GENETIC CONNECTIVITY, POPULATION DYNAMICS AND HABITAT SELECTION OF THE SOUTHERN GROUND HORNBILL (*BUCORVUS LEADBEATERI*) IN THE LIMPOPO PROVINCE

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Genetic connectivity, population dynamics and habitat selection of the Southern Ground Hornbill (*Bucorvus leadbeateri*) in the Limpopo Province

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DECLARATION

I declare that the dissertation hereby handed in for the qualification *Magister Scientiae* at the University of the Free State is my own independent work and that I have not previously submitted the same work for a qualification at/in any other University/Faculty.

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13 March 2011

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ABSTRACT

Southern ground hornbills (*Bucorvus leadbeateri)* (SGH) are co-operative breeders that occur in groups of 2-9 individuals. Long life spans, large territory sizes (100km²), and low reproductive rates render these birds vulnerable to threats such as loss of habitat, persecution for their habit of breaking windows through territorial aggression, poisoning and loss of suitable nesting sites. As a result, SGH are listed as vulnerable in the red data book of South Africa as well as globally.

The main objective of this study was to contribute to our overall understanding of the ecology and biology of the SGH for conservation planning. Data collection was completed in the non-protected, semi-arid landscape of the Limpopo Valley from June 2008 - September 2009. The seasonal habitat use by a group of SGH, seasonal abundance (numbers) and biomass (volume) of invertebrates using pitfall and sweep net methods was investigated. Furthermore, a total of eight groups and 23 birds were captured in the Limpopo Valley and different statistical analysis were performed to investigate levels of inbreeding, relatedness, sex-biased dispersal and the effects the recent re-colonisation has had on the genetic structure of SGH in the Limpopo Valley. Finally the genetic variation of the species in the rest of Africa was determined using samples from Kenya, Tanzania and three populations in South Africa namely the Limpopo Valley, Kruger National Park (KNP) and Kwa Zulu-Natal (KZN).

Genetic analysis revealed SGH have retained comparatively high levels of genetic diversity, even though there are indications of genetic bottlenecks in the Limpopo, KNP and Kenyan populations. The SGH populations studied were grouped into two clusters corresponding to the geographic origin of samples. The birds from Tanzania and Kenya clustered together while the KNP and KZN birds clustered together with the Limpopo population grouping more or less equally between the Kenyan/Tanzanian and South African populations. A large percentage of genetic variation was found within populations while among population variation was low, indicating there is little molecular evidence for the presence of SGH sub-species.

The overall home range of one group was approximately 20 000 ha while seasonal home ranges varied between 5000 ha in winter to 13 500 ha in summer. The response of organisms

to environmental variables in this extremely seasonal habitat was further revealed by the positive correlations found between the number of invertebrates with mean monthly maximum and minimum temperatures, and the volume of invertebrates with mean monthly rainfall. No significant differences were found between numbers and volume of invertebrates per order, between sites, which was expected in this homogenous vegetation type dominated by mopani shrub and trees (*Colophospermum mopane*).

The re-colonisation of the Limpopo Valley was shown to have occurred by a number of unrelated individuals. This was demonstrable by very low levels of inbreeding and average relatedness of the population, as well as the favourable levels of heterozygosity across age and sex categories. Within group relatedness was high with juveniles related to at least one parent from their natal group. Insights were also gained into the breeding behaviour of SGH, providing evidence for the first time that SGH are not as monogamous as previously thought, with two instances of extra pair copulations recorded between four groups.

This study shows that a holistic approach combining genetic techniques, radio telemetry studies and ecological principles has great potential to further investigate SGH, thereby contributing to the preservation of this enigmatic species of the savannah biome.

OPSOMMING

Bromvoëls (*Bucorvus leadbeateri)* kom voor in groepe van twee tot nege en broei koöperatief. Bromvoëls se lang lewensduur, groot territoriale gebiede (100 km²) en lae voortplantingtempo stel hierdie voëls bloot aan bedreigings soos verlies aan habitat, vervolging a.g.v. hulle gewoonte om vensters te breek, en vergiftiging. Bromvoëls is dus as kwesbaar gelys in die rooi data boek van Suid Afrika, met dieselfde status in die res van Afrika.

Die hoof doel van hiedie studie was om tot die algehele begrip van die ekologie en biologie van bromvoëls by te dra ten bate van hul bewaring. Data was versamel in die nie-bewaarde, semi-droë landskap van die Limpopo Vallei in Junie 2008 – September 2009. Seisonale habitat gebruik deur ‘n groep bromvoëls, seisonale oorvloed (getalle) en biomassa (volume) van ongewerwelde diere is deur die gebruik van vanggate en sleepnet metodes ondersoek. Verder is ‘n totaal van agt familiegroepe en 23 voëls in die Limpopo Vallei gevang. Statistiese analieses is gedoen op die genetiese struktuur van die bromvoëls in die Limpopo Vallei om die vlakke van inteling, verwantskappe, geslags ge-oriënteerde verspreiding, en die effek van die onlangse her-bevolking te ondersoek. Laastens is die genetiese variasie van die spesie in Afrika bepaal deur monsters van Kenia, Tanzanië en drie populasies in SuidAfrika naamlik, Limpopo Vallei, Kruger Nasionale Park (KNP) en Kwa Zulu-Natal (KZN) te gebruik.

Genetiese analise toon dat bromvoëls relatief hoë vlakke van genetiese diversiteit behou het, alhoewel daar aanduidings is van genetiese bottelnekke in die bevolkings van die Limpopo Vallei, KNP, en Kenia. Die bromvoël bevolkings was verdeel in twee groepe wat ooreenstem met die geografiese ligging van die steekproewe. Die voëls van Tanzanië en Kenia het saam gegroepeer terwyl die KNP en KZN voëls een groep gevorm het, met die Limpopo Vallei ‘n groep tussen die ander twee groepe. Betekenisvolle genetiese diversiteit is binne populasie groeppe gevind. Die laë diversiteit wat gevind is tussen populasies dui aan dat daar nie molekulêre bewyse vir bromvoël sub-spesies is nie.

Die totale gebied van een groep was ongeveer 20 000 ha terwyl die seisoenale gebiede gewissel het tussen 5000 en 13 500 ha in die winter en somer onderskeidelik. Die reaksie van

organismes op veranderlikes in hierdie seisoenale habitat is waargeneem aan die hand van die positieve korrelasies tussen die aantal invertebrate en die gemiddelde maandelikse maksimum en minimum temperature. Hierbenewens is positiewe korrelasies tussen die volume invertebrate en die gemiddelde maandelikse reënval ook gevind. Geen betekenisvolle verskille tussen die getalle en volume van invertebrate per orde, tussen stasies is gevind nie. Die afwesigheid van betekenisvolle verskille kan verwag word in die lig van die homogene plantegroei wat oorheers word deur mopanie bome (*Colophospermum mopane*).

Die her-kolonisering van bromvoëls in die Limpopo Vallei het plaasgevind deur ‘n aantal nie-verwante individue. Dit kan gewys word deur die baie laë vlakke van inteling en die laë gemiddelde verwantskap tussen individue binne die bevolking. Heterosigositeit vlakke was hoog en eweredig versprei oor geslag- en ouderdoms groepe. Binne-groep verwantskappe was relatief hoog, jong voëls was gekoppel aan ten minste een ouer van die natale groep. Daar is tot nuwe insigte gekom oor die broeigedrag van bromvoëls. Bewyse is gevind dat bromvoëls soms ontrou is aan hul maat met twee buite-paar parings aangeteken tussen die vier groepe voëls.

Die studie wys dat ‘n holistiese benadering wat gebruik maak van molekulêre tegnieke, radio telemetrie, en ekologiese beginsels groot potensiaal het om bromvoëls verder te bestudeer en bydrae tot die bewaring van hierdie soms mistieke spesie van die savana bioom.

CHAPTER 1

INTRODUCTION

CHAPTER 1: INTRODUCTION

General introduction

Our natural heritage is increasingly under threat and mankind faces what many biologists call the 'sixth extinction' rivalling all other extinctions evident in the fossil record (Soulé, 1985). The other five mass extinctions were caused by physical events, this is the first to be caused entirely by the actions of another species – man. Currently the worldwide human population is estimated at 6 billion and this is projected to increase to 12.8 billion people by the year 2050 (Cohen, 2003). With increased population growth is an increased need for resources. Natural habitat is destroyed to make way for farmland, building sites, mines and hydro-electric power which increases human effluent contributing to global warming, acid deposition and species loss (Brussard & Erlich, 1992).

In the past 500 years an estimated one species of bird has become extinct every year and this is no doubt an underestimate (Pimm *et al*., 2006). The single most common reason for these extinctions is due to habitat loss from burgeoning human populations. It is no wonder therefore, that the science of conservation biology has been called a 'crisis discipline' (Soulé, 1985) and exists, sadly, as a consequence of man's modification and destruction of his environment. Conservation biologists are often faced with the dilemma of having to make decisions and act, often, with whatever existing data is obtainable, which is not an ideal situation when faced with the extinctions of a species. Fortunately, due to advancements in modern science over the past 20 years, the tools available to quickly and efficiently collect data relating to problems that need rapid and immediate attention has greatly increased with the availability of geographic positioning system (GPS) technology, mathematical advances and genetics, being prime examples (DeSalle & Amato, 2004). If conservation biologists are to succeed an integrated, holistic approach that includes techniques and methods from a broad range of fields is needed. As such, conservation biology can be defined as a synthetic discipline that focuses on the application of biological principles to the preservation of biodiversity; it represents a fusion of relevant ideas from ecology, genetics, biogeography, behaviour, reproductive biology, and a number of applied disciplines such as wildlife management and forestry (Brussard, 1991). It then remains the responsibility of conservation scientists to ensure research outcomes are based on providing data for the implementation of conservation measures. In other words as mentioned by Schaal & Leverich (2005), one of the

challenges for conservation biology is to relate the processes that are of interest for research scientists to the practical application of these issues by conservation managers.

The biology and ecology of the southern ground hornbill

Of the 54+ hornbill species represented by the order *Bucerotiformes* only two occur in the family *Bucorvidae* and these are collectively known as the ground hornbills (Kemp, 1995). All other hornbill species are grouped into the family *Bucerotidae* or the typical hornbills. Of the two B*ucorvidae* species, one occurs on each side of the equator in the savannahs of sub Saharan Africa (Figure 1).

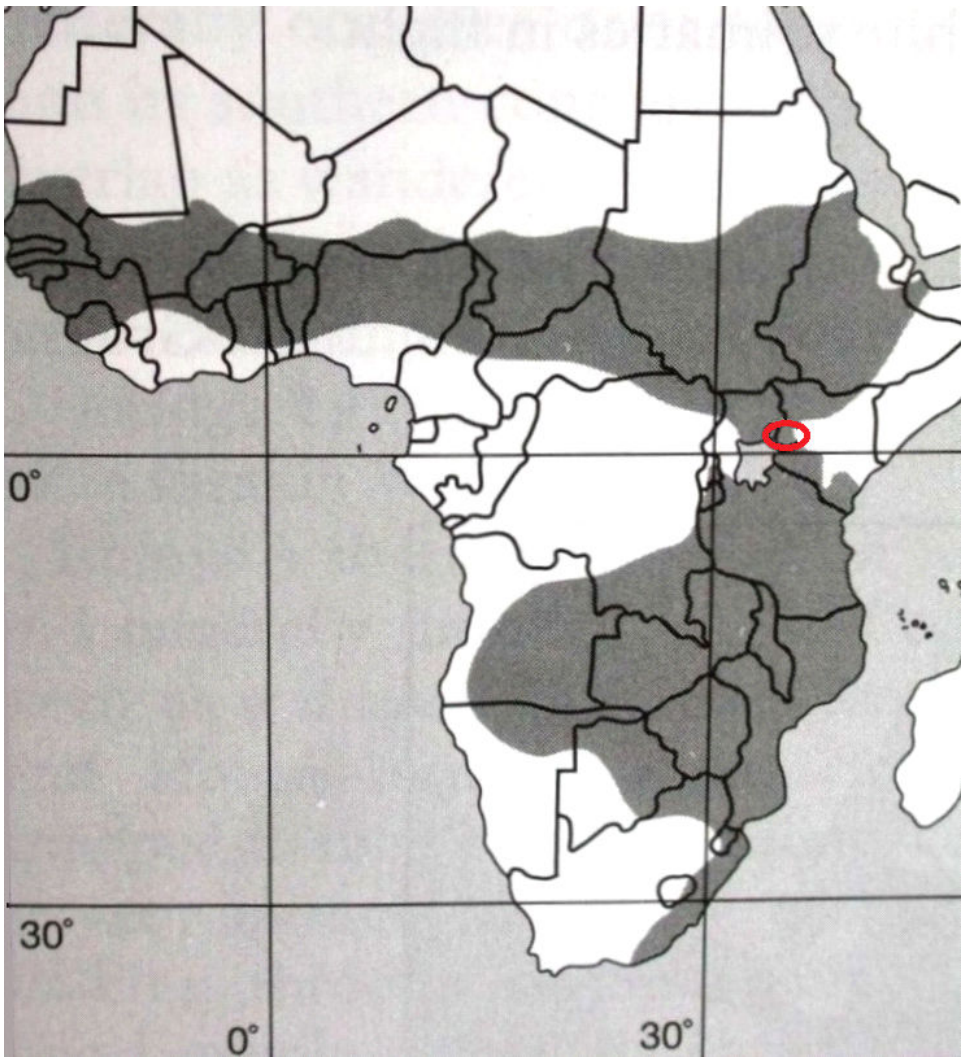

Figure 1. The distribution of SGH and NGH in Africa (adapted from Kemp, 1995). The red circle indicates the only area where SGH and NGH populations overlap with SGH distribution extending south of the circle and NGH distribution north of the circle.

The northern ground hornbill (B*ucorvus abyssinicus*) (NGH) inhabits drier steppe habitats north of the equator (Kemp, 1995) while the southern ground hornbill (*Bucorvus leadbeateri*) (SGH) occurs from the equator south, in savannah and grassland habitat, to the southern extremity of its range in the Eastern Cape Province of South Africa (Kemp, 1995). Currently, within South Africa the species extends from the Limpopo Valley of the Limpopo Province, through the lowveld regions of the Kruger National Park (KNP) and adjacent reserves (Figure 1). It is now extinct in the Mpumalanga escarpment grasslands, presumably extinct in Swaziland, and occurs at low densities in Kwa Zulu-Natal (KZN) and the Eastern Cape Provinces. Southern ground hornbills can occur throughout grassland and savannah habitat below 3000 m above sea level as long as roosting and suitable nest sites are available. Southern ground hornbill's are sedentary, occupying mutually exclusive territories containing groups of between 2-11 individuals (Kemp & Kemp, 1980) with an average group size of between 3-4 individuals recorded in the KNP (Kemp, 1988), the Kwa Zulu-Natal midlands (Knight, 1990) and the Eastern Cape (Vernon, 1984). Social bonds within the group are strong and interactions include allo-preening, allo-feeding, play, co-operative hunting and co-operative breeding (Kemp & Kemp, 1980).

Ground hornbills are obligate co-operative breeders (Du Plessis *et al*., 1995) and the largest co-operative breeding bird species in the world (Kemp, 1988). Groups consist of an alpha breeding pair, with mostly male adult helpers, occasionally adult females and juveniles of various ages. The KNP population is comprised almost exclusively of individuals forming groups, with occasional lone adult birds moving between territories (Kemp & Kemp, 1980). Juveniles are dependent on the group for food and protection for at least their first 6 months but feeding continues until juveniles are approximately two years old. Maturity is only reached at an estimated age of around 5-6 years for both males and females but breeding attempts may only occur much later on in the lifecycle of individuals (Morrison *et al*., 2005). The species has a prolonged breeding cycle of 42 days incubation and an 86 day nestling period which is restricted to the wetter summer months (Kemp & Kemp, 1991). Breeding success depends on the onset of the first rains and consequent availability of prey items, with late rains often resulting in missed breeding attempts. Only one chick is reared per season with the second chick dying after a few days from dehydration and starvation. Nest sites occur in suitably large cavities in trees and cliff faces with an internal tree diameter of at least 40cm recorded in the KNP (Kemp & Begg, 1995) and these sites can be limiting in natural environments. Ground hornbills are the largest, most carnivorous hornbill species and one of

the largest avian predators in African savannahs (Kemp & Kemp, 1980). Invertebrates form the bulk of their diet (Kemp & Kemp, 1978) but they will eat anything they can overpower including hares, squirrels, tortoises, snakes and rodents (Kemp, 1995). Southern ground hornbills group densities vary between approximately 100km² in the KNP (Kemp & Kemp, 1980), KZN midlands (Knight, 1990) and the Eastern Cape (Vernon, 1984) and 20km² in the Mana pools region of Zimbabwe (Kemp, 2005).

Conservation challenges facing the southern ground hornbill

Southern ground hornbills are long lived, monogamous co-operative breeders that are slow to reach maturity (estimated at six years of age) and have low reproductive success (Kemp & Kemp, 1980) which hampers the species' ability to adapt, recover from threats and re-establish breeding populations. SGH in South Africa once enjoyed a wider distribution in the Gauteng, North-west, Limpopo and Mpumalanga provinces (Kemp, 2000). Currently the species is only considered common in the Kruger National Park and adjacent private nature reserves, which contain an estimated half of the South African population. Outside of the KNP populations have become increasingly fragmented and isolated due to habitat loss, loss of nesting sites, secondary poisoning when they scavenge off carcasses laced with poisons meant for predators, persecution for window breaking and use in the traditional medicine trade (Kemp, 2000). Most notable is the recently emerged gap in Swaziland separating the Kruger and Kwa Zulu-Natal populations. The further fragmentation of the population in the rest of Africa and the probable consequent loss of genetic diversity; and inbreeding and outbreeding depressions, could severely impact on the species' ability to survive future stochastic events. Currently the species is listed as vulnerable in the South African Red Data Book (Kemp, 2000) but work since then has highlighted the possibility that the species in South Africa should be re-classified as endangered or possibly critically endangered as its range has declined by up to 66% in the last 115 years or three SGH generations (Kemp & Webster, 2008). A recent review of the IUCN global status has since precipitated a change in the species' status from least concern to vulnerable by BirdLife International (BirdLife International, 2010) due to an increased knowledge of the threats facing the species in Africa.

Southern ground hornbill conservation strategy in South Africa

Conservation measures over the past 15-20 years in South Africa have included: education and awareness campaigns specifically among farmers, children and the general public; harvesting and hand-rearing of second hatched chicks; re-introductions into historical

habitats; erection of artificial nests; and captive breeding programs. During February 2005 a population habitat viability assessment workshop was held (Morrison *et al*., 2005) and over 30 stakeholders met to discuss the future conservation of the species in South Africa. During this time the lack of scientific data was highlighted as well as the need to further investigate the ecology and biology of the species to focus and improve conservation decision making. Ultimately this workshop steered the integration of research and conservation by identifying gaps in our knowledge and prioritising research objectives in order to streamline SGH conservation efforts. This led to two major advancements in the field of SGH research. First, was the development of a capture technique for SGH developed by the Percy Fitzpatrick Institute of African Ornithology (University of Cape Town), in the Associated Private Nature Reserves (APNR) adjacent to the KNP. This made the fitting of transmitters, collection of morphometric data and genetic material possible. Second, was the development of 12 polymorphic micro-satellite markers (Aggarwal *et al*., 2010) making it possible, for the first time, to investigate the status and genetic diversity of the species throughout Africa, and various other questions. This is especially relevant with a species such as the SGH whose prolonged life histories and behavioural ecology make them a difficult research subject. Southern ground hornbill occur at low densities; family groups are shy avoiding humans and vehicles; habitat is often very thick and groups traverse multiple farms moving over game fences, which makes following groups with a vehicle extremely difficult. As a consequence few new studies, even with the high profile of the species, have been completed since the 1990's.

The population of SGH in the Limpopo Valley provides the opportunity to investigate the species using a complementary conservation biology approach. To conserve species and their associated habitats a sound understanding of their biology, ecology, habitat requirements and genetic structure/diversity is required.

Southern ground hornbills in the Limpopo Valley

In the Limpopo Province outside of formerly protected areas such as the KNP, SGH are restricted to the Limpopo Valley, north of the Soutpansberg Mountain range, west of Musina and east of the Platjan border post (Figure 2), although isolated birds and groups are occasionally reported by farmers elsewhere. This Limpopo Valley population has been the focus of conservation efforts by the Mabula Ground Hornbill Project over the past five years, specifically involving an extensive awareness campaign with local farmers and rural

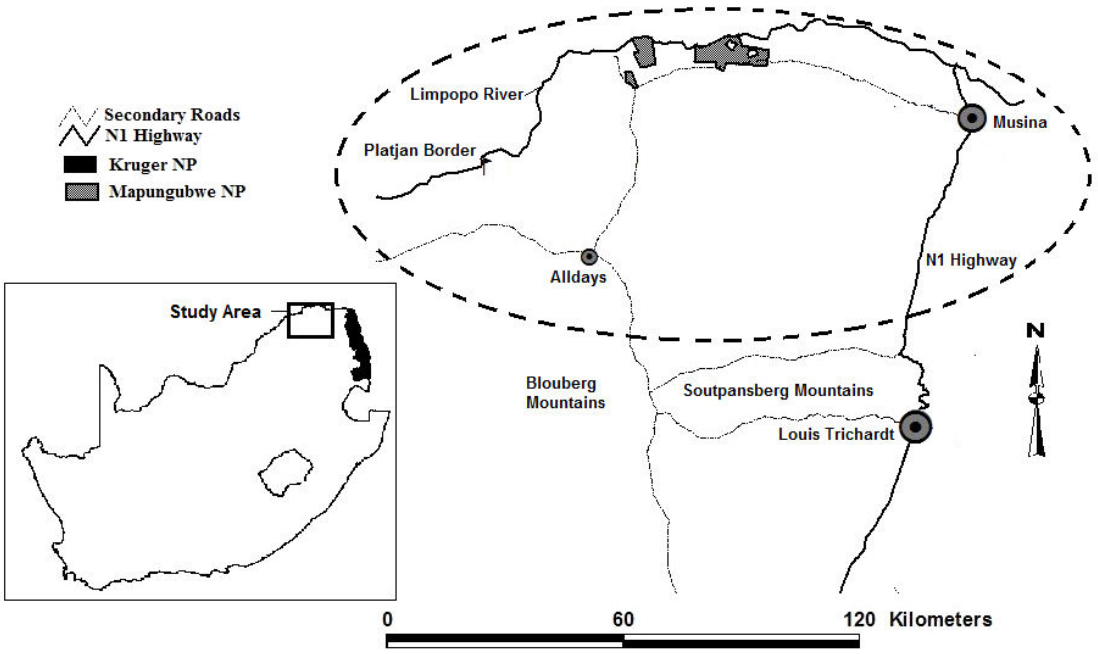

Figure 2. The location of the Limpopo Valley study area in South Africa, denoted by dotted area on map

communities, monitoring of SGH as part of a population study to determine status and distribution and the erection of artificial nests to encourage breeding.

From interviews with farmers in the area many reported the presence of the species during the 1960's to 1970's but this seemed to change approximately 20-30 years ago when ground hornbills all but disappeared from the area. Reasons for the disappearance of the species is most likely due to direct persecution for their habit of breaking windows, which SGH regularly do when hammering at their reflections as a result of territorial aggression, and secondary poisoning when SGH happen to feed on carcasses laced with poisons meant for 'problem animals'. Drought may also be a serious threat to the species in this habitat type and a possible reason for population declines. There is evidence however, that remnant groups or single birds persisted and on at least one occasion a single group close to the town of Alldays (Figure 2) has been breeding in the same nest site since the 1950's (local farmers, pers. comm). Over the past 10 years SGH have been re-colonising the area and sightings collected from 2004 to 2009 suggests that SGH groups are being established and breeding successfully based on the presence of juveniles sighted within groups (Mabula Ground Hornbill Project database, 2010). The reasons for the recovery of the SGH population is difficult to ascertain but is likely due in part to a shift from stock farming to wildlife ranching, due to decreased

profitability in cattle farming (Cousins *et al*., 2008) and increased awareness on issues such as the responsible use of poisons. Out of this work grew the need to gain a better understanding of the species in the Limpopo Valley. This is especially important because this is the only sub-population of SGH in the Limpopo Province outside of the KNP, within a non-protected conservation area and is, therefore, of high conservation priority. With almost 80% of natural habitats being privately owned in South Africa (Patterson & Khosa, 2005) the role private landowners must play in conserving biodiversity is an important one.

Southern ground hornbill genetic diversity

The theory of natural selection can be described as the differential perpetuation of genes in successive generations caused by different degrees of adaptation to the environment (Brewer, 1994). Like many species SGH have lost a large percentage of their habitat in South Africa due to the activities of man and populations are becoming increasingly fragmented as shown by the recent geographic separation of the population in the southern parts of South Africa. The only genetic study on hornbills in South Africa focused on the determination of diversity at the mitochondrial control region of six species including the SGH (Delport *et al*., 2002). Mitochondrial DNA's main conservation uses are in resolving taxonomic uncertainties and defining management units. These regions are highly variable, have high mutation rates and can be used to specifically trace female lines of descent (Frankham *et al*., 2002). In contrast microsatellite loci are known to evolve rapidly, are highly variable and are useful in population studies as they reveal much higher levels of genetic diversity per locus than allozymes (Frankham *et al*., 2002).

Southern ground hornbill habitat requirements

Habitat can basically be described as the resources and conditions that allow an organism to survive, reproduce and persist (Hall *et al*., 1997) while habitat use is the manner in which a species uses a collection of environmental components to meet life requisites and may include specific functions such as foraging, nesting or roosting (Block & Brennan, 1993). For SGHs to successfully establish territories a habitat will need to meet these specific requirements. Some of the factors that may limit populations of birds and make a habitat unsuitable are varied but may include food supply, lack of nest sites, predation, disease, pesticides and pollutants (Newton, 1980). Availability of nest sites were shown to be the principle factor limiting SGH populations in the KNP (Kemp, 1995) which is a consequence

of the large size of the species where SGH are possibly the largest cavity nesting bird species in the world and would require suitably large nesting cavities to reach their breeding needs.

Schoener (1968) found that there was a strong positive correlation between the body weight of land birds and the size of their territories and carnivorous species tended to have larger territories than herbivores or omnivores. SGH exhibit disproportionately large territories for their size (Kemp, 1988). This is possibly an adaptation due to fluctuating environments with limited, unpredictable and seasonal invertebrate prey resources distinctive of the savannah biome. Understanding how SGHs utilise their habitat may provide important insights into the factors that limit them and affect territory size where the suitability of habitat can be seen to contribute to the overall fitness and survival of an individual (Block & Brennan, 1993). Habitat suitability changes in time and space and is affected by a number of factors that are not only environmental but may also be related to the morphology and physiology of a species and intra- and inter specific competition (resulting from population densities). For instance, SGHs have long powerful bills that allow them to dig and access subterranean food resources that are often unavailable to other avian species or pry tortoises out of their shells. Intra-specific competition is also an important factor limiting SGH populations and Kemp & Kemp (1980) noted that population densities seemed to be influencing breeding with a density dependent form of population control being operative in the KNP.

Habitat use is a dynamic concept interacting with other biotic and abiotic factors that helps explain patterns and processes such as evolutionary history that contribute to the fitness of birds at the individual, community and population level (Block & Brennan, 1993). The specific habitat requirements for SGH outside of protected areas have not been investigated. It is therefore crucial that SGH habitat use within these agricultural environments be investigated in developing a national conservation action plan for the species within this savannah ecosystem. A powerful tool to aid such a study and with a species as difficult to study as SGH is radio telemetry. Although there are limitations to the use of radio telemetry such as the potential to affect behaviour due to the device being carried by the animal, intraspecific reactions, incurred energy costs, predation risk and possibly reduced foraging efficiency (Wolcott, 1995). It remains the only way to effectively follow a species such as the SGH, although recent advances mean devices are now extremely light weight and can be fitted onto the tail deck feathers of the bird.

Study area

The study was undertaken in the Limpopo Valley which is a semi-arid landscape that forms part of the savannah biome (Figure 2). The main vegetation types, as classified by Mucina & Rutherford (2006) is the Musina Mopane Bushveld consisting of undulating plains with altitudes ranging between 300 m and 800 m and Limpopo Ridge Bushveld, which occurs mostly on the hills and ridges dotted throughout the area, ranging between 300 m and 1000 m above sea level. Most of the area is dominated by mopane trees (*Colophospermum mopane*) and other broad leaved deciduous species such as baobabs (*Adansonia digitata*), lowveld cluster leaf (*Terminalia prunioides*) and various species of *Commiphora*. The Limpopo Valley is a low rainfall area with an average of 341.6 mm per annum (Jordaan *et al*., 2004) with an average maximum temperature of 29.9˚C and a minimum of 15.5˚C per year (SA Weather Bureau, 1980-2009). Rainfall is very seasonal with 79.7% of the total precipitation falling during summer (SA Weather Bureau, 1980-2009). Furthermore, rainfall is extremely sporadic (Pers. obs.) and Jordaan *et al*. (2004) in a study spanning almost 40 years, described veld conditions ranging between extremely bad (bare soil and forbs dominate), bad (annual grasses such as *Aristida spp*. and *Enneapogon cenchroides* dominate) to good (grasses such as *Aristida spp*. and *Enneapogon cenchroides* and perennial grasses such as *Eragrostis lehmanniana* dominate) depending on precipitation volume. The reasons for this severely degraded landscape can mostly be attributed to overgrazing and drought, especially the drought of the late 1950's to early 60's where the herbaceous layer was completely lost (Cunningham, 1996). As such, the main land-use types in this area consist of commercial cattle and game ranching with only 3% and 1% of Musina Mopane Bushveld and Limpopo Ridge Bushveld transformed respectively (Mucina & Rutherford, 2006). Reasons for the low levels of transformation can be attributed to the low average rainfall, shallow nutrient poor soils and to gravelly and severely eroded soils with low moisture retention (Jordaan *et al*., 2004).The area is also very rich in mineral deposits with coal and diamond mines being established in recent years. The mining potential of the area poses a great threat to the future preservation of this habitat type.

The expansion of conservation genetics

With the introduction of high-throughput DNA sequencing, PCR techniques, non-invasive sampling techniques, improved analytical software, user-friendly software, the development of new classes of genetic markers and genotyping technology, the scope and usefulness of conservation genetics over the past decade has expanded significantly (DeSalle & Amato,

2004). Genetic data has immense potential in conservation and research as explored by DeYoung & Honeycutt (2005) to assess mating systems, hybridization, gene flow, effective population size, population viability, define management units, identification of individuals, sex ratios, speciation, and to provide insights into demographic patterns associated with the reduction and expansion of populations. These data form an important component to conservation planning and strategy and have been crucial to identifying species, sub-species and units for conservation. Furthermore, the preservation of genetic diversity is one of the three key aims of the world conservation strategy developed by the IUCN. The reason that genetic diversity features so strongly in conservation strategy is that it has been shown that genetic changes in small populations result in inbreeding and reduced fitness and survival (Frankham *et al*., 2002). Even though genetics is recognised with such importance, wildlife managers and conservationists often do not incorporate genetic principles into planning strategies. This can be attributed to the barriers of terminology, access to instrumentation, laboratory skills and cost associated with genetic analysis, although these barriers are steadily eroding with the ever-increasing number of laboratories that focus on genetic analyses of wildlife (DeYoung & Honeycutt, 2005). The important role that is played by conservation genetics can easily be seen from the number of genetic studies focusing on endangered species with management implications and recommendations.

Genetic diversity and species persistence

Genetic diversity can basically be defined as the extent of genetic variation in a population, or a species, or across a group of species and can be measured in terms of heterozygosity (the average number of individuals heterozygous for a locus) or allelic diversity (Average number of alleles per locus) (Frankham *et al*., 2002). These measures provide a means to document loss of genetic diversity or adaptive evolutionary changes in a species. When individuals in a population are lost specific genes that contributed to the evolutionary persistence of those species is also lost, negatively impacting on the species ability to adapt and survive further environmental change. Conservation genetics theory suggests that most large, widespread species have high levels of genetic diversity while smaller populations, island populations and endangered species exhibit much lower levels. This loss of genetic diversity occurs due to population declines and the fragmentation of populations. The impact of population fragmentation on genetic diversity depends on the resulting population structure and gene-flow between population fragments, which, in turn, is affected by factors such as the dispersal ability of a species, number of population fragments, distance between fragments and time

since fragmentation (Frankham *et al.,* 2002*)*. Some forms of population fragmentation are shown in Figure 3.

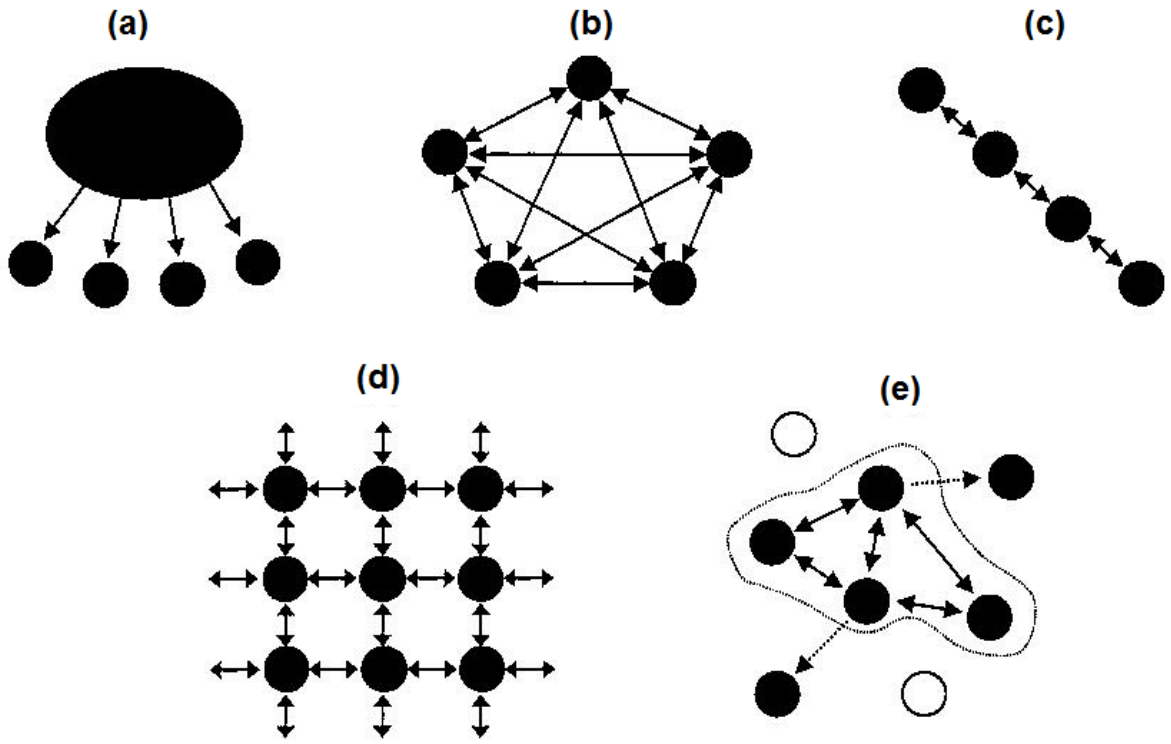

Figure 3. Five different fragmented population structures: (a) a mainland-island situation where the mainland is the source of genetic material (b) an island model where migration is equal between fragments (c) a linear stepping stone model where only neighbouring populations exchange migrants (d) a two dimensional stepping stone model where neighbouring populations exchange migrants and (e) a meta-population model (adapted from Frankham *et al*., 2002).

When gene flow between populations is high, gene flow has the effect of homogenising genetic variation over the populations. Conversely when gene flow is low genetic drift and selection may lead to genetic differentiation. In many cases this is a natural process that has been played out throughout the history of the earth resulting in the array of species present today and is the basis for Darwin's theory of evolution through natural selection. In most species therefore populations are often divided into smaller units because of geographic, ecological or behavioural factors resulting in genetic sub-structure which is the differences in genetic variation among a population's constituent parts (Hedrick, 2000).

In theory animals with high dispersal ability would ensure genetic mixing and panmixia between sub-populations where birds are the obvious candidates for such a scenario due to their abilities of long distance flight. In reality though, birds are in some cases constrained by factors such as social structure, behaviour and specific habitat requirements which limits their dispersal ability such as in the case of the red grouse (*Lagopus lagopus scoticus*). This species shows varying levels of population structure and although it is distributed throughout northern and western Britain it is restricted to moorland areas, which provides its main food source (Piertney *et al*., 1998). Another example where behavioural differences have led to differences in genetic structure to the point that subspecies are recognized, is shown in the sandhill crane (*Grus canadensis*). Five subspecies are currently accepted of which two are migratory, breeding in northern America/Canada and Siberia respectively while three subspecies are non-migratory and resident in Cuba, Florida and Mississippi (Rhymer *et al*., 2001). Understanding population sub-structures of a species is doubly important where habitat fragmentation is caused by human activities. In many cases birds do not have the ability to move between fragments, or behaviour limits this and in these cases it is up to conservationists and wildlife managers to identify these populations and determine the levels of genetic diversity and gene-flow between population fragments. Greater-prairie chickens (*Tympanuchus cupido pinnatus)* for example were once widely distributed across the tall grassprairies of the American Midwest but due to habitat transformation populations of this bird are highly fragmented and small, which has led to a loss of genetic variation and reproductive success (Johnson *et* al., 2003).

Genetic techniques are also essential because they provide estimates of gene flow between populations and thus guide efforts to maintain historical levels of genetic exchange between populations (Crandell *et al*., 2000) through concepts such as the evolutionary significant units (ESU) and minimum viable population size. ESU's can be defined as a population of organisms that is reproductively isolated from other populations of the same species, and represents an important component in the evolutionary legacy of the species, while minimum viable population size is an estimate of the smallest number of individuals in a population that is capable of maintaining that population without significant manipulation (DeSalle & Amato, 2004).

A short overview of the savannah biome in South Africa

The savannah biome is the most widely distributed biome in South Africa comprising almost 47% of the total surface area and can be described as vegetation with an herbaceous layer and an upper layer of woody plants (Rutherford and Westfall, 2004). This biome is characterised by seasonal rainfall and a pronounced dry winter (Tainton, 1999). The savannah biome is second only to the fynbos biome in plant species richness with over 5788 species recorded, but unlike the fynbos biome savannah's are also rich in mammal, reptile, bird, fish and amphibian species (Venter *et al*., 2003). The savannahs of Africa are among the bird richest habitats in the world and have a great morphological diversity of birds because of their variable habitat (Maclean, 1990). Typical to the savannah biome is a number of bird taxa and African savannahs support a relatively high terrestrial biomass of birds. It is notable that a carnivorous diet may be the only one that can support large birds in African savannah's with no frugivores over 500g represented (Kemp & Kemp, 1980). In terms of biomass African savannahs are said to support between 2.2 and eight times more insectivorous than granivorous-frugivorous birds (Tarboton, 1980). As such a number of mostly carnivorous terrestrially-feeding bird species are represented including a heron (*Ardea melanocephala*), stork (*Leptoptilos crumeniferus*), crane (*Terapteryx paradise*), a number of large *Otis* bustards and two unique species namely the secretary bird (*Sagittarius serpentarius*) and ground hornbill (*Bucorvus leadbeateri and Bucorvus abyssinicus*) (Kemp & Kemp, 1978). With such a high diversity of species the conservation of this biome is important. Currently, 8.5% of the biome is formerly conserved (Rutherford & Westfall, 2004) and although this is considered a good percentage it highlights the importance private landowner's play in maintaining the ecological integrity of vast tracts of this biome. Most of the savannah biome is used for grazing purposes while urbanisation is mostly in the form of small towns. Parts of the biome are becoming increasingly used for game ranching with the tourism potential linked to wildlife appreciation (Rutherford & Westfall, 2004). These factors provide an important economic incentive for the future conservation of savannah, and its associated biodiversity, by private landowners.

Objectives and scope of the study

Broadly, the objective of this study was twofold: firstly, to contribute to our overall understanding of the ecology and biology of the SGH, and secondly, to benefit the conservation of this species in the Limpopo Valley as well as South Africa. This was achieved by focusing on important aspects of the habitat requirements, genetic diversity and

population dynamics of SGH in the non-protected, agricultural, semi-arid landscape of the Limpopo Valley; an area with a history of SGH population declines. An outline of each chapter and the research focus are described below:

Dissertation outline

This dissertation is presented as a number of chapters focussed on specific yet interrelated aspects of the biology and ecology of SGH.

Chapter 2: An investigation into the ecological requirements and associated habitat utilisation of a group of Southern Ground Hornbill in the Limpopo Valley

The specific habitat requirements of SGH and what constitutes ideal SGH habitat is poorly understood. Understanding the continuity of suitable or unsuitable habitat has implications for the continued re-colonisation (gene-flow) and persistence of groups within this area; and would form an important component of a conservation strategy to extend the current range of the species in non-protected areas of the Limpopo Province. Furthermore, the Limpopo Valley is a semi-arid region and probably does not constitute ideal SGH habitat. I therefore hypothesise that territory sizes will be larger than those previously observed and is a response to scarce, unpredictable food resources. Other factors that will be considered during this part of the study are the availability of nesting sites, SGH densities and the movement of the group in response to various landscape features.

Chapter 3: A preliminary analysis of the genetic structure of the Southern Ground Hornbill in Africa

The aim of this chapter was to investigate the genetic diversity and genetic structure of SGH throughout their range in Africa. Microsatellite loci will be used to assess the genetic diversity between Kenyan and South African populations, which represent the northern and southern most extent of the species range. Variations within the South African population will also be analysed from samples collected in the Kruger National Park, the Limpopo Valley and Kwa Zulu-Natal. Understanding genetic structure will play a crucial role in conservation planning and implementation in that possible sub-species and genetic isolation due to fragmentation can be identified within the species range.

Chapter 4: A fine scale investigation into aspects of the biology of the Southern Ground Hornbill in the Limpopo Valley, with the aid of microsatellite markers

The gene-flow, relatedness, parentage, sex ratios, age structure, productivity and genetic diversity within the Limpopo Valley population will be investigated. Analysis of microsatellite data presents an opportunity to gain insights into the nature of the re-colonisation of the Limpopo Valley and to document a 'snapshot' in time of the genetic structure of this population and make inferences on the dispersal behaviour of the species. The potential of genetic tools as a means to further investigate difficult aspects of SGH biology, ecology and population dynamics will also be explored.

Chapter 5: Conclusion and Management Recommendations

Finally, the outcomes of the above research will be discussed with recommendations and a way forward for future SGH conservation efforts in the Limpopo Valley and South Africa.

CHAPTER 2

AN INVESTIGATION INTO THE ECOLOGICAL REQUIREMENTS AND ASSOCIATED HABITAT UTILISATION OF A GROUP OF SOUTHERN GROUND HORNBILL IN THE LIMPOPO VALLEY

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Introduction

Understanding the ecological requirements of a species is important to implement conservation strategies. Southern ground hornbills are a territorial species with groups remaining in these fixed territories throughout the year (Kemp & Kemp, 1980). A territory can be defined as a fixed space from which an individual or group of mutually tolerant individuals of the same species actively excludes competitors from a specific resource or resources (Maher & Lott, 2000). Kemp (1988) states that while nesting sites may be the primary resources in SGH territories food resources may be secondary as boundaries extend well beyond their core, which is the nesting site. Other factors do not seem to influence the distribution and spacing of territories. By analysing data from ground and aerial counts of SGH spanning 20 and nine years respectively, Kemp *et al*. (1989) found no relationship between the frequency with which groups were encountered and group size with the distribution of rainfall, landscape, geology, geomorphology, drainage lines, soil types, climate, vegetation types or vegetation structures. However, data was accumulated through general movement locality fixes by observers and not radio telemetry plots. Furthermore, the summer rainfall season is the proximate factor affecting the seasonal availability of food for hornbill species (Kemp, 1976).

Studies on the diet of SGH have shown the importance of invertebrate prey abundance (Kemp & Kemp, 1978; Knight, 1990) particularly Orthoptera species. These studies were mainly concentrated during the wetter months (August – March) of the year by direct observation. These studies also did not investigate the foraging behaviour of groups during winter when food resources would be limiting and have the most pronounced affect on the species ability to maintain their energy requirements and secure territories. Quantitative seasonal observations on the general availability of prey resources, that include invertebrate abundance, are still poorly understood. Studies in the Kruger National Park suggest that SGH adapt their behaviour and react to periods of lower food resources by concentrating in areas around waterholes that have higher densities of ungulates (Kemp *et al*., 1989) and observations indicate SGH dig more during the drier months of the year when surface prey is

less abundant, often in and around piles of elephant dung (Kemp, 1995) and in rhino middens.

This study aims to investigate: (1) the seasonal availability of invertebrates and the effects these have on the habitat utilisation of a specific group of SGH and (2) the factors that may be limiting and influencing territory size in the Limpopo Valley. This is the first attempt to study the SGH outside of formally protected areas, particularly in rangeland agriculture systems within a semi-arid savannah habitat with a history of rainfall variability, drought and land degradation. A better understanding of the ability of SGH to adapt to seasonal environmental changes and secure food resources would be critical to their long term survival in this non-protected area and further provide important ecological information on what constitutes ideal SGH habitat.

Materials and Methods

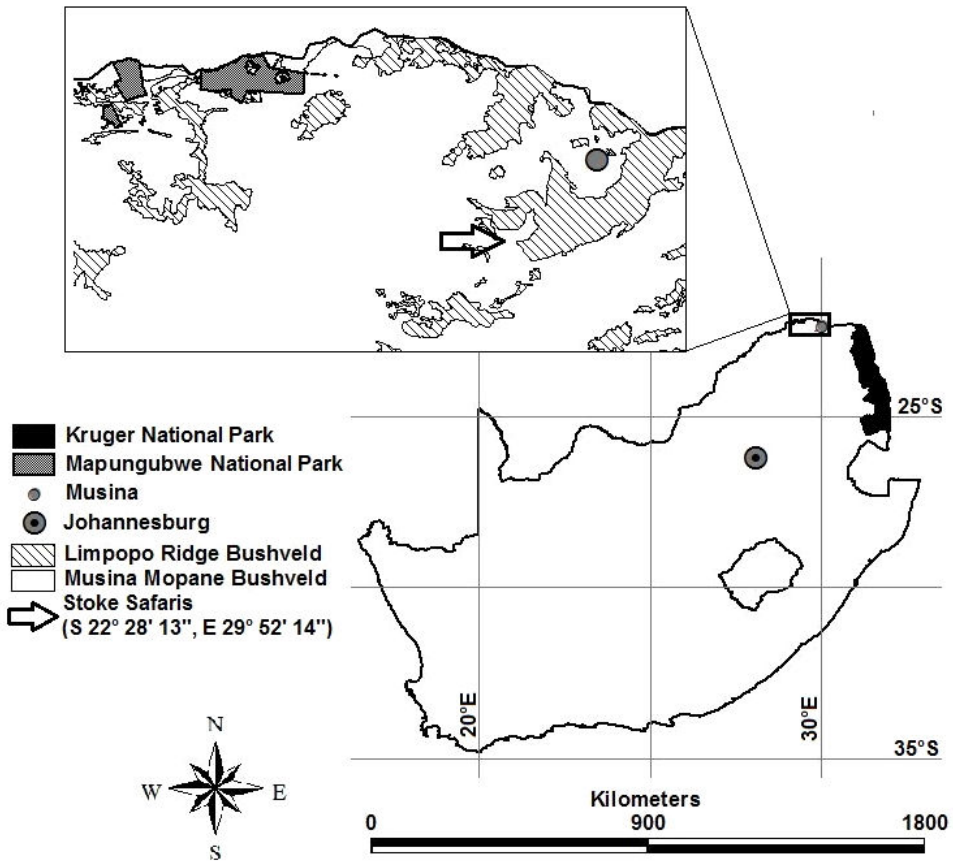

Figure 1. Map of South Africa with the location of the study site on the farm Stoke Safaris in the Limpopo Valley. Vegetation data by the South African Biodiversity Institute (SANBI) (Mucina *et al*., 2005).

Vegetation and invertebrate survey sites

Four study sites within the group's territory were randomly chosen on the farm Stokes Safaris (S 22° 28' 13", E 29° 52' 14") to collect vertebrate and vegetation data (Figure 1). The farm has a diverse history of management and land-use practises which is a common trend throughout the Limpopo Valley. Cattle were historically the dominant herbivores farmed with and now the farm has a mixed ranching approach, with cattle and game species present on parts of the farm. Site one fell in a breeding camp for nyala (*Tragelaphus angasii*) and sable (*Hippotragus niger*), but in previous seasons had stocked cattle. Sites two and three were in areas of veld that were historically grazed by cattle and were now stocked with indigenous game species. Umbrella thorn trees (*Acacia tortillis*) and various forb species dominate the fourth site, historically a small agricultural field abandoned more than 15 years ago. Sites 1-3 were very similar structurally, dominated by mopane scrub and trees (*Colophospermum mopane*) and are representative of the Limpopo Valley veld type which occurs throughout the group's territory.

Vegetation sampling

A visual estimate of cover to describe temporal changes in vegetation was conducted monthly at ten geo-referenced points per site the same time invertebrates were sampled. Canopy cover of grass species formed the focus of this estimate by making use of a 1m² metal quadrant according to the following categories: <10%; 10-25%; 25-50%; 50-75% and 75-100% each month during invertebrate sampling. Canopy cover of grass species formed the focus of this estimate and this was considered an adequate reflection as opposed to basal cover due to the low leaf production of pioneer species that dominate the area. Each category was allocated a score with one the lowest and five the highest grass-cover to compile a monthly score ranging between 10 and 50. In this way the seasonal change in vegetation per site could be monitored and modelled against other variables. Each replicate was allocated an overall condition according to categories described by Jordaan *et al*. (2004). Fixed point photographs were taken every month from the centre of each site in both a northerly and southerly direction.

Invertebrate sampling

Pitfall traps and sweep netting methods were employed monthly from October 2008 to September 2009 to determine seasonal availability of invertebrates. Pitfall traps and sweep netting methods were chosen because they would target groups of invertebrate fauna mostly encountered by a ground hornbill's mode of foraging behaviour. Pitfalls sample ground living

while sweep nets sample invertebrates mostly found on surface vegetation (Standen, 2000). No prior studies on the seasonal prevalence of invertebrate fauna in the Limpopo Valley have been undertaken. At each site 13 pitfall traps were set out using the nested cross array method (Perner & Schueler, 2004). A cross shaped trap arrangement with distances between traps doubling with increased distance from the central trap. The first traps were set out 5 m from the centre then 10 and 20 m. This method is particularly useful for larger arthropods (Perner & Schueler, 2004). Drainpipes with a 10 cm diameter were cut into 15 cm sections and buried flush with the soil surface. During each sampling period plastic containers with a 10 cm diameter were dropped into the drainpipes and filled with 3 cm of propylene glycol. Propylene glycol is safer than other chemicals which may be toxic to animals when captured invertebrates are fed upon. Pitfalls were left out for a sampling period of four days each month. Pitfalls disturbed by animals were not included in the analysis. Invertebrates were washed and stored in polytop vials with a 75% concentrated ethanol solution. Sweep netting was performed monthly by walking the same geo-referenced transect of 200 m in length at each of the four sites, within the same week pitfalls were sampled. A 45 cm diameter net was used to take sweep net samples where 200 sweeps were performed per line transect (one sweep for each step taken) before 10 am each morning. Invertebrates were transferred to a plastic bag and left in a freezer overnight before being transferred to polytop vials with a 75% concentrated ethanol preservative. All invertebrates were identified up to the level of order, counted and measured volumetrically using the volumetric water displacement method and rounded off to the nearest relative number on the volumetric flask. Invertebrates were divided into two size classes namely small (<1 ml) and large (>1 ml) to compare the monthly numbers and volume of large and small invertebrates across all four sites. Invertebrates were donated to the Agricultural Research Council's invertebrate collection.

Radio telemetry observations

A group of SGH consisting of an alpha breeding pair and three immatures were captured on the farm Lucern (See chapter 4 for capture method). A Holohil® tail transmitter was fitted onto the main tail deck-feather of the alpha female from the group and released. Transmitters attached by harness with Teflon ribbon were not considered due to the real danger of the birds becoming entangled. Harnesses can shift on the bird becoming loose, when first fitted, allowing the bird to get its long bill caught under the harness which immobilises it (Pers. obs.; Mabula Ground Hornbill Project database, 2010). After a 30-day settling period the group was tracked using a handheld yagi antennae and an AOR® receiver. Locality data was

collected every 3-4 hours, seven days a month from August 2008 to September 2009. GPS readings were recorded using a Garmin® GPS that included roost sites after sunset where possible. The ability of the group to traverse farm boundaries made following groups difficult as this could not be done on foot due to their shy nature and low visibility in thick vegetation. When the group moved to adjacent farms researchers had to drive to get access to this farm due to large game fences and locality fixes were therefore reduced on occasion due to time constraints emanating from access hindrance.

Data analysis

Ranges VII software (South *et al*., 2008) was used to analyse home range data. Two methods, the harmonic mean and kernel home range analysis were used to estimate seasonal home range size. Harmonic mean home range estimates are highly sensitive to outlying observations and thus forces the inclusion of many grid points. As such, the outcome of the home range size is an overestimate of true size whereas kernel estimators are well defined and tractable (Seaman & Powell, 1996). For comparison purposes both methods will be represented. GPS co-ordinates were loaded into ARCVIEW GIS 3.2 (Environmental Systems Research Institute, Inc) and overlayed over 1:10 000 high resolution orthophotos and spatial layers representing rivers, roads and vegetation types to further note if any associations exists with group movements and structural habitat features.

Statistical data analysis was undertaken using the software program STATISTICA (Statsoft, 2009). Kruskal-Wallis one way analyses of variance (ANOVA) was used to test for any variations between sites with regards to invertebrate prevalence and vegetation cover. A Kruskal-Wallis one way ANOVA was preferred as it is a non-parametric method and it does not make the assumption that standard deviations do not differ between groups or that samples were taken from a normally distributed population. Correlations were performed against invertebrate data and relevant meteorological data (rainfall as well as maximum and minimum temperatures) where $P < 0.05$ (95%) denotes significance. A *t*-test was further employed to test whether there was any significant difference between invertebrate sampling methods.

To more accurately represent the relationship between rainfall, vegetation and invertebrate abundance it was necessary to subjectively group rainfall into months by combining rainfall data from the last two weeks of a month with the beginning two weeks of the following

month. For example the daily rainfall values from the last two weeks of December and the first two weeks of January gave a total of 272.6 mm which was then compared to the fieldwork collected the beginning of January where a high abundance of invertebrates and the best veld condition were recorded. By contrast if the usual monthly rainfall approach is applied then December recorded 195.9 mm and is compared to the December fieldwork which was collected before these critical rains occurred resulting in a very low invertebrate abundance and a poor veld condition score being recorded, which fails to reveal the reaction of the environment to rainfall. Temperature was grouped the usual way as per calendar month. It was not deemed necessary to group temperature in the same way as rainfall because monthly temperature data are based on daily measurements and are therefore more evenly distributed across the months.

PC-Ord software (McCune & Mefford, 1999) was used to complete a Bray-Curtis cluster analysis in order to reveal the level of relationships between sites with regards to invertebrate orders expressed as a two-dimensional dendrogram. A *t*-test was further employed to test whether the observed large bias between the means of the sweep net and pitfall data were significant.

Results

Vegetation analysis

Monthly estimates of cover were very similar across all four sites and the average overall monthly score for all four sites was very low at 17.7 representing an average cover during the study of between 10-25% (Table 1). Categorisation of veld condition according to criteria determined by Jordaan *et al*. (2004) described each site as extremely bad throughout the study period. Graphical representation of grass cover and rainfall reveals the close relationship between precipitation and vegetation with vegetation responding to rainfall after a slight lag period (Figure 2). Vegetation growth was only stimulated following the December/January rains (272.6 mm) with the highest grass cover scores peaking during February and already dropping during March which were the only two months with an estimated cover of between 25-50%. The total annual rainfall during the study was above average recorded at 477.7 mm of which 460 mm (94.6%) fell during spring and summer (Figure 3). The seasonal changes in vegetation growth associated with rainfall are visually presented in Figure 4.

Table 1. Vegetation score estimates per month per site with averages and grouped into monthly categories of overall veld condition as described by Jordaan *et al*. (2004).

Month (Oct 2008 – Sept 2009)	Site 1	Site 2	Site 3	Site 4	Average monthly score	Estimated grass cover (%)	Veld condition (Jordaan *et al*. 2004)
Oct	10	10	10	10	10	>10%	Extremely bad
Nov	10	12	10	10	10.5	10-25%	Extremely bad
Dec	10	12	10	10	10.5	10-25%	Extremely bad
Jan	20	17	17	11	16.25	10-25%	Extremely bad
Feb	23	23	24	28	24.5	25-50%	Extremely bad
Mar	21	14	22	24	20.25	25-50%	Extremely bad
Apr	16	14	22	18	17.5	10-25%	Extremely bad
May	15	18	22	16	17.75	10-25%	Extremely bad
Jun	12	14	18	13	14.25	10-25%	Extremely Bad
Jul	12	11	16	14	13.25	10-25%	Extremely bad
Aug	10	10	16	10	11.5	10-25%	Extremely bad
Sep	10	10	12	10	10.5	10-25%	Extremely bad
Total average scores	16.9 (10-25%)	16.5 (10-25%)	19.9 (10-25%)	17.4 (10-25%)	**17.7 (10-25%)**	10-25%	

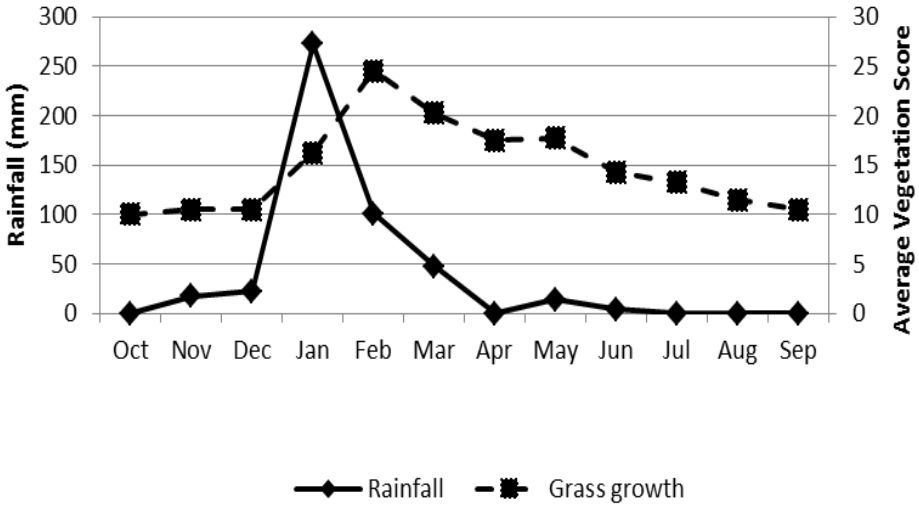

Figure 2. Seasonal comparison of rainfall (grouped from the 16th until the 15th of the following month) and grass cover scores.

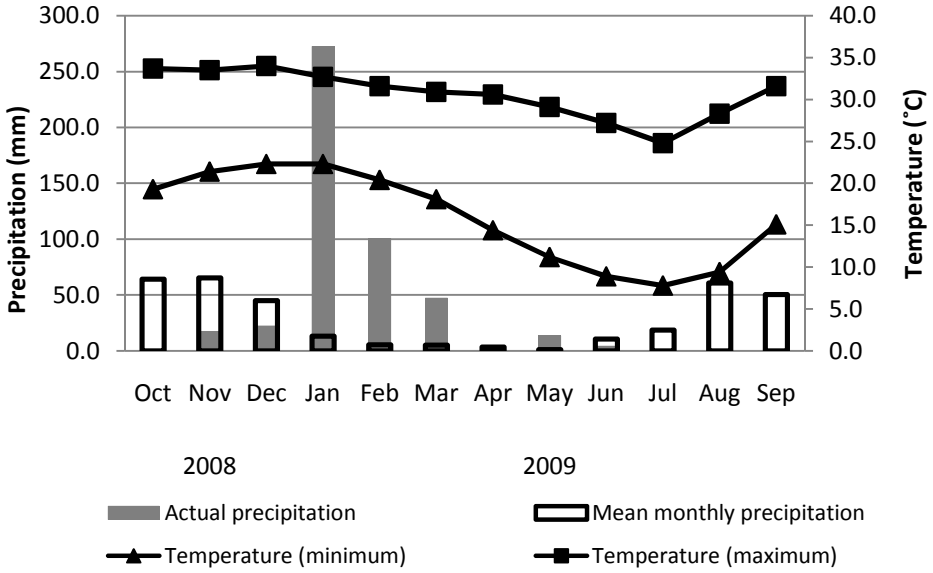

Figure 3. Mean monthly precipitation (grouped from the 16th until the 15th of the following month) at Musina, Macuville Weather Station during the period 1979-2007 (SA Weather Bureau, 1980-2009) including actual precipitation with maximum and minimum temperature means during the study (October 2008 – September 2009).

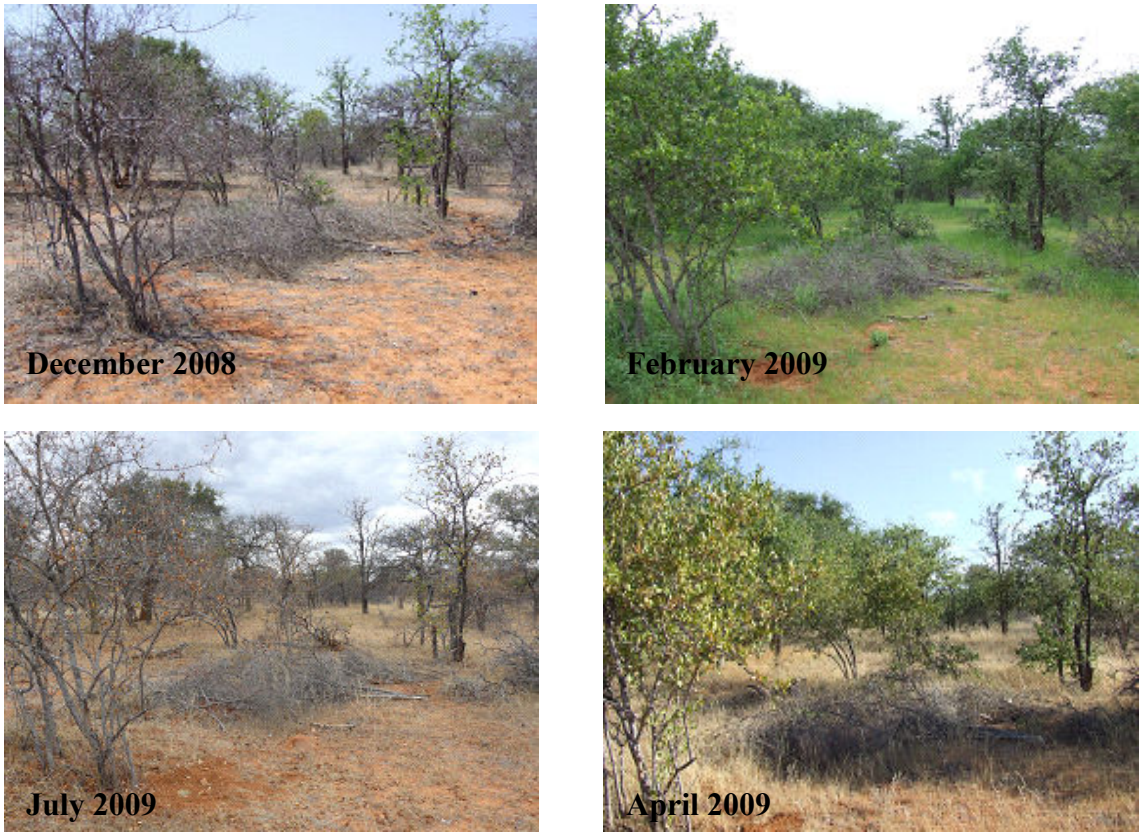

Figure 4. Examples of monthly changes in veld condition. Photos taken in a northerly direction from the centre of site 1. (Clockwise from top left to bottom left): December 2008 grass cover score 10 (<10%); February 2009 grass cover score 23 (25-50%); April 2009, grass cover score 16 (10-25%); July 2009, grass cover score 12 (10-25%).

Invertebrate analysis

The two sampling methods differed significantly with the pitfall traps being the most successful (t = 6.25, df = 21, P < 0.001). Sweep netting yielded a total of only 1193 individuals while pitfalls provided a total of 18272 individuals (Table 2).

Table 2. Total numbers and volume of invertebrates captured from October 2008 – September 2009 using two sampling methods

	Sweep nets		**Pitfalls**	
Month	**Invertebrate numbers**	**Invertebrate volume (ml)**	**Invertebrate numbers**	**Invertebrate volume (ml)**
October	0 (0)	0.00 (0)	2554 (13.98)	44.66 (2.56)
November	81 (6.79)	1.38 (0.82)	1174 (6.43)	250.53 (14.37)
December	0 (0)	0.00 (0)	1833 (10.03)	44.31 (2.54)
January	194 (16.26)	21.99 (13.01)	2078 (11.37)	383.45 (21.99)
February	177 (14.84)	16.32 (9.66)	2794 (15.29)	256.97 (14.73)
March	211 (17.69)	28.96 (17.14)	2037 (11.15)	339.98 (19.49)
April	207 (17.35)	36.55 (21.63)	1041 (5.70)	126.42 (7.25)
May	64 (5.36)	2.69 (1.59)	1028 (5.63)	142.77 (8.19)
June	99 (8.3)	28.29 (16.74)	773 (4.23)	50.16 (2.88)
July	46 (3.86)	12.95 (7.66)	462 (2.53)	26.40 (1.51)
August	66 (5.53)	18.15 (10.74)	846 (4.63)	27.99 (1.6)
September	48 (4.02)	1.34 (0.79)	1652 (9.04)	50.66 (2.9)
TOTAL	**1193 (100)**	**169 (100)**	**18272 (100)**	**1744 (100)**

The results of the one – tailed t-test revealed highly significant differences between the two sampling methods (t = 6.25, df = 21, p < 0.001) and indicate that sweep netting as a method of sampling invertebrates in this habitat type is not suitable. As such, sweep nets were not included in the Kruskall-Wallis analysis as this data was not considered representative of the sample sites. The low results of the sweep nets was also reflected in the correlation statistics as no correlations were identified between sweep net data and meteorological variables. Positive correlations where found between the number of invertebrates with both mean monthly maximum temperatures (r^2 = 0.531, P < 0.05) and minimum temperatures (r^2 = 0.612, P < 0.05) and the volume of invertebrates with mean monthly rainfall (r^2 = 0.563, P < 0.05) (Table 3).

Table 3. Results of invertebrate numbers and volume statistically tested for correlations with various important meteorological variables. Bold indicates significance ($p<0.05$)

Correlations	p value	r^2 value	r(x, y) value
rainfall vs pitfall no	0.137	0.207	0.455
rainfall vs pitfall vol	**0.005**	0.563	0.75
rainfall vs sweep no	0.077	0.28	0.529
rainfall vs sweep vol	0.495	0.048	0.219
temp max vs pitfall no	**0.007**	0.531	0.728
temp max vs pitfall vol	0.232	0.139	0.373
temp max vs sweep no	0.999	0	-0.000001
temp max vs sweep vol	0.232	0.139	-0.373
temp min vs pitfall no	**0.003**	0.612	0.782
temp min vs pitfall vol	**0.047**	0.339	0.582
temp min vs sweep no	0.586	0.03	0.175
temp min vs sweep vol	0.421	0.066	-0.256

Kruskall-Wallis ANOVA's for pitfall sites showed no significant difference between sites as measured by the numbers of invertebrates per order ($p = 0.9102$) and the volume of invertebrates per orders ($p = 09441$). Results of the Bray-Curtis cluster analysis are represented as a dendrogram in Figure 5. The Bray-Curtis cluster analyses grouped similar sites together according to invertebrate orders which showed slight differences in the results between the pitfall and sweep net sampling methods, highlighting the differences in the assemblages of ground dwelling arthropods and orders associated with surface vegetation.

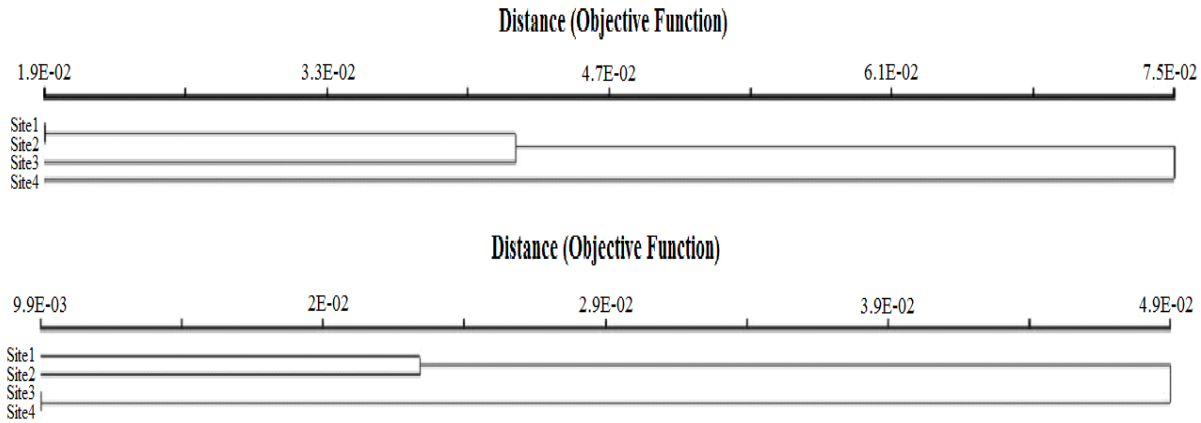

Figure 5. Dendrogram based on Bray-Curtis similarity matrix of invertebrate orders between each site for sweep nets (top) and pitfalls (bottom).

Small invertebrates dominated all sample sites (97% and above) (Figure 6). Large invertebrates only made up a fraction of the total invertebrates sampled per month (0 – 2.7%) but they represent an important proportion of the volume in certain months, most noticeably November (77.8%) and January to April (27.5 to 30.9% of the total volume). Large invertebrates were not recorded during October 2008, December 2008 and July 2009. The influence rainfall plays in this eco-system and the sudden rainfall-induced response of invertebrates was demonstrated when the first rains fell at the study site in November causing the emergence of a species of monster tiger beetle (Coleoptera: *Manticora sp*.). The importance of this species is further depicted in November (Figure 6) where they accounted for 77.8% of the invertebrates collected for that month and may very well account for the correlation between rainfall and the prevalence of invertebrates.

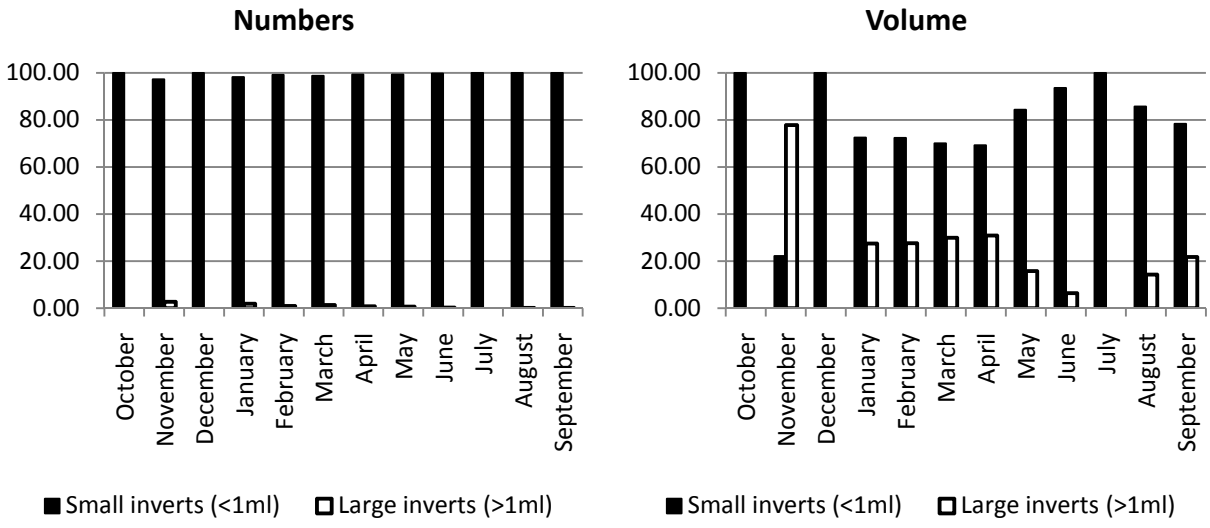

Figure 6. Percentage of large (>1ml) and small (<1ml) individual invertebrates that make up the total numbers and volume per month captured with pitfall traps.

Even though the sweep nets were shown to be an unsuitable sampling method for the area important differences between the order compositions of the two sampling methods were apparent (Figure 7). Coleopterans dominate pitfall sampling and make up 62% of the numbers and 76% of the total volume. Orthopterans were the most important order sampled with sweep nets for both numbers and volume representing 62% and 76% of the totals respectively. Orthoptera were also the second most important order in terms of pitfall volume with 6% of the total volume. Other notable differences in the most dominant orders collected for the two sampling methods were Hemiptera which represent a significant

proportion of the invertebrates captured by sweep net but not the pitfall traps for both numbers and volume. The converse was found for the order of Hymenoptera, strongly represented in the pitfall but not the sweep net sample set.

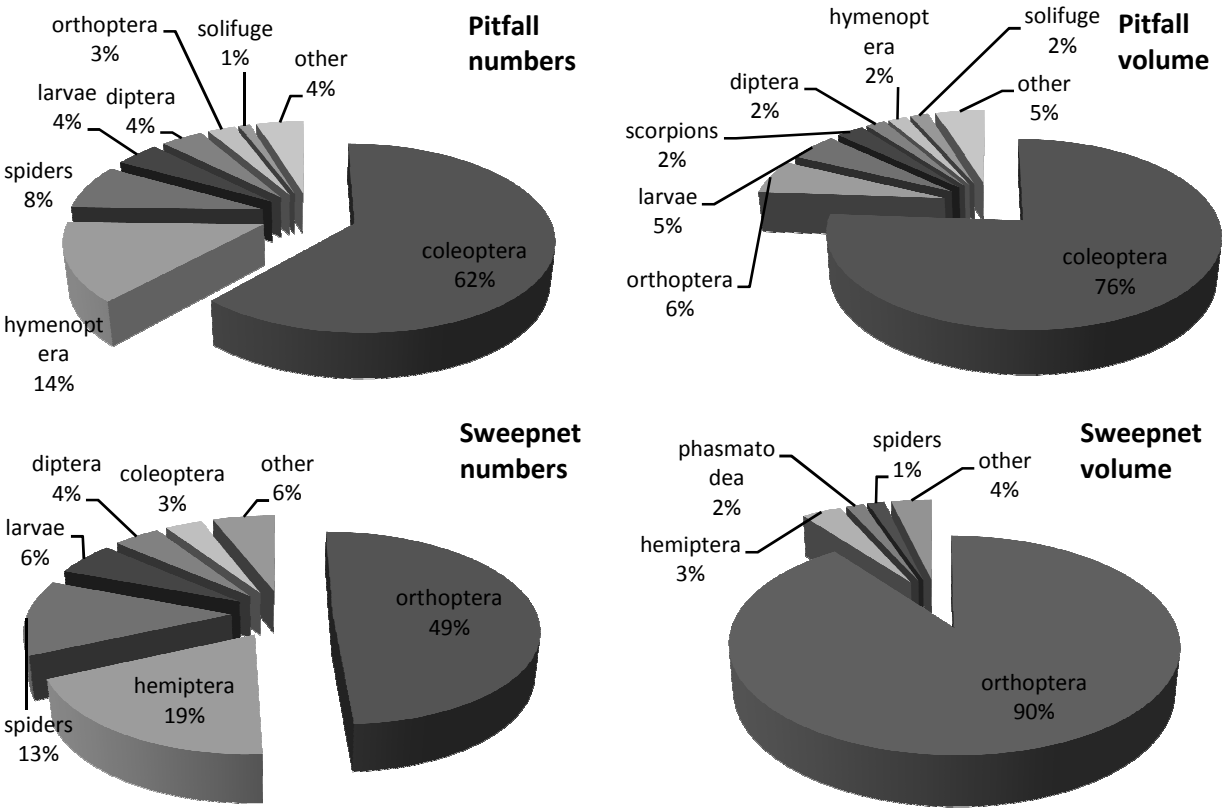

Figure 7. Numbers and volume of invertebrate orders captured by pitfall and sweep net methods.

Home range size and habitat usage

A total of 201 fixes were recorded (Figure 8). This revealed a territory size for the group of between 19 372 and 22 731 ha by the kernel and harmonic means respectively. The results of the kernel home range indicated a winter (March to August) and summer (September to February) size of 5280 ha and 13409 ha respectively (Figure 9). Overlaying orthophotos (e.g. vegetation types, roads, large trees, ridges, rocky outcrops, river courses or seasonal streams) with home range fixes indicated no specific habitat features were associated with the groups movements. It was clear that the group utilises specific areas that change seasonally (Figure 8). Although the group would generally avoid areas with human activity, no structure seemed to restrict movement of the group in any way (Figure 8).

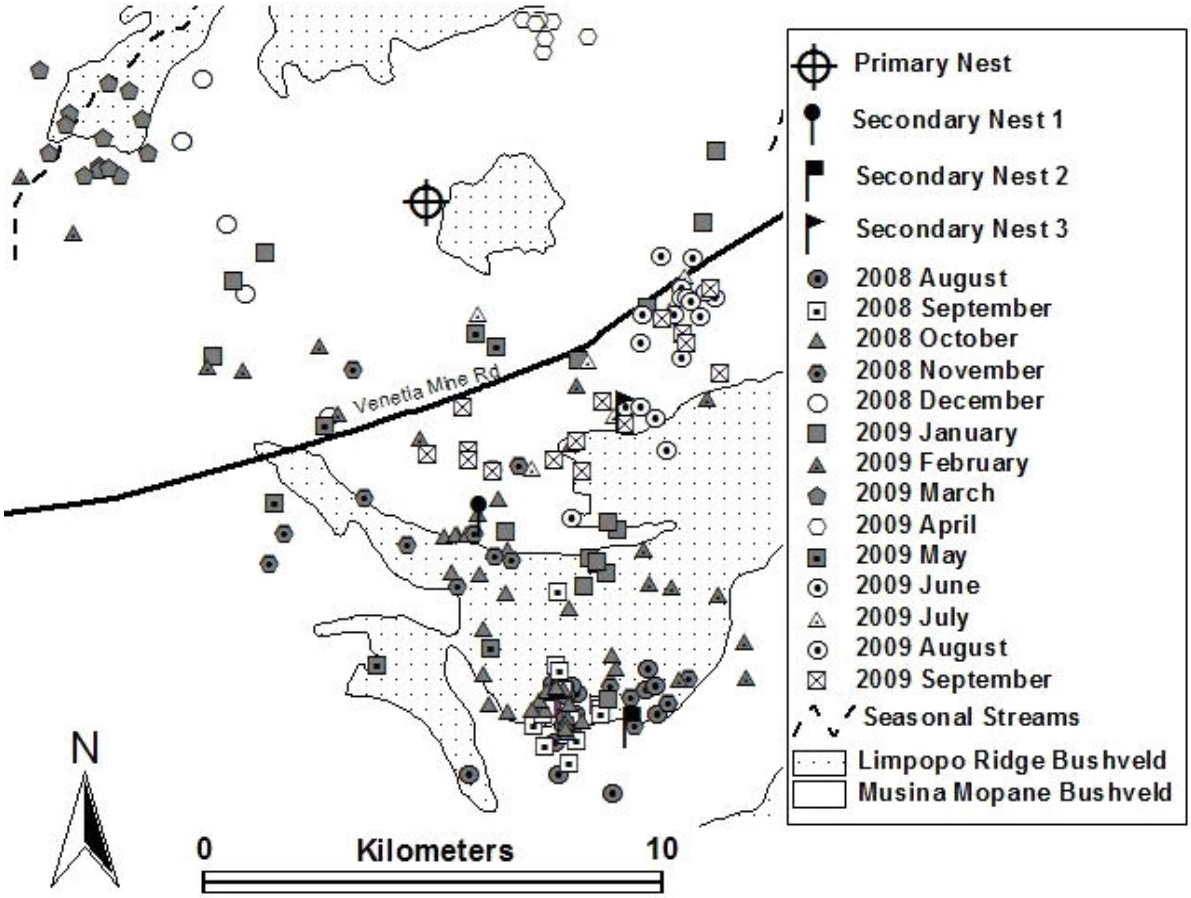

Figure 8. Seasonal movements of the group with all fixes included (n=201) as well as primary and secondary nests and various environmental and landscape features.

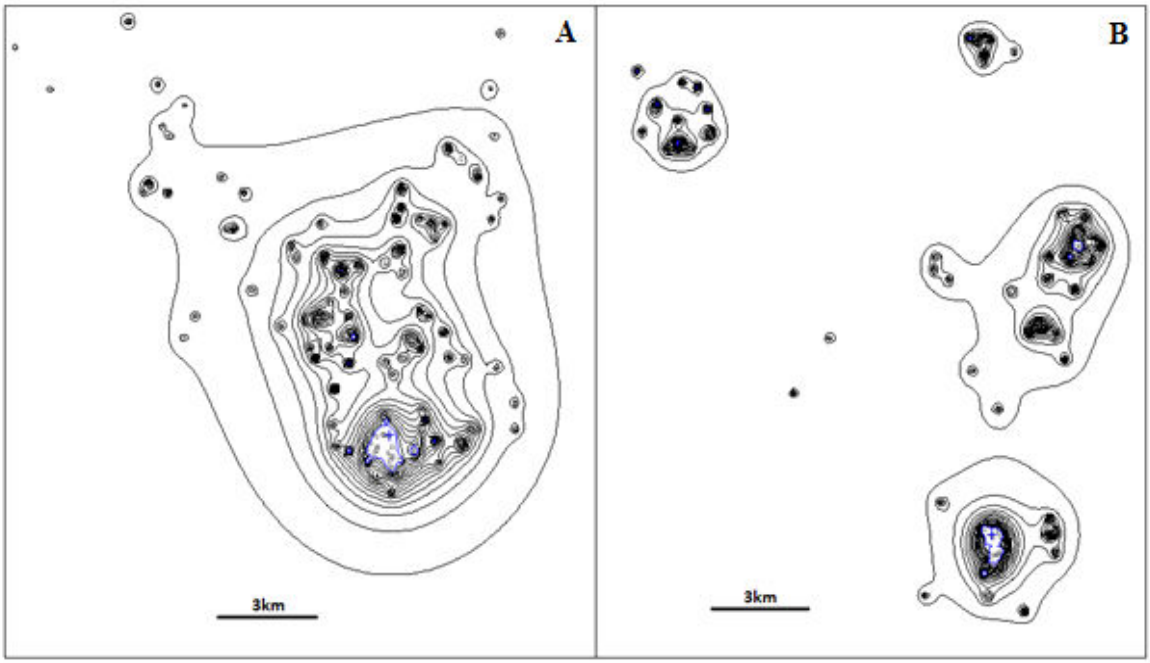

Figure 9. Seasonal summer (A) and winter (B) harmonic mean home ranges of the group during the study period.

Other layers used to help interpret the habitat utilisation of the group were rivers and seasonal streams. Small highly seasonal streams occur throughout the area but trees large enough to contain suitable cavities are rare along their banks (Pers. obs.). Large baobabs (*Adansonia digitata*) on the other hand are not restricted to the banks of rivers and are large enough to map using orthophotos. Scrutiny of the orthophotos revealed that roosting sites were not lacking and, at least for this group, neither were nest trees. Three baobab trees that contained suitably large cavities for nesting were identified with the female sitting in each hollow for a full morning on at least one occasion per tree. All the nests were in *Adansonia digitata*. The primary nesting tree used historically by the female for nesting was not visited by the group nor was the surrounding habitat. Although farmers did report the group in the area it did not seem to be favoured for foraging by the group. The group subsequently bred in this nest during the 2009/2010 season. Other factors investigated included the influence of human structures such as buildings, roads and fences on the group and although they avoid areas with human activity they would remain nearby (sometimes less than 100 m) for extended periods of time with humans mostly unaware of their close proximity. Highways also did not pose a problem with the group easily traversing highways, sometimes on a regular basis.

Discussion

Analysis of GIS data and orthophotos revealed no obvious patterns with regards to movements of SGH and landscape features as found in the KNP (Kemp *et al*., 1989) while the availability of nests were not a limiting factor for this group in contrast to areas of the Kruger National Park where they are the main factor limiting SGH. There were no obvious variations in tree density between areas where SGH concentrated and areas where they did not and this observed homogenous nature of the vegetation was supported by the Kruskall-Wallis ANOVA's which showed no statistically significant variations between the numbers and volume of invertebrate orders. Rivers-Moore & Samways (1996) found that multivariate site classification using arthropods gave a much finer classification than those using grasses and with the lack of grass cover in the Limpopo Valley may provide an alternative means to investigate habitat variations.

This study demonstrated the positive correlations between insect activity and emergence with temperature and rainfall. However, large sporadic outbreaks of certain invertebrate species such as mopani worms (*Imbrasia belina*), were not adequately recorded due to the unpredictable temporal and spatial nature of these events. It is these large invertebrates (Such

as the emergence of monster tyrant beetles) that seem to be the reason for the significant correlations between invertebrate volume and rainfall. The most important invertebrate in the area is undoubtedly the mopane worm, (Lepidoptera: Saturniidae) which is characterised by unpredictable large scale population outbreaks in the drier parts of its range that are dominated by the mopane tree (Oberprieler, 1986). Mopane worms form the prey of at least 20 insect, four reptile, 34 bird and 10 mammal species (Gaston *et al*., 1997). The behavioural response of the SGH group to rainfall was recorded during December when a thundershower occurred on the far side of the territory and the group immediately moved into this area the following day. These movements were likely in anticipation of the emergence of invertebrates triggered by rainfall. In March the group spent the entire week in the same region where the thundershower occurred during December. The rainfall in this area had precipitated the only large scale emergence of mopani worms in the group's territory. During March the mopani larvae were in the 4^{th} and 5^{th} instar stage, which occurs just before they bury themselves in the ground and pupate. Gaston *et al*. (1997) found SGH feeding on mopani worms during the 5^{th} instar stage in a study that indicated a strong positive correlation between the body weights of avian predators feeding on mopani worms and mopani worm instar weights. Although direct observations could not be made it seems likely that the group was in the area utilising this important food resource before the onset of the dry season.

During winter many species of holo-metabolic insects pupate and survive underground and for an animal that can find them represents a potentially rich food source. Southern ground hornbills have the adaptive ability of utilizing food resources that other avian species might not have access to by digging, turning objects over or breaking objects up. In a previous winter a group of SGH was monitored by a farmer for approximately a two month period during which time they were seen almost daily digging up mopani worm pupae in an area where a large outbreak of the worms occurred. This adaptable behaviour is possibly the key to the species survival in this harsh unpredictable habitat and although extra time and effort would be spent digging the energy gains would be great. It is interesting to note that species such as secretary birds (*Sagittarius serpentarius*) and kori bustards (*Ardeotis kori),* which have similar feeding and habitat requirements to SGH, are uncommon. In the case of the secretary bird only one was seen in five years while kori bustards are restricted to certain areas and migrate locally, utilising areas that had good rain and grass cover or staying in close proximity to irrigated lands (Pers. obs.). Southern ground hornbills on the other hand were not once recorded in irrigated lands and seemed to prefer areas of natural bush. Besides

rainfall invertebrate availability may also be influenced by high densities of ungulates where large deposits of dung are present. In the Kruger National Park, Kemp & Kemp (1980) noted that during winter SGH concentrated in areas surrounding waterholes where large densities of ungulates were present possibly due to the dung deposits attracting invertebrates. Similarly from August to November 2008 the group mostly concentrated in the sable/nyala breeding camp and a cattle camp on the farm Stokes Safaris. On the few occasions where observations were possible the group was observed turning over and digging through dung piles or digging under bushes.

The territory size of this group in the Limpopo Valley are almost double the 100 km² density of groups observed in the Kruger National Park (Kemp & Kemp, 1980) and Natal (Knight, 1990) and almost 10 times the 25 km² densities reported in the Mana pools in Zimbabwe (Kemp, 2005). The home range of the group during summer was recorded as 13 409 ha or approximately 134 km² which is similar to other group densities recorded in South Africa. The winter home range is about 60% smaller than the summer home range but includes areas not visited during summer. Winter foraging was concentrated in very specific areas, which were often up to 20 km apart on opposite ends of the territory. During the last few months, of winter before the first rains in both 2008 and 2009 when the lowest invertebrate abundance was recorded and grass cover was at the lowest, the movement of the group was especially restricted with daily movements of sometimes less than 500 m recorded. This behaviour seems to be a strategy whereby the group is focusing on sites that are the most productive and consequently saving more energy. It can therefore be expected that the large territory size of this group of SGH is necessary during the winter months when food resources are scarce. At this time behaviour switches from defence and provisioning of females and young in the nest, to locating suitable patches for foraging and maintaining energy requirements. This study, therefore lends support to the hypotheses that food availability is the primary factor determining territory size and an important factor determining habitat quality.

At first glance the apparent contradiction of a larger summer home range can be explained due to the fact that the group did not breed during the study and foraging was therefore not restricted to the habitat surrounding the nest. This still fails to explain why the winter foraging area represents only a fraction of the total territory and why winter foraging areas are almost 20 km apart. A number of reasons may be proposed to explain this apparent inconsistency. A possible explanation lies in the sampling design. The group was only

followed one week every month and it is likely that certain areas were utilised by the group that were not recorded. This is more likely in summer where the group's movements were less restricted. For instance, the group was not recorded in the vicinity of the nest although farmers did report the birds moving through this area. During the last months of the 2008 winter, farmers reported regularly observing the birds, which seems to indicate the continued presence of the group in this area all through the driest months of the year as reflected by this study. A more likely explanation for the comparatively small winter territory size may be a behavioural adaptation and response to the extremely unpredictable productivity of the environment regarding invertebrates. With the observed sensitive interplay between rainfall, vegetation growth and invertebrate abundance a large territory would in effect act as an insurance buffer during years when small localised showers occur. The larger the territory the greater the chance a rainfall event will happen in the territory as occurred with the outbreak of mopani worm larvae in the north of the territory. These localised outbreaks of invertebrates would not only be available in summer but also in winter to any species able to access them.

Another aspect that may be influencing the extremely large territory size and needs to be considered is the low density of SGHs in the Limpopo Valley. Although only one group was radio-tracked, extensive work in the area over the past 5 years indicates that other groups have similar territory sizes (Chapter 4) often with large gaps between them. The possibility of maintaining such an enormous territory are in all likelihood possible only due to minimal competition present in the area in part influenced by the low number of groups and the apparent high density of nests due to the abundance of baobab trees. Interactions between other groups were only noted during a week in April 2009 on the northern boundary of the territory. Neither group ventured too close to each other but spent extended periods calling during the mornings. No interactions with a southern neighbour were noted and the area to the south seems devoid of other groups after extensive groundwork. Similarly no reactions were noted with an established eastern group or a reported western neighbour although the exact status of this group is not known. It remains to be seen what influence the arrival of other SGHs and the establishment of other groups in the area will have on the overall territory size of groups.

An extremely worrying factor in the Limpopo Valley is the highly degraded veld condition. In an investigation of the proximate factors influencing veld degradation in the north eastern

parts of South Africa, Wessels *et al*. (2007) found that the grazing-induced degradation caused a substantial reduction in long-term vegetation productivity, despite a strong short-term influence of inter-annual variation in rainfall. The low productivity, poor grass cover and dominance of annual grass species, when present, have also been noted by Jordaan *et al*. (2004) and were further highlighted during this study. Annual grasses are characterised by having low leaf production because of the need to invest energy in rapid growth and the production of multiple seeds that are easily dispersed and long lived (Brown, 1984). This provides better chances of establishment in an environment such as the Limpopo Valley where precipitation is sporadic, localised and confined to a small time period. The lowered productivity and in some areas the almost complete removal of the ground vegetation layer has far reaching consequences for all biota and the poor results from sweep netting exercises are immediately apparent. Sweep nets and pitfalls target different invertebrate fauna with different habitat requirements (Standen, 2000) and the unsuccessful results of the sweep netting method can be attributed to a lack of surface vegetation and associated invertebrates. Although the study did not focus on other vertebrate groups that would constitute an important part of SGHs diet the lack of invertebrates can be expected to filter into the other levels of the food chain. Pringle *et al.* (2007) documented the strong cascading effects of ungulates on an ecosystem which negatively affected cover and tree density resulting in the regulation of lizard and coleopteran abundance with low productive systems identified as being most at threat.

Compounded by the above, the Limpopo Valley is highly susceptible to climatic phenomenon such as El Niño which results in drier spells and many models predict lower rainfall for the area due to global warming (Meadows & Hoffman, 2003). Lowered rainfall or more sporadic rainfall could have a catastrophic effect on the area that will affect farmers and biodiversity alike. Reduced rainfall would affect SGH with regards to important sources of invertebrate food and ironically if farmers are forced to decrease animal stocking rates this could further impact on the invertebrate prey of SGHs that utilise or are associated with dung piles although lower stocking rates have a positive effect in improving veld condition via lower grazing pressure. It is highly likely that the extreme drought experienced during the 1950s - 1960's would have impacted on the availability of invertebrates and therefore species that rely on invertebrates as a food source. It is interesting to note that this drought corresponds more or less to the period when many farmers noted the disappearance of SGHs from the area. Drought conditions exasperated by threats such as poisoning and persecution

for window breaking, is the most likely cause for the almost complete extirpation of the species from the Limpopo Valley. It is possible that a period of drought where little or no rain occurs may again result in the sudden collapse of this SGH population. Due to the sensitive nature of this veld type and the lowered productivity the protection of a relatively large area needs to be secured to support a viable population of SGH. Habitat loss due to mining operations and associated activities therefore constitute an additional threat and could have far reaching negative impacts in one of the last wilderness areas left in South Africa.

CHAPTER 3

A PRELIMINARY ANALYSIS OF THE GENETIC STRUCTURE OF THE SOUTHERN GROUND HORNBILL IN AFRICA

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Introduction

Understanding the extent and pattern of genetic differentiation and structure has become an essential part of conservation biology and is an important tool to provide baseline data guiding conservation decisions. As noted by Prior *et al*. (1997) data on the genetic structure of populations are of particular interest because they may reveal evidence of restricted gene flow that is undetectable through traditional demographic studies. Populations of SGH have become increasingly fragmented due to habitat loss and other factors such as poisoning and loss of large nesting trees. The most notable is a geographic divide that now exists extending from the south of the Kruger National Park through Swaziland, and northern Kwa Zulu-Natal (Kemp, 2000). Furthermore the dispersal behaviour of the species is not fully understood and the ability of individuals to move between isolated fragments is not known. If gene flow between population fragments does not occur or is restricted it may lead to serious deleterious effects such as inbreeding and loss of genetic diversity which would have negative consequences on the species evolutionary potential and ability to adapt and survive in an ever changing environment. Hedrick and Miller (2002) state that one of the major long-term goals of conservation genetics is the retention of enough genetic variation so that future adaptation, successful expansion, or re-establishment in natural populations is possible. This is the first attempt to investigate the genetic structure of the SGH in Africa.

The specific aims were to: (1) provide preliminary results regarding the extent of genetic differentiation and gene flow among SGH populations in Africa; (2) to compare levels of genetic diversity within separate populations; (3) record the current genetic diversity of the species in light of the possible future effects of reduced connectivity and inbreeding as a consequence of habitat loss and population declines; and (4) make recommendations regarding future research and conservation of the species, especially in South Africa. Understanding the genetic structure and genetic diversity is especially pertinent in South Africa where the most drastic declines have been recorded in the species' range and where conservation measures are currently being implemented. This study is the first to report preliminary findings on the genetic status of SGH in Africa based on 12 newly developed microsatellite markers. Microsatellite markers were the method of choice and show high

levels of polymorphism and many alleles per locus, which is a biologically useful measure of genetic diversity and structure (Hedrick, 2000).

Material and methods

Sample collection

A total of 79 SGH samples were included in the study constituting 12 from Kenya (KEN), six from Tanzania (TAN) and 61 from South Africa (Figure 1). The South African samples can further be divided up as follows: 35 from the Kruger National Park (KNP); 23 from the Limpopo Valley (LIM); and three from the province of Kwa Zulu-Natal (KZN) (Figure 1).

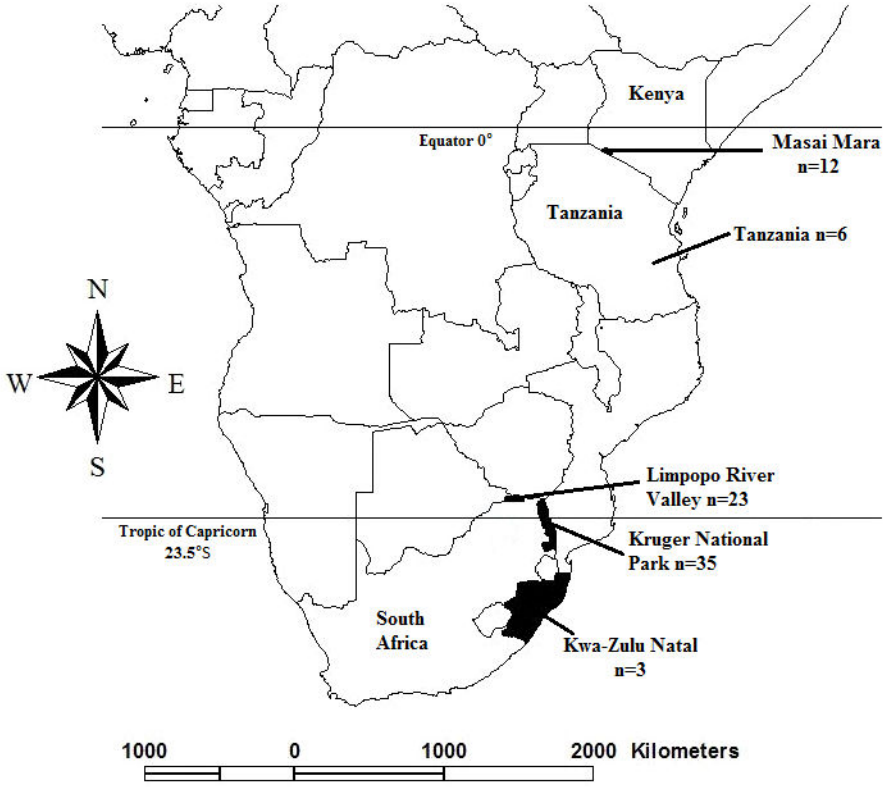


Figure 1. Samples were collected from five locations in three countries including the Masai Mara in Kenya; Tanzania; the Limpopo Valley, Kruger National Park, and Kwa Zulu-Natal in South Africa.

Because of the difficulty of capturing wild SGHs the project relied heavily on blood samples donated from zoos and private collections in South Africa, which were collected over a three year period. Only captive birds with known origins were included in the analysis namely six Tanzanian, three from KZN, and 18 KNP birds. Seventeen of the samples were donated by the Percy FitzPatrick Institute (University of Cape Town) and were collected from nestlings

sampled in artificial nests adjacent to the Kruger National Park. One northern ground hornbill (*Bucorvus abyssinicus*) (NGH) sample was included as reference. Sampling kits were distributed to all parties concerned and included EDTA and blood buffer storage vials as well as FTA paper. Sub-samples of all individuals were sent for biomaterial banking at the National Zoological Gardens of South Africa. The Kenyan birds are all from a population in the Masai Mara Nature Reserve and were captured over 10 days in October/November 2009 in collaboration with Kenyan Wildlife Services. These Kenya birds were targeted as they represent the most northern extent of the species range in Africa. The Limpopo Valley population was sampled from July 2008-July 2009 and the 23 samples represent almost 60% of the known population from this area (Chapter 4). The capture method is further explained in chapter 4.

DNA extraction

All DNA extraction and genotyping of SGH samples were undertaken at the laboratories at the National Zoological Gardens of South Africa. DNA extraction was conducted using the Qiagen DNeasy® Blood and Tissue Kit.

Microsatellite analysis

Microsatellite markers were used to analyse all genetic samples collected. DNA microsatellites are widely distributed in the genome of eukaryotic organisms. Their high variability and selective neutrality make them an extremely useful tool for population and evolutionary studies (Neef & Gross, 2001). Genotypes were assessed using 12 polymorphic microsatellite markers that were isolated in the SGH (Aggarwal *et al*., 2010). Amplification was carried out using 15 µl reaction volumes and PCR was conducted with Promega go *Taq* DNA polymerase, and the associated buffer containing 10 milli molar (mM) Tris®-HCl (pH 9.0), 50 mM potassium chloride (KCl) and 0.1% Triton® X-100. The final reaction conditions were as follows: 1 X PCR buffer, 1.5 - 2.5 mM $MgCl_2$, 200 (M) of each 2'-deoxynucleotide triphosphate (dNTP), 10 (pmol) of each of the forward and reverse primer, 1 unit (U) *Taq* DNA polymerase and 50 (ng) genomic DNA template. The conditions for PCR amplification were as follows; 15 (min) at 95°C denaturation, 30 cycles for 30 (sec) at 95°C, 30 sec at 50-65°C and 30 sec at 72°C, followed by extension at 72°C for 20 min. Non-overlapping PCR products were pooled for running, with Genescan™ 500 LIZ™ as internal size standard. An ABI 3130Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA) was used to separate

fragments, and samples were genotyped using GeneMapper v. 4.0 software (Applied Biosystems, Inc., Foster City, CA).

Statistical analysis

MS Toolkit (Park, 2001) was used to quantify the following for all populations: genetic diversity expressed as observed heterozygosity (Ho) and Nei's unbiased heterozygosity [(Hz) (Nei, 1987)]; mean numbers of alleles per locus; and to identify unique alleles. Input files for all other genetic programs were also prepared in MS Toolkit. BOTTLNECK (Cornuet & Luikart, 1996) was used to test for recent population size reductions in the KNP, Limpopo and Kenya populations, based on the principle of mutation-drift equilibrium which states that there is an approximately equal probability that a locus shows a gene diversity excess or a gene diversity deficit (Cornuet & Luikart, 1996). Arlequin (Excoffier *et al*., 2005) was used to calculate pairwise *Fst* values (Wright, 1965); within and among populations, AMOVA's (Michalakis & Excoffier, 1996); and to test for significant variations from Hardy Weinberg Equilibrium ($P < 0.05$). Fst values are a useful measure of drift and differentiation among populations and provide an indication of gene flow, while AMOVA's provide a hierarchical analysis of genetic differentiation within and among populations. A model based clustering method using individual multilocus genotype data was used to estimate population structure and assign individuals to inferred populations (Pritchard *et al*., 2000). STRUCTURE uses a Bayesian approach useful for identifying populations and assigning individuals in situations where there is little structure information (Pritchard *et al*., 2000). Samples were subjectively assigned to six populations (K=6) according to the geographical origin of samples. The program was then set to make ten independent runs for each K value with a burnin period of 100000 with Markov Chain Monte Carlo (MCMC) iterations set to run at 1000000 repetitions after burnin period. Pr(*X/K*) values were estimated for K and the best model with admixture of allele frequencies was chosen. Genetic distance (Dn) among populations was determined using Nei's standard genetic distance (Nei, 1972) as implemented in DISPAN (Ota, 1993) to gain an indication of the differentiation among populations especially due to the varying geographic gaps between sample populations. This method can be applied to any population without regard to the number of alleles at a locus or to the pattern of evolutionary forces such as mutation, selection, and migration (Nei, 1973). In order to visualise these general relationships neighbour joining trees with standard genetic distances were generated with 1000 bootstrap replications.

Results

A possible sampling bias was noted during the study due to the high level of juveniles included in the sample set, for both the captive bred and wild birds, except the Tanzanian birds, where all were adults. For this reason it was decided to complete all statistical analysis twice, once including the juveniles and once excluding them except for the assignment tests to infer population structure. Juveniles from the Limpopo Valley represent half of the population and are therefore an important part of the genetic diversity in this area, where there are a small number of groups each with an alpha breeding pair and between two and three juveniles. Southern ground hornbills are unusual among birds as offspring remain as juveniles for up to four years and are easily identified as such. Where noteworthy differences were obtained both sets of results are reported in the text, otherwise, only results including juveniles are reported, to ensure ease of reading and to prevent the results becoming too cumbersome. Figures and tables representing results both including and excluding juveniles are represented throughout.

The average observed and unbiased heterozygosities and average alleles per locus are represented in Table 1 & 2. No significant differences were noted when juveniles were excluded or not. All five populations exhibited favourable levels of genetic diversity with similar average observed (0.5694–0.6941) and unbiased (0.500–0.7140) heterozygosities. The KNP showed the highest average number of alleles per locus with 6.25 with juveniles and 6.17 without. Limpopo and Kenya were very similar, with an average of 5.17 and 5.25 respectively with juveniles and 5.00 and 4.83 respectively without juveniles.

Table 1. Average number of alleles, observed and unbiased heterozygosities for five populations of SGH including juveniles.

Population	**Unbiased heterozygosity**	**Observed heterozygosity**	**Average no. of alleles per locus**
KNP (n=35)	0.6697 (±0.0520)	0.6597 (±0.0240)	6.25 (±3.47)
LIM (n=23)	0.6623 (±0.0461)	0.6560 (±0.0295)	5.17 (±1.90)
KZN (n=3)	0.5000 (±0.0918)	0.5694 (±0.0862)	2.17 (±0.83)
TA (n=6)	0.6832 (±0.0416)	0.6292 (±0.0640)	3.58 (±1.38)
KE (n=12)	0.7140 (±0.0301)	0.6941 (±0.0398)	5.25 (± 1.48)

Table 2. Average number of alleles, observed and expected heterozygosities for three populations of SGH excluding juveniles.

Population	Unbiased heterozygosity	Observed heterozygosity	Average no. of alleles per locus
KNP (n=28)	0.6751 (±0.0535)	0.6494 (±0.0270)	6.17 (±3.38)
KZN (n=3)	0.5694 (±0.1055)	0.5833 (±0.1028)	2.08 (±0.79)
LIM (n=11)	0.6884 (±0.0492)	0.6763 (±0.0418)	5.00 (±1.91)
TAN (n=6)	0.6832 (±0.0416)	0.6292 (±0.0640)	3.58 (±1.38)
KEN (n=10)	0.7101 (±0.0370)	0.7083 (±0.0426)	4.83 (±1.53)

Table 3. Number of alleles (A), single locus observed (ho) and unbiased (hez) heterozygosities at 12 microsatellite loci for five populations of southern ground hornbills including juveniles. Significant deviations from Hardy Weinberg equilibrium are represented by bold underlined numbers.

	Kruger NP (n=35)			Limpopo (n=23)			Kwa Zulu Natal (n=3)			Tanzania (n=6)			Kenya (n=12)		
Locus	**A**	**He**	**Ho**	**A**	**He**	**Ho**	**A**	**He**	**Ho**	**A**	**He**	**Ho**	**A**	**He**	**Ho**
GHB19	6	0.7002	0.7273	6	0.7169	0.7826	2	0.5333	0.6667	6	0.8444	0.6000	5	0.5978	0.5833
GHB11	10	0.8120	0.8000	8	0.8580	0.8261	3	0.7333	0.6667	4	0.7333	0.6000	6	0.7754	0.9167
GHB15	8	0.7831	0.9118	5	0.6609	0.5652	3	0.7333	0.6667	3	0.6786	0.5000	7	0.8225	0.9091
GHB14	6	0.7580	0.8182	7	0.7952	0.9130	3	0.6000	0.6667	3	0.6071	0.7500	6	0.6407	0.6364
GHB17	15	0.8960	0.9412	8	0.8309	0.8261	2	0.5333	0.6667	4	0.7778	0.8000	8	**0.8478**	**1.0000**
GHB26	4	0.6062	0.7000	5	0.7155	0.7895	3	0.8333	1.0000	2	0.4667	0.2000	4	0.7283	0.7500
GHB29	4	**0.7432**	**0.5000**	3	**0.6226**	**0.4545**	1	0.0000	0.0000	4	0.7121	0.5000	5	**0.7319**	**0.2500**
GHB20	3	0.2875	0.2941	3	0.3391	0.3478	1	0.0000	0.0000	2	0.5357	0.7500	3	**0.4891**	**0.2500**
GHB16	5	0.3626	0.3750	3	0.3938	0.3846	1	0.0000	0.0000	2	0.4286	0.5000	5	0.7879	0.6667
GHB21	7	0.7748	0.7188	6	0.6010	0.6522	3	0.8333	0.5000	3	0.7500	0.7500	5	0.6993	0.7500
GHB22	3	0.6198	0.7059	3	0.6596	0.5909	2	0.6000	1.0000	4	0.8000	0.6000	3	0.6522	0.9167
GHB23	4	**0.6932**	**0.4242**	5	0.7536	0.7391	2	0.6000	1.0000	6	0.8636	1.0000	6	0.7947	0.7000
Polymorphic Loci		100%			100%			75%			100%			100%	

Significant (P<0.05) single locus deviations from Hardy Weinberg equilibrium were not noted in the Tanzanian or Kwa Zulu-Natal populations; but were noted for the Kruger,

Limpopo and Kenyan populations (Table 3 & 4). When juveniles were included significant deviations were noted in Kruger at locus GHB23 and Kenya at locus GHB17 and GHB 20; and for all three populations at GHB29 (Table 3). When juveniles were excluded there was no difference for the Kruger population, while the Limpopo showed an additional deviation at GHB17 and Kenya showed an additional variation at locus GHB22 while GHB20 was no longer significant (Table 4). Single locus observed and expected heterozygosity values remained similar whether juveniles were included or excluded for all populations (Table 3 & 4).

Table 4. Number of alleles (A), single locus observed (ho) and unbiased (hz) heterozygosities at 12 microsatellite loci for five populations of SGH excluding juveniles. Significant deviations from Hardy Weinberg equilibrium are represented by bold numbers.

	Kruger NP (n=28)			**Limpopo (n=11)**			**Kwa Zulu Natal (n=2)**			**Tanzania (n=6)**			**Kenya (n=10)**		
Locus	A	He	Ho	A	He	Ho	A	He	Ho	A	He	Ho	A	He	Ho
GHB19	6	0.7184	0.7407	6	0.7706	0.9091	2	0.6667	1.0000	6	0.8444	0.6000	5	0.5105	0.5000
GHB11	9	0.8195	0.8214	8	0.8701	0.9091	3	0.8333	0.5000	4	0.7333	0.6000	5	0.7579	1.0000
GHB15	8	0.7750	0.8889	5	0.6753	0.5455	3	0.8333	0.5000	3	0.6786	0.5000	6	0.8105	0.9000
GHB14	6	0.7638	0.8148	7	0.8139	0.9091	3	0.8333	1.0000	3	0.6071	0.7500	6	0.6797	0.6667
GHB17	15	0.9050	0.9259	8	**0.8658**	**0.7273**	2	0.5000	0.5000	4	0.7778	0.8000	8	**0.8842**	**1.0000**
GHB26	4	0.6338	0.6957	5	0.7778	0.8889	2	1.0000	1.0000	2	0.4667	0.2000	4	0.7368	0.8000
GHB29	4	**0.7463**	**0.4444**	3	**0.6364**	**0.3636**	1	0.0000	0.0000	4	0.7121	0.5000	5	**0.7895**	**0.3000**
GHB20	3	0.2383	0.2593	3	0.2554	0.2727	1	0.0000	0.0000	2	0.5357	0.7500	2	0.4421	0.2000
GHB16	5	0.4053	0.4286	3	0.5303	0.5000	1	0.0000	0.0000	2	0.4286	0.5000	5	0.7879	0.6667
GHB21	7	0.7747	0.7600	5	0.6277	0.7273	3	0.8333	0.5000	3	0.7500	0.7500	4	0.6947	0.8000
GHB22	3	0.6157	0.6667	3	0.6753	0.7273	2	0.6667	1.0000	4	0.8000	0.6000	3	**0.6368**	**1.0000**
GHB23	4	**0.7051**	**0.3462**	4	0.7619	0.6364	2	0.6667	1.0000	6	0.8636	1.0000	5	0.7908	0.6667
Polymorphic Loci		**100%**			**100%**			**75 %**			**100%**			**100%**	

The total number of alleles detected at each locus ranged between 1 and 15 (Table 3 & 4). The most polymorphic locus was GHB17 which had the highest number of alleles for the KNP, Limpopo and Kenya populations (8 – 15) while the highest number of alleles was recorded at locus GHB 19 & 23 for the Tanzanian population (six alleles). The locus with the

lowest allele diversity when juveniles were included or excluded remained the same except for Kenya and were as follows: GHB 20 and 22 for the Kruger (three alleles); GHB 29, 20, 16 and 22 for the Limpopo (three alleles); GHB 29, 20 and 16 for KZN (one allele); GHB 26, 20, 16 for Tanzania (two alleles); and GHB 20 for Kenya excluding juveniles (two alleles) and GHB 20 and GHB 22 including juveniles (three alleles). Single loci observed heterozygosities when juveniles were included and excluded remained similar, the results including juveniles ranged between: 0.2941 - 0.9412 in Kruger; 0.3478 - 0.9130 in Limpopo; 0.000 – 1.000 in KZN; 0.2000 – 1.000 in Tanzania; and 0.2500 - 1.000 in Kenya.

Scrutiny of allele frequency distribution per locus was only considered with sample sets including juveniles as these represent the complete possible allele diversity of the populations. Out of a possible 97 alleles, 13 were shared by all five populations constituting 13.4% of the total. Out of the 12 loci, 11 had at least one allele common to all 5 populations with GHB 17 the exception. The loci GHB 21 and GHB 22 had two alleles each, shared by all five populations. A total of 17 alleles of the possible 97 were unique to only one population constituting 17.5% of the total (Figure 2). A total of 11 unique alleles were identified for the Kruger, three for the Limpopo, two for the Kenyan, one for the Tanzanian and zero for Kwa Zulu-Natal populations respectively (Figure 2). No more than two unique alleles were recorded per locus except for GHB 17 where six unique alleles were recorded all for the KNP population (Figure 2). Unique alleles with a per locus frequency of greater than 10% were noted only once in the Kruger (10.94%) and Limpopo (10.87%) populations respectively.

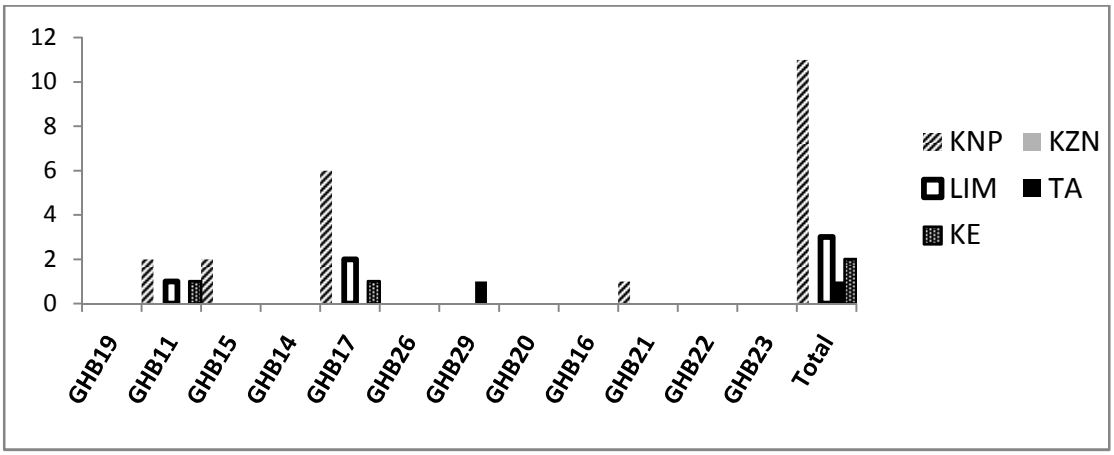


Figure 2. Distribution of unique alleles for five populations of southern ground hornbills.

Under the assumptions of the infinite alleles model (IAM) results from BOTTLENECK showed deviations from mutation drift equilibrium with heterozygosity excess highly significant under the Wilcoxon two tailed test for the Limpopo (P=0.003); Kruger (P=0.013) and Kenyan (P=0.034) populations respectively.

Structure calculations of the posterior probabilities of *K* showed the highest probability for a real structure consisting of two populations (Figure 3). All populations except the Limpopo, group strongly into one of the two clusters clearly showing the northern and southern origin of the samples collected (Table 5). The Tanzanian, Kenyan and NGH group are the most defined cluster showing 85.7, 91.7 and 97.1% affinity to cluster 1 respectively. Cluster 2 is defined by 61.4% of the KNP sample and 94.2% of the KZN sample. The Limpopo population seems to be intermediate between the two clusters but shows a stronger affinity with the northern birds with 56.2% of individuals grouping into this cluster. Bar plots showing the proportion of membership of each individual to one or more of the two real clusters identified are presented in Figure 4.

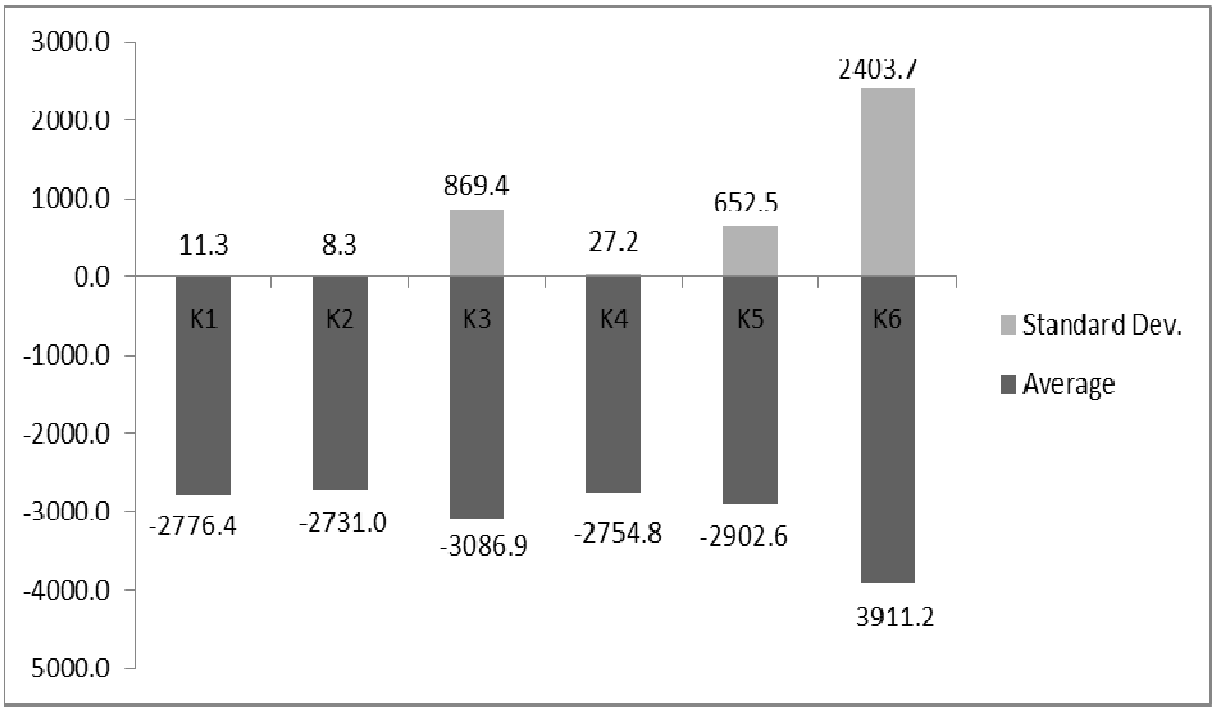


Figure 3. Results from STRUCTURE showing the average and standard deviations of the posterior probability values, Ln P(D), where K is possibly equal to 1-6 populations. K=2 is statistically the most likely number of populations.

Table 5. Proportion of membership of 5 populations of SGH and the NGH assigned to two nominal clusters, based on Bayesian analysis. Blocked values indicate dominant populations assigned to each cluster.

	Clusters	
	1	**2**
KNP	0.386	0.614
KZN	0.058	0.942
LIM	0.562	0.438
TAN	0.857	0.143
KEN	0.917	0.083
NGH	0.971	0.029

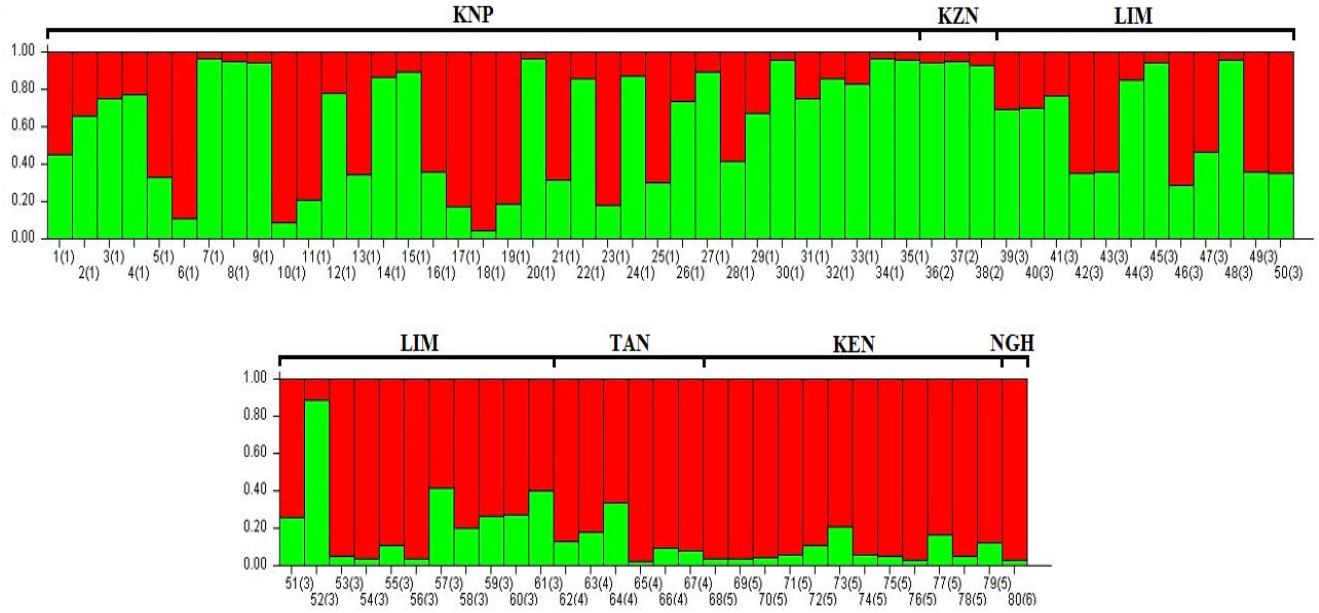


Figure 4. Bar plot showing individual SGH by sampled population. Coloured zones on each vertical bar show the proportion of membership of an individual to each of one of two clusters identified.

A large percentage of genetic variation was within populations while among population variation (AMOVA's) was low with little variation noted between results including and excluding juveniles (Table 6). Pairwise comparisons of population differentiation (Fst's) revealed significant differentiation for seven combinations highlighted in bold for the group including juveniles and only two for the group without juveniles (Table 7).

Table 6. Relative contributions of different hierarchical levels of diversity to total genetic diversity within and among SGH populations including and excluding juveniles. The NGH outgroup was not included.

Source of Variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among Populations including Juveniles	4	19.88	0.11289 Va	5.61
Among Populations excluding Juveniles	4	14.232	0.09261 Va	4.98
Within populations including juveniles	153	290.557	1.89906 Vb	94.39
Within populations excluding juveniles	109	192.662	1.76754 Vb	95.02

Table 7. Pairwise Fst values comparing sampled populations of SGH. Results including juveniles are below diagonal and those excluding juveniles above the diagonal with P values in brackets and significant levels of genetic differentiation highlighted in bold (P<0.008, after correction for multiple pairwise comparisons)

	KNP	KZN	LIM	TAN	KEN	NGH
KNP	***	**0.119**	0.028	0.002	**0.071**	0.078
		(0.004)	(0.035)	(0.446)	**(0.000)**	(0.999)
KZN	**0.104**	***	0.117	0.163	0.243	0.111
	(0.005)		(0.032)	(0.074)	(0.014)	(0.999)
LIM	**0.037**	**0.108**	***	0.010	0.040	0.081
	(0.000)	**(0.000)**		(0.360)	(0.021)	(0.999)
TAN	0.014	**0.153**	0.017	***	0.044	0.142
	(0.220)	**(0.008)**	(0.188)		(0.052)	(0.999)
KEN	**0.064**	**0.199**	**0.056**	0.003	***	0.145
	(0.000)	**(0.003)**	**(0.000)**	(0.379)		(0.999)
NGH	0.192	0.307	0.206	0.189	0.189	***
	(0.999)	(0.999)	(0.999)	(0.999)	(0.999)	

Based on Wright's (1978) guidelines for interpretation of Fst results, 0-0.05 = little differentiation; 0.05-0.15 = moderate differentiation; 0.15-0.25 = great differentiation; and an

Fst above 0.25 = very great differentiation. The only significant differentiation for the group excluding juveniles were between Kruger and KZN (0.119) and between Kenya and Kruger (0.070) which is similar to the results obtained for the data set including juveniles. For the group including juveniles the following was found: the Limpopo and Kruger populations seem to share the closest relationship overall (Fst=0.037); The highest level of differentiation occurs between KZN and the Kenyan and Tanzanian populations, 0.199 and 0.153 respectively; KZN's closest association is with Kruger (0.104) and Limpopo (0.108) although this is a relatively distant relationship in comparison with the pairwise combinations shown between Limpopo and Kruger; and Kenya's closest relation is Limpopo (0.056) followed by Kruger (0.064).

Table 8. Genetic distances between populations sampled with juvenile samples included below the diagonal and excluded above the diagonal.

	KNP	KZN	LIM	TAN	KEN
KNP	***	0.084	0.083	0.129	0.173
KZN	0.155	***	0.128	0.224	0.307
LIM	0.073	0.173	***	0.084	0.085
TAN	0.128	0.235	0.099	***	0.076
KEN	0.162	0.378	0.116	0.055	***

Genetic distance results are summarised in Table 8. Although values changed between KZN and other populations when juveniles were excluded the general trends between the various populations remained the same and only results including juveniles are listed below. The greatest genetic distance was between the northern (Kenya and Tanzania) and KZN samples (0.235-0.378). Populations that are closer geographically also showed the lowest D values notably Tanzania and Kenya (0.055), Limpopo and Kruger (0.073) but Tanzania and Limpopo also showed strong identity (0.099). The Limpopo population grouped closer to the northern samples (0.099-0.116) than KZN (0.173) while Kruger is intermediate between KZN (0.155) and the northern populations of Tanzania (0.128) and Kenya (0.162). These results are further represented in Figure 5 where the trends between populations remain the same regardless of whether juveniles are included or not. The most significant relationship were shown between Tanzania and Kenya with bootstrap support of 90% and 80% for sample sets with juveniles and without juveniles respectively; as well as between the Limpopo and

northern samples with bootstrap support of 69% and 88% including and excluding juveniles respectively.

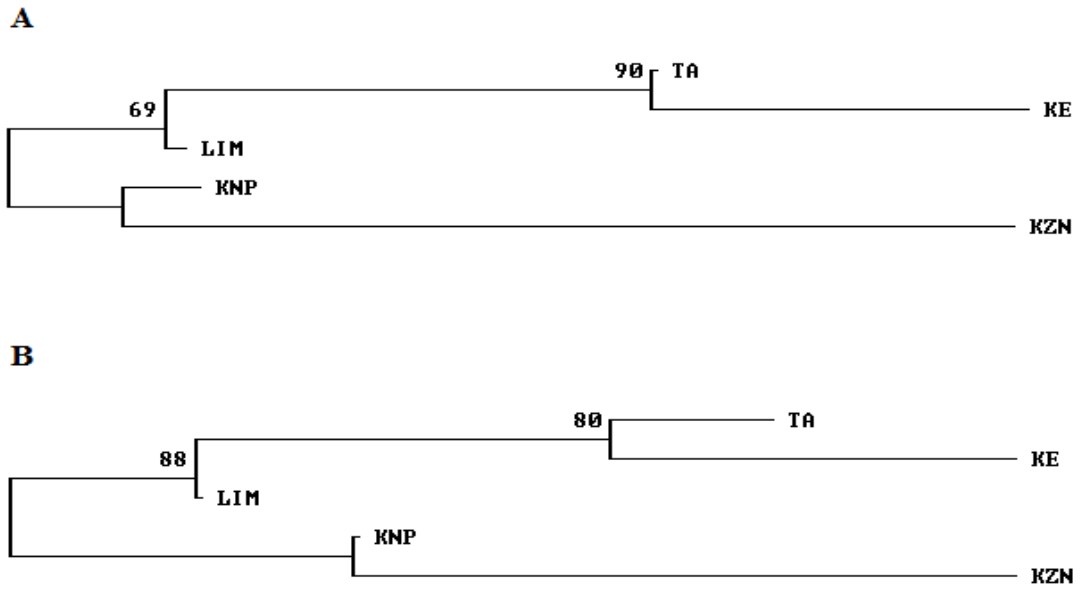


Figure 5. Neighbour joining tree displaying standard genetic distances among SGH populations sampled during the study, including juvenile samples (A) and excluding juvenile samples (B). Numbers are values from Bootstrap replicates with support lower than 50% not shown.

Discussion

Overall, the inclusion or exclusion of juveniles did not have a significant effect on the results although with regards to Hardy Weinberg equilibrium juveniles should strictly speaking not be included as Hardy Weinberg is based on a random mating population of which juveniles are not part.

The genetic diversity of the five SGH populations surveyed (56 – 69% observed heterozygosity) compares favourably to other k-selected, threatened and mobile avian species. Observed heterozygosity found in other studies varied between 46 – 59% for northern goshawks (Sonsthagen *et al*., 2004), 48 – 56% for Spanish imperial eagles (Martínez-Cruz *et al*., 2004) and 28 – 57% for wood storks (Van Den Busschel *et al*., 1999). These favourable levels of genetic diversity have been maintained despite indications from this study that SGHs have experienced a recent bottleneck. Hailer *et al*. (2006) found that levels of genetic diversity were retained in populations of the white tailed eagle (*Haliaeetus*

albicilla) with the long generation times of this species acting as a buffer to the effects of the severe population declines recorded. The estimated generation time of SGH is 50 years and the same buffering effects may have occurred, although the dispersal ability of the species is little understood (this subject is further explored in Chapter 4) and may be a contributing factor to the maintenance of gene flow and genetic diversity.

Genetic structuring correlated with geographic distance is evident in the populations sampled as shown by the KZN and Kenya/Tanzania populations, which share the least genetic similarity and are over 3000 km apart, at the southern and northern extremes of the species range respectively. Geneflow is shown to be greater with adjacent populations and the low values represent populations in panmixia as indicated by the Fst results. Initial indications therefore are, that the KZN population is isolated from populations to the north of South Africa and may be the single most threatened population of SGHs in Africa. Differentiation based on genetic distance (Dn) produced results that indicate populations that are different from each other but with similar trends as exhibited by the Fst's. A possible explanation for the exaggerated genetic distance results (Dn) could be as a consequence of recent habitat loss and population fragmentation as expressed by the BOTTLENECK results. Hedrick (1999) states that genetic distance may increase very quickly with a substantial reduction in population size and/or a genetic bottleneck. This is corroborated by the evidence we have in South Africa of population declines and the same situations also seem to be occurring in Kenya in the face of human induced pressures on the environment.

Contrary to what would be expected all the SGH populations did not group into one cluster with the NGH as an outlier but this may be an inaccurate result due to the single NGH sample used in the analysis. Southern ground hornbills are similar in size, colouration, diet and basic nesting cycle to its congener the NGH (Kemp & Kemp, 1980) but the genetic relationships between these two species have yet to be explored. The distribution of the two species of ground hornbill were known to overlap historically although this seems to no longer be the case and the changes in genetic relationship between the NGH and the SGH with increased geographic distance is a possibly fascinating topic of research.

The above preliminary results suggest that there is not enough genetic differentiation to indicate the existence of two sub-species in SGH although genetic sub-structure is evident. Only 5.6% of the genetic variation was recorded among populations with 94.4% within

populations, which is a relatively minimal variation especially in light of the geographic distances between the populations sampled. It was also notable that during capture in the Masai Mara, Kenyan birds responded immediately to playbacks from recordings of birds from South Africa.

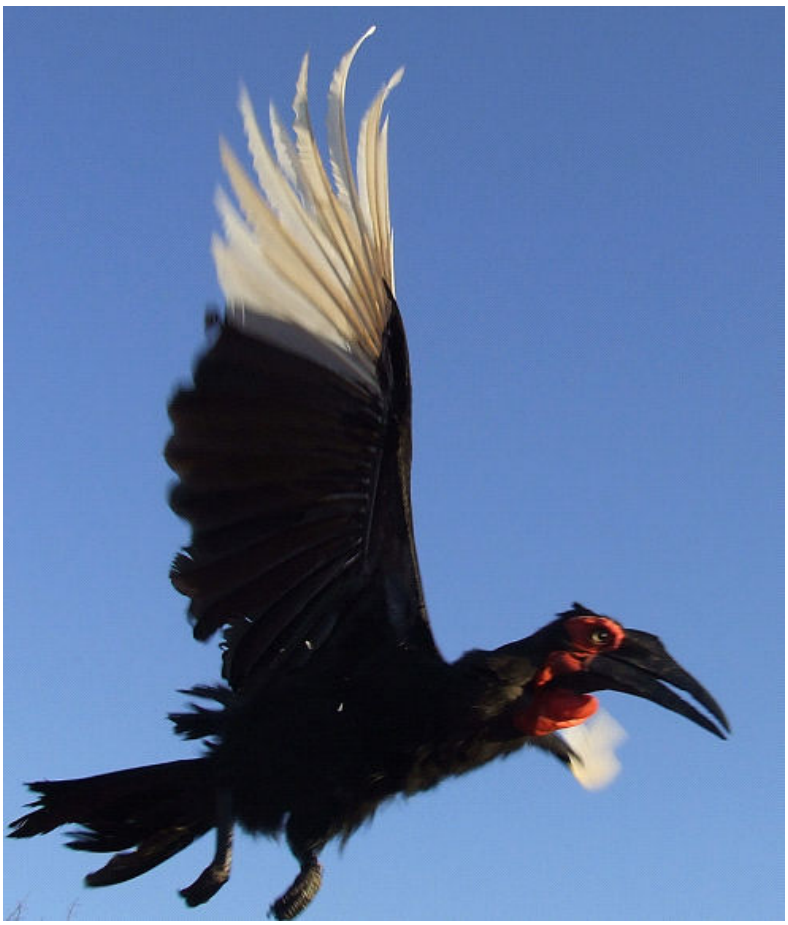
Conservation implications

Low sample sizes especially for the Kwa Zulu-Natal and Tanzania populations are immediately apparent in the study and results need to be interpreted with a certain amount of caution. For example, when significant deviations from Hardy Weinberg equilibrium were compared, the KNP was the only population to show significance at the same loci regardless of whether juveniles were excluded or not. This is in all likelihood due to the larger sample size representing this population. Furthermore, the populations sampled constitute a small percentage of the total SGH range in Africa although samples from both the northern and southern extent of the species range were included in the analysis. Consequently, direct gene flow between these populations is highly unlikely and the current estimates are therefore a good representation of the maximum degree of genetic differentiation possible for the species throughout its range.

Of special importance to this study and the Limpopo Valley population are the results which indicate that the Kruger population does not seem to be the only source of birds for the recently colonised Limpopo population but also birds from further north. This is unexpected in light of the fact that the closest population of SGHs to the Limpopo Valley is in the KNP which is less than 100 km away. The Kruger National Park and adjacent reserves are well populated with SGHs and helpers are strongly represented in groups. Immediately outside the park, on the other hand, the sudden absence of SGHs is glaringly apparent due to the high densities of farms and human habitation's encroaching on the park along most of the borders (Pers. obs.; Kemp & Webster, 2008), which may be a reason for the limited dispersal apparently displayed by this population. It is also possible that the SGH populations from the Limpopo Valley are closer linked to populations in Botswana and western Zimbabwe than the KNP populations, which are connected to the eastern half of Zimbabwe, Mozambique and historically Natal. Further research is needed to elucidate these trends and results and is a priority for the conservation of the species.

CHAPTER 4

A FINE SCALE INVESTIGATION INTO ASPECTS OF THE BIOLOGY OF THE SOUTHERN GROUND HORNBILL IN THE LIMPOPO VALLEY, WITH THE AID OF MICROSATELLITE MARKERS



CHAPTER 4: A FINE SCALE INVESTIGATION INTO ASPECTS OF THE BIOLOGY OF THE SOUTHERN GROUND HORNBILL IN THE LIMPOPO VALLEY, WITH THE AID OF MICROSATELLITE MARKERS

Introduction

Female-biased dispersal occurs in the majority of bird species (Greenwood, 1980; Clarke *et al*., 1997). Greenwood (1980) hypothesised that sex biased dispersal is due to the type of mating system employed by a species and birds (such as the SGH) mostly employ a resource defence mating system. As such, competition between males is aimed at establishing territories while females would benefit from choosing superior resources and related higher quality mates. In these systems, males generally settle closer to or inherit natal territories whilst females disperse in search of suitable mates and territories. The dispersal behaviour of SGH is not properly understood although it is believed that females more commonly disperse from natal territories (with movements of 60 km recorded), whilst males remain behind becoming helpers resulting in skewed male to female sex ratios in the KNP, and the presence of more lone females than males (Kemp & Kemp, 1980; Kemp, 1988). By using genetic markers it was explored whether the current Limpopo Valley population of SGH grew due to migration into the area from other populations or whether remnant groups re-established themselves gradually. Furthermore sex based genetic variation within the Limpopo Valley populations provided insights into sex-biased dispersal behaviour. For example a study on the relatedness of white-throated magpie-jays (*Calocitta Formosa*) found that mean genetic relatedness was higher for females than males, which was consistent with observational data indicating males disperse upon maturity while females remain in their natal territories (Berg *et al*., 2009).

Microsatellites, singlenucleotide polymorphisms (SNPs), and amplified fragment length polymorphisms (AFLPs) provide an opportunity to infer pedigrees for wild populations for which relationship information would be difficult, or impossible, to achieve by observation alone (Jones & Wang, 2010). In many cases genetic analysis has highlighted alternative mating systems and many assumed monogamous species have been shown to be polygamous through genetic analysis and therefore this approach was taken to investigate this difficult-to-study Limpopo Valley population. Numerous methods have been proposed to determine coefficients of relatedness between individuals and the relative precision and accuracy of these methods depends on allele-frequency distributions and the true relationships among

individuals (Blouin, 2003). These have been reviewed extensively in the literature (Wang, 2010; Thomas, 2005; Blouin, 2003; Van de Casteele *et al*., 2001). In parallel with the development of analytical approaches, a number of different statistical packages have been developed to generate coefficients of relatedness (Blouin, 2003).

Southern ground hornbill are categorised as obligate (regular) co-operative breeders (Du Plessis *et al*., 1995) and no observations exists of SGH populations exhibiting non- and/or facultative co-operative breeding behaviour. Pairings are assumed to be long term with the species' estimated longevity of between 25 – 50 years (Morrison *et al*., 2005) and in the longest study copulation was only ever observed between the alpha breeding pair (Kemp & Kemp, 1980), which seems to indicate SGH are monogamous with high mate fidelity.

The main objectives of the work described in this chapter were to investigate the following: (1) Aspects of the population dynamics in the Limpopo Valley with regards to age and sex structures and productivity. (2) The accuracy of traditional sexing methods using field characteristics by comparing these to lab results. (3) Recording the possible loss of genetic diversity from one generation to the next. (4) Levels of relatedness within and among SGH groups in the Limpopo Valley. (5) Characteristics of the re-colonisation of the Limpopo Valley. (6) The utility of microsatellite markers in studies of SGH social and breeding systems.

Materials and methods

Sample collection and southern ground hornbill capture method

From July 2008 until August 2009 eight groups of SGH from the Limpopo Valley were captured (Figure 1). Capture operations were always undertaken in the mornings with the trap ready by first light and in the afternoons from approximately 14:00 until the birds left to roost. The groups were not disturbed during the day from approximately 10:30 – 14:00 to allow time to recharge electrical equipment as well as to give the group a chance to settle due to stress induced by call-ups and model SGHs. Birds were located early in the mornings when SGH perform territorial duets that are audible for up to 3km (Kemp, 2005). Call-ups were then initiated from a vehicle to attract groups. The alpha pair always responded and flew in while juveniles hid and avoided the capture site. Responses from sub-adults were also positive. Once a group responded the capture trap was set up nearby. The trap measured 3×6 meters and is made of strong nylon netting. Meat scraps were left inside the trap as well as a

model SGH. To further entice the birds into the trap a speaker system is connected to a wireless digital receiver and is hidden in the trap in a wooden box while researches in a vehicle approximately 50 meters away can manipulate the calls with an MP3 player and a wireless digital transmitter. A length of nylon rope was connected to the door of the trap, which was pulled shut from the vehicle when target individuals entered the trap. The alpha pair from each group were targeted but as many individuals as possible were always captured (Figure 2). Captured birds were banded using steel metal rings obtained from SAFRing (Avian demography unit). Various morphometric measurements were completed, with birds weighed and bled from leg veins with 3 ml blood stored in EDTA storage vials and kept refrigerated (Figure 3). GPS readings for all groups were recorded using a Garmin® GPS.

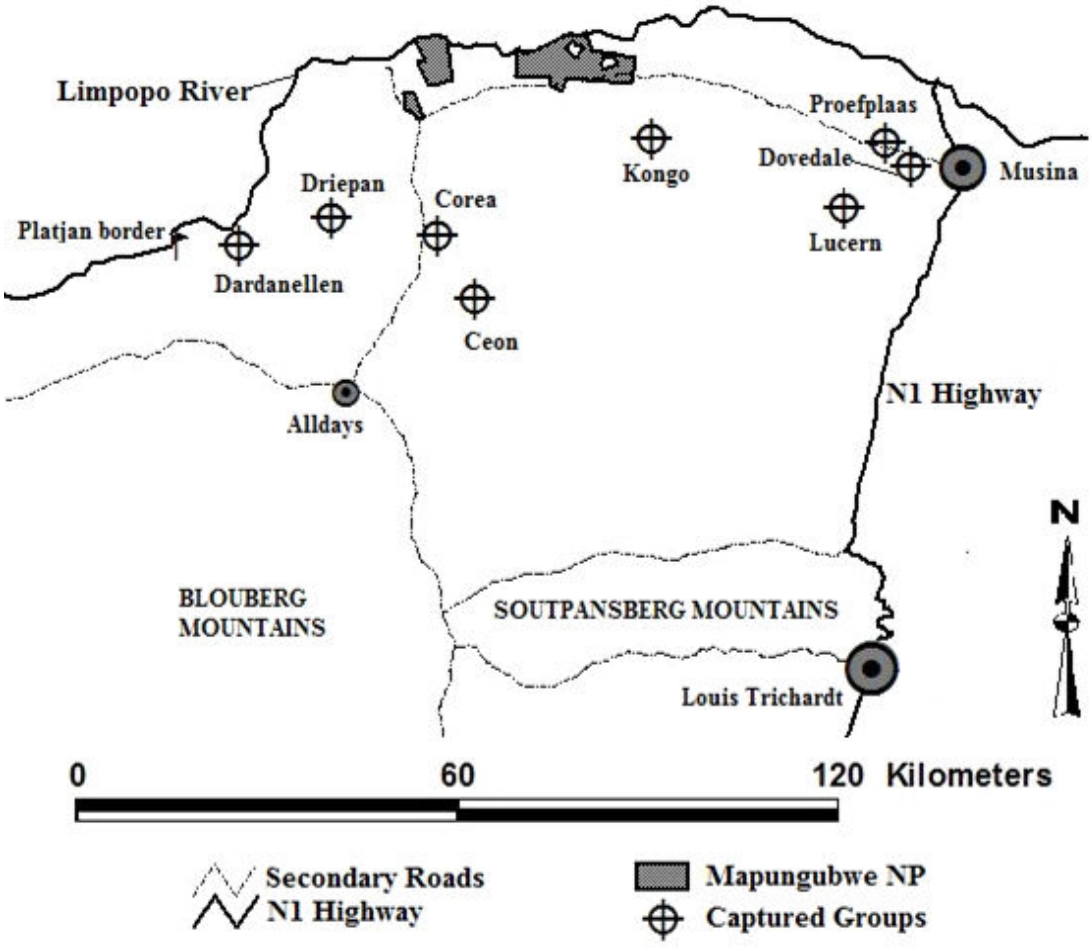


Figure 1.Capture location of eight groups in the Limpopo Valley, namely: Dardanellen (Dar); Driepan (Dri); Ceon (Ceo); Corea (Cor); Kongo (Kon); Lucern (Luc); Dovedale (Dov); and Proefplaas (Pro).

Figure 2. Southern ground hornbill juvenile and adult in trap with fibreglass model (left) and male SGH showing territorial aggression and hammering on wooden box housing wireless receiver and speakers (right).

Figure 3. Southern ground hornbill being weighed (left) and juvenile being bled from leg vein for genetic analysis (right).

Age, sex structure and productivity

During capture the opportunity arose to carefully observe and record group characteristics. Although it could not be confirmed whether all the individuals were related to the group an estimate of the productivity, and age and sex structure of the population could be made. Southern ground hornbills fledge with a cream coloured facial skin, which only begins to colour between one and one–and-a-half years of age. Estimates of the age of the chick can be

made by considering the development of colour on the throat pouch (Kemp & Kemp, 1980). The following ageing categories were used: Fledglings (>1 year old); immatures (1 -3 years old), sub-adults (3-6 years old) and adults (sexually mature birds <6 years old). By determining the age of all non-adult birds and the approximate seasons these individuals would have fledged the productivity of the eight groups could then be estimated. Where records of groups successfully fledging chicks were known these were included. Estimates did not take into account mortality rates and the natural dispersal of sub-adults, and possibly immature birds, from their natal territories. Sexing is usually only possible from the sub-adult stage with females displaying a violet blue patch on the throat pouch extending from under the base of the lower mandible, sometimes with violet blue patches on the circumorbital skin while males on the other hand have a pure red throat pouch and circumorbital skin (Kemp & Kemp, 1980). Although this is the accepted means of sexing SGHs the presence of males in captivity exhibiting the blue throat pouch characteristic of females has been recorded, while the presence of red females in captivity, as far as can be determined, is not known. This study is the first attempt to investigate the colour of individuals in the field and compare these to sexing results using microsatellite markers. As such photographs of all captured birds displaying throat pouches in left and right profiles, as well as portraits with the bills lifted were taken (Figure 4). DNA sexing of SGH samples was contracted to the National Zoological Gardens laboratories in Pretoria.

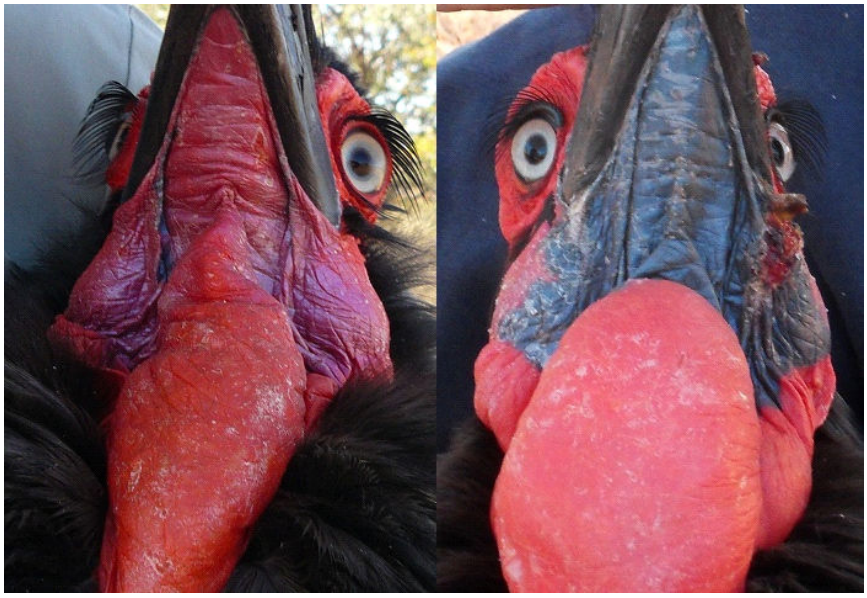


Figure 4. Male SGH on the left with pure red throat pouch and circumorbital skin and female on the right with red circumorbital skin and violet blue patch on the throat pouch extending from under the bill.

Genetic analysis

Building on from the overall genetic structure of the SGH as explored in Chapter 3 the genetic variation of the juveniles within the Limpopo Valley was compared to that of the adults. Sex-biased dispersal was also explored by comparing levels of genetic variation of adult males and females in the population. Juveniles represent the genetic potential of the population if they disperse from their natal territories to form new groups while measuring levels of sex based genetic variation provided insights into which sexes were most likely to disperse. Overall observed heterozygosity (Ho) and Nei's unbiased heterozygosities (Nei, 1987) were also explored as well as locus by locus expected and observed heterozygosities.

Methods to determine the genetic relationships between individuals and groups can be divided into two categories, namely (i) relatedness estimation, which is the measure of the fraction of alleles shared identically by descent among individuals (dyads); (ii) and the assignment of pairs or groups of individuals too categories of relationships, such as siblings or parent offspring (Blouin, 2003). Both methods were implemented using two programs to determine levels of relatedness between individuals within a group, between separate 'family' groups and between individuals in the Limpopo Valley.

The program COLONY (Jones & Wang, 2010) was used to categorise and infer sibship and parentage among individuals in the population. The program uses a full likelihood pedigree method whereby parentage and sibships are inferred jointly with likelihood considered over the entire pedigree configuration, rather than for pairs of individuals or dyads (Jones & Wang, 2010). The full pedigree likelihood method is seemingly more accurate than for pairwise methods (Wang & Santure, 2009). Twenty-three individuals from the Limpopo Valley were aged and grouped into candidate father, candidate mother and offspring sub-sample sets. All immature and sub-adults were classified as offspring. The algorithm implemented then partitions the individuals from the sub-samples into a number of family clusters where individuals within a cluster are related via sibship or shared parentage, while individuals in different clusters are unrelated (Jones & Wang, 2010). Probability values for each assignment are also estimated. The program parameters were set to make one independent, medium run with the random number seed generator, which is a Monte Carlo method. Although SGH are classified as monogamous the mating system parameter was specified as polygamous to screen for the possibilities of extra pair copulations or movements of individuals between groups. The probabilities of male or female parents included in the

candidate parent file was assumed to be highly likely and therefore set as 95% because of the high likelihoods of juveniles being produced by at least one adult in the group. To provide for the possibility of genotyping errors a conservative estimate of 0.05 per loci was assumed according to recommendations made by Wang (2004) where it was shown that allowing for genotyping errors in group likelihood approaches dramatically improved relationship inference. Variations in genotyping error rates (0.001–0.40) also did not seem to negatively affect data sets where 100% correct assignments were obtained (Wang, 2004).

To test the performance of relationship inference a captive sample set of SGHs where relationships were known from studbook records, was first analysed in COLONY. This was done to determine the accuracy of results because the relationships between samples in the Limpopo Valley could not be verified due to lack of observational data to support the findings of COLONY. Unfortunately the captive dataset only consisted of known parent offspring and full sibling relationships and therefore half sibling relationships could not be tested. Where captive birds were known to be unrelated to known parent offspring relationships these were also included in the dataset as a control.

The software package COANCESTRY (Wang, 2010) was used to determine relatedness coefficients as it implements seven methods simultaneously. Five of these are moment estimators and two are likelihood estimates. The same captive sample set, as above, was once again used to compare the results of the seven methods implemented in COANCESTRY, where relationships between individuals were known. As such, the best estimator could be identified and used for the Limpopo Valley sample set where relationships are not known. This was done by scrutinising all dyads were the relationship were either parent/offspring or full siblings. As a guide the co-efficient of relatedness (*r)* for some common relationships are as follows: parent sibling and full sibling (0.50); half siblings, avuncular and grandparent-grandchild (0.25); first cousins (0.125); and unrelated (0). Where relatedness values were below 0.400 these were discarded (No half sibling or other known relationships were represented in the captive sample set) and all dyads 0.400 and above were kept and counted and as such a score could be given for the dyad assignments that performed the best per method for each relationship dyad. The method that performed the best was then used to interpret the results from the Limpopo Valley sample set.

Because adults represent the current gene pool that will influence diversity in the population in the future, the level of relatedness between adults was investigated to determine the possible relationship of the founders in the Limpopo Valley Population. Consequently, the relatedness levels between all SGH adults were determined as well as the level of relatedness between every group of SGH in the Limpopo Valley. The average within-group relatedness and inbreeding was also determined.

The program DISPAN (Ota, 1993) was used to generate neighbour joining trees using the method of determining genetic distance with 1000 bootstrap replications implemented (Nei, 1972). Finally, sex biased dispersal of individuals into the Limpopo Valley was explored where the average relatedness between adult male and then adult female dyads was tested using 1000 bootstrap replications to test for significance at the 95% confidence level in COANCESTRY.

Estimates of effective population size (*Ne*) were obtained utilising a bias correction method based on linkage disequilibrium data as implemented in the program LDNe, which is a method especially useful for polymorphic markers such as microsatellites (Waples & Do, 2008). A jackknife method was used to obtain 95% confidence intervals (CI) on loci, (Waples & Do 2008).

Results

Age, sex structure and productivity

The mean group size in the Limpopo Valley is 4.0 (SD 1.89; range 2.0–8.0) birds per group, with all groups consisting of on average one adult male, one adult female, 1.2 (SD 1.23) immatures and 0.7 (SD 0.95) sub-adults per group (Table 1). The ratio of adults to immatures was 1.6:1 which decreases to 1.05:1 when sub-adults are grouped with immatures. Verification of sexing methods of adult and sub-adults displaying sex specific colouration with lab results were as expected with all captured females (n=8) and males (n=7), correctly sexed in the field using characteristics as defined by Kemp & Kemp (1980). No males with violet blue or females with pure red throat pouches were identified. The sex ratio in the Limpopo Valley when all observed adults are considered, assuming visual sexing criteria were correct, was 1:1 because no mature adult helpers were identified in any of the groups. A total of 19 immature and sub-adult birds from eight groups were recorded in the population representing a six year breeding period (Table 2).

Table 1. Summary of the population composition of SGH groups in the Limpopo Valley. Numbers in brackets represent individuals captured during the study period.

Group name	Group size	Adult males	Adult females	Immatures	Sub-adults	Unknowns
Driepan	8	1	1 (1)	3	1 (1)	2
Lucern	5	1	1 (1)	1 (1)	2 (2)	0
Ceon	3	1 (1)	1 (1)	1	0	0
Dovedale	4	1 (1)	1 (1)	0	2 (1)	0
Dardanellen	2	1 (1)	1 (1)	0	0	0
Kongo	5	1 (1)	1	3 (2)	0	0
Proefplaas	4	1	1 (1)	2 (2)	0	0
Corea	5	1 (1)	1 (1)	3 (2)	0	0
Rhodesdrift	2	1	1	0	0	0
Hartz	2	1	1	0	0	0
Total	40	10 (5)	10 (7)	13 (7)	5 (4)	2
Average	4	1	1	1.3	0.5	0.2
Standard Dev.	1.89	0.00	0.00	1.34	0.85	0.63

Table 2. The composition of groups over a five year period recorded during capture operations. Numbers with (*) indicate individual from the group Ceon that fledged but died soon after. Unconfirmed records of juveniles present in the group Dardanellen were not considered. The Rhodesdrift and Hartz groups were not included due to lack of records.

Group	Breeding season						Total chicks fledged	Total years per fledged
Name	2003/ 2004	2004/ 2005	2005/ 2006	2006/ 2007	2007/ 2008	2008/ 2009	per group	chick
Driepan	0	1	0	1	1	0	3	2.00
Lucern	1	1	0	1	0	0	3	2.00
Ceon	0	0	0	1	1	1	3	2.00
Dovedale	0	1	1	0	0	0	2	3.00
Dardanellen	0	0	0	0	0	0	0	6+
Kongo	0	0	0	1	1	1	3	2.00
Proefplaas	0	0	0	0	1	1	2	3.00
Corea	0	0	0	1	1	1	3	2.00
Total	1	3	1	5	5	4	19	2.53

The oldest individual recorded was approximately 5 years old and fledged during the 2003/2004 season. Breeding records from one nest monitored over the past four years were also included (Table 2). The average number of non-adult birds per group was 2.38 (SD 1.06; range 0–3) of which only five were sub-adults and 14 were juveniles while average number of chicks fledged per season is 3.17 (SD 1.83; range 1–5). According to these results 19 chicks were fledged by eight groups over a six year period which equates to one chick fledged per group on average every 2.53 years (19 chicks over 48 group breeding seasons).

Captive sample set

Relationship inference with COLONY for the captive sample set where an error rate of 0.05 was included performed best. A total of 10 captive offspring were correctly assigned to their associated parents with probability values varying between 0.926 and 1.000 and all captive siblings were correctly assigned with probability values of 1.000. Only one incorrect sibling relationship was noted with a probability of 0.986, where both individuals were unrelated to all other individuals in the sample set. These results were then used to help interpret the relationship inference results from the Limpopo Valley sample set where an error rate of 0.05 was employed and where probability values ranging between 0.900 and 1.000 were considered to be suitably high indication of relationship.

Results of the relatedness between dyads from the captive sample set as generated in COANCESTRY differed. The moment estimator of Wang (2002) fared the best with 74% of dyads with known full sibling and parent offspring relationships assigned relatedness values ≥0.400 while the moment estimator of Lynch (1988) and the likelihood estimator of Milligan (2003) performed less well assigning relatedness values ≥0.400 to 61% and 52% of the related dyads respectively.

Genetic variation in the Limpopo Valley

Levels of genetic variation were similar for individuals grouped according to age and sex (Table 3). Adult females showed the highest level of unbiased heterozygosity with 0.699 while adult males showed the highest level of observed heterozygosity with 0.711. Juveniles showed the lowest level of unbiased heterozygosity with 0.655, and adult females the lowest observed heterozygosity with 0.633. A total of 3-8 alleles per locus were recorded for adult, juvenile and female groupings while 2-6 were recorded for the male group. No significant

loci by loci deviations from Hardy Weinberg equilibrium were observed for any of the delineated groupings (Table 3).

Table 3. Number of alleles (A), single locus observed (Ho) and expected (He) heterozygosities at 12 microsatellite loci for adult, Juvenile, adult male and adult female delineated groupings from the Limpopo river valley.

	Adults		**Juv**		**Adult Males**		**Adult Females**	
Locus	**He**	**Ho**	**He**	**Ho**	**He**	**Ho**	**He**	**Ho**
GHB19	0.7681	0.9167	0.6753	0.6364	0.7778	0.8000	0.7692	1.0000
GHB11	0.8768	0.9167	0.8701	0.7273	0.9111	1.0000	0.9011	0.8571
GHB15	0.6739	0.5833	0.6580	0.5455	0.6444	0.6000	0.7143	0.5714
GHB14	0.8007	0.8333	0.7965	1.0000	0.8000	0.8000	0.8352	0.8571
GHB17	0.8406	0.6667	0.8398	1.0000	0.8889	0.8000	0.8132	0.5714
GHB26	0.7737	0.8000	0.6732	0.7778	0.8214	1.0000	0.7727	0.6667
GHB29	0.5942	0.3333	0.6579	0.6000	0.5556	0.2000	0.6484	0.4286
GHB20	0.3659	0.3333	0.3247	0.3636	0.5111	0.4000	0.2747	0.2857
GHB16	0.4725	0.4286	0.3182	0.3333	0.3333	0.3333	0.6071	0.5000
GHB21	0.6232	0.7500	0.5974	0.5455	0.7111	1.0000	0.5604	0.5714
GHB22	0.6812	0.7500	0.6526	0.4000	0.6444	0.8000	0.7143	0.7143
GHB23	0.7464	0.6667	0.7922	0.8182	0.7333	0.8000	0.7802	0.5714
Unbiased heterozygosity	0.6848		0.6547		0.6944		0.6992	
Observed heterozygosity	0.6649		0.6456		0.7111		0.6329	
Alleles per locus	5.00		4.50		3.83		4.42	

Within group relatedness

Results from COLONY assigned 23 individuals from eight groups to four clusters with probabilities ranging between 0.605 and 0.998 (Table 4). Within groups all offspring were assigned to alpha females sampled from their natal groups with probability values between 0.945 and 1.000 except in the case of Luc 4. Offspring were assigned to alpha males sampled from their natal groups on two occasions (Kongo and Corea) with probability values of 0.963 – 1.000, with Dri 1 the only juvenile assigned a father (Dar 1) outside of its natal group with

a 0.960 probability rate. The offspring Luc 2, Luc 4 and Dov 3 were all assigned the same father (Dov 3) but with lower probability rates (0.818 – 0.882) while Luc 1, Pro 1 and Pro 2 are assumed to have the same father although he was not sampled during the study. All full sibling assignments with probabilities greater than 0.900 were between individual offspring sampled from the same group (0.943–0.999). The only important half sibling assignment was between offspring from two separate groups namely Luc 2 and Dov 1 (0.921). A number of assignments were noted between individuals with probabilities varying between 0.679 and 0.897. Interpreting these results was problematic as it wasn't clear whether these were actual first order relationships with lower probabilities due to typing errors or if they were due to avuncular relationships.

Table 4. Results of the assignment of individuals from eight groups in the Limpopo Valley to categories of relationship as well as kinship clusters. Numbers in brackets are probability values and those above 0.900 are represented in bold type.

Offspring ID	Inferred father	Inferred mother	Full siblings	Half siblings	Cluster Index	Cluster probability
Luc 1	*1	Luc 3 (**0.995**)	-	Luc 2 (0.816)	1	0.6045
Luc 2	Dov 3 (0.882)	Luc 3 (**0.995**)	-	Luc4 (0.825)	1	0.6045
Luc 4	Dov 3 (0.851)	Dov 2 (0.793)	-	-	1	0.6045
Dov 1	Dov 3 (0.818)	Dov 2 (**0.998**)	Luc 4 (0.679)	Luc 2 (**0.921**)	1	0.6045
Pro 1	*1	Pro 3 (**1.000**)	Pro 2 (**0.989**)	Luc 1 (0.896)	1	0.6045
Pro 2	*1	Pro 3 (**0.998**)	Pro 1 (**0.989**)	Luc 1 (0.897)	1	0.6045
Cor 3	Cor 1 (**1.000**)	Cor 2 (**0.998**)	Cor 4 (**0.998**)	-	2	0.9978
Cor 4	Cor 1 (**1.000**)	Cor 2 (**1.000**)	Cor3 (**0.999**)	-	2	0.9978
Dri 1	Dar 1 (**0.960**)	Dri 2 (**0.945**)	-	-	3	0.8029
Kon 1	Kon 2 (**1.000**)	Ceo 1 (0.824)	Kon3 (**0.9430**)	-	4	0.8883
Kon 3	Kon 2 (**0.963**)	Ceo 1 (0.841)	Kon1 (**0.9430**)	-	4	0.8883

The within-group relatedness and inbreeding coefficients as generated in COANCESTRY are represented in Table 5. Levels of inbreeding for all groups was low and varied slightly (-0.139-0.173) while levels of relatedness within groups showed greater variation (-0.362-0.413). For groups with juveniles the most related group was Corea (0.413), followed by Kongo (0.328), while the least related group was Dovedale (0.186). The adult pair in the group Dardanellen was related at the level of half sibs (0.255) while the Ceon adult pair was unrelated (-0.362). The average relatedness of the population was 0.053 (Variance -0.045).

Table 5. Levels of within group relatedness (r) as defined by Wang (2002); and inbreeding as defined by Ritland (1996); for eight groups of SGH in the Limpopo River Valley.

Group name	Relatedness (Wang, 2002)	Inbreeding (Ritland, 1996)
Driepan (n=2)	0.295 (0.000)	0.173 (0.015)
Lucern (n=4)	0.245 (0.021)	-0.007 (0.016)
Ceon (n=2)	-0.362 (0.000)	0.041 (0.032)
Dovedale (n=3)	0.186 (0.041)	-0.133 (0.014)
Kongo n=3)	0.328 (0.001)	-0.018 (0.000)
Corea (n=4)	0.413 (0.039)	0.048 (0.005)
Dardanellen (n=2)	0.255 (0.000)	-0.139 (0.004)
Proefplaas (n=3)	0.150 (0.028)	-0.073 (0.004)
Average	0.053 (-0.045)	-0.016 (0.018)

Table 6. Pairwise values of (r) for individuals sampled from eight groups of SGH in the Limpopo Valley. Numbers in brackets are variance values.

	Driepan	Lucern	Ceon	Dovedale	Kongo	Corea	Dardanellen
Lucern	-0.16038 (0.03935)	*					
Ceon	-0.1257 (0.06134)	-0.0896 (0.0136)	*				
Dovedale	-0.0569 (0.04889)	0.20607 (0.03178)	-0.02497 (0.00874)	*			
Kongo	-0.0888 (0.01081)	-0.0775 (0.04542)	0.02748 (0.04259)	0.01919 (0.01722)	*		
Corea	-0.09976 (0.01467)	-0.29488 (0.05944)	-0.1676 (0.04554)	-0.21587 (0.02631)	-0.18108 (0.02778)	*	
Dardanellen	0.20188 (0.0499)	-0.12275 (0.02088)	0.04488 (0.07196)	-0.06858 (0.01045)	0.01233 (0.01676)	0.02184 (0.01196)	*
Proefplaas	-0.08353 (0.03431)	-0.06943 (0.02914)	-0.08425 (0.02435)	-0.00987 (0.01866)	-0.02154 (0.0246)	-0.17767 (0.01508)	0.03865 (0.01061)

Between group relatedness

A stepwise graph of the means and variances of relatedness estimates between all group dyads are represented in Table 6. The highest levels of relatedness were between Dardanellen and Driepan (mean 0.202; variance 0.050) as well as Lucern and Dovedale (mean 0.206; variance 0.032). No other significant levels of relatedness between groups were noted.

A neighbour joining tree showing genetic distance between groups allows visualisation of the relationships between groups (Figure 5). The most significant relationship were the following: Lucern and Dovedale (70%) with the Proefplaas group (50%); Ceon and Kongo (38%); Driepan and Dardanellen (28%).

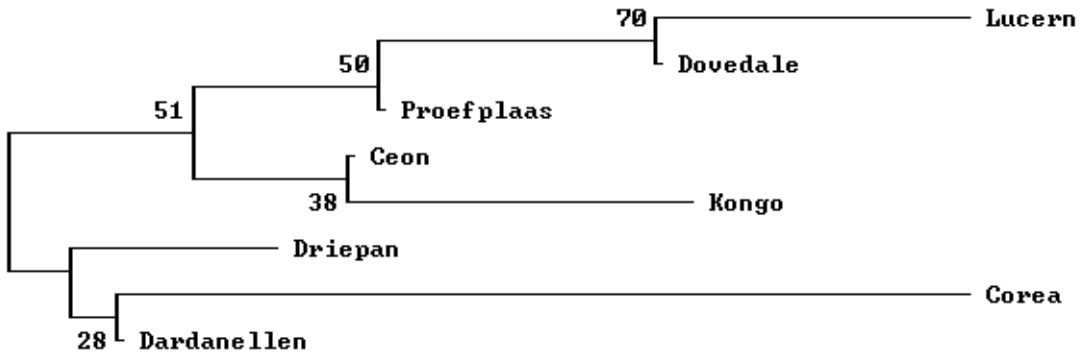


Figure 5. Neighbour joining tree with the standard genetic distances of eight groups of SGH from the same population in the Limpopo Valley. Numbers at nodes are values from Bootstrap replicates.

The average relatedness between adults in the Limpopo Valley is -0.092 (Variance 0.037) and the individual per dyad relationship is represented in Figure 6, with the most important relationship dyads labelled. Only six dyads exhibited relatedness levels of approximately 0.250 equivalent to a half sib or grandparent/grandchild relationship (0.261 – 0.322). On two occasions both adults sampled from the same group exhibited a relationship equivalent of a second degree relationship namely Corea and Dardanellen. The mean relatedness per sex for male and female adults in the Limpopo Valley was -0.101 (Variance 0.044) and -0.160 (Variance 0.030) respectively. Similarly the mean inbreeding for males and females was very low at -0.096 (Variance 0.008) and 0.024 (Variance 0.019) respectively. Furthermore, bootstraping results showed that there was no significant difference between male and female relatedness and inbreeding levels at the 95% confidence level.

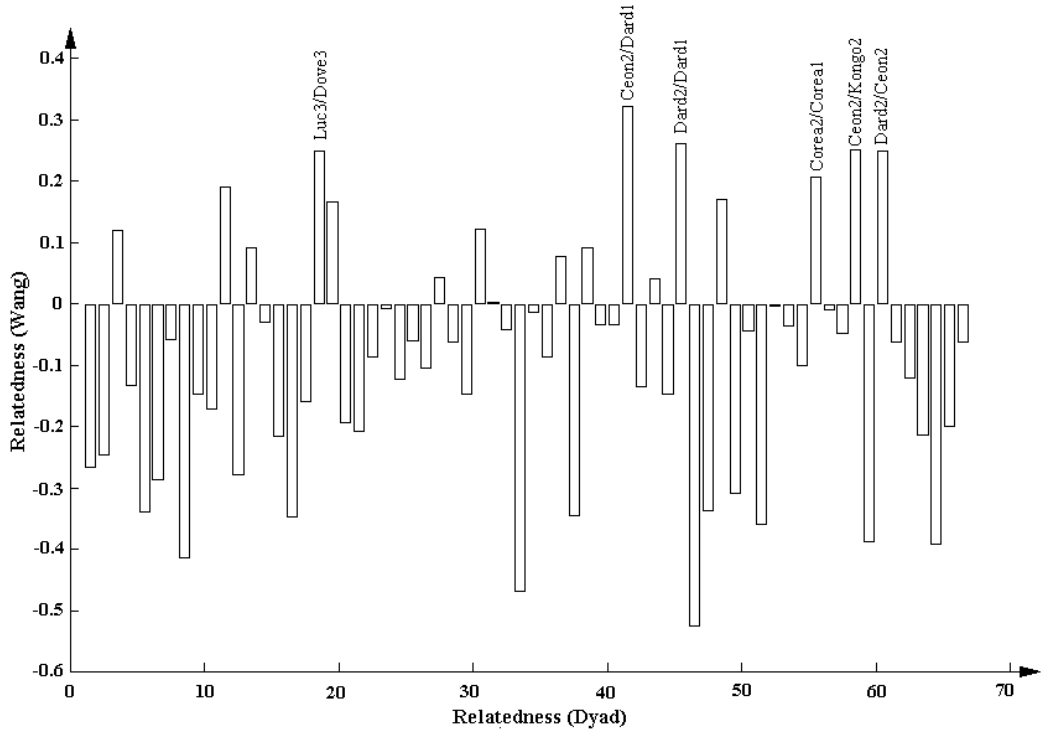


Figure 6. Bar graph showing relatedness levels between all adult dyads in the Limpopo Valley with important relationships labelled showing names of the individuals concerned.

Estimates of the effective population size (*Ne*) for the Limpopo Population was 55 (95% CI 28.0-290.9) based on linkage disequilibrium data (Waples & Do, 2008).

Discussion

Population dynamics and behaviour

Southern ground hornbill groups proved a challenge to capture due to the difficulty of locating birds, which occur at very low densities in the Limpopo Valley. The territory of at least one group in the Limpopo Valley is approximately 200 km² (Chapter 2) and although this was only confirmed for one group the spacing of groups from capture operations, examination of sighting data collected over the past 5 years, as well as spacing of identified nests seems to indicate comparable territory sizes for all groups. The study area, north of the Soutpansberg and bounded by the N1 highway to the East and the Platjan border post in the West (Figure 1) is approximately 6,000 km² in size which equates to a density of 600 km² per group, which includes tracts of habitat devoid of established groups. Individual SGH behaviour within groups during capture varied and at best shy individuals would eventually enter the trap, such as in the case of the Dovedale alpha male after a total of 7.48 hours or

never as is in the case of the Proefplaas alpha male (A total of 19.27 hours over 4 days). Juveniles responded differently and during capture would avoid the capture site and when territorial playbacks were used would immediately lie down. Juveniles were eventually lured to the capture site by not using call-ups in the early morning after the previous afternoon sessions. Adults would fly in at first light to investigate the trap and juveniles would follow. Certain juveniles were easily lured into the trap with food while others remained at a distance possibly due to the models in the trap.

The average group size in the Limpopo Valley (n=4) differs slightly from the 3.5 recorded in the KNP (Kemp & Kemp, 1980), 3.7 from the Natal midlands (Knight, 1990) and 3.5 in the Eastern Cape (Vernon, 1984). The main difference between groups in the Limpopo Valley and other populations is in the age and sex structure, although comprehensive data is only available for the Satara section of the KNP (Kemp & Kemp, 1980). Male to female ratios in the Limpopo Valley are 1:1 and the main reason for this is the absence of adult helpers in the population. Kemp & Kemp (1980) found that adult males outnumbered females by 1.4 due to two or three adult males being present in 18 group combinations out of a total of 28 (14 groups monitored over two separate years) while two females were only present in six group combinations out of a total of 28. The ratio of immatures (not yet displaying sexual maturity) to adults in the Limpopo Valley is also extremely high. 55% of the Limpopo Valley population were immatures in comparison to the Satara section of the KNP where only 20% of the population were immatures (Kemp & Kemp, 1980). The productivity in the Limpopo Valley is almost four times those recorded in the KNP where groups fledged on average one chick every 9.3 years (Kemp & Kemp, 1980). The average in the Limpopo Valley was determined according to the structure of the population as viewed over a one year period and only includes fledging rates from one nest. As such the estimated group average of 2.53 years per chick fledged is most likely an underestimate. The reasons for higher productivity in the Limpopo Valley is most likely a consequence of low densities of SGHs in the area and possibly the productivity of the environment over the last few years, which has experienced a higher average rainfall than normal (SA Weather Bureau, 1980-2009).

Southern ground hornbill dispersal and the re-colonisation of the Limpopo Valley

Microsatellite data assigned all offspring to the alpha females from their group of capture except in one case, indicating that offspring are inclined to stay in their natal territories at least until the age of approximately 3-5 years. The lack of 4–6 year old sub-adults seems to

be as a consequence of the dispersal of these individuals as they reach sexual maturity, rather than mortality rates, due to the high proportion of 0–3 year olds present in groups. Higher immature vs. sub-adult survival rates is unlikely or alternatively groups have only been successfully breeding in the past 6 years. Observational data supports the proposed high dispersal of individuals because during the study period the Lucern group was monitored (Chapter 2) and the sub-adult female and male would intermittently disappear and then return to their natal group. Both birds seemed to have left before the start of the 2009/2010 breeding season but this could not be confirmed. The dispersal of sub-adults are very little understood with regards to the movements these individuals make, survival and success rates of these individuals and the percentage of individuals that successfully establish new groups and breed.

The overall low levels of relatedness between adult males, adult females and all adults show that the recent colonisation of the Limpopo Valley was undertaken by a number of generally unrelated individuals of which no bias for any one particular sex could be detected as reflected by bootstrapping results which were not significant. This demonstrates the possibility that both sexes of SGH are able to disperse from their natal territories and both sexes may contribute equally to gene-flow within and between populations.

Genetic diversity as measured by observed and expected heterozygosities; and alleles per locus compare favourably to the genetic diversity measured in the much larger KNP population of SGH (Chapter 3). Genetic diversity is high among adults and remains so in the juvenile population. This is indicative of an outbreeding population where re-colonisation would have occurred from another source population/s and not by the gradual population growth from a few resident groups that survived in the Limpopo Valley. It is likely that some remnant groups or individuals have contributed to this gene pool. One such possible group is Driepan that have been breeding in the same tree for at least the last 40 years (Farmers, pers. comm.). Inbreeding levels were also very low for such a small population but the levels of relatedness that occurred between the adults in the Corea and Kongo groups indicate the strong possibility that these birds arrived from the same group or natal area where neighbouring groups are related. Furthermore, the estimated effective population size of 55 is much higher than the number of individuals that can be contributing offspring to the next generation, where only 10 groups consisting of a total of 40 individuals have been identified

(Table 1). These results once again indicate that individuals from other populations contributed to the gene pool in the Limpopo Valley.

Relatedness among groups

Kinship relationships between groups of SGHs were also studied in the Limpopo Valley. The two neighbouring groups, Lucern and Dovedale, were calculated as sharing an average relationship equivalent to that of half siblings (0.250). The history of the kinship links between these two groups is difficult to determine but it seems likely that the original ancestor/s of these groups were related on some level. This is supported by the second degree relationships detected between the alpha pairs of the groups Corea and Dardanellen. Whether these related individuals are remaining members of SGH groups before the historical extirpation of the population in the Limpopo Valley or whether similarly related individuals moved into the area as related coalitions after dispersing from their natal territories is open to conjecture. Very little supporting observational data is available but movement of individuals as well as pairs of SGH have been reported in the Limpopo Valley. The longevity of the species would further support the possible persistence of individuals for such a prolonged period of time in territories, before possible mates arrived from other populations. The establishment of kinship groups in the Limpopo Valley is a recent phenomenon but in established populations this structure may be much more defined with groups becoming less related by distance and with time the Limpopo Valley may show more defined genetic similarities between neighbouring groups.

SGH breeding behaviour

Genetic evidence for extra pair copulations were recorded and indicates that SGHs are not as monogamous as previously expected. The Lucern female produced two offspring with two separate mates and the Driepan female mated with the male from the Dardannelen group producing offspring. Whether this is a case of females or males moving between territories or male competition ousting males from groups is difficult to determine. It can be argued that the apparent facultative monogamy exhibited by the species is due to loose social bonds due to the recent establishment of the population, but further monitoring of this aspect of SGH behaviour is necessary in the Limpopo Valley as well as other more stable populations.

Use of microsatellite markers in southern ground hornbill behavioural studies

Demonstrating the potential for using microsatellite markers to study aspects of the SGHs mating behaviour, population dynamics, kinship, dispersal and relatedness are an important contribution of this study, especially in light of the difficulties of studying SGHs by more traditional means such as observational studies. Koening *et al*. (1992) suggested the importance of studying non-cooperatively breeding populations to contribute to our understanding of what causes co-operative breeding within a species, of which the Limpopo Valley population is the only known example within SGH, the largest co-operative breeding bird species in the world.

CHAPTER 5

SUMMARY, RECOMMENDATIONS AND FINAL CONCLUSIONS

CHAPTER 5: SUMMARY, RECOMMENDATIONS AND FINAL CONCLUSIONS

Following is a chapter by chapter summary of this study with conclusions and recommendations that can be made regarding SGH conservation and research in the Limpopo Valley and South Africa, and management recommendations.

Chapter 2: An investigation into the ecological requirements and associated habitat utilisation of a group of Southern Ground Hornbill in the Limpopo Valley.

In Chapter 2 the seasonal influence of environmental variables on invertebrate abundance and how this in turn affected the habitat usage of a single group of SGH was reported. It was shown that the Limpopo Valley is an extremely variable environment with sporadic and localised rainfall playing a pivotal role in sustaining ecological processes. The territory size of the SGH group is estimated to be approximately 200 km², the largest recorded for the species to date with SGH densities reported in the area being approximately 600 km² per group. This is six times lower than the density of SGH groups observed in the Kruger National Park (Kemp & Kemp, 1980) but includes habitat devoid of SGH groups. Although nests are not a limiting factor for the SGH groups monitored, the variability of this environment may lead to the uneven distribution of resources over space and time. For instance, all the nests recorded in the Limpopo Valley (n=7) are in Baobab trees (*Adansonia digitata*) and there seems to be a pattern with SGH distribution related to the presence of large Baobab trees (Pers. obs.). This observation, however, needs to be verified by future on-going monitoring of these groups. In this semi-arid landscape, with few rivers, the conditions for the growth of suitably large trees containing nest holes is severely restricted with baobabs the only common tree, not restricted to water courses, that can reach large enough dimensions for SGH nesting requirements. Baobabs are not evenly distributed within the Limpopo Valley and there are areas where they are rare and/or even absent which may preclude the establishment of SGH groups due to a lack of nesting sites. It is therefore plausible that the lack of large nest sites is the primary reason for the absence of SGHs in parts of the Limpopo Valley. As such, groups are distributed at low densities when compared to population densities in areas such as the KNP. Anthropogenic factors such as poisoning or persecution of SGH for window breaking may also contribute to the absence of groups in specific areas, even in situations where all other habitat requirements for the species are met, and such factors can thus not be ignored.

If nest sites and/or poisoning and/or persecution are the main factors limiting SGH, then availability of food resources is probably the main factor determining territory quality and size. The sporadic spatial and temporal nature at which food becomes available leads to food insecurity and the consequent adaptation of territory size by SGH. Rainfall plays a crucial role in the life cycles of specific large invertebrates such as mopani worms (*Imbrasia belina*), armoured ground crickets (*Acanthoplus sp.*) and monster tyrant beetles (*Manticora sp*.) which are dormant for most of the year and emerge with the first rains. These invertebrates in turn are an important food resource available to any animal able to access them throughout the wet and dry seasons. If rainfall were to be absent over a number of successive years, the survival of a species such as the SGH may be severely compromised due to a lack of invertebrate prey items which rely on the onset of rains. It is possible that the high productivity recorded by the current study, with groups fledging on average 1 chick every 2.53 years, is due to favourable environmental conditions over the past 5-10 years. The current situation is in stark contrast to the population crash which seemed to correspond with the severe drought conditions of the 1950's to 1960's. If drought conditions reoccur (which they invariably will) population declines may once again be experienced, negatively affecting the preservation of the species in the Limpopo Valley.

The following recommendations can be made:

1. Ideally on-going research in the Limpopo Valley should be designed over multiple seasons and years in order to better understand processes in this very seasonal environment and the ways in which SGH adapt to these changes. Specific objectives that could be pursued include: The seasonal abundance of invertebrates incorporating sampling techniques such as pitfalls and sweep netting at fixed sites. This can then be compared to results of spot sampling where seasonal outbreaks of invertebrates are sampled and/or areas where SGH are observed foraging. In addition, this can be compared to the breeding success of groups over time in relation to climatic events and food availability.
2. Further monitoring of the Limpopo Valley population is essential. Monitoring can focus on breeding success, successful recruitment and distribution patterns. The most practical means of achieving this is by:
    a. Continued monitoring of the breeding success of groups in the Limpopo Valley over time. Productivity rates can then be modelled against important

environmental variables such as rainfall and seasonal abundance of invertebrates. Permanent rain gauges can be erected at nests.

b. Identification, plotting and monitoring of new nest sites and potential nest sites.
c. Continued encouragement of farmers to report sightings of SGHs in order to understand changes in the distribution of the species in the Limpopo Valley and the effects this has on territory size.

3. Further home range and radio tracking studies of other groups in the area are encouraged in order to gain a better overall picture of the territory sizes of groups in the Limpopo Valley.
4. Artificial nests remain an important management tool in areas where natural nests are limiting and in the Limpopo Valley is a likely cause of the absence of groups from specific areas. There may be a correlation between the distribution of groups and large baobab trees. Only by mapping the distribution of baobab trees (which is possible with high resolution orthophotos) and by comparing this to sighting data can this hypothesis be tested. This would provide important baseline data in designing a plan for the erection of artificial nests which would be important to encourage the expansion of the current population into areas devoid of natural nest sites.
5. The mitigation of threats such as the persecution of SGH for window breaking and incidences of secondary poisoning will always remain a challenge for conservationists. With the expansion of SGHs in the Limpopo Province the emergence of window breaking has resurfaced. At least four groups in the Limpopo Valley were reported breaking windows (Local farmers, pers. comm.) and if this problem is not carefully monitored it may lead to these birds once again being persecuted. Farmers have shown a strong willingness to find solutions to this problem but the relationships formed between conservationists and farmers needs to be maintained. Regarding the responsible use of poisons, SGH may play an ambassadorial role due to their positive profile within farming communities in the Limpopo Valley, with farmers aware of the harmful effects of poisoning. Two farmers reported not using poisons as a means of controlling predators due to their fear of inadvertently poisoning SGHs and other bird species (Taute & Elsa, pers. comm.)
6. It is further recommended that to fully understand the factors that influence territory quality in relation to important resource requirements, studies need to be undertaken

in other parts of the species' range, especially in regions where smaller territory sizes are recorded so that the diversity of habitats in which SGHs occur are adequately represented. With a better understanding of the ecological and habitat requirements of the SGH, suitable areas can be identified and mapped to support conservation decisions in South Africa and further afield as well as to conserve important habitats associated with the species.

Chapter 3: A preliminary analysis of the genetic structure of the Southern Ground Hornbill in Africa.

A preliminary investigation into the levels of genetic diversity within and among populations of SGHs in Africa was reported on in Chapter 3. Fragmentation of SGH habitat is occurring and this is potentially affecting gene-flow between sub-populations. Genetic differentiation between populations was however shown to be minimal and although populations of SGH are becoming increasingly fragmented, genetic diversity has been maintained and gene-flow between populations still seems to be occurring or occurred recently. Initial results indicate that there is not enough differentiation between the populations to indicate the existence of sub-species although certain populations may be classified as sufficiently unique to warrant special conservation status. The KZN population, although only based on three samples, was the only population that seems to be genetically isolated but this needs further investigation.

From the above, the following recommendations on genetic management can be made:

1. It is critical that sampling is continued, to fill distribution gaps in Africa. Where samples are lacking these should be collected, such as in the KZN population to increase the statistical reliability of results. Although declines have been experienced throughout the species' range, the southern African populations seem to be most at threat and as such sampling should be concentrated in the neighbouring South African countries of Zimbabwe, Botswana and southern Mozambique.
2. The collection of samples from the KZN and the Eastern Cape provinces is urgent in order to clarify the genetic status of these birds and determine whether these populations are distinct and isolated enough, and whether priority should be given to the management of these populations.
3. Further genetic approaches need to be employed to confirm the preliminary results obtained and the addition of data from mitochondrial markers should strongly be considered.

4. Phenological studies should be undertaken to investigate whether there are differences in morphology between different populations of SGH in Africa.
5. More NGH samples also need to be included in the study. The NGH is a species which in the past has received very little research effort.

Chapter 4: A fine scale investigation into aspects of the biology of the Southern Ground Hornbill in the Limpopo Valley, with the aid of microsatellite markers.

Facets of the history, biology, social structure and breeding systems of a population of SGH in the Limpopo Valley, using microsatellite markers, were explored. Aspects focused on included: age and sex structures; productivity; the accuracy of field sexing criteria; the loss of genetic diversity (inbreeding) from one generation to the next; levels of relatedness within and among SGH groups; dispersal behaviour; the mode of re-colonisation in the Limpopo Valley; and mating behaviour. The suitability of microsatellite markers as a means to investigate these factors was also explored in the context of the practical (field) difficulties of researching SGH and the consequent lack of research conducted on the species in the past 10-15 years. Using molecular techniques, insights were gained into the biology and social behaviour of the species, in the Limpopo Valley, that would not easily have been detected or discovered by traditional observational methods. Molecular analysis is therefore a powerful tool in the research and conservation of SGHs that can be undertaken relatively quickly with high levels of output. Genetic analysis can easily contribute to studies of the species where access to SGH biological samples are available and should be encouraged.

The following recommendations can be made:

1. Further genetic studies in other parts of the species' range where co-operative breeding successfully occurs; and/or adult helpers are present in the group but breeding is not occurring, needs to be completed in order to better understand SGH breeding behaviour. This will also contribute to a better understanding of co-operatively breeding bird species in general. It is hoped that further insights will be gained on the apparent polygamy, dispersal of individuals from groups and the kinship structure within populations using genetic markers. Much remains to be discovered about this enigmatic species which is the largest co-operative breeding bird species in the world and a species under increasing threat.

2. The development of other markers such as mitochondrial DNA sequences would greatly enhance the potential of parentage analyses and therefore our understanding of this species.
3. Genetic methods also have a huge amount of potential for use in the management of captive SGH populations and should be used to supplement studbook information and help guide captive management policy and objectives.

Conservation recommendations

The recovery and persistence of SGHs in South Africa is largely dependent on: (1) the continued identification and conservation of SGH populations and their associated habitat; and (2) the re-establishment of populations in areas where SGHs historically occurred and where suitable habitat remains. Because of the large territory sizes of SGH in South Africa, co-operative breeding strategies and low population turnovers, the preservation of viable populations will always be difficult to attain. Even the KNP population, which resides in the largest protected area in South Africa (at over 2.2 million hectares in extent), shows signs of a genetic bottleneck. Although fragmentation has occurred populations are often separated by large areas of suitable habitat and re-colonisation does occur naturally as displayed in the Limpopo Valley (Chapter 4). Re-establishment of groups in these areas is also possible by re-introduction as achieved on Mabula Private Game Reserve (Theron & Turner, 2008). The loss of SGHs from areas where suitable habitat still remains shows the sensitivity of the species to human induced threats such as loss of large nesting trees, poisoning and persecution. Nevertheless where these threats can be mitigated rendering territories safe, an important opportunity exists where populations can be established. This has in fact been done with varying degrees of success in South Africa where a combination of hand reared and wild rehabilitated birds have been released (Theron & Turner, 2008). An option that has not fully been explored though is the translocation of wild birds. This can be achieved by moving individuals from areas of high SGH density such as in the KNP where suitable habitat seems to be saturated (Kemp, 1988) to areas where SGH do not occur but occurred historically. Possible areas for these translocations include parts of the Limpopo Valley or areas surrounding the currently established group on Mabula Private Game Reserve. Young adults within groups from the KNP could be considered for translocation within the Limpopo Province. The KNP represents the closest stable population of SGHs to the Limpopo Valley and is the largest population of SGH in South Africa. Viable populations to the north only seem to occur in the northern parts of Zimbabwe and Botswana and parts of Mozambique,

but this needs to be further investigated. Therefore the KNP population is the only available source of individuals for consideration in a translocation process in the Limpopo Province. In essence the future conservation of SGHs may be characterised by an integrated approach where isolated populations are managed as a meta-population and the dispersal of individuals is artificially maintained into areas of suitable habitat where SGH historically occurred.

REFERENCES

REFERENCES

Aggarwal, R., Allainguillaume, J., Bajay, M.M., Barthwal, S., Bertolino, P., Chauhan, P., Consuegra, S., Croxford, A., Dalton, D., Belder, D., Di´az-ferguson, E., Douglas, R., Drees, M., Elderson, J., Esselink, D., Fernandez-manjarre, J.F., Frascaria-lacoste, N., Gabler-Schwarz, S., Garcia de Leaniz, C., Ginwal,H.S., Goodisman, M.D., Guo, B., Hamilton, M.B., Hayes, P.K. Hong, Y., Kajita, T.,Kalinowski, S.T., Keller, L., Koop, B.F., Kotze, A., Lalremruata, A., Leese, F., Li, C., Liew, W.Y., Martinelli, S., Matthews, E.A., Medlin, L.K., Messmer, A.M., Meyer, E.I., Monteiro, M., Moyer, G.R., Nelson, R.J., Nguyen, T.T., Omoto, C., Ono, J., Pavinato, V.A.C., Pearcy, M., Pinheiro, J.B., Power, D., Rawat, A., Reusch, T.B.H., Sanderson, D., Sannier, J., Sathe, S., Sheridan, C.K., Smulders, M.J.M., Sukganah, A., Takayama, K., Tamura, M., Tateishi, Y., Vanhaecke, D., Vum, N.V., Wickneswari, R., Williams, A.S., Wimp, G.M., Witte, V. and Zucchi, M.I. 2010. Permanent genetic resources added to molecular ecology resources database 1 August 2010 – 30 September 2010. *Molecular Ecology Resources*. Manuscript submitted for publication.

Berg, E.C., Eadie, J.M., Langen, T.A. and Russels, A.F. 2009. Reverse sex-biased philopatry in a cooperative bird: Genetic consequences and a social cause. *Molecular Ecology*. 18: 3486–3499.

BirdLife International [Online]. 2010. BirdLife International. Available on the internet at: www.birdlife.org.za (01-03-2010).

Block, W.M. and Brennan, L.A. 1993. The habitat concept in ornithology: Theory and applications. *Current Ornithology*. 11: 35-91.

Blouin, M.S. 2003. DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. *Trends in Ecology and Evolution*. 18(10): 503-511.

Brewer, R. 1994. *The Science of Ecology. 2nd Edition*. Saunders College Publishing, Orlando. 773 pp.

Brown, V.K. 1984. Secondary succession: Insect-plant relationships. *Bioscience*. 34(11): 710-716.

Brussard, P.F. 1991. The Role of Ecology in Biological Conservation. *Ecological Applications*. 1(1): 6-12.

Brussard, P.F and Ehrlich, P.R. 1992. The Challenges of Conservation Biology. Ecological Applications. 2(1).

Clarke, A.L., Sæther, B.E. and Røskaft, E. 1997. Sex Biases in Avian Dispersal: A reappraisal. *Oikos*. 79(3): 429-438.

Cohen, J.E. 2003. Human Population: The Next Half Century. *Science.* 302: 1172-1175.

Cornuet J.M. and Luikart, G. 1996. Description and power analysis of two tests for detecting recent demographic bottlenecks from allele frequency data. *Genetics*. 144: 2001–2014.

Cousins, J. A., Sadler, J.P. and Evans, J. 2008. Exploring the role of private wildlife ranching as a conservation tool in South Africa: Stakeholder perspectives. *Ecology and Society.* 13(2): 43.

Crandell, K.A., Bininda-Emonds, O.R.P., Mace, G.M. and Wayne, R.K. 2000. Considering evolutionary processes in conservation biology. *Tree*. 15(7): 290 – 295.

Cunningham, P.L. 1996. Prospects for sustained harvesting of mopane (*Colophospermum mopane*) on the Venitia Limpopo Nature Reserve and its implications for browsing ungulates. Unpublished MSc dissertation, University of Stellenbosch, South Africa.

Delport, W., Ferguson, J.W. and Bloomer, P. 2002. Characterization and evolution of the mitochondrial DNA control region in hornbills (Bucerotiformes). *Journal of Molecular Evolution*. 54(6): 794 – 806.

DeSalle, R. and Amato, G. 2004. The expansion of conservation genetics. *Nature Reviews Genetics*. 5: 702-712.

DeYoung, R.W. and Honeycutt, R.L. 2005. The Molecular Toolbox: Genetic techniques in wildlife ecology and management. *The Journal of Wildlife Management*. 69(4): 1362-1384.

Du Plessis, M.A., Siegfried, W.R. and Armstrong, A.J. 1995. Ecological and life-history correlates of cooperative breeding in South African birds. *Oecologia*. 102(2): 180-188.

Excoffier, L., Laval, G., and Schneider, S. 2005. Arlequin, Version 3.0: An Integrated software package for population genetics data analysis. Computational and Molecular Population Genetics Laboratory (CMPG). Institute of Zoology, University of Berne, Switzerland.

Frankham, R., Ballou, J.D. and Briscoe, D.A. 2002. *Introduction to Conservation Genetics*. Cambridge University Press, Cambridge. 616 pp.

Gaston, K.J., Chown, S.L. and Styles, C.V. 1997. Changing size and changing enemies: The case of the mopane worm. *Acta Ogcologica*. 18(1): 21-26.

Greenwood, P.J. 1980. Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour*. 28: 1140-1162.

Hailer, F., Helander, B., Folkestad, A.O., Ganusevich, S.A., Garstad, S., Hauff, P., Koren, C., Nygard, T., Volke, V., Vila, C. and Ellegren, H. 2006. Bottlenecked but long-lived: High genetic diversity retained in white-tailed eagles upon recovery from population decline. *Biology Letters*. 2: 316-319.

Hall, L.S., Krausman, P.R. and Morrison, M.L. 1997. The habitat concept and a plea for standard terminology. *Wildlife Society Bulletin*. 25(1): 173-182.

Hedrick, P.W.1999. Perspective: Highly variable loci and their interpretation in evolution and conservation. *Evolution*. 53(2): 313-318.

Hedrick, P.W. 2000. *Genetics of Populations*. *2nd Edition*. Jones and Bartlett. Sudbury, Massachusetts. 553 pp.

Hedrick, P.W. and Miller, P.S. 2002. Conservation genetics: techniques and fundamentals. *Ecological Applications*. 2: 30-46.

Johnson, J.A., Toepfer, J.E. and Dunn, P.O. 2003. Contrasting patterns of mitochondrial and microsatellite population structure in fragmented populations of greater prairie-chickens. *Molecular Ecology*. 12: 3335-3337.

Jones, O.R. and Wang, J. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources*. 10: 551–555.

Jordaan, J.J., Wessels, D.C.J., Dannhauser, C.S. and Rootman, G.T. 2004. Secondary succession in the Mopani Veld of the Limpopo Valley, South Africa. *African Journal of Range and Forage Science*. 21(3): 205-210.

Kemp, A.C. 1976. Factors affecting the onset of breeding in African hornbills. *Proceedings of the 16th International Ornithological Congress*. Canberra City, Australia. 248-257.

Kemp, A.C. 1988. The behavioural ecology of the Southern Ground Hornbill: Are competitive offspring at a premium? *Proceedings of the International 100 DO-G Meeting, Current Topics in Avian Biology*. Bonn: 267-271.

Kemp, A.C. 1995. *The Hornbills, Bucerotiformes*. Oxford University press, Oxford.

Kemp, A.C. 2000. Southern Ground Hornbill. *In*: Barnes, K.N. (Ed). *The Eskom Red Data Book of Birds of South Africa, Lesotho and Swaziland*. BirdLife South Africa, Johannesburg. Pp. 117-119.

Kemp, A.C. 2005. Ground Hornbills. *In*: Hockey, P.A.R., Dean, W.A.J and Ryan, P.G. (Eds). *Roberts Birds of Southern African*. *7th edition*. John Voelcker Bird Book Fund, Cape Town. Pp. 158-159.

Kemp, A.C. and Begg, S.G. 1995. Nest sites of the Southern Ground Hornbill *Bucorvus leadbeateri* in the Kruger National Park, South Africa, and conservation implications. *Ostrich*. 67: 9-14.

Kemp, A.C., Joubert, S.C.J. and Kemp, M.I. 1989. Distribution of Southern Ground Hornbills in the Kruger National Park in relation to some environmental features. *Southern African Journal of Wildlife Research.* 19(3): 93-98.

Kemp, A.C. and Kemp, M.I. 1980. The biology of the Southern Ground Hornbill *Bucorvus leadbeateri.*(Vigors) (Aves: Bucerotidae). *Annals of the Transvaal Museum*. 32(4): 65-100.

Kemp, A.C. and Kemp, M.I. 1991. Timing of egg laying by Southern Ground Hornbills *Bucorvus leadbeateri* in the central Kruger National Park, South Africa. *Ostrich*. 62: 80-82.

Kemp, A.C. and Webster, R. 2008. Latest analysis of Southern Ground Hornbill (SGH) distribution and population in South Africa. *Unpublished Report.* Mabula Ground Hornbill Project.

Kemp, M.I. and Kemp, A.C. 1978. Bucorvus and Sagittarius: Two modes of terrestrial predation. *In*: Kemp, A.C. (Ed). *Proceedings of the Symposium on African Predatory Birds*. Northern Transvaal Ornithological Society, Pretoria. Pp. 13-16.

Knight, G.M. 1990. Status, distribution and foraging ecology of the Southern Ground Hornbill (*Bucorvus cafer*) in Natal. Unpublished MSc dissertation, University Natal, Durban

Koening, W.D., Pitelka, F.A., Carmen, W.J., Mumme, R.L. and Stanback, M.T. 1992. The evolution of delayed dispersal in cooperative breeders. *The Quarterly Review of Biology*. 67(2): 111-150.

Lynch, M. 1988. Estimation of relatedness by DNA fingerprinting. *Molecular Biology and Evolution*. 5: 584–599.

Maclean, G.L. 1990. *Ornithology for Africa*. University of Natal Press, Pietermaritzburg. 270 pp.

Maher, R. and Lott, D.F. 2000. A Review of ecological determinants of territoriality within vertebrate species. *American Midland Naturalist.* 143(1): 1-29.

Martínez-Cruz, B., Godoy, J.A and Negro, J.J. 2004. Population genetics after fragmentation: the case of the endangered Spanish imperial eagle (*Aquila adalberti*). *Molecular Ecology*. 13: 2243-2255.

McCune, B and Mefford, M.J. 1999. PC-Ord. Multivariate analyses of ecological data, (Ver. 4). MjM Software Design, Gleneden Beach, Oregon, USA.

Meadows, M.E. and Hoffman, T.M. 2003. Land degradation and climate change in South Africa. *The Geographical Journal*. 169(2): 168-177.

Michalakis, Y. and Excoffier, L. 1996. A genetic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. *Genetics.* 1061–1064.

Milligan, B.G. 2003. Maximum-likelihood estimation of relatedness. *Genetics*. 163: 1153-1167.

Morrison, K., Daly, B., Burden D., Engelbrecht, D., Jordan M., Kemp, A., Kemp, M., Potgieter, C., Turner A. and Friedmann, Y., (Eds). 2005. *Southern Ground Hornbill (Bucorvus leadbeateri) PHVA Workshop Report*. Conservation Breeding Specialist Group (SSC / IUCN) /CBSG South Africa: Endangered Wildlife Trust.

Mucina, L. and Rutherford, M.C. (Eds). 2006. The vegetation of South Africa, Lesotho and Swaziland. *Strelitzia 19.* South African National Biodiversity Institute, Pretoria.

Mucina, L., Rutherford, M.C. and Powrie, L.W. 2005. Vegetation map of South Africa, Lesotho and Swaziland. South African National Biodiversity Institute, Pretoria.

Neef, B.D. and Gross, M.R. 2001. Microsatellite evolution in vertebrates: Inference from AC dinucleotide repeats. *Evolution*. 55(9): 1717-1733.

Nei, M. 1972. Genetic distances between populations. *Amateur Naturalist.* 283–292.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences*. 70(12): 3321-3323.

Nei, M. 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York, NY, USA.

Newton, I. 1980. The role of food in limiting bird numbers. *Ardea*. 68: 11-30.

Oberprieler, R. 1986. *Pinus radiata*. Don: A new foodplant record for *lmbrasia (Gonimbrasia) belina* (Westwood) (Lepidoptera: Saturniidae). *Journal of the Entomological Society of Southern Africa*. 49: 168-170.

Ota, T. 1993. *DISPAN: A software for genetic distances and neighbor-joining calculations*. Institute of Molecular Evolutionary Genetics. University Park, PA: The Pennsylvania State University.

Park, S. 2001. Microsatellite Toolkit. http://oscar.gen.tcd.ie/sdepark/ms-toolkit. (5 March 2008)

Patterson, C. and Khosa, P. 2005. *A status quo study on the professional and recreational hunting industry in South Africa.* Report to the Minister of Environmental Affairs and Tourism, Panel of Experts on Professional and Recreational Hunting in South Africa. Pretoria, South Africa.

Perner, J and Schueler, S. 2004. Estimating the density of ground-dwelling arthropods with pitfall traps using a nested-cross array. *Journal of Animal Ecology.* 73: 469-477.

Piertney, S.B., MacColl, A.D.C., Bacon, P.J. and Dallas, J.F. 1998. Local genetic structure in red grouse (*Lagopus lagopus scoticus*): evidence from microsatellite DNA markers. *Molecular Ecology*. 7: 1645-1654.

Pimm, S., Raven, P., Peterson, A., Sekerciog, C.H. and Ehrlich, P.R. 2006. Human impacts on the rates of recent, present, and future bird extinctions. *Proceedings of the National Academy of Sciences*. 13(29): 10941-10946.

Pringle, R.M., Young, T.P., Rubenstein, D.I. and McCauley, D.J. 2007. Herbivore-initiated interaction cascades and their modulation by productivity in an African savanna. *Proceedings of the National Academy of Sciences. 104(1): 193-197.*

Prior, K.A., Gibbs, H.L. and Weatherhead, J. 1997. Population genetic structure in the Black Rat Snake: Implications for management. *Conservation Biology*. 11(5): 1147-1158.

Pritchard, J. K., Stephens, M. and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics*. 155: 945–959.

Rhymer, J.M., Fain, M.G., Austin, J.E., Johnson, D.H. and Krajewski, C. 2001. Mitochondrial phylogeography, subspecific taxonomy, and conservation genetics of sandhill cranes (*Grus canadensis*; Aves: Gruidae). *Conservation Biology*. 2: 203-218.

Ritland, K. 1996. Estimators for pairwise relatedness and inbreeding coefficients. *Genetical Research*. 67: 175–186.

Rivers-Moore, N.A. and Samways, M.J. 1996. Game and cattle trampling, and impacts of human dwellings on arthropods at a game park boundary. *Biodiversity and Conservation* 5: 1545-1556.

Rutherford, M.C. and Westfall, R.H. 1994. Biomes of southern Africa: An objective categorization. *Memoirs of the Botanical Survey of South Africa no. 63.* National Botanical Institute, Pretoria.

Schaal, B.A. and Leverich, W.J. 2005. Conservation Genetics: Theory and Practice. *Annals of the Missouri Botanical Garden.* 92(1) 1-11.

Schoener, T.W. 1968. Sizes of feeding territories among birds. *Ecology*. 49(1): 123-141.

Seaman, A.D. and Powell, R.A. 1996. An evaluation of the accuracy of kernel density estimators for home range analysis. *Ecology*. 77(7): 2075-2085.

Sonsthagen, S.A., Talbot, S.L. and White, C.M. 2004. Gene flow and genetic characterization of Northern Goshawks breeding in Utah. *The Condor*. 106(4): 826-836.

Soulé, M.E. 1985. What Is Conservation Biology? *BioScience*. 35(11) 727-734.

South, A.B., Kenward, R.E. and Walls, S.S. 2008. *Ranges 7: For the analysis of tracking and location data*. Online Manual. Anatrack Ltd. Wareham, UK.

Standen, V. 2000. The adequacy of collecting techniques for estimating species richness of grassland invertebrates. *Journal of Applied Ecology*. 37(5): 884-893.

StatSoft Inc. 2009. STATISTICA (Data analysis software system). Version 9.

Tainton, N.M. 1999. The savanna biome. *In*: Tainton, N.M. (Ed). *Veld Management in South Africa*. University of Natal Press, Pietermaritzburg. Pp. 33-36.

Tarboton, W.R. 1980. Avian populations in Transvaal savanna. *In*: Johnson, D.N. (Ed). *Proceedings of the 4th Pan-African Ornithological Congress*. Southern African Ornithological Society, Johannesburg. Pp. 113-124.

Theron, N. and Turner, A. 2008. Ten years on: a re-introduction of Southern Ground Hornbill on Mabula Private Game Reserve in the Limpopo Province of South Africa. *In*: Soorae, P.S. (Ed). *Global Re-introduction Perspectives. Re-introduction Case Studies from Around the World.* IUCN/SSC Re-introduction Specialist Group. Abu Dhabi, UAE. Pp. 104-107.

Thomas, S.C. 2005. The estimation of genetic relationships using molecular markers and their efficiency in estimating heritability in natural populations. *Philosophical Transactions: Biological Sciences*. 360: 1457-1467.

Van De Casteele, T., Galbusera, P. and Matthysen, E. 2001. A comparison of microsatellite-based pairwise relatedness estimators. *Molecular Ecology*. 10: 1539-1549.

Van Den Busschel, R.A., Harmon, S., Baker, R.J., Lawrence, A., Ryan, A.L., Rogers, J.R., Harris, M.J. and Brisbin, I.L. 1999. Low levels of genetic variability in North American populations of the Wood Stork (*Mycteria americana*). *The Auk*. 116(4): 1083-1092.

Venter, J.F., Scholes, R.J. and Eckhardt, H.C. 2003. The abiotic template and its associated vegetation pattern. *In*: Du Toit, J.T., Rogers, K.H. and Biggs, H.C. (Eds). *The Kruger Experience: Ecology and Management of Savannah Heterogeneity*. Island Press, Washington. Pp. 83-129.

Vernon, C.J. 1984. The Ground Hornbill at the Southern extremity of its range. *Ostrich*. 57: 16-24.

Wang, J. 2002. An estimator for pairwise relatedness using molecular markers. *Genetics*. 160: 1203–1215.

Wang, J. 2004. Sibship reconstruction from genetic data with typing errors. *Genetics*. 166: 1963–1979.

Wang, J. 2010. COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Molecular Ecology Resources*. Computer Program Note.

Wang, J. and Santure, A.W. 2009. Parentage and sibship inference from multilocus genotype data under polygamy. *Genetics*. 181: 1579-1594.

Waples, R.S. and Do, C. 2008. LDNE: a program for estimating effective population size from data on linkage disequilibrium. *Molecular Ecology Resources*. 8: 753-756.

Wessels, K.J., Prince, S.D., Carroll, M. and Malherbe, J. 2007. Relevance of rangeland degradation in semi-arid north eastern South Africa to the non-equilibrium theory. *Ecological Applications*. 17(3): 815-827.

Wolcott, T.G. 1995. New options in physiological and behavioural ecology through multichannel telemetry. *Journal of Environmental Marine Biology and Ecology*. 193: 257-275.

Wright, S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution*. 19(3): 395–420.

Wright, J. S. 1978. *Evolution and the genetics of natural populations*. *Volume 4*. University of Chicago Press, Chicago, Illinois.