Who Calls the African Wild Dog?

– *De novo* SNP discovery and genotyping in the *Lycaon pictus*.

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Abstract

Large carnivores, especially the African wild dog, *Lycaon pictus*, are vulnerable to human-mediated changes and climate effects. As they are apex predators, they also affect lower trophic levels. Due to anthropogenic land-use changes affecting habitat, prey, and population densities, large carnivore populations are declining. Thus, accurate population demographic estimates are required for conservation efforts to sustain carnivore populations. Genetic data are of high importance when analysing population demographics as they allow the study of oftentimes cryptic effects (loss of genetic diversity, inbreeding depression, and genetic drift). As next-generation sequencing techniques advance, genetic markers as single nucleotide polymorphism (SNP) can provide key ecological information. This information can be used to implement conservation efforts to impede the negative effects on populations. The aim of this study was to develop a highly informative SNP panel through *de novo* SNP discovery and genotyping in the non-modeled African wild dog. A total of 74 SNP markers were validated and 83 individuals were identified. The SNP chip provides a foundation for further research on relatedness, parental linkage, dispersal patterns, population size estimates, and the discovery of cryptic effects.

Keywords: SNP, Single nucleotide polymorphism, African wild dog, *Lycaon pictus*, Conservation

Preface

When African wild dogs call for attention, they hoo-call. I call a hoo to save this species!

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Abbreviations

SLU	Swedish University of Agricultural Sciences
ZCP	Zambian Carnivore Programme
ACCP	African Carnivore Connectivity Project
SNP	Single Nucleotide Polymorphism
PCR	Polymerase Chain Reaction
RADseq	Restriction site Associated DNA sequencing

1. Introduction

We have today entered a new epoch of Earth recognised as the Anthropocene, where human induced-ecological changes are the primary cause of planetary change (Crutzen 2006). For the past three centuries, developing more convenient living standards, healthcare, and access to resources have outcompeted the need to preserve natural resources on our planet (Crutzen 2002; Whitmee et al. 2015). This development has a negative effect on the worlds' biodiversity (Rockström et al. 2017). Anthropogenic land-use changes result in the declination of species in dynamics, densities, and social structures resulting in the prediction that we are at the beginning, or in the middle of a sixth mass extinction (Wake & Vredenburg 2008).

Large carnivores are species particularly susceptible to biodiversity threats caused by human-altered changes (Ripple et al. 2014). The two main threats are habitat loss and fragmentation (Watson et al. 2015). The degradation of viable habitats results in smaller, more isolated populations, patched in-between a matrix of human cultivated land (Crooks et al. 2011). Other negative impacts on large carnivores are human-wildlife conflicts, poaching, diseases, and illegal trading (Cushman et al. 2016; Wolf & Ripple 2018). Roughly 80 % of the terrestrial large carnivore species show population declines and 64 % of the species are listed as threatened with extinction (Wolf & Ripple 2018). Their excessive vulnerability is caused by their occurrence in low densities, ranging over large areas, low reproductivity rate, large body mass, and high confliction with humans over resources (Crooks et al. 2011; Ripple et al. 2014). Additionally, they are apex predators, i.e. are at the top of the food chain, having a major impact on lower trophic levels. Further studies are required on endangered large carnivores with large home range, strong social structure, and dispersal dependent on maintained corridors (Marsden et al. 2012). Conservation efforts to sustain viable carnivore populations are vital, long-term investments to sustain overall biodiversity (Ray et al. 2013).

The African wild dog, *Lycaon pictus*, (hereafter wild dog), is a carnivore listed by the International Union for Conservation of Nature's (IUCN) Red Data List as Endangered with only 6,679 adults and yearlings left

(Woodroffe & Sillero-Zubiri 2020). The wild dog has a social pack structure and is a widespread species with a large home ranges (Creel & Creel 2002), although in 2015 estimated to only persist approximately 17% of its historic range (RWCP & IUCN/SSC 2015). Woodroffe & Sillero-Zubiri (2020) identifies they are highly sensitive to habitat fragmentation due to their wide-ranging and social behaviour. Other negative impacts on their population densities are human-wildlife conflicts, diseases, poaching, road accidents, and snaring. The negative effects on wild dogs have been greater compared with other large carnivores (Milner-Gulland & Woodroffe 2001). This is partly due to historical persecution of wild dogs (Creel & Creel 2002). They were considered vermin during parts of the 20th century and were exposed to organised eradication campaigns (Milner-Gulland & Woodroffe 2001). They are also subordinate competitors, making them susceptible to dominant competitors such as lions and hyenas (Creel & Creel 1996; Marsden et al. 2012; Creel et al. 2019). Subsequently, anthropogenic land-use changes and competition with dominant competitors negatively affect the wild dog population resulting in a declining population trend. Additionally, because of their scarcity and evasiveness, reliable population estimates of the wild dog populations are difficult to predict, yet important for conservation and management planning (Creel & Rosenblatt 2013). There is an urgent need to establish reliable population size estimates and geographic distribution of the species.

A declining population trend results in a loss of genetic diversity, negatively affecting survival and reproductive success within a species (Norman 2016). Inbreeding, demographic- and environmental stochasticity can result in the further reduction of genetic diversity eventually leading to the extinction of that population (Cushman et al. 2018). Through identifying genetic variation, accurate estimations of populations, migration patterns, and trophic interactions can delimitate the negative effects that result in reduced genetic representation (Luikart et al. 2003). Studying population demographics (i.e., population size, dispersal, and translocation) enhances knowledge of species, particularly endangered species (Tende et al. 2010). Greater knowledge of population demographics provide valuable support for conservation strategies and guidelines of future management to support the long-term health of species (Tensen et al. 2019). Genetic data can contribute to the knowledge of species ecology and demographics, of the environmental changes affecting species, (human-mediated changes), and prove useful for conservation efforts to sustain species (Swift & Hannon 2010; Marsden et al. 2012). Genetic markers are today used for studying population demographics as a part of enhancing conservation efforts to impede the declining population trends of species (Luikart et al. 2003; Ruegg et al. 2014).

The single nucleotide polymorphism (SNP) marker is an increasingly popular choice of genetic markers when analysing species' genomic data (Vignal et al. 2002). SNPs are especially used in kinship and pedigree reconstruction as they provide high genomic resolution but can also be used to identify diseases, identify hybridised individuals, and overall study of species (Shaw 2013; Norman & Spong 2015; Norman et al. 2019). Although in some cases, they can also be multi-allelic, SNPs are generally bi-allelic, occurring only in two variants within a population (Krawczak 1999). As they are bi-allelic they contain little information per SNP, compared with microsatellites, indicating it would require significantly more SNPs to gain the same result as microsatellites (Norman et al. 2019). However, with the advancements in Next-Generation Sequencing techniques, such as the Restriction site Associated DNA sequencing (RAD-seq), this argument is subdued. RAD-seq enables screening tens to hundreds of thousands of SNPs across the entire genome in hundreds of individuals in both non-modeled and modeled organisms (Etter et al. 2012; Hohenlohe et al. 2012). SNP markers are with next-generation sequencing techniques more cost-effective, less error-prone, faster, and easily understandable in comparison with microsatellites (Vignal et al. 2002; Marguerat et al. 2008; Williams et al. 2010; von Thaden et al. 2017). Additionally, the SNP sequencing techniques require shorter DNA sequences and perform better in genotyping compared with microsatellites when studying highly contaminated DNA samples (von Thaden et al. 2017).

Since they require shorter sequencing reads, SNP markers are a suitable choice when studying non-modeled organisms where no prior genomic information has yet been developed (Hohenlohe et al. 2011). Instead, developing high-throughput sequences and SNP discovery can be performed *de novo*, by sequencing fragments of the genome gathered from multiple individuals (Morin et al. 2004; Seeb et al. 2011).

In this study, *de novo* SNP discovery and genotyping of wild dog samples were conducted. The aim was also to develop a SNP panel that contains 96 highly informative SNPs. In order to develop an assay of high quality, SNPs with maximum allelic representation within the population and show no linkage to one another are selected (Krawczak 1999; Norman et al. 2013). A highly informative SNP assay can be used for further studies on relatedness, geographical distribution, diseases, and individual identification to improve preservation efforts in wild dogs.

2. Material and Methods

This study is part of a larger research project, the African Carnivore Connectivity Project (ACCP). The research project is a collaboration between Swedish University of Agricultural Science's Molecular Ecology Group, Montana State University, and Zambian Carnivore Programme. The sample collection, DNA extraction, DNA sequencing, and library preparation in this study follow the procedures presented in Creel et al. (2019).

2.1. Sample Collection and DNA Extraction

Samples from 94 wild dogs were collected in three ecosystems, South Luangwa, Kafue, and Liuwa, Zambia. Field data collection methods were approved by the Zambia Department of National Parks and Wildlife. Biopsy darts (PneuDart) fired from parked vehicles were used to collect tissue using a Dan-Inject JM Standard air rifle. Tissue biopsies were also collected from anaesthetized wild dogs for radio-collaring or de-snaring. Immediately after collection, samples were stored in containers with ethanol. All sample collection was performed by licensed wildlife veterinarians. The collection was performed following protocols produced by the Department of National Parks and Wildlife in Zambia and approved by the Montana State University Animal Care and Use Committee (protocol 2010-39). DNA was extracted following the manufacturer's instructions at the Zambian Carnivore Programme's Luangwa field site using a QIAsymphony DNA kit (Qiagen). Thereafter, samples were sent to the laboratory in Umeå, Sweden for assessing nucleotide quantity and purity using a spectrophotometer, (NanoDrop, Thermo Fisher Scientific). Kodak Electrophoresis Documentation and Analysis System 120 (Eastman Kodak) was used for visualising DNA quality for sequencing by gel electrophoresis and final screening used Qubit. Library preparation and sequencing were assessed at the National Genomics Institute, Stockholm.

2.2. Library Preparation and DNA Sequencing

DNA extracts were digested into sequences with the EcoRI restriction enzyme. This was done to optimise the trade-off between coverage of breadth (approximately 1% of the genome) and read depth (minimum 30 x/nucleotide). EcoRI was chosen as the restriction enzyme according to the manufacturer's instructions, to digest 0.5 µg of each DNA extract. In two elutions, activated enzymes were removed with the MinElute Reaction Cleanup kit (Qiagen). The second elution was visualised by gel electrophoresis for a quality assessment of the digestion. After the removal of the EcoRI enzyme, the National Genomics Institute in Stockholm received the samples for library construction and preparation. By sample, excised and blunt end repaired 400-700 base pairs fragments were ligated with paired-end, multiplexed adapters. Paired-end reads of 2 x 150 base pairs were developed when equimolar concentrations were sequenced on an Illumina Hiseq. 2500 or Novaseq. The barcode-splitter option of the FASTX Toolkit (v 0.0.13) demultiplexed the RAD-seq Illumina reads and cutadapt (v 0.93; Martin 2011) removed the adapters. FASTX Toolkit trimmer and quality_filtered trimmed, and quality filtered the reads with the settings: q 10, p 70 and FastQC (version 0.9; Babraham Bioinformatics) then accessed quality sequences. The resulting 151 base pairs long Illumina sequences were trimmed down to 140 and later used as input to detect and call SNPs using Stacks (version 2.55; Catchen et al. 2013).

2.3. Quality Filtering and Alignment

Reads were trimmed at the enzyme recognition site, to remove known Illumina adapters, (five first base pairs), and trimmed to remove the three base pairs of both ends of the reads. The trimming restrained false negatives associated with the cut site and removed base pairs of bad quality. Only reads with pairs of full length were kept, resulting in 140 base pairs. The remaining reads were used as input when running *process_radtags* in Stacks (Catchen et al. 2013), to be examined, cleaned and quality filtered. Since the enzyme recognition site had been trimmed, *process_radtags* run with the 'disable-rad-check' option. This option tells the program to assume that every read is correct and starts at the right cut site. Thereafter, the sequences were used as input in a pipeline for SNP detection. The pipeline started with *ustacks*, creating a catalogue in *cstacks*, matched samples to the catalogue (*sstacks*), transposed data to be re-organised by samples instead of by locus (*tsv2bam*), and called sites in the population to genotype samples at certain cites (*gstacks*). A population genomics analysis was

performed in *populations*, the final step of the pipeline. The pipeline was executed *de novo*, as no reference genome was used, and executed with the default settings in the programs except for in *cstacks*, where –n was set to 2 as it matches the default settings of *ustacks* and is generally recommended.

2.4. SNP Calling and Validation

The output of Stacks was imported into RStudio (RStudio Team 2020) for filtering and validating putative SNPs, and remove SNPs of low quality. The first filter ensured that only one SNP per read was present by removing all SNPs found on the same locus. This was performed to minimise the risk of linkage between SNPs, with close physical distance from one another. Since paired-end reads with sequences that had been aligned were used, the filtering of reads were set to only include SNPs located in the middle of each part of a pair. This was performed to ensure sufficient flanks of either side of the SNP for the ensuing development of the SNP assay.

Since the SNP assay only required 96 high-quality SNPs, (96 samples x 96 markers, Fluidigm Corporation, San Fransisco, USA) it allowed me to be very stringent in filtering. To ensure a high minor allele frequency and homozygosity, filters should contain SNPs highly represented among individuals, with reduced major allele frequency and observed heterozygosity. Lastly, SNPs had to contain all genotypes (i.e. XX, YY, XY) and removed otherwise. To secure a buffer for the verification of designable SNPs, 150 SNPs were selected. The verification was performed *in silico* at Fluidigm Corporation, San Francisco, USA. Thereafter, Fluidigm Corporation developed assays of 96-well plate SNPs. These assays were tested on 93 samples for validation of the selected SNPs.

3. Results

3.1. RAD sequencing

RAD sequencing resulted in a total of 1.9 billion paired-end, 140 base pair long reads from 94 sampled individuals. The quality filtering and drop-out of unfit reads from *process_radtags* resulted in the removal of 0.04% reads. The Stacks pipeline (Catchen et al. 2013) attempted to assemble and align paired-end reads for 1,181,091 loci. Out of these loci, 18,506 loci had paired-end reads that could not assemble into a contig (1.6%). Of the paired-end contigs, 137,997 loci were overlapping in the forward region (11.9%). In the remaining 1,162,585 loci, the total successfully aligning reads resulted in 99.8 million reads (99.7%) with a mean of 858.7 reads per locus.

3.2. SNP Calling

The Stacks pipeline genotyped 1,162,559 loci and identified 340,691 putative SNPs from 94 individuals. The putative SNPs were filtered down to 150 candidate SNPs for assay development (Figure 1). The first filter selected loci with only one SNP per loci, reducing the number of SNPs to 80,186. Reads with SNPs between flanks of the 40th and 100th base pairs, and between the 190th and 250th base pairs, were included and SNPs present in less than 50 individuals were removed. This reduced the number of SNPs to 5,937. The major allele frequency was set to be less than 65%, and observed heterozygosity between 25-65% to ensure a high minor allele frequency and homozygosity, resulting in 622 SNPs. An additional filter limited the difference between observed and expected heterozygosity between -0.015-0.05. A final selection confirmed that the remaining 168 SNPs contained all genotypes and none were removed. Instead, the 168 SNPs were randomly reduced to 150 candidate SNPs. The *in silico* verification resulted in 140 designable SNPs which were used for assay development (Fluidigm Corporation, San Francisco, USA).

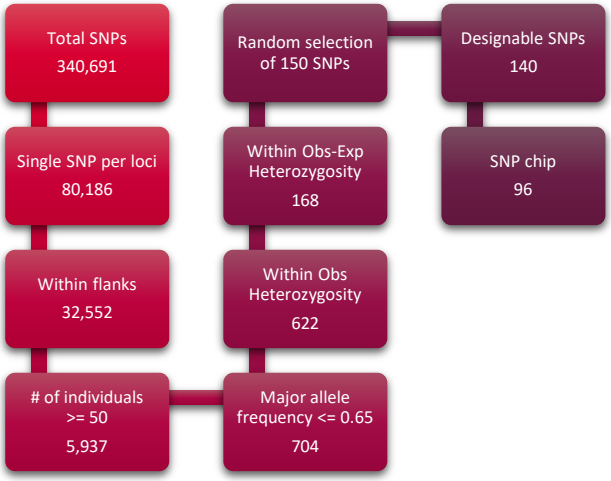

Figure 1. Filtering steps of SNPs to be selected for the development of high-quality SNP assay.

3.3. SNP Validation and Genotyping

Out of the 140 designable candidate SNPs, 96 were tested on a panel to be validated by genotyping 93 individuals. A total of 74 autosomal SNPs were validated, and 83 individuals were identified (Figure 2). The 22 invalid SNPs failed to cluster properly: poor template control (NTC) in three, ten were too near one another, eight were either exclusively homozygote or heterozygote, and one was not working, a “no call” caused by probable contamination (Figure 2). Norman et al. (2013) suggest scatterplots without heterozygosity and missing a second allele would indicate a Y-chromosome marker. Furthermore, scatterplots with two separated clusters representing each allele with no heterozygosity displayed would indicate mitochondrial markers as the mitochondria are haploid. None of the SNPs validated in the current study yield any indication of representing the Y chromosome or mitochondria based on the aforementioned criteria, indicating they are autosomal SNPs (Figure 2).

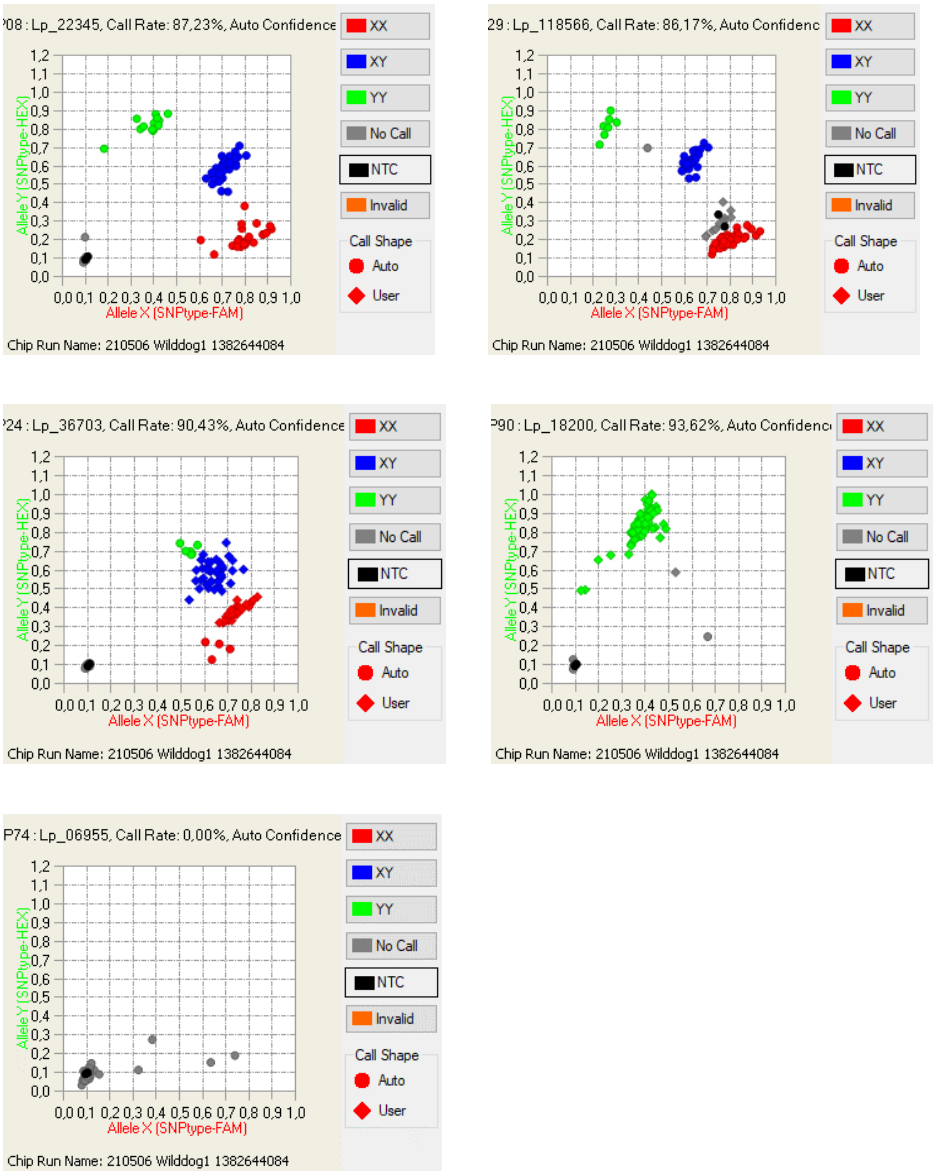

Figure 2. Scatterplots demonstrating clusters of alleles visualised with fluorescent for SNPs produced with Biomark system (Fluidigm, San Francisco, USA). a) Scatterplot generating good results, b) generally okay but no template control (NTC) is not good, c) too closely connected, d) homozygote, and e) "no call" providing no results.

4. Discussion

Developing a SNP assay of highly informative SNPs requires a sufficient number of individuals to ensure high minor allele frequency and SNPs with no linkage to one another (Catchen et al. 2013; Hand et al. 2015). In this study, putative SNPs were discovered and genotyped using RAD-seq to develop a high-qualitative SNP chip of the African wild dog. Validation genotyping resulted in verifying 74 SNP markers and the identification of 83 individuals. Similar to Spitzer et al. (2020), the unsuccessfully identified individuals might be identified by a second genotyping. An adjustment of more preamplification cycles added in the polymerase chain reaction (PCR) might result in a second genotyping with higher success rate in identifying the ten currently unidentified individuals. However, the genotyping performed in this paper resulted in high discriminatory success validation of SNPs, ranging throughout the genome. This success rate suggests the SNPs are suitable for continued genetic monitoring of wild dogs using non-invasive samples.

Successfully validated SNP markers can be used to study evolutionary and ecological processes (Luikart et al. 2003; Hohenlohe et al. 2012; Larson et al. 2014). In this study, the SNP discovery and genotyping were performed *de novo* and thus, the development of SNP markers lay a foundation for increased genetic knowledge of the wild dog population. Additionally, the targeted approach was to identify markers across the entire genome, focusing on autosomal SNPs. Further improvements to the panel would be to add Y-chromosome sex-determination markers and mitochondrial markers. The 74 verified autosomal markers are selected for the final panel. With a second chip run, the remaining designable candidate SNPs produced in this paper can be validated, and a total of 85-90 autosomal SNPs be selected. The complete set of validated autosomal SNPs including Y-chromosome sex-determination markers and mitochondrial markers constitute a final 96 high-qualitative SNP panel.

Continuous genetic monitoring of an endangered species can access signals of the negative impact on their viability at an early stage which can provide valuable conservational recommendations to sustain the species (Roques et al. 2016). Developing a SNP chip in this paper enables

opportunities for further research on the endangered African wild dog. In further monitoring exercises, the SNP chip can be used for relatedness analysis, detecting diseases, dispersal patterns, and overall population size estimations (Norman & Spong 2015). It can also be a valuable asset to study cryptic effects such as inbreeding, gene flow, and limitations in genetic diversity in the wild dog (Ferreira et al. 2018). Genetic data can increase knowledge of population demographics valuable for conservation efforts to sustain the species and identify potential threats (Taylor et al. 2017). Endangered species populations are inherently unstable as the genetic variation quickly can change (Marsden et al. 2012). Long-term projects are necessary to implement for monitoring changes in genetic variation for endangered species. With a changing climate and increasing human-mediated changes, it is essential to identify potential adaptive genetic traits (Allendorf et al. 2010). It is also important for management concerns to detect and counteract negative genetic effects, such as bottleneck, inbreeding depression, or genetic drift. The study of genetic data is a conservational key factor in a changing environment and increasing anthropogenic activity.

Conclusion

Although bi-allelic, SNP markers provide highly informative, fast, and inexpensive genetic identification of individuals. Next-generation sequencing enables discovery of tens to hundreds of thousands of SNPs represented across the entire genome and with a high minor allele frequency in both model and non-model organisms. Validation of 74 high-qualitative autosomal SNP markers allows genotyping of a large set of individuals and from highly contaminated DNA based on non-invasive sampling techniques. Further research opportunities are given with a developed SNP assay of the African wild dog which is advantageous to provide long-term conservational monitoring of this endangered species.

References

Allendorf, F.W., Hohenlohe, P.A. & Luikart, G. (2010). Genomics and the future of conservation genetics. *Nature Reviews Genetics*, 11 (10), 697–709. https://doi.org/10.1038/nrg2844

Catchen, J., Hohenlohe, P.A., Bassham, S., Amores, A. & Cresko, W.A. (2013). Stacks: an analysis tool set for population genomics. *Molecular Ecology*, 22 (11), 3124–3140. https://doi.org/10.1111/mec.12354

Creel, S. & Creel, N.M. (1996). Limitation of African Wild Dogs by Competition with Larger Carnivores. *Conservation Biology*, 10 (2), 526–538

Creel, S. & Creel, N.M. (2002). *The African Wild Dog: Behavior, Ecology, and Conservation*. Princeton: Princeton University Press.

Creel, S. & Rosenblatt, E. (2013). Using pedigree reconstruction to estimate population size: genotypes are more than individually unique marks. *Ecology and Evolution*, 3 (5), 1294–1304. https://doi.org/10.1002/ece3.538

Creel, S., Spong, G., Becker, M., Simukonda, C., Norman, A., Schiffthaler, B. & Chifunte, C. (2019). Carnivores, competition and genetic connectivity in the Anthropocene. *Scientific Reports*, 9 (1), 1–8. https://doi.org/10.1038/s41598-019-52904-0

Crooks, K.R., Burdett, C.L., Theobald, D.M., Rondinini, C. & Boitani, L. (2011). Global patterns of fragmentation and connectivity of mammalian carnivore habitat. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 366 (1578), 2642–2651. https://doi.org/10.1098/rstb.2011.0120

Cushman, S.A., Elliot, N.B., Bauer, D., Kesch, K., Bahaa-el-din, L., Bothwell, H., Flyman, M., Mtare, G., Macdonald, D.W. & Loveridge, A.J. (2018). Prioritizing core areas, corridors and conflict hotspots for lion conservation in southern Africa. *PLOS ONE*, 13 (7), e0196213. https://doi.org/10.1371/journal.pone.0196213

Cushman, S.A., Elliot, N.B., Macdonald, D.W. & Loveridge, A.J. (2016). A multi-scale assessment of population connectivity in African lions (Panthera leo) in response to landscape change. *Landscape Ecology*, 31 (6), 1337–1353. https://doi.org/10.1007/s10980-015-0292-3

Etter, P.D., Bassham, S., Hohenlohe, P.A., Johnson, E.A. & Cresko, W.A. (2012). SNP Discovery and Genotyping for Evolutionary Genetics Using RAD Sequencing. *Molecular Methods for Evolutionary Genetics*. Totowa, NJ: Humana Press, 157–178. https://doi.org/10.1007/978-1-61779-228-1_9

Ferreira, C.M., Sabino-Marques, H., Barbosa, S., Costa, P., Encarnação, C., Alpizar-Jara, R., Pita, R., Beja, P., Mira, A., Searle, J.B., Paupério, J. & Alves, P.C. (2018). Genetic non-invasive sampling (gNIS) as a cost-effective tool for monitoring elusive small mammals. *European Journal of Wildlife Research*, 64 (4), 1–15. https://doi.org/10.1007/s10344-018-1188-8

Hand, B.K., Hether, T.D., Kovach, R.P., Muhlfeld, C.C., Amish, S.J., Boyer, M.C., O'rourke, S.M., Miller, M.R., Lowe, W.H., Hohenlohe, P.A. & Luikart, G. (2015). Genomics and introgression: Discovery and mapping of thousands of species-diagnostic SNPs using RAD sequencing. *Current Zoology*, 61 (1), 146–154. https://doi.org/10.1093/czoolo/61.1.146

Hohenlohe, P.A., Amish, S.J., Catchen, J., Allendorf, F.W. & Luikart, G. (2011). Next-generation RAD sequencing identifies thousands of SNPs for assessing hybridization between rainbow and westslope cutthroat trout. *Molecular Ecology Resources*, 11 (s1), 117–122. https://doi.org/10.1111/j.1755-0998.2010.02967.x

Hohenlohe, P.A., Catchen, J. & Cresko, W.A. (2012). Population Genomic Analysis of Model and Nonmodel Organisms Using Sequenced RAD Tags. I: Pompanon, F. & Bonin, A. (red.) *Data Production and Analysis in Population Genomics: Methods and Protocols*. Totowa, NJ: Humana Press, 235–260. https://doi.org/10.1007/978-1-61779-870-2_14

Krawczak, M. (1999). Informativity assessment for biallelic single nucleotide polymorphisms. *ELECTROPHORESIS*, 20 (8), 1676–1681. https://doi.org/10.1002/(SICI)1522-2683(19990101)20:8<1676::AID-ELPS1676>3.0.CO;2-D

Larson, W.A., Seeb, J.E., Pascal, C.E., Templin, W.D. & Seeb, L.W. (2014). Single-nucleotide polymorphisms (SNPs) identified through genotyping-by-sequencing improve genetic stock identification of Chinook salmon (Oncorhynchus tshawytscha) from western Alaska. *Canadian Journal of Fisheries and Aquatic Sciences*, 71 (5), 698–708. https://doi.org/10.1139/cjfas-2013-0502

Luikart, G., England, P.R., Tallmon, D., Jordan, S. & Taberlet, P. (2003). The power and promise of population genomics: from genotyping to genome typing. *Nature Reviews Genetics*, 4 (12), 981–994. https://doi.org/10.1038/nrg1226

Marguerat, S., Wilhelm, B.T. & Bähler, J. (2008). Next-generation sequencing: applications beyond genomes. *Biochemical Society Transactions*, 36 (5), 1091–1096. https://doi.org/10.1042/BST0361091

Marsden, C.D., Woodroffe, R., Mills, M.G.L., McNutt, J.W., Creel, S., Groom, R., Emmanuel, M., Cleaveland, S., Kat, P., Rasmussen, G.S.A., Ginsberg, J., Lines, R., André, J.-M., Begg, C., Wayne, R.K. & Mable, B.K. (2012). Spatial and temporal patterns of neutral and adaptive genetic variation in the endangered African wild dog (Lycaon pictus). *Molecular Ecology*, 21 (6), 1379–1393. https://doi.org/10.1111/j.1365-294X.2012.05477.x

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal*, 17 (1), 10–12. https://doi.org/10.14806/ej.17.1.200

Milner-Gulland, E.J. & Woodroffe, R. (2001). Mammals, Conservation Efforts for. I: Levin, S.A. (red.) *Encyclopedia of Biodiversity*. New York: Elsevier, 811–824. https://doi.org/10.1016/B0-12-226865-2/00181-4

Morin, P.A., Luikart, G., Wayne, R.K., & the SNP workshop group (2004). SNPs in ecology, evolution and conservation. *Trends in Ecology & Evolution*, 19 (4), 208–216. https://doi.org/10.1016/j.tree.2004.01.009

Norman, A.J. (2016-02-26). *Genomic studies of contemporary processes in wild populations*. [Doctoral thesis]. https://pub.epsilon.slu.se/13114/ [2020-10-01]

Norman, A.J., Putnam, A.S. & Ivy, J.A. (2019). Use of molecular data in zoo and aquarium collection management: Benefits, challenges, and best practices. *Zoo Biology*, 38 (1), 106–118. https://doi.org/10.1002/zoo.21451

Norman, A.J. & Spong, G. (2015). Single nucleotide polymorphism-based dispersal estimates using noninvasive sampling. *Ecology and evolution*, 5 (15), 3056–3065. https://doi.org/10.1002/ece3.1588

Norman, A.J., Street, N.R. & Spong, G. (2013). De Novo SNP Discovery in the Scandinavian Brown Bear (Ursus arctos). *PLOS ONE*, 8 (11), e81012. https://doi.org/10.1371/journal.pone.0081012

Ray, J., Redford, K.H., Steneck, R. & Berger, J. (2013). *Large Carnivores and the Conservation of Biodiversity*. Island Press.
Ripple, W.J., Estes, J.A., Beschta, R.L., Wilmers, C.C., Ritchie, E.G., Hebblewhite, M., Berger, J., Elmhagen, B., Letnic, M., Nelson, M.P., Schmitz, O.J., Smith, D.W., Wallach, A.D. & Wirsing, A.J. (2014). Status and Ecological Effects of the World's Largest Carnivores. *Science*, 343 (6167). https://doi.org/10.1126/science.1241484
Roques, S., Sollman, R., Jácomo, A., Tôrres, N., Silveira, L., Chávez, C., Keller, C., do Prado, D.M., Torres, P.C., dos Santos, C.J., da Luz, X.B.G., Magnusson, W.E., Godoy, J.A., Ceballos, G. & Palomares, F. (2016). Effects of habitat deterioration on the population genetics and conservation of the jaguar. *Conservation Genetics*, 17 (1), 125–139. https://doi.org/10.1007/s10592-015-0766-5
RStudio Team (2020). *RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.*
Ruegg, K.C., Anderson, E.C., Paxton, K.L., Apkenas, V., Lao, S., Siegel, R.B., DeSante, D.F., Moore, F. & Smith, T.B. (2014). Mapping migration in a songbird using high-resolution genetic markers. *Molecular Ecology*, 23 (23), 5726–5739. https://doi.org/10.1111/mec.12977
RWCP & IUCN/SSC (2015). *Regional Conservation Strategy for the cheetah and African Wild Dog in Southern Africa*
Seeb, J.E., Carvalho, G., Hauser, L., Naish, K., Roberts, S. & Seeb, L.W. (2011). Single-nucleotide polymorphism (SNP) discovery and applications of SNP genotyping in nonmodel organisms. *Molecular Ecology Resources*, 11 (s1), 1–8. https://doi.org/10.1111/j.1755-0998.2010.02979.x
Shaw, G. (2013). Polymorphism and single nucleotide polymorphisms (SNPs). *BJU International*, 112 (5), 664–665. https://doi.org/10.1111/bju.12298
Spitzer, R., Norman, A.J., Königsson, H., Schiffthaler, B. & Spong, G. (2020). De novo discovery of SNPs for genotyping endangered sun parakeets (Aratinga solstitialis) in Guyana. *Conservation Genetics Resources*, 12 (4), 631–641. https://doi.org/10.1007/s12686-020-01151-x
Taylor, H.R., Colbourne, R.M., Robertson, H.A., Nelson, N.J., Allendorf, F.W. & Ramstad, K.M. (2017). Cryptic inbreeding depression in a growing population of a long-lived species. *Molecular Ecology*, 26 (3), 799–813. https://doi.org/10.1111/mec.13977
Tende, T., Ottosson, U., Hansson, B., Åkesson, M. & Bensch, S. (2010). Population size of lions in Yankari Game Reserve as revealed by faecal DNA sampling: Population size from faecal lion DNA. *African Journal of Ecology*, 48 (4), 949–952. https://doi.org/10.1111/j.1365-2028.2009.01196.x
Tensen, L., van Vuuren, B.J., du Plessis, C. & Marneweck, D.G. (2019). African wild dogs: Genetic viability of translocated populations across South Africa. *Biological Conservation*, 234, 131–139. https://doi.org/10.1016/j.biocon.2019.03.033
von Thaden, A., Cocchiararo, B., Jarausch, A., Jüngling, H., Karamanlidis, A.A., Tiesmeyer, A., Nowak, C. & Muñoz-Fuentes, V. (2017). Assessing SNP genotyping of noninvasively collected wildlife samples using microfluidic arrays. *Scientific Reports*, 7 (1), 10768. https://doi.org/10.1038/s41598-017-10647-w
Vignal, A., Milan, D., SanCristobal, M. & Eggen, A. (2002). A review on SNP and other types of molecular markers and their use in animal genetics. *Genetics Selection Evolution*, 34 (3), 275–305. https://doi.org/10.1186/1297-9686-34-3-275
Wake, D.B. & Vredenburg, V.T. (2008). Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the*

National Academy of Sciences, 105 (Supplement 1), 11466–11473. https://doi.org/10.1073/pnas.0801921105

Watson, F.G.R., Becker, M.S., Milanzi, J. & Nyirenda, M. (2015). Human encroachment into protected area networks in Zambia: implications for large carnivore conservation. *Regional Environmental Change*, 15 (2), 415–429. https://doi.org/10.1007/s10113-014-0629-5

Williams, L.M., Ma, X., Boyko, A.R., Bustamante, C.D. & Oleksiak, M.F. (2010). SNP identification, verification, and utility for population genetics in a non-model genus. *BMC Genetics*, 11 (1), 32. https://doi.org/10.1186/1471-2156-11-32

Wolf, C. & Ripple, W.J. (2018). Rewilding the world's large carnivores. *Royal Society Open Science*, 5 (3), 172235. https://doi.org/10.1098/rsos.172235

Woodroffe, R. & Sillero-Zubiri, C. (2020). *African Wild Dog Lycaon pictus (amended version of 2012 assessment)*. *IUCN Red List of Threatened Species*. https://www.iucnredlist.org/en [2021-01-11]

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