# Population genetics of the bearded vulture

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**Submitted in fulfilment of the academic requirements for the degree of**

**Master of Science**

**in the Discipline of Genetics**

**School of Life Sciences**

**College of Agriculture, Engineering and Science**

**University of KwaZulu-Natal**

**Pietermaritzburg Campus**
**2018**

1                                             **ABSTRACT**

2   The bearded vulture (*Gypaetus barbatus*) is a large, long-lived bird which inhabits high-
3   altitude mountains across Africa, Europe and Asia. Low breeding rates and heightened
4   persecution over the last two centuries have left many populations severely diminished or
5   extinct. The species is globally at risk and is thus listed as 'Near Threatened' by the
6   International Union for Conservation of Nature (IUCN). This global conservation status may
7   under-represent the threats faced by local populations. The southern African population,
8   which is the most geographically isolated, is estimated at 100 breeding pairs, and is classified
9   regionally as Critically Endangered.

10   This thesis aims to describe the genetic status of *G. barbatus* populations using a panel of 14
11   microsatellite loci. In the first chapter, the phylogeographic structure and genetic connectivity
12   among *G. barbatus* populations across Africa and Europe will be assessed. In particular, this
13   chapter focusses on determining if the geographically isolated southern Africa population is
14   genetically unique. In the second chapter, the genetic diversity of the southern African *G.*
15   *barbatus* population is compared to that of the Cape Vulture (*Gyps coprotheres*), Hooded
16   Vulture (*Necrosyrtes monachus*) and the African White-backed Vulture (*Gyps africanus*).
17   These vulture species are ecologically similar and also occur in southern Africa. In the third
18   data chapter, the genetic data is combined with ecological and behavioural data to determine
19   the southern African population's future in a population viability analysis.

20   My results show that the presence of spatial genetic differentiation and the low level of gene
21   flow into the southern African population supports the management of this population as a
22   separate entity. The genetic assessment of the global and southern African *G. barbatus*
23   populations is useful in revising and updating the current conservation management for this
24   species. Although the southern African population harbours more homozygosity in
25   comparison to two other vulture species occurring in southern Africa and beyond, these
26   bearded vultures had comparatively high allelic richness. The expected high inbreeding found
27   in the southern African *G. barbatus* population highlights the need for conservation
28   programmes to effectively manage and maintain the extant genetic diversity.

29    Recommendations reported in this thesis will contribute significantly towards conservation

30    efforts to ensure the sustainability of this emblematic species.

31 **PREFACE**

32 The data described in this thesis were processed in Pietermaritzburg, Republic of South Africa

33 from March 2016 to October 2017. Laboratory work was carried out while registered at the

34 School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, under the supervision

35 of Dr Sandi Willows-Munro and Dr Sonja Krüger.

36

37 This thesis, submitted for the degree of Master of Science in the College of Agriculture,

38 Engineering and Science, University of KwaZulu-Natal, School of Life Sciences,

39 Pietermaritzburg campus, represents original work by the author and has not otherwise been

40 submitted in any form for any degree or diploma to any University. Where use has been made

41 of the work of others, it is duly acknowledged in the text.

42

43

44 …………………………

45 Melanie Burke

46 March 2018

47

48 I certify that the above statement is correct and as the candidate's supervisor I have

49 approved this thesis for submission.

50

51 ………………………….

52 Doctor Sandi Willows-Munro and Dr Sonja Krüger

53 Supervisor

54 March 2018

55

## COLLEGE OF AGRICULTURE, ENGINEERING AND SCIENCE
### DECLARATION 1 - PLAGIARISM

I, Melanie Burke, declare that

1.  The research reported in this thesis, except where otherwise indicated, is my original research.

2.  This thesis has not been submitted for any degree or examination at any other university.

3.  This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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    a.  Their words have been re-written but the general information attributed to them has been referenced
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5.  This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

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90 **COLLEGE OF AGRICULTURE, ENGINEERING AND SCIENCE**
91 **DECLARATION 2 - PUBLICATIONS**

92 DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research
93 presented in this thesis.
94
95 Publication 1- in prep.

96 **MB Burke, S Krüger and S Willows-Munro**

97 **Genetic structuring in the endangered bearded vulture (*Gypaetus barbatus, L.*) as revealed**

98 **by microsatellite DNA: Placing the South African population in a global context.** In review

99 *Author contributions:*

100 MBB conceived paper with SK and SWM. MBB collected and analysed data, and wrote the

101 paper. SWM contributed valuable comments to the manuscript.

102

103 Publication 2- in prep.

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106 **microsatellite DNA: Placing the isolated southern African population in context of the**

107 **African Vulture Crisis.** In review

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109 MBB conceived paper with SK and SWM. MBB collected and analysed data, and wrote the

110 paper. SWM contributed valuable comments to the manuscript.

111

112 Publication 3- in prep.

113 **MB Burke, S Krüger and Willows-Munro**

114 **A genetically informed population viability analysis of the southern African bearded vulture**

115 ***Gypaetus barbatus* population.** In review

116 *Author contributions:*

117 MBB conceived paper with SK and SWM. MBB collected and analysed data, and wrote the

118 paper. SWM contributed valuable comments to the manuscript.

119 Signed: …………………………………………

120 Melanie Burke March 2018

121

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**ACKNOWLEDGMENTS**

123 I appreciate all who enabled this reserach, and showed their support throughout the duration
124 of this project. I extend gratitude to those institutes which loaned samples from their valuable
125 specimens, and to all who gave of their time in assisting me.

126 1 Corinthians 2v5 "that your faith should not be in the wisdom of men but in the power of
127 God."



128 A near-adult bearded vulture *Gypaetus barbatus*, KwaZulu-Natal Midlands South Africa
129 (Photo credit JP Streicher).

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**CHAPTER 1: Literature review and aims of the study**

**Introduction**

1    There is now no doubt that the recent acceleration in anthropogenic activity has impacted natural

2    systems, and it is widely believed that a geological epoch named the "Anthropocene" has been

3    entered into (Barnosky et al. 2011; Ripple et al. 2017; Steffen et al. 2015). The Anthropocene is

4    marked by a sixth mass extinction which has been predicted to culminate in the loss of half the

5    world's biodiversity (Barnosky et al. 2011; Koh et al. 2004). Rapid population and species' declines

6    logically follow the destruction of natural habitats. However, where the loss of a species which is

7    integral to its habitat occurs, it has the potential to elicit a cascading effect of 'secondary extinctions'

8    either directly (eg. obligate mutualism) or indirectly (eg. Loss of ecosystem engineers whose presence

9    significantly modifies an ecosystem, loss of a service) (Groom 2006).

10    Recent declines in vultures are an example of this. The rapid extirpation of vultures in India as a

11    consequence of using the veterinary drug diclofenac (Paudel et al. 2016; Prakash et al. 2012) had a

12    dramatic effect on the rate of rabies incidents between 1992-2006. The epidemic was attributed to

13    the increase in decaying carcasses which supported an unprecedented surge in the population of

14    feral dogs (Markandya et al. 2008). The "Asian Vulture Crisis" is comparable to the "Pan-African

15    Vulture Crisis". African vulture population declines reported as well as the associated ecological,

16    economic and human costs are concomitant with the loss of vultures (Ogada et al. 2016). Six of

17    Africa's 11 vulture species are now on the edge of extinction. On a global scale, "the vulture crisis"

18    describes the concomitant extinction of local vulture populations. The reasons behind vulture

19    declines are varied. Species in natural habitats are subjected to factors such as pollution, over-

20    exploitation (Ogada and Buij 2011), invasive species and the influx of infectious diseases

21    (Cunningham et al. 2003) and to a lesser extent habitat loss (Pain et al. 2003; Thewlis et al. 1998).

22    Poisoning, collisions, electrocutions and habitats loss account for the primary factors which have led

23    to the African vulture crisis (Ogada et al. 2016).  Ecological conservation efforts primarily focus their

24    efforts on improving these factors. However, while improving those external deterministic factors-

25    which have historically driven vulture species to critical population numbers- may help initially,

26    threatened populations are subject to genetic stochastic factors (such as inbreeding, heightened

27    genetic drift) which are unavoidable in small, isolated populations. As both the deterministic and

28    stochastic factors are inextricably linked, it is the interaction between them which will determine the

29    success of any conservation scheme. The current project will be examining the genetic stochastic

30    factors, using the bearded vulture *Gypaetus barbatus* as its subject.

31    **The study species: *Gypaetus barbatus***

32        The bearded vulture, *Gypaetus barbatus*, is a large, osteophagous old-world species, which

33    belongs to the family Accipitridae (Donázar et al. 1993; Hiraldo et al. 1979; Margalida and Bertran

34    2000). This species has a long lifespan (20 years in the wild and up to 40 years in captivity) but

35    breeding rates are extremely low (Brown 1997; Brown 1977). Both the abundance and breeding rate

36    of the species have diminished in the last century owing to direct (Hernández and Margalida 2009)

37    and indirect anthropogenic causes (Hernández and Margalida 2008). Similar to other vulture species,

38    the bearded vultures' persistence is threatened and the species is thus of considerable conservational

39    concern. They are integral to ecosystem health by, amongst others, controlling pests, aiding nutrient

40    recycling and feeding off carrion and thereby reducing the potential of disease spread (DeVault et al.

41    2016). Vultures are also ecologically sensitive predators and are thus valuable indicators of habitat

42    quality (Lerner and Mindell 2005).

43    The use of population genetics to determine the spatial distribution of genetic variation and

44    gene flow among fragmented populations is a vital component of conservation planning (Frankham

45    et al. 2002). Endangered species are characterized by a low number of individuals distributed across

46    a few populations. Fragmented populations may have limited contact, which would accelerate

47    genetic drift and inbreeding (Méndez et al. 2011; Willi et al. 2007). Consequently, this leads to a

48    reduction in genetic variation present in small, isolated populations. Even if the number of individuals

49    in a population were to increase owing to ecological conservation (thereby improving life span), the

50    level of genetic variation will not necessarily increase as drastically as mature individuals may

51    continue to dominate the breeding pool. Thus the maintenance of genetic variation in natural

52    populations is a key component of any conservation strategy. Genetic variation is also a direct

53    prediction of population fitness. Without genetic variation, a population cannot respond accordingly

54    (evolve) in response to a change in their external environment (climate and emerging disease) (Hartl

55    and Clark 1997). This study aims to provide a better understanding of the population structure and

56    connectivity between the contemporary isolated populations of the bearded vulture.


57    **Distribution and habitat**


58    The bearded vulture is sparsely distributed across a wide geographic range across the

59    Palearctic, Afrotropic and Indomalay regions (Margalida et al. 2003). Historically the populations

60    were abundant, but have become locally extinct or survive in highly habitat-specific isolated refuges

61    across their former range (Brown 1997; Gautschi et al. 2000). The Pyrenees, the southern Balkans,

62    the islands of Corsica and Crete, and a re-established population in the Alps currently comprise the

63    European populations; while the Maloti-Drakensberg (Lesotho boarder), Ethiopia and Morocco are

64    the refuges for the African bearded vulture populations (Brown 1997; Godoy et al. 2004).

65    Nests are situated preferentially on large, rocky outcrops and cliff ledges. Home range size

66    differs between the age classes with the highest being recorded for nonbreeding adults (Margalida

67    et al. 2008). Territory size is primarily dependent on food supply and available nesting sites, rather

68    than on a dominant patriarchal hierarchy system as this species is typically monogamous. Usually,

69    successful reproduction requires the significant contribution of both sexes as food can be scarce and

70    unpredictable, and this precludes the raising of two chicks (Margalida et al. 2003). In areas with

71    artificial feeding sites however, the increased food abundance has permitted polyandry where

72    reproductive output is maximised by an additional individual assisting with parenting the chick

73    (Heredia and Donázar 1990; Margalida et al. 2003). A polyandrous trio usually consists of a breeding

74    female and two males; one subordinate and one dominant. Studies by Bertran and Margalida (2004)

75    suggest that these are unrelated individuals, where females have the potential to maximise the males'

76    help (nest building, territory defence, food) via copulation.  The precise niche requirements and spatial

77    distribution of sub-adults, who don't require nesting sites, differs slightly from mature individuals as

78    they exhibit a nomadic-style existence dependent on food supply (Brown 1997; Milanesi et al. 2016).

79    Unlike other vultures, bearded vultures' diet consists almost exclusively of carrion bone (Brown and

80    Plug 1990; Roberts et al. 2005). Only in the early fledgling stages will breeding pairs sustain their

81    offspring with a soft tissue diet (Thibault et al. 1993).


82    **Population genetics**

83    *Genetic diversity*

84    Understanding the genetic variation present within a species is crucial. The need to conserve

85    genetic diversity is one of the top conservation priorities recognised by the International Union for

86    Conservation of Nature (IUCN). It is assumed that large populations harbour a healthy level of genetic

87  variation, one that would support the species' existence sustainably. Loss of genetic variation through

88  bottlenecking, founder effects and subsequent inbreeding are consequences that small populations

89  inevitably have to bear (Alcaide et al. 2010). Usually, the loss of genetic variation correlates to a

90  decrease in adaptive potential, which could jeopardise the species long-term survival. Threatened

91  species are intrinsically more prone to extinction and should therefore come under different

92  management strategies in comparison to larger, panmictic populations. As such, monitoring effective

93  population size is a pervasive issue in conservation genetics, not only due to the immediate effects

94  of limited genetic diversity, but more so because evolutionary processes are fundamentally different

95  between small and large populations (Frankham et al. 2002). Stochastic effects (genetic drift, fixation

96  of advantageous/disadvantageous alleles) rather than natural selection prevails in small populations.

97  These stochastic effects reduce polymorphism and hastens the loss of genetic variation.

98  Reintroducing and re-establishing populations of bearded vultures with limited founder individuals,

99  such as is occurring in the Alps and Andalucía (southern Spain) (Negro and Torres 1999), emphasizes

100  the need not only to have a thorough understanding of the genetics of the species (Bretagnolle et al.

101  2004), but to incorporate genetic information (with the biology and ecology of the species) in

102  population viability analyses.

103     The genetic processes referred to above are termed "extinction vortices" where mutual

104  reinforcement occurs between demographic and genetics processes. The interaction between these

105  processes could drive populations to extinction. Conservation of small populations should attempt

106  to disrupt the vortex by using population genetics to predict and potentially reverse the extinction

107  process.  Although this has not been documented in the wild, wild species studied under natural

108  conditions frequently display moderate to high levels of inbreeding depression in fitness traits which

109  reduces reproductive output and ultimately impacts the population's dynamics.

110    *Inbreeding*

111    An immediate ramification of small populations is inbreeding, as the number of reproductive

112    individuals to choose from is reduced. Inbreeding is marked by the accrual of disadvantageous traits:

113    a reduction in offspring number, reduced juvenile survival, poor sperm quantity and quality among

114    others (Keller 1998; Keller and Waller 2002). Inbreeding depression is syndrome marked by an accrual

115    of these traits. This has been documented in numerous studies examining populations of captive and

116    bottle-necked populations (Keller and Waller 2002; Laikre and Ryman 1991). This grouping of

117    deleterious effects in small populations is again, a cause for concern when implementing

118    conservation strategies on endangered species with declining populations. The inbreeding coefficient

119    is the unit, which is used to measure the probability that both alleles at a locus are identical by

120    descent (autozygous). Inbreeding increases over time in a close, finite population and decreases

121    population viability (Frankham 2005).

122    *Migration*

123    Fragmentation, and its effects on small populations, depends on time since 'isolation' and

124    migration rates between fragmented populations. This in turn depends on how isolated populations

125    are, as well as meta-population structure. Isolation in an already threatened population further

126    diminishes its viability and accentuates the evolutionary processes (genetic drift, divergence rates)

127    at work in it. Population differentiation is measured by F-statistics (Fit, Fis and Fst). Gene flow

128    between sub-populations lessens the adverse effects of isolation by introducing new genes into the

129    breeding population. Ideally, the effects of inbreeding and extinction risk can be alleviated in

130    fragmented populations if they are genetically connected by migration. Migration rates can depend

131    on the species biology and the distance between isolated populations and are therefore often

132  difficult to quantify. Gene flow, then, is used as an indication of migration. In the case of the Bearded

133  vulture, it would be erroneous to believe that birds actively flew between the populations, and this

134  thesis does not intend to claim that active migration is presently occurring between the populations.

135  However, migration rates perhaps present the closest proxy of past levels of genetic mixing.

136  **Molecular markers used in conservation genetics**

137  Despite their conservation status, vultures have been the subject of only a few phylogenetic

138  studies (Arshad et al. 2009; Lerner and Mindell 2005). Studies examining genetic diversity below the

139  species level are also limited, but have used mitochondrial DNA (Seibold and Helbig 1995), nuclear

140  sequence markers (Lerner and Mindell 2005) and allozymes (van Wyk et al. 1992) to examine

141  population level processes. Conclusions drawn from these few studies are alike - low genetic variation

142  with minimal hope of adaptability, all predicting a dire future for the species studied. Several genetic

143  studies have been conducted on the bearded vulture (Gautschi 2001; Gautschi et al. 2003b; Godoy

144  et al. 2004). Most of these studies have focused on the captive and European populations (Gautschi

145  et al. 2003a; Negro and Torres 1999). Those studies that have included African, Asian and European

146  samples (Godoy et al. 2004; Krüger et al. 2015) analysed mitochondrial DNA markers only and thus

147  the conclusions drawn are limited to the maternal inheritance pattern. Godoy et al. (2004) examined

148  the phylogeographic structure of bearded vultures using mitochondrial control region sequences. By

149  using museum specimens, they were able to include many historic populations, which are now

150  extinct. Despite this, the sampling used in the study was biased towards European and Asian

151  populations with only a few samples from Ethiopia, one sample from Yemen, one sample from Algeria

152  and two samples from South Africa. The study noted especially low levels of genetic diversity in

153  Ethiopian bearded vulture population when compared to samples gathered from other regions

154 across the species range. Unfortunately, the limited sample size of the South African population

155 precluded any accurate genetic assessment. This study also revealed significant genetic structuring

156 across the distribution of the species, which is unexpected in a species that travels distances that

157 should permit gene transfer. The authors found Spanish and Sardinian bearded vultures to be

158 significantly different from all other populations, but the remaining populations were not significantly

159 differentiated. Their median joining network reinforced this result; the eastern samples grouped

160 strongly together, while western populations formed their own cluster. Central Asian and roughly

161 grouped eastern European samples did not differ considerably and may therefore have come from

162 the peripheral habitat of the same broad population. In addition to this, they found low levels of gene

163 flow and thus haplotype differentiation between even geographically close populations. A study by

164 Krüger et al. 2015, also used two mitochondrial markers (control region and cytochrome *b*) to

165 examine the genetic diversity and connectivity present within the Sub-Saharan population. Here

166 again, the analyses detected reduced genetic variation and no significant differentiation between the

167 southern African and Ethiopian population. The study confirmed Godoy et al. (2004) assertion that

168 the global population should be managed as a single entity as the African samples clustered with

169 those from Europe, Asia and the Middle East. Moreover, the absence of vast differences in life history

170 traits, morphology, habitat and behaviour support this conclusion.

171     Mitochondrial DNA provides a good first assessment of phylogeographic patterns (Godoy et

172 al. 2004; Seibold and Helbig 1995). The use of a single locus system may, however, not be able to

173 answer evolutionary questions that require genome-wide information, or that require molecular

174 markers of a higher mutation rate that detect diversity below the species level. Revealing recent

175 (contemporary) genetic trends necessitates fast-evolving sections of the genome that are not

176 evolutionarily constrained. Population-level questions are amenable to applications of

177 microsatellites or SNPs (single nucleotide polymorphisms). Microsatellites are sensitive to genetic

178 changes such as those in effective population size fluctuations and low rates of migration.

179 Microsatellites may reveal crucial demographic information that other markers (e.g. mitochondrial

180 analyses- containing less genetic resolution and reduced sensitivity) may not detect.

181       Microsatellites are typically located ubiquitously throughout the non-coding regions of a

182 genome, and less so in coding segments (Chistiakov et al. 2006; Primmer et al. 1997). Microsatellites

183 are small (short tandem repeats- STRs) codominant markers which exhibit high levels of allelic

184 polymorphism (Callen et al. 1993), and undergo polymerase chain reaction (PCR) for amplification

185 with relative ease (Chistiakov et al. 2006).  A prominent feature of microsatellites as molecular

186 markers is their high mutational rate and consequent high variability within populations and species

187 (Chistiakov et al. 2006). The mutational rate of microsatellites is approximately $10^{-2}$ to $10^{-6}$ per locus

188 per generation (Ellegren 2000) whilst non repetitive DNA is approximately $10^{-9}$ per locus per

189 generation.

190       Microsatellites have proven to be an exceptional tool in the field of molecular ecology (Arshad

191 et al. 2009; Chistiakov et al. 2006). They are widely used in population and conservation genetics

192 (Arshad et al. 2009) and have been used to quantify genetic variation within a species as well as

193 deduce evolutionary origins within and among species (Chistiakov et al. 2006; Coetzer et al. 2015;

194 Ellegren 2000). Due to their versatility to monitor and direct conservation efforts, and to inform

195 reintroduction programmes of threatened species, microsatellites have been a prized breakthrough

196 in biological studies (Mira et al. 2002). Not only can they be implemented to predict future population

197 structure, but because they can be amplified from historic tissue one can also use them to study the

198 past genetic structure of a population in the study of extinct species, or comparing genetic diversity

199 over a wide temporal scale. The short length of microsatellites allows them to be readily amplified in

200 archival museum tissues (Chistiakov et al. 2006; Seutin et al. 1991).

201      Accordingly, microsatellites are regularly used in population biology (Primmer et al. 2005) and

202 are recommended for conservation efforts to assess the relatedness between individuals (Arshad et

203 al. 2009; Coetzer et al. 2017), as  well as to address questions of effective population size, population

204 structure, reproductive systems, migration and colonization rates (Gautschi et al. 2003; Banhos et al.

205 2008). For this type of study to be relevant for contemorary conservation practises, a reference point

206 is needed to add context and to make practical conclusions. Ideally, we would like to compare current

207 levels of diversity to that of the same species a generation or two ago. These are unfortunately

208 unavailable. The closest comparison we can achieve, is to compare amongst species which have

209 suffered like declines and are expected to respond similarly. In this way, we can guage to what extent

210 a species has suffered, and how intensively and urgently conservation would need to act.

211      Gautschi et al. 2003 developed and used a suite of 14 microsatellites to compare between the

212 variation present in captive and natural European populations (Pyrenees, Sardinia, Alps) of bearded

213 vulture. Here the authors found the observed heterozygosity (~0.56) to be slightly lower than should

214 be expected (~0.60) if the population conformed to expectation of Hardy-Weinberg (Gautschi et al.

215 2003a). The captive population studied maintained an high level of diversity ($H_o = 0.68$; No. of alleles

216 = 8.5) in comparison to wild populations.

217 **Main aims of study**

218      This study aims to provide a better understanding of the genetic structure among

219 contemporary populations of bearded vulture across Africa, Europe and Asia, but will also focus on

220 the southern African population. This study extends the work of Godoy et al. (2004) and Krüger et

221     al. (2015) by increasing the number of bearded vulture individuals included and loci analysed, and

222     by using microsatellite loci as compliment to mitochondrial data.

223         In chapter 2, microsatellite data will be used to establish whether genetic structuring has

224     resulted from prolonged isolation of small populations following the global collapse of bearded

225     vulture populations. Several indices of genetic diversity will be estimated to provide information on

226     the extent of genetic drift and inbreeding in isolated populations across the range of the species.

227     Migration rates among African and European populations will also be estimated. Estimating

228     population structure and genetic status of disjunct populations will provide conservation

229     authorities with the necessary information needed to make, and implement the appropriate

230     conservation measures to protect the bearded vulture. In particular, the information from this

231     chapter will be used to determine if global bearded vulture populations should be managed as a

232     single unit or if isolated population should be regarded as different management units.

233         In chapter 3, I will focus on the southern African bearded vulture population. In this chapter,

234     the genetic diversity of the southern African population will be compared to that of three

235     ecologically similar sub-Saharan vulture species.

236         In chapter 4, a population viability analysis will be conducted by combing the specie's

237     biology and life history traits with genetic data (allelic frequencies). There should not be a vast

238     difference in the population trajectories if the bearded vulture's genetics are still diverse and stable

239     enough. However, if the population's genetics are severely reduced, the probabilistic trajectory for

240     this species will be negatively altered.

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**CHAPTER 2**

*Evidence of genetic structure across African and European populations of the bearded vulture*

*(Gypaetus barbatus, L.): Placing the southern African population in a global context*

**Abstract**

387    The bearded vulture *Gypaetus barbatus* has a wide and intermittent distribution across the

388    Palearctic, Afrotropical and Indomalay regions. Despite this wide distribution, some local populations

389    have undergone collapses because of several compounding anthropogenic threats. Continued

390    persecution and prolonged isolation has resulted in a decline in the species. Genetic depauperation

391    and geographic isolation are known to adversely affect the evolutionary potential of a species in the

392    long term and this is the impetus behind conducting research on isolated and dwindling populations

393    of threatened species. Here, the genetic structure of the bearded vulture population on a global scale

394    is investigated. Specifically, this chapter seeks to determine whether the southern African population

395    is disjunct and genetically separate from the other African and European populations. Analyses

396    reveals that the global Beaded vulture population is genetically structured. The southern Africa

397    population is genetically different from European/Asian populations, and to a lesser extent is also

398    different from eastern and western Africa population of bearded vultures. Population differentiation

399    statistics confirm that the genetic structuring is geographically correlated, with low levels of

400    migration detected between some populations. The northern African population seems to be the

401    meeting point between African and European/Asian populations. The southern and eastern African

402    populations are considerably isolated and analysis suggests very limited genetic exchange has

403    occurred between these populations in the recent past. Continued geographical isolation,

404    particularly in the southern African population, may in coming generations exacerbate the population

405    differentiation. As the populations can be considered management units, I argue for a revised

406    assessment of the current management of the bearded vulture species as separate populations, and

407    not as a single, panmictic global population.


**Introduction**

408        Historically bearded vulture, *Gypaetus barbatus*, populations were considerably more

409    abundant, albeit sparsely distributed across a wide geography ranging from Palearctic, through

410    Neotopic and into Indomalay regions (Godoy et al. 2004).  In the past century however, they have

411    become locally extinct, or survive in highly habitat-specific isolated refuges across their former range

412    (Brown 1997; Gautschi et al. 2000). The Pyrenees, the southern Balkans, the islands of Corsica and

413    Crete, and a re-established population in the Alps currently comprise the European populations;

414    while southern Africa, Ethiopia and Morocco are the remaining habitats for the African bearded

415    vulture population (Brown 1997; Godoy et al. 2004).

416

417        In the southern hemisphere, electrocution and unintentional poisoning through veterinary

418    drug-use are the primary culprits for population decline in vultures (Ledger and Annegarn 1981;

419    Ogada 2014). Other factors such as intentional poisoning for the use of vulture body parts in the

420    illegal wildlife and Muthi trade are also having an impact on the population (Ogada et al. 2016). The

421    Northern hemisphere populations also suffered collapses through ingestion of heavy metals

422    (Hernández and Margalida 2009; Margalida et al. 2008). In addition, as a consequence of the EU

423    sanitary legislation of 2000 which progressively limited the unauthorised disposal of carcasses, the

424    vulture populations also suffer from lack of food (Donázar et al. 2010) and the European populations

425    now require supplemental feeding (Hille et al. 2016; Oro et al. 2008; Piper 2005).

426      Reduced population numbers have led to the rapid demise and extinction of local populations

427    across the distribution of bearded vulture (Mingozzi and Estève 1997). African populations of the

428    species have been particularly hard hit (Figure 2.1). This has led to the fragmentation of the African

429    population into several isolated regions. Ethiopia is believed to hold a few hundred pairs, however

430    reliable data are not available and these values are therefore thought to be considerably

431    overestimated  (Ash and Atkins 2010; Brown 1977). A 2011-survey identified only three nest-sites in

432    Kenya and roughly double that in Tanzania (Krueger et al. 2014). Morocco is home to fewer than 20

433    individuals and the species is considered extinct in Tunisia (Godino et al. 2003). The entire southern

434    hemisphere population is isolated to the south eastern regions of South Africa, where they roost and

435    breed on high altitude cliffs of the Maloti-Drakensberg mountains which form the border between

436    KwaZulu-Natal, South Africa and Lesotho (Brown 1997). Current estimates suggest that this

437    population consists of between 368-408 individuals (109 - 221 breeding pairs) and occupies a

438    breeding range of 28 125 km$^2$ (Krueger et al. 2014).

439

440      Small, disjunct populations- such as the southern African one- are of conservation concern for

441    several reasons. Due to the reduced effective population size, small populations experience amplified

442    genetic drift, inbreeding and a greater loss of genetic diversity (Allendorf 1986; Frankham 1995; Lacy

443    1987). Although the inherent effect of low genetic diversity on species persistence was long

444    underestimated (Lande and Barrowclough 1987) there is now no doubt that genetic variation

445    correlates to individual fitness and population stability (Reed and Frankham 2003).

446

447      Following severe population collapses, studies on bearded vultures have found a reduced

448    level of genetic diversity in the wild populations of Europe and southern Africa (Gautschi et al. 2003;

449    Godoy et al. 2004; Krüger et al. 2015). Past studies based on mitochondrial data found evidence for

450    two distinct lineages: one occurring predominantly in western Europe, and the other extending over

451    Africa, eastern Europe and central Asia. This latter study detected high levels of inter-population

452    differentiation, with the maximum admixture between the two lineages occurring in Greece and the

453    Alps (Godoy et al. 2004). Godoy et al. (2004) found the populations to be genetically structured as a

454    consequence of the extinction of the central European population. The authors suggest that the

455    lineages underwent allopatric differentiation (Mediterranean vs. Africa/Asian), and range expansion

456    in the African/Asian cohort which resulted in secondary contact zones in central Europe and northern

457    Africa (Godoy et al. 2004). However, these remnant populations were found to be ecologically

458    interchangeable, even between vastly segregated populations. Additional studies have confirmed

459    reduced genetic diversity in bearded vulture populations using both mitochondrial (Krüger et al.

460    2015) and microsatellite loci (Godoy et al. 2004). However, the overriding conclusion is that the global

461    population essentially behaves as a panmictic species (genetic admixture) and all stocks are therefore

462    interchangeable for the purposes of conservation (Godoy et al. 2004; Krüger et al. 2015). These

463    studies however have focussed their analyses either primarily on the European population (with low

464    representation from southern Africa), or on slow-evolving parts of the genome. In this chapter, I use

465    microsatellite-derived data to confirm and to test for resultant structuring of the global population

466    as a consequence of fragmented populations. Microsatellite markers evolve at a faster rate than the

467    mitochondrial genome, and should therefore provide greater resolution of potential fine-scale

468    genetic structuring (Froeschke and von der Heyden 2014). This should elucidate contemporary

469    factors rather than deep-wrought evolutionary events. This study also expands on previous research

470    by increasing the sample size used in analyses, particularly the southern African population's

471    representation in the data set is increased to adequately and accurately place it in a global context.

472    The broad aim of the research presented here is to test for the presence of genetic structuring

473    in the global bearded vulture population using microsatellites. I test specifically whether the

474    geographically isolated population in southern Africa has resulted in genetic isolation from other

475    bearded vulture populations. This was done by testing for the presence of migration across Africa

476    and by examining the migratory links between African and European populations. Understanding the

477    genetic structure across a species with such a wide distribution is essential in establishing proper

478    conservation management programs.
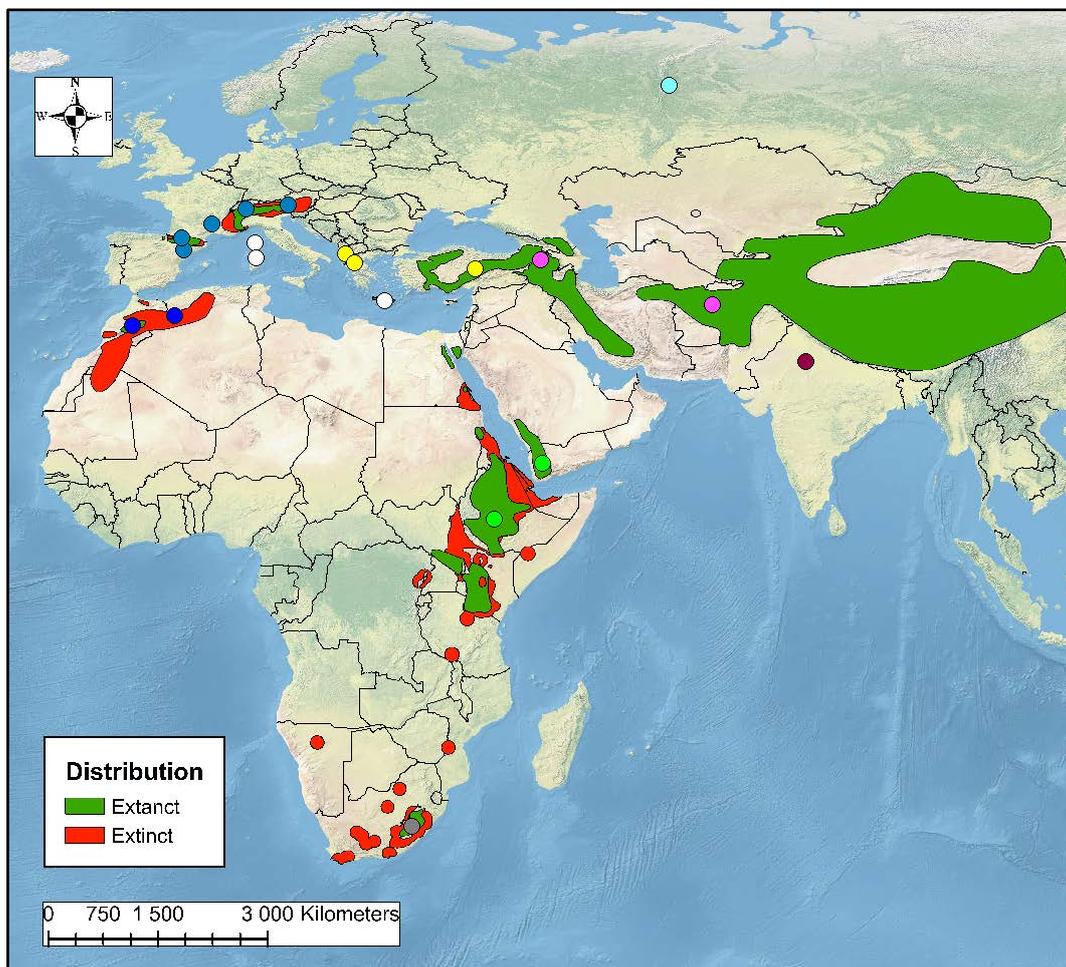


Figure 2.1: The bearded vulture's *Gypaetus barbatus* global distribution across the Palearctic,
Afrotropical and Indomalay regions. Green shading indicates the extant range and red shading where
populations have become locally extinct. Nine coloured circles indicate the locality of samples
included in this study (see Appendix Table 2.1.).

**Materials and methods**

479    *Samples*

480    A total of 217 samples of bearded vulture (*Gypaetus barbatus*) comprise the sample set for

481    this chapter. The countries from which these samples were gathered include: southern Africa (n= 52),

482    Ethiopia (n= 16), Yemen (n= 2), Morocco (n= 1), Algeria (n= 5), Albania (n= 1), Greece (n= 14), Turkey

483    (n= 1), Caucasus (n= 7), central Asia (n= 4), Turkestan (n= 5), Kyrgyzstan (n= 2), central Russia (n= 11),

484    India (n= 5), Crete (n= 1), Corsica (n= 3), Sardinia (n= 30), Switzerland (n= 35), Austria (n= 1), the

485    Pyrenees (n= 9), France (n= 2), Spain (n= 10). To avoid grouping bias, samples were grouped rigidly

486    by the locality information attached to the samples, which in cases was very broad. Sample details

487    are given in Appendix Table 2.2. These samples came in various tissue forms: blood, feather, and

488    archival tissue (toepad and dried skin). The use of toepad tissue and feathers is known to be a reliable

489    source for retrieving genetic information from deceased individuals and extinct populations (Horváth

490    et al. 2005). Archival samples were received from the Durban Natural Science Museum, the American

491    Museum of Natural History, the Naturalis Biodiversity Centre in Leiden, and from the Zoology

492    Museum at the University of Zurich. Contemporary samples came from the work of Dr. Sonja Krüger.

493    Samples varied in age. The earliest sample was gathered in 1793 and the most recent samples came

494    from feathers collected in 2012.

495    *DNA extraction*

496    The NucleoSpin® Tissue kit (Macherey-Nagel, Nucleic acid and protein purification, South

497    Africa) was used for all DNA extractions for this study. The standard protocol for extracting genetic

498    material from blood was followed for blood samples. The protocol was modified as coagulation had

499    occurred during transportation in some samples, and liquid blood could not be pipetted. Whatman®

500 FTA® Elute cards were immersed in the coagulated blood mass until some had transferred to the

501 card. Drying of the FTA cards took ~2 days after which the standard protocol for blood extractions

502 was followed. The feather extractions were done using the standard protocol for muscle tissue with

503 the following modifications: incubation of the ~5mm feather tip and proteinase K for 48 hours, lysate

504 was then incubated in B3 (buffer) for 45 minutes (70°C), the final volume of pre-warmed Buffer BE

505 was decreased to 80µl, incubation at 70°C for 20 minutes followed by centrifuging and then

506 reapplication of the solution onto the membrane and a final incubation at 70°C for an additional 5

507 minutes. This was followed by a final centrifugation step. Extractions from archival toepad or dried

508 skin were performed using the standard protocol for muscle tissue modified to optimize DNA

509 extraction. In this case, the final volume of pre-warmed Buffer BE was decreased to 80µl and included

510 an incubation step at 70°C for 10 minutes followed by centrifuging and then reapplication of the

511 solution onto the membrane and a final incubation at 70°C for an additional 10 minutes followed by

512 a final centrifuging step to obtain the DNA extract. DNA concentrations were determined using the

513 ThermoScientific NanoDrop 2000 spectrophotometer (Inqaba Biotec, South Africa). All DNA extracts

514 were stored at -20°C. Dr Willows-Munro laboratory has a long and successful history of working with

515 archival samples. Contamination was avoided by following all the necessary and standard laboratory

516 procedures when processing contemporary and historic smaples. In addition, exceptionally old

517 samples were processed twice for confirmation.


518 *Microsatellite amplification*

519 Fourteen microsatellite loci (Table 2.1) were chosen from a potential suite of 18 markers used

520 in previous studies. These microsatellite primers were developed specifically for European

521 populations of *Gypaetus barbatus* (Gautschi et al., 2000) and for *Gyps* species (Mira et al., 2002). The

522 vulture genera *Gyps* and *Gypaetus* both belong to the Accipitridae family. Each microsatellite locus

523 was amplified independently after which loci were combined to form multiplexes. Four of the primers

524 were problematic in amplifying (BV 1, BV13, BV 16 and BV 20). The remaining primers were amplified

525 in four multiplex reactions, and one (BV 17) was run in isolation (Multiplex 1 through 4, Table 2.1)

526 using KAPA2G Fast multiplex PCR Kit. The KAPA2GT Fast Multiplex PCR Kit (KAPA Biosystems, Cape

527 Town, South Africa) contains KAPA2G Fast HotStart DNA Polymerase, reaction buffer, dNTPs and

528 MgCl$_2$. The multiplex PCR reactions consisted of 5µl KAPA2G Fast Multiplex mix, 0.1- 0.2 µM of each

529 primer, 0.5-2.0µl of template DNA (muscle tissue or blood) or 3.5µl of template DNA (dried skin or

530 feather), purified water was added to each reaction to make up a final reaction volume of 10µl. The

531 following thermocycler cycle parameters were used for PCR of Multiplex 2 and 3: 95°C for 3 minutes

532 as the initial denaturation step, 30 cycles at 95°C for 15 seconds, 60°C for 30 seconds, 72°C for 1

533 minute, with a final elongation step at 72°C for 10 minutes. The samples were held at 4°C once the

534 cycle had completed running. Multiplex 1 and 4 PCR cycling parameters remained the same as above,

535 but the annealing temperature was decreased to 58°C. The amplified products were sent to the

536 Central Analytical Facilities (Stellenbosch University, South Africa) for fragment analysis. To ensure

537 correct genotype scoring, roughly 20% of samples were reamplified and negative controls were also

538 sent for genotyping to confirm no contamination occurred. Chromatograms of each microsatellite-

539 genotyped individual were analysed using the software package GeneMarker® v2.4.0 (Soft Genetics).

540 Genotype scores were documented on Microsoft Excel.

Table 2.1: Microsatellite marker multiplex combinations, and associated information for the selected primers.

| Multiplex | Loci | Expected product size | Dye Used | Annealing temperature | Amplified (Yes/No) | Citation |
|---|---|---|---|---|---|---|
| 1 | BV 9* | 212 | Hex | 58°C | Yes | Gautschi et al. 2000 |
| | BV14 | 162-164 | Fam | 58°C | Yes | Gautschi et al. 2000 |
| | Gf11a | 130-147 | Hex | 58°C | Yes | Mira et al. 2002 |
| 2 | BV2* | 115-120 | Hex | 60°C | Yes | Gautschi et al. 2000 |
| | Gf8G | 274 | Fam | 60°C | Yes | Mira et al. 2002 |
| | Gf3f3 | 177-179 | Tet | 60°C | Yes | Mira et al. 2002 |
| 3 | BV6* | 106-119 | Hex | 60°C | Yes | Gautschi et al. 2000 |
| | Gf3h3 | 137-141 | Fam | 60°C | Yes | Mira et al. 2002 |
| | Gf9C | 262-268 | Hex | 60°C | Yes | Mira et al. 2002 |
| 4 | BV5* | 178 | Tet | 58°C | Yes | Gautschi et al. 2000 |
| | BV8* | 105 | Fam | 58°Cs | Yes | Gautschi et al. 2000 |
| | BV11 | 160-162 | Hex | 58°C | Yes | Gautschi et al. 2000 |
| | BV12 | 256-269 | Fam | 58°C | Yes | Gautschi et al. 2000 |
| ** | BV17 | 186 | Hex | 62°C | Yes | Gautschi et al. 2000 |

*species-specific loci, the remaining are family specific

**BV17 was run in isolation

541     *Microsatellite analysis*

542     A prevalent problem with using microsatellites designed for different species is the potential

543     for allelic drop out, strand-mispairing and null alleles. This is caused by inefficient amplification due

544     to mutations in the primer binding site (Kalinowski and Taper, 2006) or low quality DNA (Sefc et al.

545     2003). Both of these factors may be an issue in this study, particularly as some microsatellite loci

546     were designed for other species of vulture and many of the samples included in the data set come

547     from considerably old, archival material where the DNA may be degraded. Different methods of

548     taxidermy preparation and preservation are also known to influence the quality of the DNA, and this

549     may also be a limiting factor in the quality of the final extracted nucleic acids (Burrell et al. 2015).

550     The presence of null alleles can lead to an excess of homozygotes in a data set, which could

551     potentially cause deviations from Hardy-Weinberg (HWE) predictions. Deviations from HWE reduce

552     the similarity between populations (greater Fst values) and therefore erroneously overestimate the

553     level of differentiation between populations (Chapuis and Estoup, 2006). Subsequent population

554     analyses, which rely on HWE as a baseline comparison, are therefore compromised when high

555     frequencies of null alleles are included in the data. The effect that null alleles have on biasing, and

556     over predicting population differentiation is well understood and methods have been developed to

557     correct for these (Carlsson, 2008; Chapuis and Estoup, 2006; Kalinowski and Taper, 2006).

558     The effect of over predicting homozygosity on downstream assignment tests was examined

559     by Carlsson (2008) across a number of programmes, including the programmes I have used in this

560     chapter. He found that null alleles, slightly increased the percentage of incorrectly assigned

561     individuals, but overall population structure estimation was unaffected (Carlsson, 2008). The

562     presence of null alleles in microsatellite data generated in this study was tested for in ML-Null Freq

563     (Kalinowski and Taper 2006) and FreeNA (Chapuis and Estoup 2006).  Both programmes detected

564     significant levels of null alleles at multiple loci (Table 2.5). Population differentiation (Fst) values

565    reported are thus those calculated in FreeNA using the ENA-corrected method, which accounts for

566    null alleles.  Pairwise Fst values were calculated using two grouping schemes. Individuals were

567    grouped by country of origin, and then because some countries have very low sample sizes,

568    individuals were also grouped by region (n = 9; Appendix Table 2.1).

569

570    *Population structure and migration analysis*

571        Population structure across the global distribution of the bearded vulture was performed

572    using a Bayesian clustering method implemented in the software program STRUCTURE version 2.3.4

573    (Falush et al. 2003; Pritchard et al. 2000a). For this analysis, the model with correlated allele

574    frequencies was used (Pritchard et al. 2000b). The analysis was performed using sampling localities

575    as prior information to direct and confirm the clustering. I selected the LOCPRIOR option in Structure

576    for this. This is useful for data sets where the signal of structure is relatively weak, or where there is

577    true population structure (e.g. significant Fst between sampling locations), but standard structure

578    models are unable to detect this. This may occur for data sets with few markers, few individuals, or

579    generally weak structure.

580        Analyses were run using both the *Admixture* and *No Admixture* model parameters. If there is

581    reason to consider each population as completely discrete, the *No Admixture* model is appropriate.

582    In contrast, the *Admixture* model allows for a large proportion of sampled individuals to have recent

583    ancestors from multiple populations. The wide geographic distribution of the bearded vulture

584    favours the *No Admixture* model (suggesting that each regional population is discrete), but previous

585    molecular work (Godoy et al. 2004) suggests that the Africa, European and Asian populations are

586    closely genetically linked. For this reason, both models were implemented.

587        All analyses were performed with 100 000 Markov-Chain Monte Carlo (MCMC) replicates with

588    a burnin of 10 000. The number of iterations for all assignments was 25 with K ranging from 1 to 10.

589    Once all the simulations were completed, STRUCTURE Selector (Li and Liu 2017) was used to

590    determine the optimum ΔK value for each assignment using the Puechmaille method (Puechmaille

591    2016). Detecting the optimal number of genetic clusters were also run through STRUCTURE Harvester

592    (Earl 2012) using the Evanno method (Evanno et al. 2005) to estimate optimal K, although the

593    inherent bias of the Evanno model has recently come under scrutiny (Puechmaille 2016). I ran both

594    STRUCTURE Selector and STRUCTURE Harvester to check for concordance. The online software

595    package Pophelper (Francis 2016) was used to compile bar plots of the optimum K- value (ΔK).


596          Genetic distances were computed and subjected to a Principal Coordinates Analysis (PCoA) in

597    GenAlEx version 6.1 software (Peakall and Smouse 2006). The patterns of clustering from the PCoA

598    were compared to clustering output from EDENetworks v2.18 (Kivelä et al. 2015). EDENetworks when

599    run without prior assumptions of population identity or sampling location, plots nodes (individuals

600    or populations) in a network graph with edges (genetic distance) which are weighted by pairwise

601    genetic distance. EDENetworks uses percolation theory to establish the most suitable network by

602    removing links until the 'super structure' eventually disappears. The point where this occurs is called

603    the 'percolation threshold'. For analysis in EDENetworks, I used the Fst distance measure and

604    automatic thresholding option that allows the programme to detect the optimal threshold. The final

605    clustering pattern was recalculated 10 times to test for alternate placement of nodes or connections.

758          An Analysis of Molecular Variance (AMOVA) was performed to ascertain whether the genetic

759    variation was arising from between or within populations, or primarily from within individuals. For

760    this, the sample set (n=217) was divided into nine different populations based on geographic

761    proximity (Appendix Table 2.1).

762          Testing for potential migration among geographically segregated population was performed

763    using BayesAss 3.0 which implements a Bayesian, assignment test-based method (Wilson and

764 Rannala 2003). This programme uses the Markov chain Monte Carlo (MCMC) procedure to estimate

765 the probability of an individual belonging to the local population, being an immigrant, or whether the

766 individual is of immigrant ancestry. A migration rate is calculated based on the proportion of

767 immigrants in the present and previous generations. I used the recommended settings of 60 000 000

768 MCMC iterations with a burnin of 20 000 steps (Wilson and Rannala 2003).  Migration was tested by

769 dividing populations into regionals groups.

**Results**

770    Null alleles were present in all loci with frequencies ranging from 0.02 to 0.12, but for nine

771 loci out of fourteen, the frequency of null alleles was particularly high (>0.14). FreeNA and MN-Null

772 Freq confirmed this (Table 2.2), though ML-Null Freq predicted consistently higher levels of null

773 alleles than FreeNA. Simulation studies suggest that null alleles with frequencies between 5% and 8%

774 should have only minor effects on standard estimates of population differentiation, but that higher

775 frequencies would bias such parameters (Chapuis and Estoup 2006).

776    I tested for deviations from Hardy-Weinberg equilibrium at each locus, across all samples.

777 Significant departures ($p < 0.05$) from HW equilibrium were obtained for all loci. This was not

778 unexpected as the populations naturally deviate from the assumptions of HWE. These samples were

779 derived from small populations which have presumably been isolated for several generations.

Table 2.2: Null allele frequencies detected across 14 loci.

| | Locus | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BV9 | Gf11a4 | BV14 | BV2 | Gf3f3 | Gf8g | BV6 | Gf3h3 | Gf9c | BV5 | BV8 | BV11 | BV12 | BV17 |
| ML-Null Freq | 0.03 | 0.11 | 0.02 | 0.23 | 0.45 | 0.35 | 0.14 | 0.14 | 0.40 | 0.23 | 0.19 | 0.16 | 0.02 | 0.12 |

| FreeNA | 0.03 | 0.06 | 0.01 | 0.17 | 0.28 | 0.13 | 0.11 | 0.10 | 0.23 | 0.18 | 0.09 | 0.13 | 0.02 | 0.09 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

*Population structure*

The analysis using the *Admixture* parameter detected seven genetic clusters (ΔK = 7, Figure 2.2 and 2.3). The Bayesian STRUCTURE cluster plot revealed some genetic admixture between the populations from different countries. The lowest admixture was evident in the southern African population (Q=0.83), followed by the central Russian and Ethiopian populations (Q= 0.80 and 0.78, respectively). The highest admixture was detected in the Kyrgyzstan, Corsican and Spanish populations (Q = 0.30, 0.30, and 0.31 respectively, Table 2.4). The genetic clustering was closely linked to geography (Figure 2. 2.3). In particular, the isolated southern African and central Russian bearded populations are genetically distinct from the other bearded vulture populations, with genotypes collected from these regions belonging to unique genetic clusters. The genetic composition of the remaining geographic regions is predominantly a combination of four genetic clusters (dark blue, light blue, red and orange cluster in Figure 2.3).
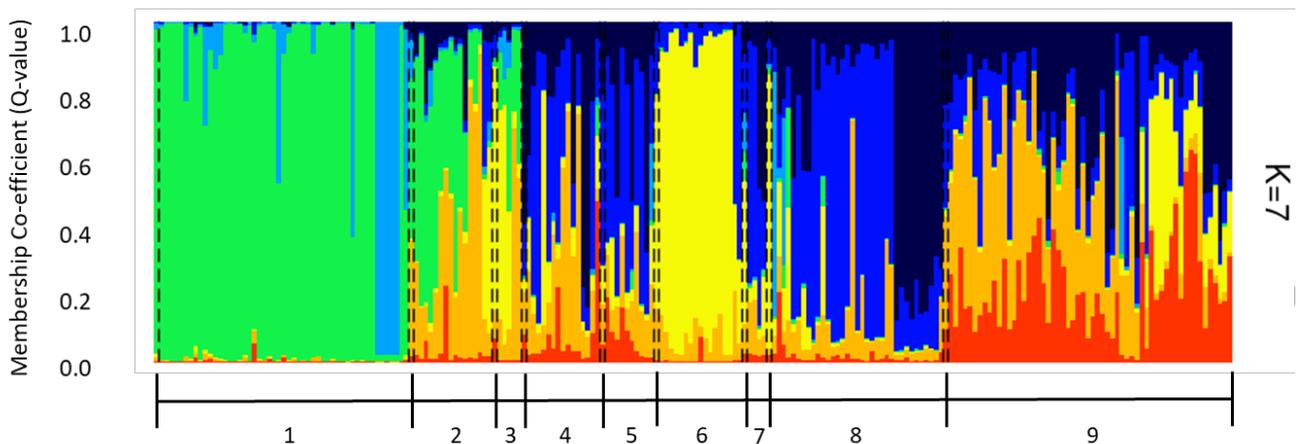


Figure 2.2: Probabilistic Admixed population structure given for 217 Gypaetus barbatus collected from across the distribution of the species. Individual vultures have been grouped into broad geographically delineated regions (1- southern Africa; 2- eastern Africa; 3- western Africa; 4- south-east Europe; 5- central Asia; 6- central Russia; 7- northern India, 8- Mediterranean islands; 9-

European countries) and are represented by vertical lines which is partitioned into (K = 7) coloured segments that represent the estimated membership coefficients (Q-value).

792        When running the programme using the *No Admixture* parameter, the model detected either

793    seven genetic clusters (Figure 2.4) when run on the Puechmaille clustering method, or two (Evanno)

794    genetic clusters (not presented here). Given the tendency of the Evanno model to bias toward K = 2

795    (Puechmaille 2016), and in view of the *Admixture* model detecting 7 genetic clusters, the K = 2 result

796    is not presented here. The results from the *No Admixture* parameter setting differ only slightly from

797    those returned from the *Admixture* model (Table 2.3). The *No Admixture* model more frequently

798    assigned individuals to a specific genetic cluster (with chiefly higher or similar Q-values, Table 2.4)

799    whereas the *Admixture* model predicted an individual to have ancestry from several genetic clusters.

800    Understandably, in comparison to the *Admixture* model, less mixing of genetic clusters is apparent.

801    Nevertheless, there is a clear distinction between the southern African population and the remaining

802    populations. Individuals collected from southern Africa also belong to three different genetic clusters.

803    Two of these clusters were only present in the southern African population.
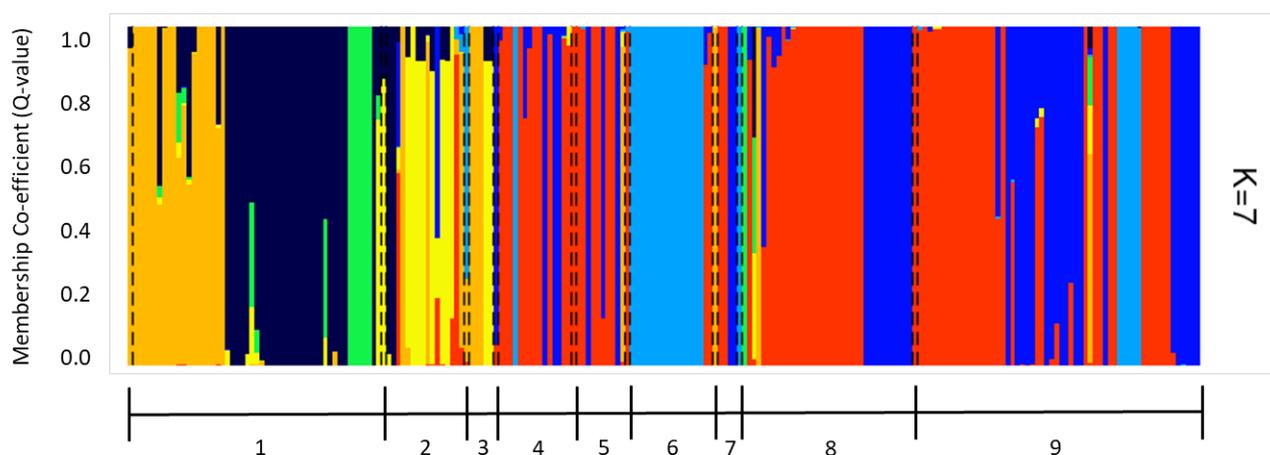


Figure 2.3: Probabilistic population structure under the No Admixture model given for 217 Gypaetus barbatus samples collected from across the global distribution of the species. Individual vultures have been grouped into broad geographically delineated regions (1- southern Africa; 2- eastern Africa; 3-

western Africa; 4- south-east Europe; 5- central Asia; 6- central Russia; 7- northern India, 8- Mediterranean islands; 9- European countries) and are represented by vertical lines which are partitioned into (max K = 7) coloured segments which represent the estimated membership coefficients (Q-value) of each cluster represented in their genetics.

Table 2.3: Comparison of membership coefficients (Q-values) for both *Admixture* and *No Admixture* models; summary statistics provided

| Region | Country | | Admixture K = 7 | No Admixture K = 7 | $N_A$ | $H_o$ | $H_E$ |
|---|---|---|---|---|---|---|---|
| South Africa | South Africa | 1 | 0.83 | 0.75 | 6.57 | 0.49 | 0.60 |
| Eastern Africa | Ethiopia | 2 | 0.43 | 0.14 | 4.21 | 0.39 | 0.48 |
| | Abyssinia | 3 | 0.78 | 0.25 | | | |
| | Yemen | 4 | 0.41 | 0.61 | | | |
| Western Africa | Morocco | 5 | 0.62 | 0.75 | 2.86 | 0.56 | 0.44 |
| | Algeria | 6 | 0.44 | 0.78 | | | |
| South-east Europe | Albania | 7 | 0.61 | 0.85 | 4.0 | 0.46 | 0.48 |
| | Greece | 8 | 0.35 | 0.62 | | | |
| | Turkey | 9 | 0.48 | 0.98 | | | |
| Central Asia | Caucasus | 10 | 0.56 | 0.79 | 3.93 | 0.38 | 0.49 |
| | Central Asia | 11 | 0.44 | 0.96 | | | |
| | Kazakhstan | 12 | 0.47 | 0.61 | | | |
| | Kyrgyzstan | 13 | 0.30 | 0.57 | | | |
| Central Russia | Central Russia | 14 | 0.80 | 0.84 | 3.45 | 0.36 | 0.44 |
| India | India | 15 | 0.41 | 0.40 | 2.50 | 0.44 | 0.40 |
| Mediterranean Islands | Crete | 16 | 0.76 | 1.00 | 4.21 | 0.45 | 0.43 |
| | Corsica | 17 | 0.31 | 0.49 | | | |
| | Sardinia | 18 | 0.51 | 0.60 | | | |
| Europe | Switzerland | 19 | 0.42 | 0.57 | 5.57 | 0.47 | 0.53 |
| | Austria | 20 | 0.74 | 1.00 | | | |
| | Pyrenees | 21 | 0.35 | 0.45 | | | |
| | France | 22 | 0.61 | 0.94 | | | |
| | Spain | 23 | 0.31 | 0.36 | | | |

804    *Genetic differentiation and connectivity*

805    Genetic differentiation of bearded vulture populations was detected using the ENA-corrected

806    Fst values. Levels of genetic differentiation as delineated by Hartl and Clark (1998), and Frankham et

807    al. (2002) were followed for interpretation of results. Evidence for genetic differentiation was found

808    between populations from different countries (Fst > 0, Table 2.2). Pairwise comparisons revealed that

809    the South African population of bearded vultures is moderately different from populations in

810    Ethiopia, Algeria and Crete (Fst ≤ 0.05 – 0.15, Table 2.2) and was found to be genetically different

811    from all other populations (Fst > 0.15). The Abyssinian population was also found to have moderate-

812    to great genetic differentiation from all other populations (Fst 0.14 – 0.59, Table 2.2).

813    Given the limited sample sizes available in some countries, samples were also grouped

814    according to region (n=9). In this analysis pairwise Fst values support moderate genetic

815    differentiation of southern African populations from eastern and western Africa (Fst = 0.10, 0.11

816    respectively, Table 2.3b). The southern African populations was found to be strongly differentiated

817    from all populations outside of Africa (Fst > 0.15). Interestingly, there is little genetic differentiation

818    between populations of bearded vultures in eastern and western Africa (Fst = 0.01).

819    AMOVA results conducted when grouping individuals into the nine regions, showed that 50%

820    (p < 0.001) of variation was attributed to within individual differences, while differences among

821    individuals and among populations explained only 33% (p < 0.001) and 17% (p < 0.001) of the

822    diversity, respectively.

Table 2.4: Estimates of pairwise Fst using first uncorrected frequencies (Table 2.4a) and ENA-corrected allelic frequencies (Table 2.4.b). Country numbers derived from Table 2.3. Values < 0.05 indicate little genetic differentiation, 0.05-0.15 = moderate differentiation, 0.15-0.25 = great differentiation, and >0.25 = very great differentiation (given in bold).

*Table 2.4a. Fst without ENA*

| Pop | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 0.06 | | | | | | | | | | | | | | | | | | | | | |
| 3 | 0.22 | 0.06 | | | | | | | | | | | | | | | | | | | | |
| 4 | 0.15 | 0.15 | **0.58** | | | | | | | | | | | | | | | | | | | |
| 5 | 0.12 | 0.05 | **0.52** | 0.27 | | | | | | | | | | | | | | | | | | |
| 6 | 0.10 | -0.02 | 0.11 | 0.09 | -0.02 | | | | | | | | | | | | | | | | | |
| 7 | 0.21 | 0.23 | **0.53** | **0.46** | - | 0.21 | | | | | | | | | | | | | | | | |
| 8 | 0.22 | 0.14 | 0.19 | 0.17 | 0.07 | 0.14 | -0.05 | | | | | | | | | | | | | | | |
| 9 | 0.24 | 0.19 | **0.41** | **0.58** | - | 0.20 | - | 0.15 | | | | | | | | | | | | | | |
| 10 | 0.23 | 0.15 | 0.19 | **0.26** | 0.24 | 0.17 | 0.21 | 0.04 | 0.23 | | | | | | | | | | | | | |
| 11 | 0.10 | -0.03 | **0.56** | **0.26** | - | 0.01 | - | 0.09 | - | **0.31** | | | | | | | | | | | | |
| 12 | 0.24 | 0.18 | **0.26** | **0.31** | **0.33** | 0.20 | 0.09 | 0.00 | 0.24 | 0.07 | 0.17 | | | | | | | | | | | |
| 13 | **0.26** | **0.30** | **0.53** | 0.25 | - | **0.33** | - | 0.13 | - | 0.24 | - | 0.12 | | | | | | | | | | |
| 14 | 0.22 | 0.17 | **0.34** | 0.14 | 0.14 | 0.15 | **0.40** | 0.16 | **0.31** | 0.19 | **0.33** | 0.25 | **0.39** | | | | | | | | | |
| 15 | 0.19 | 0.11 | 0.23 | 0.06 | 0.13 | 0.10 | **0.32** | 0.11 | 0.21 | 0.06 | -0.01 | 0.05 | **0.37** | 0.18 | | | | | | | | |
| 16 | 0.06 | -0.05 | **0.46** | **0.27** | - | -0.03 | - | 0.04 | - | 0.14 | - | 0.22 | - | -0.14 | 0.03 | | | | | | | |
| 17 | 0.19 | 0.20 | **0.27** | 0.06 | -0.15 | 0.19 | -0.13 | 0.13 | 0.03 | **0.26** | -0.14 | 0.13 | -0.32 | **0.33** | 0.17 | -0.13 | | | | | | |
| 18 | 0.19 | 0.13 | 0.19 | 0.15 | 0.17 | 0.13 | 0.08 | 0.03 | 0.21 | 0.04 | 0.10 | 0.00 | 0.25 | 0.15 | 0.08 | 0.07 | 0.18 | | | | | |
| 19 | 0.24 | 0.14 | 0.14 | 0.24 | 0.20 | 0.16 | 0.12 | 0.02 | 0.22 | 0.06 | 0.12 | 0.04 | **0.27** | 0.20 | 0.11 | 0.16 | 0.23 | 0.04 | | | | |
| 20 | 0.18 | 0.02 | 0.22 | **0.41** | - | 0.05 | - | 0.00 | - | -0.01 | - | 0.10 | - | 0.21 | 0.08 | - | -0.11 | 0.03 | -0.05 | | | |
| 21 | 0.21 | 0.13 | 0.21 | **0.41** | **0.44** | 0.17 | **0.38** | 0.02 | **0.28** | 0.06 | 0.17 | 0.02 | **0.41** | 0.25 | 0.08 | **0.30** | 0.18 | 0.00 | 0.01 | 0.06 | | |
| 22 | 0.19 | 0.08 | 0.20 | **0.31** | **0.27** | 0.09 | 0.23 | -0.02 | 0.14 | 0.03 | 0.12 | 0.04 | 0.09 | 0.19 | 0.00 | 0.14 | 0.00 | 0.00 | 0.03 | 0.08 | 0.00 | |
| 23 | 0.20 | 0.12 | 0.21 | 0.10 | 0.00 | 0.12 | 0.08 | 0.03 | 0.17 | 0.08 | 0.12 | 0.07 | 0.17 | 0.10 | 0.09 | -0.05 | 0.15 | 0.05 | 0.07 | 0.05 | 0.10 | 0.00 |

1

*Table 2.4b. Fst with ENA*

| Pop | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 0.07 | | | | | | | | | | | | | | | | | | | | | |
| 3 | 0.24 | 0.10 | | | | | | | | | | | | | | | | | | | | |
| 4 | 0.19 | 0.21 | **0.59** | | | | | | | | | | | | | | | | | | | |
| 5 | 0.18 | 0.13 | **0.54** | **0.27** | | | | | | | | | | | | | | | | | | |
| 6 | 0.10 | 0.00 | 0.14 | 0.17 | 0.09 | | | | | | | | | | | | | | | | | |
| 7 | **0.26** | 0.31 | **0.55** | **0.46** | - | **0.30** | | | | | | | | | | | | | | | | |
| 8 | 0.20 | 0.14 | 0.20 | 0.21 | 0.13 | 0.15 | 0.03 | | | | | | | | | | | | | | | |
| 9 | **0.29** | 0.26 | **0.43** | **0.58** | - | 0.25 | - | 0.21 | | | | | | | | | | | | | | |
| 10 | 0.22 | 0.16 | 0.20 | **0.29** | **0.28** | 0.18 | 0.24 | 0.04 | 0.25 | | | | | | | | | | | | | |
| 11 | 0.19 | 0.10 | **0.59** | **0.26** | - | 0.13 | - | 0.17 | - | **0.36** | | | | | | | | | | | | |
| 12 | **0.25** | 0.20 | **0.26** | **0.32** | **0.33** | 0.22 | 0.10 | 0.03 | 0.25 | 0.08 | 0.19 | | | | | | | | | | | |
| 13 | **0.30** | **0.32** | **0.56** | 0.25 | - | **0.37** | - | 0.19 | - | 0.24 | - | 0.14 | | | | | | | | | | |
| 14 | 0.20 | 0.15 | **0.32** | 0.14 | 0.15 | 0.15 | **0.39** | 0.14 | **0.31** | 0.17 | **0.34** | 0.23 | **0.38** | | | | | | | | | |
| 15 | 0.19 | 0.13 | **0.27** | 0.11 | 0.20 | 0.13 | **0.37** | 0.13 | **0.26** | 0.09 | 0.11 | 0.08 | **0.38** | 0.16 | | | | | | | | |
| 16 | 0.13 | 0.05 | **0.47** | 0.27 | - | 0.06 | - | 0.09 | - | 0.18 | - | 0.22 | - | -0.09 | 0.09 | | | | | | | |
| 17 | 0.18 | 0.21 | **0.29** | 0.10 | -0.04 | 0.20 | 0.02 | 0.13 | 0.10 | 0.25 | -0.01 | 0.14 | -0.17 | **0.29** | 0.17 | -0.05 | | | | | | |
| 18 | 0.18 | 0.13 | 0.20 | 0.17 | 0.19 | 0.14 | 0.11 | 0.03 | 0.21 | 0.05 | 0.14 | 0.02 | 0.25 | 0.13 | 0.09 | 0.09 | 0.17 | | | | | |
| 19 | 0.22 | 0.12 | 0.15 | 0.24 | 0.21 | 0.16 | 0.15 | 0.03 | 0.24 | 0.06 | 0.15 | 0.05 | **0.28** | 0.16 | 0.12 | 0.16 | 0.21 | 0.04 | | | | |
| 20 | 0.24 | 0.12 | 0.25 | **0.41** | - | 0.13 | - | 0.05 | - | 0.04 | - | 0.10 | - | 0.21 | 0.16 | - | -0.01 | 0.07 | -0.01 | | | |
| 21 | 0.23 | 0.16 | 0.23 | **0.43** | **0.46** | 0.19 | **0.38** | 0.06 | **0.30** | 0.09 | 0.21 | 0.04 | **0.41** | 0.24 | 0.12 | **0.32** | 0.20 | 0.03 | 0.04 | 0.09 | | |
| 22 | 0.21 | 0.12 | 0.21 | **0.31** | **0.26** | 0.13 | 0.23 | 0.01 | 0.14 | 0.06 | 0.12 | 0.05 | 0.09 | 0.17 | 0.05 | 0.14 | 0.03 | 0.03 | 0.05 | 0.08 | 0.02 | |
| 23 | 0.18 | 0.12 | 0.23 | 0.14 | 0.07 | 0.13 | 0.14 | 0.03 | 0.22 | 0.08 | 0.20 | 0.09 | 0.21 | 0.09 | 0.10 | 0.03 | 0.16 | 0.05 | 0.06 | 0.11 | 0.13 | 0.04 |

Table 2.5: Estimates of pairwise Fst using first uncorrected frequencies (Table 2.5a) and ENA-corrected allelic frequencies (Table 2.5.b). Country numbers derived from Table 2.4. Values < 0.05 indicate little genetic differentiation, 0.05-0.15 = moderate differentiation, 0.15-0.25 = great differentiation, and >0.25 = very great differentiation (given in bold).

*Table 2.5a. Fst without ENA*

|  | SAfrica | EAfrica | WAfrica | SEEurope | CAsia | CRussia | India | MIslands |
|---|---|---|---|---|---|---|---|---|
| EAfrica | 0.09 |  |  |  |  |  |  |  |
| WAfrica | 0.12 | 0.00 |  |  |  |  |  |  |
| SEEurope | 0.22 | 0.13 | 0.14 |  |  |  |  |  |
| CAsia | 0.20 | 0.09 | 0.12 | 0.03 |  |  |  |  |
| CRussia | 0.21 | 0.15 | 0.14 | 0.18 | 0.12 |  |  |  |
| India | 0.19 | 0.08 | 0.10 | 0.12 | 0.03 | 0.17 |  |  |
| MIslands | 0.18 | 0.11 | 0.13 | 0.03 | 0.01 | 0.14 | 0.07 |  |
| Europe | 0.22 | 0.10 | 0.13 | 0.01 | 0.02 | 0.16 | 0.09 | 0.03 |

*Table 2.5b. Fst with ENA*

|  | SAfrica | EAfrica | WAfrica | SEEurope | CAsia | CRussia | India | MIslands |
|---|---|---|---|---|---|---|---|---|
| EAfrica | 0.10 |  |  |  |  |  |  |  |
| WAfrica | 0.11 | 0.01 |  |  |  |  |  |  |
| SEEurope | 0.20 | 0.14 | 0.14 |  |  |  |  |  |
| CAsia | 0.18 | 0.10 | 0.12 | 0.03 |  |  |  |  |
| CRussia | 0.19 | 0.15 | 0.13 | 0.16 | 0.10 |  |  |  |
| India | 0.19 | 0.11 | 0.12 | 0.13 | 0.05 | 0.16 |  |  |
| MIslands | 0.16 | 0.12 | 0.13 | 0.03 | 0.02 | 0.13 | 0.08 |  |
| Europe | 0.20 | 0.09 | 0.12 | 0.02 | 0.02 | 0.14 | 0.09 | 0.03 |

Principal coordinates analysis (PCoA) of Fst pairwise values generally clustered individuals by geography. The first and second dimensions explain 19.73% (Axis 1) and 8.98% (Axis 2) of variation. Together they account for 28.22% of the variation present in the microsatellite data. The analysis revealed tight clustering of individuals from the Mediterranean Island (MIsland) populations, and to a lesser extent, the European grouping (Figure 2.4).

820  Principal coordinates analysis (PCoA) of Fst pairwise values generally clustered

821 individuals by geography. The first and second dimensions explain 19.73% (Axis 1) and 8.98%

822 (Axis 2) of variation. Together they account for 28.22% of the variation present in the

823 microsatellite data. The analysis revealed tight clustering of individuals from the

824 Mediterranean Island (MIsland) populations, and to a lesser extent, the European grouping

825 (Figure 2.4). The PCoA clustered all African samples together, with almost no overlap with

826 other European or Asian populations. The southern African cluster overlapped with both the

827 eastern and western African clusters (Figure 2.4). There is a slight overlap between the

828 western African population and the south-east Europe (SEEurope) cluster (35 shared alleles,

829 Figure 2.4 & 2.5). There was a high degree of overlap between the central Asian (CAsia) and

830 European clusters, the majority of the Asian samples fell within the European cluster (Figure

831 2.4). No distinct clusters were detected in the central Russian (CRussia) samples. The Indian

832 samples clustered tightly together, but overlap with the MIslands and Europe clusters (Figure

833 2.4). Interestingly, there was no overlap between samples from India and those from central

834 Asia, as would have been expected given geographic proximity.
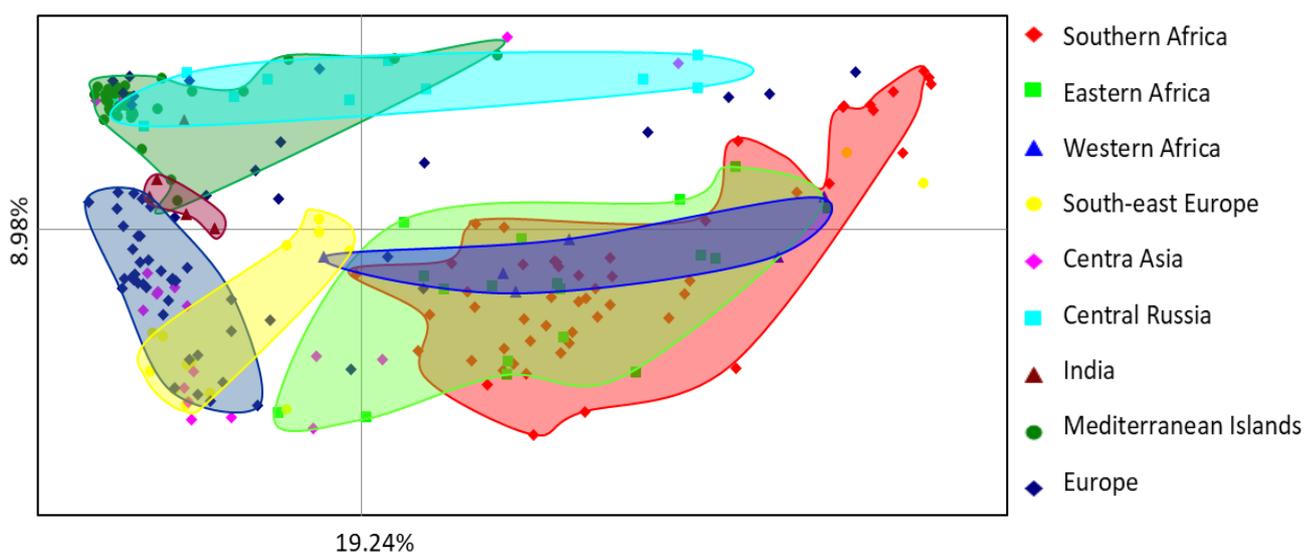
Figure 2.4: Principal coordinate analysis based on 14 microsatellite markers showing affinity between the populations. Each dimension explains 19.24% (Axis 1) and 8.98% (Axis 2) of variation. The axes together explained 28.22% of total variation present in the data set.

835        The clustering patterns which emerge from the EDENetworks output concur with the

836    PCoA analysis. EDENetwork analysis revealed two major clusters which are clearly segregated

837    and share only a weak genetic linkage. In the network, the thickness of the connecting lines

838    indicate levels of genetic connectivity. Populations from Southern Africa, Ethiopia and Algeria

839    are clustered together in the network, while all European and Asian populations clustered

840    together in a separate lineage (Figure 2.5). Genetic connectivity is much stronger with more

841    genetic exchange predicted between the European and Asian populations (as seen by the

842    thicker links). The connections among the African populations were less pronounced in the

843    EDENetwork output than in the PCoA.

844
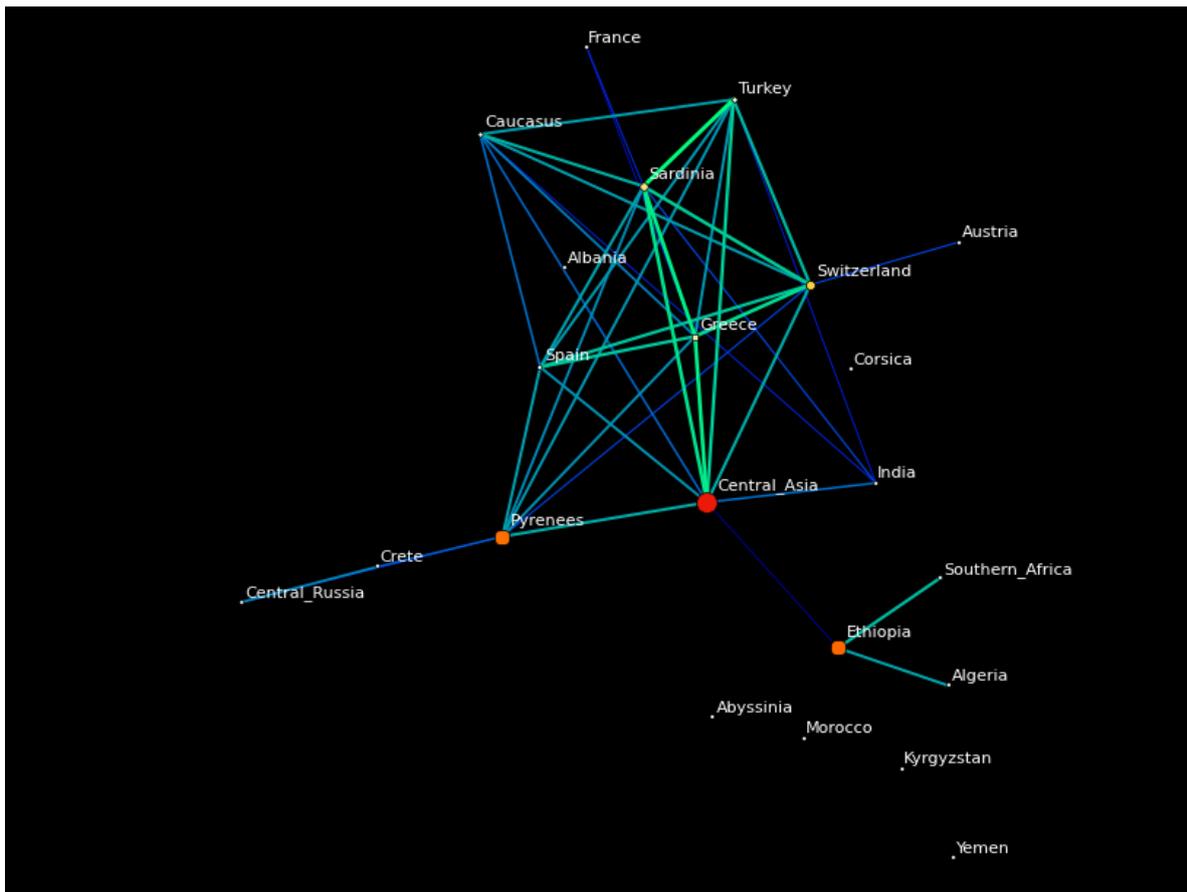
Figure 2.5: Network constructed of all samples (n = 217, grouped by regional population) in EDENetworks. Populations (nodes) are named based on their region of origin and are linked by edges which are weighted in proportion to the strength of the genetic linkage.

845    When individuals are used as nodes (Appendix Figure 2.1), one cluster is comprised

846    almost entirely of the South African samples, whereas the second cluster contains all samples

847    collected from outside of Africa (Appendix Figure 2.1). The Ethiopian individuals overlap

848    slightly with South African and Algerian samples. The predominant cluster in Appendix Figure

849    2.1 is a clustering together of all non-African samples. The individuals belonging to this cluster

850    are highly interconnected although the strength of the linkages is not as strong as those

851    existing between the nodes of the African cluster. A sub-cluster consisting mostly of Russian

852       samples emerges from the larger European cluster. The remainder of this large cluster

853       contains the European and Mediterranean country samples.

854          Although there is disparity between the PCoA and EDENetwork clustering output, both

855       predict a distinct segregation of the southern African, and to a lesser extent- African, samples

856       from all other bearded vulture populations. Statistical outliers are inevitable (missing

857       microsatellite data, limited samples, and vague specimen information may well explain

858       incongruence between analyses). Appendix figure 2.1 shows the vastly connectedness

859       between samples and here one could argue again for 4 clusters. Despite this, southern African

860       samples again, cluster predominantly apart from other genetic clusters.

861       *Migration analysis*

862          The BayesAss 3.0 analysis indicates that bearded vulture populations in India and the

863       Mediterranean (countries and islands) appear to be closed systems which do not send off any

864       emigrants (Figure 2.6 and 2.7). They do, however receive small proportions of immigrants

865       from Europe, central Asia and central Russia. Interestingly, all populations contain between

866       3% and 8% genetic signature of the eastern African population. This would point toward the

867       eastern African population being an important source population for migrants. On the

868       contrary, western Africa is a sink population as it receives migrants from southern and eastern

869       Africa, Europe and central Russia, but no migrants leave this population (Figure 2.7). The

870       strongest flow of migration occurs between central Asian to European populations (11%; 41

871       shared alleles), and between Europe to the populations on the Mediterranean Islands (12%;

872       48 shared alleles). This is also substantiated by the high number of shared alleles along these

873       migration routes (Figure 2.6). Considering their geographical proximity, and despite sharing

874       many alleles (45), analysis did not detect any migration between south-east Europe and

875 Mediterranean Islands (Figure 2.7). While movement between central Asia to Europe might

876 be indicative of past migration of individuals. In contrast, migration from south-east Europe

877 to the islands might possibly reflect human-mediated movement caused by conservation

878 efforts in reintroducing individuals to previously extinct populations. These reintroduced

879 populations are also relatively small and possibly inbred, which would bias the results of this

880 migration analysis (Biebach and Keller 2010).

881     The southern and eastern African populations are entirely genetically isolated from

882 the European and Asian populations. The southern African and western African populations

883 share equal rates of migration per year (4%; Figure 2.7). Eastern Africa appears to be an

884 important sink population (92% non-migrants, <10% private alleles) for individuals from both

885 southern and western Africa. There is greater migration to eastern Africa (1% migration, 100%

886 shared alleles) than what is emigrated in return (1%). Eastern Africa (~10% locally common

887 alleles) could potentially act as a 'stepping stone' population for the transfer of genetic

888 material between western and southern Africa. Emigration rates are low between the African

889 populations, in particular the southern African population is crucial in preserving genetic

890 diversity within the species as it contains the greatest percentage of private alleles (Figure

891 2.6).
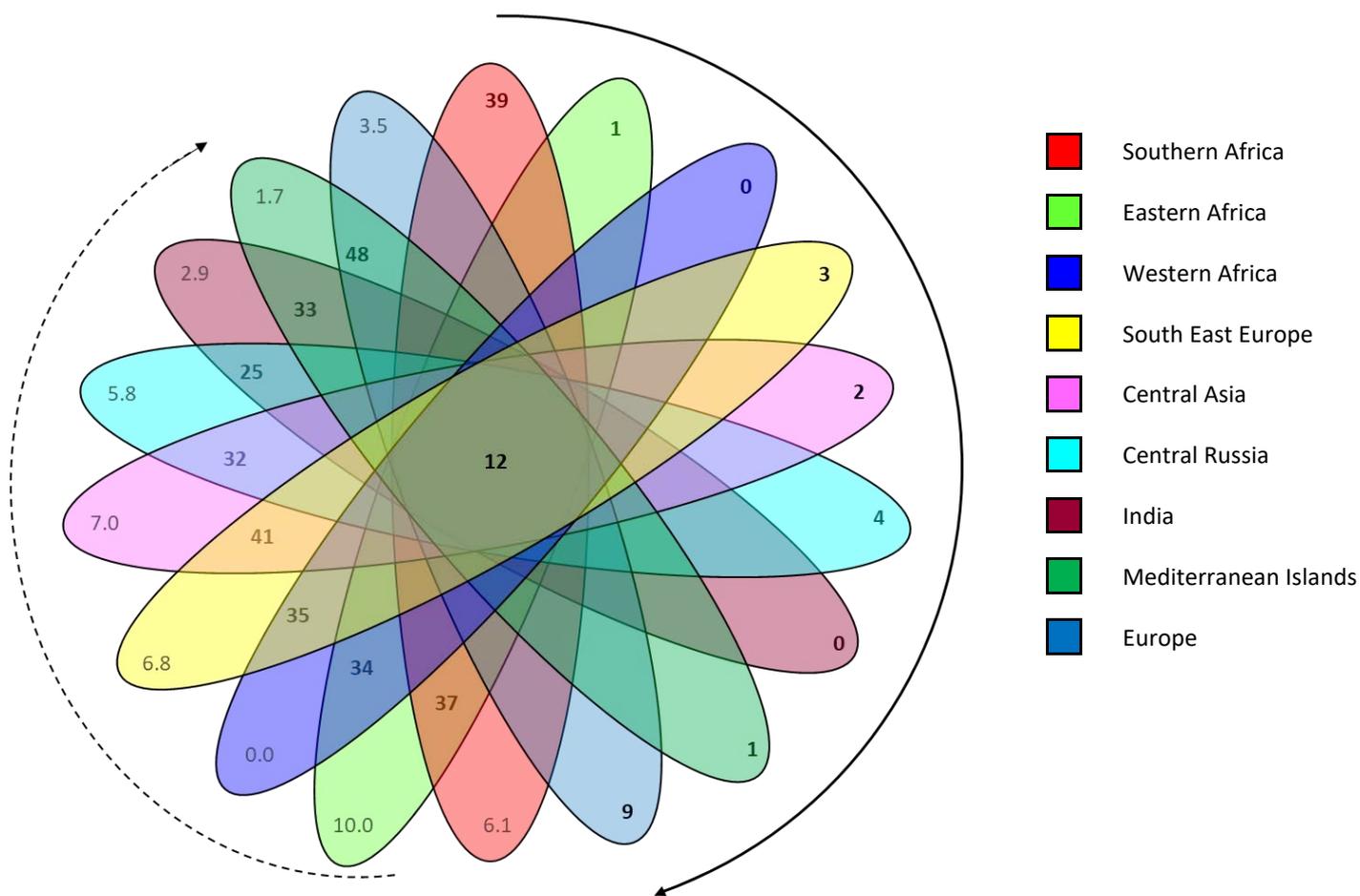
Figure 2.6: Depicting private alleles (solid arrow), and common (dashed arrow) and locally shared alleles (outer value). Locally common alleles (present in <25% of the population) given as a percentage of total alleles in population.
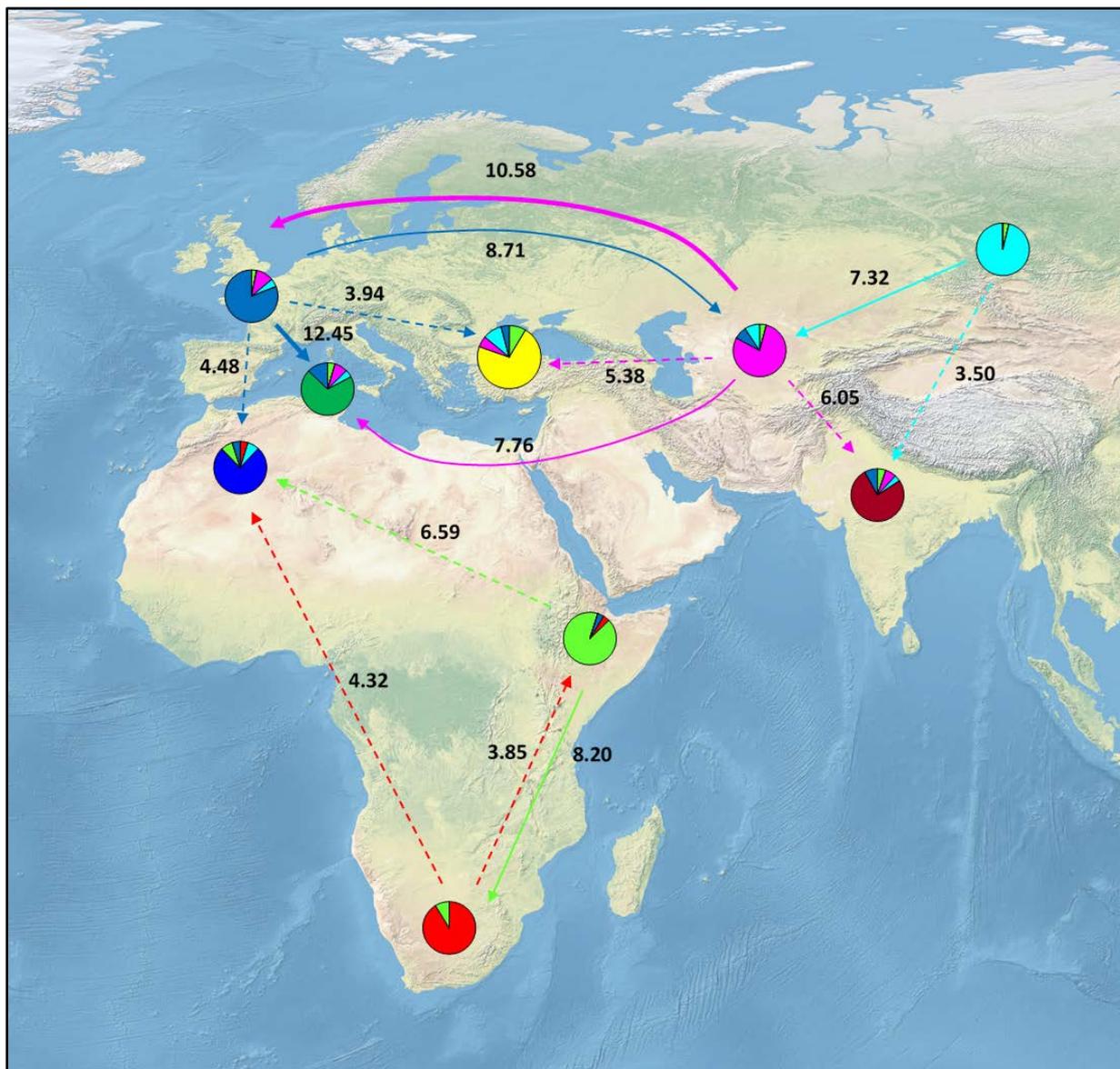
Figure 2.7: Global migration analysis showing routes of past transferral of alleles. Migration is given in the percentage of 'migrants' in the population and is segregated into high (10-15%; bold arrows), medium (thin arrows, 6-9%) and low (3-5%; stippled arrows). Migration calculated as a percentage of the population per generation that are 'immigrants'. Pie charts show proportion of migrants to non-migrants, while arrows show the direction of gene flow between populations. Only immediate 'neighbour' migration routes, and not presumed 'stepping stone' routes, indicated.

**Discussion**

892     Population structure can be the result of population collapses, bottlenecking events

893     and isolation of populations in endangered species (Lande and Barrowclough 1987; Vucetich

894     and Waite 1999; Wilcox and Murphy 1985). Genetic variability is generally a good indicator of

895     species' evolutionary persistence, as it is expected that a healthy level of variation will always

896     allow for a proportion of the population to respond to natural selection (Frankham et al. 2014;

897     Gilpin 1996; Shaffer 1987). The associated loss of genetic variability in small isolated

898     populations through genetic drift and inbreeding is believed to adversely affect the remnant

899     species adaptive potential and their evolutionary persistence (Bretagnolle et al. 2004; Soulé

900     1986; Vucetich and Waite 1999). This is particularly true if remaining populations are

901     segregated with little immigration and poor dispersal ability.

902     This present study adds to the body of research on genetics of the endangered

903     bearded vulture. Past studies have focussed primarily on mitochondrial markers (Godoy et al.

904     2004; Krüger et al. 2015). These papers suggest that there is a lack of mitochondrial genetic

905     diversity within the species. Based on the lack of adequate mitochondrial differentiation

906     between the geographically disjunct populations in these studies and apparent ecological and

907     genetic interchangeability, both research papers recommended that the southern

908     hemispheric and northern hemispheric populations of bearded vultures be managed as one

909     (Godoy et al. 2004; Krüger et al. 2015). In the research presented here, I investigated

910     population structuring by using microsatellite fragment data. These markers are distributed

911     on different chromosome of the nuclear genome and are typically noncoding and as such are

912     subject to higher mutation rates which should therefore render them considerably more

913     informative than coding regions which are evolutionarily constrained, and as such should give

914    an improved resolution of fine-scale structuring of the species (Ellegren 2000; Primmer et al.

915    1997).

*Population structure*

917        A key consideration when using microsatellite data, however, is allelic drop out,

918    strand-mispairing and null alleles which are caused by inefficient amplification due to

919    mutations in the primer binding site (Kalinowski and Taper 2006) or degraded DNA. Excess

920    homozygotes, and thus deviations from HWE predictions, can result in the misidentification,

921    and overestimation, of false null alleles. This is particularly relevant in this study where some

922    bearded vulture populations have very small population size and would be expected to

923    experience high levels of inbreeding leading to increased homozygosity.  Deviations from

924    HWE necessarily reduce the similarity between populations (greater Fst values), but the

925    inclusion of null alleles over predicts the level of differentiation (Chapuis and Estoup 2006).

926    Population analyses which rely on HWE as a baseline comparison are therefore compromised

927    when high frequencies of null alleles are detected. Where data sets have few loci or missing

928    data, several programmes (FSTAT, FreeNA, and ML-Null Freq) which differ in their level of

929    statistical rigor have been developed to specifically deal with these problems. The effect that

930    null alleles have on biasing, and over predicting population differentiation is well understood

931    and methods have been developed to correct for these (Carlsson 2008; Chapuis and Estoup

932    2006; Kalinowski and Taper 2006). Testing for genetic structuring and assigning individuals to

933    source populations is a well-used application of microsatellites, especially when concerning

934    conservation of a threatened species. Statistically correcting for increased null allele

935    frequency and population differentiation is simple (Carlsson 2008; Van Oosterhout et al.

936    2004). There may not be associated species-threatening ramifications to over-predicting

937    population differentiation. Rather, one anticipates the conservation steps taken (e.g.

938    translocation, restocking) to remedy the population structuring to be an unnecessary

939    spending of available funds. However, from a scientific point of view, over predicting

940    population diversity and differentiation leads to biased, compromised results and

941    conclusions. Carlsson (2008) found that null alleles slightly increases the percentage of

942    incorrectly assigned individuals in programs such as STRUCTURE. This conclusion held true for

943    null allele frequencies of up to 0.913, which is well beyond those encountered in this study. I

944    believe that the population structure presented in this chapter has not been biased by the

945    inclusion null alleles.

946    Genetic structuring is often prevalent in populations which are widely distributed and

947    display strong natal philopatric behaviour (Gautschi et al. 2003; Haig et al. 1997; Wenink et

948    al. 1993). Given that the bearded vulture populations are isolated by vast geographic

949    distances, and philopatric behaviour has been documented in the species, it would be

950    reasonable to expect genetic structuring (Gautschi et al. 2003; López-López et al. 2013).

951    Segregating an ideally panmictic population into several isolated populations should

952    result in less heterozygosity than if the population interbred freely and randomly. In such ideal

953    panmictic populations, one expects that variation would arise chiefly from within the samples

954    and not between individual populations. Here, AMOVA revealed that the genetic variation

955    was greatest between individuals, rather than between populations. This is evidence against

956    strong population structuring, which is in contrast to clustering pattern observed in

957    STRUCTURE and PCA. This could be a result of unbalanced sample size of populations

958    examined or linked to the unusually long generation time of the bearded vultures and time

959    since the bottleneck event. Alleles can take many generations to become fixed in populations.

960    This takes longer if individuals have extended generation time. The time since the collapse of

961     bearded vulture populations and subsequent isolation of populations has occurred within the

962     last 100 years, which has not allowed sufficient time for complete allele fixation to occur.

963     Incomplete lineage sorting or "deep coalescence" can be invoked to explain the lack of among

964     population differences (Maddison 1997). Given enough time and continued isolation I expect

965     the genetic differentiation of the southern African population to be more pronounced.

966        Low to moderate genetic differentiation was detected in the global population of

967     bearded vultures. European population differed only slightly from populations in Asia. Vast

968     topographical features such as the Pyrenees and Alps mountain ranges are used to explain

969     expansion limitations in terrestrial mammals (Munguía et al. 2008). However for long,

970     distance flighted species which specifically inhabits high altitude mountains, this should not

971     be a hindrance to gene flow (Godoy et al. 2004). The pattern of pronounced genetic

972     differentiation among African populations, and much shallower differentiation found in the

973     Eurasian population (Europe and Asia) is concordant with this knowledge. The pattern of

974     population differentiation found here corroborate previous work (Godoy et al. 2004). They

975     describe the dispersal and separate diversification of two lineages of bearded vultures,

976     *Gypaetus barbatus barbatus*, and *Gypaetus barbatus meridionalis. Gypaetus barbatus*

977     *barbatus* dominates Eurasia and northern Africa, while *Gypaetus barbatus meridionalis* is

978     found in eastern and southern Africa. The diversification of these two lineages was

979     hypothesises to be linked with the expanding Sahara Desert which acted as a physical barrier

980     during the last glacial maximum (Coetzee 1993; Godoy et al. 2004). When the Sahara receded,

981     bearded vultures were able to disperse back into eastern Africa and this formed a 'stepping

982     stone' allowing for admixture between the two subspecies. The highest levels of admixture in

983     this study were found in the eastern and western African populations, and this could be

984    reflective of these past dispersal events, and the subsequent isolation that followed in the

985    recent past.

986    *Migration*

987        Based on the species philopatric dispersal behaviour and population differentiation,

988    genetic differentiation between regional populations is expected to increase with geographic

989    distance following an isolation-by-distance model (Hartl and Clark 1998; Slatkin 1987),

990    especially in the absence of translocations or supplementations to the population. Here

991    analyses found little migration between the populations, with higher movement rates

992    between the European and Asian populations, and little to no migration to the more southern

993    African populations. This supports the hypothesis that the southern African population is

994    indeed isolated, both geographically and genetically.

995    *Further considerations*

996        The research presented here adds to past studies by providing an extensive evaluation

997    of the genetic status of the southern African bearded vulture population. Further, it supports

998    the conclusion of these studies that there is reduced genetic variation and inbreeding in

999    natural populations of bearded vultures. This research detected significant structuring in the

1000   bearded vulture population. Management would indeed have to consider this in their actions.

1001   In this case, analysis found that the southern African population is distinct in its genetic

1002   makeup. Considering that no active migration is occurring between the subpopulations, it

1003   seems intuitive to manage them as separate units. Additionally, the threats faced by these

1004   populations (Europe, northern Africa, southern Africa) may be vastly different and as such, a

1005   blanket    management    strategy,    which    governs    all    populations,    would    appear

1006 counterproductive to conservation. Given the isolation and genetic uniqueness of the

1007 southern African population, giving this population conservation priority could lead to a

1008 reassessment of the funds allocated to its conservation. Genetic diversity will continue to be

1009 lost, and population sub-structuring can be expected to become more pronounced, with

1010 geographic isolation of regional populations in the absence of active conservation.

1011 Conservation efforts in the form of captive breeding and release programmes have

1012 been successful (Antor et al. 2007; Gautschi et al. 2003; Schaub et al. 2009) in restocking

1013 diminishing and extirpated populations (Krüger et al. 2005; Tewes et al. 2004). The genotypes

1014 of founder individuals needs to be monitored to ensure the founders are adequately

1015 representative of the population's genetic variability. Given that the southern African

1016 population appears to be the least inbred and most diverse of the African populations

1017 supplementation should be done such that the maximum genetic variation is introduced. This

1018 should be managed on a case-by-case basis, as required. However, caution and timely

1019 research should be exercised before supplementing with individuals from the Eurasian

1020 population, lest these separate lineages be already too locally adapted and outbreeding

1021 depression be a concern (Frankham et al. 2011). That being said the diverged lineages

1022 proposed by Godoy et al. (2004) would have merged naturally and without adverse effects

1023 following the glacial maximum (Godoy et al. 2004).

1024 Further research should be aimed to increase sample sizes from central Asia, eastern

1025 and western Africa to confirm whether these populations are truly genetically isolated, or

1026 whether this is merely an artefact of sampling effort. Should these populations already be

1027 genetically distinct as a result of their past isolation due to population collapse, as well as

1028 continued persecution and decline, the potential a significant proportion of the global species

1029 genetic diversity being lost through the process of genetic erosion, is increased.

1030       The several bearded vulture populations are clearly subject to different environmental

1031    and legal forces. Drawing comparisons between these then may not present an honest

1032    reflection of the health of the species. This research confirmed the isolation of the southern

1033    African bearded vulture population as well as the presence of clear genetic structuring in the

1034    global population.

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**CHAPTER 3**

***Genetic diversity in the endangered South African bearded vulture (Gypaetus barbatus, L.)***

***population as revealed by microsatellite DNA***

**Abstract**

1204  The southernmost remnant bearded vulture *Gypaetus barbatus* population exists in the

1205  isolated mountainous regions of South Africa and Lesotho. A decline in population numbers

1206  and territory occupancy of this population is well documented, with breeding productivity

1207  and survival rate also known to be diminishing. Severe population collapse is acknowledged

1208  to accelerate genetic deterioration, which is known to impact on species' vital survival rates

1209  and directly impact its evolutionary persistence. Given that the southern African population

1210  is isolated and shows limited genetic connectivity with other bearded vulture populations

1211  (chapter 2), in this study I aim to assess the diversity present in the southern African

1212  population at the microsatellite-level and compare these values to that observed in other

1213  isolated bearded vulture populations. I will then also use the diversity found in other southern

1214  African vulture species (Cape vultures *Gyps coprotheres*, Hooded vultures *Necrosyrtes*

1215  *monachus*, and African White-backed vultures *Gyps africanus*) as a comparison. These three

1216  vulture species fulfil a similar ecological role. Results established that the southern African

1217  bearded vulture population harbours less diversity than would be expected under Hardy-

1218  Weinberg predictions ($H_O < H_E$). Results indicate that the mean genetic diversity of the South

1219  African populations to be similar to that seen in the East African and West African populations.

1220  The southern African population, however, contained the greatest allelic richness. Despite

1221  this, inbreeding seemed to be greatest within the southern African population. In comparison

1222  to other southern African vulture species, the southern African bearded vulture population

1223  appears to have higher levels of genetic variation, particularly in comparison to the Cape

1224    vulture. This is surprising given low population numbers, diminished range and limited

1225    breeding dispersal (natal philopatry), and it was expected that the bearded vultures should

1226    be more inbred and have higher levels of homozygosity than the other Africa vulture species.

**Introduction**

1227        Vultures are synonymous with the iconic 'African Experience'. Folk law, traditional

1228    stories and the museum artefacts are all testimony to this. However, of Africa's 11 vulture

1229    species, six are now classified as Threatened on the IUCN red data list (IUCN Species Survival

1230    Commission 2001; Ogada and Buij 2011; Ogada et al. 2016). Sudden reduction in numbers,

1231    diminishing habitat quality and low breeding rates are distinguishing red-flag features of

1232    species, which are deemed as 'threatened' (Brown 1997; Donázar et al. 1993; IUCN Species

1233    Survival Commission 2001; Krüger et al. 2014).

1234        Chief causes in vulture population declines are known to be a reduction in habitat

1235    quality and available breeding ground, due to anthropogenic sprawl. Electrocution and

1236    unintentional poisoning through veterinary drug-use, and intentional poisoning for the use of

1237    vulture body parts in the illegal wildlife trade has worsened the situation (Ogada et al. 2012;

1238    Rushworth and Krüger 2014). This targeted persecution, compounded by several other

1239    ecological and seemingly benign anthropogenic factors, has led to the rapid demise of most

1240    of Africa's vultures (Ogada et al. 2016). The Cape Griffon *Gyps coprotheres* (Boshoff and

1241    Anderson 2007), the Hooded vulture *Necrosyrtes monachus* (Ogada and Buij 2011), the

1242    White-headed vulture *Trigonoceps occipitalis*, the Rűppell's vulture *Gyps rueppelli* and

1243    Egyptian vulture *Neophron percnopterus* are species which have suffered a decline of 4.6 %

1244    per annum, which equates to 41- 50 % decline per decade (Ogada et al. 2016).

1245    Historically bearded vulture populations were considerably more abundant, albeit

1246    distributed across a wide geographic range from Palearctic, through Afrotropical and to

1247    Indomalay regions (Figure 3.1) (Godoy et al. 2004).  In the past century however, the species

1248    has become locally extinct, or survives in highly habitat-specific isolated refuges across their

1249    former range (Brown 1997; Gautschi et al. 2000). The Maloti-Drakensberg (Lesotho boarder)

1250    is home to the most southern population of African bearded vultures (Brown 1997). A recent

1251    estimate placed this breeding population at a meagre 109 pairs (Krüger et al. 2015). With

1252    foreboding statistics of diminishing territory occupancy, abandonment of suitable territories

1253    and ever decreasing breeding rates (Brown 1997; Krüger et al. 2014), the future of the South

1254    African bearded vultures is fragile.


1255    A prevalent and persistent concern in all species in which a population collapse has

1256    been documented, is the effect of a rapid genetic bottlenecking event. A drastic cut in the

1257    population numbers is often thought to be correlated to the depauperation and

1258    impoverishment of their genetic variability which directly impacts long-term persistence

1259    (Frankham 2005; Frankham et al. 2014; Tollington et al. 2013; Vucetich and Waite 1999).
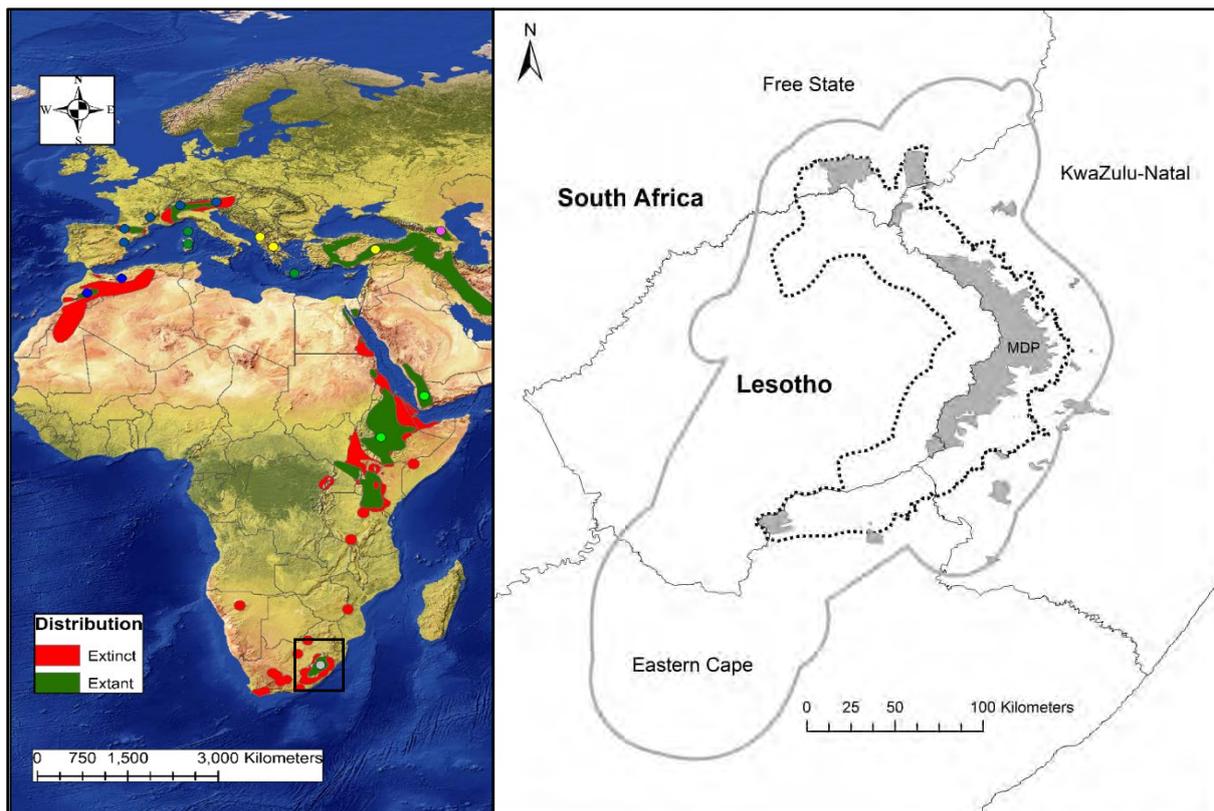
Figure 3.1: African distribution of bearded vulture Gypaetus barbatus (a), with samples assigned to three populations (South African, East African and West African) denoted by coloured circles. Boxed and enlarged (b) is the southern African range of the bearded vulture (stippled line) between 27°00"– 30°00" E and 28°00"–31°40" S. Protected areas are shaded in grey with the Maloti Drakensberg Park being the largest protected area along the South Africa/Lesotho border (Krüger et al. 2014).

1260    Despite the belief that the genetic component does not contribute meaningfully to a

1261    threatened species ultimate fate in the short term (Coulson et al. 2001; Lande 1999; Lande

1262    and Barrowclough 1987), the concomitant loss of diversity in bottlenecked vulture

1263    populations has been the impetus behind genetic studies for a number of species (Arshad et

1264    al. 2009; Frankham et al. 2014; Godoy et al. 2004; Krüger et al. 2015). Previous research on

1265    the genetics of the bearded vulture has revealed low levels of genetic diversity based on

1266    multilocus DNA fingerprinting (Negro and Torres 1999) and microsatellite markers (Gautschi

1267    et al. 2003). Both these studies confirmed the suspected lack of genetic diversity in European

1268    populations. A further study specifically on the southern African population of bearded

1269    vultures detected little differentiation and reduced genetic diversity compared to northern

1270    hemisphere populations (Krüger et al. 2015). Krüger and co-workers used mitochondrial DNA

1271    fragment analyses to detect this lack of genetic variation, and concluded that there is little to

1272    no differentiation between the southern African populations and populations in North Africa

1273    and Europe. Data presented in chapter 2, supports the hypothesis that the southern African

1274    population of bearded vultures is isolated and has limited genetic contact with other

1275    populations. Migrants comprise fewer than 9% of the total population per generation.


1276        In the present study, I augmented the work done by Krüger et al. (2015) by using

1277    nuclear markers to scan for genetic diversity and potential genetic structuring in the isolated

1278    southern African population. The aim of the research presented here is to confirm the lack of

1279    genetic diversity in the southern African bearded vulture population using a suite of

1280    microsatellite markers. The results from the southern African population are compared to

1281    those found in western and eastern African populations of bearded vultures. The genetic

1282    diversity present in the southern African bearded vulture is then compared to diversity values

1283    estimated from other southern African vulture species (Cape vulture *Gyps coprotheres*,

1284    Hooded vulture *Necrosyrtes monachus*, and White-headed vulture *Trigonoceps occipitalis*)

1285    which are similar in ecology and have also suffered population collapses. Confirmation of

1286    reduced microsatellite diversity would necessitate drastic conservation measures for the

1287    management of the isolated southern African population.

**Materials and methods**

1288   *Southern African bearded vultures*

1289        A total of 52 samples of southern African bearded vulture (*Gypaetus barbatus*)

1290   comprise the sample set for this chapter. The vast majority (51) of these are contemporary

1291   samples (2001 – 2012) while one historic sample was from the Durban Museum of Natural

1292   History (collected in 1961). Sample details given in Table 3.1. The individuals sampled belong

1293   to the southern African bearded vulture population which inhabits the Drakensberg mountain

1294   range between Lesotho and the KwaZulu-Natal province. The population is largely restricted

1295   to the highlands of Lesotho and immediately adjacent areas of the Maloti-Drakensberg

1296   mountains (Brown 1990).

Table 3.1: Details of the 52 bearded vulture samples derived from the southern African population.

| Provided by | Sample Name | Catalogue No. | Country | Precise Locality if available | Collection Year |
|---|---|---|---|---|---|
| UKZN, Krüger Pet al. 2015 | BV001 | G22911 | South Africa | Southern Drakensberg | 2006 |
| | BV002† | G27343 | South Africa | Southern Drakensberg | 2007 |
| | BV003 | G27341 | South Africa | Southern Drakensberg | 2007 |
| | BV004† | G27307 | South Africa | Southern Drakensberg | 2009 |
| | BV005† | G27313 | South Africa | Southern Drakensberg | 2010 |
| | BV006† | G27306 | South Africa | Southern Drakensberg | 2009 |
| | BV007† | G27378 | Lesotho | Lesotho | 2012 |
| | BV008 | G27308 | South Africa | Southern Drakensberg | 2009 |
| | BV009† | G27376 | South Africa | Northern Drakensberg | 2011 |
| | BV010 | G27314 | South Africa | Northern Drakensberg | 2010 |
| | BV011 | BV14 | South Africa | NE Free State | 2004 |
| | BV012 | BV2002 | South Africa | Central Drakensberg | 2002 |
| | BV013† | G27302 | Lesotho | Lesotho | 2008 |
| | BV014 | BV2009 | South Africa | Central Drakensberg | 2009 |
| | BV015† | G27375 | South Africa | Southern Drakensberg | 2011 |
| | BV016† | G27305 | South Africa | Southern Drakensberg | 2009 |
| | BV017 | G27303 | Lesotho | Lesotho | 2008 |

| | | | | |
|---|---|---|---|---|
| BV018 | G27309 | South Africa | Southern Drakensberg | 2009 |
| BV019 | 12 | South Africa | Southern Drakensberg | 2003 |
| BV020 | 6 | Lesotho | Lesotho | Not provided |
| BV021 | 25 | South Africa | Northern Drakensberg | 2011 |
| BV022 | 13 | South Africa | Central Drakensberg | 2008 |
| BV023 | 19 | South Africa | Northern Drakensberg | 2009 |
| BV024 | 27 | South Africa | Southern Drakensberg | 2010 |
| BV025 | 11 | South Africa | Southern Drakensberg | 2000 |
| BV026 | 10 | South Africa | Southern Drakensberg | 2007 |
| BV027 | 31.1 | South Africa | Southern Drakensberg | 2010 |
| BV028 | 31.2 | South Africa | Southern Drakensberg | 2010 |
| BV029 | 30 | South Africa | Northern Drakensberg | 2010 |
| BV030 | 9 | South Africa | Southern Drakensberg | 2009 |
| BV034 | G27382 | South Africa | Central Drakensberg | 2012 |
| BV035 | 02/08/2012 | South Africa | Eastern Cape | 2012 |
| BV036 | G27377 | South Africa | Northern Drakensberg | 2012 |
| BV037† | G27379 | South Africa | Central Drakensberg | 2012 |
| BV038 | G27381 | South Africa | Northern Drakensberg | 2012 |
| BV039 | G27383 | South Africa | Central Drakensberg | 2012 |
| BV040† | G27384 | South Africa | Central Drakensberg | 2012 |
| | G27380 | South Africa | Not provided | 2011 |
| | 34 | South Africa | Not provided | Not provided |
| | G27319 | South Africa | Northern Drakensberg | 2010 |
| | G27310 | South Africa | Central Drakensberg | 2010 |
| | G27311 | South Africa | Northern Drakensberg | 2010 |
| | G27315 | South Africa | Northern Drakensberg | 2010 |
| | G27316 | South Africa | Northern Drakensberg | 2010 |
| | G27342 | South Africa | Southern Drakensberg | 2007 |
| | BV6 | South Africa | Lesotho | 2009 |
| | BV7 | South Africa | Lesotho | 2009 |
| | BV8 | South Africa | Lesotho | 2009 |
| | BV10 | South Africa | Lesotho | 2009 |
| | BV12 | South Africa | Lesotho | 2009 |
| | G | South Africa | Drakensberg | Not provided |
| DNSM | DNSM 2 | South Africa | Central Drakensberg | 1961 |

1297    *African bearded vultures*

1298        Samples grouped into the eastern (n = 18) and western (n = 6) African bearded vulture

1299    populations were derived from specimens collected from these broad geographic areas. The

1300    eastern grouping consists of samples collected from Ethiopia, Abyssinia (northern Ethiopia)

1301    and Yemen; while samples collected from Algeria and Morocco formed the western grouping.

1302    Details for these samples are provided in Table 3.2.

Table 3.2: Details of the 24 bearded vulture samples, broadly grouped into eastern and western African populations.

| Sample Name | Catalogue No. | Origin | Precise Locality if available | Collection Date |
|---|---|---|---|---|
| A1 | Skin 268806 | Ethiopia | Kaka Mt., Arussi | 1929 |
| A13 | SKIN 535830 | Yemen | Sôk al Khamis | 1913 |
| A14 | SKIN 535829 | Yemen | Sôk al Khamis | 1913 |
| A17 | SKIN 535836 | Morocco | Djebel Tixa (Atlas) | 1905 |
| A18 | SKIN 535837 | Algeria | Djebel Taya, Meskoutine | 1909 |
| A19 | SKIN 535839 | Algeria | Kerrata | 1904 |
| A20 | SKIN 535840 | Algeria | El Kantara | 1909 |
| A21 | SKIN 535848 | Ethiopia | Yeka Hill, Addis Ababa | 1926 |
| A22 | SKIN 535855 | Ethiopia | Addis Ababa | 1914 |
| A23 | SKIN 535856 | Ethiopia | Addis Ababa | 1914 |
| A25 | SKIN 535858 | Ethiopia | Addis Ababa | 1914 |
| BV031 | Kolo | Ethiopia | Not provided | 2001 |
| BV032 | Mutt | Ethiopia | Not provided | 2001 |
| BV033 | Putin | Ethiopia | Not provided | 2001 |
| S1.14 | 14 | Algeria | Algeria | Not provided |
| S1.16 | 16 | Ethiopia | Abyssinia | 1959 |
| S1.17 | 17 | Ethiopia | Abyssinia | 1959 |
| S1.50 | 50 | Ethiopia | Ethiopia | 1887 |
| S1.60 | 60 | Ethiopia | Abyssinia | 1900 |
| S2.113 | 113 | Ethiopia | Abyssinia | 1911 |
| S3.19 | 19 | Algeria | Algeria | 2000 |
| S3.27 | 27 | Ethiopia | Abyssinia | 1959 |
| S3.29 | 29 | Ethiopia | Abyssinia | 1959 |
| S3.49 | 49 | Ethiopia | Abyssinia | 1990 |

1303    *Other Southern African vulture species*

1304        Cape vultures *Gyps coprotheres* (n= 547), Hooded vultures *Necrosyrtes monachus* (n=

1305    54; Table 3.3), and African White-backed vultures *Gyps africanus* (n= 31), are used to draw

1306    reasonable, context-dependent comparisons. With the exception of the African White-

1307    backed vulture which can be found through much of Africa, these species occur in southern

1308    Africa and belong to the Old-world bird of prey family, Accipitridae. As they exhibit similar

1309    life-history traits and are subject to the same environmental and anthropogenic threats in

1310    South Africa. All four vulture species are facing population declines and are currently classified

1311    by the IUCN as Threatened to Critically Endangered. The statistical values for comparison

1312    between species are taken chiefly from unpublished work on Cape (Kleinhans 2018) and

1313    Hooded vultures (Willows-Munro *pers. coms.* 2017) that has been conducted in Dr. Willows-

1314    Munro's laboratory, and from Arshad et al*.* 2009 (African White-backed vulture).


Table 3.3: Details of the 54 Hooded vulture samples derived from the southern African population.

| Sample Name | Country | Province | Precise Locality | Collection Year |
|---|---|---|---|---|
| HV2 | South Africa | Limpopo | Cleveland | 2015 |
| HV3 | South Africa | Limpopo | Cleveland | 2015 |
| HV4 | South Africa | Limpopo | Tulloh Farm | 2015 |
| HV5 | South Africa | Limpopo | Ndlovumzi Nature Reserve | 2015 |
| HV6 | South Africa | Limpopo | Ndlovumzi Nature Reserve | 2015 |
| HV7 | South Africa | Limpopo | Ndlovumzi Nature Reserve | 2015 |
| HV8 | South Africa | Limpopo | Ndlovumzi Nature Reserve | 2015 |
| HV9 | South Africa | Limpopo | Kruger Ntional Park | 2015 |
| HV10 | South Africa | Limpopo | Kruger Ntional Park | 2015 |
| HV11 | South Africa | Limpopo | Kruger Ntional Park | 2015 |
| HV12 | South Africa | Limpopo | Pidwa Game Reserve | 2017 |
| HV13 | South Africa | Limpopo | Ndlovumzi Nature Reserve | 2015 |
| HV14 | South Africa | Limpopo | Baluleni Lodge | 2015 |
| HV15 | South Africa | Limpopo | Baluleni Lodge | 2015 |

| HV16 | South Africa | Limpopo | Baluleni Lodge | 2015 |
|---|---|---|---|---|
| HV17 | South Africa | Limpopo | Baluleni Lodge | 2015 |
| HV18 | South Africa | Limpopo | Baluleni Lodge | 2015 |
| HV19 | South Africa | Limpopo | Baluleni Lodge | 2015 |
| HV20 | South Africa | Limpopo | Cleveland | 2015 |
| HV21 | South Africa | Limpopo | Damian's, Grietjie Game Reserve | 2015 |
| HV22 | South Africa | Limpopo | Tulloh Farm | 2015 |
| HV23 | South Africa | Limpopo | Wild Rivers Nature Reserve | 2015 |
| HV24 | South Africa | Limpopo | Mfubu Lodge, Grietjie Nature Reserve | 2017 |
| HV25 | South Africa | Limpopo | Mfubu Lodge, Grietjie Nature Reserve | 2015 |
| HV26 | South Africa | Limpopo | Damian's, Grietjie Game Reserve | 2015 |
| HV27 | South Africa | Limpopo | Ukhozi Nature Reserve | 2017 |
| HV28 | South Africa | Limpopo | Damian's, Grietjie Game Reserve | 2015 |
| HV29 | South Africa | Limpopo | Baluleni Lodge | 2015 |
| HV30 | South Africa | Limpopo | Ukhozi Nature Reserve | 2017 |
| HV31 | South Africa | Limpopo | Wild Rivers Nature Reserve | 2015 |
| HV32 | South Africa | Limpopo | Mfubu Lodge, Grietjie Nature Reserve | 2015 |
| HV33 | South Africa | Limpopo | Cleveland Island | 2015 |
| HV34 | South Africa | Limpopo | Grietjie Nature Reserve | 2015 |
| HV36 | South Africa | Limpopo | Uni-Fattoria Farm | 2015 |
| HV38 | South Africa | Limpopo | Cleveland, Phalaborwa | 2015 |
| HV39 | South Africa | Limpopo | Cleveland, Phalaborwa | 2015 |
| HV40 | South Africa | Limpopo | Ukhozi Nature Reserve | 2015 |
| HV41 | South Africa | Limpopo | Ukhozi Nature Reserve | 2015 |
| HV42 | South Africa | Limpopo | Kruger National Park | 2015 |
| HV43 | South Africa | Limpopo | Grietjie Game Reserve | 2015 |
| HV44 | South Africa | Limpopo | Grietjie Game Reserve | 2015 |
| HV45 | South Africa | Limpopo | Ndlovumzi Nature Reserve | 2015 |
| HV46 | South Africa | Limpopo | Grietjie Game Reserve | 2015 |
| HV47 | South Africa | Limpopo | Grietjie Game Reserve | 2015 |
| HV48 | South Africa | Limpopo | Grietjie Nature Reserve | 2015 |
| HV49 | South Africa | Limpopo | Wild Rivers Nature Reserve | 2015 |
| HV50 | South Africa | Limpopo | Timbavati | 2016 |
| HV51 | South Africa | Limpopo | Uni-Fattoria Farm | 2016 |
| HV52 | South Africa | Limpopo | Cleveland | 2015 |
| HV53 | South Africa | Limpopo | Blyde River | 2015 |
| HV54 | South Africa | Limpopo | Cleveland | 2015 |
| HV55 | South Africa | Limpopo | Wild Rivers Nature Reserve | 2016 |
| HV56 | South Africa | Limpopo | Cleveland | 2015 |
| HVF103 | South Africa | Limpopo | Hoedspruit | 2015 |

1315    *DNA extraction and microsatellite amplification*

1316    DNA extraction and purification was performed followed the same protocol described

1317    in chapter 2. The microsatellite loci chosen are the same as for chapter 2. The details of

1318    primers used can be found in chapter 2 Table 2.1. All products were run at the Central

1319    Analytical Facility (CAF) at Stellenbosch University. Just as in the previous chapter, 20% of

1320    specimens were re-genotyped to ensure accuracy of genotype scoring.


1321    *Microsatellite analysis*

1322    The presence of null alleles was tested for in ML-Null Freq (Kalinowski and Taper 2006)

1323    and FreeNA (Chapuis and Estoup 2006).  Both programmes detected significant levels of null

1324    alleles in multiple loci. Null alleles may bias towards low-levels of heterozygosity and

1325    increased population differentiation. The presence of scoring errors, such as incorrect

1326    assigning of genotypes and fractions in genotypes, was accessed using GenAlex v6.5 (Peakall

1327    and Smouse 2006) and FSTAT (Goudet 1995). These were also used to calculate summary

1328    statistics (average number of alleles at each locus ($N_A$), allelic richness ($A_R$), inbreeding

1329    coefficient (Fis) and gene diversity) for South African bearded vulture population as well as

1330    the three other species. Effective alleles at each locus ($N_E$) and observed ($H_O$) and expected

1331    (He) heterozygosity were calculated in GenAlex. FSTAT was used to determine allelic richness

1332    ($A_R$) and gene diversity. The fixation indices (inbreeding coefficient, Fis and population

1333    differentiation, Fst) (Saitou and Nei 1987; Weir 1996) were calculated in FSTAT (Raymond and

1334    Rousset 1995). Fis and Fst explain the deviations seen from Hardy-Weinberg Equilibrium

1335    (HWE). Positive Fis values suggest higher inbreeding values (due to higher observed

1336    homozygosity) than would be expected under Hardy-Weinberg equilibrium, and vice versa.

1337    Fst shows the genetic differentiation between two populations which no longer conform to

1338    the HWE assumptions. This analysis was conducted by comparing the eastern, western and

1339    southern African bearded vulture populations.


**Results**

1340    FSTAT detected high (> 8%) null allele frequencies across 11 loci (GF11a4, Gf8g, Gf3f3,

1341    Gf3h3, Gf9c, BV2, BV6, BV5, BV8, BV11, BV17). FreeNA and ML-Null Freq confirmed this,

1342    though ML-Null Freq predicted higher frequencies of null alleles (up to 22%). The age of the

1343    sample was found to negatively affect the efficiency of amplification, and this could have

1344    biased the proportion of null alleles detected. This could have been because of low DNA

1345    concentration or highly fragmented DNA typical of dated and degraded museum samples.

1346    The spurious scoring of null alleles as homozygous individuals is known to exaggerate

1347    population differentiation (Chapuis and Estoup 2006) without radically influencing

1348    assignment test interpretation (Carlsson 2008). For this reason, null-allele frequencies

1349    reported and used here are the corrected values.


1350    *Southern African bearded vultures*

1351    All bearded vulture samples (52) were genotyped using 14 microsatellite loci.

1352    Complete amplification across all primers was problematic; missing data was encountered

1353    (11% - 37%) across all loci. The average number of alleles across all loci was 6.57 although

1354    effective alleles were lower ($N_E$ =2.77) (Table 3.4). Bearded vultures in South Africa show

1355    moderate levels of genetic diversity ($H_O$ = 0.49) when compared to other southern African

1356    vulture species (Table 3.2) but have a comparatively high allelic richness (= 6.57, Table 3.4).

1357    Table 3.4: Estimates of genetic diversity across 14 amplified loci in the southern African

1358    population of bearded vultures *Gypaetus barbatus*.

| Locus | Observed allele size | $N_A$ | $N_E$ | $H_O$ | $H_E$ | Gene Diversity | $F_{IS}$ |
|---|---|---|---|---|---|---|---|
| *Gypaetus barbatus* | | | | | | | |
| BV2 | 114 - 136 | 6 | 2.88 | 0.57 | 0.65 | 0.66 | 0.14 |
| BV5 | 153 - 187 | 3 | 1.61 | 0.00 | 0.38 | 0.41 | 1.00 |
| BV6 | 105 - 201 | 10 | 5.37 | 0.52 | 0.81 | 0.84 | 0.43 |
| BV8 | 103 - 117 | 5 | 2.49 | 0.45 | 0.60 | 0.61 | 0.25 |
| BV9 | 209 - 219 | 5 | 2.65 | 0.68 | 0.62 | 0.63 | -0.05 |
| BV11 | 143 - 187 | 5 | 1.73 | 0.13 | 0.42 | 0.44 | 0.71 |
| BV12 | 231 - 257 | 5 | 2.89 | 0.87 | 0.65 | 0.66 | -0.32 |
| BV14 | 157 - 189 | 9 | 3.86 | 0.90 | 0.74 | 0.75 | -0.21 |
| BV17 | 183 - 193 | 5 | 2.10 | 0.40 | 0.52 | 0.53 | 0.25 |
| Gf3f3 | 138 - 180 | 8 | 2.34 | 0.45 | 0.57 | 0.58 | 0.28 |
| Gf3h3 | 111 - 139 | 8 | 2.64 | 0.49 | 0.62 | 0.63 | 0.23 |
| Gf8g | 164 - 280 | 7 | 2.23 | 0.37 | 0.55 | 0.56 | 0.34 |
| Gf9c | 220 -264 | 10 | 3.96 | 0.46 | 0.75 | 0.76 | 0.39 |
| Gf11a4 | 125 - 160 | 6 | 2.01 | 0.52 | 0.50 | 0.51 | -0.01 |
| Total | | 6.57 | 2.77 | 0.49 | 0.60 | 0.61 | 0.22 |

Key: $N_A$, average number of alleles; $H_O$, observed heterozygosity; $H_E$, expected heterozygosity; $A_R$, Allelic richness; $F_{is}$, inbreeding coefficient.

1359 *African populations of bearded vultures*

1360       This data set (n=70) includes the populations of bearded vultures which are located in

1361 southern Africa (n=52), eastern Africa (chiefly Ethiopia) (n=7) and western Africa (Algeria,

1362 Morocco, Tunisia) (n=11). The mean genetic diversity was similar for all three populations

1363 (0.51-0.61), and expected heterozygosities ranging from 0.45-0.60 (Table 3.2). All populations

1364 reveal a lower than expected level of heterozygosity ($H_o < H_E$, Table 3.2). The southern African

1365 population harbours the greatest allelic richness, with western Africa showing less than half

1366 this (Table 3.2). Quite possibly this could be attributed to the difference in sample size. All

1367 three populations show slight to intermediate inbreeding levels. The southern African

1368 population appears to be the least inbred (Fis = 0.22), followed by eastern (Fis = 0.29) and

1369 then western populations (Fis= 0.30, Table 3.2).

1370 Table 3.5: Estimates of genetic diversity across 14 amplified loci in the African population of
1371 bearded vultures *Gypaetus barbatus*.

| Locus | $N_E$ | $A_R$ | $H_O$ | $H_E$ | Gene Diversity | $F_{IS}$ | % PML |
|-------|-------|-------|-------|-------|----------------|----------|-------|
| *Gypaetus barbatus* | | | | | | | |
| S. Africa | 2.77 | 6.57 | 0.49 | 0.60 | 0.61 | 0.22 | 100.00 |
| E. Africa | 2.72 | 3.57 | 0.41 | 0.51 | 0.56 | 0.29 | 92.86 |
| W. Africa | 2.32 | 3.07 | 0.36 | 0.45 | 0.51 | 0.30 | 85.71 |

Key: $N_E$, expected no. alleles; $A_R$, Allelic richness; $H_O$, observed heterozygosity; $H_E$, expected heterozygosity; $F_{is}$, inbreeding coefficient; %PML: percentage polymorphic loci

1372    *Other southern African vulture species*

1373    The bearded vulture population reveals high allelic richness in the suite of

1374    microsatellites chosen for this research ($A_R$ = 6.57, Table 3.6). The Hooded and African White-

1375    backed vulture populations contain a proportion of heterozygous individuals, which is

1376    expected under Hardy-Weinberg, whereas the bearded vulture population has higher

1377    proportions of homozygosity (Table 3.6). The Cape vultures, despite the large sample size and

1378    loci tested, have little allelic richness ($A_R$ = 4.49, Table 3.3) and low observed heterozygosity

1379    ($H_O$ = 0.31, Table 3.6).

1380    Table 3.6: Estimates of genetic diversity across 8 to 14 amplified loci in populations of four
1381    African vulture species.

| Species | Sample Size | No. of loci | $H_O$ | $H_E$ | $A_R$ | Reference |
|---|---|---|---|---|---|---|
| Bearded | 52 | 14 | 0.49 | 0.60 | 6.57 | Current study |
| Hooded | 54 | 12 | 0.74 | 0.69 | 4.82 | Willows-Munro *pers. coms* |
| Cape | 547 | 13 | 0.31 | 0.41 | 4.49 | Kleinhans 2018 |
| White-backed | 31 | 8 | 0.67 | 0.69 | 5.00 | Arshad *et. al*, 2009 |

Key: $A_R$, Allelic richness; $H_O$, observed heterozygosity; $H_E$, expected heterozygosity; $F_{is}$, inbreeding coefficient

**Discussion**

1382 *Diversity*

1383        A significant loss of genetic diversity is expected to occur following population collapse

1384 and bottlenecking events (Lande and Barrowclough 1987; Vucetich and Waite 1999; Wilcox

1385 and Murphy 1985). The associated loss of genetic variability is believed to adversely affect the

1386 adaptive potential and evolutionary persistence (Bretagnolle et al. 2004; Soulé 1986; Vucetich

1387 and Waite 1999). The southern African bearded vulture population is geographically and

1388 genetically isolated (chapter 2). Isolated populations are at risk of increased genetic drift,

1389 inbreeding and loss of genetic diversity. Despite the smaller population and sample size in

1390 comparison to Cape vultures, the southern African bearded vultures appear to maintain

1391 greater genetic variation as regards allelic richness and heterozygosity levels.

1392 *Southern African bearded vulture population*

1393        Apart from past persecution, the southern African population of bearded vultures

1394 continues to suffer threats from several sources, adding to their continued decline (Krüger et

1395 al. 2006; Krüger et al. 2014). Past studies on the global and local bearded vulture populations

1396 have raised concern for their management based on the reduced genetic variability

1397 (mitochondrial, microsatellite and multilocus DNA fingerprinting) found (Gautschi et al. 2003;

1398 Godoy et al. 2004; Krüger et al. 2006; Negro and Torres 1999). Given that the southern African

1399 bearded vulture population is geographically and genetically isolated (chapter 2), it was

1400 expected that this population should be genetically at risk.

1401        The analysis presented in this chapter found the southern African population of

1402 bearded vultures to harbour less genetic diversity than would be expected, with observed

1403   heterozygosity values lower than expected under Hardy-Weinberg. The drastic population

1404   decline and reduced genetic status of the bearded vulture places the southern African

1405   population alongside other ecologically and behaviourally similar vulture species. The

1406   bearded vultures however, seem to be maintaining their genetic diversity as seen by a rich

1407   allele count despite having lower levels of observed heterozygosity than either Hooded or

1408   African White-backed vultures. When compared to Cape vultures however, which have

1409   overlapping ranges and similar roosting requirements, bearded vultures appear to be more

1410   genetically diverse. The severe genetic loss that the Cape vultures have experienced in

1411   comparison to bearded vultures is alarming considering the difference in sample size (547 vs.

1412   52) for these studies, and breeding pair numbers in southern Africa (1450 vs. 109-221 pairs)

1413   (Brown 1997; Krüger 2014). That the bearded vultures have maintain more diversity may well

1414   be because Cape vultures are still recovering from a more pronounced bottleneck event

1415   (Ogada et al. 2016). Cape vultures may be experiencing greater genetic drift and this has lead

1416   to an accelerated loss of variation.


1417   *African bearded vulture populations*

1418      In comparison to the other African bearded vulture populations, analysis of the

1419   southern population returned the highest average number of effective alleles, highest

1420   observed heterozygosity and the highest allelic and genetic richness. Correspondingly, the

1421   inbreeding coefficient (Fis) was lowest within the southern African population when

1422   compared to the eastern and western populations.

1423      The bearded vultures have undergone a global population decline over a relatively

1424   short duration. This population collapse, coupled with the isolated nature of the southern

1425  African population, raises concern for the long-term population health and persistence of the

1426  species in the southern African region. However, the microsatellite data showed estimates of

1427  genetic variation greater than that recorded for the Cape vulture. This could be because

1428  bearded vultures are long-lived and as such can retain levels of genetic diversity in small

1429  populations over longer time periods than species with faster generation time (Johnson et al.

1430  2008; Kuo and Janzen 2004). Nonetheless, diversity levels will continue to decline if the

1431  population bottleneck is indefinitely sustained. The genetic variation seen in contemporary

1432  populations is a reflection of the diversity in the previous generations. Should conservation

1433  seek to preserve this gene-assemblage, active management would need to firstly minimise

1434  external threats, and mitigate factors leading to population structuring as this amplifies

1435  genetic drift. Specifically, this research found the southern African population to be

1436  genetically distinct from other populations of bearded vultures. For conservation, it would be

1437  senseless to divide a species into multiple management units if migration and breeding still

1438  actively occurred between the subspecies. However, in the absence of evident interbreeding

1439  between subpopulations, each separate population should be conserved as an entity. Given

1440  this fact, the southern African population should be managed as such and not be subject to a

1441  global 'blanket' management. It would not be wise to disrupt the unique genetic composition

1442  of the southern African population, which appears to contain diversity levels abnormal for

1443  such a small population.

1444      These results highlight the necessity to integrate genetic data into conservation

1445  strategies as it elucidates information that is not readily perceivable. Here, it suggests that

1446  the southern African population, being distinct from other such African populations, may

1447  represent a reservoir of genetic variation.

1448       The research presented contributes to past and ongoing bearded vulture studies by

1449    providing an overview of the genetic status of the southern African population. Further, it

1450    supports the caution of these studies that there is reduced genetic variation and inbreeding

1451    in naturally breeding populations of the bearded vulture. Genetic diversity will continue to be

1452    lost, and population sub-structuring can be expected to become more evident with

1453    geographic isolation of populations in the absence of active conservation.

1454       Further research, and continued sampling are needed to definitively confirm whether

1455    the southern African population is isolated, or whether this is merely an artefact of sampling

1456    effort. Should these populations already be structured as a result of their past population

1457    collapse, as well as continued persecution and decline, the potential for a significant

1458    proportion of the global species genetic diversity being lost through the process of genetic

1459    erosion, is increased.

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**CHAPTER 4**

***A genetically informed population viability analysis of the southern African bearded vulture***

***Gypaetus barbatus population.***

**Abstract**

1559    Population viability analysis (PVA) is an adaptable tool used to predict the probabilistic future of

1560    a species. PVAs have found particular popularity in informing the management of endangered

1561    species. Over the last century, vultures have been under sever persecution, and have suffered

1562    severe population declines. Six of Africa's 11 species are subsequently at risk of extinction. The

1563    southern African bearded vulture *Gypaetus barbatus* population has suffered major declines since

1564    1700-1970s, and continues to decline due to indiscriminate use of poison, electrocutions and

1565    harvesting. In this study, the PVA software VORTEX is used to predict the viability of the southern

1566    African *G. barbatus* population by combining life history data with genotypic information derived

1567    from 14 polymorphic microsatellite markers sampled from 52 individuals belonging to isolated

1568    southern African bearded vulture population. The simulations tracked a hypothetical population

1569    that had a high probability of extinction over the next 100 years. The probabilistic outcome of the

1570    population was not markedly different when the genetics data was included in the model. This

1571    points toward a species that, although characterised by a small census population size is still

1572    genetically variable enough to delay genetic erosion and the negative impacts this would have on

1573    demographic vital rates. However, this is not intended to lessen the urgency of actively conserving

1574    the species. To our knowledge this study, on the management of an endangered southern African

1575    vulture, is amongst one of the first where the genetic data is incorporated into the PVA model to

1576    predicted future trends in species survival.

**Introduction**

1577    Several decades of evidence refutes any doubt that indigenous plant and animal species'

1578    populations are not under threat (Ehrlich and Ehrlich 1981). Concomitant population declines,

1579    associated with ongoing habitat fragmentation (Wilcox and Murphy 1985), necessitates a means

1580    of estimating the persistence of populations under particular scenarios (Oostermeijer et al. 2003).

1581    Conservation biologists in the 1980s coined the term 'population viability' and developed

1582    theoretical, and statistical means of measuring a populations risk of extinction (Shaffer 1979;

1583    Soulé 1986; 1987; Soulé and Wilcox 1980). Population viability analyses (PVAs) predict the

1584    effective population number (individuals who contribute to the reproductive output of the

1585    species) required to maintain the species persistence over a given time. Or alternatively, PVA

1586    predicts the likelihood of extinction by incorporating potential threats to the population into the

1587    model (Baral et al. 2013; Velevski et al. 2014).

1588    PVA came as an improvement to the rudimentary 50/500 rule which simplified species'

1589    conservation by providing basic threshold numbers of the number of individuals need for a

1590    species to survive (Baral et al. 2013; Gilpin 1996). The rule stated that a population of less than

1591    50 individuals would go extinct in the short-term due to inbreeding depression, and a size less

1592    than 500 would not contain enough genetic variability for adaptation, which would lead to

1593    extinction in the face of environmental change (Gilpin 1996).

1594    Since then, alternate means of approaching population conservation have emerged as it

1595    was recognised that prescribing a generalised number was overly simplistic as life history traits

1596    differ vastly between species which could influence population viability. The practical problems

1597    of conservation and wildlife management, however, require knowledge of statistical viability of a

1598    population and the risks affecting species. Because of the inherent variation and confounding

1599    factors associated with ecological data, PVAs implement a risk-based approach (Akçakaya and

1600 Sjögren-Gulve 2000). In this, the feasibility of survival is expressed as the likelihood of a

1601 population's extinction (or recovery) over a given time (Akçakaya and Sjögren-Gulve 2000; Brook

1602 et al. 2000). Habitat suitability, GAP analysis, reserve selection algorithms, landscape indices

1603 among others are alternative conservation strategies which are applicable under certain

1604 scenarios, but for several distinct advantages PVA is the chosen tool in conservation (Akçakaya

1605 and Sjögren-Gulve 2000).

1606      The predictive power of PVA is continuously under scrutiny. The intent of the PVA needs

1607 to be clearly defined for this concern to be resolved. As PVAs are used to select among several

1608 management options, the argument is that there need not be exact precision as long as the

1609 comparison is valid (McCarthy et al. 2003; Velevski et al. 2014). However, Brook et al. (2000) argue

1610 for the qualitative reliability of a PVA, as the conservation costs entailed must be justified by the

1611 anticipated benefits. Legally, the 'fallibility' of PVAs has led to management decisions in favour of

1612 conservation being refuted in courts (Brook et al. 2000). The predicative power and accuracy of

1613 regularly used PVA programmes has been tested and published (McCarthy et al. 2003). Such

1614 studies tested between the various programmes which implement PVA across a variety of taxa

1615 and families. Where sufficient data was available from past extinction events, the simulated

1616 results were compared to the eventual (real) outcome. Research has also refined the conditions

1617 under which the programmes perform best. The results showed that the simulated results

1618 modelled the historical outcomes with surprising accuracy, although there was a tendency of

1619 predictions to be marginally pessimistic. The ultimate findings of the comparative studies

1620 contradict the belief that PVAs are poor predictors of a populations fate (Brook et al. 2000). PVAs

1621 have since been used in numerous studies used to inform conservation decisions (Baral et al.

1622 2013; Tollington et al. 2013; Velevski et al. 2014).

1623       Populations that have undergone a severe, or continual decline, are considered at higher

1624       risk of extinction, especially if the decline prohibits movement between neighbouring populations

1625       (Gaston 1994). Further, large populations are known to buffer against random variation in

1626       demography, unpredictable environmental catastrophes, genetic effects (inbreeding and drift)

1627       and slow rates of adaptation (Brown 1995; Lande 1999; Lande and Barrowclough 1987).

1628       Populations of long-lived raptors are particularly susceptible to variability in birth and

1629       death rates (Smart et al. 2010). In species where the reproductive rate is notably low- such as in

1630       vultures- susceptibility to demographic stochasticity is heightened (Ogada et al. 2012). Most

1631       Southern African vulture species are thus listed as Endangered or Critically Endangered by IUCN.

1632       The bearded vulture population of the Maloti-Drakensberg in southern Africa is

1633       geographically and genetically isolated from other populations in Africa (Ethiopia and Algeria) and

1634       Europe (chapter 2). The population is under threat of extinction as a result of declines both in

1635       number of individuals and occupied territory, as well as its continued persecution (Krüger et al.

1636       2014).

1637       The contribution of a specie's genetics to its ultimate survival or extinction has been

1638       neglected in the past (Frankham et al. 2002). The belief was that small populations would be

1639       driven to extinction by environmental catastrophes and demographic stochasticity before genetic

1640       factors would have a chance to exert their effect (Lande and Barrowclough 1987; Shaffer 1987),

1641       and therefore including genetic data in PVA would have little impact on the predicted outcomes.

1642       Genetic effects however, are unavoidable and amplified in small populations. Factors such as

1643       inbreeding and loss of heterozygosity further reduces the population's reproductive output, even

1644       after the initial cause for decline has been mitigated. Even when the genetic component is

1645       acknowledged to impact a specie's extinction probability, it is still unclear how this probability

1646       relates to population size (Wittman et al. 2018). It is also unclear if the extinction probability

1647    increases in a linear function and if recovery is also linear. Extinction probability may instead

1648    follow a sigmoidal function where a sharp transition exists from which recovery could be more

1649    complicated. Not including genetic effects in PVA models may thus lead to biased predictions.

1650        In this paper, I aim to add to the bearded vulture population viability analyses of

1651    Rushworth and Krüger (2014) to compare the extent to which the incorporation of genetic data

1652    affects the model. I conducted a PVA in the modelling programme VORTEX using identical

1653    parameters (Rushworth and Krüger 2014b), but also include allele frequencies of the southern

1654    African population as an additional parameter.

**Methods**

1655    *Simulation model*

1656        I used the VORTEX 10 simulation software (Lacy 1993; 2000) to model the future

1657    population trend of the southern African bearded vulture population. VORTEX is an individual-

1658    based simulation model for PVA that models the effect of deterministic and stochastic processes

1659    on the population dynamics of species, both wild and captive (Miller and Lacy, 2005). This

1660    programme was developed for avian and mammalian species with typically low fecundity

1661    (Keedwell 2004; Lacy 1993). VORTEX is advantageous as it has the ability to model population

1662    trends with more acuity by taking into consideration the species biology (such as behaviour and

1663    mating systems) (Duca et al. 2009) as well as genetic data (Lacy 1993). Brook et al. (2000)

1664    performed a comprehensive analysis in which they evaluated both the strengths and failing of the

1665    various PVA programmes. VORTEX is best able to model a populations growth trend for long-lived

1666    species with low-fecundity, such as mammals, birds and reptiles (Lacy 1993). Thus, VORTEX is

1667    often the chosen PVA program used in conservation studies.

1668 *Simulation model parameters*

1669    The baseline scenario was modelled using the biological data given in Krüger et al. (2014).

1670    The number of lethal equivalents and recessive alleles was kept at the model's default values

1671    (3.14 and 50 respectively). Although, it has been suggested that the lethal equivalents can

1672    sometimes be quadruple (~12) this value in wild populations (Keller 1998; Spielman et al. 2004),

1673    I did not conduct analyses varying these values. Extinction was defined as the point at which the

1674    remnant population is comprised of only one sex.

1675    The parameters used in this study are given in Appendices 4.1 and 4.2. Several simulations

1676    were run to model the hypothetical future population trajectory. These are summarised in two

1677    broad scenarios. The scenarios always compare four situations: a- only life-history trait data, b-

1678    life-history trait data with inbreeding incorporated, c- life-history trait data with inbreeding and

1679    genetic data incorporated, d- life-history trait data with genetic data only.

1680    For incorporating genetic data into the PVA, I used allele frequencies derived from the

1681    empirical microsatellite data estimated directly from the southern African population. The details

1682    of these samples can be found in chapter 3, Table 3.1. Small populations are assumed to show

1683    evidence of inbreeding depression, thus I used a value of 6.29 lethal equivalents to simulate the

1684    adverse effect of inbreeding on the hypothetical population's persistence (Lacy and Pollak 2014).

1685    Scenario 1a-d makes use of species trait data from the Krüger 2014 paper. Scenario 2a-d

1686    tracks a similar trajectory, however in this case several of the biological settings are altered. The

1687    lifespan is decreased from 32 to 21 years, and the age at last reproductive output is reduced to

1688    19 years, accounting for variation seen in these traits (Brown 1997). For each scenario, 1000

1689    simulations were run and the time span was set to 250 years; extinction was defined as the

1690    absence of at least one sex. The initial population size was set at 408 individuals according to the

1691    latest census data and this variable was set using stable-age distribution (Rushworth and Krüger

1692    2014b).

**Results**

1693        Scenario 1 and 2 both predict hypothetical populations which initially increase in size

1694    within the first decade, but then follow a steep decline for three decades after which they

1695    continue to decrease until extinction is reached (Figure 4.1 a and b).
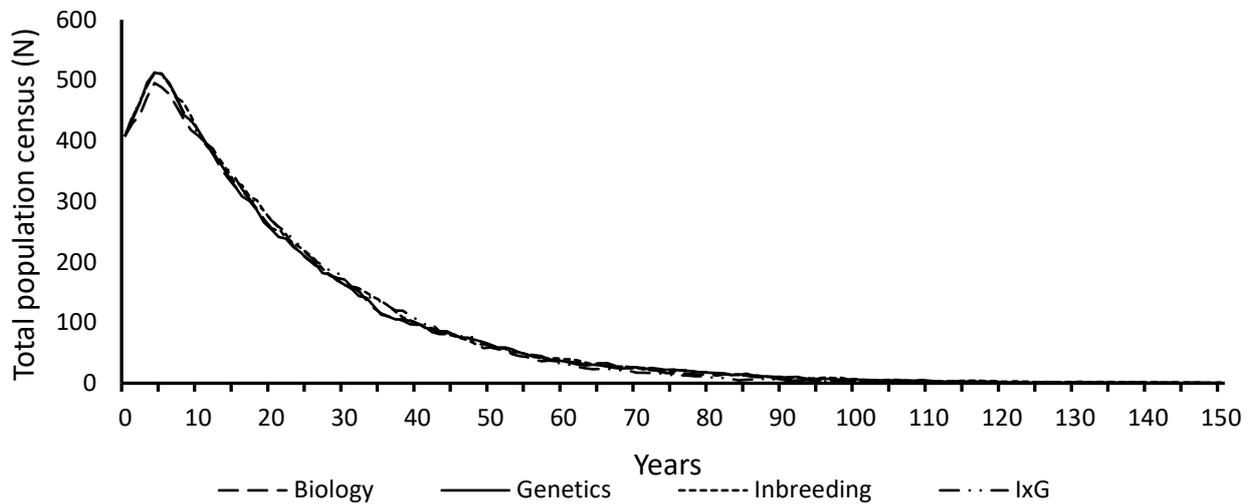


Figure 4.1.a: Bearded vulture population numbers contrasting the trajectories of different models run under scenario 1.
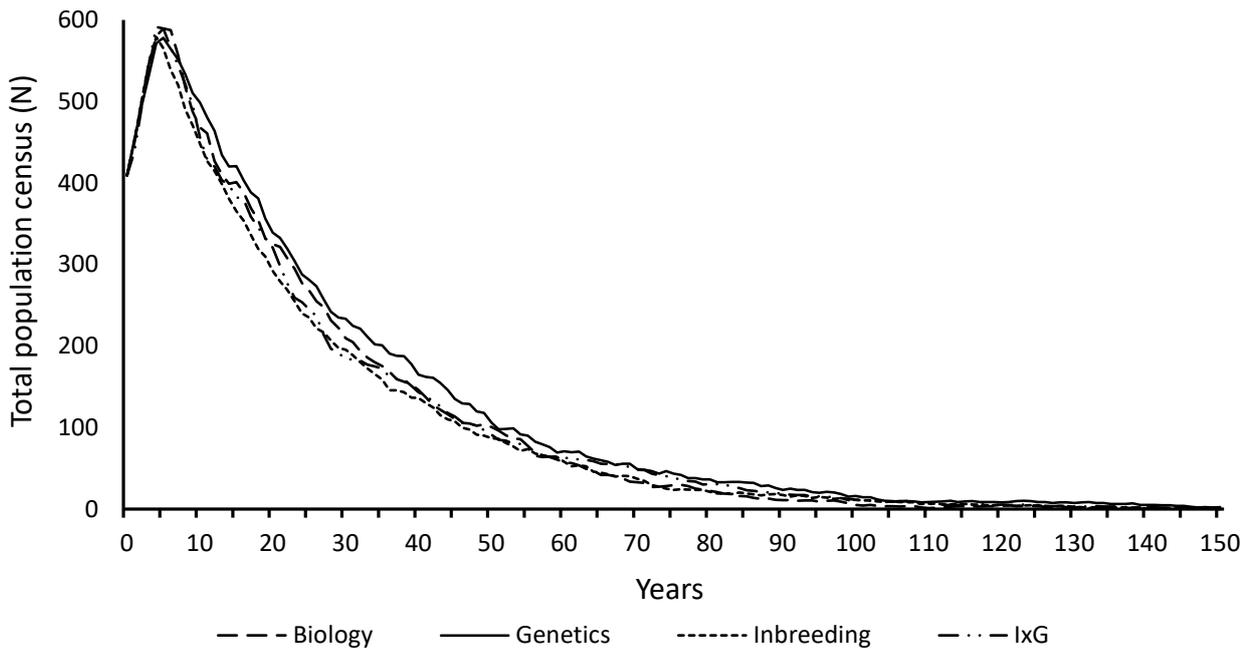
Figure 4.1.b: Bearded vulture population numbers contrasting the trajectories of different models run under scenario 2.

1696    Scenario 1 reports initial population size to be slightly higher than for scenario 2 (Table 4.1 and

1697    2). For both scenarios, the population trend tracks the same path even after the incorporation of

1698    additional genetic data into the simulation (Figure 4.1a and b; Tables 4.1 and 2). For scenario 1a,

1699    in which only the population parameters are included, the southern African bearded vulture

1700    population decreased to 101 individuals (100.56 +- 8.85) after 50 years. Adding genetic data to

1701    the simulation results in the highest population census, which is expected to reach 106 individuals

1702    (105.99 +- 9.06; Table 4.1). Lowest numbers (N = 87) are predicted by the model which includes

1703    only the inbreeding depression parameter (Table 4.1). Although all models predict a collapsing

1704    population, adding genetic data appears to improve the probabilistic population census slightly

1705    in scenario 2 (Figure 4.1b). However, this distinction is obscured in scenario 1 (Figure 4.1a).

1706    All genetic indicators show deterioration after 50, 100 and 150 years. The number of alleles

1707    present and heterozygosity levels within the population are diminished, while inbreeding

1708  becomes more pronounced (Table 4.1). Although the number of alleles remains low, for both

1709  scenarios, incorporating the allelic frequencies as a parameter (with or without inbreeding),

1710  results in a controlled and linear decline in the number of alleles (Table 4.1).

1711  The population's growth rates (scenario 1 and 2) appear not to be largely altered by the addition

1712  of genetic data. The deterministic growth rates of the population is decreased slightly when

1713  modelled under scenario 2 (0.05 vs. 0.09). The stochastic growth rates increased minimally after

1714  50 years when the baseline model parameters were changed from those of Krüger et al. (Table

1715  4.1) to the modified parameters (Figures 4.2 a and b; Table 4.1). This suggests that the modelled

1716  predictions of the PVA, in this case, is not biased by a particular data type (demographic versus

1717  genetic). Both scenarios predict a consistently low, positive growth rate over the next 50 years,

1718  after which the growth rates follow an unpredictable and irregular pattern (Figure 4.2 a and b).
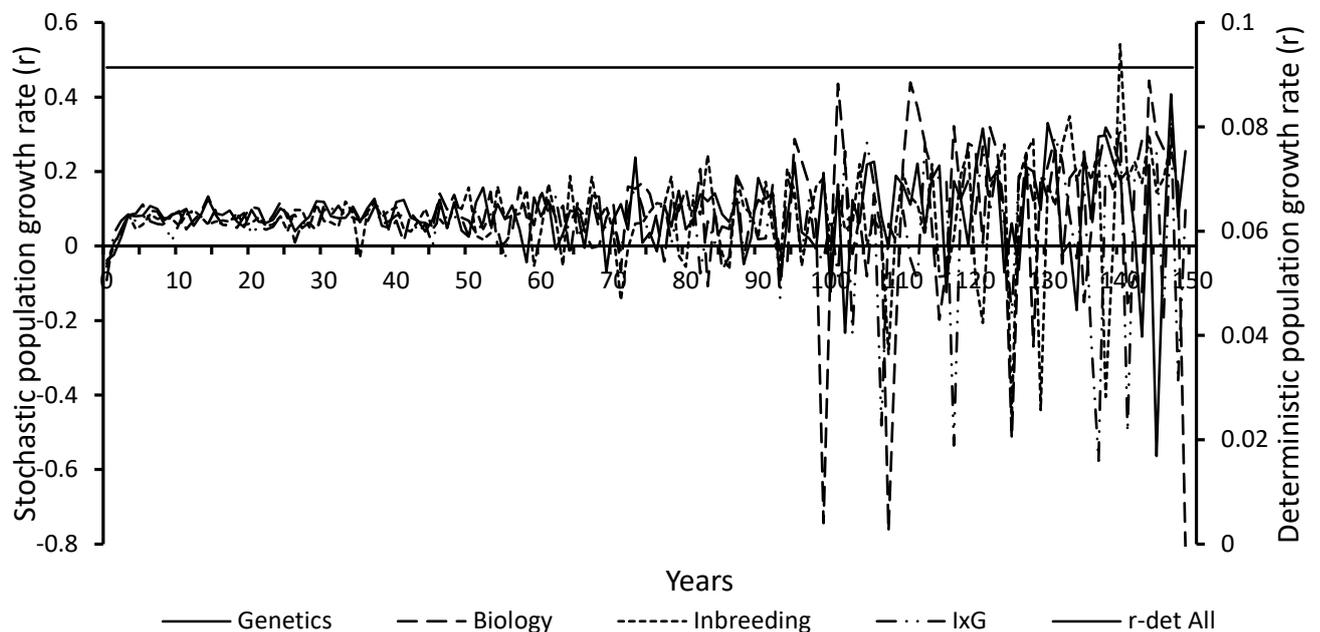


Figure 4.2.a: Bearded vulture projected (stochastic and deterministic) population growth rates contrasting the incorporation of life-history data (biology) against genetic data; simulated under default inbreeding levels (scenario 1).

1719    Scenario 1 predicts greater negative growth rates after ~95 years in the baseline model whereas

1720    the IxG model only reaches highly negative growth rates after ~108 years (Figure 4.1a). Scenario

1721    2, although predicting lower growth rates over the first 50 years, models a delay in the highly

1722    negative growth rates, which are expected to manifest only after ~120 years.
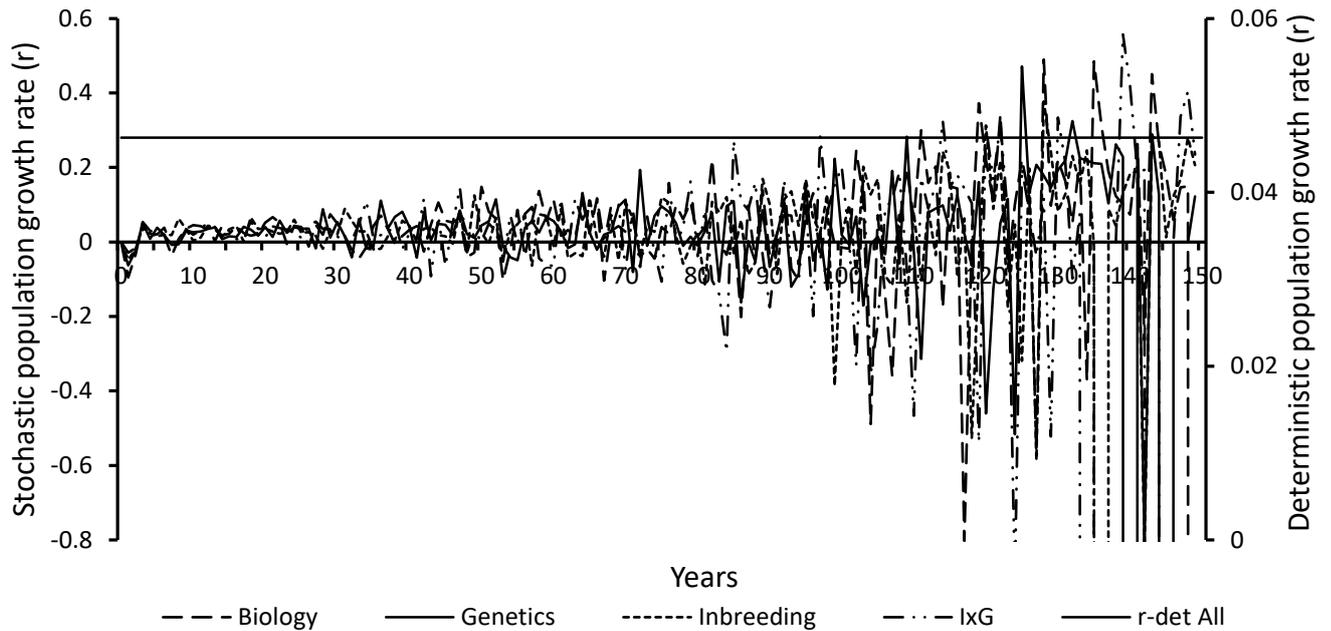


Figure 4.2. b: Bearded vulture projected (stochastic and deterministic) population growth rates contrasting the incorporation of life-history data (biology) against genetic data; simulated under default inbreeding levels (scenario 2).

1723    Table 4.1: Summary statistics of the modelled bearded vulture population trajectory under scenario 1 after 50, 100 and 150 years.

| | After 50 yrs | | | | | | After 100 yrs | | | | | | After 150 yrs | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | stoch-r | $F_{IS}$ | $A_R$ | $H_0$ | MTE | N | stoch-r | $F_{IS}$ | $A_R$ | $H_0$ | MTE | N | stoch-r | $F_{IS}$ | $A_R$ | $H_0$ | MTE |
| Baseline | 101 | 0.08 | 0.01 | 122 | 0.98 | 19 | 5 | -0.74 | 0.01 | 76 | 0.97 | 25 | 0 | -1.09 | 0.05 | 40 | 0.96 | 26 |
| Inbreeding | 87 | 0.10 | 0.01 | 120 | 0.98 | 18 | 11 | 0.18 | 0.03 | 66 | 0.97 | 23 | 0 | 0.00 | 0.05 | 33 | 0.96 | 25 |
| IxG | 91 | 0.07 | 0.37 | 15 | 0.63 | 19 | 11 | -0.04 | 0.37 | 11 | 0.63 | 25 | 2 | 0.10 | 0.37 | 10 | 0.63 | 26 |
| Genetic | 106 | 0.04 | 0.37 | 14 | 0.63 | 20 | 16 | 0.20 | 0.37 | 11 | 0.63 | 26 | 2 | 0.25 | 0.39 | 11 | 0.61 | 28 |

1724    Table 4.2: Summary statistics of the modelled bearded vulture population trajectory under scenario 2 after 50, 100 and 150 years.

| | After 50 yrs | | | | | | After 100 yrs | | | | | | After 150 yrs | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | stoch-r | $F_{IS}$ | $A_R$ | $H_0$ | MTE | N | stoch-r | $F_{IS}$ | $A_R$ | $H_0$ | MTE | N | stoch-r | $F_{IS}$ | $A_R$ | $H_0$ | MTE |
| Baseline | 59 | 0.15 | 0.02 | 91 | 0.97 | 18 | 4 | -0.02 | 0.06 | 40 | 0.93 | 23 | 0 | 0.62 | 0.06 | 29 | 0.94 | 24 |
| Inbreeding | 62 | 0.02 | 0.02 | 85 | 0.97 | 19 | 7 | 0.02 | 0.06 | 39 | 0.94 | 23 | 1 | 0.17 | 0.13 | 12 | 0.87 | 24 |
| IxG | 60 | 0.00 | 0.37 | 3 | 0.62 | 19 | 4 | 0.20 | 0.38 | 3 | 0.62 | 24 | 1 | 0.26 | 0.41 | 3 | 0.59 | 24 |
| Genetic | 63 | 0.04 | 0.37 | 13 | 0.62 | 19 | 6 | 0.02 | 0.38 | 9 | 0.61 | 23 | 0 | 0.72 | 0.37 | 8 | 0.63 | 24 |

**Discussion**

1725      A chief limitation to the versatility of PVA lies in obtaining reliable input parameters

1726      on the demography and life-history traits of species, especially if the species is IUCN-classified

1727      and prioritised (Boyce 1992). Despite this limitation and other many objections to PVA, it is a

1728      widely used tool to advise and direct the management of threatened species (Brook et al.

1729      2000; Olsen et al. 2014). In this study, I was able to draw on the extensive and thorough

1730      research of previous studies (Brown 1997; Krüger et al. 2014; Rushworth and Krüger 2014a)

1731      and include novel genetic data to provide a comprehensive PVA for the Southern African

1732      bearded vulture population.

1733      It is well known that small and threatened populations are subject to inbreeding

1734      depression and accordingly the loss of genetic variation, which is further exacerbated by

1735      genetic drift (Boyce 1992; Lande and Barrowclough 1987). In turn, this is known to reduce the

1736      populations viability and increase its risk of extinction (Boyce 1992; Lande and Barrowclough

1737      1987). However, it is often not known to what extent the incorporation of genetic data in a

1738      PVA will affect the population's future trajectory. Often, the contribution of a populations'

1739      genetics is thought to be insignificant when compared to the effects of the specie's life-history

1740      traits (Coulson et al. 2001). This could be true in cases where the species has a high level of

1741      genetic variation and where external factors place the greater pressure on survival. Several

1742      papers assert that either stochastic events (typically environmental catastrophes), or the

1743      species life-history traits will far outweigh the detrimental effects of a species impoverished

1744      genetics (Coulson et al. 2001; Lande 1999; Lande and Barrowclough 1987). Threatened

1745      species, whose extinction is purportedly imminent, fall under this school of thought.

1746 Nonetheless, including all available data, especially genetic data, can improve the predication

1747 made by PVA.

1748    In this study, I performed a genetically informed PVA for the bearded vulture

1749 population of southern Africa. This geographically and genetically distinct population is

1750 restricted to the high-altitude mountainous borders of Lesotho and KwaZulu-Natal, South

1751 Africa. The confirmation of the genetic uniqueness of this population (chapter 2) means that

1752 the population is best modelled and managed as a single entity. It is also important that the

1753 life history traits and genetic data used in the model are records from the southern African

1754 population as local adaptation may have an effect on population survival.

1755    In contrast to other genetically informed PVAs (Kirchhoff et al. 2016; Ogden et al.

1756 2005), the current PVA reveals no substantial additive effect of incorporating the specie's

1757 genetics on the ultimate fate of the population. The future population size modelled by

1758 VORTEX did not show a more exaggerated population decline when informed by the current

1759 level of genetic variation, as would be expected if the population suffered severely under the

1760 effects of a impoverished genetic status (high inbreeding, low heterozygosity).  On the

1761 contrary, the stochastic growth showed less variation when the population's genetics were

1762 added as a parameter. That the incorporation of a population's genetics into the model did

1763 not alter the census outcome vastly even after 150 years simulation, indicates that, despite

1764 the isolated nature of this population, the population exhibits sufficient genetic variation to

1765 buffer against the negative effects of inbreeding depression. In this particular case then,

1766 including genetic data appears not to deteriorate the viability of this population. This result

1767 supports the finding of chapter 3 where I found that the southern African bearded vulture

1768 population has higher levels of heterozygosity and allelic richness, and lower levels of

1769 inbreeding in comparison to the ecologically similar Cape vulture (Kleinhans 2018).

1770    In the same way that our conservation efforts will be enjoyed by future generations,

1771    present day allele frequencies contained within the genome of extant populations can be

1772    viewed as the genetic 'heritage' of past generations, as they reflect the genetic status of their

1773    ancestors. Conservation plans that do not specifically address the preservation of genetic

1774    diversity may unintentionally underestimate the risk of extinction. This holds particularly true

1775    for the bearded vultures as the population is geographically and genetically isolated (chapter

1776    3) and the census population remains small. So over time this population may begin to suffer

1777    the consequences of inbreeding depression, and with no intervention genetic deterioration

1778    will be amplified in this isolated population. Especially in such populations, genetic erosion

1779    poses an unavoidable risk, which can only be compensated for through active genetic

1780    management (supplementations/translocations simulating immigration even when the

1781    immigrant gene pool is similar to the focus population; selective breeding where possible).

1782    From the results of the PVA, I can conclude that the southern African bearded vulture

1783    population is genetically stable. The probabilistic outcome of their future census numbers is

1784    in no way diminished by their current genetic health. This is corroborated by the population's

1785    comparatively diverse genetics as seen by a higher than expected heterozygosity and low

1786    levels of inbreeding (chapter 3). This stands in contrast to the Cape vulture *Gyps coprotheres*,

1787    where the incorporation of genetic data in to the PVA undermined the population's trajectory

1788    (Kleinhans 2018). The vastly different outcomes of the modelled future of these two species,

1789    despite being alike in life history traits and ecological requirements, reinforces the need for

1790    conservation to be species- and context-dependent, and more importantly not to disregard

1791    the genetic component of the population.

1792    The bearded vulture population of southern Africa does not appear to be genetically

1793    disadvantaged as a result of its isolation. Often populations with underlying genetic problems

1794 would appear to grow initially, but then tend to crash. The interaction between ecological and

1795 genetic allee effects should be taken into consideration when assessing a species viability, as

1796 well as when drawing conclusions between species. In the case of the current population of

1797 southern African bearded vultures, it is clear that the species will benefit more under

1798 management which is targeted at improving external factors which will increase the

1799 population size (improve fecundity and survival rates). Based on the findings of my research,

1800 I advise contemporary management to monitor genetic variation but chief focus and funding

1801 need not be invested in intense genetic management of the current population. Rather, it

1802 should be targeted at increasing the population size by improving external factors (which

1803 would improve the reproductive output) and eliminating direct threats from either

1804 environmental or anthropogenic origin.

1805       There is immense value in the use of genetic data in conservation efforts, and it should

1806 not be negated or neglected. It is true that to fully understand the effects of genetics and

1807 reproductive ecology on population viability, one needs to assess their relative impacts on

1808 demographic vital rates (Lande 1999). Yet, one should not then seek only to improve the

1809 demographic rates or ecological requirements without understanding the genetic factors that

1810 govern them. This is particularly true in the conservation of threatened and diminishing

1811 populations. More attention needs to be given to, and better communication is a necessity,

1812 understanding the interconnectedness of these three facets of conservation.

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1907

**CHAPTER 5**

**Summary and conclusion**

1908    The vast acceleration in human population growth and the associated demands this

1909    places on the environment, have led to increased pressure on natural ecosystems (Barnosky

1910    et al. 2011; Ripple et al. 2017; Steffen et al. 2015). The multifaceted threats that are imposed

1911    on natural systems more often than not, compromise the efficient and healthy running of

1912    ecosystem function (Hansen et al. 2012).   Avian scavengers, often because of their

1913    mythological associations, are the most threatened and persecuted group of birds (Ogada et

1914    al. 2016). Vultures are integral to the ecosystem within which they are found. They are vital

1915    in nutrient cycling and are involved indirectly in mitigating the spread of disease and potential

1916    pathogen transfer by removing decaying carcasses. The rapid extirpation of vultures in India

1917    as a consequence of using the veterinary drug diclofenac (Paudel et al. 2016; Prakash et al.

1918    2012) had a dramatic effect on the rate of rabies incidents between 1992 and 2006. The

1919    epidemic was attributed to the increase in decaying carcasses that supported an

1920    unprecedented surge in the population of feral dogs (Markandya et al. 2008). This also reveals

1921    the huge economic value of vultures as India ultimately spent $34 billion on humanitarian

1922    related costs. Likewise revised sanitation laws in Europe subsequent to the outbreak of bovine

1923    spongiform encephalopathy resulted in huge environmental (77,344 metric tons of GHG

1924    released into the atmosphere per year) and economic costs ($50 million to insurance

1925    companies) (Morales-Reyes et al. 2015). Vultures, and the ecosystem function they perform,

1926    are indispensable and it should thus be high-priority to curb the rate at which vultures are

1927    declining.

1928    The bearded vulture is widely distributed with remaining populations sparsely and

1929    disjunctly distributed across the Palearctic, Afrotropic and Indomalay regions (Margalida et

1930    al. 2003). Historically the populations were abundant, but have become locally extinct or

1931    survive in highly habitat-specific isolated refuges across their former range (Brown 1997;

1932    Gautschi et al. 2000). The Pyrenees, the southern Balkans, the islands of Corsica and Crete,

1933    and a re-established population in the Alps currently comprise the European populations;

1934    while the Maloti-Drakensberg (Lesotho boarder), Ethiopia and Morocco are the refuges for

1935    the African bearded vulture populations (Brown 1997; Godoy et al. 2004).

1936    It is common to believe that the genetic component does not contribute significantly

1937    to a threatened specie's ultimate outcome in the short term (Coulson et al. 2001; Lande 1999;

1938    Lande and Barrowclough 1987). However, the concomitant loss of diversity in a bottlenecked

1939    population is the impetus behind numerous genetic studies (Arshad et al. 2009; Frankham et

1940    al. 2014; Godoy et al. 2004; Krüger et al. 2015). Small and dwindling populations are known

1941    to suffer genetic consequences through lack of diversity and a gradual increase in inbreeding

1942    (Alcaide et al. 2010). Opinions appear to be segregated into two general lines of thought. One

1943    advocating to increase reproductive output and demographic rates (Lande 1988) (perhaps by

1944    protection) irrespective of genetic consequences in the short term (inbreeding depression if

1945    mating between relative is inevitable), and the other which seeks to conserve the genetic

1946    diversity perhaps by captive and intentional breeding such that the maximum diversity is

1947    conserved (Frankham 2005). I tend to think that these should operate at different points in the

1948    species recovery. If the population number is low, I believe one should aim to eliminate

1949    repercussions of inbreeding which would lead to lower reproductive output and of poorer

1950    quality. At some point there should be a threshold where the population is genetically stable

1951    enough, at this point one can 'neglect' the genetic component and focus of boosting population

1952 numbers. At this point, population numbers will buffer against natural selection and one won't

1953 be at threat of losing a species due to natural selection/inbreeding depression. Previous research

1954 on the genetics of the bearded vulture has revealed low levels of genetic diversity based on

1955 multilocus DNA fingerprinting (Negro and Torres 1999), microsatellite markers (Gautschi et

1956 al. 2003) and mitochondrial data (Godoy et al. 2004; Krüger et al. 2015). These studies suggest

1957 a lack of genetic diversity post-population decline in European populations. A further study,

1958 specifically targeting the southern African population of bearded vultures detected little

1959 differentiation and reduced genetic diversity compared to northern hemisphere source'

1960 populations (Krüger et al. 2015).

1961 This MSc study aims to provide a better understanding of the population structure

1962 between the contemporary populations of bearded vultures across Africa, Europe and Asia.

1963 This study extends the work of Godoy et al. (2004) and Krueger et al. (2015) by increasing the

1964 number of bearded vultures and loci analysed, and by using microsatellite loci as a

1965 compliment to mitochondrial data. These non-coding markers provide a higher resolution

1966 assessment of the current diversity present as they evolve at a higher rate than mitochondrial

1967 markers previously used.


**Research objectives**

1968 Three objectives were set to address the genetic state of the bearded vulture species:

1969 The first objective was to use microsatellite data to establish whether genetic

1970 structuring has resulted from prolonged isolation of populations following the global collapse

1971 of bearded vulture numbers (chapter 2). Estimating population structure and genetic status

1972 of disjunct populations will provide conservation authorities with the necessary information

1973    needed to make, and implement the appropriate conservation measures to protect the

1974    bearded vulture. My research established that the southern African bearded vulture

1975    population is genetically isolated and subsequently holds a unique genetic assemblage as a

1976    consequence of being geographically isolated. This finding contradicts past work on

1977    mitochondrial data which concluded that the bearded vulture species should be managed as

1978    a global population due to insufficient genetic differentiation between the several

1979    populations.

1980        The second objective was to focus on the southern African bearded vulture population

1981    (chapter 3). Indices of genetic diversity derived from the microsatellite data were used to

1982    assess the genetic health of the southern African population following its decline and

1983    isolation. Small isolated populations are expected to have reduced genetic diversity, and

1984    higher levels of inbreeding. The genetic diversity of the southern African bearded vulture

1985    population was compared to that of eastern and western African bearded vulture population

1986    as well as to that of three sympatric, and ecologically similar species (Cape vultures *Gyps*

1987    *coprotheres*, Hooded vultures *Necrosyrtes monachus*, and African White-backed vultures

1988    *Gyps africanus*). Analysis revealed that, despite the 5-fold difference in population size

1989    between the eastern and southern African bearded vulture populations, and despite the

1990    connectivity that the western population holds with European populations, the southern

1991    African population contains the greatest amount of genetic variation and unique genetic

1992    identity.

1993        In comparison to ecologically similar species, the southern African bearded vulture

1994    population, here too, appears to have suffered less of a genetic impoverishment because of

1995    the African vulture persecution crisis. Comparing against the Cape vulture, which is

1996    ecologically and behaviourally most similar but has a far larger population size, we expected

1997     the bearded vulture to show relative lower genetic diversity, yet the analysis contradicted this

1998     prediction.

1999         In chapter 4, I tested for the effect of decreased genetic diversity on the bearded

2000     vulture's future persistence. Standard population viability analysis (PVA) was conducted, but

2001     supplemented the specie's biology and life history traits with genetic data (allelic frequencies)

2002     generated in chapter 2 and 3. Few PVA studies incorporate genetic data as some literature

2003     suggests that endangered species will likely go extinct due to threats such as habitat

2004     destruction and hunting before experiencing any detrimental effects of reduced genetic

2005     diversity. Previous work on another South African species, Cape vulture, has shown this

2006     assumption not to be true (Kleinhans 2018). When including genetic data as parameter in the

2007     Cape vulture PVA, Kleinhans (2018) illustrated that including genetic data in PVA models

2008     decreases the predicted time to extinction as well as decreases genetic diversity estimations.

2009     As seen in chapter 3, the Cape vulture population in South Africa has the lowest genetic

2010     diversity estimates. If the genetic diversity of southern African bearded vulture population is

2011     sufficient to buffer the population against the effects of genetic drift, despite low population

2012     numbers, then it is expected that there not be a vast difference in the population's trajectory

2013     when the genetic data is added to the PVA. However, if the population's genetics are severely

2014     reduced, the PVA including genetic data should have altered probabilistic population

2015     trajectory. Here the results show that the incorporation of the genetic data did not result in

2016     a lower estimation of population numbers.

**Major findings and future work**

2017        The first data chapter established that, based on microsatellite data, there is genetic

2018   structuring present in the global bearded vulture population. The African populations, and

2019   specifically the southern African population, are different from European and Asian

2020   populations and presents a unique genetic assemblage. Additionally, the detected migration

2021   rates between populations were low in the recent past. Outside of actively translocating

2022   individuals between populations, there is low connectivity between disjunct populations and

2023   the exchange of genetic material is limited. Is this impetus for a revision in the management

2024   of the species as a single population? Should each population be conserved individually? A

2025   first step would be to clarify the trends by increasing sample sizes where sub-populations

2026   consisted of only a few samples and thus were not accurately portrayed.

2027        The second data chapter confirmed that the southern African population has reduced

2028   genetic diversity when compared to other vulture species (Cape vultures, Hooded vultures,

2029   and African White-backed vultures) in southern Africa. However, the southern African

2030   bearded vulture population did not have the lowest genetic diversity estimate despite having

2031   the smallest population size. What are potential ecological reasons for this? Could this be an

2032   inherent biological capacity of the species, or has the conservation merely been more

2033   successful for the bearded vulture? Moreover, how can we learn from this to improve

2034   protection of these other sympatric species?

2035        The last chapter incorporated genetic data into PVA. This sought to determine what

2036   effect genetic variation may have on the evolutionary persistence of the species. This

2037   information can be used to direct conservation efforts as one can identify whether focus

2038   should be placed primarily on conserving external factors to rapidly increase population size,

2039   or whether one should focus on increasing the genetic health of the species first

2040   (translocations, managed breeding).

2041       I believe the research presented here would stand to benefit by being supplemented

2042   in two respects. One, to evaluate the non-neutral (functional) diversity within the species;

2043   and secondly by ascertaining stress levels of individuals. Examining functional genetic

2044   diversity would involve genotyping genes involved in traits closely linked to survival and

2045   fitness of individuals and populations. Understanding functional genetic diversity, and the

2046   significance that various evolutionary forces have had in modifying this, is central to

2047   conservation (Gonzalez-Quevedo et al. 2015). Particularly so as the variation present in the

2048   gene pool of a population represents the inherent capability to respond to a changing

2049   environment, a loss of which could undermine the adaptive potential of that population and

2050   ultimately the species as a whole (Martinez-Cruz 2011). The host-pathogen interaction

2051   exposes the susceptibility of a population to environmental change due to depauperate

2052   genetic variation (Martinez-Cruz 2011). The most well studied immunological system relates

2053   to the major histocompatibility complex (MHC) which is primarily responsible for encoding

2054   proteins that perform this vital task. The vast diversity exhibited in MHC genes conveys

2055   considerable importance due to their direct impact on the immune function and thus the

2056   viability of individuals and populations (Brownlie and Allan 2011). Immune genes are ideal for

2057   studying the evolutionary processes affecting functional genetic diversity of wild populations

2058   as they represent the most rapidly evolving genes within the genome. Especially so for

2059   vultures, due to their feeding habits and wide range they cover, are exposed to a plethora of

2060   noxious factors (Apanius et al. 1983), and the findings of immunological studies would

2061   contribute to our understanding of the selective pressures imposed by coevolving pathogens.

2062 As regards the second recommendation, the prevailing crisis of biodiversity loss has affected

2063 the old-world vultures to a devastating extent. Populations that thrived prior to their

2064 persecution in the early to mid-1900 over much of Asia and Africa are today close to

2065 extinction. Over the last three generations, populations have declined, and in several regions

2066 of their distribution, they have become locally extinct. One of the chief causes has been listed

2067 as intentional poisoning and unintentional poisoning through veterinary drug use in treating

2068 livestock, or by heavy metal poisoning used in hunters' bullets (Hernández and Margalida

2069 2009). The vultures are exposed to the poison when they feed on carcasses which had

2070 consumed the veterinary drug (Green et al. 2016), or they unintentionally consume metal

2071 fragments with which the animal had been shot. This is either fatal, or if consumed in low

2072 amounts, may have a chronic effect on the health of vulture. Conservation efforts need to be

2073 made aware of those threats which are not immediately perceptible, and which could render

2074 any other form of outward conservation (physical protection, habitat improvement, artificial

2075 food supply) futile. A good indicator of health would be to measure levels of certain stress

2076 hormones (in particular cortisol) and to examine general blood chemistry. Endocrinology is a

2077 useful tool for conservation biologists and animal managers, and measuring glucocorticoids

2078 can help understand biological mechanisms associated with species decline and animal

2079 welfare. Enzyme immunoassays for the non-invasive assessment of glucocorticoid

2080 metabolites have been developed and are known to work successfully over a range of

2081 taxonomic groupings. As few studies have researched this for the endangered bearded

2082 vulture, the aim of a preliminary investigation into this would be to establish some baseline

2083 biochemical and haematological parameters.

**Concluding remarks**

2084        The thesis presented here analysed and described the current genetic status of the

2085    global bearded vulture population, and focused specifically on placing the southern African

2086    population in context following global vulture decline. The results support the need for a

2087    revision in the conservation of the southern African bearded vultures as being managed as an

2088    isolated population rather than as an entire global population. Furthermore, it contributes to

2089    the genetic knowledge we have of this species, which is vital in guiding and directing any

2090    conservation efforts of the species (Alcaide et al. 2010).

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**Appendix**

2172    Appendix Table 2.1: Segregation, origin and number of samples used for STRUCTURE analysis;

2173    groupings given for indication on figures.

| Grouping | | Pop | Country | Sample Count |
|---|---|---|---|---|
| 1 | SA | 1 | SA | 52 |
| 2 | Eastern Africa | 2 | Ethiopia | 13 |
| | | 3 | Abyssinia | 3 |
| | | 4 | Yemen | 2 |
| 3 | Western Africa | 5 | Morocco | 1 |
| | | 6 | Algeria | 5 |
| 4 | South-east Europe | 7 | Albania | 1 |
| | | 8 | Greece | 14 |
| | | 9 | Turkey | 1 |
| 5 | Central Asia | 10 | Caucasus | 7 |
| | | 11 | Central Asia | 4 |
| | | 12 | Turkestan | 5 |
| | | 13 | Kyrgyzstan | 2 |
| 6 | Central Russia | 14 | Central Russia | 11 |
| 7 | India | 15 | India | 5 |
| 8 | Mediterranean Islands | 16 | Crete | 1 |
| | | 17 | Corsica | 3 |
| | | 18 | Sardinia | 30 |
| 9 | Europe | 19 | Switzerland | 35 |
| | | 20 | Austria | 1 |
| | | 21 | Pyrenees | 9 |
| | | 22 | France | 2 |
| | | 23 | Spain | 10 |

2174    Appendix Table 2.2: Details of 217 bearded vulture samples used in the study. Fix this table,

2175    the field samples and PhD samples need to be put into same category.

| Provided by | Sample name | Catalogue no. | Sample Origin | | Collection date |
|---|---|---|---|---|---|
| | | | Broad Locality | Precise locality if available | |
| Historic Samples from American Museum of Natural History | A1 | SKIN 268806 | Ethiopia | Kaka Mt., Arussi | 1929 |
| | A4 | SKIN 535811 | Sardinia | Urgulei, Ogliastra | 1902 |
| | A5 | SKIN 535812 | Sardinia | Iglesias | 1905 |
| | A6 | SKIN 535813 | Sardinia | Ascuentu (Guspini) | 1903 |
| | A7 | SKIN 535815 | Sardinia | Aritzo | 1903 |
| | A8 | SKIN 535817 | Greece | Parnes, Attica | 1900 |
| | A9 | SKIN 535821 | Greece | Parnass, Velitza | 1900 |
| | A11 | SKIN 535824 | Russia | Goudan, Transcaspian | 1900 |
| | A12 | SKIN 535826 | Kyrgyzstan | Tian-Shan Mts. | 1911 |
| | A13 | SKIN 535830 | Yemen | Sôk al Khamis | 1913 |
| | A14 | SKIN 535829 | Yemen | Sôk al Khamis | 1913 |
| | A16 | SKIN 535832 | India | Simla, N.W. Himilayas | 1913 |
| | A17 | SKIN 535836 | Morocco | Djebel Tixa (Atlas) | 1905 |
| | A18 | SKIN 535837 | Algeria | Djebel Taya, Meskoutine | 1909 |
| | A19 | SKIN 535839 | Algeria | Kerrata | 1904 |
| | A20 | SKIN 535840 | Algeria | El Kantara | 1909 |
| | A21 | SKIN 535848 | Ethiopia | Yeka Hill, Addis Ababa | 1926 |
| | A22 | SKIN 535855 | Ethiopia | Addis Ababa | 1914 |
| | A23 | SKIN 535856 | Ethiopia | ddis Ababa | 1914 |
| | A25 | SKIN 535858 | Ethiopia | Addis Ababa | 1914 |
| S. Krüger | BV001 | G22911 | South Africa | Southern Drakensberg | 2006 |
| | BV002† | G27343 | South Africa | Southern Drakensberg | 2007 |
| | BV003 | G27341 | South Africa | Southern Drakensberg | 2007 |
| | BV004† | G27307 | South Africa | Southern Drakensberg | 2009 |
| | BV005† | G27313 | South Africa | Southern Drakensberg | 2010 |
| | BV006† | G27306 | South Africa | Southern Drakensberg | 2009 |
| | BV007† | G27378 | Lesotho | Lesotho | 2012 |
| | BV008 | G27308 | South Africa | Southern Drakensberg | 2009 |
| | BV009† | G27376 | South Africa | Northern Drakensberg | 2011 |
| | BV010 | G27314 | South Africa | Northern Drakensberg | 2010 |
| | BV011 | BV14 inside | South Africa | NE Free State | 2004 |
| | SBV012 | BV2002 | South Africa | Central Drakensberg | 2002 |
| | BV013† | G27302 | Lesotho | Lesotho | 2008 |
| | BV014 | BV2009 | South Africa | Central Drakensberg | 2009 |
| | BV015† | G27375 | South Africa | Southern Drakensberg | 2011 |
| | BV016† | G27305 | South Africa | Southern Drakensberg | 2009 |
| | BV017 | G27303 | Lesotho | Lesotho | 2008 |
| | BV018 | G27309 | South Africa | Southern Drakensberg | 2009 |

| | | | | |
|---|---|---|---|---|
| BV019 | 12 | South Africa | Southern Drakensberg | 2003 |
| BV020 | 6 | Lesotho | Lesotho | Not provided |
| BV021 | 25 | South Africa | Northern Drakensberg | 2011 |
| BV022 | 13 | South Africa | Central Drakensberg | 2008 |
| BV023 | 19 | South Africa | Northern Drakensberg | 2009 |
| BV024 | 27 | South Africa | Southern Drakensberg | 2010 |
| BV025 | 11 | South Africa | Southern Drakensberg | 2000 |
| BV026 | 10 | South Africa | Southern Drakensberg | 2007 |
| BV027 | 31.1 | South Africa | Southern Drakensberg | 2010 |
| BV028 | 31.2 | South Africa | Southern Drakensberg | 2010 |
| BV029 | 30 | South Africa | Northern Drakensberg | 2010 |
| BV030 | 9 | South Africa | Southern Drakensberg | 2009 |
| BV034 | G27382 | South Africa | Central Drakensberg | 2012 |
| BV035 | 2.8.12 | South Africa | Eastern Cape | 2012 |
| BV036 | G27377 | South Africa | Northern Drakensberg | 2012 |
| BV037† | G27379 | South Africa | Central Drakensberg | 2012 |
| BV038 | G27381 | South Africa | Northern Drakensberg | 2012 |
| BV039 | G27383 | South Africa | Central Drakensberg | 2012 |
| BV040† | G27384 | South Africa | Central Drakensberg | 2012 |
| BV031 | Kolo | Ethiopia | Not provided | 2001 |
| BV032 | Mutt | Ethiopia | Not provided | 2001 |
| BV033 | Putin | Ethiopia | Not provided | 2001 |
| | G27380 | South Africa | Not provided | 2011 |
| | 34 | South Africa | Not provided | Not provided |
| | G27319 | South Africa | Northern Drakensberg | 2010 |
| | G27310 | South Africa | Central Drakensberg | 2010 |
| | G27311 | South Africa | Northern Drakensberg | 2010 |
| | G27315 | South Africa | Northern Drakensberg | 2010 |
| | G27316 | South Africa | Northern Drakensberg | 2010 |
| | G27342 | South Africa | Southern Drakensberg | 2007 |
| | BV6 | Lesotho | Lesotho | 2009 |
| | BV7 | Lesotho | Lesotho | 2009 |
| | BV8 | Lesotho | Lesotho | 2009 |
| | BV10 | Lesotho | Lesotho | 2009 |
| | BV12 | Lesotho | Lesotho | 2009 |
| | g | South Africa | Drakensberg | Not provided |
| DNSM | | DNSM 2 | South Africa | KZN, Kamberg | 1961 |
| Naturalis Leiden | L1 | L1 | Switzerland | Switzerland | Not provided |
| | L2 | L2 | Sardinia | Sardinia | Not provided |
| | L3 | L3 | Sardinia | Sardinia | Not provided |
| | L4 | L4 | Sardinia | Sardinia | Not provided |
| | L5 | L5 | Pyrenees | Not provided | Not provided |
| | L6 | L6 | Turkey | Not provided | Not provided |
| | L7 | L7 | Greece | Not provided | Not provided |
| Vulture Conservation | SB1 | BG178 | Greece | Greece | Not provided |
| | SB2 | BG725 | Pyrenees | Pyrenees | Not provided |
| | SB3 | BG131 | Russia | Former Soviet Union | Not provided |

| | | | | |
|---|---|---|---|---|
| SB4 | BG680 | Pyrenees | Aragon Pyrenean | Not provided |
| SB5 | BG065 | Crete | Crete | Not provided |
| SB6 | BG132 | Russia | Former Soviet Union | Not provided |
| SB7 | BG652 | Pyrenees | Aragon Pyrenees | Not provided |
| SB8 | BG551 | Pyrenees | Spanish Pyrenees | Not provided |
| SB9 | BG151 | Russia | Former Soviet Union | Not provided |
| SB10 | BG482 | Kyrgyzstan | Kyrgyzstan | Not provided |
| SB13 | BG022 | Russia | Former Soviet Union | Not provided |
| SB16 | BG201 | Turkestan | Tadzhikistan | Not provided |
| SB18 | BG003 | Central Asia | West-middle Asia | Not provided |
| SB19 | BG199 | Russia | Former Soviet Union | Not provided |
| SB20 | BG232 | Pyrenees | Spanish Pyrenees | Not provided |
| SB21 | BG014 | Russia | Not Provided | Not provided |
| SB22 | BG204 | Russia | Not provided | Not provided |
| SB23 | BG153 | Russia | Not provided | Not provided |
| SB24 | BG009 | Russia | Not provided | Not provided |
| S1.1 | 1 | Spain | Sierra Magina | Not provided |
| S1.2 | 2 | Spain | Sierra Magina | 1930 |
| S1.3 | 3 | Switzerland | Glarus | 1830 |
| S1.4 | 4 | Caucasus | Caucasus, North Side | 1900 |
| S1.6 | 6 | Greece | Greece | 1905 |
| S1.7 | 7 | Pyrenees | Pyrenees | 1904 |
| S1.9 | 9 | Switzerland | Basel Zoo | 1987 |
| S1.10 | 10 | Switzerland | Oberhasli | 1805 |
| S1.11 | 11 | Switzerland | Brienzersee | 1823 |
| S1.12 | 12 | Switzerland | Zuoz | 1854 |
| S1.14 | 14 | Algeria | Algeria | Not provided |
| S1.15 | 15 | Russia | Russia | 1979 |
| S1.16 | 16 | Ethiopia | Abyssinia | 1959 |
| S1.17 | 17 | Ethiopia | Abyssinia | 1959 |
| S1.18 | 18 | Corsica | Asco, Haute Corse | 1957 |
| S1.19 | 19 | Albania | Lamentite | 1920 |
| S1.20 | 20 | Caucasus | Petrowsk | 1900 |
| S1.24 | 24 | Greece | Parnio Chasia Ciocha | 1899 |
| S1.25 | 25 | Greece | Parnis Chasia Ciocha | 1899 |
| S1.26 | 26 | India | Khalatase Ladakh | 1929 |
| S1.27 | 27 | India | Ladka | 1930 |
| S1.29 | 29 | Caucasus | Nislam | 1900 |
| S1.30 | 30 | Sardinia | Ogliastra | 1902 |
| S1.31 | 31 | Sardinia | Sardinia | 1905 |
| S1.32 | 32 | Sardinia | Sardinia | 1901 |
| S1.33 | 33 | Sardinia | Uzzulei | 1911 |
| S1.36 | 36 | Central Asia | Thian Shan | 1900 |
| S1.39 | 39 | France | Bagnerre de bigore | Not provided |
| S1.43 | 43 | Spain | South Spain "gaitanes" | 1869 |
| S1.44 | 44 | Switzerland | Ftan, Val Tasua | 1859 |
| S1.50 | 50 | Ethiopia | Ethiopia | 1887 |

| | | | | |
|---|---|---|---|---|
| S1.51 | 51 | Sardinia | Sardegna | Not provided |
| S1.52 | 52 | Sardinia | Colle di Tena, Conoraza | 1885 |
| S1.54 | 54 | Sardinia | Sardegna | 1901 |
| S1.56 | 56 | Sardinia | Sardegna | 1907 |
| S1.57 | 57 | Sardinia | Sardegna | 1907 |
| S1.60 | 60 | Ethiopia | Abyssinia | 1900 |
| S1.61 | 61 | Greece | Greece | 1899 |
| S1.63 | 63 | Spain | Sierra | 1899 |
| S1.66 | 66 | Pyrenees | Tardet | 1896 |
| S1.71 | 71 | Sardinia | Sardinia | 1906 |
| S1.73 | 73 | France | Presles. Pont en Royans | Not provided |
| S1.74 | 74 | Spain | Sierra Magina | 1920 |
| S1.77 | 77 | Switzerland | Tessin | 1903 |
| S1.78 | 78 | Switzerland | Zermatt, Wallis | 1839 |
| S1.79 | 79 | Switzerland | Wallis | 1886 |
| S1.80 | 80 | Sardinia | Not provided | 1915 |
| S1.81 | 81 | Spain | Not provided | 1940 |
| S1.84 | 84 | Switzerland | Oberland | 1850 |
| S1.85 | 85 | Switzerland | Brig, Wallis | 1833 |
| S1.88 | 88 | Caucasus | Caucasus | 1905 |
| S1.89 | 89 | Turkestan | Naryn | 1913 |
| S1.90 | 90 | Turkestan | Naryn | 1910 |
| S2.92 | 92 | Switzerland | Switzerland | 1881 |
| S2.93 | 93 | Switzerland | Switzerland | 1891 |
| S2.95 | 95 | Switzerland | Not provided | 1981 |
| S2.98 | 98 | Greece | Naundes, Tirol | 1871 |
| S2.99 | 99 | Greece | Parnassus | 1885 |
| S2.101 | 101 | Sardinia | Not provided | 1917 |
| S2.105 | 105 | Caucasus | Caucasus | 1904 |
| S2.106 | 106 | Austria | Vorarlberg | Not provided |
| S2.107 | 107 | Sardinia | Not provided | Not provided |
| S2.109 | 109 | Switzerland | Andeer | Not provided |
| S2.110 | 110 | Switzerland | Wattensburg | 1852 |
| S2.111 | 111 | Switzerland | Winterthur | 1850 |
| S2.113 | 113 | Ethiopia | Abyssinia | 1911 |
| S2.114 | 114 | Sardinia | Sardinia | 1911 |
| S2.115 | 115 | Corsica | Corsica | Not provided |
| S2.116 | 116 | Switzerland | Not provided | Not provided |
| S3.2 | 2 | Switzerland | Andeer | 2000 |
| S3.6 | 6 | Switzerland | Wattensburg (GR) | 1852 |
| S3.7 | 7 | Switzerland | Prättigau | 1850 / 1851 |
| S3.8 | 8 | Switzerland | Not provided | 2000 / 2001 |
| S3.10 | 10 | Switzerland | Glarus | 1830 |
| S3.15 | 15 | Switzerland | Brienzersee | 1823 |
| S3.17 | 17 | Switzerland | Zuoz | 1854 |
| S3.19 | 19 | Algeria | Algeria | 2000 |
| S3.27 | 27 | Ethiopia | Abyssinia | 1959 |

| S3.29 | 29 | Ethiopia | Abyssinia | 1959 |
|-------|-----|--------------|----------------------|--------------|
| S3.30 | 30 | Corsica | Asco, Haute Corse | 1957 |
| S3.32 | 32 | Switzerland | Brig, Wallis | 1833 |
| S3.35 | 35 | Switzerland | Oberland, GR | 1850 |
| S3.36 | 36 | Switzerland | Switzerland | 1881 |
| S3.39 | 39 | Switzerland | Switzerland | 1891 |
| S3.45 | 45 | Spain | Sierra nevada | 1879 |
| S3.49 | 49 | Ethiopia | Abyssinia | 1990 |
| S3.53 | 53 | Spain | Sierra, Spain | 1899 |
| S3.55 | 55 | Greece | Greece | 1899 |
| S3.58 | 58 | Switzerland | Lausanne, Wallis | 1886 |
| S3.60 | 60 | Switzerland | Zermatt, Wallis | 1839 |
| S3.62 | 62 | Switzerland | Not provided | 1886 |
| S3.65 | 65 | Spain | Benasque | 1966 |
| S3.69 | 69 | Sardinia | Sardinia | 1915 |
| S3.72 | 72 | Greece | Greece | 1904 |
| S3.74 | 74 | Caucasus | Caucasus, North | 1900 |
| S3.77 | 77 | Switzerland | Tessin | 1903 |
| S3.87 | 87 | Spain | South Spain "gaitanes" | 1869 |
| S3.88 | 88 | Greece | Greece | 1904 |
| S3.90 | 90 | Pyrenees | Pyrenees | 1904 |
| S4.97 | 97 | Switzerland | Ftan, Val Tasua | 1859 |
| S4.99 | 99 | India | Kashmir, Ladakh | 1929 |
| S4.100 | 100 | India | Kashmir, Ladka | 1930 |
| S4.101 | 101 | Sardinia | Sardinia | 1905 |
| S4.105 | 105 | Greece | Smolika | 1921 |
| S4.106 | 106 | Sardinia | Sardegna | 1901 |
| S4.107 | 107 | Sardinia | Sardegna | 1907 |
| S4.108 | 108 | Turkestan | Naryn | 1910 |
| S4.109 | 109 | Central Asia | Caucasus | 1905 |
| S4.120 | 120 | Caucasus | Dagestan | 1900 |
| S4.122 | 122 | Sardinia | Not provided | Not provided |
| S4.125 | 125 | Sardinia | Uzulei | 1911 |
| S4.130 | 130 | Central Asia | Not provided | Not provided |
| S4.133 | 133 | Turkestan | Naryn | 1913 |
| S4.138 | 138 | Sardinia | Ogliastra | 1902 |
| S4.145 | 145 | Sardinia | Sardinia | 1901 |
| S4.147 | 147 | Sardinia | Sardinia | 1885 |
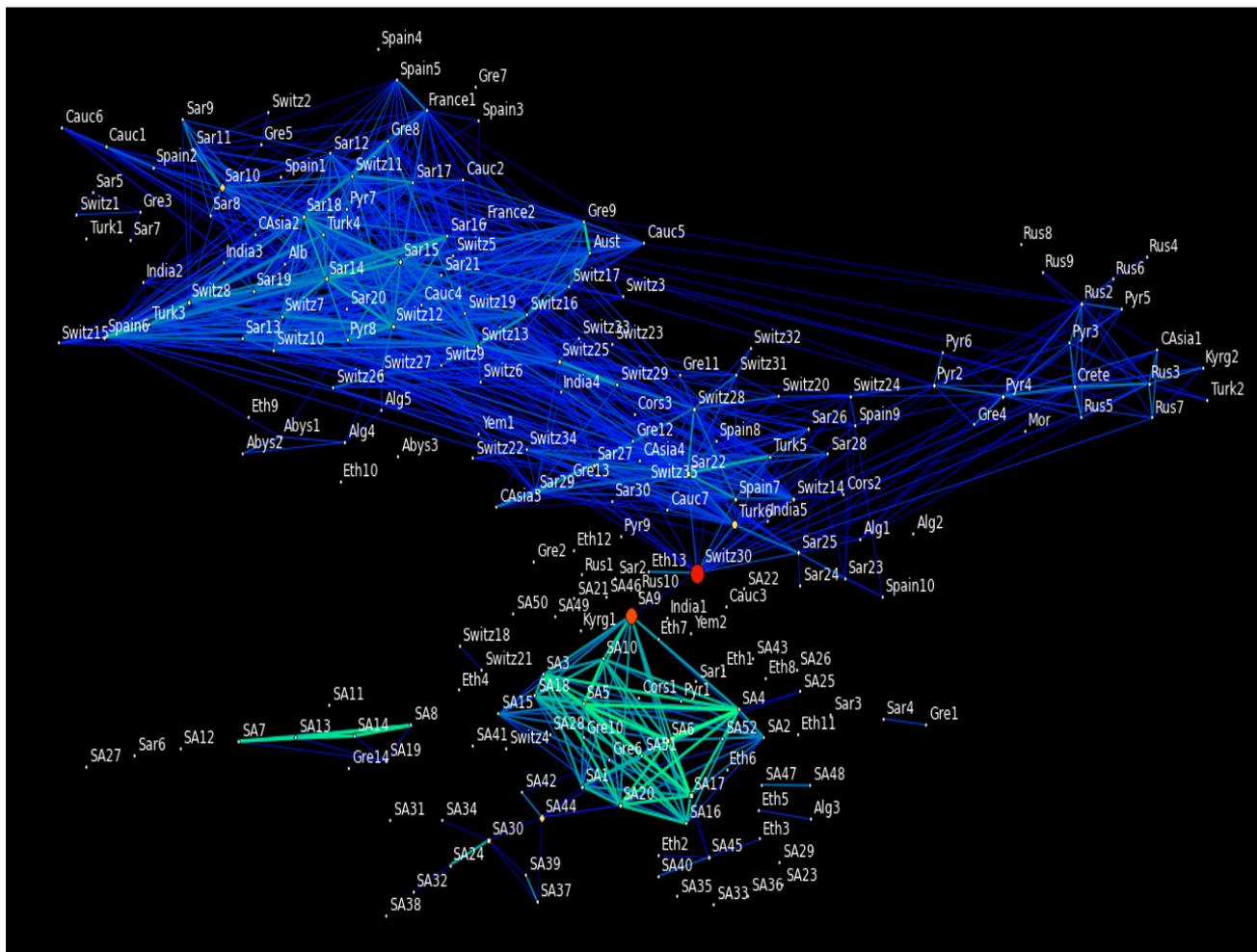
Appendix Figure 2.1: Network constructed of all individual samples (n = 217) in EDENetworks.

Populations (nodes) are named based on region of origin and are linked by edges which are

weighted in proportion to the strength of the genetic linkage.

2176      Appendix Table 4.1: Input parameters for the Vortex model for the southern African bearded

2177      vulture population

| Parameter | Value | Notes |
|---|---|---|
| Length of simulation | 500 | |
| Iterations | 1000 | |
| Extinction depression | One sex remains | |
| Inbreeding definition | None | |
| Age at first reproduction | 7 | Tracking data from one juvenile indicates that reproduction may be initiated at 7 years, but this may also be an indication of a declining population |
| Maximum breeding age | 32 years | Assumed, no data for Maluti-Drakensberg population |
| Sex ratio at birth | 50:50 | Assumed |
| Breeding strategy | Long-term monogamy | |
| % of adult males in breeding pool | 98% | A few nest sites observed with trios, assumed to be two males and one female |
| % of adult females in breeding pool | 72% (Brown 1988; SK unpublished data) | Pairs do not breed every year |
| Distribution of number of seperately sired broods produced by a female in a year | 100% of females produce a single clutch; maximum of one chick produced per nesting attempt (Brown 1988) | |
| Initial population size | 408 | |
| Carrying capacity | 1000, assumed to remain constant over time with 10% environmental variation | Although the original population was substantially larger, it is unlikely that given food and habitat constraints the population could ever recover to above 1000 birds; nest sites are not limiting; realistically the carrying capacity is likely to continue decreasing because of habitat change and livestock practices, but there will be an increased food provisioning to offset |

| | these reductions in food availability |
|---|---|
| Mortality | Age-specific mortality assumed equal in males and females; environmental variation in reproduction and mortality concordant |
| Harvest | None |

See Appendix 4.2 for mortality rates used in baseline and wind farm scenarios

2178    Appendix Table 4. 2: Age class mortality parameters used in the Vortex model for bearded

2179    Vulture

| Age Class (years) | Mortality (%) | SD |
|---|---|---|
| 0-1 | 25.80 | 38.18 |
| 1-2 | 5.40 | 37.48 |
| 2-3 | 15.00 | 42.14 |
| 3-4 | 25.20 | 47.05 |
| 4-5 | 22.80 | 41.57 |
| 5-6 | 0.00 | 0.00 |
| 6-7 | 8.40 | 30.70 |
| 7+ | 8.40 | 30.70 |
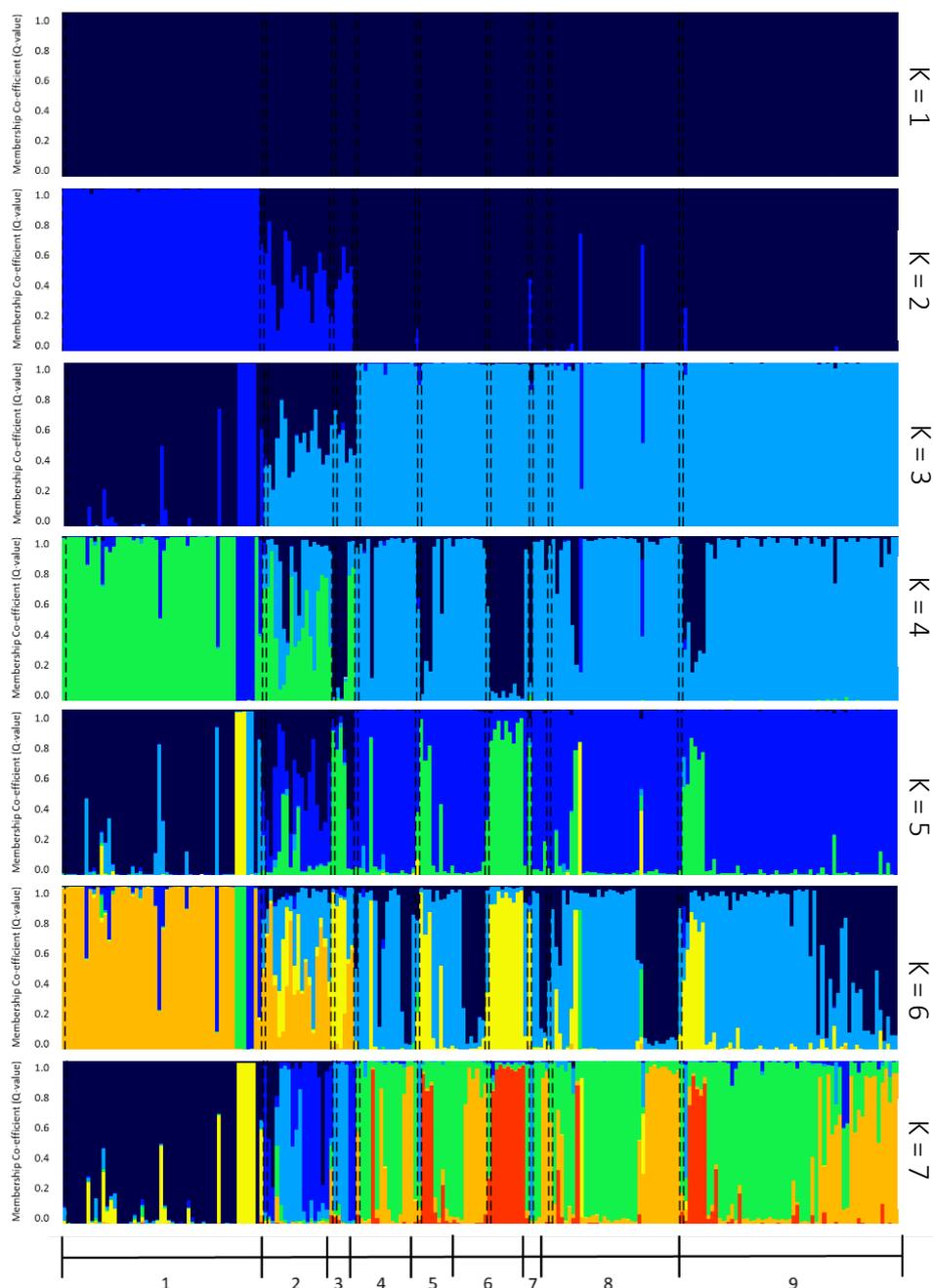
Appendix 4.3: A contrast of selected K = 7 against lower K-values from the probabilistic Admixed population structure given for 217 Gypaetus barbatus collected from across the distribution of the species. Individual vultures have been grouped into broad geographically delineated regions (1- southern Africa; 2- eastern Africa; 3- western Africa; 4- south-east Europe; 5- central Asia; 6- central Russia; 7- northern India, 8- Mediterranean islands; 9- European countries) and are represented by vertical lines which is partitioned into (K = 7) coloured segments that represent the estimated membership coefficients (Q-value).

Appendix Table 4.3. Raw data for 217 samples run against 14 microsatellite loci primers

| | BV9 | | Gf11a4 | | BV14 | | BV2 | | Gf3f3 | | Gf8g | | BV6 | | Gf3h3 | | Gf9c | | BV5 | | BV8 | | BV11 | | BV12 | | BV17 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G22911 | 217 | 219 | 130 | 135 | 181 | 183 | 136 | 136 | 172 | 172 | 270 | 270 | ? | ? | 117 | 121 | 220 | 228 | ? | ? | 105 | 107 | ? | ? | 251 | 253 | 191 | 193 |
| G27343 | 217 | 219 | 135 | 135 | 183 | 185 | 134 | 136 | 170 | 172 | 258 | 270 | ? | ? | 115 | 115 | ? | ? | ? | ? | 103 | 105 | ? | ? | 251 | 253 | 183 | 193 |
| G27341 | 215 | 217 | 130 | 135 | 183 | 185 | 136 | 136 | 172 | 172 | 258 | 270 | ? | ? | 117 | 121 | 220 | 228 | ? | ? | 103 | 105 | ? | ? | 251 | 253 | 185 | 193 |
| G27307 | 217 | 219 | 130 | 135 | 183 | 185 | 134 | 136 | 170 | 172 | 258 | 270 | ? | ? | 117 | 121 | 220 | 228 | ? | ? | 103 | 105 | ? | ? | 251 | 253 | 183 | 193 |
| G27313 | 215 | 217 | 130 | 135 | 183 | 185 | 134 | 136 | 170 | 172 | 258 | 270 | ? | ? | 111 | 117 | 220 | 228 | ? | ? | 103 | 105 | ? | ? | 251 | 253 | 185 | 193 |
| G27306 | 217 | 219 | 130 | 135 | 183 | 185 | 134 | 136 | 170 | 172 | 258 | 270 | ? | ? | 111 | 117 | 220 | 228 | ? | ? | 105 | 107 | ? | ? | 251 | 253 | 183 | 193 |
| G27378 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | 105 | 107 | ? | ? | 253 | 255 | 187 | 193 |
| G27308 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | 105 | 107 | ? | ? | 251 | 253 | 185 | 193 |
| G27376 | 215 | 217 | 130 | 135 | 179 | 183 | 130 | 132 | 170 | 172 | 258 | 270 | ? | ? | 117 | 121 | 220 | 228 | ? | ? | 105 | 107 | ? | ? | 253 | 257 | 185 | 193 |
| G27314 | 215 | 217 | 135 | 135 | 183 | 185 | 132 | 134 | 170 | 172 | 258 | 270 | ? | ? | 111 | 117 | 220 | 228 | ? | ? | 103 | 105 | ? | ? | 253 | 257 | 185 | 193 |
| BV14 inside | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | 191 | 193 |
| BV2002 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | 105 | 107 | ? | ? | ? | ? | ? | ? |
| G27302 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | 105 | 107 | ? | ? | 251 | 253 | 187 | 193 |
| BV2009 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | 105 | 107 | ? | ? | 251 | 253 | 191 | 193 |
| G27375 | 215 | 217 | 130 | 135 | 183 | 185 | ? | ? | ? | ? | ? | ? | ? | ? | 111 | 117 | 220 | 228 | ? | ? | 105 | 107 | ? | ? | 251 | 253 | 185 | 193 |
| G27305 | 215 | 217 | 135 | 135 | 185 | 189 | 136 | 136 | 170 | 172 | 258 | 270 | ? | ? | 117 | 117 | 220 | 228 | ? | ? | 105 | 107 | ? | ? | 251 | 253 | 185 | 193 |
| G27303 | 217 | 219 | 130 | 135 | 185 | 189 | 134 | 136 | 170 | 172 | 258 | 270 | ? | ? | 111 | 117 | 220 | 228 | ? | ? | 105 | 107 | ? | ? | 251 | 253 | 185 | 193 |
| G27380 | 215 | 217 | 135 | 135 | 185 | 189 | 130 | 136 | 172 | 172 | 258 | 270 | ? | ? | 111 | 117 | 226 | 228 | ? | ? | 103 | 105 | ? | ? | 251 | 253 | 185 | 193 |
| 34 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | 105 | 107 | ? | ? | ? | ? | 193 | 193 |
| G27309 | 217 | 219 | 135 | 140 | 183 | 185 | 136 | 136 | 170 | 172 | 258 | 270 | ? | ? | 117 | 121 | 220 | 228 | ? | ? | 105 | 107 | ? | ? | 251 | 253 | 185 | 193 |
| 12 | ? | ? | ? | ? | ? | ? | ? | ? | 172 | 172 | ? | ? | 117 | 121 | 117 | 121 | 230 | 230 | ? | ? | 107 | 107 | ? | ? | ? | ? | ? | ? |
| 6 | 217 | 219 | ? | ? | 183 | 189 | ? | ? | 172 | 172 | 270 | 270 | ? | ? | 117 | 121 | 228 | 228 | 187 | 187 | 107 | 107 | 187 | 187 | 255 | 255 | 193 | 193 |
| 25 | 217 | 219 | 135 | 135 | 185 | 183 | ? | ? | 172 | 172 | ? | ? | ? | ? | 117 | 121 | ? | ? | 187 | 187 | 107 | 107 | 187 | 187 | 251 | 253 | 193 | 193 |
| 13 | 217 | 219 | 135 | 135 | 179 | 189 | 134 | 136 | 172 | 172 | 270 | 270 | 117 | 117 | 117 | 117 | 220 | 228 | ? | ? | 107 | 107 | 187 | 187 | 251 | 253 | 193 | 193 |
| 19 | 217 | 219 | ? | ? | 183 | 185 | 134 | 136 | 172 | 172 | 270 | 270 | 117 | 121 | 117 | 121 | 254 | 256 | ? | ? | ? | ? | ? | ? | ? | ? | 193 | 193 |
| 27 | 217 | 219 | ? | ? | 183 | 185 | ? | ? | 178 | 180 | 274 | 274 | 117 | 121 | ? | ? | 254 | 256 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| 11 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | 117 | 121 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | 133 | 135 | 117 | 117 | 226 | 226 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| 31.1 | 217 | 219 | ? | ? | 185 | 183 | ? | ? | 172 | 172 | 270 | 270 | ? | ? | 117 | 117 | 230 | 230 | 187 | 187 | 107 | 107 | 187 | 187 | 253 | 257 | 193 | 193 |
| 31.2 | 217 | 219 | 135 | 135 | 183 | 185 | 134 | 136 | 172 | 172 | 270 | 270 | 117 | 117 | 117 | 117 | 220 | 228 | ? | ? | 107 | 107 | 187 | 187 | 253 | 257 | 193 | 193 |
| 30 | 217 | 219 | ? | ? | 183 | 185 | 134 | 136 | 172 | 172 | 270 | 270 | 201 | 201 | 117 | 117 | 230 | 230 | ? | ? | 107 | 107 | 187 | 187 | 251 | 253 | 193 | 193 |
| 9 | 217 | 219 | 135 | 135 | 185 | 189 | 134 | 136 | ? | ? | 270 | 270 | 193 | 193 | 117 | 117 | 220 | 228 | ? | ? | 107 | 107 | 187 | 187 | 251 | 253 | 193 | 193 |
| G27382 | 217 | 217 | 125 | 135 | 183 | 185 | 136 | 136 | 172 | 174 | 270 | 270 | 133 | 135 | 137 | 139 | 230 | 230 | 187 | 187 | 117 | 117 | 187 | 187 | 253 | 251 | 193 | 193 |
| Dead_2/08/2012 | 217 | 219 | 135 | 135 | 183 | 185 | 134 | 136 | 172 | 172 | 270 | 270 | 107 | 107 | 117 | 121 | 230 | 230 | ? | ? | 107 | 107 | 187 | 187 | 251 | 257 | 193 | 193 |
| G27377 | 217 | 217 | 135 | 135 | 185 | 183 | ? | ? | ? | ? | ? | ? | 199 | 201 | 117 | 121 | 220 | 220 | 187 | 187 | 107 | 107 | 187 | 187 | 251 | 253 | 193 | 193 |
| G27379 | 217 | 217 | 135 | 160 | 185 | 185 | 136 | 136 | 172 | 172 | 270 | 270 | ? | ? | 117 | 117 | 220 | 228 | 187 | 187 | 107 | 107 | 185 | 187 | 253 | 253 | 193 | 193 |
| G27381 | 217 | 219 | ? | ? | 183 | 185 | 136 | 136 | 172 | 174 | 270 | 270 | 117 | 117 | 117 | 117 | 228 | 228 | ? | ? | 107 | 107 | 187 | 187 | 251 | 253 | 193 | 193 |
| G27383 | 217 | 219 | 135 | 135 | 185 | 189 | 136 | 134 | ? | ? | 270 | 270 | ? | ? | 117 | 117 | 228 | 228 | 187 | 187 | 107 | 107 | 187 | 187 | 251 | 253 | 193 | 193 |
| G27384 | 217 | 217 | ? | ? | 183 | 185 | 134 | 136 | 172 | 172 | 270 | 270 | 117 | 117 | 117 | 117 | 228 | 228 | ? | ? | 107 | 107 | 187 | 187 | 251 | 253 | 193 | 193 |
| G27319 | 217 | 219 | 160 | 160 | 185 | 189 | 134 | 136 | 168 | 172 | 260 | 270 | 121 | 121 | 117 | 117 | 220 | 220 | 187 | 187 | 107 | 107 | 187 | 187 | 251 | 253 | 193 | 193 |
| G27310 | 217 | 217 | 135 | 135 | 185 | 187 | ? | ? | ? | ? | ? | ? | 121 | 121 | 137 | 139 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | 187 | 187 |
| G27311 | 217 | 217 | 135 | 160 | 183 | 185 | 134 | 136 | 172 | 172 | 270 | 270 | ? | ? | 117 | 117 | 228 | 228 | ? | ? | 107 | 107 | 187 | 187 | 253 | 253 | 193 | 193 |
| G27315 | 217 | 217 | ? | ? | 183 | 189 | 136 | 136 | 172 | 172 | 270 | 270 | ? | ? | 117 | 117 | 228 | 228 | ? | ? | ? | ? | ? | ? | ? | ? | 193 | 193 |
| G27316 | 217 | 219 | 135 | 160 | 183 | 185 | 136 | 136 | 168 | 172 | 270 | 270 | ? | ? | 117 | 117 | 228 | 228 | ? | ? | 107 | 107 | 187 | 187 | 253 | 255 | 193 | 193 |
| G27342 | 217 | 217 | 135 | 160 | 187 | 189 | 134 | 136 | 166 | 172 | 260 | 270 | 133 | 135 | 117 | 121 | 220 | 220 | 187 | 187 | 107 | 107 | 185 | 187 | 251 | 253 | 193 | 193 |
| BV6 | 213 | 213 | 135 | 135 | 157 | 157 | 114 | 114 | 170 | 170 | 280 | 280 | 109 | 109 | 129 | 129 | 260 | 260 | 153 | 153 | 109 | 109 | 159 | 159 | 255 | 257 | 191 | 191 |
| BV7 | 213 | 213 | 130 | 135 | 157 | 157 | 114 | 114 | 170 | 170 | 280 | 280 | 105 | 109 | 123 | 129 | 230 | 230 | ? | ? | 109 | 109 | 143 | 143 | 231 | 231 | 191 | 191 |
| BV8 | 213 | 213 | 135 | 135 | 157 | 157 | 114 | 114 | 170 | 170 | 280 | 280 | 105 | 109 | 129 | 129 | 230 | 230 | ? | ? | 109 | 109 | 143 | 143 | 231 | 231 | 191 | 191 |
| BV10 | 209 | 209 | 125 | 140 | 163 | 165 | 120 | 120 | 180 | 180 | 274 | 276 | 121 | 121 | 139 | 139 | 262 | 262 | 179 | 179 | 105 | 105 | 159 | 161 | ? | ? | 187 | 187 |
| BV12 | 213 | 213 | 130 | 155 | 163 | 165 | 120 | 120 | 178 | 178 | 274 | 274 | 121 | 121 | 139 | 139 | 262 | 264 | 179 | 179 | 105 | 105 | 161 | 161 | ? | ? | 187 | 187 |
| g | ? | ? | ? | ? | ? | ? | 134 | 136 | 166 | 172 | 270 | 270 | ? | ? | 117 | 117 | 222 | 222 | 187 | 187 | 107 | 117 | 187 | 187 | 251 | 253 | ? | ? |
| DNSM 2 | 217 | 217 | 135 | 135 | 163 | 185 | 120 | 120 | 138 | 138 | 164 | 164 | 117 | 121 | 121 | 121 | 228 | 228 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| Kolo | 217 | 217 | 125 | 135 | 181 | 183 | 134 | 136 | 180 | 180 | ? | ? | ? | ? | 117 | 121 | 230 | 230 | ? | ? | ? | ? | ? | ? | ? | ? | 193 | 193 |
| Mutt | 217 | 217 | 135 | 135 | 181 | 183 | 124 | 136 | 168 | 174 | 270 | 270 | 133 | 135 | 121 | 121 | 220 | 220 | ? | ? | 107 | 107 | 187 | 189 | 251 | 253 | 193 | 193 |
| Putin | 217 | 219 | 135 | 135 | 181 | 183 | 134 | 136 | 168 | 174 | 260 | 270 | ? | ? | ? | ? | 220 | 220 | 187 | 187 | 107 | 107 | 187 | 187 | 251 | 253 | 193 | 193 |
| A1 | ? | ? | 125 | 135 | 185 | 187 | 128 | 132 | ? | ? | ? | ? | 117 | 119 | 117 | 117 | 228 | 228 | ? | ? | ? | ? | ? | ? | 251 | 251 | ? | ? |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A13 | 217 | 219 | 135 | 135 | 177 | 179 | 132 | 132 | ? | ? | 270 | 270 | 113 | 113 | ? | ? | 228 | 228 | ? | ? | ? | ? | 175 | 177 | ? | ? | 193 | 193 |
| A14 | 219 | 219 | 135 | 135 | 177 | 179 | 130 | 132 | 172 | 172 | 270 | 270 | 111 | 113 | 117 | 121 | 228 | 228 | ? | ? | ? | ? | ? | ? | ? | ? | 191 | 193 |
| A17 | 217 | 217 | 135 | 135 | 179 | 179 | 130 | 130 | 172 | 172 | 270 | 270 | 111 | 113 | 121 | 121 | 228 | 228 | ? | ? | 107 | 107 | 173 | 175 | 251 | 253 | 193 | 195 |
| A18 | 217 | 219 | 135 | 135 | 183 | 185 | 128 | 130 | 172 | 172 | 270 | 270 | 113 | 113 | 117 | 121 | 228 | 228 | 179 | 179 | 107 | 107 | 173 | 175 | 253 | 255 | 193 | 193 |
| A19 | ? | ? | ? | ? | ? | ? | 130 | 130 | 172 | 172 | 274 | 274 | 113 | 113 | 117 | 121 | 228 | 228 | 179 | 179 | 107 | 107 | 173 | 175 | ? | ? | 193 | 193 |
| A20 | ? | ? | ? | ? | ? | ? | 130 | 136 | 172 | 172 | 270 | 270 | 111 | 111 | ? | ? | 228 | 228 | 179 | 179 | 107 | 107 | 181 | 183 | 251 | 253 | 193 | 193 |
| A21 | ? | ? | ? | ? | ? | ? | 130 | 136 | 172 | 172 | 270 | 270 | ? | ? | ? | ? | 228 | 228 | 179 | 179 | 107 | 107 | 181 | 183 | 251 | 253 | 191 | 193 |
| A22 | ? | ? | ? | ? | ? | ? | 134 | 136 | 172 | 172 | 270 | 270 | 123 | 123 | 117 | 121 | 228 | 228 | ? | ? | 107 | 107 | 179 | 181 | 251 | 253 | 193 | 193 |
| A23 | 217 | 217 | ? | ? | 181 | 183 | 124 | 136 | 172 | 172 | ? | ? | ? | ? | 121 | 121 | ? | ? | 178 | 178 | 107 | 107 | 175 | 177 | 271 | 271 | 193 | 193 |
| A25 | ? | ? | ? | ? | 179 | 183 | 134 | 136 | 172 | 172 | ? | ? | 111 | 113 | 121 | 121 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| A4 | ? | ? | 135 | 135 | 183 | 185 | 124 | 124 | 172 | 172 | ? | ? | ? | ? | 117 | 121 | ? | ? | ? | ? | ? | ? | ? | ? | 247 | 247 | ? | ? |
| A5 | ? | ? | 135 | 135 | 177 | 179 | 128 | 132 | ? | ? | ? | ? | 115 | 115 | 117 | 117 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| A6 | ? | ? | 135 | 140 | 183 | 185 | 128 | 132 | 172 | 172 | ? | ? | 113 | 115 | 121 | 121 | 228 | 228 | ? | ? | ? | ? | 171 | 173 | 247 | 249 | ? | ? |
| A7 | 217 | 217 | 135 | 135 | 183 | 185 | 130 | 132 | ? | ? | ? | ? | 113 | 115 | 117 | 121 | 228 | 228 | ? | ? | ? | ? | 171 | 173 | 247 | 247 | ? | ? |
| A8 | 217 | 217 | 135 | 135 | 181 | 183 | 128 | 130 | ? | ? | ? | ? | 115 | 115 | 121 | 121 | 228 | 228 | ? | ? | ? | ? | 173 | 175 | 245 | 247 | ? | ? |
| A9 | 217 | 219 | ? | ? | 185 | 187 | 130 | 132 | ? | ? | 270 | 270 | 115 | 115 | 115 | 115 | ? | ? | ? | ? | ? | ? | 171 | 175 | 249 | 257 | 193 | 195 |
| A11 | 217 | 219 | 135 | 135 | 179 | 183 | 128 | 132 | ? | ? | 270 | 270 | 113 | 115 | 121 | 121 | ? | ? | ? | ? | ? | ? | 169 | 171 | 249 | 251 | 193 | 193 |
| A12 | ? | ? | ? | ? | 183 | 185 | 132 | 132 | ? | ? | ? | ? | 111 | 113 | 121 | 121 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | 191 | 191 |
| A16 | ? | ? | ? | ? | ? | ? | 130 | 134 | 172 | 172 | 274 | 274 | 111 | 113 | 117 | 121 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | 193 | 193 |
| L1 | 217 | 217 | ? | ? | 177 | 181 | 130 | 132 | ? | ? | 270 | 270 | 113 | 115 | 117 | 121 | 228 | 230 | 182 | 188 | 107 | 107 | 175 | 179 | 249 | 249 | ? | ? |
| L2 | 217 | 219 | 125 | 125 | 177 | 177 | 130 | 132 | ? | ? | 270 | 270 | 113 | 113 | 117 | 117 | 224 | 230 | 188 | 190 | 107 | 109 | 173 | 179 | 247 | 249 | ? | ? |
| L3 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | 115 | 117 | 117 | 121 | ? | ? | ? | ? | 107 | 109 | ? | ? | ? | ? | ? | ? |
| L4 | 217 | 217 | 130 | 130 | 181 | 185 | 128 | 130 | ? | ? | 270 | 270 | 113 | 113 | 117 | 121 | 230 | 230 | 182 | 190 | 107 | 107 | 173 | 175 | 251 | 253 | ? | ? |
| L5 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | 274 | 274 | ? | ? | 117 | 121 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| L6 | 135 | 135 | 125 | 130 | 183 | 185 | 130 | 132 | ? | ? | 270 | 270 | 117 | 119 | 121 | 121 | 230 | 230 | 182 | 192 | 107 | 107 | 173 | 173 | 239 | 241 | ? | ? |
| L7 | 217 | 219 | ? | ? | 185 | 191 | 128 | 132 | ? | ? | 270 | 270 | 113 | 117 | 117 | 117 | 230 | 230 | 182 | 190 | 107 | 107 | 173 | 175 | 247 | 249 | ? | ? |
| SB1 | 217 | 219 | 135 | 135 | 179 | 185 | 109 | 109 | 172 | 172 | 270 | 270 | 113 | 115 | 121 | 121 | 228 | 228 | 182 | 188 | 107 | 107 | 171 | 173 | 257 | 257 | 195 | 195 |
| SB2 | 217 | 219 | 135 | 135 | 179 | 187 | 107 | 109 | 172 | 172 | 270 | 270 | 115 | 117 | 121 | 121 | 226 | 228 | 182 | 182 | 107 | 107 | 173 | 175 | 241 | 249 | 195 | 197 |
| SB3 | 215 | 217 | 135 | 135 | 179 | 185 | 109 | 109 | 172 | 172 | 270 | 270 | 113 | 115 | 117 | 117 | 228 | 230 | 182 | 182 | 107 | 107 | 175 | 175 | 253 | 255 | 193 | 193 |
| SB4 | 217 | 219 | 135 | 135 | 179 | 185 | 109 | 109 | 172 | 172 | 270 | 270 | 113 | 115 | 117 | 121 | 228 | 230 | 182 | 182 | 107 | 107 | 173 | 173 | 249 | 249 | 193 | 193 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SB5 | 217 | 219 | 135 | 135 | 183 | 187 | 109 | 109 | 172 | 172 | 270 | 270 | 111 | 113 | 117 | 121 | 226 | 228 | 182 | 190 | 107 | 107 | 171 | 173 | 249 | 251 | 193 | 193 |
| SB6 | 217 | 219 | 135 | 135 | 179 | 185 | 109 | 109 | 172 | 172 | 270 | 270 | 113 | 113 | 121 | 121 | 230 | 230 | 182 | 188 | 107 | 107 | 171 | 173 | 257 | 257 | 193 | 193 |
| SB7 | 217 | 219 | 135 | 135 | 179 | 183 | 109 | 109 | 172 | 172 | 270 | 270 | 113 | 119 | 121 | 121 | 228 | 228 | 182 | 190 | 107 | 107 | 173 | 175 | 249 | 257 | 193 | 195 |
| SB8 | 217 | 219 | 135 | 135 | 185 | 191 | 109 | 109 | 172 | 172 | 270 | 270 | 113 | 113 | 121 | 121 | 230 | 230 | 182 | 184 | 107 | 107 | 173 | 175 | 249 | 263 | 193 | 197 |
| SB9 | 217 | 219 | 135 | 135 | 183 | 185 | 109 | 109 | 172 | 172 | 270 | 270 | 113 | 115 | 117 | 121 | 228 | 228 | 188 | 188 | 107 | 107 | 171 | 173 | 249 | 251 | 193 | 193 |
| SB10 | 217 | 219 | 135 | 135 | 179 | 183 | 109 | 109 | 172 | 172 | 270 | 270 | 111 | 113 | 117 | 121 | 228 | 228 | 188 | 188 | ? | ? | 173 | 175 | 249 | 251 | 193 | 193 |
| SB13 | 215 | 217 | 135 | 135 | 181 | 183 | 109 | 109 | 172 | 172 | 270 | 270 | 113 | 115 | 117 | 117 | 228 | 228 | ? | ? | 107 | 107 | ? | ? | ? | ? | 193 | 195 |
| SB16 | 217 | 219 | 135 | 135 | 183 | 185 | 109 | 109 | 172 | 172 | 270 | 270 | 115 | 115 | 117 | 121 | 230 | 230 | 188 | 188 | 107 | 107 | 173 | 173 | 249 | 249 | 193 | 193 |
| SB18 | 217 | 219 | 135 | 135 | 183 | 185 | 109 | 109 | 172 | 172 | 270 | 270 | 113 | 113 | 121 | 121 | 228 | 228 | 188 | 188 | 107 | 107 | 175 | 177 | 245 | 251 | 193 | 195 |
| SB19 | 217 | 219 | 135 | 135 | 181 | 185 | 109 | 109 | 172 | 172 | 270 | 270 | 113 | 113 | 117 | 121 | 228 | 228 | 182 | 182 | 107 | 107 | 169 | 171 | 249 | 251 | 193 | 193 |
| SB20 | 217 | 219 | 135 | 135 | 179 | 185 | 109 | 109 | 172 | 172 | 270 | 270 | 117 | 117 | 121 | 121 | 226 | 228 | 182 | 182 | 107 | 107 | 175 | 175 | 263 | 265 | 195 | 195 |
| SB21 | 215 | 217 | 135 | 135 | 179 | 181 | 109 | 109 | 172 | 172 | 270 | 270 | 113 | 113 | 117 | 121 | 228 | 230 | ? | ? | 107 | 107 | ? | ? | 249 | 257 | 193 | 193 |
| SB22 | 215 | 217 | 135 | 135 | 183 | 185 | 109 | 109 | 172 | 172 | 270 | 270 | 113 | 115 | 117 | 121 | 228 | 228 | 188 | 188 | 107 | 107 | 175 | 175 | 243 | 249 | 193 | 195 |
| SB23 | 159 | 159 | 130 | 135 | 181 | 185 | 109 | 109 | 172 | 172 | 270 | 270 | 113 | 113 | 117 | 121 | 228 | 228 | ? | ? | 107 | 107 | 175 | 175 | 245 | 249 | 193 | 193 |
| SB24 | 159 | 159 | 135 | 135 | 179 | 185 | 109 | 109 | 172 | 172 | 270 | 270 | 113 | 113 | 117 | 117 | 228 | 228 | 188 | 194 | 107 | 107 | 173 | 175 | 251 | 257 | 193 | 193 |
| S1 | 217 | 219 | 135 | 135 | 179 | 191 | 124 | 124 | 172 | 172 | 270 | 270 | 117 | 119 | 121 | 121 | 230 | 230 | 180 | 180 | 107 | 107 | 171 | 173 | 249 | 251 | 193 | 193 |
| S2 | 215 | 217 | 135 | 135 | 183 | 187 | 128 | 132 | 172 | 172 | 270 | 270 | 115 | 115 | 117 | 121 | 230 | 230 | 180 | 182 | 107 | 107 | 173 | 173 | 249 | 251 | 193 | 193 |
| S3 | 215 | 217 | 135 | 135 | 183 | 187 | 126 | 126 | 172 | 172 | 270 | 270 | 113 | 115 | 117 | 121 | 230 | 230 | 182 | 182 | 107 | 107 | 173 | 173 | 249 | 251 | 195 | 195 |
| S4 | 217 | 221 | 135 | 135 | 181 | 187 | 130 | 132 | 172 | 172 | 270 | 270 | 115 | 115 | 117 | 121 | 230 | 230 | 188 | 190 | 107 | 107 | 171 | 173 | 245 | 249 | 193 | 193 |
| S6 | 217 | 219 | 135 | 135 | 185 | 187 | 128 | 130 | 172 | 172 | 270 | 270 | 115 | 115 | 115 | 121 | 230 | 230 | 182 | 182 | 107 | 107 | 173 | 173 | 245 | 251 | 195 | 195 |
| S7 | 217 | 219 | 135 | 135 | 185 | 191 | 128 | 128 | 174 | 174 | 270 | 270 | 115 | 115 | ? | ? | 230 | 230 | 182 | 194 | 107 | 107 | 173 | 177 | 247 | 249 | 193 | 193 |
| S9 | 215 | 217 | 135 | 135 | 179 | 185 | 130 | 130 | 174 | 174 | 270 | 270 | 115 | 115 | ? | ? | 230 | 230 | 188 | 188 | 107 | 107 | 177 | 179 | 249 | 257 | 193 | 193 |
| S10 | ? | ? | 135 | 135 | 183 | 185 | ? | ? | ? | ? | ? | ? | 117 | 119 | ? | ? | 230 | 230 | ? | ? | ? | ? | ? | ? | ? | ? | 193 | 195 |
| S11 | ? | ? | ? | ? | ? | ? | 124 | 134 | 174 | 174 | ? | ? | 115 | 115 | ? | ? | 226 | 230 | 188 | 188 | 107 | 107 | 171 | 179 | ? | ? | ? | ? |
| S12 | 215 | 217 | 135 | 135 | 179 | 181 | 128 | 128 | 174 | 174 | 270 | 270 | 111 | 117 | ? | ? | 230 | 230 | 188 | 188 | 107 | 107 | ? | ? | 251 | 257 | 193 | 195 |
| S14 | 215 | 217 | 135 | 135 | 181 | 183 | 136 | 136 | 174 | 174 | 270 | 270 | 123 | 125 | ? | ? | 230 | 230 | 186 | 188 | 107 | 107 | ? | ? | 251 | 253 | 193 | 193 |
| S15 | ? | ? | ? | ? | ? | ? | 132 | 132 | 174 | 174 | 270 | 270 | 115 | 115 | ? | ? | | | | | | | | | | | 193 | 193 |
| S16 | 215 | 217 | 135 | 135 | 181 | 183 | 130 | 136 | 174 | 174 | ? | ? | 123 | 125 | ? | ? | 230 | 230 | 186 | 188 | 107 | 107 | 177 | 179 | 253 | 255 | 193 | 193 |
| S17 | ? | ? | 135 | 135 | 181 | 183 | 136 | 136 | 174 | 174 | ? | ? | 123 | 125 | ? | ? | 230 | 230 | 186 | 188 | 107 | 107 | ? | ? | 251 | 253 | 193 | 193 |
| S18 | ? | ? | ? | ? | 157 | 157 | 116 | 116 | 170 | 170 | ? | ? | 113 | 115 | ? | ? | 230 | 230 | ? | ? | 109 | 109 | ? | ? | ? | ? | 191 | 191 |

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| S19 | 175 | 175 | 135 | 135 | 185 | 187 | 128 | 130 | 174 | 174 | ? | ? | 113 | 115 | ? | ? | 228 | 230 | | | 107 | 107 | ? | ? | ? | ? | 195 | 195 |
| S20 | 215 | 217 | 135 | 135 | 179 | 185 | 132 | 132 | 174 | 174 | 270 | 270 | 115 | 117 | ? | ? | 230 | 230 | 182 | 188 | 107 | 107 | 171 | 171 | 241 | 249 | 193 | 193 |
| S24 | ? | ? | ? | ? | 175 | 175 | ? | ? | 174 | 174 | ? | ? | 113 | 115 | ? | ? | 230 | 240 | | | ? | ? | | | 243 | 245 | ? | ? |
| S25 | ? | ? | 135 | 135 | 179 | 185 | 132 | 132 | 174 | 174 | 270 | 270 | 113 | 119 | ? | ? | ? | ? | 182 | 182 | 107 | 107 | | | ? | ? | 193 | 193 |
| S26 | 215 | 217 | 135 | 135 | 179 | 185 | 130 | 132 | 174 | 174 | 270 | 270 | 113 | 113 | ? | ? | 230 | 230 | 182 | 182 | 107 | 109 | 179 | 179 | 249 | 255 | 193 | 193 |
| S27 | 217 | 219 | 135 | 135 | 179 | 181 | 132 | 132 | 174 | 174 | 270 | 270 | 113 | 113 | ? | ? | 230 | 230 | ? | ? | 107 | 107 | 175 | 177 | 247 | 249 | 193 | 193 |
| S29 | 217 | 217 | 135 | 135 | 179 | 183 | 130 | 130 | 172 | 172 | 270 | 270 | 113 | 115 | ? | ? | ? | ? | 182 | 188 | 107 | 107 | 171 | 171 | 247 | 249 | 193 | 195 |
| S30 | 215 | 217 | 135 | 135 | 185 | 191 | 132 | 132 | 172 | 172 | 270 | 270 | 115 | 115 | ? | ? | 230 | 230 | 188 | 190 | 107 | 107 | 171 | 177 | 247 | 249 | 193 | 195 |
| S31 | 217 | 219 | 135 | 135 | 185 | 191 | 124 | 128 | 172 | 172 | 270 | 270 | 113 | 117 | ? | ? | 230 | 230 | 182 | 190 | 107 | 109 | 173 | 173 | 245 | 247 | 193 | 193 |
| S32 | 217 | 219 | 135 | 135 | 185 | 191 | 128 | 132 | 172 | 172 | 270 | 270 | 115 | 117 | ? | ? | 230 | 230 | 180 | 182 | 107 | 107 | 173 | 175 | 247 | 249 | 193 | 195 |
| S33 | 217 | 219 | 135 | 135 | 185 | 191 | 124 | 132 | 172 | 172 | 270 | 270 | 113 | 115 | ? | ? | 230 | 230 | 182 | 182 | 107 | 109 | 175 | 177 | 245 | 247 | 193 | 195 |
| S36 | 217 | 219 | 135 | 135 | ? | ? | 132 | 132 | 174 | 174 | ? | ? | 113 | 115 | ? | ? | 230 | 230 | 182 | 182 | 107 | 107 | ? | ? | 249 | 249 | 193 | 193 |
| S39 | 217 | 219 | 135 | 135 | 183 | 185 | 124 | 132 | 174 | 174 | 270 | 270 | 117 | 119 | ? | ? | 228 | 230 | 182 | 182 | 107 | 107 | 173 | 177 | 249 | 251 | 193 | 197 |
| S43 | 217 | 219 | 135 | 135 | 179 | 185 | 130 | 130 | 174 | 174 | 270 | 270 | 113 | 119 | ? | ? | 230 | 230 | 182 | 182 | 107 | 107 | 173 | 177 | 239 | 241 | ? | ? |
| S44 | 215 | 217 | 135 | 135 | 187 | 191 | 128 | 128 | 174 | 174 | 270 | 270 | 113 | 115 | ? | ? | 230 | 230 | 186 | 188 | 107 | 107 | 173 | 177 | 245 | 247 | 193 | 195 |
| S50 | 217 | 217 | 135 | 135 | 179 | 183 | 136 | 136 | 174 | 174 | ? | ? | ? | ? | ? | ? | 230 | 230 | 184 | 184 | 107 | 107 | ? | ? | ? | ? | 193 | 193 |
| S51 | 217 | 219 | 135 | 135 | 179 | 183 | 128 | 128 | 174 | 174 | 270 | 270 | 115 | 117 | ? | ? | 230 | 230 | 182 | 190 | 107 | 109 | 173 | 173 | 247 | 257 | 193 | 195 |
| S52 | 215 | 217 | 135 | 135 | 173 | 187 | 128 | 128 | 174 | 174 | 270 | 270 | 115 | 115 | ? | ? | 230 | 230 | 188 | 194 | 107 | 107 | 175 | 177 | 243 | 251 | 193 | 195 |
| S54 | 217 | 219 | 135 | 135 | 185 | 191 | 124 | 128 | 174 | 174 | 270 | 270 | 113 | 115 | ? | ? | 230 | 230 | 188 | 190 | 107 | 107 | 173 | 175 | 245 | 247 | 193 | 195 |
| S56 | 217 | 219 | 135 | 135 | 183 | 185 | 124 | 132 | 174 | 174 | 270 | 270 | 113 | 115 | ? | ? | 230 | 230 | 188 | 190 | 107 | 107 | 177 | 179 | 255 | 247 | 193 | 195 |
| S57 | 217 | 219 | 135 | 135 | 183 | 185 | 132 | 132 | 174 | 174 | 270 | 270 | 113 | 113 | ? | ? | 230 | 230 | 188 | 190 | 107 | 107 | 173 | 175 | 245 | 247 | 193 | 195 |
| S60 | 217 | 217 | 135 | 135 | 181 | 183 | 124 | 124 | 174 | 174 | 270 | 270 | ? | ? | ? | ? | 230 | 230 | 186 | 188 | 107 | 107 | ? | ? | 251 | 251 | 193 | 193 |
| S61 | 215 | 217 | 135 | 135 | 181 | 183 | 128 | 132 | 174 | 174 | 270 | 270 | 111 | 115 | ? | ? | 230 | 230 | 182 | 182 | 107 | 107 | 177 | 179 | 249 | 251 | 193 | 195 |
| S63 | 217 | 217 | 135 | 135 | 177 | 179 | 124 | 124 | 174 | 174 | 270 | 270 | 117 | 117 | ? | ? | 230 | 238 | 182 | 182 | 107 | 107 | 171 | 171 | 249 | 251 | 195 | 195 |
| S66 | 217 | 219 | 135 | 135 | 183 | 185 | ? | ? | 174 | 174 | 270 | 270 | 115 | 117 | ? | ? | 230 | 230 | 186 | 188 | 107 | 107 | 175 | 177 | 245 | 247 | 193 | 195 |
| S71 | 217 | 219 | 135 | 135 | 179 | 191 | 132 | 132 | 174 | 174 | 270 | 270 | 115 | 117 | ? | ? | 230 | 230 | 182 | 182 | 107 | 107 | 171 | 173 | 245 | 247 | 193 | 195 |
| S73 | 217 | 217 | 135 | 135 | 183 | 185 | 128 | 132 | 174 | 174 | ? | ? | 113 | 113 | ? | ? | 230 | 230 | 182 | 190 | 107 | 107 | 173 | 175 | 245 | 247 | 193 | 193 |
| S74 | 217 | 219 | 135 | 135 | 179 | 183 | 124 | 124 | 174 | 174 | 270 | 270 | 117 | 119 | ? | ? | 230 | 230 | 182 | 182 | 107 | 107 | 173 | 175 | 251 | 253 | 193 | 195 |
| S77 | 217 | 219 | 135 | 135 | 179 | 185 | 128 | 132 | 174 | 174 | 270 | 270 | 113 | 113 | ? | ? | 230 | 230 | 188 | 192 | 107 | 109 | 171 | 173 | 247 | 249 | 193 | 195 |
| S78 | 217 | 217 | 135 | 135 | 181 | 189 | 128 | 128 | 174 | 174 | 270 | 270 | 115 | 115 | ? | ? | 230 | 230 | 182 | 188 | 107 | 107 | 173 | 177 | 241 | 243 | 193 | 193 |

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| S79 | 215 | 217 | 135 | 135 | 179 | 183 | 128 | 128 | 174 | 174 | 270 | 270 | 113 | 115 | ? | ? | 230 | 238 | 182 | 188 | 107 | 107 | 175 | 177 | 243 | 247 | 193 | 193 |
| S80 | 217 | 219 | 135 | 135 | 189 | 191 | 128 | 132 | 174 | 174 | 270 | 270 | 113 | 115 | ? | ? | 230 | 230 | 182 | 190 | 107 | 107 | 175 | 179 | 247 | 249 | 193 | 195 |
| S81 | 217 | 219 | 135 | 135 | 179 | 183 | 128 | 130 | 174 | 174 | 270 | 270 | 113 | 119 | ? | ? | 230 | 230 | 186 | 188 | 107 | 107 | 169 | 171 | 249 | 255 | 193 | 195 |
| S84 | 215 | 217 | 135 | 135 | 179 | 181 | 128 | 132 | 174 | 174 | 270 | 270 | 115 | 117 | ? | ? | 230 | 230 | 182 | 182 | 107 | 107 | 173 | 175 | 247 | 249 | 193 | 195 |
| S85 | 217 | 219 | 135 | 135 | 181 | 183 | 128 | 132 | 174 | 174 | 270 | 270 | 111 | 115 | ? | ? | 230 | 230 | 186 | 188 | 107 | 107 | 173 | 175 | 245 | 247 | 193 | 195 |
| S88 | 217 | 219 | 135 | 135 | 185 | 187 | 132 | 132 | 174 | 174 | 270 | 270 | 111 | 115 | ? | ? | 230 | 230 | 180 | 182 | 107 | 107 | 173 | 173 | 247 | 249 | 195 | 195 |
| S89 | 217 | 219 | 135 | 135 | 179 | 185 | 128 | 132 | 174 | 174 | 270 | 270 | 113 | 113 | ? | ? | 230 | 230 | 186 | 188 | 107 | 107 | 169 | 171 | 255 | 257 | 193 | 195 |
| S90 | 217 | 219 | 135 | 135 | 185 | 191 | 128 | 128 | 174 | 174 | 270 | 270 | 113 | 113 | ? | ? | 230 | 230 | 190 | 194 | 107 | 107 | 171 | 179 | 247 | 249 | 193 | 195 |
| S92 | 217 | 219 | 135 | 135 | 179 | 181 | 124 | 128 | 174 | 174 | 270 | 270 | 111 | 115 | ? | ? | 230 | 230 | 182 | 188 | 107 | 107 | 171 | 177 | 241 | 243 | 193 | 195 |
| S93 | 215 | 217 | 135 | 135 | 183 | 191 | 124 | 132 | 174 | 174 | 270 | 270 | 113 | 115 | ? | ? | 228 | 228 | 188 | 194 | 107 | 109 | 171 | 173 | 247 | 249 | 193 | 195 |
| S95 | 215 | 217 | 135 | 135 | 181 | 183 | 126 | 126 | 174 | 174 | 270 | 270 | 113 | 113 | ? | ? | 230 | 230 | 186 | 188 | 107 | 107 | 171 | 175 | 243 | 249 | 193 | 193 |
| S98 | 215 | 217 | 135 | 135 | 177 | 179 | 124 | 128 | 174 | 174 | 270 | 270 | 115 | 115 | ? | ? | 230 | 230 | 186 | 188 | 107 | 107 | 173 | 175 | 255 | 257 | 193 | 193 |
| S99 | 217 | 217 | ? | ? | 183 | 183 | 124 | 128 | 174 | 174 | 270 | 270 | 115 | 115 | ? | ? | 230 | 230 | | | ? | ? | ? | ? | ? | ? | ? | ? |
| S101 | 217 | 219 | 135 | 135 | 185 | 191 | 124 | 132 | 174 | 174 | 270 | 270 | 113 | 113 | ? | ? | 230 | 230 | 182 | 188 | 107 | 107 | 171 | 175 | 247 | 249 | 193 | 193 |
| S105 | 215 | 217 | 135 | 135 | 177 | 179 | 132 | 132 | 174 | 174 | 270 | 270 | 113 | 117 | ? | ? | 230 | 230 | 188 | 190 | 107 | 107 | 173 | 175 | 241 | 243 | 193 | 193 |
| S106 | 215 | 217 | 135 | 135 | 177 | 179 | 124 | 128 | 174 | 174 | 270 | 270 | 115 | 117 | ? | ? | 230 | 230 | 188 | 188 | 107 | 107 | 173 | 175 | 255 | 257 | 193 | 193 |
| S107 | ? | ? | 135 | 135 | 177 | 185 | 124 | 128 | 174 | 174 | 270 | 270 | 115 | 117 | ? | ? | 230 | 230 | ? | ? | 107 | 107 | ? | ? | 255 | 257 | 193 | 195 |
| S109 | 217 | 219 | 135 | 135 | 177 | 179 | 124 | 128 | 174 | 174 | 270 | 270 | 115 | 115 | ? | ? | 230 | 230 | 188 | 188 | 107 | 107 | 175 | 177 | 239 | 241 | 193 | 195 |
| S110 | 215 | 217 | 135 | 135 | 177 | 179 | 124 | 132 | 174 | 174 | 270 | 270 | 115 | 117 | ? | ? | 230 | 230 | 188 | 188 | 107 | 107 | 177 | 177 | 255 | 257 | 193 | 195 |
| S111 | 217 | 219 | 135 | 135 | 179 | 181 | ? | ? | ? | ? | ? | ? | | | | | | | | | | | | | | | 195 | 195 |
| S113 | 215 | 217 | 135 | 135 | 181 | 183 | 136 | 136 | 174 | 174 | 270 | 270 | 121 | 121 | ? | ? | 228 | 228 | 188 | 188 | 107 | 107 | ? | ? | 251 | 255 | 193 | 193 |
| S114 | 217 | 219 | 135 | 135 | 179 | 183 | ? | ? | 174 | 174 | 270 | 270 | 113 | 115 | ? | ? | 230 | 230 | 188 | 190 | 107 | 109 | 175 | 177 | 255 | 257 | 195 | 195 |
| S115 | 217 | 219 | 135 | 135 | 183 | 185 | 124 | 124 | 174 | 174 | 270 | 270 | 113 | 113 | ? | ? | 228 | 228 | ? | ? | 107 | 107 | 173 | 173 | 243 | 249 | 195 | 195 |
| S116 | 217 | 219 | 135 | 135 | 179 | 181 | 124 | 128 | 174 | 174 | 270 | 270 | 117 | 117 | ? | ? | 230 | 230 | 186 | 188 | 107 | 107 | 175 | 177 | 239 | 241 | 193 | 193 |
| S2 | 217 | 219 | 135 | 135 | 179 | 179 | 124 | 128 | 174 | 174 | 270 | 270 | 115 | 121 | 121 | 121 | 228 | 228 | 182 | 182 | 107 | 107 | 181 | 187 | 241 | 241 | 193 | 195 |
| S6 | 217 | 219 | 135 | 135 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | 193 | 195 |
| S7 | 217 | 219 | 135 | 135 | 179 | 181 | 128 | 134 | 172 | 174 | 270 | 270 | 115 | 115 | 117 | 121 | 228 | 228 | 170 | 174 | ? | ? | 185 | 187 | 245 | 247 | 195 | 195 |
| S8 | 217 | 219 | 135 | 135 | ? | ? | 124 | 128 | 174 | 174 | 270 | 270 | 117 | 117 | 117 | 121 | 230 | 230 | 174 | 176 | ? | ? | 185 | 187 | 239 | 241 | 193 | 193 |
| S10 | 217 | 219 | 135 | 135 | 173 | 179 | 124 | 128 | 172 | 172 | 270 | 270 | 113 | 115 | 121 | 121 | 228 | 228 | 182 | 184 | 105 | 107 | 181 | 183 | 241 | 241 | 193 | 195 |
| S15 | 217 | 219 | 135 | 135 | 179 | 181 | 124 | 128 | 174 | 174 | 270 | 270 | 113 | 115 | 117 | 121 | 230 | 230 | 182 | 184 | 107 | 107 | 181 | 187 | 241 | 247 | 193 | 193 |

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| S17 | 215 | 217 | 135 | 135 | 179 | 181 | 128 | 128 | 174 | 174 | 270 | 270 | 111 | 111 | 117 | 121 | 230 | 230 | 186 | 188 | 107 | 107 | 187 | 187 | 251 | 257 | 193 | 195 |
| S19 | 215 | 217 | 135 | 135 | 183 | 183 | 134 | 136 | 174 | 174 | 270 | 270 | 117 | 125 | 117 | 121 | 230 | 230 | 186 | 188 | 107 | 107 | 187 | 187 | 251 | 253 | 193 | 193 |
| S27 | 217 | 219 | 135 | 135 | ? | ? | ? | ? | ? | ? | 270 | 270 | ? | ? | ? | ? | ? | ? | ? | ? | 103 | 105 | ? | ? | ? | ? | 193 | 193 |
| S29 | 217 | 219 | 135 | 135 | ? | ? | ? | ? | ? | ? | 270 | 270 | ? | ? | ? | ? | ? | ? | 186 | 188 | 107 | 107 | 187 | 187 | 253 | 255 | 193 | 193 |
| S30 | 217 | 219 | 135 | 135 | ? | ? | 130 | 136 | 172 | 174 | 272 | 276 | 105 | 111 | 119 | 119 | 228 | 228 | 182 | 182 | 109 | 109 | 181 | 187 | ? | ? | 191 | 191 |
| S32 | 217 | 219 | 135 | 135 | 181 | 183 | 128 | 132 | 174 | 174 | 270 | 270 | 111 | 115 | 121 | 121 | 230 | 230 | 186 | 188 | 107 | 107 | 185 | 187 | 253 | 257 | 193 | 195 |
| S35 | 217 | 219 | 135 | 135 | 179 | 181 | 128 | 132 | 174 | 174 | 270 | 270 | 115 | 117 | 117 | 121 | 228 | 228 | 182 | 182 | 107 | 107 | 181 | 187 | 245 | 247 | 193 | 195 |
| S36 | 217 | 219 | 135 | 135 | 179 | 181 | 124 | 128 | 174 | 174 | 270 | 270 | 111 | 115 | 117 | 117 | 230 | 230 | 182 | 182 | 107 | 107 | 181 | 187 | 243 | 247 | 193 | 195 |
| S39 | 215 | 217 | 135 | 135 | 183 | 191 | 124 | 132 | 172 | 172 | 270 | 270 | 113 | 115 | 117 | 121 | 226 | 228 | 186 | 188 | 107 | 107 | 187 | 187 | 247 | 249 | 193 | 195 |
| S45 | 215 | 217 | 135 | 135 | 181 | 183 | 128 | 132 | 174 | 174 | 270 | 270 | 115 | 115 | 117 | 121 | 228 | 228 | 188 | 190 | 107 | 107 | 187 | 189 | 247 | 249 | 193 | 195 |
| S49 | 217 | 217 | 135 | 135 | 183 | 183 | 122 | 124 | 172 | 172 | 270 | 270 | 113 | 115 | 117 | 121 | ? | ? | 186 | 188 | 107 | 107 | 187 | 187 | 247 | 249 | 193 | 193 |
| S53 | 215 | 217 | 135 | 135 | 183 | 191 | 124 | 124 | 174 | 174 | 270 | 270 | 113 | 115 | 121 | 121 | 228 | 228 | 182 | 182 | 107 | 107 | 181 | 187 | 247 | 249 | 195 | 195 |
| S55 | 215 | 217 | 135 | 135 | 183 | 183 | 128 | 132 | 174 | 174 | 270 | 270 | 111 | 115 | 121 | 121 | 228 | 228 | 182 | 182 | 107 | 107 | 181 | 181 | 249 | 251 | 193 | 195 |
| S58 | 215 | 217 | 135 | 135 | 179 | 183 | 128 | 128 | 174 | 174 | 270 | 270 | 113 | 115 | 121 | 121 | 228 | 228 | 182 | 182 | 107 | 107 | 181 | 187 | 243 | 247 | 193 | 193 |
| S60 | 217 | 219 | 135 | 135 | ? | ? | 128 | 128 | 174 | 174 | 270 | 270 | 115 | 115 | 121 | 121 | 228 | 228 | 182 | 182 | 107 | 107 | 181 | 187 | 241 | 243 | 193 | 193 |
| S62 | 217 | 217 | 135 | 135 | 181 | 183 | 128 | 128 | 174 | 174 | 270 | 270 | 113 | 115 | 121 | 121 | 230 | 230 | 182 | 182 | 107 | 107 | 185 | 187 | 243 | 247 | 193 | 193 |
| S65 | 215 | 217 | 135 | 135 | 179 | 185 | 128 | 132 | 172 | 172 | 270 | 270 | 113 | 117 | 121 | 121 | 228 | 228 | 182 | 182 | 107 | 107 | 181 | 181 | 249 | 251 | 193 | 195 |
| S69 | 217 | 219 | 135 | 135 | 189 | 191 | 128 | 132 | 174 | 174 | 270 | 270 | 113 | 115 | 117 | 121 | 228 | 228 | 178 | 182 | 107 | 107 | 187 | 189 | 247 | 249 | 193 | 195 |
| S72 | 215 | 217 | 135 | 135 | 181 | 187 | 130 | 132 | 174 | 174 | 270 | 270 | 113 | 113 | 117 | 117 | 228 | 228 | 172 | 172 | 107 | 107 | 181 | 187 | 245 | 249 | 193 | 195 |
| S74 | 217 | 221 | 135 | 135 | 181 | 187 | 130 | 132 | 174 | 174 | 270 | 270 | 115 | 117 | 117 | 121 | 230 | 230 | 170 | 172 | 107 | 107 | 189 | 189 | 245 | 249 | 193 | 193 |
| S77 | 217 | 219 | 135 | 135 | 179 | 185 | 128 | 132 | 174 | 174 | 270 | 270 | 113 | 113 | 117 | 121 | 228 | 228 | 170 | 172 | ? | ? | 181 | 189 | 247 | 249 | 193 | 195 |
| S87 | 217 | 219 | 135 | 135 | 179 | 185 | 130 | 130 | 172 | 172 | 270 | 270 | 113 | 119 | 121 | 121 | 228 | 228 | 172 | 172 | 107 | 107 | 181 | 181 | 239 | 241 | 193 | 195 |
| S88 | 217 | 217 | 135 | 135 | 185 | 187 | 128 | 130 | 174 | 174 | 270 | 270 | 115 | 115 | 121 | 121 | 228 | 228 | 170 | 172 | 107 | 107 | 181 | 181 | 245 | 249 | 195 | 195 |
| S90 | 217 | 219 | 135 | 135 | ? | ? | 128 | 132 | 174 | 174 | 270 | 270 | ? | ? | ? | ? | ? | ? | 172 | 174 | 107 | 107 | 181 | 187 | 247 | 249 | 193 | 193 |
| S97 | 215 | 217 | 135 | 135 | 187 | 191 | 128 | 128 | 174 | 174 | 270 | 270 | 113 | 115 | 121 | 121 | 228 | 230 | 172 | 176 | 107 | 107 | 187 | 187 | 245 | 247 | 193 | 195 |
| S99 | 217 | 217 | 135 | 135 | 179 | 185 | 130 | 132 | 174 | 174 | 270 | 270 | 113 | 113 | 117 | 117 | 230 | 230 | 172 | 176 | 107 | 107 | 181 | 187 | 247 | 249 | 193 | 193 |
| S100 | 217 | 219 | 135 | 135 | 179 | 181 | 132 | 132 | 172 | 172 | 270 | 270 | 113 | 113 | 121 | 121 | 228 | 228 | ? | ? | 107 | 107 | 187 | 187 | 247 | 249 | 193 | 193 |
| S101 | 217 | 219 | 135 | 135 | 185 | 191 | 124 | 128 | 172 | 172 | 270 | 270 | 113 | 117 | 121 | 121 | 228 | 228 | 172 | 172 | 107 | 107 | 181 | 189 | 245 | 247 | 193 | 193 |
| S105 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | 107 | 109 | ? | ? | ? | ? | 195 | 195 |
| S106 | ? | ? | ? | ? | 185 | 185 | 124 | 128 | 172 | 172 | 270 | 270 | 113 | 115 | 117 | 117 | 228 | 228 | 172 | 174 | 107 | 107 | 187 | 189 | 245 | 247 | 193 | 195 |

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| S107 | 217 | 219 | 135 | 135 | 185 | 185 | 130 | 132 | 172 | 172 | 270 | 270 | 113 | 113 | 117 | 121 | 228 | 230 | 172 | 174 | 107 | 107 | 187 | 189 | 245 | 247 | 193 | 195 |
| S108 | 217 | 219 | 135 | 135 | 185 | 191 | 128 | 128 | 174 | 174 | 270 | 270 | 113 | 115 | 117 | 121 | 226 | 228 | 170 | 178 | 107 | 107 | 189 | 189 | 247 | 249 | 193 | 195 |
| S109 | 217 | 219 | 135 | 135 | 185 | 187 | 132 | 132 | 174 | 174 | 270 | 270 | 111 | 115 | 117 | 121 | 228 | 230 | 172 | 172 | 107 | 107 | 181 | 181 | 247 | 249 | 195 | 195 |
| S120 | 217 | 219 | 135 | 135 | ? | ? | 128 | 132 | 174 | 174 | 270 | 270 | 113 | 115 | 117 | 121 | 226 | 228 | 170 | 170 | 107 | 107 | 181 | 187 | 249 | 249 | 193 | 193 |
| S122 | 217 | 219 | 135 | 135 | 179 | 183 | 128 | 128 | 172 | 174 | 270 | 270 | 115 | 115 | 117 | 121 | 226 | 268 | 172 | 172 | 107 | 107 | 181 | 189 | 245 | 247 | 193 | 195 |
| S125 | 217 | 219 | 135 | 135 | 179 | 185 | 124 | 132 | 174 | 174 | 270 | 270 | 113 | 115 | 117 | 121 | 228 | 228 | 174 | 176 | 107 | 107 | 181 | 181 | 247 | 249 | 193 | 195 |
| S130 | ? | ? | ? | ? | ? | ? | 128 | 128 | 174 | 174 | 270 | 270 | 113 | 117 | 117 | 117 | 228 | 228 | 180 | 182 | 107 | 107 | 187 | 187 | ? | ? | 193 | 193 |
| S133 | 217 | 219 | 135 | 135 | 179 | 185 | 128 | 132 | 172 | 174 | 270 | 270 | 113 | 113 | 117 | 121 | 228 | 230 | 168 | 170 | 107 | 107 | 187 | 187 | 249 | 251 | 193 | 195 |
| S138 | 215 | 217 | 135 | 135 | 185 | 191 | 130 | 132 | 172 | 174 | 270 | 270 | 115 | 115 | 117 | 117 | 228 | 230 | 170 | 170 | 107 | 107 | 189 | 189 | 247 | 249 | 193 | 195 |
| S145 | 217 | 219 | 135 | 135 | 185 | 191 | 128 | 132 | 174 | 174 | 270 | 270 | 115 | 117 | 117 | 117 | 228 | 230 | 172 | 174 | 107 | 107 | 181 | 181 | 247 | 249 | 193 | 195 |
| S147 | 215 | 217 | 135 | 135 | 187 | 187 | 128 | 128 | 172 | 174 | 270 | 270 | 115 | 115 | 117 | 117 | 228 | 230 | 174 | 176 | 107 | 107 | 187 | 187 | 241 | 243 | 193 | 195 |