids in the ecosystem can enhance numerous ecosystem services such as providing long distance seed dispersal (Sarasola et al., 2016), additional resources to scavengers shaping their diversity - be it in insects or birds (Elbroch et al., 2017b), biological pest control in agricultural landscapes (Silmi et al., 2021) and intangible benefits to humans including cultural values and spirituality in almost every continent (Herrmann et al., 2013; Menéndez, 2019). Alongside horses, mammoths, and rhinoceroses, big cats were among the most frequently depicted animals as engraved in Chauvet-Pont-d'Arc Cave (Clottes and Azéma, 2005). An ivory sculpture resembling a composite of a felid-human being has also been recovered in south-western Germany (Conard, 2003). These examples of figurative artwork indicate one of the earliest cultural endowments by humans more than 30,000 years ago outside of Africa. Today the presence of many felid species is unfortunately persecuted due to conflicts with humans, raising a myriad of challenges for species and landscape conservation (Inskip and Zimmermann, 2009; Macdonald and Loveridge, 2010).

**The Cat Is Out Of The Den**

The evolutionary history of Felidae is a fascinating subject with abundant in-depth research inquiries. The phylogenetic tree derived from molecular data of extant species and dated based on fossil records, allows to determine major evolutionary events in Felidae, such as a rapid and recent lineage diversification (Johnson et al., 2006), the origin of cat domestication (Driscoll et al., 2007), as well as complex patterns of ancient hybridization events (Li et al., 2016a)(Figure 4).

Felids are very similar in their general body morphology and musculature, but rather diverse in terms of body size - ranging from 1 kg in the Rusty-spotted cat (*Pristailurus rubiginosus*) to more than 300 kg in Siberian tigers (*Panthera tigris altaica*) (Cuff et al., 2015), coat pattern (Werdelin and Olsson, 1997) and hunting techniques - be it from trees, in water as well as in a pack (Kitchener et al., 2010). Carnivores exhibit varying degrees of sociality and in felids, lions are the only truly social species that live in prides (Nowak, 1983). Although most felids are known to be solitary, a formation of coalitions among adult male individuals has been observed in cheetahs, wherein females showed promiscuity (Caro and Collins, 1987; Gottelli et al., 2007).
General Introduction

Pumas in Wyoming and feral cats on a small island in Japan have also exhibited social organization while procuring resources (Elbroch et al., 2017a; Yamane et al., 1996). The benefits of social spatial-groupings in carnivores have been considered as “equally perhaps the evolutionary origins of contemporary highly social groups” (Macdonald, 1983). The felids’ evolution into a broad range of predatory strategies adapted to their prey preference and availability may have been catalysed by their agility to disperse strategically into new environments and by their capacity for interspecific hybridization facilitating functional novelty (Kiltie, 1988; Li et al., 2016a; Pfennig et al., 2016).

Felidae had likely originated and diverged from its sister group, Prionodontidae - a monogeneric family with two extant species, some time after the Eocene–Oligocene extinction event, termed as ‘Grande Coupure’ (Zhou et al., 2017; Hassanin et al., 2021). Then, they expanded its range from South Asia into the East Palaearctic in the late Miocene, during which the radiation into 8 living lineages had occurred within a short burst of time between 10 and 6 Mya (Johnson et al., 2006; Li et al., 2016a; Zhou et al., 2017). Diversification analyses through fossils and molecular data have suggested that this rapid radiation is associated with multiple extinctions in the temperate regions and dispersal events into the tropics within 6 of these lineages (Rolland et al., 2015a). As the resource availability increases in the tropics during the late Miocene cooling, the increase in diversity of prey species in the tropics had likely contributed to their long-distance dispersal, partitioning into various feeding strategies and tenability to coexist with other members of the same guild (Ray and Sunquist, 2001). The evolutionary history of an extinct lineage, Machairodontinae based on molecular data has recently unravelled. Also known as the ‘sabre-toothed cats’, these taxa once coexisted with the ancestors of living felids but went extinct in the Late Pleistocene extinction, leaving extensive fossil traces across the continents (Werdelin et al., 2010). The molecular divergence between the wide-ranging Homotherium and Smilodon – that lived in America only, was estimated to occur between 18 and 22.5 Mya (Barnett et al., 2020; Paijmans et al., 2017; Westbury et al., 2021).
Figure 4. Dated phylogenetic tree of Felidae as inferred from nuclear DNA (Li et al. 2016), and their geographic distributions.

Distribution range of each species are depicted in the rectangles at the edge of the branches (Johnson et al. 2006, Li et al. 2016), with each color represents the area as shown on the map. Species with white colored square are distributed in all areas. Vertical lines between branches represent some major dispersal and hybridization events in the lineages. The divergence time between Felidae and Prionodontidae is based on mtDNA data (Hassanin et al., 2021), between Felinae and Macahroidontinae from Pajjmans et al. (2017) and Westbury et al. (2021). Evolutionary events represented on the branches such as expansion and extinction were adapted from Rolland et al. (2015), whereas hybridization events taken from Li et al. (2019). Illustrations of felids are from Ward (1899, 1900) and Voyage dans l’Amérique Méridionale (1835-47), taken from Biodiversity Heritage Library, and phylopic. Figure is author’s own work.
The evolutionary history of Panthera, a genus well recognized by the large body size, is still ambiguous and inconsistent in some studies (Davis et al., 2010). This great uncertainty is partly because of the “ghost lineage” (i.e. cladistically inferred lineage undocumented by fossils) lasting 7 Mya (Werdelin et al., 2010). Although some missing links have been found and provided some improvements on the crown age estimation of Panthera (e.g. Tseng et al., 2014), the difficulties of identifying and attributing fossils to species due to their gross morphological similarities, as well as the occurrence of introgression between lineages, have led to contrasting patterns of divergence time within some big cat species. For instance, the initial out-of-Africa expansion of leopards (Panthera pardus) into Asia is contentious being around 2 Mya based on old fossils from Pakistan, or later: some time between 710 and 483 kya based on mitogenome phylogenetic (Paijmans et al., 2018), or between 500 and 600 kya using historical and ancient samples (Paijmans et al., 2021), or even earlier, between 400 and 300 kya based on changes in effective population size inferred using nuclear genomic data (Pečnerová et al., 2021).

In spite of the ability of these apex predators to disperse over large distances, the only extant species in the big cat genus living in the New World is the jaguar (Panthera onca). This species has probably diverged from the common ancestor of Panthera and entered America between 3 and 2 Mya (Johnson et al., 2006; Li et al., 2016a) or probably later near 1.5 Mya (Christiansen and Harris, 2009). Similarly, the timing of colonisation of the New World in other felids is complicated. Based on the molecular estimation, the ancestral areas reconstructed on the phylogenetic tree of Felidae revealed the common ancestor of Leopardus, Puma and Lynx lineages have reached America around 8 Mya (Johnson et al., 2006), whereas Zhou et al. (2017) inferred the dispersal of the common ancestor of puma and cheetah into America occurred in a similar time period but the ancestor of Lynx arrived 4 My later from Eurasia. Despite these inconsistencies, the recent lineage diversification within felids may have been accelerated during the Pliocene and Pleistocene epochs and similar trends have been found in other carnivores (Hassanin et al., 2021).
Phylogeographic Patterns In Felidae

Phylogeographic studies on felids have yielded interesting and significant findings regarding how the climatic and environmental changes that occurred during the Pleistocene contribute to the shaping of genetic lineages. Some examples from phylogeographic studies given in each continent are as follows:

- In Asia, the sea level changes during glacial/interglacial periods, and the forest/savanna expansions in the wake of the Toba super volcanic eruption 74,000 years ago, have been suggested as the cause of population differentiation in *Catopuma* (Luo et al., 2014; Patel et al., 2016), *Prionailurus* (Patel et al., 2017a, 2017b), *Neofelis* (Buckley-Beason et al., 2006; Wilting et al., 2011, 2007) and tigers (Luo et al., 2004; Liu et al., 2018). Luo et al. (2014) compared the phylogeographic patterns across 6 species in Southeast Asia, which all have significant disjunct patterns between Indochina and Sundaland, except for the leopard. This study also revealed that the timing of differentiation within tigers into these regions was more recent (72 kya) than that of smaller felids, such as: the Asiatic golden cat, marbled cat and leopard cat (2.7 - 0.68 Mya).

- The disjunct genetic pattern of the southern and northern African lion lineages at some point in the Late Pleistocene has been linked to the expansion of the Sahara desert and rain forest in the central African region, acting as geographic barriers for dispersal (Barnett et al., 2006b; Bertola et al., 2016; Manuel et al., 2020). A similar population split has been attributed to the cheetahs (Charreau et al., 2011; Rai et al., 2020).

- The European wildcat (*Felis silvestris*) ancestors had suspected to arrive on this continent between 230 to 173,000 years ago. Sometime in the Late Pleistocene to early Holocene multiple population groups dispersed out of their glacial refugia throughout western Europe, even into Sicily benefiting from the fluctuation of the Mediterranean sea level (Driscoll et al., 2007; Mattucci et al., 2016; Sommer and Benecke, 2006). Similarly, the Eurasian populations have been suggested to diverge from different glacial refugia, followed by population expansions into distinct phylogroups across Europe, however, the timing predates the Last Glacial Maximum (Rueness et al., 2014). One of these phylogroups had probably differentiated in southern Europe and gave rise to
the Iberian lynx around the Pleistocene/Holocene boundary, when the gene flow ceased (Rodríguez Varela et al., 2016).

- The dry/humid cycles leading to the expansion and contraction of the Andean forest and savanna, the rapid uplift during the last phase of Andean Cordillera formation, and oscillations of large river banks during the Pleistocene in South America are among the potential cause of population genetic structuration and hybridization in *Leopardus* (Cossios et al., 2009; da Silva Santos et al., 2018; Ruiz-García et al., 2018; Trigo et al., 2013). Other felids such as jaguarundi, jaguar and puma ancestors may have participated in the Great American Biotic Interchange via the Panamanian land bridge (Moreno et al., 2006; Ruiz-García and Pinedo-Castro, 2013; Webb, 2006). During the Pleistocene, the pumas from South America have probably recolonized and expanded into North America multiple times, with the latest replacement of the earlier founder populations occurring near 10 kya (Culver et al., 2000). This timing coincides with the late Pleistocene extinction in North America.

Empirical studies based on microsatellite markers and genomic data have also inferred ancient bottlenecks across felids. Some examples include the populations of Iranian and African cheetahs (Charrua et al., 2011; Dobrynin et al., 2015), ocelots from South America to Texas in the USA (Figueiredo et al., 2015; Janečka et al., 2007), Bengal tigers in Nepal (Thapa et al., 2018) and India (Cho et al., 2013; Singh et al., 2017; Armstrong et al., 2021), and the Amur tigers in the Russian Far East (Cho et al., 2013; Sugimoto et al., 2014), the Iberian lynx in Spain (Abascal et al., 2016; Casas Marce et al., 2013), the Scandinavian lynx (Hellborg et al., 2002), jaguars (Figueiró et al., 2017), and pumas in South and North America (Castilho et al., 2012; Culver et al., 2008; Ernest et al., 2014; Gustafson et al., 2019; Holbrook et al., 2012). Conversely, some studies found no evidence of bottlenecks in the Indian (Dutta et al., 2013) and African leopards (Spong et al., 2000).

Comparative demography approaches have the potential to infer whether, where and when, distinct genealogical lineages have undergone shared or opposed episodes of population growth and contraction (Hecht et al., 2020). Despite these extensive studies on felids in each region, the demographic history for many other species is still unknown. To my knowledge, the evolutionary history of the *Lynx* throughout its entire distribution (Europe, Asia and North America) has never been examined.
together either. While there is clear evidence from fossil records of an evolutionary shift towards smaller-bodied carnivores and other mammals towards the late Pleistocene (Lyons et al., 2004; Prevosti and Soibelzon, 2012; Middleton et al., 2020; F. A. Smith et al., 2018), the genetic variations of small cats have rarely been compared to those of big cats. There is sparse evidence from Southeast Asia (Luo et al., 2014), and ocelots in South America (Ruiz-García et al., 2013; Wulfsch et al., 2016). The lack of study on smaller felids could hamper quantifying the magnitude of the impact of past climate change on the genetic variation of Felidae of different body sizes and ecological traits. Comparative demographics using the PSMC approach have also been applied to multiple big cat species (Cho et al., 2013). However the findings in this study concerning their shared or unique trend in population size over time and among species were not heavily discussed.

The causes of reproductive isolation and population divergence during felid evolutionary history, however, cannot be appointed alone to the complex history of geological processes, or sea-level and climatic oscillations during the Pleistocene leading to ecological changes. Certainly the evolutionary pathway in felids is in part shaped by our fascination towards, and fear of them, since the rise of modern humans during the Pleistocene (Daujeard et al., 2016; Treves and Palmqvist, 2007). Some of the phylogeographic studies above have suggested that the timing of genetic divergence and population declines overlapped with early human migration during the Pleistocene. However, the distinctive genetic and demographic patterns left in surviving carnivores during the climatic changes of the Pleistocene, before, and after human arrival, have never been quantified on a global scale. One study has attempted to correlate the extinction rate of carnivores to the past anthropogenic impacts in East Africa (Faurby et al., 2020). They found that the extinction rate of large carnivores was not affected by climate change but was in fact best explained by changes in human brain size or forest cover, likely since 4 Mya. Small carnivores, however, showed no changes in extinction rate (Faurby et al., 2020).

Quantifying the differential rate of demographic changes from the Pleistocene to the Holocene across species could identify the possible mismatches between the effects of climate change and the amplifying feedback from past to recent human interactions, which is of unequivocal importance for conservation management. Cardillo et al. (2004) evaluated the relationship of carnivores’ extinction risk defined by IUCN
Red List with biological and anthropogenic factors associated with high human population density (HPD) on a global-scale. Providing that there was a weak HPD effect on carnivores’ extinction risk after accounting for biological factors, the authors suggested amongst other explanations, that “species' current extinction risk status may reflect patterns of human impact in the past more closely than it does current impact”. Observations of the relationship of humans and felids in archaeological remains and current practices can elaborate to what extent ancient human influence may have left imprints in the genetic structure across felids of different body size since the Pleistocene.

**Anthropogenic Impact On The Genetics Diversity And Population Size Of Felids**

Throughout the vast majority of the history of our kind we have facilitated the cat population and tamed about 40% of felid species (Faure and Kitchener, 2009; Inskip and Zimmermann, 2009). During the medieval era, humans kept cheetahs and caracals as aides in hunting for imperial sport (Akhtar, 1996; Masseti, 2009). Similarly, jaguar and puma have been bred in captivity in Mexico since the ancient Maya (A.D. 1–550)(Sugiyama et al., 2018, 2015). Today almost all felid species can be found in ex-situ populations (Amstislavsky et al., 2017), and one can visit 30 species from all 4 continents in one zoological park in France, “Le Parc des Félins”.

Although large felids have been considered vermin, keeping a tame and small carnivore species during seafaring trips, for instance, may help reduce rodent pests in transit or at the destination (Vigne et al., 2014). Unintentionally our fascination with cats as a beneficial companion used as a biological control is probably the major reason for their domestication that began 9000 years ago in the historical Fertile Crescent, one of the cradle of human civilization (Driscoll et al., 2007). The actual number of domestic cats in the world is hard to pinpoint, but one attempt in 1986 has estimated it to be around 400 millions (Legay, 1986). The PSMC analyses of the domestic cat revealed that their ancestral populations, conspecific to the African wildcat, had experienced a drastic rise around 100 to 400 kya during the Middle to Late Pleistocene (Yu et al., 2021), which coincides with the timing of differentiation between the African and non-African modern human populations (Li and Durbin, 2011). Other felid species have also been postulated to disperse via human introduction where predators were
previously absent, but no robust evidence from molecular data has yet come to light (Suzuki et al., 1994; Kitchener et al., 2017).

Due to human expansion, felids have been forced to adapt to a different path throughout the course of evolution, in ways to ensure their survival in an ever-changing human altered landscape across the world. Some carnivores may have responded to human arrival or climatic changes differently, either by shifting their distribution (Pushkina and Raia, 2008) or changing their dietary strategy (DeSantis and Haupt, 2014). Many of those that did not have the opportunity to compete successfully against humans are ending up close to, or are already extinct, as classified in the IUCN Red List of Threatened Species (2020)(Table 1) and among those that survived, 73% are in population decline. For instance, about 11,650 tigers (Panthera tigris) have been estimated to be present in Southeast Asia (Malaya, Sumatra, Java and Bali) around 1820 (Boomgaard, 1994), and 80,000 tigers and 150,000 leopards (Panthera pardus) were killed between 1875 and 1925 in Central India (Rangarajan, 2005). Notably, perhaps less than 5% of these numbers remain today whereas Javan and Balinese tigers have been officially declared extinct since 2003, but may have succumb even earlier, either by the end of World War II or in the early 1950s (Jackson and Nowell, 2008).

Human-induced habitat fragmentation and urbanization have also affected the genetic connectivity of felids at population-level (Kozakiewicz et al., 2019; Lewis et al., 2015; O. Smith et al., 2018; Trumbo et al., 2019) as well as increased inter-specific encounters between felids (Cruz et al., 2018; Lewis et al., 2015). Human altered landscapes have also brought opportunities for smaller carnivores to exploit where access to anthropogenic food sources is easier. For example: bobcats in urbanised areas (Lombardi et al., 2017), servals in abandoned industrial sites (Loock et al., 2018), or the leopard cat preferring monoculture environments like palm plantations over secondary forests (Chua et al., 2016; Jennings et al., 2015; Silmi et al., 2021).

Where habitat loss and land-use for agriculture and human development is more pervasive, human contact in the remaining wildlife habitats is increased, adding to the woes of felids by hunting and exploitation (Symes et al., 2018). From early human history, carnivores could not escape being hunted for protection and for cultural values, such as use of pelt or for traditional rituals (Cueto et al., 2016). Large-scale hunting of large felids had increased dramatically during imperialism and colonial expansion in the 19th century, as rewards were also given by colonized governments
General Introduction

(Kang et al., 2010; Storey, 1991). Due to globalization more felids are poached in the wild and commercially bred for traditional medicine, luxury products and as exotic pets to satisfy the illegal market (Davis et al., 2020; D’Cruze and Macdonald, 2015; Nijman et al., 2019a, 2019b). The Afro-Tropic and Indo-Malay ecoregions have lost relatively fewer large and mega-carnivores in the past (Dalerum et al., 2009; Louys, 2014; Malhi et al., 2016; Stuart, 2015). However, today the global epicentres of traded animals, mostly large-bodied carnivores, are located in these regions where high land-use intensity coincides with high endemism (Hughes, 2017; Kehoe et al., 2015; Schefvers et al., 2019).
### Table 1. The distribution and conservation status of 41 felid species (in bold) and subspecies.

Subspecies and population follow those recognized in Kitchener et al. (2017). Red list categories were sourced from the IUCN Red List of Threatened Species (IUCN 2020).

<table>
<thead>
<tr>
<th>Species</th>
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<th>Red List status</th>
</tr>
</thead>
<tbody>
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<tr>
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<tr>
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<td>SW Asia and India</td>
<td>CR</td>
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<td><strong>3 Caracal caracal</strong></td>
<td>Africa and Asia</td>
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</tr>
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<td>NT</td>
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<td>Nepal to N Burma, China, Tibet and SE Asia</td>
<td></td>
</tr>
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<td></td>
</tr>
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<td><strong>6 Felis bieti</strong></td>
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<td>VU</td>
</tr>
<tr>
<td><strong>7 Felis catus</strong></td>
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</tr>
<tr>
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<td><strong>9 Felis lybica</strong></td>
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</tbody>
</table>
### General Introduction

<table>
<thead>
<tr>
<th>Species</th>
<th>Remarks</th>
<th>Status</th>
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</thead>
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<td>Africa and Asia</td>
<td>VU</td>
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<td></td>
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</tbody>
</table>
### General Introduction

<table>
<thead>
<tr>
<th>Species</th>
<th>Region</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Panthera pardus delacouri</em></td>
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| *Panthera tigris*                                                       | Asia                          | EN
| *Panthera tigris altaica*                                              | East Asia                     | EN
| *Panthera tigris amoyensis*                                            | SE Asia                       | CR
| *Panthera tigris balica*                                               | Bali                          | EX
| *Panthera tigris corbetti*                                             | SE Asia                       | EN
| *Panthera tigris jacksoni*                                             | Peninsular Malaysia           | CR
| *Panthera tigris sondaica*                                             | Java                          | EX
| *Panthera tigris sumatrae*                                             | Sumatra                       | CR
| *Panthera tigris tigris*                                               | South Asia                    | EN
| *Panthera tigris virgata*                                              | C Asia                        | EX
| *Panthera uncia*                                                       | C Asia                        | VU
| *Pardofelis marmorata*                                                 | SE Asia                       | NT
| *Pardofelis marmorata longicaudata*                                    | Indochina                     |
| *Pardofelis marmorata marmorata*                                       | Borneo, Sumatra, Malay Peninsula, S of Isthmus of Kra |
| *Prionailurus bengalensis*                                             | Asia                          | LC
| *Prionailurus bengalensis bengalensis*                                 | S to SE Asia                  |
| *Prionailurus bengalensis euptilurus*                                  | East Asia                     | CR
| *Prionailurus javanensis*                                              | SE Asia                       |
| *Prionailurus javanensis sumatranus*                                   | Sumatra, Borneo, Philippines  | VU
| *Prionailurus javanensis javanensis*                                   | Java and Bali                |
| *Prionailurus planiceps*                                               | SE Asia                       | EN
| *Prionailurus rubiginosus*                                              | S Asia                        | NT
### General Introduction

<table>
<thead>
<tr>
<th>Species</th>
<th>Distribution</th>
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<td>S and SE Asia</td>
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<tr>
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<td><em>Puma concolor couguar</em></td>
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Aims of this thesis

During the last three years I have decided to focus my research on better understanding how humans have interfered in the evolution of extant carnivores since the Quaternary by linking local to global scale ecological processes.

In order to answer several questions highlighted in the introduction, my research work began with a review on the population genetic studies of Southeast Asian Carnivorans in Chapter I – Part of the impetus of this review arose from my primary interest in gaining an in-depth knowledge of the role of genetics in conservation biology, and conducting field work in this region which is close to my heart. In this review I collated the current knowledge of genetic diversity of carnivores in Southeast Asia, searched for evidence of low genetic diversity caused by human activities, and highlighted some insights for future studies. This review was also written as a contribution chapter for a book entitled “On the edge of sixth extinction in biodiversity hotspots in Southeast Asia” edited by Julien Claude (UM). Together with his colleagues in Thailand and its neighbouring countries, we presented the state of knowledge on the ongoing actions, needs, and opportunities for biodiversity studies. This book targets to inform the local decision makers and regional scientific communities for a more efficient biodiversity management in this critical time. Our current understanding of carnivoran genetic diversity in this region is still poor, which is likely caused by the combined result of species elusiveness and lack of research funding, both of which are crucial to generate reliable genetic data.

Since the global scale of human impact on carnivores had mostly focused on large species, I questioned whether small carnivores had been affected in the same manner. To do so I established a database of the population genetic studies of Felidae. This database was also built to ascertain how other species traits, including life-history traits, current human population density, and geographical regions can affect felids’ level of genetic diversity derived from neutral microsatellite markers. This part of my thesis - Chapter II, has been published in Biodiversity and Conservation journal.

My database has led to an interdisciplinary collaboration with Stefano Anile and Sébastien Devillard in order to test one of the most important (and challenged) hypothesis in evolutionary biology (or as some have referred to it: the central dogma of
General Introduction

conservation genetics), by using population density as the surrogate of population size. We developed a method to combine independent population genetic and density studies from the collecting localities in our database, so that we are able to examine the pattern on a local scale. Chapter III has reached the stage of a manuscript.

While building my database I realized that microsatellite genotype data and mitochondrial sequences used to estimate genetic diversity in published studies, are not always publicly available or in good resolution (see Annexe 2). This insufficient data has impaired my work to estimate other genetic diversity measures and to infer past demographic history derived from molecular data following a standardized approach for a comparative study across multiple felids. Fortunately, there has been a steep increase in felids’ whole genome sequences since I’ve started my PhD, and this has caught my interest to become more involved. To gain first-hand experience in genomic studies, I initiated a collaboration with the zoo of Montpellier to acquire samples to generate molecular data of felid species, which I used to investigate whether genomic diversity of captive populations has been compromised by human intervention. By combining my newly generated data with those available online, this dataset enables the opportunity to examine the uncertainties related to reference genome and SNV variants, and to have sufficient confidence on the reconstructed demographic history, in anticipation of a full set data for comparative genomics in Felidae. Given that I only managed to acquire the sheer volume of data earlier this year, I was not able to complete this study thus far and there are many other genomic analyses I would like to pursue. Nonetheless, some promising results from my preliminary analyses are presented in Chapter IV. Each chapter follows the scientific manuscript format of the journal in which we had intended to submit our results.
Chapter I

Genetic Erosion In Carnivores’ Evolutionary Hotspot?

Research Trends And Opportunities In Southeast Asia


“We live in a zoologically impoverished world, from which all the hugest, and fiercest and strangest forms have recently disappeared; and it is, no doubt, a much better world for us now they have gone.”

Alfred Russell Wallace
The Geographical Distribution of Animals, 1876
Summary

Southeast Asia has a very rich carnivoran diversity. Many of these species play a significant role in ecosystem services and fascinate society. However, they are also potentially threatened by genetic erosion due to an unprecedented scale of habitat destruction and overexploitation in this rapidly developing region. Understanding their genetic differences within a population provides valuable insights into how they have adapted and will adapt to a changing environment. Despite their highly threatened status and the increasing efforts on monitoring activities, the intraspecific genetic diversity - the fundamental source to sustaining biodiversity - is understudied. This chapter provides an overview of current knowledge and gaps in population genetic studies of carnivorans in Southeast Asia to facilitate further research initiatives and appropriate conservation policies.
Introduction

The mammalian order Carnivora (Bowdich, 1821) is an ecologically and morphologically diverse group, occupying a remarkable range of ecosystems across the globe. There are about 304 living species classified into 16 families within this order (Burgin et al., 2018; Hunter and Barrett, 2019). Hereafter, I refer to species within this order as carnivorans to avoid all ambiguity with the term carnivore related to the feeding regime which is not exclusive in this group.

It is generally known that the removal of large sized carnivorans can disrupt the top-down regulation in the food chain, triggering a trophic cascade to the detriment of the ecosystem (Estes et al., 2011; Ripple et al., 2014). Despite increasing human conflicts with carnivorans, many carnivorans provide ecosystem services for the benefit of human well-being through pest control, carcass removal, seed dispersal, provision of ornamental materials, ecotourism and spiritual value (Estes et al., 2011; Williams et al., 2018). The order Carnivora has remarkably low genetic diversity among mammals, arguably due to their small populations and low reproductive rate (Merola, 1994; Naghash et al., 2008; Romiguier et al., 2014). These species would therefore be highly susceptible to genetic erosion, through a sharp population reduction caused by human activities with potentially unforeseen consequences on the functioning of ecosystems and its services (Hughes et al., 2008).

Within Asia, Southeast Asia (SEA) is among the top hotspot for the evolutionary history of carnivorans where about 60 out of 104 species occur here (Sechrist et al., 2002). As shown in Figure 1, up to 39 species are found in Myanmar and half of the species occurring in each country are threatened with the population of more than half of the carnivorans (63%) are decreasing due primarily to habitat loss. SEA carnivorans are represented by the linsang family (Prionodontidae), the cat family (Felidae), the civet family (Viveridae), the mongoose family (Herpestidae), the dog family (Canidae), the bear family (Ursidae), the skunk family (Mephitidae), the otter family (Mustelidae) as well as the herbivorous red panda, the sole representative of the family Ailuridae. SEA hosts the last populations of giant hyenas, *Pachy cercus* and *Pliocroc- cuta*, which disappeared around the Late Pleistocene, with large carnivores such as tigers, leopards and dholes having lived through the extinction (Louys, 2014; Louys et
al., 2007). Palaeontologists believe that there could be more carnivoran species across SEA in the past than was previously known (Grohé et al., 2020).

Carnivorans are also one of the most internationally traded animals to be used for commercial purposes such as medicine, pet trade and trophy collections (Krishnasamy and Zavagli, 2020; Scheffers et al., 2019). In addition to this, SEA is one of the regions with the greatest wildlife trade across the world (Krishnasamy and Zavagli, 2020; Scheffers et al., 2019). Needless to say, the population losses of carnivorans are emblematic of the harmful effect of deforestation and wildlife harvesting, which continue to occur extensively in SEA (Cardillo et al., 2004; Hughes, 2017; Sodhi et al., 2004).

Carnivora species in their natural habitats are elusive despite their great contribution to maintaining healthy ecosystems. They are highly mobile, territorial, nocturnal hunters, living in low population density, and sometimes in a complex social organization (Boitani et al., 2012). Monitoring the populations of carnivorans to ensure proper management in SEA, a region rich in dense tropical forest and human population, has undoubtedly many challenges. In such circumstances, population genetic methods relying on molecular markers have the potential as a key tool to assess and monitor wildlife indirectly and thereby mitigate the detrimental anthropogenic impact in SEA (Allendorf et al., 2008; Goossens et al., 2006; Schwartz et al., 2007). Where a small and isolated population is reduced in size, inbreeding and loss of genetic variability through random changes of gene frequencies become more likely (Frankham et al., 2002). This process could lead to an erosion of gene variants and subsequently contribute to an increase of extinction risk in threatened species before they are affected by demographic, environmental and catastrophic factors (Spielman et al., 2004). By characterizing the polymorphism within a population at DNA level, the population genetics approach can inform us about the evolutionary process and geographic boundaries throughout a species history (Avise, 2009; Moritz and Faith, 1998) as well as making predictions on species capacity to adapt to environmental and climatic changes (Frankham et al., 2002; Razgour et al., 2019), and to control disease resistance and spread (Cardillo et al., 2004; Hughes, 2017). There remains much research to be done using genetic approach to demystifying the history of carnivorans in the world (Pérez-Irineo and Santos-Moreno, 2013).
Figure I.1. Number of Carnivora species in each Southeast Asian country.

In this chapter, I review the current knowledge based on population genetic studies of carnivorans in SEA to inform two questions: *What is known about the genetic diversity of carnivorans in SEA? Do carnivorans in SEA have low genetic diversity due to human activities?* I will highlight the opportunities and challenges for future research in hope that this will accelerate conservation policies to preserve the adaptive potential of threatened carnivoran species in SEA.

**Literature search**

I searched for empirical studies on conservation genetics in SEA from 1991 to 2020 using the search strings “population* AND (*genetic* OR *genomic* OR molecular)” followed by country names and biogeographical regions in SEA as well as taxonomic groups. Literature searches were done via Web of Science (WoS), Google and PubMed. I referred to Burgin et al. (2018), Hunter and Barrett (2019) and Kitchen et al. (2017) for taxonomic names and species’ geographic distribution to get the most comprehensive and recent list. Review papers, cytogenetic studies, methodology papers (e.g. development and characterization of molecular markers for species identification), studies related to infectious disease and reproductive biology, as well as those focusing on domestic animals (i.e. cats and dogs) were discarded. Additional studies were located after cross-referencing with the remaining articles. Finally, I categorized these articles as follows: i) genetic diversity within populations/subspecies with comparison of polymorphism indexes (i.e. heterozygosity, allelic richness, nucleotide diversity) among populations/subspecies, ii) genetic diversity among captive populations either within or outside of a species natural distribution and iii) taxonomic, systematics and phylogeography (i.e. species geographic delimitation, demographic history and dating biogeographical events). I identified 42 articles published internationally through the literature search. This could possibly be a subset of available empirical studies, as I have no access to the collections within the university libraries in SEA. Although the number of articles has increased since 1996 (Figure 2), the number of species identified from these articles remains low (34 out of 61 or 44%).
Figure I.2. Trend of population genetics study for Southeast Asian carnivores since 1995.

The line represents the cumulative number of study. There were no publication between 1991 to 1995, 1997 to 2000, 2002, 2003 and 2019.

Figure I.3. The relationship between the rate of publication per species and the percentage of species in each family / total carnivores in SEA.

The number below the family name correspond to the number of studied species. The number in the brackets refers to percentage of species present in SEA relative to all carnivores in the world. Size of the circle reflects total number of publication in the family. Squares indicate no available studies.
What is known about the genetic diversity of carnivorans in SEA?

The genetic variability and structure at population-level (cat. 1) has not been investigated in 43 species (72%), including two families Mephitidae and Prionodontidae, both representing 2 species each (Table 1). Three threatened species (Endangered and Vulnerable status) have never been studied previously in either of the three categories. They are the hog badger (*Arctonyx collaris*), the Bornean ferret-badger (*Melogale everetti*) and the large-spotted civet (*Viverra megasplia*). In addition to these three, I identified eight species occurring within one country in SEA which should be prioritised for future genetic studies (Table 1). These are the Vietnamese ferret-badger (*Melogale cucphuongensis*) and the Tonkin weasel (*Mustela tonkinesis*) in Vietnam; the Palawan stink-badger (*Mydaus marchei*) in the Phillipines; the Sumatran hog badger (*Arctonyx hoevenii*), the Javan ferret badger (*Melogale orientalis*), and the Indonesian Mountain weasel (*Mustela lutreolina*) in Indonesia. Interestingly, given their endemic status, these species are listed as either: Least Concern or Data Deficient according to the IUCN Red List (2020). The red fox (*Vulpes vulpes*) and the Siberian weasel (*Mustela sibirica*) have a wide distribution across the Northern Hemisphere but their populations in SEA, especially in Northern Indochina, have rarely been studied.

Across all of the identified studies, the primary source of specimens are zoos (either captive-born or wild-born) followed by museums (Table 2). Sampling molecular data from the wild remains an open challenge. Mitochondrial (mtDNA) sequences are the most frequent molecular markers used and only 17 studies (40%) applied both mtDNA and nuclear DNA data (Table 2). It also appears as if researchers in SEA institutes are less interested, or perhaps have not taken full advantage of population genetic approaches for carnivorans. Out of the identified articles, 18 publications (43%) have at least one contributing author affiliated with research or conservation organizations located in SEA countries, resulting in an average of 3.2 authors per study.

There is a certain bias towards the cat family (Felidae) which makes up only 19% of all carnivorans in SEA (Figure 3). Eighteen studies (42%) on felid species in SEA have been conducted with the exception of the jungle cat (*Felis chaus*) and the Javan leopard cat (*Prionailurus javanensis*). The combination of articles for Mustelidae and Viverridae which represent the majority of carnivorans in SEA, 29% and 24% re-
spectively, are still below the number for Felidae with 16 studies. The cat family has many species of top conservation concerns compared to other carnivoran families (Nowell, 2002). They are often maintained in private or public zoo facilities outside of their natural distribution to serve as flagship species for conservation, which may have contributed to more attainable genetic sampling. Members of Felidae were among the earliest reported molecular analysis of genetic variations within populations or subspecies occurring in Southeast Asia. These are the captive leopards published in Miththapala et al. (1996) and both captive and wild tigers by Luo et al. (2004). Nevertheless, estimating genetic variability within captive populations (cat. 2) has only been carried out for the Asiatic Golden cat (Phandee et al., 2016), tigers (Luo et al., 2008) and binturongs (Cosson et al., 2007).

Finally, the majority of the studies were aimed to delineate species or subspecies, and to infer their geographic origin and history (cat. 3, no. of publications = 30, no. of species = 33). These studies provide insights into taxonomy and systematic status of understudied species relying on museum specimens, which are valuable in order to set conservation priorities in SEA. For instance, comparing intraspecific genetic structure between closely related species with their geographic distribution has led to the recognition of new species that were morphologically indistinguishable in the field. This was the case for *Neofelis* (Buckley-Beason et al., 2006; Wilting et al., 2007, 2011) and *Paradoxurus* (Patou et al., 2010; Veron et al., 2015). The applications of molecular genetics have been able to recognize evolutionarily significant units (ESU) in the binturong (*Arctictis binturong*) (Cosson et al., 2007), the smooth-coated otter (*Lutrogale perspicillata*) (Moretti et al., 2017) and the Malayan sun bear (*Helarctos malayanus*) (Onuma et al., 2006). Although the methods of identification and the application of ESU in conservation have been debated, incorporating species evolutionary process, intraspecific adaptive differences and genetic exchangeability in conservation decision-making remain essential to preserve the evolutionary potential of species (Moritz, 1994; Crandall et al., 2000; Carvalho et al., 2017).
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| [42] | √ | mtDNA sequences |
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List of publications in table 1 and 2.


Koeppfli, K.-P. et al. (2008) Establishing the foundation for an applied molecular taxonomy of otters in Southeast Asia. Conserv Genet 9, 1589


Kim, Y.-K. et al. (2011) Genetic Status of Asiatic Black Bear (Ursus thibetanus) Reintroduced into South Korea Based on Mitochondrial DNA and Microsatellite Loci Analysis. J. Hered. 102, 165–174


Veron, G. et al. (2017) Molecular systematics and biogeography of the Hemigalinae civets (Mammalia, Carnivora). European Journal of Taxonomy 0,


Do carnivores in SEA have low genetic diversity caused by human activities?

Theoretically, when a subset of individuals from a parent population has established a new population following a long distance dispersal into a new niche, the founding population would represent a smaller gene pool compared to the parent population if no more migration occurred (Mayr, 1954). A significant reduction of a population caused by a catastrophic nature or overexploitation would lead to a decline in genetic variation altering the adaptive potential of said population. In such bottleneck events, the founding and recovered population would experience strong genetic drift which could lead to genetic consequences such as an increase in the risk of inbreeding depression and reduced long-term adaptability of the population (Frankham et al., 2002).

Reviews and empirical studies have demonstrated that forest fragmentation and anthropogenic barriers such as roads are limiting animal dispersal and reproduction, causing restricted gene flow among carnivore populations in the western part of the world (Riley et al., 2006; Dixon et al., 2007; McManus et al., 2015; Hartmann et al., 2013). Furthermore, there is growing evidence linking population decline near urban settlements to additional roadkill incidents, nuisance, and spreading of disease, which may have genetic impacts (Jackson and Fahrig, 2011; Bateman and Fleming, 2012).

However, the impact of expanding human activities on populations’ genetic variation and structure has not been adequately addressed across carnivores in SEA, with only two studies have investigated empirically. Across Sumatra, tigers in the southern populations have reduced gene flow and low migration rates and similar scenario had been suggested to other tiger populations around national parks in Sumatra (O. Smith et al., 2018). Similarly, Kunde et al. (2020) investigated the impact of landscape features on the Malayan sun bear in Cambodia using nuclear genetic markers in which the genetic exchange and structure between sun bear populations were more affected by agricultural mosaics than geographic distances only when they included highly developed areas. Local studies such as these have the strength to provide a clear interpretation of genetic consequences due to human-carnivore conflicts and to inform landscape development before irreversible changes occur (McManus et al., 2015; Williams et al., 2018). For instance, Kaszta et al. (2020, 2019) used simulated genetic es-
estimates and habitat selection as a proxy, combined with population data to map and project the potential impacts of ongoing and future landscape changes under different conservation scenarios on the distribution of clouded leopard and its habitat in Borneo and Myanmar.

Based on the shared haplotype (or group of alleles) and the indicated locality, Human-mediated dispersal has been suggested in the dholes (Cuon alpinus) and the Malayan civet (Viverra tangalunga) from either South Asia, East Asia or Myanmar into Indonesia and from the Sundaland to Sulawesi, respectively (Iyengar et al., 2005; Veron et al., 2014). However, this hypothesis of species introduction by humans remains speculative. Further testing using molecular dating and more empirical data is required to clarify the timing of these species’ dispersals across Southeast Asia. Similarly, Moretti et al. (2017) found an admixture of Asian small-clawed otters DNA among the smooth-coated otters in Singapore where the former species is rarely present. Secondary contact from one established population to another through purposeful introduction by humans has the potential for introgression hybridization. The admixture or introgression between populations producing novel gene variants was once thought to increase the risk of extinction (Rhymer and Simberloff, 1996). More recently, population geneticists have argued that when hybridization produces fertile offspring, this has the potential to increase the adaptation of threatened species up to 148% in terms of the composite fitness in stressful conditions (Frankham, 2015; Whiteley et al., 2015), and species’ distribution range (Pfennig et al., 2016). However, due to a lack of sampling size it remains unknown whether i) the dispersal was human mediated, ii) the hybridization is more widespread, or iii) the hybridization has increased genetic variation and species range.

Ancestral area reconstruction revealed that the order Carnivora originated in the temperate regions of Eastern Palaeartic, part of today’s East Asia, with multiple independent lineages dispersed across Africa, SEA and America (Rolland et al., 2015b). The diversification of Carnivora is distinct to other mammals, specifically in that the high species richness in the tropics was not attributed to a latitudinal diversity gradient but to the repeated colonization into low-latitude regions when the Tethys Sea was closed, since the collision of the Indian Plate, and via land bridges connection linking Eurasia to North America, and North to South America (Rolland et al., 2015b). Therefore, it could be hypothesized that carnivorans with wide distribution across Asia
have lower genetic variability in SEA due to bottleneck events as the subsequent of vicariance or dispersal driven by the dynamic geological and climatological history of SEA (Pfennig et al., 2016).

This low genetic diversity pattern compared to populations outside of SEA is consistent with the Javan dholes (Iyengar et al., 2005), the Vietnamese raccoon dog (*Nyctereutes procyonoides*) (Hong et al., 2018), the Javan leopard (*Panthera pardus melas*) (Wilting et al., 2016), and the Sundaic masked palm civet (*Paguma larvata*) (Patou et al., 2010). However, the type of bottleneck events leading to the reduced genetic diversity, either related to founder effects since the populations originated or enhanced population reduction after the establishment of these populations, could not be clearly identified. This would require estimating divergence times and dating genetic bottleneck events using ample molecular data by increasing population sampling throughout species distribution and in pre-bottleneck populations to examine whether it was caused by past environmental changes or the anthropogenic events.

Theory predicts close associations between genetic variability and body size, as large species have lower population turnover, and thereby a lower molecular evolutionary rate (Frankham, 1996). Furthermore, large predators have been suggested to experience substantial loss since Pleistocene due to a combination of human induced and climatic factors compared to smaller sized carnivores (Louys, 2014; Malhi et al., 2016). Although previous studies did not corroborate to this theoretical expectation (Frankham, 1996; Wilting et al., 2016) the only megacarnivora (> 100 kg) represented in SEA revealed no variability in the Y chromosome when compared to smaller sympatric species (Luo et al., 2004). Increasing numbers of species in comparative genetic studies could provide further insights on this relationship, as the influence of body mass and extinction risk among mammals is more relevant in the tropics (Fritz et al., 2009). In an attempt to solve this, more populational-level genetic studies are needed for the large sized carnivors in SEA such as the leopard, the Malayan sun bear and the Asiatic black bear (*Ursus thibetanus*). With the addition of this data, other factors determining genetic variability such as the mating structure, distribution range and exploitation history can be tested among closely-related species (Flight, 2010; Malhi et al., 2016; Rolland et al., 2015b)

When more sufficient data are available, examining the temporal changes between historical and contemporary population genetics of SEA carnivorans can be-
come attainable to address i) the anthropogenic impact such as: habitat fragmentation, road barriers, and human-mediated dispersal, ii) the genetic impact of hybridization and iii) the relationship of genetic diversity with carnivoran traits and its implication to sympatric species.

**Challenges in sampling genetic data and recent progress**

Methods to screen genetic variants of an individual through non-invasive sampling from faecal or hair samples have made considerable advances in recent years. Faecal samples from these elusive, rare, and nocturnal hunter animals are extremely valuable but locating and preserving them in the tropical regions were deemed difficult in practice (Goossens and Salgado-Lynn, 2013). Relatively few of these studies identified above have discussed the efficacy of non-invasive sampling methods in collecting genetic data from wild populations.

In general, non-invasive sampling has the ability to increase sample size, but the DNA amplification and species identification from faecal samples are still limited. Smith et al. (2018) have been able to match 25% of the 148 faecal samples to tiger DNA and identified 25 individuals including a pair of siblings in Sumatra. Thirty of the 51 collected faecal samples in Java, Indonesia by Iyengar et al. (2005) have revealed 17 individuals of dholes. Both studies have a moderate species identification success, 68% and 57% respectively, which is probably caused by the high DNA degradation and contamination in tropical environment. Opportunistic sampling of carnivoran faecal marked by the presence of animal matter (e.g. bones, hairs, scales) could also increase the number of species for population genetic studies. Of the 48 faecal samples successfully extracted, three species were identified in a study across Peninsular Malaysia (Rosli et al., 2014).

Dogs have been recommended as an efficient tool to locate faecal drops from single or multiple carnivoran species under field conditions in temperate areas (Hollerbach et al., 2018; Long et al., 2007) and in South America (Oliveira et al., 2012; Vynne et al., 2011). In SEA however, one study argued that using detection dogs appears to be more costly and time-consuming than using human forces to increase faecal sample detection because the hot and humid climate have weakened their Labrador Retriever’s ability (O. Smith et al., 2018).
Museum samples can be used as a reference group to provide evidence of temporal loss of genetic variability caused by recent human activities (Allendorf et al., 2008; Holbrook et al., 2012; Janečka et al., 2014). Such patterns could aid in elucidating genetic consequences of historical processes such as selection, drift, mutation and migration. Most of the identified studies here included museum samples to increase sampling size, but whether the genetic diversity pattern is historic or recent has never been directly investigated. There are also challenges in sampling ancient and historic DNA from tropical regions as the DNA quality is highly linked to the environment in which samples were retrieved, as well as to the collection treatment and storage rather than with the age of specimen (Gutiérrez-García et al., 2014; Kehlmaier et al., 2017; Mason et al., 2011).

Museums associated with university faculties have the potential to support the exchanges of these underused resources among local and international researchers. Digital access of national biodiversity data promoting visibility and availability of its collection have made recent progress (Table 3). Furthermore, Vietnam and Cambodia have recently joined the Global Biodiversity Information Facility as direct associate country participants (GBIF, https://www.gbif.org/the-gbif-network/asia). Museums have the opportunities to play an important role as biobanks for the cryopreservation of endangered animal specimens such as tissue, blood and germ cells, which could elevate molecular data sampling for genetic studies (Yeates et al., 2016). As listed in Lee et al. (2016), only one biobank for non-human animal specimens in SEA was mentioned, that of Vietnam (http://www.iebr.ac.vn/index.asp?prgID=100). Given this progress, significant improvement on a standardized documentation of these collections following technological advancements should be adhered to, to effectively disseminate and reduce knowledge gaps across SEA (Webb et al., 2010).

Genetic sampling from seized animals, either dead or alive, is permissible by CITES but surprisingly rare in practice. Some studies have sourced genetic data from confiscated animals (Onuma et al., 2006; Veron et al., 2004) and at local markets in SEA (Patou et al., 2010; Veron et al., 2015). Species identification and geographic origin assignment of confiscated samples using DNA based methods can contribute to ascertaining legal status, as well as to identifying the scale and location of poaching hotspots by wildlife authorities (Ogden and Linacre, 2015). Another way to sample opportunistically is through dead animals due to road accidents which have increased im-
mensely. One study has included roadkill sampling to investigate the genetic variation within the hairy-nosed otters (*Lutra sumatrana*) (Rosli et al., 2015). However, they did not assess the impact of the tissue’s DNA quality. Similar to the faecal samples, it remains unknown whether the DNA extracted from roadkill has been degraded rapidly due to the environmental conditions and rapid decomposition in the tropical regions, which could cause bias in downstream analyses.

Table I.3. List of national biodiversity information online database in Southeast Asia.

<table>
<thead>
<tr>
<th>Country</th>
<th>Website</th>
<th>Collections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaysia</td>
<td><a href="https://www.mybis.gov.my/">https://www.mybis.gov.my/</a></td>
<td>published books, journals, expert checklists and specimen databases</td>
</tr>
<tr>
<td>Singapore</td>
<td><a href="https://singapore.biodiversity.online/">https://singapore.biodiversity.online/</a></td>
<td>museum specimens in Lee Kog Chian Natural History Museum’s (LKC NHM) collections</td>
</tr>
<tr>
<td>Indonesia</td>
<td><a href="http://indobiosys.org/">http://indobiosys.org/</a></td>
<td>fieldwork, DNA barcoding, museum specimens in Berlin</td>
</tr>
<tr>
<td>Thailand</td>
<td><a href="http://www.thaiscibiodiversity.org/">http://www.thaiscibiodiversity.org/</a></td>
<td>museum specimens in Thailand Natural History Museum</td>
</tr>
<tr>
<td>Philippines</td>
<td><a href="http://www.philippineplants.org">http://www.philippineplants.org</a> (plants only)</td>
<td>published books, journals, and expert checklists</td>
</tr>
</tbody>
</table>
Conclusion and future recommendations

The usage of molecular markers to study population diversity has rapidly evolved from basic markers to large polymorphic data within 30 years, and has been considered a cost-effective tool to monitor populations indirectly. Population genetic methods should be considered as a key step in conservation policies to set a baseline of a healthy and viable populations for the long-term survival of carnivorans in Southeast Asia. While there has been considerable progress in addressing the intraspecific genetic diversity of carnivorans, there is still limited knowledge whether populations of threatened and endemic species in SEA are affected by recent human-driven threats.

As resources for conservation can be limited in tropical regions, genetic monitoring can be achieved by sustained efforts using non-invasive sampling based on: faecal samples, museum specimens, confiscated animals, and roadkill to increase molecular data. Besides this, there is room for further improvement on the methods relying on non-invasive sampling of carnivorans that would minimise impacts on biodiversity across SEA landscapes. While museum and zoo specimens outside SEA have contributed to species recognition and delineation, there are substantial opportunities for collaborations among the natural history conservatories in SEA to ensure common guidelines for documentation and preservation of the genetic material of species that recognize no borders. Insights from genetic studies could provide vigorous support to promote long-term behavioural and ecological studies, for example, on the social organization, the movement patterns, the predator-prey relationships, which are urgently needed for the conservation in this changing region. Finally, taking into account the nature of the human-carnivoran relationship can lead to enhancing practical solutions in conservation policies, aimed to revise species conservation status, to deter illegal and unsustainable harvesting of valuable exploited populations, to mitigate wildlife conflicts, and finally, to improve landscape management in SEA.

Acknowledgment
I thank the editors for inviting me to contribute in this book. Many thanks to Emmanuel Paradis, Professor Benoît Goossens and the two anonymous reviewers for providing helpful comments on earlier drafts of this chapter.
Chapter II

Patterns And Drivers Of Genetic Diversity Among Felidae Species


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Supplementary information available online

“Are cats crazy? Well, if they are, that makes them more powerful.
Which of us doesn’t dream of doing just what we want, when we want, how we want, and as much as we want?”

Fernand Mery (1897-1984)

*a French veterinarian and great advocate for the animal cause*
Patterns and drivers of genetic diversity among Felidae species
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Patterns and drivers of genetic diversity among Felidae species
Chapter III

Population Density And Genetic Diversity Are Positively Correlated In Wild Felids

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† Co-last authors

Supplementary materials available in Annexe 3

“The combination of some data and an aching desire for an answer does not ensure that a reasonable answer can be extracted from a given body of data.”

John W. Tukey

Exploratory Data Analysis, 1977
Population density and genetic diversity are positively correlated in wild felids

Summary

The relationship between genetic diversity and population density is one of the most important hypotheses in evolutionary biology namely that the reduction of genetic diversity is related to the decrease in population size. Felids are one of the most conservation-sensitive and charismatic taxon and we collected population density estimates from camera-trap studies and population genetic metrics based on microsatellites across 18 species throughout five continents to test this hypothesis. We found that felids with low population density and with short generation length show depauperate genetic diversity. Preserving healthy populations size of felids are crucial to preserve the genetic diversity. Our framework provides the basis to identify threatened felid populations at higher risk of facing the extinction vortex.
**Introduction**

The maintenance of high genetic diversity within populations is an important consideration in conservation measures to ensure that species can face changes in the ecosystem. Similarly, maintaining population size and density above a viable threshold is crucial to any threatened species. When genetic diversity or population density is rapidly lost, species are expected to face an elevated risk of extinction. Genetic diversity and population density are expected to covary positively. Much attention has been paid to demonstrate the positive association between genetic variation and indicators of population size such as species conservation status, distribution range size, body mass, longevity and fecundity (Bazin et al., 2006; Doyle et al., 2015; Frankham, 1996; Romiguier et al., 2014), as well as that human activities can also lead to depauperate genetic diversity (Leigh et al., 2019; Lino et al., 2019; Miraldo et al., 2016). However, relatively little consideration on the relationship between population genetic diversity and population density involving multiple species have been given. Few studies have assessed this relationship within a single community (Gram and Sork, 1999) and the same geographic range (Hague and Routman, 2016), and both studies have given conflicting results. Population density within a species can vary widely over time and space, driven primarily by the interaction between ecological laws, habitat quality and resource availability, while accounting for species-specific metabolic and dispersal needs (Anile and Devillard, 2020; Carbone and Gittleman, 2002; Stephens et al., 2019). As a result, the levels of genetic diversity within a species with large distribution can have a wide variation among populations in that using an average estimate per species could potentially underestimate the effect of population size on genetic diversity when gaining insight into the impact of human activities at large spatial scale.

Felidae is a taxon particularly susceptible to genetic erosion and being trapped in an extinction vortex caused by demographic and environmental stochasticity due to their intrinsic traits such as low population density, slow reproductive rate and large home range requirement (Johnson, 2002; Tanaka, 2000). A lot of conservation efforts have targeted on felids across the globe because it is one of the most charismatic taxa with a large and broad interest from society (Brodie, 2009; Macdonald et al., 2015; Tensen, 2018b), but also many felids are often in conflict with human activities (Inskip
and Zimmermann, 2009). The loss of these species will inevitably induce a devastating effect of trophic cascades on ecosystems and evolutionary processes (Estes et al., 2011). Obtaining data of both population size and genetic variation for felids in the wild is, however, a challenging task due to their elusiveness, nocturnality, extensive territory and their remote habitats often inaccessible for monitoring (Anile et al., 2014; Goossens and Salgado-Lynn, 2013). In this study, we combined independent data from field surveys of genetic diversity derived from presumably neutral nuclear microsatellite (or simple sequence repeat) markers and population density (PD) estimates from camera-trapping studies to examine to what extent within-population genetic diversity (GD) can be predicted by PD across species. Our response variables were the three most often reported estimators of GD in population genetic studies: the observed heterozygosity (Ho), the expected heterozygosity (He), and the allelic richness (AR; or the mean number of alleles across loci examined within a population).

**Material and methods**

**Data building**

We used genetic data collected and presented in Azizan and Paradis (2021), with 6 additional studies published in 2020. We selected GD estimates estimated using at least 5 individuals per population, and at least 5 autosomal loci. In cases where studies used a combination of both domestic cat and other species-specific markers, we recalculated the GD estimates across loci using the largest set to increase the precision of the estimation (Figure S1). We acknowledged that microsatellite markers employed on the same species in which it has been isolated, it could lead to greater polymorphism (Ellegren et al., 1995). However, since 94% of the GD estimates were derived from domestic cat microsatellite markers and the genomic architecture of the cat family is highly conserved (Cho et al., 2013; Menotti-Raymond and O’Brien, 1995), we assumed the effect of microsatellite marker choices to be negligible in our dataset. The PD data (number of individuals per 100 km²) are a subset of those collected in Anile and Devillard (2020). The PD estimates were mostly from spatially explicit capture-recapture (SECR) approach, either via Maximum Likelihood or Bayesian, and CR records were rescaled to SECR, with the exception of some lion data. Where PD estimates were collected over multiple years within a specific study, we calculated the mean density per population.
**Population density and genetic diversity are positively correlated in wild felids**

The majority of data were not sampled at the same sites (i.e. a precise overlap between the geographic coordinates of the populations whenever provided or extracted by ourselves) and the same time we hence joined GD and PD datasets using two spatial approaches, the cluster and the country one. The cluster approach is based on the spatial proximity of the populations within the species-dispersal distance range by using the K-means clustering approach. The country approach involves combining populations within the political borders as reported in the studies. We refer to these two datasets as cluster and country datasets and compared the relationship between GD and PD at these different spatial scales under the assumption that species populations within a country are more continuous than those in the cluster data.

Cluster dataset: The first approach consisted of using K-means clustering algorithms to assign GD (the response variable) to PD estimates (the independent variable) based on spatial proximity and similarities following these steps:

1. Each population was geo-referenced with coordinates in decimal degrees, as provided in the studies or approximated using the given sample localities, sampling areas, study maps, or country centroid geolocation.

2. We overlaid biogeographic regions (7) over our data points (both GD and PD) so that the K-means clustering can be applied within each region for each species to ensure greater ecological similarities between the data points.

3. The K-means algorithm requires two parameters, the number of clusters and the initial data point at the center of a cluster (initial centroid). To get the most realistic and greatest number of clusters, we visually compared results between separate runs when GD or PD population coordinates were implemented as initial centroids using the `kmeans` function in the ‘stats’ R package (Figure S2).

4. We inspected the unassigned points and where possible, these were manually joined to the nearest neighbors located within the median distance between all GD and PD points (62 km) in the database. Distances between data points were calculated using the `pointDistance` function in the ‘raster’ R package.

5. Finally, we only considered clusters with PD and GD data that fell within each species’ dispersal distances in km. Species dispersal distances were estimated following MacDonald et al. (8): the calculation of the dispersal distance from the $\sigma$ values whenever this parameter was provided. The spatial parameter $\sigma$ computed in SECR
Population density and genetic diversity are positively correlated in wild felids

analysis is a proxy of the home range under the assumption of a circular area. After this step, three species, Felis chaus in India, Leopardus geoffroyi in Argentina, Brazil and Bolivia, and Leopardus tigrinus in Brazil were excluded from the cluster dataset since the distances between the sampling location of the GD and PD were greater than their dispersal distance. The number of clusters per species is given in Table S1.

Country dataset: To test whether the clustering approach had any influence on the relationship between GD and PD, we also grouped GD and PD estimates to the country reported in the studies. The differences in the number of observations between the cluster and country dataset were due to unassigned estimates after clustering (see above), studies lacking geographic coordinates and to the averaging of GD estimates across each country. The number of countries per species is given in Table S1.

To find outliers in the response variables (Ho, He, AR), we plotted boxplots of all 3 GD estimates and the PD estimates. We observed 2 points in Ho (Panthera leo and Puma concolor), 5 points of He (in Panthera tigris, Panthera pardus and Puma concolor) and three points of AR (in Panthera onca and Puma concolor) fell outside of the distribution within each species in the cluster dataset (Figure S3a). The identification and discarding of outliers in the response variables were performed on the both dataset, leading to the removal of n=15 and n=55 for the cluster and country datasets, respectively (Figure S3b). We also inspected the relationship of DP and GD within these 5 species to check the subsequent effect of these outliers (Figure S3). In the final datasets, GD were sampled from 1972 to 2018 while PD were surveyed between 1995 and 2017. PD estimates in the cluster dataset ranged from 0.2 to 78 individuals / 100 km² (median = 3.3 and mean = 6.4), whereas PD in the country dataset varied between 0.3 and 55 individuals / 100 km², with a higher mean (11.1) and median (4.3).

The PD estimates (n = 399) over different time periods and at close proximity were averaged within each cluster (n = 103) and each country (n = 69) for each felid species (Table S1). The joining of these PD with the GD estimates resulted in 156 and 449 records from 18 felid species for the cluster and the country datasets, respectively. Our records covered 41 countries in five continents: North America, South America, Europe, Africa and Asia (Figure 1).
Figure III.1 Locations of the n = 399 records of population density and n = 156 records of population genetic from cluster data set across 15 Felid species.

Lines linking the points indicate cluster groups (n = 103). Grey shaded countries are areas covered in the country data set. Species names in the legend are ranked by body mass, from small (blue) to large (red). The map was generated using ggplot2 and ggrepard libraries in R.
Statistical Analyses

In the cluster dataset, PD estimates fluctuated more frequently than GD as camera trapping surveys at a given location occurred during multiple periods within a given year (e.g. winter/summer, wet/dry). Although we acknowledged this uncertainty, GD estimates were also expected to vary by generation, but given the time interval between GD estimates was fewer than 2 generations and more than a third of the GD estimates came from a single study, we further assumed this variation to be negligible. Moreover, GD and PD estimates were independent within each dataset. During preliminary analyses, we modeled the averaged GD and PD estimates within each species and found no significant effect of PD using a standard linear model. We also fitted the averaged GD and PD estimates within each cluster and country using standard linear models, including species as fixed effect, and found both variables were significant for AR only (DP F-value = 13.88, species F-value = 3.38, both p-values < 0.001, adjusted R-squared = 0.32, Residual standard error = 0.16). Finally, to estimate the common slope of the PD effect, we performed linear mixed effects (LME) modeling, using the ‘lme4’ package (9) (Bates et al., 2015), with species set as random intercepts to capture between-species variability (i.e. the effect of explanatory variable was allowed to vary between species). Occasionally, some groups, either from the cluster or country dataset, had several GD estimates, leading to unbalanced data, thus we considered these multiple y-values in each explanatory variable as repeated estimates (i.e. each observation row of each cluster had the same PD value). We hence recognized the estimates within a species were more correlated to each other as they had similar effective population sizes and evolutionary history, irrespective if they belong to the cluster or country dataset.

Since GD estimates were not corrected for sample size, and standard error ranges were not reported for all the studies, we have further investigated the effect of sample size as a covariate. We examined whether the covariates GL, extracted from Pacifici et al. (2013), and BS, obtained from Johnson et al. (2017), could have increased the predictive power of our models by comparing the coefficient of determination, the marginal R² between models. The marginal R² explains the variance arisen by the fixed effects only (Nakagawa et al., 2017), in addition with the comparison of the Akaike information criterion, AIC. We used the dredge function in ‘MuMin’ package (Barton,
Population density and genetic diversity are positively correlated in wild felids

2020) to compare summary statistics of models containing every possible combination of the explanatory variables. In the final datasets, allelic richness (AR), PD (individuals per 100 km²), body mass (BS) and sample size were log₁₀ transformed prior to model fitting to approach normality in the residuals. The P-value for each covariate was computed using the ‘ImerTest’ package which applies the Satterthwaite’s method (Kuznetsova, Brockhoff, & Christensen, 2017). Assumptions of the LME models were checked visually using the relevant plots. All statistical analyses were performed using R software version 3.6.3.

Results and discussion

Our analyses provide compelling evidence that dense populations harbour higher GD across felid species for both cluster and country datasets, in agreement with the prediction of neutral theory (Figure 2). For some species with several records in the cluster dataset, we observed a consistent relationship with a moderate to high index of Pearson’s correlation, R (Figure 3). However, this coefficient was significantly greater than 0 only in tigers (Ar = 0.4, p-value = 0.02) and leopards (Ho = 0.6, p-value = 0.004; He = 0.49, p-value = 0.023) after outliers were removed. When compared to previous similar studies across woody plant species our findings are in contrast, whereas they (Gram and Sork, 1999), concur with those found for four lizard species based on sequence data (Hague and Routman, 2016) likely, the greater and faster capacity of dispersal in animals when compared to plants may explain this contradiction. Our results further stress the importance of assessing the relationship between of GD and PD using closely related species, as the strength of the effect could indeed be specific to taxa with similar historical, demographic, ecological factors and mutation rate (Leffler et al., 2012). The variance accounted for by PD in the models were low, ranging from 3% to 8% for Ho, 1% to 6% for He and 6% to 17% for AR, across the two datasets (Table 1 and Table S2). We identified that Amur tiger populations specifically in north-east China and Hunchun Nature Reserve have both depauperate genetic diversity and low population density in both cluster and country datasets (Figure 2), which raises the possibility of high risk of extinction vortices in this subspecies driven by a strong genetic Allee effect as reviewed in (Luque et al., 2016). From the few fitness-related data collected in the wild, the reproductive rate of Amur tigers is expected to be much lower than those in Bengal tigers (Kerley et al., 2003).
Population density and genetic diversity are positively correlated in wild felids

Figure III.2 Positive relationship between within-population genetic diversity and population density from camera-trap surveys across wild felids using (a-c) cluster and (d-f) country data sets.

Regression lines were estimated from the best-fit linear mixed models with species included as a random effect. Log-transformed population density represents the number of individuals within a 100 km² area. Colored squares are the median values for each species calculated from the data used in the models, which are represented by outlined circles in each plot. Colored circles are species populations with extreme low PD values.
Population density and genetic diversity are positively correlated in wild felids

Figure III.3. Correlation coefficient between within-population genetic diversity (GD) and population density (PD) from camera-trapping surveys in some species with several records from the cluster datasets.

Red circles are populations identified as outliers by the boxplots shown on the right and at the bottom of the scatter plot. Pearson’s correlation (one-tailed) and standard regression were applied after outliers were removed (in black solid) and with outliers (in blue dashed lines). Animal illustrations are from freesvg.org.
However, the tiger populations in Royal Manas National Park and Bhutan, as well as puma populations in the Rio Grande do Sul, Brazil and in north-east Argentina have similar PD estimates, but with almost doubled GD estimates tigers in China. We suspect these records might be potential outliers due to the uncertainties in the cluster approach that were not revealed during the preliminary analyses; excluding them from the models increased the strength of the effect, such as those shown in Figure 3 (with an exception to tigers’ He).

In addition to the PD, we included other covariates that are known to influence GD, such as species body mass (BM, kg), generation length (GL, in years), and sample size within GD populations (e.g. (Smith and Wang, 2014) to examine whether the confounding effects of these covariates could increase the fit of our models. GL can be considered as a life history proxy given that it is related with several demographic parameters (e.g. population growth rates, age- and sex-specific fecundity and survival rates). In a previous study GL but not BM, has been shown to be important for explaining the variation in GD across felids (Azizan and Paradis, 2021). We re-examined the effect of log10-transformed BM on GD using the data provided in Johnson et al. (Johnson et al., 2017) which have shown to be inversely scaled with PD in Anile et al. (Anile and Devillard, 2020). All LME models indicated that the inclusion of the GL improved the prediction of the GD (Table 1 and Table S2). The inclusion of species-specific GL estimates increased the marginal R2 (i.e. the variance explained by fixed effects only). Moreover, sample size was also an important predictor, but the effect is rather weak and closer to 0 in Ho and He than in AR (Table 2). This is as expected since we did not use AR calculated by using a standardised method such as using the rarefaction method to estimate the number of alleles for one unique sample size (Leberg, 2002; Smith and Wang, 2014).

BM did not show a significant effect on the GD, nor it improve the predictions of the He in the AR models. Furthermore, including BM in Ho models showed little improvement in the variance accounted for by the model, and indeed the BM effect was never significant. We further observed that smaller cats have high median PD and high GD, but medium size cats had the lowest median estimates for both GD and PD than expected. We believe that the lack of GD and PD records for the smaller cats – which is result of a strong bias in the conservation effort towards larger species evident
Table III.1 Linear mixed model comparisons for predicting genetic diversity estimates using the cluster dataset.

The models presented here have less than 2 differences in AIC compared to the model with the smallest AIC, thus are almost as good as the best-fit model (in bold). Full model indicates all four covariates are included as fixed terms. Allelic richness, population density, body mass and sample size are in log10 scale. K = number of parameter.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Fixed term</th>
<th>Marginal R²</th>
<th>Conditional R²</th>
<th>k</th>
<th>log-Likelihood</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed heterozygosity</td>
<td>Full model</td>
<td>0.31</td>
<td>0.44</td>
<td>7</td>
<td>119.9</td>
<td>-225.8</td>
</tr>
<tr>
<td>Population density + generation length + sample size</td>
<td>0.29</td>
<td>0.46</td>
<td>6</td>
<td>118.77</td>
<td>-225.53</td>
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<tr>
<td>Generation length + sample size</td>
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<td>0.41</td>
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<td>117.53</td>
<td>-225.06</td>
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<tr>
<td>Population density only</td>
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<td>0.56</td>
<td>4</td>
<td>111.73</td>
<td>-215.5</td>
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<tr>
<td>Null model</td>
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<td>0.46</td>
<td>3</td>
<td>107.59</td>
<td>-209.18</td>
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<tr>
<td>Expected heterozygosity</td>
<td>Generation length + sample size</td>
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<td>0.45</td>
<td>5</td>
<td>126.04</td>
<td>-242.08</td>
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<tr>
<td>Generation length + sample size + body mass</td>
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<td>0.44</td>
<td>6</td>
<td>126.21</td>
<td>-240.41</td>
<td></td>
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<tr>
<td>Population density + Generation length + sample size</td>
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<td>0.45</td>
<td>6</td>
<td>126.12</td>
<td>-240.23</td>
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<tr>
<td>Allelic richness</td>
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<td>0.57</td>
<td>6</td>
<td>126.04</td>
<td>-242.08</td>
</tr>
<tr>
<td>Full model</td>
<td>0.44</td>
<td>0.56</td>
<td>7</td>
<td>126.21</td>
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<td></td>
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<tr>
<td>Population density only</td>
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<td>54.06</td>
<td>-100.12</td>
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<tr>
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<td>49.3</td>
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Table III.2 Summary statistics and parameters from the best-fit linear mixed models in cluster (L) and country (C) data sets. 
*Values in the bracket are the 95% confidence intervals of the fixed effect estimate.  
***p < 0.001; **p < 0.01; *p < 0.05

<table>
<thead>
<tr>
<th>Dataset (n. obs)</th>
<th>Observed heterozygosity</th>
<th>Expected heterozygosity</th>
<th>Allelic richness</th>
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<tr>
<td></td>
<td>L (134)</td>
<td>C (377)</td>
<td>L (142)</td>
</tr>
<tr>
<td>Estimates of fixed effects</td>
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<td>0.1</td>
<td>0.22*</td>
<td>0.22**</td>
</tr>
<tr>
<td></td>
<td>[-0.102; 0.300]</td>
<td>[0.046; 0.402]</td>
<td>[0.061; 0.385]</td>
</tr>
<tr>
<td>Population density</td>
<td>0.05*</td>
<td>0.07***</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>[0.008; 0.086]</td>
<td>[0.038; 0.108]</td>
<td>[0.016; 0.079]</td>
</tr>
<tr>
<td>Generation length</td>
<td>0.04***</td>
<td>0.03**</td>
<td>0.03***</td>
</tr>
<tr>
<td></td>
<td>[0.016; 0.059]</td>
<td>[0.010; 0.052]</td>
<td>[0.014; 0.053]</td>
</tr>
<tr>
<td>Sample size</td>
<td>0.06*</td>
<td>-</td>
<td>0.1*</td>
</tr>
<tr>
<td></td>
<td>[0.008; 0.108]</td>
<td></td>
<td>[0.059; 0.150]</td>
</tr>
<tr>
<td>Body mass</td>
<td>0.05</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>[-0.023; 0.128]</td>
<td></td>
<td>[-0.021; 0.123]</td>
</tr>
<tr>
<td>Marginal R²</td>
<td>0.3</td>
<td>0.23</td>
<td>0.28</td>
</tr>
<tr>
<td>Conditional R²</td>
<td>0.49</td>
<td>0.54</td>
<td>0.4</td>
</tr>
<tr>
<td>Variance of the random effects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species (n)</td>
<td>0.003</td>
<td>0.005</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>(14)</td>
<td>(17)</td>
<td>(15)</td>
</tr>
<tr>
<td>Residual</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Population density and genetic diversity are positively correlated in wild felids.
Population density and genetic diversity are positively correlated in wild felids

in our datasets (Brodie, 2009; Tensen, 2018a; Zanin et al., 2015), might have limited our attempt to correlate BM to GD. Large felids including lynx have been frequently reintroduced to compensate the loss (Hayward and Somers, 2009) or translocated to mitigate perceived risks to humans or their livestock (Fontúrbel and Simonetti, 2011). These human interventions could sufficiently change the genetic structure through population mixing (e.g. Diefenbach et al., 2015; Mueller et al., 2020) or the population density estimates in other species (Mondal et al., 2012), both could potentially introduce an artefact in our data. In addition, our study shows that the PD records outweigh the number of GD records (212 vs. 116) and 40% of the records were excluded in the cluster approach, underlying that PD and GD have not been sufficiently studied closely together within the species distribution, despite the evident importance as confirmed by our study.

We demonstrated that our experimental framework of combining estimates from camera-trapping surveys and population genetic studies has the potential to uncover the eco-evo interactions and to evaluate species extinction risk from local populations across species (Lowe et al., 2017). A recent debate whether neutral genetic diversity metrics should be incorporated in conservation assessment has risen (DeWooody et al., 2021; Teixeira and Huber, 2021). Indeed, species risk assessment for imperative conservation action often requires holistic solutions. However, detailed information of PD estimates for many endangered felid species is still lacking and the genetic information is still rarely considered within the IUCN RedList species assessments (Garner et al., 2020). Combining PD and GD following our framework with already existing database of PD (i.e. TetraDENSITY (Santini et al., 2018a) or GD (i.e. MacroPopGen (Lawrence et al., 2019) and VarVer (Yashima and Innan, 2017)) across various species is a promising opportunity to explore the relationship between GD and PD across different taxa, as well as identifying suitable areas for conservation planning. Finally, in the spirit of improving both the framework and the datasets, we provide an interactive map of the PD and GD records including those which were not used in the analysis, to encourage the efforts worldwide (see Interactive_Map).
Population density and genetic diversity are positively correlated in wild felids

Acknowledgments
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Methodology: AA, EP, SA, SD
Investigation: AA, SA
Visualization: AA
Supervision: EP, SD
Writing – original draft: AA, SA
Writing – review & editing: CN, EP, SD
Chapter IV

Comparative Genomic Studies Among Captive And Wild Individuals

The Case Study Of African Cheetahs And Asiatic Lions

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Supplementary materials available in Annexe 3

“"But the tiger you see madly pacing its cage is nevertheless preoccupied with something that a human would certainly recognize as a thought. And this thought is a question: “Why?”

“Why, why, why, why, why, why?””

Daniel Quinn
Ishmael (1992)
Summary

The cheetah and the Asiatic lion are flagship species in European breeding programme, albeit with different management histories. While the genetic management of captive cheetahs has been considered successful, this was not the case for captive Asiatic lions in which consanguineous matings from the small founder populations could not be completely avoided. The genetic profiles of the descendants of these founder populations in captivity under the European Endangered species Programme (EEP) have not been characterised sufficiently. Here we used whole-genome sequence data to evaluate the genomic variability in these two species housed in Montpellier Zoo and compared them with genomic data from wild congeners and other available captive individuals. We found that the captive cheetahs have higher heterozygosity compared to the wild individual. However, the inbreeding measured by runs of homozygosity in the genome increases with the number of generations in captivity. Surprisingly, the Asiatic lions in Montpellier Zoo have similar genomic diversity with the one individual kept in Gir Forest Sanctuary. Although Asiatic lions have the least contemporary diversity, the inferred effective population size at 10,000 years ago was the lowest in the cheetahs based on conspecific reference genome. We also showed that the phylogenetic distance of the reference genomes to the targeted species can have a significant impact on the measures of individual genome-wide heterozygosity, the inbreeding coefficient based on identity-by-descent segments and the demographic inferences.
Introduction

Cheetahs (*Acinonyx jubatus*) and Asiatic lions (*Panthera leo persica*) are textbook examples of species in genetic peril (Frankham et al., 2002). These species provide the earliest empirical observations of low genetic variation associated with low fitness affecting spermatogenesis and perinatal mortality in captivity (O’Brien et al., 1985; Wildt et al., 1987a). Captive populations of endangered species are generally initiated from a small number of founders and there is a strong agreement that inbreeding depression coupled with decreasing fitness in captivity can substantially increase the species extinction risk even within a single generation (Boakes et al., 2007; Christie et al., 2012; Der Sarkissian et al., 2015b). Maintaining the genetic insurance of these species in case of an extinction throughout its natural distribution has thus become a critical issue in ex situ breeding programmes. The concerns of genetic changes or adaptations in captive breeding programmes have been widely addressed which has led to several recommendations to minimise the deleterious effects of genetic adaptation, such as minimising inbreeding by the exchange of individuals between zoos with sufficient pedigree information, or by supplementing new founders from the wild, as well as minimising the number of generations by delaying reproduction and long-term preservation of genetic material (e.g. Frankham, 2008; Williams and Hoffman, 2009; Witzenberger and Hochkirch, 2011; Willoughby et al., 2017). However, predicting genetic responses to captivity for threatened carnivores which have typically small populations, large home-range size and slow generation length is challenging (Clubb and Mason, 2003).

Several studies have compared the genetic diversity among wild and captive populations across carnivore species using various molecular markers (Table 1). Remarkably, most of these studies reported that captive populations have higher than or equal genetic diversity to their wild congeners suggesting that either the breeding programmes have been successful in maintaining genetic diversity or that these captive individuals may not yet display genetic adaptation to captivity. Since most of these studies used a small number of genetic markers which may not be representative of the whole genome, there is an urgent need to compare the genomic variability between captive and wild populations, and across generations in captivity in order to improve the maintenance of genetic diversity in managed captive breeding programmes. One
recent study has measured the genomic diversity from both mitochondrial and nuclear high-coverage genomes and found low genetic diversity but no evidence of inbreeding within a captive brown hyena (*Parahyaena brunnea*) (Westbury et al., 2018). However, no comparison of genomic diversity was made with the other wild individuals sampled in the aforementioned study.

The cheetahs and Asiatic lions have contrasting history of captive breeding management under the European Endangered species Programme (EEP). Cheetahs have been kept in zoos since 1829 but successful breeding of cheetahs in captivity has only started around the mid 1950s with a continuing supply of captive stocks from wild populations (Marker et al., 2018). Captive individuals’ information including their pedigrees has been documented in studbooks since the late 1980s and there had been an average of 1% annual population growth of cheetahs in captivity until 2014 (Marker et al., 2018). As of 2019, there are about 1820 living captive cheetahs in 46 countries but only 300 individuals are potential breeders (or the effective population size) (Marker and Johnston, 2019). In spite of the extensive studies of genetic diversity in cheetahs (Terrell et al., 2016; reviewed in Schmidt-Küntzel et al., 2018), the captive populations under EEP have not been characterised genetically; therefore the genetic diversity has only quantified using substitute variables based on the pedigrees (Marker et al., 2018). The captive breeding programme for the Asiatic lions, on the contrary, started later since it had been re-established in Europe a couple of years after the first attempt to breed captive Asiatic lions outside of its natural distribution in 1981 was halted due to the suspicion of introgression of the Africa subspecies among the founders (O’Brien et al., 1987). Microsatellite marker assessments have revealed that the genetic variation of the nine founders that were donated for the EEP population was lower than those reported from the populations in India, implying that the genetic consequences of inbreeding had already occurred before they were even transferred from India (Atkinson et al., 2018; Gaur et al., 2006; Singh et al., 2002).

Whole-genome sequences (WGS) provide opportunities to accurately quantify the impact of reduced population size on genetic diversity and to make inference about species demographic history (Beichman et al., 2018; Díez-del-Molino et al., 2018). Any comparative study of genome-wide heterozygosity across species requires a high-quality assembly of reference genome to accurately map targeted WGS. Given that the cost of sequencing and annotating high-quality reference genomes can be limited by
**Comparative genomic studies among captive and wild individuals**

available funding and computational resources in conservation, studies on non-model organisms may benefit from the genome assembly of a closely related species with important applications in biomedical research (e.g. canids in Gopalakrishnan et al., 2017). An important aspect to consider is that the choice of the reference genome could cause discrepancies in downstream analyses such as estimating heterozygosity, inbreeding coefficients, and effective population sizes (Armstrong et al., 2020; Gopalakrishnan et al., 2017; Günther and Nettelblad, 2019; Samaha et al., 2021). The influence of the phylogenetic distance between the reference genome’s taxon and the studied taxon (or taxa) on the downstream analyses have been recently addressed in humans (Yang et al., 2019), canids (Gopalakrishnan et al., 2017), cetaceans and birds (Prasad et al., 2021), and across various bacterial species (Valiente-Mullor et al., 2021). Genomic studies in Felidae have exploited the continuously improved and revised genome of the domestic cat, which is currently at the 9<sup>th</sup> version since its release in 2002 (Buckley et al., 2020; O’Brien et al., 2002). This has paved ways to genomic sequencing of other felid species enabling comparison studies of their genome structure. One of the general findings is that felid genomes have a strong level of synteny thus it can provide opportunities for high alignment quality for comparative analyses and to detect genomic signatures of adaptation with high certainties (Cho et al., 2013; Figueiró et al., 2017; Samaha et al., 2021). Furthermore, the availability of chromosome-level genomes recently assembled with high levels of contiguity in Felidae (Table 2) offers a rich source to further investigate the relative importance of the quality of the reference genome and the phylogenetic distance for the genome-wide diversity analyses of targeted species with high conservation priorities for which the reference genome is not available.

To examine the genomic consequences of captivity in South African cheetahs (referred as cheetahs throughout this chapter) and Asiatic lions, we characterise the pattern of genomic diversity which includes the genome-wide heterozygosity and the inbreeding coefficients quantified using the number, length and proportion of runs of homozygosity in the genome of the individuals from the Montpellier zoo (south of France) and those with published genomes. Given that cheetahs had a larger founder population with frequent mating with wild individuals compared to Asiatic lions (Figure S1-S2), we expected that the impact of genetic adaptation to captivity to be lower in cheetahs than in the Asiatic lions. We also compared the demographic history
between these two species to distinguish the relative effect of past climatic changes and recent captivity on their genomic diversity. We also assessed the influence of reference genomes derived from the targeted species and the domestic cat on these genome-wide diversity measures and the demographic inferences.

**Figure IV.1 Phylogenetic relationship, based on biparental nuclear genome, between the cross-species reference genomes and the sampled species, cheetah (*Acinonyx jubatus*) and Asiatic lion (*Panthera leo persica*).**

The dated tree is adapted from Liu et al. 2016 and the maximum estimated time for splitting between African lion and Asiatic lion (67 kya) was taken from Manuel et al. (2020). The time scale, in million years, is represented below the phylogenetic tree. The grey rectangles at the nodes are the range of inferred divergence time.
Table IV.1 A non-exhaustive compilation of studies investigating the differences of wild vs. captive genetic diversity in carnivores.  
Highlighted key findings are studies showing similar or greater genetic diversity in captive populations than in the wild. (ND: No differences, CBD: conservation breeding programme, EEP: European Endangered species Programme, MHC: Major histocompatibility complex.)

<table>
<thead>
<tr>
<th>Species (or subspecies)</th>
<th>Natural range</th>
<th>Zoo locations</th>
<th>Is the zoo located within sp. natural distribution?</th>
<th>Molecular markers</th>
<th>Key findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheetah</td>
<td>Africa</td>
<td>North America, Europe, South Africa</td>
<td>Yes</td>
<td>Microsatellites</td>
<td>Genetic diversity increases in captive but decrease in wild. Significant inbreeding in wild only.</td>
<td>(Terrell et al., 2016)</td>
</tr>
<tr>
<td>Asiatic lion</td>
<td>Asia</td>
<td>EEP</td>
<td>No</td>
<td>Microsatellites</td>
<td>GD in Captive (contemporary) &lt; EEP founders &lt; Indian population</td>
<td>(Atkinson et al., 2018)</td>
</tr>
<tr>
<td>Asiatic lion</td>
<td>Asia</td>
<td>Unknown</td>
<td>Unknown</td>
<td>MHC</td>
<td>GD in captive-bred &lt; Wild</td>
<td>(Sachdev et al., 2005)</td>
</tr>
</tbody>
</table>
| Tiger (Panthera tigris) | Eurasia       | USA Zoos     | No                    | MHC             | Pooled samples: Captive > Wild  
Siberian tigers only: Captive < Wild | (Hendrickson et al., 2000) |
<p>| Tiger                   | Eurasia       | Multiple     | Some                  | Microsatellites and mtDNA | Captive samples have novel variants not identified in voucher samples. ND in haplotypes among Siberian tigers | (Luo et al., 2008) |
| Siberian tiger (Panthera tigris altaica) | Eurasia       | North America and Chengdu Zoos | No and possibly extirpated | Microsatellites and mtDNA | Captive have novel variants GD in Captive &gt; Wild, and Captive &lt; Wild in inbreeding | (Henry et al., 2009) |</p>
<table>
<thead>
<tr>
<th>Species</th>
<th>Continent</th>
<th>Location</th>
<th>Population</th>
<th>Marker</th>
<th>Coefficient (Fis)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bengal tiger <em>(Panthera tigris tigris)</em></td>
<td>Asia</td>
<td>Chandigarh and Delhi Zoo</td>
<td>Yes</td>
<td>mtDNA</td>
<td>Captive samples have novel variants</td>
<td>(Sharma et al., 2009)</td>
</tr>
<tr>
<td>Bengal tigers</td>
<td>Asia</td>
<td>Corbett Tiger Reserve, Delhi and Hyderabad Zoo</td>
<td>Yes</td>
<td>MHC</td>
<td>ND in allelic variability</td>
<td>(Pokorny et al., 2010)</td>
</tr>
<tr>
<td>African lion <em>(Panthera leo leo)</em></td>
<td>Africa</td>
<td>Ukutula GR, South Africa</td>
<td>Yes</td>
<td>Microsatellites</td>
<td>Captive samples have novel variants</td>
<td>(Miller et al., 2014)</td>
</tr>
<tr>
<td>Iberian lynx <em>(Lynx pardinus)</em></td>
<td>Europe</td>
<td>Spain</td>
<td>Yes</td>
<td>Microsatellites</td>
<td>Captive &gt; Wild in observed heterozygosity. Captive &lt; Wild in allelic diversity. Wild have private alleles</td>
<td>(Kleinman-Ruiz et al., 2019)</td>
</tr>
<tr>
<td>Indian leopard <em>(Panthera pardus fusca)</em></td>
<td>Asia</td>
<td>Various zoos under CBP of the Central Zoo Authority of India</td>
<td>Yes</td>
<td>MHC-I and II DRB</td>
<td>Captive &lt; Wild in rate of non-synonymous substitution</td>
<td>(Parmar et al., 2017)</td>
</tr>
<tr>
<td>Iberian wolf <em>(Canis lupus signatus)</em></td>
<td>Spain</td>
<td>EEP and other institutions</td>
<td>Yes</td>
<td>Microsatellites</td>
<td>ND</td>
<td>(Ramirez et al., 2006)</td>
</tr>
<tr>
<td>Fosa <em>(Cryptoprocta ferox)</em></td>
<td>Madagascar</td>
<td>Multiple</td>
<td>Both</td>
<td>MtDNA and one nuclear sequence</td>
<td>GD in captive &gt; wild</td>
<td>(Veron et al., 2018)</td>
</tr>
</tbody>
</table>
Table IV.2 Chromosome-level assembly of felid genomes. Genome assembly marked with an asterisk were used in this study for short-read alignment.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>ID</th>
<th>Reference</th>
<th>Sequencing technology</th>
<th>Genome coverage</th>
<th>Total length (Gb)</th>
<th>Number of scaffold</th>
<th>Scaffold N50</th>
<th>Number of contigs</th>
<th>Contig N50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domestic cat*</td>
<td>F</td>
<td>Felcat9</td>
<td>(Buckley et al., 2020)</td>
<td>PacBio; 454 Titanium; Illumina; Sanger dideoxy sequencing</td>
<td>72</td>
<td>2.5</td>
<td>4525</td>
<td>83 Mb</td>
<td>4909</td>
<td>41 Mb</td>
</tr>
<tr>
<td>Cheetah*</td>
<td>F</td>
<td>aciJub1</td>
<td>(Dobrynin et al., 2015) and DNAzoo</td>
<td>HiSeq X Ten, Hi-C</td>
<td>48</td>
<td>2.37</td>
<td>13047</td>
<td>145 Mb</td>
<td>197914</td>
<td>28 kb</td>
</tr>
<tr>
<td>Canadian lynx*</td>
<td>M</td>
<td>LynCan4</td>
<td>Vertebrate genome project by G10K Consortium (Rhie et al., 2021)</td>
<td>PacBio Sequel I; 10X genome; Bionano Genomics; Arima Genomics Hi-C</td>
<td>72</td>
<td>2.4</td>
<td>54</td>
<td>147 Mb</td>
<td>894</td>
<td>7.5 Mb</td>
</tr>
<tr>
<td>Lion*</td>
<td>F</td>
<td>PanLeo1</td>
<td>(Armstrong et al., 2020)</td>
<td>10x Genomics Chromium, Hi-C, Nanopore</td>
<td>46</td>
<td>2.4</td>
<td>8061</td>
<td>136 Mb</td>
<td>23775</td>
<td>286 kb</td>
</tr>
<tr>
<td>Puma</td>
<td>F</td>
<td>PumCon1</td>
<td>(Saremi et al., 2019) and DNAzoo</td>
<td>Illumina; Oxford Nanopore, Hi-C</td>
<td>47</td>
<td>2.4</td>
<td>2370</td>
<td>149 Mb</td>
<td>181487</td>
<td>27 kb</td>
</tr>
<tr>
<td>Jaguarundi</td>
<td>M</td>
<td>PumYag</td>
<td>(Tamazian et al., 2021)</td>
<td>10x Genomics Chromium</td>
<td>53</td>
<td>2.6</td>
<td>10947</td>
<td>49 Mb</td>
<td>53446</td>
<td>100 kb</td>
</tr>
<tr>
<td>Tigers</td>
<td>F</td>
<td>Pti1_mat1.1</td>
<td>Bredemeyer and Murphy (GenBank, unpublished, 2021)</td>
<td>PacBio Sequel 2</td>
<td>78</td>
<td>2.4</td>
<td>75</td>
<td>147 Mb</td>
<td>140</td>
<td>75 Mb</td>
</tr>
</tbody>
</table>

Note: DNAzoo is a chromosome-length genome database assembled using high-throughput chromosome conformation capture (Hi-C) library developed and released by Dudchenko et al. (2017, 2018)
Materials and methods

Whole-genome sequencing

Whole blood samples (2.5 to 3.5 ml / individual) of 2 female Asiatic lions (samples ‘Surina’ 14114 and ‘Kiran’ 14116) and 4 Southeast African cheetahs (‘Ajabu’ 18020, ‘Aywa’ 18021, ‘Azrael’ 18022, and ‘Bappe’ 18036) were obtained from Montpellier Zoo (Parc de Lunaret, France) on the 25th May and 17th June 2020. The two female lions are by the same sire and their dams are siblings. The cheetahs came from two different parents: Ajabu, Aywa and Azrael are from the same clutch, and Bappe shares maternal grandparents with them (Figure S1-S2). The samples were collected in PAXgene Blood DNA Tubes and high-quality DNA was purified using the associated PAXgene Blood DNA Kit (Qiagen, Hilden, Germany). The extracted DNA, approximately 2000 ng, were stored at –20°C before being sent to Montpellier GenomiX (CNRS) sequencing platform through two batches (14114, 18020 & 18036 and 14116, 18021 & 18022). Library preparation was done using TruSeq Nano DNA Library Preparation Kit with an average insert size of 550 bp. The size and quantity of the constructed libraries were assessed using High Sensitivity NGS kit on a Fragment Analyzer™ and by qPCR (Roche LightCycler® 480). The libraries were then sequenced using a 2 x 150 bp paired-end method by Illumina Novaseq 6000 platform on 2 lanes of SP flowcells. The Illumina sequences, with 50 Gb of raw reads per sample in average, was quality-checked and trimmed in order to remove PCR adapters.

WGS data from GenBank

We downloaded WGS data from Sequence Read Archive (SRA) database of Asiatic lion, ‘Atul’ (SRX2725780:SRX2725782) and cheetah, ‘Chewbaaka’ (SRR2737519:SRR2737521), of whom one of the parents was presumably taken from wild populations (Table 3). For these two individuals, we only selected sequences with insert size 500 bp. We also included WGS data of ‘Rico’ the cheetah which were sequenced using Illumina NovaSeq (SRR9951918:SRR9951920), as well as two unnamed Asiatic lions with low genomic coverage (SRR11286170,SRR11286171) and ‘Brooke’ the African lion (SRX6748111) to increase the sample size in this study.
Mapping short-reads to reference genome

To compare how different reference genome assemblies may affect variant calling and further downstream analysis, all Montpellier Zoo samples were mapped to the NCBI genomic assembly Felcat9. We also aligned these samples to their respective species (conspecific) and to the closest-species reference genome (i.e., Cheetah to aciJub1 and LynCan4, and Asiatic lions to PanLeo1, Figure 1). Other cheetah sequences were mapped to aciJub1 only whereas for the other three Asiatic lions, we mapped their sequences on both Felcat9 and PanLeo1 assemblies. The reference genome with the highest quality in terms of contiguity based on the number of scaffolds and the median size of contiguous sequences of nucleotide bases (contig N50) is LynCan4, followed by Felcat9, PanLeo1 and aciJub1. When considering for the ‘completeness’ of the contigs assembly, the median scaffold size (scaffold N50) decreases from LynCan4, aciJub1, Panleo1 to Felcat9 (Table 2). Each sequence in Fasta format was mapped individually with BWA-MEM algorithms in bwa v.0.7.17 (Li and Durbin, 2009). For individuals with multiple files sequences, we annotated the read groups using the SRA identifier before merging the the mapped reads into a single BAM file. Since the BAM file of ‘Rico’ produced an average depth coverage of 75 after mapping, we subsampled the mapped reads towards a similar coverage as our samples (44x). The aciJub1 genome assembly was aligned to Felcat9 genome using Basic Local Alignment Search Tool, ‘BLAST’ (Altschul et al., 1990) to localise the chromosome-length scaffold positions.

Variant calling and filtering

To ensure that the inbreeding and demographic history analyses were not heavily compromised by the choice of the variant caller (Pighton et al., 2015), we ran single-sample variant calling using two approaches, i) site align-based algorithms implemented in ‘samtools mpileup’ v.1.12 and ‘bcftools call’ v.1.7 (Li et al., 2009; Li, 2011), referred to bcf-vc herein, and ii) haplotype-based algorithms implemented in freebayes (Garrison and Marth, 2012). Both variant callers were run using the default parameters. The detected single nucleotide variants (SNVs) were then filtered using ‘vcftools’ v.1.15 (Danecek et al., 2011) as follows: only reads mapped on the 19 chromosome-length scaffolds were retained, indels were filtered out, minimum depth and average depth were set to 10, and minimum mapping quality and minimum genotype assign-
ment quality (GQ) were set to 30. Additionally, the depth cut-off was set to the average depth plus 4 times the root-mean-square of depth to remove false heterozygote sites (Li, 2014).

**Genome-wide heterozygosity, inbreeding coefficient and demographic history**

We calculated genome-wide heterozygosity or the proportion of heterozygous genotypes from the mapped reads (.bam files) as implemented in ‘angsd’ v.0.935 (Korneliussen et al., 2014). This programme estimates the sample allele frequency likelihoods using genotype likelihood model from the parameters obtained through read mapping, instead of the detected SNVs, and then estimates the single frequency spectrum using the Expectation Maximization (EM) algorithm. Bases with qscore (-minQ) and mapping quality (-minmapq) less than 30 were removed from the input BAM files and we also included adjustment for excessive mismatches (-C 50). We averaged the values across 100 bootstraps calculated in each chromosome-length scaffold individually.

To estimate the inbreeding coefficients, we use a hidden Markov model as implemented in ‘bcftools’ to detect tracts of autozygous regions or runs of homozygosity (ROH) (Narasimhan et al., 2016) using the filtered SNV data identified in the chromosome-length scaffolds. The parameters were set to “-G 30” to specify the use of genotype calls with a quality of 30 or more, using default allele frequency since we only used single-sample, and a recombination rate of 1.9 cM/Mbp (Li et al., 2016b). We then calculated the individual inbreeding coefficient, $F_{ROH}$, as the proportion of ROH or contiguous homozygous regions of the genome similar to that of in (McQuillan et al., 2008), $F_{ROH} = \text{Sum of the ROH length} / \text{Length of autosomal genome}$. The $F_{ROH}$ was calculated separately and then averaged across 18 autosomal-scaffolds. ROHs with phred score lower than 60 and less than 50 SNVs were removed. Moreover, we recalculated the $F_{ROH}$ using 2 minimum specified length of ROH thresholds that is 500 kb and 1 Mb. Shorter ROHs are more likely to have arisen from a strong linkage disequilibrium whereas long tracts of homozygosity indicate a recent close-relative inbreeding (Kirin et al., 2010).

To estimate the effective population size changes ($N_e$) trajectories in cheetahs and Asiatic lions, we used the pairwise sequentially Markovian coalescent (PSMC) ap-
proach (Li and Durbin, 2011) as implemented in ‘psmcR’ (Paradis and Li, 2021) on the SNVs detected on the autosomal scaffolds. PSMC plots can indicate the point at which gene flow ceased between a pair of populations (Li and Durbin, 2011; Mather et al., 2020). To identify whether the $N_e$ in Asiatic and African lions would converge looking backwards in time owing to a shared population history, we also run the PSMC using variants from mapping on Felcat9 assembly for comparison with conspecific reference genome and from African lion ‘Brooke’ SNV data. Since the nuclear mutation rate in cheetahs and lions have not been described, we used 3 different mutation rates to calibrate the time and population size in the PSMC outputs. These mutation rates, per base pair per year, correspond to the averaged mammalian mutation rates, $2.2 \times 10^{-9}$ (Kumar and Subramanian, 2002), the neutral mutation rate, $0.9 \times 10^{-9}$ as used in (Cho et al., 2013; Manuel et al., 2020) and $3 \times 10^{-9}$ based on the pairwise alignment differences between the cheetah and domestic cat (Felcat 6.2) assembly divided by their divergence time, 7 million years ago (mya) (Dobrynin et al., 2015). The generation time for cheetah was set to 3 years and to 5 years for Asiatic lions as in previous studies (Armstrong et al., 2020; Dobrynin et al., 2015; Manuel et al., 2020). Panleo_India_1 and Panleo_India_2 samples were excluded from the ROH and PSMC analyses due to their low genome coverage. To statistically compare the genome-wide heterozygosity and inbreeding among the individuals sampled here and among the reference genomes, we used a nonparametric one-sided Wilcoxon rank sum test of the estimates in each chromosome set (e.g. Figure S3) from the ‘stats’ package as implemented in R v.3.6.3.
Table IV.3 Information on the nuclear genome sequences sampled and their coverage for this study.
* subsampled SRA data from the original coverage given in the brackets. $ sequences with insert size 500bp. Parent populations were based on the pedigree information acquired from studbook keepers and online resources (a) Marker & Johnston (2019) and (b) Wildlife Institute of India (2018). For the individuals indicated with ‘?’ in parent population column, we were not able to retrieve their pedigree information. See supplementary figures for the pedigree charts.

<table>
<thead>
<tr>
<th>Species</th>
<th>House name (ID)</th>
<th>Sex</th>
<th>Date of birth</th>
<th>Parent population</th>
<th>Sample number / Biosample</th>
<th>Cov.</th>
<th>Study</th>
</tr>
</thead>
<tbody>
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<td>Ajabu</td>
<td>F</td>
<td>14/05/18</td>
<td>Captive</td>
<td>18020</td>
<td>33</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>Aywa</td>
<td>F</td>
<td>14/05/18</td>
<td>Captive</td>
<td>18021</td>
<td>45</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>Azrael</td>
<td>M</td>
<td>14/05/18</td>
<td>Captive</td>
<td>18022</td>
<td>45</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>Bappé</td>
<td>M</td>
<td>03/07/18</td>
<td>Captive</td>
<td>18036</td>
<td>38</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>Rico</td>
<td>M</td>
<td>28/10/09</td>
<td>Captive*</td>
<td>SAMN09699341</td>
<td>44$ (75)</td>
<td>Felidae consortium (GenBank, unpublished, 2018)</td>
</tr>
<tr>
<td></td>
<td>Chewbaaka</td>
<td>M</td>
<td>07/95</td>
<td>Wild*</td>
<td>SAMN04127588</td>
<td>21$</td>
<td>(Dobrynin et al., 2015)</td>
</tr>
<tr>
<td>Asiatic lion</td>
<td>Surina</td>
<td>F</td>
<td>15/02/13</td>
<td>Captive</td>
<td>14114</td>
<td>34</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>Kiran</td>
<td>F</td>
<td>14/04/13</td>
<td>Captive</td>
<td>14116</td>
<td>37</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>Atul</td>
<td>M</td>
<td>07/09/99</td>
<td>Captive dam and Wild sireb</td>
<td>SAMN06606823</td>
<td>22$</td>
<td>(Mitra et al., 2019)</td>
</tr>
<tr>
<td></td>
<td>Panleo_India_1</td>
<td>?</td>
<td></td>
<td>“Present-day”</td>
<td>SAMN14352183</td>
<td>6</td>
<td>(Manuel et al., 2020)</td>
</tr>
<tr>
<td></td>
<td>Panleo_India_2</td>
<td>?</td>
<td></td>
<td>“Present-day”</td>
<td>SAMN14352184</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>African lion</td>
<td>Brooke</td>
<td>F</td>
<td></td>
<td></td>
<td>SAMN12375718</td>
<td>41</td>
<td>(Armstrong et al., 2020)</td>
</tr>
</tbody>
</table>
Results

Genome Mapping and Variant Calling

Our samples obtained from the Montpellier Zoo had coverage ranging from 32x to 45x and more than 98% of the reads were successfully mapped to the reference genome (Figure 2). The error rates were less than 1% when mapped to the reference genome of the same species and increase with the phylogenetic distance of the closest-species. For the cheetahs, mapping to LynCan4 produces slightly lower error rate than when mapping to Felcat9. The percentage of mapped bases also appears to decrease with sequencing error rates.

The differences in terms of percentage of SNVs retained, number of SNV loci, average depth and Ts/Tv ratio after filtering, among the reference genomes used and between bcf-vc and freebayes callers are shown in Figure 2. The percentage of SNVs detected in the Montpellier Zoo samples when using the conspecific reference genome, ranging from 12-22 % in cheetahs and 18 - 24% in Asiatic lions, were higher than when using Felcat9 (i.e. less than 8% were removed for both species)(Figure 3a-b). For the cheetahs, mapping with LynCan4 produced higher average depth and Ts/Tv than mapping with Felcat9 followed by acJub1 (Figure 3c-d). In lions, we see a reverse trend in that mapping with PanLeo1 gave a slightly higher average depth.

After filtering, bcf-vc produced higher total SNV loci than in freebayes, the latter performed better in terms of average read depths and Ts/Tv ratio. The total number of SNV loci obtained in low-coverage samples, Panleo_India 1 and Panleo_India_2, were greater with freebayes than with bcf-vc, even though the filtering removed more than 90% of the SNVs. Mapping with Felcat9 produced the most SNVs in both species. We did not statistically test the effect of reference genome quality on the mapping quality of short reads, however, the quality of the reference genome measured by its ‘contiguity’ or ‘completeness’ does not appear to show any detectable correlations.
Figure IV.2. Summary statistics of the reads mapped on different reference genomes in cheetahs and lions. The names in the x-axis represent the individuals examined.

Figure IV.3. Summary statistics of the filtered single nucleotide variant (SNV) called using ‘bcftools call’ and ‘freebayes’ based on different reference genomes in cheetahs and lions. The names in the x-axis represent the individuals examined.
**Genome-Wide Heterozygosity**

In general, the Asiatic lions had up to 5.8 times lower heterozygosity compared to the cheetahs (Table 4 & Figure 3). The heterozygosity of the Montpellier cheetahs obtained using aciJub1 reference genome was larger than those obtained using Felcat9, but only statistically significant in ‘Ajabu’ (average $H_{\text{auto}} = 5.9 \times 10^{-4}$ vs $5.4 \times 10^{-4}$, Wilcoxon rank sum test $p$-value = 0.001). In contrast, the heterozygosity in each Asiatic lion individuals using PanLeo1 was significantly lower than those obtained using Felcat9 (Wilcoxon test $p$-values < 0.001). In cheetahs, the heterozygosity of ‘Chewbaaka’ ($H_{\text{auto}} = 4.9 \times 10^{-4}$) is 17% and 14% lower than the heterozygosity in the average Montpellier Zoo individuals ($H_{\text{auto}} = 5.8 \times 10^{-4}$) and in Rico ($H_{\text{auto}} = 5.7 \times 10^{-4}$) respectively. These differences were statistically significant (Wilcoxon test $p$-values < 0.001).

The genome-wide heterozygosity based on Felcat9 between ‘Atul’, ‘Surina’ and ‘Kiran’ were comparable (Figure 4). However, when mapped to PanLeo1 assembly, ‘Atul’ had significantly lower heterozygosity compared to our samples ($H_{\text{auto}} = 1.04 \times 10^{-4}$ vs $1.23 \times 10^{-4}$ to $1.26 \times 10^{-4}$, Wilcoxon test $p$-values < 0.02). Moreover, the two samples with low-genomic coverage appear to have the lowest genomic diversity in both reference genomes mapping.

In general, all females in our samples showed higher heterozygosity in X chromosome than in autosomal chromosomes, with an exception to ‘Chewbaaka’ whose heterozygosity is disproportionally greater than the other cheetah samples. Given the low heterozygosity rate in X chromosome of ‘Panleo_India_1’ and ‘Panleo_India_2’ similar to ‘Atul’ than to the two females from Montpellier Zoo, we deduced the sex of these individuals as males (Figure S3).
Comparative genomic studies among captive and wild individuals

Table IV.4 Mean and standard deviation (sd) genome-wide heterozygosity based on autosomal chromosomes of each species.  
*The second heterozygosity values in square brackets Asiatic lions only include high-coverage samples.*

<table>
<thead>
<tr>
<th>Species</th>
<th>Reference genome</th>
<th>Mean heterozygosity (sd)</th>
<th>N sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheetah</td>
<td>aciJub1</td>
<td>5.53×10⁻⁴ (0.97×10⁻⁴)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Felcat9</td>
<td>5.5×10⁻⁴ (0.84×10⁻⁴)</td>
<td>4</td>
</tr>
<tr>
<td>Asiatic lion</td>
<td>Panleo1</td>
<td>0.95×10⁻⁴ (0.39×10⁻⁴)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>[1.18×10⁻⁴ (0.3×10⁻⁴)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Felcat9</td>
<td>1.58×10⁻⁴ (0.52×10⁻⁴)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>[1.9×10⁻⁴ (0.34×10⁻⁴)]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure IV.4. Distribution of heterozygosity from 18 autosomal chromosomes for each cheetah (red) and Asiatic lions (blue) according to various reference genomes: aciJub1, Felcat9 and PanLe01.

*The heterozygosity in the X chromosome was not included in the boxplot and is represented by the crosses.*

![Boxplot of heterozygosity distribution](image)
**Runs of homozygosity**

We detected the ROH stretches in each chromosome relative to aciJub1, Felcat9 and PanLeo1 reference genomes. The distribution of the ROH along the genomes, \( F_{ROH} \) are shown in Figure 5 with all samples displayed at least one ROH longer than 1Mb (ROHs > 1Mb) (Table S1). The level of \( F_{ROH} \) corresponds well to the one estimated using the pedigree (Table 5). In general, the Asiatic lions had the most inbreeding burden as indicated by higher \( F_{ROH} \) and longer ROHs than the cheetahs. Among the cheetahs, ‘Bappe’ had the greatest number of ROHs > 1Mb, \( n = 53 \) (Table 5, Table S1). All 3 Asiatic lions had about 50% of ROHs > 1Mb with the longest ROHs among the Asiatic lions were represented in ‘Atul’ (31.5 Mb using Panleo1 and 19.6 Mb using Felcat9). We found that the \( F_{ROH} \) among the cheetahs born in Montpellier Zoo were significantly higher than in ‘Chewbaaka’, although the proportion of ROHs > 1Mb among all the detected ROHs is greater in the latter. When \( F_{ROH} \) with length smaller than 500 kb or 1 Mb were removed, these differences were not significant, except for ‘Bappe’. Furthermore, this individual has the greatest \( F_{ROH} > 1 \) Mb (6.5%) among the cheetahs. The differences between Atul and the Asiatic lions housed in Montpellier zoos were not statistically different (Wilcoxon test \( p-values > 0.2 \)).

There is a great discrepancy in ROHs among the results depending in reference genome used and the difference is more pronounced in cheetahs. Mapping to Felcat9 assembly produced greater numbers of ROHs and numbers of SNVs but the length of ROHs tends to be shorter than when the sequences were mapped to the conspecific reference genome (Table S1). Using the aciJub1 assembly, the cheetahs have mostly short ROHs with the average ROH length being about 298,000 base pairs and less than 10% of the genomes were covered by ROHs. Where all ROH lengths were accounted for, about 98% of the genomes were covered by ROHs in both species when using Felcat9 assembly. After removing these short ROHs (less than 1 Mb), the \( F_{ROH} \) in cheetahs decreased to about 50%.
Comparative genomic studies among captive and wild individuals

Figure IV.5. Distribution of inbreeding coefficient based on runs of homozygosity (F_{ROH}) in cheetahs (red) and lions (blue).

Different reference genomes were used for mapping and two minimum length of ROH segment thresholds (500 kb and 1Mb). ROHs on the X chromosome are not included. Scales in Y-axis are not identical.
Table IV.5 Averaged inbreeding coefficients in cheetahs and Asiatic lions from Montpellier Zoo based on pedigree and genome-wide run of homozygosity (ROH)
Values were averaged across 18 autosome chromosomes using own species genome reference (aciJub1 and Panleo1). The p-values calculated based on Wilcoxon test represent evidence of differences in $F$ between the captive individuals and ‘Chewbaaka’ in cheetahs and between Asiatic lions from Montpellier Zoo and ‘Atul’. FROH and the proportion of ROH (number of ROH tracts / total number of ROH) restricted to two minimum ROH lengths 500 kb and 1 Mb were also presented.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sample</th>
<th>$F_{pedigree}$</th>
<th>$F_{ROH}$</th>
<th>$p$-value</th>
<th>Prop ROH $&gt; 500$ kb</th>
<th>$F_{ROH-500}$ kb $p$-value</th>
<th>Prop ROH $&gt; 1$ Mb</th>
<th>$F_{ROH-1}$ Mb $p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheetah</td>
<td>Ajabu</td>
<td>0.02</td>
<td><strong>0.094</strong></td>
<td>&lt;0.001</td>
<td>0.1</td>
<td>0.048</td>
<td>0.260</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Aywa</td>
<td>0.02</td>
<td><strong>0.098</strong></td>
<td>&lt;0.001</td>
<td>0.11</td>
<td>0.041</td>
<td>0.110</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Azrael</td>
<td>0.02</td>
<td><strong>0.099</strong></td>
<td>&lt;0.001</td>
<td>0.13</td>
<td>0.049</td>
<td>0.184</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Bappe</td>
<td>0.03</td>
<td><strong>0.111</strong></td>
<td>&lt;0.001</td>
<td>0.17</td>
<td><strong>0.073</strong></td>
<td><strong>0.026</strong></td>
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<td></td>
<td>Rico</td>
<td>NA</td>
<td>0.089</td>
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<td>0.044</td>
<td>0.177</td>
<td>0.04</td>
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<tr>
<td></td>
<td>Chewbaaka</td>
<td>NA</td>
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<td>0.63</td>
<td>0.033</td>
<td>0.177</td>
<td>0.32</td>
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<tr>
<td>Lion</td>
<td>Surina</td>
<td>0.11</td>
<td>0.945</td>
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<td>0.900</td>
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<tr>
<td></td>
<td>Kiran</td>
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<td>0.901</td>
<td>0.864</td>
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<tr>
<td></td>
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<td>0.906</td>
<td>0.61</td>
<td>0.861</td>
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Past Population Size Changes

Here we report the results of PSMC using the SNVs from the freebayes caller only. The PSMC results from different individuals were consistent within each species. Using the averaged mammalian mutation rate, the African lion and the cheetahs showed a more drastic decline after 200 thousand years ago (kya) (Figure 6-7). Cheetahs showed a gradual population effective size reduction after the first decline, especially around 100, 50, and 10 kya towards an average $N_e$ of 16,910. As for the lions, the most ancient $N_e$ in the African lion was estimated at 780 kya, with an increase in $N_e$ from 51,800 to 62,800 around 200 kya. One hundred ky later, the $N_e$ dropped to 38,203. During this period, the averaged $N_e$ in the Asiatic lions (51,259) is slightly higher than in the African lion (38,203). Furthermore, there is one large population decline, between 55 and 35 kya, and then the population stabilised towards 19 kya followed by a slight increase in $N_e$ near 10 kya. The $N_e$ of African lions at 10 kya is about 17132 whereas it is higher in the Asiatic lions, ranging between 22,706 and 26,676. We could not pinpoint the timing when the $N_e$ in African and Asiatic lions converged as the most ancient $N_e$ inferred in the latter is at 150 kya.

We found that the various mutation rates tested to calibrate the PSMC output had a strong effect on the timing and magnitude of $N_e$ changes, especially in the lions. For instance, the PanLeo1 assembly showed between 37% to 49% increase after the Last Glacial Maximum (LGM), whereas this increase was more subtle using the Felcat9 assembly, between 13% to 18% (Figure 6). This result suggests that caution should be taken in interpreting the correlation between the Ne trajectories with major events through time. In cheetahs, there is no significant discrepancy in the timing of Ne changes when using Felcat9 assembly (Figure 7). The estimated $N_e$ near 10 kya based on conspecific reference genome is the lowest in cheetahs, $N_e = 11,868$ whereas the Asiatic lion has the least $N_e$ when using Felcat9 assembly in either mutation rates (Table 6). The $N_e$ of African lion near 10 kya is lower than the Asiatic lions.
Table IV.6 The mean effective population size, *Ne* near 10 kya averaged across 100 bootstraps inferred in PSMC and scaled to various mutation rate.

<table>
<thead>
<tr>
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<th>Reference</th>
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<tr>
<td></td>
<td></td>
<td>0.9 × 10&lt;sup&gt;−9&lt;/sup&gt;</td>
<td>2.2 × 10&lt;sup&gt;−9&lt;/sup&gt;</td>
<td>3.0 × 10&lt;sup&gt;−9&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Cho et al., 2013; Manuel et al., 2020)</td>
<td>(Kumar and Subramanian, 2002)</td>
<td>(Dobrynin et al., 2015)</td>
</tr>
<tr>
<td>Cheetahs (n = 6)</td>
<td>aciJub1</td>
<td>-</td>
<td>16183</td>
<td>11868</td>
</tr>
<tr>
<td></td>
<td>Felcat9</td>
<td>-</td>
<td>16573</td>
<td>12154</td>
</tr>
<tr>
<td>Asiatic lions (n = 3)</td>
<td>PanLeo1</td>
<td>32657</td>
<td>22984</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Felcat9</td>
<td>7349</td>
<td>14706</td>
<td>-</td>
</tr>
<tr>
<td>African lion</td>
<td>PanLeo1</td>
<td>18410</td>
<td>18218</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure IV.6. Effective population size trajectory over time among captive (Ajabu, Aywa, Azrael, and Bappé) and wild cheetahs (Chewbaaka and Rico).

PSMC plots were obtained using two reference genomes, own species (aciJub1) and domestic cat (Felcat9), and two mutation rates (μ), per base and per generation, to scale the time and population size. Vertical dark grey bars correspond to major climatic change events, YD: Younger Dryas (12.9 to 11.7 kya), LGM: Last Glacial Maximum (26.5 to 19.0 kya), Toba eruption (74 kya) LIG: Last interglacial (~125 kya). Vertical light grey bars show the timing of inferred shifts in global extinction rates by Andermann et al. 2020. Vertical lines represent the inferred population history by Dobrynin et al. 2015, I: Founder populations in Africa II: Eastern and Southern populations split, III: Most recent bottleneck and gene flow cease.
Figure IV.7. Effective population size trajectory over time among the Asiatic lion (Surina, Kiran and Atul) and African lion (Brooke).

PSMC plots were obtained using two reference genomes, own species (PanLeo1) and domestic cat (Felcat9), and two mutation rates (mu), per base and per generation, to scale the time and population size. Vertical dark and light grey bars are as represented in Figure 5. Vertical lines represent the inferred population history by Manuel et al. 2020, I: Asiatic and South African lions split, II: Asiatic and Central African lions split, III: Asiatic and Barbary lions split.
Discussion

Comparative genomic studies of captive felids to those in the wild and over generations of captive breeding are still lacking and our study has been aimed at filling this gap. In this paper, our results provide the first evidence of the genomic variation and structure of cheetahs and Asiatic lions in managed captivity under the EEP. We are unable to provide the genome-wide heterozygosity and ROH using Felcat9 assembly on ‘Chewbaaka’ and ‘Rico’ short-read data at this time therefore the discussion below is primarily based on conspecific reference genome unless stated otherwise. The comparison of genome-wide heterozygosity, ROH and demographic history between the two variant callers, ‘bcftools’ and ‘freebayes’ is still ongoing.

The average level of genomic diversity in these two species (Table 1 and Figure 1) is comparable with those calculated in previous studies using high-throughput sequences of cheetahs, 0.0002 in Dobrynin et al. (2015) 0.0004 in Kim et al. (2016) and of Asiatic lions, 0.000276 in Mitra et al. (2019) and 0.00019 in Armstrong et al. (2020). We provide additional evidence that the inbreeding coefficients derived from genomic data are more precise than those calculated based on pedigree information, and that the level of both inbreeding coefficients correlates well to some degree as previously demonstrated (Kardos et al., 2015).

Genomic Diversity of Captive Cheetahs and Asiatic Lions

Our results revealed that the captive cheetahs carried more genomic diversity than the wild individuals, corroborating the findings from a microsatellite study (Terrell et al., 2016). The generational consequence of captive breeding on the genomic structure appears to be less impacted in Asiatic lions than in cheetahs which have been managed in captivity longer. Cheetahs displayed increasing inbreeding with the number of generations in captivity, in line with previous studies showing increase in inbreeding can occur even after a single generation in captivity (Christie et al., 2012; Grueber et al., 2017). Interestingly, we found that one of the cheetahs, ‘Bappe’, has a greater number of long tracts of homozygosity than the other cheetahs in Montpellier Zoo and they differ only by the sire. ‘Bappe’’s sire is an F3 of wild ancestors whereas the closest wild ancestor in the other cheetah family can be traced up to 5 generations ago. Given our small sample size, these findings do not represent the whole captive
population of cheetahs. Despite some cheetah ancestors have been taken from the wild for captive breeding, this suggests that mating between close relatives could not be avoided in small captive populations.

We expected the genomic diversity in ‘Atul’ to be higher than in our samples since the Asiatic lions in the Montpellier Zoo are the offspring of a small founder population in which their genetic diversity derived from microsatellite markers were already at a very low level (Atkinson et al., 2018). However, the captive Asiatic lion in India ‘Atul’ revealed neither obvious differences in genomic diversity nor in inbreeding coefficients with the individuals in the EEP. The pedigree of ‘Atul’ showed that the dam is an offspring of a half-sibling mating (Figure S2) and we suspected that the sire, which has wild parents, is potentially already inbred within the Gir Forest Wildlife Sanctuary border due to long-lasting and drastic population reduction driven by predation by humans (Joslin, 1973). When the sanctuary was built in the 1972, there were about 200 individuals in Gir Forest (Singh et Gibson, 2011), which has increased 10 folds since the 1930s (Caldwell, K. (1938) in Wildt et al., 1987). Some of these individuals were 3 generations apart from ‘Atul’. Recent study has pointed that canine distemper virus has killed many Asiatic lions in the Gir Wildlife Sanctuary and this virus was not observed in other carnivores (Mourya et al., 2019). The high inbreeding based on the long tracts of ROHs may have increased the disease susceptibility among these individuals (Ku et al., 2011; Szpiech et al., 2013) and comparing the selection pressure on traits associated with these ROHs regions across other carnivores could provide insights to mitigate this problem in future conservation efforts.

Additionally, the disagreement of our results compared to those reported in Atkinson et al. (2018) might be explained by the sample type used in that study. Although they applied identical microsatellite markers, Singh et al. (2002) and Gaur et al. (2006) collected blood samples from the wild populations whereas Atkinson et al. (2018) used tissues and bone samples from deceased individuals which might have led to the high failure rate and low repeatability of microsatellite amplification. Furthermore, the high genetic diversity estimates could also be an artefact towards selecting microsatellite loci that are most variable and easiest to amplify by PCR - an ascertainment bias that is usually introduced by the limited sampling size during marker development (Rogers and Jorde, 1996). Given the increased number of wild Asiatic lions in the Gir Forest, a detail inspection using more genomic samples would be able to ascer-
tain this. The level of heterozygosity in the captive populations indicates that the breeding strategy worked well. However there needs to be more studies to identify whether the long ROHs inherited by recent common ancestors could carry disease variants detrimental to these cheetahs and Asiatic lions (e.g. Sams et Boyko, 2019).

**Demographic History Inferences**

Despite the differences in heterozygosity and ROHs between the individuals sampled in this study, the trajectories of past \( N_e \) revealed by PSMC did not show substantial difference. This confirms that the differences in genomic diversity associated with severe inbreeding between most of the individuals sampled in this study are due to a recent impact less than 10 kya. However, such recent and drastic population reduction can also bias the signals of the past \( N_e \) changes through deeper times (Nadachowska-Brzyska et al., 2015). We found that the shifts in \( N_e \) for both species can be associated to some major events during the mid-through the end of Pleistocene (Figure 4-5). The earlier reduction in \( N_e \) near 60 kya coincides with an increase in extinction rates in mammals, which has been suggested to be partially correlated with the earliest human dispersal events that spread out of Africa into new continents (Malhi et al., 2016; Andermann et al., 2020). Similar declines during this period have been observed in lions (Armstrong et al., 2020; Manuel et al., 2020), leopards (Paijmans et al., 2021; Pečnerová et al., 2021) and lynx (Abascal et al., 2016) as well as in other large-bodied herbivores (Chen et al., 2019). We showed that this reduction was more considerable in Asiatic lions than in cheetahs. Such differences may be due to more extreme climatic changes in the Northern Hemisphere or due to the longer human coexistence with wild animals in Africa than it is elsewhere (Daujeard et al., 2016; Faurby et al., 2020; Treves and Palmqvist, 2007).

\( N_e \) shifts using PSMC can also be related to changes in population structure (Mazet et al., 2016). Previously, Dobrynin et al. (2015) showed a gradual reduction of \( N_e \) without any evidence for a sharp bottleneck based on the PSMC analysis. In this study, we observed two contractions in the past that coincides with the founder population in Africa and a population split between Eastern and Southern cheetahs as inferred using another model of demographic history inference (i.e. \( \delta \alpha \delta \iota \)) (Dobrynin et al., 2015). The most ancient \( N_e \) in cheetahs showed high variance in \( N_e \) among bootstraps replicates which could reflect the large population size and admixture of cheetahs.
Comparative genomic studies among captive and wild individuals

across continents before the founder event leading to the sole living populations in Africa. This high variance in ancient $N_e$ was not observed in the Asiatic lions probably because our samples had a recent common ancestor in the 1970s (Figure S2). The reduction of $N_e$ in Asiatic lions between 30 and 50 kya observed in our analyses could also reflect the emergence of population structure where it coincides with multiple population splits during this time as approximated using various lion populations (Manuel et al., 2020). Bertola et al. (2016) estimated mitochondrial divergence between Central Africa and India to be older, 112.8 kya but with a large confidence interval (48 to 189 ky 95% highest posterior density). The matrilineal populations may have founded the population in India passing through multiple bottlenecks and these population admixture inferred by Manuel et al. (2020) could be an indication of multiple migration waves from North Africa or Europe towards some already established populations in Asia or vice versa. We could not point accurately when their $N_e$ become identical, which is a signal of population divergence, with confidence as a more ancient $N_e$ in Asiatic lions has been potentially obscured in our analysis due to the low density of heterozygous positions in the genome compared to the African lion. We observed a unique bottleneck event in the Asiatic lions, for which the $N_e$ had a modest recovery after the peak of LGM. Similar scenarios have been revealed in lynx suggesting complete isolation between the Asian and European lynx (Lucena Perez et al., 2020). During this period, the changes in temperature and precipitation after LGM had led to the forest biome’s expansion in the temperate regions towards the equator (Prentice et Jolly, 2000). This scenario could potentially favour the lions to recolonise or expand near South Asia. Additionally, given that the $N_e$ in cheetahs were lower than the Asiatic lions around 10 kya after the LGM, this suggests that the lions in Asia in which they have more reduced contemporary genomic diversity and drastic $N_e$ changes in the past, are more vulnerable to environmental variability than the cheetahs as well as the lions in Africa. Female Asiatic lions require between 32 and 50 km$^2$ (Jhala et al., 2009) whereas cheetahs have a larger home range, ranging between 170 and 306 km$^2$ (Broomhall et al., 2003; Houser et al., 2009). Furthermore, these two species differ in their habitat preferences. The core home range of cheetahs is usually situated in open savannah habitat (Broomhall et al., 2003), similar to African lions, whereas Asiatic lions have been observed more frequently in dense forests (Jhala et al., 2009). During the early Pleistocene, the expansion of savannahs in Eurasia and North of Africa might
Comparative genomic studies among captive and wild individuals

have benefited the cheetah and African lion populations to differentiate and steadily expand whereas the forest fragmentation has likely limited the Asiatic lion’s range expansion and dispersal. Increased sample size and comparisons between Asian and African cheetahs are necessary to investigate whether the patterns of population split and recolonisation observed between Asiatic and African populations are general to both and other felid species.

Influence of Reference Genome

Inaccurate mapping leading to false call of heterozygote sites in the targeted genome can be caused by the reference genome quality and/or phylogenetic distance (Prasad et al., 2021; Valiente-Mullor et al., 2021). Overall, our results support that the choice of the reference genome had a lower impact on the PSMC inference in cheetahs whom have lower phylogenetic distance to the domestic cat compared to the Asiatic lion. Furthermore, the genome-wide heterozygosity of Asiatic lions estimated using Felcat9 is significantly higher than the heterozygosity inferred using the conspecific reference genome, similarly to a previous study (Armstrong et al., 2020). Conversely, the choice of the reference genome in the ROH analyses has a more pronounced impact in the cheetahs (Figure 5 and Table S1), which indicates the genome quality could also be the potential cause as the aciJub1 assembly is more fragmented than the Pan-Leo1 based on the number of contigs (197,914 vs 22,589 in Table 2). Prasad et al. (2021) demonstrated that the average length of ROH and segments in ROH were lower and can be undetectable when mapped to the closest relative whereas our analyses in cheetahs showed a contrast pattern. The average length of ROH using aciJub1 was about 6 times greater than when using Felcat9. More analyses are required to investigate the pattern of ROH using LynCan4, which has higher contiguity and lower number of contigs. Additionally, mapping the Asiatic lion sequences onto recently published tiger genome assembly (Pti1_mat1.1, Table 2) which is more contiguous than PanLeo1, may help to disentangle the effect of spurious heterozygous calls due to phylogenetic distance. We could not examine whether the genome quality or phylogenetic distance has attributed to this discordance in lions, as the genome assembly was not available at the time of the preliminary analyses. We anticipate that the recent addition of other chromosome-level assembly genomes in Felidae will probably facilitate the characterisation of the confounding effects in cross-species alignment on the reference genome.
Conclusion

Our study confirms that comparing the heterozygosity, inbreeding and trajectory of $N_e$ between species could also determine the relative influence of Pleistocene climate change over the past 10,000 years ago and the recent captivity have on the contemporary genomic diversity of individuals. Supplementing captive stocks with wild individuals as in the case of the cheetahs can be beneficial to reduce the genetic consequences of small founder populations, however given the threatened status of both species, this should not be encouraged. Furthermore, despite the higher heterozygosity than in the wild individual, captive breeding with close-relative matings may lead to the rise of long tracts of homozygosity which were not detected previously using microsatellite markers. We recommend further assessing the genomic structure of the potential mates in captive populations before attempting the reproduction to avoid the risk of increase susceptibility to disease. The past decline in $N_e$ near 60 kya was consistent with the decline in other carnivores and the elevated rate of mammalian extinction. Since then, the cheetahs showed a gradual reduction in $N_e$ after this event whereas the Asiatic lions had recovered slowly after the LGM. Our comparative demography suggests that the magnitude of past $N_e$ shifts also depends on carnivores’ ecological features and deserve further investigation.

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Authors contribution

A.A. conceived the study, analysed and interpreted the data, took the lead in writing the manuscript. B.C. and L.V. obtained the blood samples, C.M-M. conducted the laboratory work, E.G. carried out the genomic sequencing, K.B. developed the bioinformatic pipeline, E.P. supervised the project and the methodological aspect. All authors contributed to the final version of the manuscript.
In dedication to the Asiatic lions and South African cheetahs in Montpellier Zoo (Photo taken by A.A.)
A Genetic Tale of Cats

General discussion

The question of how carnivores have responded, and will respond, to human activities is among the most intriguing in the field of conservation and evolutionary biology. The evolutionary history of felids is fascinating due to its recent and rapid adaptive radiation, despite their specialisation to hypercarnivores having been perceived as ‘an evolutionary dead end’ (Holliday and Steppan, 2004), and their geographic ranges being the most reduced among all carnivores (Middleton et al., 2020). However, not all felids respond equally and have followed the same evolutionary trajectories as a result of climate change and human activities. This makes the cat family an important model to gain insight into how species interact with their complex environment.

Considering the scale of progress in genetic studies of felids and their congeners in wild, captive and extinct populations, answering this question across an eco-evolutionary scale that is integrating both ancient and recent times, above and below species levels, on a global scale, is almost approachable. However, the genetic resources presently available are not sufficient, especially from Southeast Asia where most of them live and are under serious threat (Cardillo et al., 2004; Miraldo et al., 2016; Sodhi et al., 2004; Wolf and Ripple, 2017).

This thesis constitutes an important step towards making this possible in the near future. I have emphasised where and which felids and Southeast Asian carnivorans should be prioritised for population genetic study and several other related areas. This contributed to a necessary database of microsatellite markers across Felidae to provide the first study of genetic diversity among terrestrial (inland) carnivores on a global scale. Finally, I have examined whether genomic data of threatened felids kept in captive populations for generations could be informative to distinguish past and recent demographic changes as a useful benchmark for future comparative studies. Here, I further elaborate the major findings in my thesis, and some considerations for future research.
A Genetic Tale of Cats

Genetic Diversity in Tropical Regions is Higher but not Frequency of Studies

Tropical regions harbour rich biodiversity and a great amount of the evolutionary history of carnivores (Sechrest et al., 2002; Rolland et al., 2015). Unfortunately, these regions are still underrepresented in species population genetics and phylogeography (Beheregaray, 2008). Further evolutionary studies in this region have the potential to provide new and efficient ways for genetic sampling from the field and museums in the tropics, which can be applied to understand how carnivores have fairied with ancient humans and during the megafauna extinction, how carnivores tolerate urbanisation and the unprecedented rate of anthropogenic impacts, and the timing and effect of human-mediated dispersal and post-introduction hybridisation (Chapter I).

Such studies are still limited in number and comparative breadth in the Southern Hemisphere and developing countries. The reasons being partly due to technological challenges (Beheregaray, 2008), difficulty to access diverse biomes such as the dense tropical rain forest for data sampling (Hughes et al., 2021), and lack of conservation investments (Dickman et al., 2015). Absence of such data could hinder our knowledge about the genetic diversity patterns and speciation across latitudinal gradients, as well as the effect of past human impact on current patterns of genetic diversity in regions where humans have coexisted with nature longer than elsewhere (Leigh et al., 2021; Millette et al., 2020; Otto, 2018).

Other difficulties I encountered while building the database and literature review were: the lack of support for sharing data publicly, incomplete information about the sampled individuals who are particularly important to replicate the study, or that the collected data were withheld in the prospect of gaining more data for other analyses. This is universally acknowledged as a major problem in research and a primary threat to progressive conservation studies. Nonetheless, there is a legitimate interest underway for data sharing seeing that there is a range of initiatives being developed to facilitate exchanges in biodiversity information in Southeast Asia (e.g. Table I.3). As more fine-scale data of carnivores are collected in this region, it is likely that patterns of human-carnivore coexistence, both in the past and today, will begin to emerge, which is essential for effective mitigation in human-wildlife conflict and more resilient ecological networks (Carter et al., 2012; Nyhus, 2016).
A Genetic Tale of Cats

The Unexpected Trend of Life History Traits with Genetic Variation

Generation time determines the pace of key demographic and evolutionary processes. Small mammals tend to have more generations between the present and any past bottleneck events, resulting in higher mutation and recombination rates over a given time, thereby accumulating a lot of genetic variation compared to large mammals (Gaillard et al., 2005; Martin and Palumbi, 1993). This hypothesis appears to be supported by a large-scale comparative study using contemporary transcriptomic data at a higher level of taxonomic groups with life-history traits in the form of parental investment, that is the ratio of propagule size to adult size as a proxy to r- or K-strategists (Romiguier et al., 2014). However, different patterns emerged on a lower taxonomic scale and seemingly contradicted the theory. Neither breeding season length nor generation time were associated with allelic richness across pinnipeds (Stoffel et al., 2018). In the case of Felidae, I have revealed critical data and theory gaps, which deserve additional investigation as it can impede the conservation of carnivores when hinge on the assertion that large species with slow generation length are more vulnerable.

In Chapter II, I found that felids with fast generation time have reduced genetic variation compared to their counterparts. This positive relationship is in strong accordance with a previous study on terrestrial mammals, in which species with shorter generation times showed stronger increases in the inbreeding coefficient, while species with longer generation times showed weaker effects of fragmentation in the inbreeding coefficient (Rivera Ortíz et al., 2015). Furthermore, it has been generally assumed that large species are more vulnerable to loss of genetic variation (e.g. Frankham, 1996; Lino et al., 2019; Romiguier et al., 2014) and that smaller species are more resilient to the disturbances (Middleton et al., 2020). However, although body mass is related to generation length, it came as a surprise that this former variable was not an important predictor in explaining the genetic variation across felids. In all likelihood, this lack of correlation could be caused by the artefacts in sampling bias (i.e., towards large-bodied felids) in our data. Nonetheless our results are in accordance with previous studies which focus on taxa across mammals (Doyle et al., 2015; Johnson, 2002; Nabholz et al., 2008) and within the cetacean (Vachon et al., 2018). Our results are timely, since felid body mass has also failed to predict their effectiveness as umbrella
A Genetic Tale of Cats

species for conservation purposes (Dickman et al., 2015). Additionally, our study reinforces the view that taxon-specific comparisons with similar mutation rate would be more informative to clarify to what extent species life-history traits have influences on $Ne$ (Ellegren and Galtier, 2016).

The relationship between the population genetic structure and generation length requires further study conducted in a natural environment. It can be hypothesised that there is considerably shorter delay between the bottleneck events and negative genetic outcomes in cases where the population recovery is not permissible in species with fast generation length (Hailer et al., 2006). Another hypothesis related to this, is that population growth in felids with fast generation time is accompanied by an evolutionary trend towards inbreeding tolerance, as the benefit of tolerance through kin recognition can exceed those of inbreeding avoidance (Waser et al., 1986). This could be the case when there is limited dispersal opportunity (Dharmarajan et al., 2009; Keane et al., 1996). Clearly, these hypotheses deserve to be tested where the mating opportunities (i.e. dispersal) and population density are influenced by anthropogenic disturbances, such as in tropical landscapes along a gradient of forest growth type (i.e. old-growth, secondary growth, up to recently fragmented)(e.g. Wearn et al., 2017), or with different habitat-patch size (Dharmarajan et al., 2009). In those systems it is possible to examine whether the degree of genetic differentiation, relatedness, and fitness in species with short generation time occupying different sites, is in contrast to another sympatric carnivore with longer generation time.
A Genetic Tale of Cats

**Back to Basic: From Population size to Population density?**

Our current knowledge on the relationship between population density and genetic variation across carnivores is at best fragmentary. In chapter III, I showed that the trend across species is as expected by theoretical predictions. However, a generalisation of this positive correlation within species is still unclear and both of these variables could indeed be affected by environmental heterogeneity which we did not account for. Specifically, whether felids have higher density in the temperate and northern latitudes following previous study on mammals (Santini et al., 2018b), and if so, whether this is concurrent with higher genetic diversity. Another explanation pertaining to the lack of significant effects in other species could be mediated by varying degrees of social structure in different populations related to population density.

While most felids are solitary, it has been suggested that the maintenance of genetic variability across populations may not differ from other social species of carnivores (Schmidt et al., 2016). Furthermore, socially related features, such as the sex-ratio variation in population density, tend to vary strongly with the spatial distribution of habitat productivity, which in turn affects home-range size (Gittleman and Harvey, 1982; Lindstedt et al., 1986). High population density could also be where dense social groups are formed (Smallwood and Schonewald, 1996), and in the case of felids, large areas are associated with an increase in female density (Anile et Devillard, 2018). Thereby, considering species population size only for species extinction risk assessment, could potentially mask the relative importance of ecological mechanisms that contributes to determining genetic diversity. The database built during this thesis holds much promise to uncover the eco-evolutionary dynamics of carnivores and their responses to environmental changes, by gradually incorporating these factors, such as: the intraspecific variation of home-range size, sex ratio, and ecological attributes (e.g. human disturbance, prey abundance, latitudinal gradient, forest growth type, and distance to urbanisation).
A Genetic Tale of Cats

Stuck in a Captive loop

Genomic data from captive populations can give insights into which breeding strategies need to be improved to avoid genetic adaptation to captivity and are more suitable to be implemented into wildlife management (Willoughby et al., 2017; Willoughby and Christie, 2019; Wright et al., 2020). In chapter IV, I examined whether the genomic variation between captive and wild populations has the same pattern as observed using microsatellite markers in two species with contrasting breeding management histories. I found that the level of heterozygosity has indeed increased in captive populations for both species. This is unexpected for Asiatic lions as their founder population size was very limited. We cannot exclude the probability that our sampling size may be limited in bringing these findings to a conclusion, but the sheer size of genomic data obtained from captive individuals has enabled us to gain more accurate information on the consequence of inbreeding and the choice of the reference genome.

The occurrence of long ROHs has also been expected to have greater influence on directional selection and could increase deleterious variants (Szpiech et al., 2013). These animals have evolved in an artificial environment under the auspice of humans and may not be able to adapt to the wild (Hunter et al., 2013). The recent World Wildlife Crime Report (2020) highlighted that most of the seizures and trophy hunting of big cats involved captive-sourced animals. Unless funding is sufficient to conserve species in their natural distribution, which is not always the case in less developed countries of the tropics, the transferral of these species to zoos or releasing them back to the wild after captivity for genetic rescue or assisted migration, could represent a significant setback by exposing individuals to unnatural varieties of pathogens in areas where they are not naturally observed. The next step would be to investigate the empirical association between ROHs and putative harmful variants in this two species, which would give detailed insights into the consequences that an artificial founder population has on their genomic structure and evolution.
The (small) Rise and (big) Fall of Pleistocene Carnivores

During the Pleistocene, large-sized mammals or megafauna were ubiquitous and the early Palaeolithic humans (*Homo*) began to disperse into new habitats. Along the way humans had to face unforgiving choices for survival: to avoid, to coexist, or to confront terrestrial mammalian carnivores. The dietary preference of carnivores has likely influenced migration routes among early humans that avoided certain areas, or developed cooperative groups to increase vigilance where encountering carnivores was likely (Carotenuto et al., 2016; Treves and Palmqvist, 2007). Another scenario is that some humans may have chosen to opportunistically scavenge carcasses left behind by carnivores, as the procured protein-rich meat can be beneficial to promote human expansion and to supply additional strength for a long-distance dispersal out of the African tropics and beyond (Turner, 1992). As carnivores are pivotal top-down regulators of the ecosystem, the widespread demise of Pleistocene megafauna could have potentially changed the species assemblage and diversity of other species with functionally similar clades (Smith et al., 2016). Survived carnivores would also have had the opportunity to expand their geographic range into habitats left behind by extinct sister taxa (Waters et al., 2013).

In chapter II I revealed that the pattern of genetic variation assessed with microsatellite markers in extant felids was consistent with the consequences of large-scale changes in global climate and human expansion during the Pleistocene, and not of the recent human population density. However, this remains speculative based on the observation that the Northern Hemisphere (where extensive ice sheets largely covered the region) has significantly lower genetic diversity than Africa (where humans originated) and South America (where modern humans arrived most recently (Eriksson et al., 2012)). Additionally, the high degree of genetic diversity in South America found in our study was unexpected, as it had been suggested that the severity of carnivore and other megafauna extinction in the late Pleistocene in this region is as substantial as in North America (Lyons et al., 2004). According to one extensive review, there is no clear pattern of genetic diversity related to latitudinal gradients in America (Lawrence and Fraser, 2020). The only avenue towards verifying these hypotheses and elucidating the primary driver of these distinctive patterns of genetic diversity would depend on the availability of raw genotype data of felids, which are currently inadequate for comparative study. With more samples from different sampling
A Genetic Tale of Cats

time, we can hopefully elucidate the last signs in the genetic structure of species before they went extinct, or the early signs of population expansion due to domestication for example. Another possibility to delineate the pace and origins of specific events linked to anthropogenic impacts and climate change in the Pleistocene (Hecht et al., 2020), is to obtain high-quality DNA data from captive animals - an undervalued resource for comparative demography. In chapter IV I showed that the demographic inference based on PSMC between contemporary wild and captive individuals yielded similar results, despite the significant differences in genome-wide heterozygosity and the in-breeding coefficient. If such a case is applicable to other carnivores, sampling from captive populations would be safer, quicker and more feasible for comparative demography studies, and would help quantify the extinction risk in threatened species without harming those in the wild (Minteer et al., 2014). Such knowledge has important conservation implication, as we are heading towards future climate scenarios which are possibly similar to that in the paleoclimate context in terms of the level of CO₂ emission and the associated warmer climate (Tierney et al. 2020). Understanding how the ancestors of these species have survived in the past as well as the evolutionary genomics of species’ responses to climate change, can hopefully help to project the fate of species and conserve the future of biodiversity efficiently.
A Genetic Tale of Cats

Epilogue

To develop accurate predictions about the future status of carnivores is an urgent matter, given that animals are now facing even harder challenges. The combination of genome sequences from living felids and their extinct relatives can provide a myriad of possibilities that will enhance the knowledge about the evolutionary forces that expand, maintain, and deplete the variation within felids and other carnivores with famously low levels of genetic diversity, as well as to provide more reliable model to predict their future outcomes and enhance the conservation of species. Hopefully, this could bring us a profound lesson to our ‘predatory’ impacts on other species, overcome the human-nature dualism, or to alarming discoveries which perhaps could lead to a new Pandora’s box (or possibly even a new type of house cat). Whichever the case may be, understanding the importance of genetic diversity as the essence for maintaining adaptive capacity for all species must be the priority to promote a healthy ecosystem, one which is rich in biodiversity and therefore beneficial to all living things. Evolution will continue to push some species towards the brink of extinction and others away from it, with humans playing a continuously increasing role in the process. Therein lies greater responsibility as to how we interact and coexist with nature and how we should adapt our perspective on conservation to the current (and future) situation accordingly. Semantically conservation implies the maintaining of status, when it should rather be treated within the dynamic context of continuously evolving life. To paraphrase the late Theodosius Dobzhansky:

“nothing in conservation (biology) makes sense except in the light of evolution”
Résumé (version longue)

Introduction

Génétique des populations et génomique des espèces menacées

Tous les êtres reproducteurs sont soumis à une évolution dirigée par la sélection naturelle, depuis l’organisme entier jusqu’au niveau cellulaire, avec la variation génétique comme le schéma directive. Les espèces qui s’adaptent à l’environnement ont la possibilité de survivre et de se reproduire. La meilleure chance de poursuivre un héritage viable est de trouver des partenaires potentiels dans une grande population possédant un ensemble unique de matériel génétique. Tout pool génétique, défini ici comme la variation génétique totale présente au sein d’une population croisée d’une espèce particulière, peut fluctuer en taille grâce à l’équilibre entre la mutation, la migration et les processus aléatoires appelés "dérive génétique". Cette dernière a des conséquences importantes dans une petite population. Le rythme et la direction de la spéciation et de l’extinction sont régis par ces mécanismes au niveau génomique (Lynch et Walsh, 2007). La variation génétique mesurée au sein d’un individu ou d’une population à un moment donné est donc un instantané des forces évolutives en jeu tout au long de l’histoire de l’espèce.

La théorie, les expériences et les travaux de terrain menés dans le cadre des études de génétique des populations pour décrire l’effet des forces évolutives sur les divers modèles de la nature ont abouti à une théorie unifiée de l’évolution depuis la fondation du modèle d’hérédité des caractères de Gregor Mendel. Depuis lors, une grande diversité de méthodes statistiques a été développée pour effectuer l’estimation des paramètres et les tests d’hypothèse basés sur les modèles permanents et la structure de la variation génétique dans les populations sauvages. Ces méthodes ont également facilité et amélioré la recherche en biologie de la conservation dans le but général de maintenir la diversité génétique en tant qu’assurance de l’espèce contre les aléas futurs des phénomènes naturels (Frankham et al., 2002). Avec l’avènement de l’ère génomique, les mesures de la variation génétique peuvent être estimées avec une meilleure précision chez chaque individu, et sans nécessiter une grande taille d’échantillon, ce qui est le cas lorsqu’on étudie des espèces menacées qui sont difficiles à trouver sur le terrain ou déjà éteintes.
"Paradigme de la "petite population, génétiquement appauvrie"

Lorsque les méthodes de génétique des populations n'en étaient qu'à leurs débuts dans les études sur la conservation, le guépard (Acinonyx jubatus), le carnivore terrestre le plus rapide, était l'une des premières espèces dépourvues de ressources génétiques bien connues, ce qui souligne le rôle important de l'histoire de l'évolution dans la conservation des espèces (Frankham et al., 2002 ; O'Brien, 2003). Les premiers travaux traitant de la variation génétique chez les guépards ont été menés par le professeur Stephen O'Brien, éminent généticien, et son équipe de recherche. Après un appel alarmant du centre d'élevage en Afrique du Sud, où les guépards étaient très difficiles à reproduire en captivité, ils ont examiné la variation génétique chez ces individus (O'Brien et al., 1986, 1983). Aucun polymorphisme basé sur la variation des allozymes n'a été trouvé et les protéines détectées à l'aide d'une électrophorèse sur gel bidimensionnelle ont révélé une hétérozygotie plus faible que celle rapportée chez d'autres espèces dans cette étude. Étant donné le nombre de spermatozoïdes plus faible chez ces guépards que chez d'autres espèces, ils ont suggéré que la faible diversité génétique observée était à l'origine de la faible condition physique de ces individus (O'Brien, 2003). Les guépards dans leur habitat naturel semblent également avoir des spermatozoïdes structurellement anormaux similaires à ceux des guépards en captivité (Wildt et al., 1987b). De plus, les guépards présentent également des schémas très uniformes dans le complexe majeur d'histocompatibilité, un groupe de gènes qui sont liés au système immunitaire, ce qui les a probablement rendus extrêmement vulnérables à l'exposition et à la propagation de la péritonite infectieuse féline causée par un coronavirus (O'Brien et al., 1985).

O'Brien et al. (1986) ont également comparé la variation génétique du guépard à d'autres espèces de félinidés africains comme les léopards, les lions, les servals et les caracals en utilisant des loci allozymes supplémentaires. Ils ont constaté que la variation génétique du guépard est systématiquement inférieure à celle des autres espèces étudiées, et même à la variation génétique de l'homme. On pensait que l'extrême rareté de la variabilité génétique et un coefficient de consanguinité élevé chez les guépards par rapport aux autres félinidés et mammifères expliquaient pourquoi ils étaient sensibles aux maladies et portaient des spermatozoïdes très anormaux, autant de facteurs
A Genetic Tale of Cats

qui contribuent à la situation difficile de la reproduction en captivité. Avec un échantillonnage plus important, les lions asiatiques du zoo de Sakkarbaug en Inde ont montré un manque de polymorphisme sur l'ensemble des 50 loci allozymes testés et "produisent des ejaculats avec plus de formes de sperme pléiomorphes et moins de spermatozoïdes mobiles que même les lions de Crater dont la reproduction est compromise" (Wildt et al., 1987a). Les auteurs ont émis l'hypothèse que le manque de variation génétique neutre révélé dans leur étude pourrait être le résultat d'une contraction ancienne dans la population naturelle et non de la captivité.

L'analyse de la variation de l'ADN mitochondrial (ADNm) et des empreintes génétiques a permis Menotti-Raymond et O'Brien (1993) d'estimer le moment de cette contraction démographique. Ils estiment que le goulot d'étranglement s'est produit il y a environ 10 000 ans, ce qui suggère que la contraction démographique a coïncidé avec le dernier maximum glaciaire du Pléistocène. Ils affirment que les changements climatiques extrêmes ont poussé les populations de guépards dans des goulots d'étranglement génétiques répétés, ce qui les a amenés à être le seul représentant existant du genre. Ces interprétations semblent intuitives et ont des considérations importantes pour la conservation, mais certains les ont trouvées plutôt controversées. Par exemple, Merola (1994) a évalué cette hypothèse en comparant les variations observées chez les guépards à ceux d'autres carnivores. Elle a constaté que la faible variation génétique n’est pas propre aux guépards - certains carnivores présentent une variation plus faible que les guépards, et le groupe de carnivores terrestres présente des niveaux de polymorphisme encore plus faibles par rapport aux autres groupes de mammifères. O'Brien (1994) a critiqué cette étude, arguant que "les estimations d'une variation génétique plus faible chez huit espèces de carnivores proviennent d'études allozymes anciennes qui sont presque certainement inexactes car elles portent sur très peu de loci".

Driscoll et al. (2002) ont réexaminé les modèles génétiques des grands félinidés en utilisant 90 loci microsatellites et une approche plus robuste pour déduire l'histoire démographique. Ils ont révélé que les niveaux de variation génétique des loci allozymes et microsatellites étaient similaires, les lions asiatiques présentant la plus faible diversité génétique. Cependant, le résultat le plus frappant de cette étude est que, selon les marqueurs et la taille de l'échantillon, d'autres espèces au niveau de la population semblent présenter une diversité inférieure à celle du "modèle" guépard. Bien que le polymorphisme allozyme soit plus faible chez le guépard (2 à 4 %) que chez le puma
A Genetic Tale of Cats

d’Amérique du Nord (5 à 10 %), la diversité génétique du puma en microsatellites pré-
sentait des valeurs encore plus faibles (42,9 à 75 % contre 81 à 84 % chez le guépard) et n’était pas significativement différente de celle des lions d’Asie. Ils ont interprété les résultats de telle sorte que les trois espèces avaient subi un goulot d’étranglement génétique à des périodes et des endroits différents dans le passé. D’abord les guépards africains, puis plus récemment les pumas d’Amérique du Nord, et enfin les lions d’Asie, probablement à cause de la chasse excessive pratiquée par les humains il y a moins de 100 ans (Driscoll et al., 2002).

Il est clairement établi que la croissance inexorable des activités anthropiques ré-
centes, qui entraînent par exemple la fragmentation des habitats et la modification des paysages, a gravement compromis le patrimoine génétique des carnivores (voir par exemple Bull et Maron, 2016 ; Creel et al., 2019 ; DiBattista, 2008 ; Leigh et al., 2019 ; Lino et al., 2019 ; Miraldo et al., 2016 ; Rivera-Ortiz et al., 2015 ; Schlaepfer et al., 2018). Les diverses caractéristiques des carnivores en font un système-modèle intéressant, très pertinent pour approfondir le paradigme "petite population génétiquement appauvrie", à travers un cadre interdisciplinaire d’écologie et d’étude génétique évolu-
tive. La mise en évidence de la relation entre les schémas actuels et passés de la diversité génétique peut donc permettre de comprendre l’histoire de l’évolution des carni-
vores, les changements évolutifs induits par l’homme dans les espèces et de déterminer efficacement quels carnivores et leurs emplacements sont prioritaires en matière de conservation. Cependant, les études de génétique des populations sur les carnivores font encore défaut en Asie du Sud-Est, l’un des points chauds de l’histoire de l’évo-
lution des carnivores (Beheregay, 2008 ; Sechrest et al., 2002) et également une région extrêmement touchée par les activités humaines (Sodhi et al., 2004). Dans le chapitre I, j’ai rédigé une revue de notre connaissance sur l’impact anthropique sur la diversité génétique des carnivores dans cette région, abordé cette lacune et présenté quelques re-
commandations pour les travaux futurs.
A Genetic Tale of Cats

La variation génétique, une clé pour connaître la taille des populations passées ?

Traditionnellement, la surveillance des espèces à l'aide de techniques de capture vivante ou de pièges photographiques est appliquée pour recenser le nombre d'individus et estimer l'abondance au sein d'une population en fonction de leurs marques distinguées (Boitani et al., 2012 ; Karanth et al., 2010). La comparaison de l'abondance dans le temps, entre les espèces et dans différents endroits, peut fournir une explication des mécanismes de causalité dans la dynamique de la population. Cependant, les carnivores sont logistiquement plus difficiles à suivre que la plupart des autres animaux, car ils sont plus discrets, nocturnes, vivent dans des zones reculées inaccessibles aux chercheurs et existent à de faibles densités (Boitani et al., 2012). Obtenir des estimations fiables et précises de la dynamique ou de la fluctuation de leur population est une tâche difficile, car cela nécessiterait des enquêtes à long terme sur plusieurs années et dans de grandes zones avec des ressources logistiques considérables (Harmsen et al., 2017). En outre, il n'existe pas de données fiables ni standardisées sur l'abondance des espèces provenant de registres historiques pour estimer les tailles de population passées. Quelques rares rapports ont été trouvés principalement dans des comptes rendus d'expéditions de chasse menées par des rois et des nobles ou des chasseurs de primes à la fin du 19e et au début du 20e siècle, et sous-estiment probablement l'abondance réelle (Karanth et al., 2010).

Étant donné que chaque individu a sa propre composition génétique, il est également possible de distinguer les changements de la taille de la population au fil du temps en fonction de la diversité génétique permanente, en utilisant la taille effective de la population. Le concept de taille effective de la population, \( Ne \), a été introduit par Sewall Wright (1931) sur la base de son observation de la dérive génétique et de la consanguinité dans de petites populations réelles. Dans une petite population, la dérive génétique, c'est-à-dire la fluctuation aléatoire des allèles, conduisant à une fixation d'une génération à l'autre dans une population finie, devient importante et peut entraîner une grande perte de variation génétique. Avec Ronald A. Fisher et J.B.S. Haldane, ils ont généralisé mathématiquement le \( Ne \) s'appuyant sur le mécanisme mendélien de l'hérédité et l'évolution darwinienne pour tenir compte d'une variété d'hypothèses pratiques qui sont : la population est de taille constante avec un accouplement aléatoire, avec des générations discrètes, et sans migration ou flux de gènes, sélection ou mutation. Le \( Ne \) a donc été défini comme "le nombre d'individus reproducteurs dans une
population idéalisée qui présenterait le même degré de dispersion des fréquences allé-liques en cas de dérive génétique aléatoire ou le même degré de consanguinité que la population considérée". Des preuves empiriques tirées de données provenant de populations sauvages ont également vérifié la validité du lien entre le niveau de variation génétique et la variation de Ne entre les espèces (par exemple, Frankham, 1996 ; Hamrick et Godt, 1996 ; Gram et Sork, 1999). Reste à savoir si les traits des espèces (au chapitre II) et la densité de population provenant d'études par pièges photographiques (au chapitre III) peuvent être utilisés pour prédire le niveau de diversité génétique au sein d'une population.

Inférence démographique à partir de séquences à haut débit

La révolution technologique dans le séquençage de l'ADN depuis la découverte de Sanger et al. (1977) a offert des données puissantes et plus précises pour obtenir des informations sur la variation moléculaire, depuis une poignée de marqueurs co-dominants jusqu'au séquençage à haut débit d'un génome entier. Cette évolution a stimulé la recherche dans d'autres domaines de la génétique des populations, comme la modélisation de la démographie complexe des populations et l'évolution des séquences d'ADN en remontant dans le temps. Sur la base de la théorie de la coalescence telle que formulée mathématiquement par John Kingman (1982), la variation entre les séquences d'ADN attendue dans le cadre de la théorie neutre de l'évolution moléculaire peut être utilisée pour estimer la probabilité que deux lignées coalescent dans le passé, c'est-à-dire que deux séquences proviennent d'une seule séquence ancestrale ou de l'ancêtre le plus récent (Emerson et al., 2001 ; Wakeley, 2008). En d'autres termes, plus la variation au sein d'un locus est grande, plus la probabilité de choisir des ancêtres aléatoires par le biais d'un processus de coalescence dans les générations précédentes est faible (1 / Ne), ainsi la longueur de la branche vers l'ancêtre le plus récent tend à augmenter avec la taille de la population, alors que dans une petite population, leur ancêtre commun sera récent (c'est-à-dire des longueurs de branche seront plus courtes).

L'approche fondée sur la coalescence fournit un modèle simple et utile ayant de nombreuses applications, notamment l'évaluation de la taille d'une population variant dans le temps et ayant subi un processus historique, sur la base de la variation contemporaine des séquences d'ADN non recombinantes au sein d'une population, générale-
A Genetic Tale of Cats

tement supposée à l'équilibre mutation-dérive. C'est ainsi qu'est né un nouveau domaine de la génétique des populations : l'étude phylogéographique (Avise et al., 1987), qui examine les données empiriques pour déduire comment les grands changements géographiques et climatiques du passé ont inévitablement façonné la structure des populations des espèces. Les études phylogéographiques basées sur la comparaison entre les phylogroupes intraspécifiques et les distances génétiques peuvent permettre d'éclairer la spéciation récente des carnivores au Pléistocène, puisque 72 % de la divergence des populations de mammifères, selon une calibration standard de l'ADNmt, se situe dans les 2 derniers millions d'années (Avise et al., 1998). Cependant, la divergence de l'ADNmt ne recouvre que l'histoire matrilinéaire, de sorte que les prédictions basées sur la neutralité (c'est-à-dire la fixation aléatoire d'allèles variant en fonction de la taille de la population par rapport à la sélection) pourraient être biaisées (Bazin et al., 2006 ; Nabholz et al., 2008), et le temps déduit pourrait être entaché d'une large marge d'erreur (Lyholm et al., 1996 ; Stiller et al., 2014).

Lorsque la taille de l'échantillon est limitée à quelques individus pour des études comparatives, ce qui est le cas lors de l'étude d'espèces menacées, l'approche de coalescence séquentielle markovienne par paire (PSMC) développée par Li et Durbin (2011) serait plus appropriée, car elle nécessite au moins un seul individu diploïde. Cette méthode détecte la distribution des temps de coalescence en appliquant un modèle de Markov caché à des tronçons contigus de segments génomiques afin d'identifier la densité locale de sites polymorphes et le nombre d'événements de recombinaison méiotique, donc des séquences à haut débit assemblées à une contiguïté élevée seraient idéales (Li et Durbin, 2011 ; Mather et al., 2020). Des études antérieures ont démontré la robustesse de cette méthode pour l'analyse comparative de la variation de l'histoire démographique et des changements de structure de la population parmi les populations aviaires (Nadachowska-Brzyska et al., 2015), les parasites et leur hôte (Hecht et al., 2018), les serpents marins et terrestres (Ludington et Sanders, 2021), les espèces d'arbres forestiers (Patil et al., 2021) et les espèces de rhinocéros, y compris trois parents éteints (Liu et al., 2021). Une mise en garde s'impose : les changements dans la taille de la population dus au flux de gènes via la migration entre les populations ou les types de biais dans le choix du partenaire (par exemple, le système d'accouplement, la structure du groupe), pourraient créer des signaux confondants de
A Genetic Tale of Cats

contraction ou d'expansion de la population au fil du temps. Les résultats du PSMC nécessitent donc une interprétation prudente et un examen croisé de la structure de la population à partir d'autres analyses (Mather et al., 2020 ; Mazet et al., 2016, 2015).

L'approche PSMC fonctionne bien pour les périodes comprises entre 10 000 et 1 million d'années, mais a tendance à surestimer les tailles de population récentes et à répartir les changements soudains de taille de population sur plusieurs dizaines de milliers d'années précédentes (Li et Durbin, 2011 ; Mather et al., 2020). Une histoire démographique plus récente peut être déduite du niveau de consanguinité individuelle à partir des données du génome entier. Entre une paire de gamètes, certaines régions de son génome peuvent être une copie directe de l'ADN de leur ancêtre commun. Ces régions identiques par ascendance (IBD) ont été identifiées pour la première fois dans les familles de référence échantillonnées du Centre d'étude du polymorphisme humain, afin de construire une carte génétique du génome humain (Broman et Weber, 1999). Il a été reconnu par la suite que ces régions IBD sont communes et conservent un taux élevé d'homozgygotie même dans les populations consanguines, et peuvent donc fournir des informations sur l'histoire démographique des populations humaines (Gibson et al., 2006 ; McQuillan et al., 2008). Ces longs trajets homozygotes dans les régions IBD sont également connus sous le nom de runs of homozygosity, ROH.

Pour obtenir des données génomiques de haute qualité en termes de contiguïté et d'exhaustivité des espèces menacées et insaisissables, une quantité ample d'échantillons de sang ou de tissus est nécessaire et ceux-ci peuvent être facilement collectés sur des animaux détenus en captivité. De nombreux carnivores gravement menacés ont été sélectionnés comme espèces phares importantes pour sensibiliser, susciter un soutien et générer des fonds pour la conservation de la biodiversité (Macdonald et al., 2015 ; Consorte-McCrea et al., 2019). En outre, la recherche ex-situ réalisée sur les populations captives de carnivores peut avoir de grandes implications à la fois pour préserver les populations sauvages et pour améliorer la gestion de l'élevage avant la réintroduction. Étant donné que les populations captives sont constituées de plusieurs individus prélevés dans la nature, de nombreuses préoccupations ont été exprimées quant à l'adaptation génétique à la captivité en raison d'un phénomène connu sous le nom d'effet fondateur (figure 3) - une variation génétique réduite résultant du prélèvement de quelques individus issus d'une grande population sauvage pour établir la population
A Genetic Tale of Cats

captive (par exemple, Clubb et Mason, 2003 ; Frankham, 2008 ; Christie et al., 2012 ; Theodorou et Couvet, 2015 ; Grueber et al., 2017).

À l’aide de données génomiques, Willoughby et al. (2017 ; 2019) ont démontré que le nombre de SNP non neutres a augmenté au fil du temps en captivité chez de multiples espèces ayant des programmes d'élevage en captivité de longue date. Cependant, le niveau de variation génétique dépend étroitement de la stratégie de gestion (Willoughby et al., 2017 ; Humble et al., 2020). Certaines espèces ont également présenté des changements dans leur réponse comportementale en raison des conditions de captivité. Par exemple, les diables de Tasmanie montrent une vulnérabilité accrue à la mort par véhicules motorisés avec l'augmentation des générations en captivité (Grueber et al., 2017). Un autre aspect de l'environnement captif que nous comprenons mal est de savoir si la dépression de consanguinité a eu un effet négatif sur la structure et la fonction du génome dans la population captive. Il semble clair que la dépression consanguine peut augmenter dans les environnements stressants (Armbruster et Reed, 2005) et qu'une telle interaction est moins susceptible de se produire dans la nature, probablement en raison d'une purge génétique plus efficace (Pemberton et al., 2017). Les carnivores détenus en captivité offrent des points de vue intéressants sur le Ne de base et la variation génomique imposés par l'effet fondateur plus récent, ainsi que sur les conséquences de l'altération environnementale dans le passé sur la variation estimée du Ne. Ce dernier est l'un des objectifs que j'aborde au chapitre IV.
Objectifs de cette thèse

Au cours des trois dernières années, j'ai concentré mes recherches sur une meilleure compréhension de la façon dont les humains ont interféré dans l'évolution des carnivores existants depuis le Quaternaire en reliant les processus écologiques à l'échelle locale et globale.

Afin de répondre à plusieurs questions soulignées dans l'introduction, mes travaux de recherche ont commencé par une revue des études de génétique des populations de carnivores d'Asie du Sud-Est (chapitre I). L'idée de cette revue est née en partie de mon intérêt premier pour l'acquisition d'une connaissance approfondie du rôle de la génétique dans la biologie de la conservation et pour la réalisation de travaux de terrain dans cette région qui me tient à cœur. Dans cette revue, j'ai rassemblé et synthétisé les connaissances actuelles sur la diversité génétique des carnivores en Asie du Sud-Est, j'ai cherché des preuves de la faible diversité génétique causée par les activités humaines, et j'ai mis en évidence certaines idées pour des études futures. Cette étude a également été rédigée en tant que chapitre de contribution pour un livre intitulé "On the edge of sixth extinction in biodiversity hotspots in Southeast Asia" édité par Julien Claude (UM). Avec ses collègues de Thaïlande et des pays voisins, nous avons présenté l'état des connaissances sur les actions en cours, les besoins et les opportunités pour les études de biodiversité. Ce livre vise à informer les décideurs locaux et les communautés scientifiques régionales pour une gestion plus efficace de la biodiversité en cette période critique. Notre compréhension actuelle de la diversité génétique des carnivores dans cette région est encore faible, ce qui est probablement dû au résultat combiné de la disparition des espèces et du manque de financement de la recherche, deux facteurs cruciaux pour générer des données génétiques fiables.

Étant donné que l'impact de l'homme sur les carnivores à l'échelle mondiale s'est surtout concentré sur les grandes espèces, je me suis demandé si les petits carnivores avaient été touchés de la même manière. Pour ce faire, j'ai établi une base de données des études de génétique des populations à travers des espèces de la famille Felidae. Cette base de données a également été construite pour vérifier comment d'autres caractéristiques des espèces, y compris les traits de l'histoire de vie, la densité actuelle de la population humaine et les régions géographiques peuvent affecter le niveau de diversité génétique des félinidés dérivé des marqueurs microsatellites neutres. Cette partie
A Genetic Tale of Cats

de ma thèse - le chapitre II - a été publiée dans le journal Biodiversity and Conservation.

Ma base de données a donné lieu à une collaboration interdisciplinaire avec Stefano Anile et Sébastien Devillard afin de tester l'une des hypothèses les plus importantes (et les plus contestées) en biologie évolutive (ou comme certains l'ont appelé : le dogme central de la génétique de la conservation), en utilisant la densité de population comme substitut de la taille de la population. Nous avons mis au point une méthode permettant de combiner des études indépendantes sur la génétique et la densité des populations à partir des localités de collecte de notre base de données, de sorte que nous sommes en mesure d'examiner le modèle à l'échelle locale. Le chapitre III a atteint le stade d'un manuscrit.

En construisant ma base de données, j'ai réalisé que les données de génotype microsatellite et les séquences mitogénomiques utilisées pour estimer la diversité génétique dans les études publiées, ne sont pas toujours disponibles publiquement ou en bonne résolution (voir Annexe 2). Cette insuffisance de données a entravé mon travail d'estimation d'autres mesures de diversité génétique et d'inférence de l'histoire démographique passée dérivée des données moléculaires suivant une approche standardisée pour une étude comparative entre plusieurs félidés. Heureusement, il y a eu une forte augmentation des séquences de génomes entiers de félidés depuis que j'ai commencé mon doctorat, et cela a suscité mon intérêt pour m'impliquer davantage. Pour acquérir une expérience de première main dans les études génomiques, j'ai initié une collaboration avec le zoo de Montpellier pour acquérir des échantillons afin de générer des données moléculaires d'espèces de félin, que j'ai utilisées pour étudier si la diversité génomique des populations captives a été compromise par l'intervention humaine. En combinant mes données nouvellement générées avec celles disponibles en ligne, cet ensemble de données permet d'examiner les incertitudes liées au génome de référence et aux variants SNV, et d'avoir une confiance suffisante sur l'histoire démographique reconstruite, en prévision d'un ensemble complet de données pour la génomique comparative chez les Felidae. Étant donné que je n'ai réussi à acquérir le volume considérable de données qu'au début de l'année, je n'ai pas été en mesure de terminer cette étude jusqu'à présent et il y a beaucoup d'autres analyses génomiques que j'aimerais poursuivre. Néanmoins, certains résultats prometteurs de mes analyses préliminaires sont présentés au chapitre IV.
A Genetic Tale of Cats

Résumé de chapitre I

Érosion génétique dans le hotspot évolutif des carnivores ? :

Les tendances et opportunités de la recherche sur la génétique des populations des carnivores en Asie du Sud-Est

L'Asie du Sud-Est (ASE) possèdent une diversité de carnivores très riche. La plupart de ces espèces bénéficiant d’un fort intérêt de la part du grand public, tout en ayant un un rôle important dans les services écosystémiques. Cependant, elles sont aussi potentiellement menacées d'érosion génétique en raison d'une destruction de leur habitat et d'une surexploitation d'une ampleur sans précédent dans cette région en développement rapide. La compréhension des différences génétiques au sein d'une population fournit des informations précieuses sur la manière dont ils se sont adaptés et s'adapteront à un environnement changeant. En dépit de leur statut hautement menacé et des efforts croissants en matière de conservation, la diversité génétique intraspecifique – un facteur fondamentale pour le maintien de la biodiversité – est peu étudiée. Ce chapitre propose une vue d'ensemble des connaissances et des lacunes actuelles dans les études de génétique des populations sur les carnivores en ASE. Il fournit aussi des pistes pour améliorer les politiques de conservation et futures recherches.

Résultats principaux

Bien que des progrès considérables aient été réalisés en ce qui concerne la diversité génétique intraspecifique des carnivores, on ne sait toujours pas si les populations d'espèces menacées et endémiques de l'ASE sont affectées par les récentes menaces d'origine humaine. La recherche bibliographique a permis d'identifier 42 articles publiés au niveau international. Bien que le nombre d'articles ait augmenté considérablement depuis 1996, le nombre d'espèces identifiées à partir de ces articles reste faible (34 sur 61 espèce en ASE, soit 44 %). La variabilité et la structure génétique au niveau des populations n'ont pas été étudiées chez 43 espèces (72%). Il y a un certain biais en faveur de la famille des félins (Felidae) qui ne représente que 19% de tous les carnivores de l'ASE. L'impact de l'expansion des activités humaines sur la variation et la structure génétique des populations n'a été abordé que dans deux études, à savoir sur les tigres de Sumatra et les ours malais au Cambodge.
Résumé de chapitre II

Les patrons et les facteurs de la variation de diversité génétique chez les espèces de Felidae

Depuis la fin du Pléistocène, les espèces de l’ordre des carnivores ont connu une réduction drastique de leurs populations et de leurs aires de distributions. Cependant, aucune étude à une échelle globale n’a encore été réalisé sur la diversité génétique de cet ordre. En utilisant plusieurs espèces de félins comme modèle, nous avons tout d’abord caractérisé la variation génétique au sein des populations, puis comparé ces mesures entre les continents, entre les régions tropicales et non-tropicales, et en fonction des relations des espèces avec les activités humaines. Enfin, nous avons évalué comment les caractéristiques de ces espèces influencent les patrons de diversité génétique interspécifique. Pour cela, nous avons utilisé les données recueillies dans le cadre de 135 études de génétique des populations menées sur 28 espèces de félidés sauvages (70 % des espèces décrites). Nos résultats permettent d’identifier les espèces de félidés les plus vulnérables à la perte de diversité génétique pour de futures études de conservation, et donnent un aperçu des conséquences génétiques possibles des extinctions de la fin du Pléistocène.

Résultats principaux

Ces données analysés grâce à des modèles linéaires mixtes, ont montré que les populations d’Afrique et d’Amérique du Sud présentent une hétérozygote et une richesse allélique significativement plus élevées que celles des autres continents. Les lions d’Asie ont la plus faible diversité génétique par rapport aux autres félidés du monde. Comme prévu, les espèces menacées ont une diversité génétique significativement plus faible que les espèces à faible risque. Cependant, la diversité génétique n’était pas liée à la masse corporelle des espèces ou à leurs aires de répartition géographiques. Nous avons constaté que le temps de générations était une variable importante expliquant la variation de la diversité génétique, les espèces ayant un temps de génération court ayant une diversité génétique réduite. En outre, la combinaison du temps de génération, de l’habitat et de la taille du domaine vital permet de mieux expliquer la variation de l’hétérozygote. Les espèces vivant dans un habitat fermé et ayant un grand do-
A Genetic Tale of Cats

maine vital ont une hétérozygotie réduite par rapport aux espèces vivant dans un habitats ouvert et ayant une superficie de domaine vital réduite.

Résumé de chapitre III

La densité de population et la diversité génétique sont positivement corrélées chez les félinidés sauvages

L’hypothèse que la réduction de la diversité génétique est liée à la diminution de la taille de la population est l’une des les plus importantes hypothèses en biologie évolution. Les félinidés sont l’un des taxons les plus charismatiques et les plus sensibles aux mesures de conservation. Afin de tester cette hypothèse, nous avons recueilli des estimations de la densité de population pour 18 espèces de félinidés présentes sur cinq continents différents. Ces estimations proviennent d’études utilisant soit des piège-caméra soit des mesures génétiques basées sur des microsatellites

Résultats principaux:

Nous avons démontrés que nos données correspondent au modèle théorique – la variation génétique augmente avec la densité de population. Les félinidés ayant une faible densité de population et une durée de génération courte présentaient une diversité génétique réduite. La préservation de populations saines de félinidés est cruciale pour préserver la diversité génétique. Notre étude fournit donc une base afin d’identifier les populations de félinidés menacées qui ont le plus de risque de s’éteindre.
Résumé de chapitre IV

Études génomiques comparatives entre individus captifs et sauvages :
le cas des guépards africains et des lions asiatiques

Le guépard et le lion d'Asie sont deux espèces phares des programmes d'élevage européens ayant un historique de gestion bien différent. Si la gestion génétique des guépards en captivité a été considérée comme un succès, ce n'est pas le cas des lions d'Asie pour lesquels les accouplements consanguins issus des petites populations fondatrices n'ont pas pu être complètement évités en captivité. Les profils génétiques des descendants de ces populations fondatrices n'ont pas été suffisamment caractérisés dans le cadre du Programme Européen pour les Espèces menacées (EEP). Ici, nous avons utilisé les données de séquences du génome entier pour évaluer la variabilité génomique des individus de ces deux espèces hébergées au zoo de Montpellier, et les avons comparées aux données génomiques de congénères sauvages et de d'autres individus captifs disponibles.

Résultats principaux :

Nous avons constaté que les guépards captifs ont une hétérozygotie plus élevée que les individus sauvages. Cependant, la consanguinité mesurée par les séries d'homozygotie dans le génome augmente avec le nombre de générations en captivité. Étonnamment, les lions asiatiques du zoo de Montpellier ont une diversité génomique similaire à celle de l'individu conservé dans le sanctuaire de la forêt de Gir. Les lions asiatiques présentent aujourd'hui la plus faible diversité génétiques. Cependant, la taille effective estimée sur la base du génome de référence conspécifique pour la population de guépard d'il y a 10 000 ans était encore plus faible. Nous avons également montré que la distance phylogénétique entre les génomes de référence et les espèces ciblées peut avoir un impact significatif sur les mesures de l'hétérozygotie individuelle, sur le coefficient de consanguinité basé sur les segments d'identité par descendance et sur les inférences démographiques.
A Genetic Tale of Cats

Discussion et Conclusion Générale

La question de savoir comment les carnivores ont répondu et répondront aux activités humaines est l'une des plus importantes dans le domaine de la conservation et de la biologie de l'évolution. Les félinés ont présenté un rayonnement adaptatif au cours de leur histoire évolutive, mais ils sont aujourd'hui considérés comme une "impasse évolutive" du fait de leur spécialisation en tant qu'hypercarnivores (Holliday et Steppan, 2004), et leurs aires de répartition géographique réduites comparées à tous les autres carnivores (Middleton et al., 2020). Malgré cela, tous les félinés ne réagissent pas de la même manière et n'ont pas suivi les mêmes trajectoires face aux changements climatiques du Pléistocène et aux activités humaines. Ces caractéristiques font de cet modèle un modèle majeur pour mieux comprendre comment les espèces interagissent avec un environnement complexe.

Compte tenu de l'amplitude des progrès réalisés dans les études génétiques sur les félinés et leurs congénères dans les populations sauvages, captives et éteintes, il est aujourd'hui presque possible de répondre à cette question à une échelle mondiale, en intégrant à la fois le temps profond et le temps récent, et avec des niveaux taxonomiques infra- et supra- spécifiques. Cependant, les ressources génétiques ne sont pas suffisantes pour certaines zones, notamment en Asie du Sud-Est. Cette zone regroupe la plupart des espèces de félinés, dont beaucoup sont menacées (Miraldo et al., 2016 ; Sodhi et al., 2004 ; Cardillo et al., 2004 ; Wolf et Ripple, 2017).

Cette thèse constitue une étape importante pour comprendre comment les félinés ont été, et seront affecté par les activités humaines. J'ai souligné où et quels félinés et carnivores d'Asie du Sud-Est devraient être prioritaires pour l'étude génétique des populations et plusieurs autres domaines connexes. J'ai contribué à la création d'une base de données de marqueurs microsatellites pour les Félinés, qui m'a permis d'étudier la diversité génétique des carnivores terrestres (continentaux) à l'échelle mondiale. Enfin, j'ai examiné si les données génomiques de félinés menacés et maintenus en captivité depuis des générations, pouvaient être utiles pour distinguer les changements démographiques passés et récents et servir de référence utile pour de futures études comparatives.
Une diversité génétique plus élevée dans les régions tropicales mais un nombre d'études plus faible

Les régions tropicales abritent une biodiversité riche, ainsi qu’une grande partie de l’histoire évolutionne des carnivores (Sechrest et al., 2002 ; Rolland et al., 2015). Malheureusement, la génétique des populations et la phylogéographie sont en général mal connues pour les espèces vivant dans ces régions (Beheregay, 2008). Des études évolutive plus poussées ont le potentiel de fournir des moyens nouveaux et efficaces pour l’échantillonnage génétique, à la fois sur le terrain et dans les musées sous les tropiques. Ces nouvelles données peuvent être appliqués pour comprendre comment les carnivores ont été affecté par les humain dans les temps anciens et pendant l'extinction de la mégafaune, comment les carnivores sont affecté l'urbanisation et le taux sans précédent d'impacts anthropiques. Enfin ces nouvelles données permettent de dater et de mesurer l'effet de la dispersion médiée par l'homme et l'hybridation post-introduction (Chapitre I).

Le nombre et l’ampleur comparative de telles études sont, aujourd’hui encore, limitées en dans l'hémisphère sud et dans les pays à faible développement. Ce observation est le résultat des défis technologiques (Beheregay, 2008), de la difficulté d’échantillonnage dans certain biomes tels que la forêt tropicale dense (Hughes et al., 2021), et des investissements limités dans la conservation dans les pays du sud (Dickman et al., 2015). L’absence de telles données limite nos connaissances sur les patrons latitudinaux de diversité génétique et de spéciation. Cette absence empêche aussi d’étudier les conséquences des impacts humain passés sur les patrons actuels de diversité génétique, dans des régions où les humains coexistent avec la biodiversité depuis bien plus longtemps qu'ailleurs (Otto, 2018 ; Millette et al., 2020 ; Leigh et al., 2021).

Parmi les autres difficultés que j’ai rencontrées lors de la constitution de la base de données et de la revue de la littérature se trouvait : le manque de soutien pour le partage public des données, la présence d’informations incomplètes sur les individus échantillonnés qui étaient particulièrement importantes pour répliquer l’étude, et le fait que les données collectées n'ont pas été publiées dans la perspective d'obtenir des données supplémentaires utilisable pour une analyse plus approfondie. Néanmoins, vu l'augmentation du nombre de publications (figure I.2) et le développement d'une série d'initiatives visant à faciliter les échanges d'informations sur la biodiversité (par exemple, le tableau I.3), nous pouvons espérer que davantage de données provenant de
A Genetic Tale of Cats

cette région seront collectées ou numérisées pour être partagées en ligne au cours des prochaines années. Au fur et à mesure que des données locales sur les carnivores seront rassemblées dans cette région, il est probable que des modèles de coexistence entre l'homme et les carnivores commenceront à émerger, pour le passé et pour la période actuelle. Ces modèles sont essentiels pour atténuer efficacement les conflits entre l'homme et la faune sauvage et rendre les réseaux écologiques plus résilients (Carter et al., 2012 ; Nyhus, 2016).

Les effets inattendus des traits d'histoire de vie sur la variation génétique

Le temps de génération détermine le rythme des principaux processus démographiques et évolutifs. Les petits mammifères ont tendance à avoir plus de générations entre le présent et tout événement de goulot d'étranglement passé, ce qui entraîne un taux de mutation et de recombinaison plus élevé sur une période donnée, accumulant ainsi beaucoup de variation génétique par rapport aux grands mammifères (Martin et Palumbi, 1993 ; Gaillard et al., 2005). Cette relation négative entre la quantité de variation génétique et le temps de génération a été vérifiée dans une étude comparative à grande échelle utilisant des données transcriptomiques contemporaines et basé sur des niveau taxonomiques élevés ayant des traits d'histoire de vie sous la forme d'investissement parental, c'est-à-dire utilisant le rapport entre la taille des propagules et la taille des adultes comme un proxy pour les stratégies $r$ ou $K$ (Romiguier et al., 2014). Cependant, des modèles différents sont apparus à une échelle taxonomique inférieure et semblent contredire cette théorie. Chez les pinnipèdes, ni la durée de la saison de reproduction ni le temps de génération n'étaient associés à la richesse allélique (Stoffel et al., 2018). Dans le cas des Felidae, j'ai révélé comment certaines lacunes critiques en matière de données et de théorie pourraient accidentellement rendre les efforts de conservation inadaptés si ils se concentrent uniquement sur les espèces de grandes tailles.

Dans le chapitre II, j'ai constaté que les félinidés dont le temps de génération est rapide présentent une variation génétique réduite par rapport à leurs homologues. Cette relation positive est en accord avec une étude précédente sur les mammifères terrestres, dans laquelle les espèces avec des temps de génération plus courts ont montré
A Genetic Tale of Cats

des augmentations plus fortes du coefficient de consanguinité, tandis que les espèces avec des temps de génération plus longs présentent des effets plus faibles de la fragmentation sur le coefficient de consanguinité (Rivera-Ortíz et al., 2015). En outre, il est généralement admis que les espèces de grandes tailles sont plus vulnérables à la perte de variation génétique (par exemple, (Frankham, 1996 ; Romiguier et al., 2014 ; Lino et al., 2019) et que les espèces de petites tailles sont plus résilientes aux perturbations (Middleton et al., 2020). Cependant, bien que la masse corporelle soit liée au temps de génération, il est surprenant que cette première variable permette pas d’expliquer la variation génétique chez les félinés. Ce manque de corrélation pourrait vraisemblablement être causé par un biais d'échantillonnage (c'est-à-dire vers les félinés à grande taille), mais nos résultats sont en accord avec certaines études basées sur plusieurs taxons de mammifères (Johnson, 2002 ; Nabholz et al., 2008 ; Doyle et al., 2015) et sur les cétacés (Vachon et al., 2018).

La relation entre la structure génétique de la population et le temps de génération nécessite une étude plus approfondie. On peut supposer qu’il y a un délai considérablement plus court entre les événements de goulot d'étranglement et les conséquences génétiques négatives, pour les espèces ayant un temps de génération rapide et dans les cas où le rétablissement de la population n'est pas possible (Hailer et al., 2006). Une autre hypothèse est que la croissance de la population chez les félinés à temps de générations rapides est accompagnée d'une tendance évolutive vers la tolérance à la consanguinité, car les avantages de la tolérance par la reconnaissance de la parenté peuvent dépasser ceux de l'évitement de la consanguinité (Waser et al., 1986). Cela pourrait être le cas lorsque les possibilités de dispersion sont limitées (Keane et al., 1996 ; Dharmarajan et al., 2009). Il est clair que ces hypothèses méritent d'être testées lorsque les possibilités d'accouplement (c'est-à-dire la dispersion) et la densité de population sont influencées par des perturbations anthropiques, comme c'est le cas dans les paysages tropicaux présentant un gradient forestier (c'est-à-dire forêt ancienne, croissance secondaire, jusqu'à la fragmentation récente) (par exemple Wearn et al., 2017), ou ayant différentes tailles de parcelles d'habitat (Dharmarajan et al., 2009). Dans ces systèmes, il est possible d'examiner si le degré de différenciation génétique, de parenté et de fitness des espèces ayant un temps de génération court est différent de ceux de d'autre espèces de carnivores sympatriques ayant un temps de génération plus long.
A Genetic Tale of Cats

La densité de population un bon substitut de la taille de la population ?

Notre connaissance actuelle sur la relation entre la densité de population et la variation génétique chez les carnivores est au mieux fragmentaire. Dans le chapitre III, j’ai montré que les espèces de félinidés présentent un patron conforme aux prévisions théoriques. Cependant, ce patrons n’est pas généralisable pour toutes les espèces et le densité de population et taille de la population pourraient être affectées par l’hétérogénéité environnementale, que nous n’avons pas prise en compte. Une autre explication pour l’absence d’effets significatifs chez certaines espèces pourrait être le degré variable de structure sociale dans différentes populations du fait de variations dans la densité de population. Bien que la plupart des félinidés soient solitaires, il a été suggéré que le maintien de la variabilité génétique entre les populations ne diffère pas des autres espèces sociales de carnivores (Schmidt et al., 2016). En outre, les caractéristiques sociales, telles que la variation du sex-ratio en fonction de la densité de population, ont tendance à varier fortement avec la productivité de l’habitat, qui à son tour affecte la taille du domaine vital (Gittleman et Harvey, 1982 ; Lindstedt et al., 1986). Une forte densité de population pourrait également être le lieu de formation de groupes sociaux denses (Smallwood et Schonewald, 1996). Par exemple, dans le cas des félinidés, les zones de grandes tailles sont associées à une augmentation de la densité des femelles (Anile et Devillard, 2018). La base de données construite au cours de cette thèse est très prometteuse pour découvrir la dynamique éco-évolutive des carnivores et leurs réponses aux changements environnementaux, puisqu’elle comprend des facteurs tels que la variation intraspécifique de la taille du domaine vital, le sex-ratio, et les attributs écologiques (par exemple, les perturbations humaines, l’abondance des proies, le gradient latitudinal, le type de croissance de la forêt, et la distance à l’urbanisation).
Piégé dans la boucle de la captivité

Les données génomiques des populations captives peuvent servir la création de stratégies de reproduction plus efficaces, évitant l'adaptation génétique à la captivité, et pouvant être mises en œuvre dans la gestion de la faune sauvage pour (Wright et al., 2020 ; Willoughby et al., 2017 ; Willoughby et Christie, 2019). Dans le chapitre IV, j’ai examiné si la variation génomique entre les populations captives et sauvages présente le même patron que celui observé à l’aide de marqueurs microsatellites chez deux espèces ayant un historique de gestion de l’élevage différent. J'ai constaté que, pour les deux espèces, le niveau d'hétérozygotie a effectivement augmenté dans les populations captives. Ceci est inattendu pour les lions asiatiques car la taille de leur population fondatrice était très limitée. Nous ne pouvions pas exclure la possibilité que la taille limitée de notre échantillonnage affecte nos conclusions. Cependant, la grande quantité des données génomiques obtenues à partir d'individus captifs nous a permis d'obtenir des informations plus précises sur les conséquences de la consanguinité et le choix du génome de référence. Nous nous attendions à ce que la présence de long ROHs ait une forte influence sur la sélection directionnelle et puisse augmenter la fréquence des variants délétères (Szpiech et al., 2013). Ces animaux ayant évolué dans un environnement artificiel sous l’égide de l’homme, ils pourraient ne pas être en mesure de s’adapter à la nature (Hunter et al., 2013). Le récent Rapport mondial sur la criminalité liée à la faune sauvage (2020) a souligné que la plupart des saisies et des chasses au trophée de grands félins concernaient des animaux élevés en captivité. Il est possible que ces espèces soit transférées du milieu naturel vers les zoos, à moins que les financements soient suffisants pour conserver les espèces dans leur milieu naturelle, ce qui n’est pas toujours le cas dans les pays moins développés des tropiques. Ces transferts pourraient représenter un risque important, en exposant les individus à des variétés non naturelles d’agents pathogènes dans des zones où ils ne sont pas naturellement observés. L’étape suivante consisterait à étudier l’association empirique entre les ROHs et les variantes potentiellement dangereux, ce qui donnerait un aperçu détaillé des conséquences d’une population fondatrice artificielle sur leur structure génomique et leur évolution.
A Genetic Tale of Cats

La (petite) montée et la (grande) chute des carnivores du Pléistocène

Durant le Pléistocène, les mammifères de grande taille ou mégafaune étaient omniprésents. Ce fut aussi le moment où les premiers humains du Paléolithique (Homo) ont commencé à se disperser dans de nouveaux habitats. Afin de survivre, les humains ont dû faire face à un choix: éviter, coexister ou affronter les mammifères terrestres carnivores. Les préférences alimentaires des carnivores ont probablement influencé les itinéraires de migration des premiers humains. Ils évitaient certaines zones où le risque de rencontrer des carnivores était important, ou développèrent des groupes coopératifs pour accroître leur vigilance (Treves et Palmqvist, 2007 ; Carotenuto et al., 2016). Un autre scénario est que certains humains choisissaient de fouiller les carcasses laissées par les carnivores. La viande riche en protéines ainsi obtenue a potentiellement facilité l'expansion humaine en fournissant des forces supplémentaires pour une dispersion sur de longues distances hors des tropiques africains (Turner, 1992). Les carnivores étant des top prédateurs, leur disparition généralisée a pu modifier la diversité et la composition des assemblages d’espèces d’autres clades fonctionnellement similaires (Smith et al., 2016). Les carnivores survivants auraient également eu la possibilité d’étendre leur aire de répartition géographique aux habitats laissés par les taxons frères éteints (Waters et al., 2013).

Dans le chapitre II, j’ai montré que le patron de la variation génétique obtenu à partir des marqueurs microsatellites pouvait être corrélé aux effets négatifs du changement climatique du Pléistocène et de la migration humaine dans toutes les parties du territoire sur la mégafaune. Toutefois, cela reste spéculatif, car il semble que l’Afrique, où les humains sont apparus, et l’Amérique du Sud, où les espèces de carnivores sont arrivées le plus récemment, présentent la plus faible diversité génétique par rapport aux autres continents. La seule possibilité pour éclaircir cette question serait d’obtenir une meilleure accessibilité des données brutes de génotYPE. Les données ADN de haute qualité provenant d’animaux en captivité constituent une ressource importante et sous-évaluée pour la démographie comparative, alors qu’elles permettent de délimiter le rythme et les origines d’événements spécifiques liés aux impacts anthropogéniques et au changement climatique au Pléistocène (Hecht et al., 2020). Dans le chapitre IV, j’ai montré que l’influence démographique basée sur le PSMC entre des individus contemporains sauvages et captifs donnait des résultats similaires, malgré les différences significatives dans l’hétérozygotie à l’échelle du génome et le coefficient de consanguini-
A Genetic Tale of Cats

té. Cette méthode, si elle est appliquée à d'autres carnivores, permettrait un échantillonnage plus sûr, plus rapide et plus réalisable pour les études de démographie comparative, et faciliterait ainsi la quantification du risque d'extinction chez les espèces menacées sans nuire aux individus sauvages.

Il est urgent d'élaborer des prédictions précises sur le futur statut de conservation des carnivores, étant donné que ces animaux sont aujourd'hui confrontés à des risques toujours plus importants. La combinaison de nouvelles séquences génomiques et de données provenant de parents éteints, tels que le grand félin à dents de sabre, offre de nombreuses possibilités pour améliorer la conservation des félinés, mais peut aussi conduire à une nouvelle boîte de Pandore, voire à un nouveau type de chat domestique. Quoi qu'il en soit, comprendre l'importance qu'à la diversité génétique pour le potentiel adaptatif de toutes les espèces doit être la priorité afin de promouvoir un écosystème sain, riche en biodiversité et donc bénéfique à tous les êtres vivants. L'évolution continuera à mener au bord de l'extinction certaines espèces et à éviter l'extinction pour d'autres, alors que l'homme jouera un rôle toujours plus important dans ce processus. C'est là que réside notre grande responsabilité, à la fois quant à la manière dont nous interagissons avec la nature et à la manière dont nous devrions changer notre point de vue sur la conservation. Du point de vue sémantique, la conservation implique le maintien d'un statut, alors qu'elle devrait plutôt être traitée dans le contexte dynamique d'une vie en constante évolution. Pour paraphraser le regrette Theodosius Dobzhansky : "rien dans la conservation (biologie) n'a de sens si ce n'est à la lumière de l'évolution".
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188


192


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Appendix
## Appendix

### Annexe I Source of the genome-wide heterozygosities values in Figure 1

<table>
<thead>
<tr>
<th>Reference</th>
<th>Species</th>
</tr>
</thead>
</table>
Appendix


Appendix

Bonobo
Brown hyena
Cheetah
Chimpanzee
Human
Iberian Lynx
Island fox
Orca
Polar bear
Yellow baboon

Narwhal
Spotted Hyena
Tibetan black bear
Annexe II Distribution of population genetic studies in Felidae.

(a) The number of studies in each genus, represented by the type of molecular data used and the scale of the study (greyscale bars). The number of publications within the genus is shown on the right and the number of studied species among the total number of species within the genus are shown in the brackets.
(b) The relative frequency of available mitogenome from NCBI within genus

(c) The total number of microsatellite genotype data available either in Dryad or in the supplementary materials. The colours indicate the species distribution.
Annexe III Supplementary information for Chapter 3

Figure S1. Schematic representation of within-population genetic diversity data collection and cleaning.
Figure S2. K-means clustering results were compared between a) the coordinates of PD and b) the coordinates of GD as initial centroids. The number of records for PD and GD in each species and in each biogeographic region are presented (PA) Palearctic, (NE) Neartic, (IM) Indo-Malay, (NT) Neotropic and (AT) Afrotropic. The final clustering results using the centroids that give the most data and after removing data points with distance between GD and PD points greater than the dispersal distance of each species are shown in (c).
Acinonyx jubatus

a) n. PD : AT=12, PA=2
n. cluster = 3

b) n. GD : AT=16, PA=1
n. cluster = 3

c) dispersal distance = 1018km

Felis chaus

a) n. PD : IM=1
n. cluster = 1

b) n. GD : IM=1
n. cluster = 0

c) dispersal distance = 132km

Felis silvestris

a) n. PD : PA=17
n. cluster = 2

b) n. GD : PA=31
n. cluster = 2

c) dispersal distance = 322km
Leopardus colocolo

a) n. PD : NT=5
   n. cluster = 3
b) n. GD : NT=14
   n. cluster = 3
c) dispersal distance = 380km

Leopardus geoffroyi

a) n. PD : NT=10
   n. cluster = 3
b) n. GD : NT=5
   n. cluster = 3
c) dispersal distance = 237km

Leopardus jacobita

a) n. PD : NT=4
   n. cluster = 1
b) n. GD : NT=6
   n. cluster = 2
c) dispersal distance = 422km
Leopardus pardalis

Leopardus tigrinus

Lynx canadensis
Lynx lynx

- a) n. PD : PA=32
- b) n. GD : PA=23
- c) dispersal distance = 771km

Lynx pardinus

- a) n. PD : PA=6
- b) n. GD : PA=4
- c) dispersal distance = 658km

Lynx rufus

- a) n. PD : NE=13
- b) n. GD : NE=61
- c) dispersal distance = 452km
Panthera pardus

Panthera tigris

Panthera uncia

Prionailurus bengalensis
Figure S3. Boxplots of PD and GD estimates for each species in a) cluster and b) country datasets. Outliers are shown as circles. The x-axis is ordered following species body mass (small to largest).

a) Cluster dataset

Appendix
b) Country dataset
### Table S1. Data structure. Number of records, cluster and studies in Cluster and Country datasets for each species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Cluster data set</th>
<th>Country data set</th>
<th>Both datasets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PD records</td>
<td>GD records</td>
<td>Cluster</td>
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<td>Acinonyx jubatus</td>
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<td>2</td>
<td>2</td>
</tr>
<tr>
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</tr>
<tr>
<td>Felis silvestris</td>
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<td>4</td>
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<tr>
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<td>3</td>
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<tr>
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<td>0</td>
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<td>Leopardus jacobita</td>
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<td>2</td>
</tr>
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<tr>
<td>Leopardus tigrinus</td>
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<td>2</td>
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<td>3</td>
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<td>14</td>
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<td>Panthera uncia</td>
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<tr>
<td>Puma concolor</td>
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<td>Total</td>
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Table S2. Linear mixed model comparisons for predicting genetic diversity estimates using the country dataset. The models presented here had fewer than 2 differences in AIC compared to the model with the smallest AIC, thus they can be considered to have substantial support. The most parsimonious models are highlighted in bold, chosen on the basis of lowest AIC and least number of parameters. Null models and models fitted with PD as single fixed effect are also presented. Sample size is the number of individuals for each GD estimates. K refers to the number of parameters.

<table>
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<tr>
<th>Response variable</th>
<th>Fixed term</th>
<th>Marginal $R^2$</th>
<th>Conditional $R^2$</th>
<th>k</th>
<th>log-Likelihood</th>
<th>AIC</th>
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<td>Observed heterozygosity</td>
<td>Population density + Generation length + body mass</td>
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<td>0.49</td>
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<td>Null model</td>
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<td>Expected heterozygosity</td>
<td>Population density + Generation length + Sample size</td>
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Annexe IV Supplementary information for Chapter 4

Figure S1. Pedigree chart of the cheetahs sampled from Montpellier Zoo and ‘Rico’, highlighted in red. Wild ancestors are assigned in black filled shapes. Males are symbolised by squares; females by circles. A consanguineous marriage is indicated by two horizontal lines connecting the parents rather than one. The number beneath the last zoo location (in capital letters) is the year of birth.
Figure S2. Pedigree tree of the Asiatic lions sampled from Montpellier Zoo and ‘Atul’, highlighted in red. Wild ancestors are assigned in black filled shapes. Males are symbolised by squares; females by circles. A consanguineous marriage is indicated by two horizontal lines connecting the parents rather than one line. The number beneath the last zoo location represents the year of birth of the individual.
Figure S3. Heterozygosity rate in each chromosome based on the different reference genomes.
<table>
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<th>Number of ROH with length &gt; 1Mb</th>
<th>Prop ROH&lt;sub&gt;1Mb&lt;/sub&gt;</th>
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<th>SNV</th>
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<tbody>
<tr>
<td></td>
<td></td>
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<td>Mean</td>
<td>Min</td>
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