

EXPLORING THE MAINTENANCE OF PLUMAGE POLYMORPHISM IN THE BLACK SPARROWHAWK



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ABSTRACT

Animals often display striking variation with respect to their phenotype. Intraspecific and interspecific variation in body colour represents one of the most well studied forms of phenotypic variation. For decades evolutionary biologists have been fascinated by the mechanisms that maintain colour variation in species and traditional explanations for this diversity of colour in nature often invoke an interaction between selection for conspicuous signals and natural selection for crypsis. Colour polymorphic species have frequently been used to explore the evolutionary processes that lead to colour variation in species. Geographic variation in colour morph ratios also occurs frequently in polymorphic species and is often considered an ideal model system to examine the interplay of gene flow and local adaptation in populations. This thesis aims to explore the role and maintenance of plumage colour polymorphism in a raptor, the black sparrowhawk.

The black sparrowhawk exhibits discrete colour polymorphism, with adults occurring as either white or dark morphs. Within South Africa, the species has undergone a recent range expansion, successfully colonising the Cape Peninsula in the Western Cape. As winter breeders, black sparrowhawks in South Africa now experience two contrasting climatic regimes; dry winters in their historical north-eastern range, and wet winters in the recently colonised Western Cape region. Within this newly colonised region, the dark morph occurs in greatest frequency. Across South Africa, the species displays clinal variation, with the frequency of dark morphs declining from > 75 % in the far south-west on the Cape Peninsula, to < 20 % in the north-east of the country. Two contrasting hypotheses have been proposed for the high frequency of dark morph birds in the Cape Peninsula population; (1) that colour variation is non-adaptive and is simply due to a chance founder effect and strong genetic drift and (2) that this is reflective of local adaptation and that irrespective of the founding morph ratios, dark morphs have a selective advantage in this newly colonised environment with its novel winter rainfall regime.

The main aims of this study were to determine the (i) ecological and evolutionary mechanisms that influence the maintenance of colour polymorphism in the species and (ii) to establish explanations for the unusually high proportion of dark morphs on the Cape Peninsula. In this thesis I have used a range of ecological and genetic approaches to explore both neutral and adaptationist explanations for the high frequency of dark morphs in my study population. Data from the mitochondrial control region was used to examine the distribution of genetic diversity in several geographic populations of black sparrowhawks across South Africa, allowing the exploration of trait divergence under neutrality. Using a phylogeographic framework, genetic variation was used to (i) quantify the extent to which population structure and gene flow may influence the observed pattern of colour morphs in the focal study population on the Cape Peninsula, and (ii) explore how selection and gene flow may interact to explain the patterns of morph frequencies in my study system. I found very low genetic differentiation between sample sites across South Africa suggesting that substantial gene flow occurs among populations, supporting the hypothesis that selection, and thus local adaptation, is the primary force maintaining colour variation on the Peninsula.

I then explored why dark morphs might have a selective advantage on the Cape Peninsula. To do this I investigated various components of behaviour; including foraging success (prey provisioning) as well as foraging behaviour and habitat preference, together with key demographic fitness parameters (reproduction and survival) between the two morphs in the Cape Peninsula population. Much of this research was based around a previous hypothesis that different morphs may be better adapted to different light levels. In keeping with this hypothesis, I found that ambient light conditions strongly influenced the foraging success of the different coloured morphs; dark morphs delivered more prey during low light conditions, whereas the opposite relationship was found for white morphs, which appeared to have a foraging advantage in brighter conditions. This advantage is also reflected in the distribution of morphs across South Africa, with the proportion of dark morphs strongly correlated with breeding season light level. These findings suggest that, under disruptive selection, ambient light conditions interact with morph colour to improve foraging success, supporting the hypothesis that the two morphs may be better adapted to foraging under different light conditions. Exploring this more

directly using GPS-tagged male black sparrowhawks I found that dark morphs displayed greater foraging activity and effort in lower light conditions and showed a stronger degree of selection for closed habitats with denser vegetation cover. In contrast, white morphs displayed greater foraging activity and effort in brighter conditions and showed a stronger degree of selection for open habitat types. Morphs therefore forage during conditions, and within habitats (related to the light conditions in a specific habitat), that are likely to enhance crypsis from their avian prey and thus are investing time into foraging activities during the conditions which will maximize their foraging success. I then examined the fitness consequences of the differential adaptations to the light environment displayed in the different morphs by comparing their long-term reproductive performance, both in isolation and in combination with their breeding partner morph, as well as their survival. I found that neither morph had a specific advantage in terms of reproductive performance or survival in my study population. There did however appear to be an advantage of mating with the opposite morph, with mixed-pairs producing almost a quarter more offspring per year than pairs consisting of partners of the same morph. While my results suggest an advantage of mating with the opposite morph, there was no evidence for disassortative mating; instead breeding pair morph combinations were random with respect to the background frequencies of the two morphs. Higher productivity of mixed-pairs may be the result of the complementary nature of care provided by the different morphs. I propose that differential foraging success and behaviour between black sparrowhawk morphs under varying light conditions, as well as variable morph habitat preference, allows mixed-pairs to expand their foraging niche; therefore, mixed-pairs are able to exploit a wider range of habitat (open and closed) as well as a broader window of light conditions (cloudy vs. non-cloudy or dawn & dusk vs. midday) in which to hunt. I conclude that emergent pair-level properties may play an important role in promoting and maintaining polymorphism in the black sparrowhawk. My findings provide important supporting evidence that predator-prey interactions are a major component in the maintenance of polymorphisms in natural populations and visual detection by prey is therefore likely to be a key selective agent for colouration in polymorphic predatory hawks.

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Holding a Black Sparrowhawk in the hand is such a privilege and a great opportunity to marvel at their design; their eyes a deep red with highly responsive pupils that pick up even the slightest of movements. Their sleek aerodynamic body with short broad wings and long tail are perfectly shaped for life hunting and maneuvering in the forest. Their oversized talons and long legs are perfectly equipped for taking down their avian prey. The Black Sparrowhawk is a true “forest enigma” of Africa. Photo: Amir Rezaei.



CHAPTER 1

INTRODUCTION



Chart farm female, Constantia, Cape Town, July 2013. This dark morph individual was a very special addition to my research and well worth mentioning. She was the only Black Sparrowhawk that I've ever witnessed to stay on her nest during chick ringing and camera installments, allowing us to capture some incredible shots of her and her clutch. I grew very fond of her over the years and she was a living proof of how substantially Black Sparrowhawk individuals differ in terms of their temperament and character. Photo: Mark Cowen.

PHENOTYPIC VARIATION IN NATURE

One of the most striking features evident throughout the natural world is the extensive variation that exists within and among species. From the smallest insects to the largest plants, the elaborate shapes, designs, patterns and colours species display have presented an intellectual challenge to biologists seeking to understand and explain biodiversity within the framework of evolution by natural selection (Darwin, 1859). Across the spectra of multicellular life, species display widespread inter- and intraspecific variation in their morphology, physiology and behaviour i.e. in their phenotype, which has likely been shaped by complex processes and selective pressures over ecological and evolutionary timescales across ever-changing environments (Meyers & Bull, 2002; Forsman *et al.*, 2008). One of the most fundamental pursuits in the field of evolutionary biology is to establish the underlying mechanisms that drive and maintain phenotypic diversity in nature (Darwin, 1859; Fisher, 1930). Knowledge of the evolutionary processes that lead to phenotypic variation is essential for understanding how well a species might adapt and cope with future changes in the environment (Root *et al.*, 2003; Hubbard *et al.*, 2010). This is especially important in the light of contemporary and rapid global climate change, habitat degradation and loss, increasing urbanisation (Parmesan, 2006; Gil & Brumm, 2014) and their collective threat to biodiversity on a global scale (Hoffman *et al.*, 2010; Wennersten & Forsman, 2012).

The ecological success and evolutionary stability of species have frequently been linked to the extent of inter-individual variation present in a population (Darwin, 1859; Van Valen, 1965; Hughes *et al.*, 2008; Forsman, 2014, 2016; Forsman *et al.*, 2015). For example, phenotypic variation within populations may lead to certain individuals being better adapted to particular environmental conditions (Darwin, 1859; Van Valen, 1965; Gould & Johnston, 1972); this is widely considered to be a prerequisite for evolution by natural selection (Gray & McKinnon, 2007; Hugall & Stuart-Fox, 2012; McLean & Stuart-Fox, 2014). Species that display greater variability in their phenotype therefore generally have a higher evolutionary potential, which may facilitate divergence between populations

and thus speciation (Gray & McKinnon, 2007; Hugall & Stuart-Fox, 2012). Theory and recent reviews propose that greater heritable variation is likely to benefit both population abundance and stability (Forsman *et al.*, 2008) and thereby enhances the ecological success of populations by shielding them against environmental change; this enables growth, allowing for higher densities and reduced fluctuations in population size (Dobzhansky, 1951; Van Valen, 1965; Wennersten & Forsman, 2012; Forsman *et al.*, 2015).

Animal Body Colour and Pattern

Variation in animal body colour and pattern, both within and among species, represents one of the most taxonomically diverse and well documented forms of phenotypic variation in nature (Hingston, 1933; Cott, 1940; Endler, 1977; Forsman, 1999a; Hill & McGraw, 2006; McLean & Stuart-Fox, 2014). Although overarching explanations remain elusive (Brooke, 2010), studies have shown that colouration and pattern is under strong selective pressure from both natural and sexual selection (Darwin, 1859; Cott, 1940; Bortolotti, 2006; Roulin *et al.*, 2009; Hubbard *et al.*, 2010) and influences a variety of performance and fitness related characteristics of individuals; it is important to note that sexual selection is a mode of natural selection and is not parallel to it (Endler, 1984; Starr *et al.*, 1987; Andersson, 1994). The ecological and evolutionary function of colour and pattern has therefore been a highly attractive topic for many years amongst evolutionary biologists and, in particular, amongst ornithologists (Bortolotti, 2006).

Sexual Selection and Colouration

Most modern biologists widely consider animal colouration to be the product of sexual selection (Darwin, 1859; Bortolotti, 2006). Although there are some exceptions (i.e. Amundsen & Pärn, 2006), this mostly relates to sexually colour dimorphic species (Orians, 1969; Endler, 1991b; Seehausen & Van Alphen, 1998; Griffith & Pryke, 2006; Stoehr *et al.*, 2006). Indeed, across the vertebrates, in numerous species, colour and pattern play an important role as visual signals in mate choice (i.e. intraspecific signalling) (Endler, 1980, 1987; Seehausen & Van Alphen, 1998; Summers *et al.*, 1999; Waitt *et al.*, 2003; Hill, 2006; Senar, 2006; Hurtado-Gonzales *et al.*, 2014; League, 2016). One of the

best examples is evident in bird mating systems, where females pay close attention to subtle variations in male plumage when choosing a mate (Orians, 1969; Pruett-jones & Pruett-Jones, 1990; Griffith & Pryke, 2006). Often males with the brightest, most conspicuous colours and plumage ornaments are selected, as they may signal a variety of different benefits to their partners, with many of these having direct consequences for the subsequent life-history and reproductive success of that individual (Griffith & Pryke, 2006). Colour and pattern may therefore signal “honest measures” of fitness in mates, such as genetic or phenotypic quality; including the ability to fend off diseases and competitors, body condition, reproductive success, and survival prospects (Roulin & Bize, 2007; Schuett *et al.*, 2010; Horváthová *et al.*, 2012). In many vertebrates and invertebrates, these honest signals of greater biological fitness often lower individual quality through handicapping their behaviour or morphology. This is known as ‘The Handicap Principle’ (Zahavi, 1975, 1997) and has been argued as the most likely explanation for the conspicuous colours and ornaments in species displaying strong sexual dimorphism.

Natural Selection and Colouration

While the relative fitness advantage of maintaining colour and pattern variation within species has been explored extensively within the context of sexual selection, researchers exploring the adaptive function of colour and pattern are beginning to reveal important new findings of how natural selection has more generally shaped animal colour and pattern (Hill & McGraw, 2006). Two themes dominate the literature examining how natural selection operates in the evolution of animal colour and pattern. The first explores how colour and pattern is perceived by animals and thus involves the interaction between the signal giver (i.e. bearer of the colours) and the signal receiver (i.e. the observer) (Bortolotti, 2006). In most cases these studies involve predator and prey interactions (Endler, 1986, 1991a; Bortolotti, 2006; Dale, 2006). When examining animal colour and pattern in nature it is important to be conscious of the sensory world of one’s subject species (i.e. in the case of colour, the visual systems in play) and other animals in the community in which it lives (Stuart-Fox *et al.*, 2004; Bortolotti, 2006) and to be aware that different species often perceive colour and pattern differently in an environment (Owens, 2006; Bond, 2007). Although numerous adaptive functions have been proposed for colour and pattern in predator prey systems (Cott, 1940), concealment, i.e. camouflage or crypsis achieved by matching

against the visual background, may represent one of the major functions of colour in animals (Thayer, 1909; Preston, 1980; Götmark, 1987; Rohwer, 1990; Wilkinson & Sherratt, 2008; Dreiss *et al.*, 2012). Cryptic colouration and pattern is vital for prey to minimize their detection by visual predators (Endler, 1984; Troscianko *et al.*, 2016), and equally so, for predators to reduce their conspicuousness to their prey (Thayer, 1909; Preston, 1980; Götmark, 1987; Rohwer, 1990; Dreiss *et al.*, 2012) and thus has important implications for the survival of individuals (Troscianko *et al.*, 2016). Colour and pattern are therefore often strongly associated with habitat and background in an environment. In some species animal body colour and pattern is also used as advertisement; such warning signals may act to deter attacks from potential predators (Ruxton *et al.*, 2004; Merilaita & Lind, 2005; Forsman *et al.*, 2015), while in prey species conspicuous colours may also serve a protective function, advertising their “unprofitability” to predators (i.e. can be difficult to capture, aggressive, distasteful or poisonous) (Endler, 1988; Caro, 2005). Additionally, in some species, colour and pattern are also used to mimic the perceived colour and patterns of other groups of animals (Rettenmeyer, 1970; Endler, 1990b; Ruxton *et al.*, 2004; Trnka & Grim, 2013).

The second theme argues that colouration has evolved through natural selection acting on the non-signalling attributes of colouration (Bortolotti, 2006). For example, the colour of skin, scales and feathers may also have important functions in an individual’s physiology (Bortolotti, 2006). In many species, including humans, darker pigmentation has been found to play a role in protection against harmful oxidative stress and cell damage caused by ultraviolet radiation (Galván *et al.*, 2010; Jablonski & Chaplin, 2010; Roulin, 2014) and pathogens (i.e. bacterial degradation; Burt & Ichida, 2004, or blood parasites; Roulin *et al.*, 2001; Lei *et al.*, 2013) and has also been shown to contribute to the structural support of flight feathers, scales and claws (McGraw, 2006), reduce glare from from the sun (Burt, 1984; Varada & Alessa, 2014) and help resist abrasion of feathers (Ward *et al.*, 2002). Body colour also plays a highly important thermoregulatory role in animals, particularly in ectothermic species such as reptiles and insects that rely on external radiant energy to control their body temperatures (Rosenblum *et al.*, 2004).

PERSISTENT PHENOTYPIC COLOUR POLYMORPHISM

In addition to the wide variation in colour exhibited between different animals, across sexes and age groups, many species also display colour polymorphism. Whereby, multiple phenotypically discrete, genetically based colour variants or morphs occur within a natural breeding population, irrespective of age or sex, and the least abundant of which is present in numbers too great to be due solely to recurrent mutation (Huxley, 1955a). Persistent phenotypic colour polymorphism is one of the most distinctive sorts of variation and represents extreme intraspecific phenotypic variation (McLean & Stuart-Fox, 2014). Body colour is an easily measurable 'phenotypic marker' and colour polymorphic species have frequently been used as classic model systems in empirical studies examining evolutionary processes such as natural (Endler, 1984) and sexual selection (Endler, 1991b), frequency-dependent selection (Allen, 1988) and the evolution of alternative reproductive strategies (Sinervo & Lively, 1996). Indeed, Darwin first invoked body colour to test sexual selection as a corollary hypothesis to natural selection that could explain traits that appeared to be ornamental rather than functional (Darwin, 1859; Hill & McGraw, 2006).

The Maintenance of Polymorphism

A range of theories have been proposed for the evolution and maintenance of polymorphism and while many have suggested an adaptive function, few have identified the selective agent involved (Ducrest *et al.*, 2008). Key hypotheses explaining the maintenance of polymorphism include: i) Apostatic selection, whereby the uncommon morph gains an advantage because either the prey or predator's search/avoidance image is less familiar to the rarer form (Rohwer & Paulson, 1987; Fowlie & Kruger, 2003). In predators, under apostatic selection individuals displaying a new invading colour morph may enjoy an initial foraging advantage because prey have difficulties in learning the colour of a rare morph. This is known as the "Avoidance- Image Hypothesis" (Rohwer & Paulson, 1987; Roulin & Wink, 2004). ii) Disruptive selection, whereby different morphs have selective advantages in different habitats or environmental conditions (Mather, 1955a; Rueffler *et al.*, 2006). iii) Allopatric evolution, whereby

the two morphs have evolved in reproductive isolation, but where conditions have subsequently changed to remove this isolation (i.e. through range expansions or changes in climate) and genetic differentiation has not been sufficient to promote full speciation (Roulin, 2004b) and iv) Sexual selection, whereby colouration is used for intraspecific signaling and the maintenance of different colour morphs may result from some preferences in mate-choice (O'Donald, 1983). Underpinning all these theories is the concept that an individual's morph type is heritable, intransient and not influenced by environment variation (Ford, 1945; Huxley, 1955a; Amar *et al.*, 2013).

Balanced polymorphism occurs when two different versions of a gene are maintained in a population of organisms. This is because individuals carrying both versions are better able to survive than those who have two copies of either version alone. In other words, heterozygotes for the alleles under consideration have a higher adaptive value than the homozygote (King *et al.*, 2013). The evolutionary process that maintains the two versions over time is called balancing selection (Takahata *et al.*, 1992; Weaver & Hedrick., 1992). Balancing selection thus involves selective mechanisms that maintain variation (i.e. multiple alleles) in the gene pool of a population at frequencies above that of gene mutation (Hedrick, 1999). The two main mechanisms of balancing selection are frequency-dependent selection (which includes apostatic and disruptive selection) and heterozygote advantage (Allen, 1988; Krüger *et al.*, 2001).

Heterozygote advantage, whereby individuals that are heterozygous for an allele encoding a trait have a fitness advantage compared with homozygotes (Gray & McKinnon, 2007), is one of the simplest and well developed forms of balancing selection proposed to explain the maintenance of colour polymorphism (Krüger & Lindstrom, 2001; Briggs *et al.*, 2011a; Boerner *et al.*, 2013). Colour polymorphism may also be the result of a preference, common to both sexes, for mating with dissimilar partners i.e. disassortative mating. This nonrandom mating may indicate selection for optimal outbreeding when diverse or heterozygous young are favoured. Colour, in this case, should reflect different genotypes (Krüger *et al.*, 2001; Galeotti *et al.*, 2003).

Colour polymorphism may also be influenced and maintained by the complementarity of a breeding pair. Although this has been explored more extensively within the context of birds (Selander, 1966; Kristensen *et al.*, 2014), studies have shown that different coloured morphs are able to exploit different habitats (Preston, 1980; Rohwer, 1990), prey resources (Roulin, 2004a) and environmental conditions (Tate *et al.*, 2016). Therefore in a single species, if a breeding pair consists of mixed-morphs, the parents may have in combination, greater access to resources and optimum foraging conditions during the parental care period (Selander, 1966). In a similar manner in which sexual size dimorphism in *Accipiter* hawks enables a breeding pair to exploit a wider range of food resources during the nestling rearing period (Robert, 1966; Reynolds, 1972).

Numerous studies have also shown that adaptive colour polymorphism is found to influence various key life history and fitness related traits (reviewed in Roulin 2004) and have revealed that morph coexistence could stem from balancing selection between survival and reproduction (Stearns, 1992; Briggs *et al.*, 2011b); whereby one morph accrues an advantage through higher reproductive success but suffers from higher mortality, whereas the other morph may have lower reproductive success but higher long-term survival rates. Differences in key demographic parameters such as survival and reproductive performance might explain the underlying mechanisms which maintain or segregate morphs in populations and across species' distributions and may also explain why one morph often occurs at higher proportions over another in certain environments (Fisher, 1930; Ford, 1945; McLean & Stuart-Fox, 2014).

Although classified as generally rare, colour polymorphism is phylogenetically widespread (Hugall & Stuart-Fox, 2012), occurring throughout the animal and plant kingdoms (Kay, 1978; Forsman, 1999a; Hoffman & Blouin, 2000; Ferguson-Lees & Christie, 2001; Stuart-Fox *et al.*, 2004; Hoekstra *et al.*, 2005; Gosden *et al.*, 2011). The ecological and evolutionary mechanisms maintaining most colour polymorphisms in natural populations remain unclear. Thus the coexistence of multiple morphs in natural populations of a polymorphic species is a particularly interesting phenomenon from an evolutionary perspective. Within the context of sexual selection, the relative fitness advantage of

maintaining morphs within species has been explored broadly (Roulin & Bize, 2007); but arguably an area deserving of more attention in this field is that the presence of colour polymorphism indicates the use of alternative strategies by species, where equivalent morph fitness may be achieved in heterogeneous environments under scenarios of frequency-dependent (Allen, 1988), balancing (Losey *et al.*, 1997) and disruptive selection (Roulin & Wink, 2004). Thus the coexistence of multiple morphs and the maintenance of a permanent polymorphism within a population suggests a selective trade-off between alternative morphs, all enjoying some advantage but commonly also incurring some cost (Fisher 1930).

Given the likely adaptive value of colour (Cott, 1940) the occurrence of particular colour morphs in a habitat is thought to reflect adaptation to local selective pressures driven by prevailing environmental conditions such as climate, predator or prey abundance, substrate colour, habitat structure and vegetation cover (Endler, 1977; Forsman, 1999b; Roulin, 2004b; Dreiss *et al.*, 2012). Interestingly, in many polymorphic species the most common colour morphs in a population often closely match their local substrate. A well-studied example of this is the rock pocket mouse *Chaetodipus intermedius*, whose coat colour is strongly correlated with the surrounding substrate colour (Hoekstra *et al.*, 2005). Similarly, in the tawny lizard species complex *Ctenophorus decresii*, Stuart-Fox *et al.* (2004) found that individuals were more cryptic against their native soil background than against the background of other populations. In birds, studies on the larks (Alaudidae) also report a positive relationship between the plumage colour of individuals and the colour of the soil on which they live (Alström *et al.*, 2013). These studies reflect the important role of body colour matching background to minimize detection by visual predators (Endler, 1984; Karpestam *et al.*, 2016), but may equally function in reducing predator conspicuousness to their prey (Preston, 1980; Götmark, 1987; Rohwer, 1990; Dreiss *et al.*, 2012).

Geographic Variation in Animal Colour Polymorphism

The degree of colour variation within populations frequently varies geographically (Endler, 1977; McLean & Stuart-Fox, 2014). In colour polymorphic species, populations can differ in the number, type and frequency of coexisting morphs across their geographic range (James, 1991; McLean & Stuart-Fox,

2014). For example, in the blue-tailed damselfly *Ischnura elegans*, there are pronounced differences in morph frequencies both across their entire European geographic range, as well as at a smaller scale within regions (Gray & McKinnon, 2007; Gosden *et al.*, 2011). Because environments are rarely constant, at both the spatial and temporal scale, populations are expected to adapt to local prevailing environmental conditions over evolutionary time scales (Darwin, 1859; Antoniazza *et al.*, 2010; Emaresi *et al.*, 2014). Since local selective pressures, and hence fitness of individuals are likely to change across heterogeneous landscapes, colour polymorphism itself may predispose species to geographic variation (Roulin, 2004b; McLean & Stuart-Fox, 2014). It has been proposed that polymorphic species are more likely to display geographic variation in colour compared to monomorphic species, because their more variable phenotype allows them to utilize many different habitats across wider climatic regimes, resulting in broader ecological niches and larger range sizes (Galeotti & Rubolini, 2004; Forsman *et al.*, 2008; Forsman & Hagman, 2009). Greater phenotypic variation in polymorphic species may therefore reduce their susceptibility to environmental change and subsequently, they are predicted to experience lower extinction risks and range contractions compared to species with monomorphic phenotypes (Forsman & Hagman, 2009; Roulin *et al.*, 2011; Roulin, 2014; Forsman, 2016).

In many polymorphic species including insects (de Jong & Brakefield, 1998), reptiles (Alsteadt *et al.*, 2006), mammals (Rounds, 1987) and birds (Galeotti *et al.*, 2003; Antoniazza *et al.*, 2010; Amar *et al.*, 2014) an interesting observation is that morph ratios often vary clinally, with a gradual change in morph frequencies across the range of a species (Roulin, 2003; Antoniazza *et al.*, 2010; Amar *et al.*, 2014). Clinal variation in polymorphism is particularly common amongst birds, with at least 20% of polymorphic species exhibiting clinal variation in morph frequencies (Galeotti *et al.*, 2003). For example, the bananquit *Coereba flaveola* shows strong morph ratio clines in populations across the island of Grenada, where the frequency of dark morphs are highly correlated with annual rainfall and altitude (Wunderle, 1981). In the European barn owl *Tyto alba*, clinal variation is also extremely pronounced, with individuals being mostly reddish in North-Eastern Europe, whitish in the South-West, and highly variable in colour in Central Europe (Roulin *et al.*, 2009). Although the adaptive significance

of different morphs is poorly understood, a number of studies identify links between these clines and climate (i.e. rainfall, Wunderle, 1981; Amar *et al.*, 2014), while others have failed to find any such relationships (Galeotti & Cesaris, 1996; Atkinson & Briskie, 2011). Understanding the environmental factors that shape these ratio-clines are important in isolating the selective forces operating on the balancing mechanisms of the different morphs (Galeotti *et al.*, 2003; Amar *et al.*, 2014).

Another interesting feature, first described by the Victorian zoologist Constantin Gloger (1833), is that individuals tend to be darker in areas where relative humidity is high, and paler where it is low (Zink *et al.*, 1986). This is known as Gloger's ecogeographical or climatic rule (Gloger, 1833, cited by Mayr, 1956) and has been reported for a number of mammals (Rounds, 1987; Kamilar & Bradley, 2011) and birds (Wunderle, 1981; Antoniazza *et al.*, 2010; Amar *et al.*, 2014) and is also found in some beetles, flies and butterflies (Mayr, 1963).

Is Variation in Colour Always Adaptive?

In many biological systems spatial variation in adaptive traits such as colour cannot be explained by natural selection alone (Calsbeek *et al.*, 2009). Geographic variation in adaptive colouration can also be shaped by neutral (stochastic) evolutionary processes: Gene flow and processes such as genetic drift (Wright, 1932; Slatkin, 1973; Endler, 1977; King & Lawson, 1995; Roulin, 2004b; Gray & McKinnon, 2007; Roulin & Ducrest, 2013) as well as historical events such as population bottlenecks (Keller *et al.*, 2001) and founder events can all influence the frequency of phenotypic traits (King & Lawson, 1995; Luikart *et al.*, 1998; Tarr *et al.*, 1998; Sonsthagen *et al.*, 2012). Gene flow may promote adaptation by making genetic novelties available throughout a species range, but can also restrict differentiation among local populations (Slatkin, 1987; Merilaita, 2001). Whereas founder events generally reduce genetic variation in a population (Wright, 1932; Luikart *et al.*, 1998) and in newly colonised areas, allele frequencies can rapidly diverge from source populations via genetic drift (Sanchez-Guillen *et al.*, 2011; Hugall & Stuart-Fox, 2012).

In some polymorphic species colour appears to have no adaptive function and is therefore unlikely to be influenced by ongoing selection (Kimura, 1962, 1983; Buckley, 1987). A well-studied example of this is the lesser snow goose *Chen caerulescens*, where despite long term observations and extensive datasets, studies have failed to detect any fitness correlates with the highly conspicuous colour variability displayed by the species (Cooke, 1995). As a complement to hypotheses centered on natural selection, neutral evolutionary processes such as historical gene flow have also been proposed to explain contemporary patterns of polymorphism in some populations (Lank, 2002; Dale, 2006). For instance in species such as the snow goose, populations may have diverged where morphs could have subsequently evolved separately through allopatry followed by a secondary introgression, where the different morphs would now be brought back into contact (Cooke, 1995; Lank, 2002).

Genetic diversity in newly founded populations strongly depends on the number of initial colonisers, the duration of the process of colonisation, and whether an area was colonised via a single event or repeated recruitment from a single or multiple source populations (Slatkin, 1987). Genetic diversity in founded populations can also be influenced by species' behaviour (Sonsthagen *et al.*, 2012); those that display strong dispersal and colonisation capabilities may recover genetic diversity to founded populations through subsequent immigration (Ward *et al.*, 1994; Crochet, 2000; Keller *et al.*, 2001). This is common in species like birds (Crochet, 2000) and marine fish (Ward *et al.*, 1994) that generally have a high dispersal potential. For example, in a population of song sparrows *Melospiza melodia* on Mandarte Island, Canada, that experienced a severe population bottleneck where the majority of the population died after a heavy winter storm, steady immigration of individuals led to rapid recovery of genetic diversity (Keller *et al.*, 2001). Extensive gene flow can therefore limit the evolution and frequency of adaptive traits in populations by homogenising gene pools that would otherwise diverge in response to selection under different ecological regimes (Antonovics & Bradshaw, 1978; Slatkin, 1987; Räsänen & Hendry, 2008).

Importantly, a number of recent studies have also demonstrated the contrary, whereby adaptive traits have been shown to diverge in the presence of extensive gene flow (Merilaita, 2001; Antoniazza *et al.*,

2010; Odendaal *et al.*, 2014). Differentiation in adaptive traits between genetically-interconnected populations is often driven by strong adaptation to local selective forces such as climate (Karell *et al.*, 2011; Tate *et al.*, 2016), prey resources (Roulin, 2004a) and predator abundance (Endler, 1991b; Merilaita, 2001) and may also be influenced by habitat structure (Antoniazza *et al.*, 2010; Dreiss *et al.*, 2012; Odendaal *et al.*, 2014). For example, in the Cape horseshoe bat *Rhinolophus capensis*, significant sensory trait (echolocation frequency) differentiation has evolved in populations despite strong historical gene flow (Odendaal *et al.*, 2014). These kinds of studies highlight the importance of population level analyses; studying the relationship between gene flow and trait divergence is central to better understanding the process of local adaptation (Antonovics & Bradshaw, 1978; Bridle & Vines, 2006) and adaptive traits such as colour are clearly a valuable model (McLean & Stuart-Fox, 2014).

P LUMAGE COLOUR POLYMORPHISM

Why do polymorphic species of bird display different colour morphs? And why is there such extensive inter- and intraspecific variation in colouration amongst bird species? Since the time of Darwin the vast array of plumage colour, patterns and ornaments in birds have been a great fascination for evolutionary biologists (Darwin, 1859; Hill & McGraw, 2006). Studying the variability in bird colour, however, is by no means a simple task largely because colouration varies in so many different dimensions (Dale 2006). Examining the literature it is also evident that the function of colour varies substantially across species and from one biological system to the next. The selective mechanisms that shape such great diversity in plumage colour are therefore extremely complex and have challenged evolutionary biologists and the ideas of evolution by natural selection (Darwin, 1859; Huxley, 1955a).

Plumage colour polymorphism is amongst the most well studied systems used to examine selective evolutionary processes and their role in shaping colour and pattern in nature (Roulin, 2004b; McLean & Stuart-Fox, 2014). Indeed, Huxley (1955) was among the first to recognise the great variation in plumage colour displayed within colour polymorphic birds and there is a substantial literature on the role of polymorphism in speciation (Gray & McKinnon, 2007; Forsman *et al.*, 2008; Hugall & Stuart-

Fox, 2012), with recent evidence linking polymorphism to rapid phenotypic evolution and accelerated speciation rates across a range of species (Hugall & Stuart-Fox, 2012).

Although there is considerable variation among different bird groups, colour polymorphism occurs in species from a wide range of bird families (Galeotti *et al.*, 2003). Some of the most well studied species include the lesser snow goose *Chen caerulescens*, the Arctic skua *Stercorarius parasiticus*, the European barn owl *Tyto alba* (Owens, 2006) and the cuckoos (Cuculidae) (Galeotti *et al.*, 2003). In birds colour polymorphism is often associated with a variety of behavioural, physiological and life history traits and influences numerous fitness-related parameters (Galeotti *et al.*, 2003; Roulin, 2004b; Chakarov *et al.*, 2008). Plumage colour can vary continuously or show two or more discrete morphs. For example, the polymorphic Swainson's hawk *Buteo swainsoni* and common buzzard *Buteo buteo* show continuous polymorphism, although they are often classified as dark, light or intermediate for analyses (Krüger & Lindstrom, 2001; Briggs *et al.*, 2010). While discrete polymorphism exists in the ferruginous hawk *Buteo regalis* (Schmutz & Schmutz, 1981) and Eleonora's falcon *Falco eleonora* (Gangoso *et al.*, 2011), with either distinct dark or light morph birds.

Concealment, the Variable Light Environment and Disruptive Selection

One of the most consistent adaptive functions proposed for colouration in avian predator-prey systems, is that of concealment or camouflage (Thayer, 1909; Roulin & Wink, 2004; Bortolotti, 2006; Dale, 2006; Owens, 2006; Karpestam *et al.*, 2016). Is it not obvious that a nightjar's (Caprimulgidae) plumage colour and pattern perfectly matches its surrounds to hide it from visual predators? (Troscianko *et al.*, 2016). Similarly, the brown summer plumage of willow ptarmigans *Lagopus l. lagopus* blends in impeccably with its surrounds at the nest, while its white feathers camouflage it against winter's snow (Steen *et al.*, 1992). Camouflage, achieved by matching body colour and pattern with its surrounds is common in vertebrates (Stuart-Fox *et al.*, 2004; Hoekstra *et al.*, 2005; Behrens, 2009; Brooke, 2010). One of the most popular examples of how polymorphism is thought to be regulated by camouflage via background matching is demonstrated in the pepper moth *Biston betularia* (Kettlewell, 1958; Majerus, 2009). During the industrial revolution significant surges in pollution led to increases in the number of

dark melanistic moths, which were not known in many areas prior to the industrial revolution. By the end of the revolution the dark variety almost completely outnumbered the speckled light-coloured type. The evolutionary explanation for this was that dark moths were more cryptic to their avian predators against their habitat that had been darkened by pollution and soot, whereas the historically abundant light moths were now picked off easily (Majerus, 2009).

In their review on the topic, Galeotti *et al.* (2003) conclude that colour polymorphism in birds probably evolved under selective pressures linked to individual detectability in variable light conditions and argue that it is most likely maintained by disruptive selection. Colour patterns of both the subject and the background change in different ways when exposed to different ambient light spectra (Endler, 1991b; Endler & Théry, 1996; Théry, 2006; Gomez & Théry, 2007); therefore some colour patterns appear highly conspicuous in one light environment and may be cryptic in another. The most conspicuous colours are those that are rich in the colours of the ambient light but poor in the colours reflected by the visual background (Endler, 1990a, 1991a). Different bird species may therefore use different colours because they inhabit different light environments (Endler, 1993; Endler & Théry, 1996). Ambient light conditions are likely to be strong selective agents, since light intensity within an environment during the course of the day, between habitats or in different weather conditions can vary dramatically (Théry, 2006). For example, shading from vegetation canopy or increased cloudiness can strongly reduce ambient light intensity (Martin, 1990; Théry, 2006). Differences in light conditions therefore offer a potential mechanism under which disruptive selection could operate, favouring the maintenance of different colour morphs within a species range (Galeotti *et al.*, 2003). Central to this argument is the hypothesis that different morphs enjoy selective advantages under differing light levels (Galeotti *et al.*, 2003; Roulin & Wink, 2004). For example, dark morphs may benefit from crypsis in low light conditions i.e. on cloudier days, during dusk/dawn, or in closed habitats, and may accrue a foraging/hiding advantage via background matching (Preston, 1980; Rohwer, 1990). Conversely, light morphs may forage/hide more successfully in brighter conditions, benefitting from improved crypsis against a lighter background i.e. on sunny days, in the middle of the day, or in open habitats (Galeotti

et al., 2003; Roulin, 2004b; Roulin & Wink, 2004). Whilst these ideas are highly credible, no empirical study has yet explicitly tested these underlying assumptions.

Plumage Polymorphism in Birds of Prey

Colour polymorphism is particularly prevalent amongst raptorial species (Thayer, 1909; Fowlie & Kruger, 2003; Roulin & Wink, 2004) especially in the owls (Strigidae) and hawks (Accipitridae), where it occurs in about 39% and 22% of species respectively (Galeotti *et al.*, 2003). The high prevalence of polymorphism in birds of prey suggests that predator-prey relationships may be a key component in the evolution and maintenance of polymorphism (Galeotti *et al.*, 2003; Roulin & Wink, 2004). This makes the study of plumage colour polymorphism in raptors of great interest in avian biology (Holderby *et al.*, 2012).

In diurnal raptors, colour polymorphism is most common in the genus *Accipiter* (Fowlie & Kruger, 2003) where almost a quarter of the species are polymorphic (Ferguson-Lees & Christie, 2001). Many *Accipiters* are specialist predators of birds (Fowlie & Kruger, 2003). Given the excellent visual acuity of birds (Jacobs, 1992), their memory capacity (Clayton, 1998) and ability to detect and evade their predators (Roulin & Wink, 2004) the relatively high frequency of polymorphism within *Accipiters* may reflect selection for reduced detection by their prey under variable environmental conditions; more cryptic morphs may therefore be favoured through improved foraging success in environments with greater background matching. Under this hypothesis prey are thus considered a strong selective agent (Galeotti & Rubolini, 2004).

PLUMAGE PLOYMORPHISM IN THE BLACK SPARROWHAWK

The Study Species

The black sparrowhawk *Accipiter melanoleucus* (Accipitridae; Smith, 1830) is a polymorphic raptor that exhibits discrete polymorphism, with adults occurring as either dark or white breasted morphs with

varying degrees of white under the chin (Fig 1; Amar *et al.*, 2013). The trait is not sexually dimorphic in the species (Hockey *et al.*, 2005; Taylor & Louette, 2009).



Figure 1. The Black Sparrowhawk exhibits discrete plumage colour polymorphism, with adults occurring as dark (right) or white morphs (left). They differ in their ventral plumage colour, with dark morphs displaying black plumage on the breast and underwing coverts, and white morphs displaying white plumage on the breast and underwing coverts. Photos: Ann Koeslag

After their moult into adult plumage, morph type and plumage pattern are invariant over time for both males and females and follow typical Mendelian inheritance with an apparent one-locus two-allele system where the allele coding for the white morph is dominant (Amar *et al.*, 2013). Adult plumage colour and pattern is often similar amongst polymorphic species in the family Accipitridae, with a standard common light morph with pale breast and underwing coverts and a rarer dark adult morph, which tends to be black on the breast and underwing coverts (Ferguson-Lees & Christie, 2001). For

example, the gabar goshawk *Micronisus gabar*, booted eagle *Hieraaetus pennatus* and Wahlberg's eagle *Hieraaetus wahlbergi* are all sub-Saharan Accipitridae species that follow this adult plumage colour pattern. Throughout most of the black sparrowhawks' geographic range in South Africa, the white morph predominates (Amar *et al.*, 2014; Tate *et al.*, 2016) whereas the adult dark morph of this species is usually classed as rare (Steyn, 1982; Ferguson-Lees & Christie, 2001; Hockey *et al.*, 2005).

A Range-Expanding Species

The black sparrowhawk is broadly distributed (Fig. 2), occurring throughout much of the forested areas of sub-Saharan Africa (Ferguson-Lees & Christie, 2001).

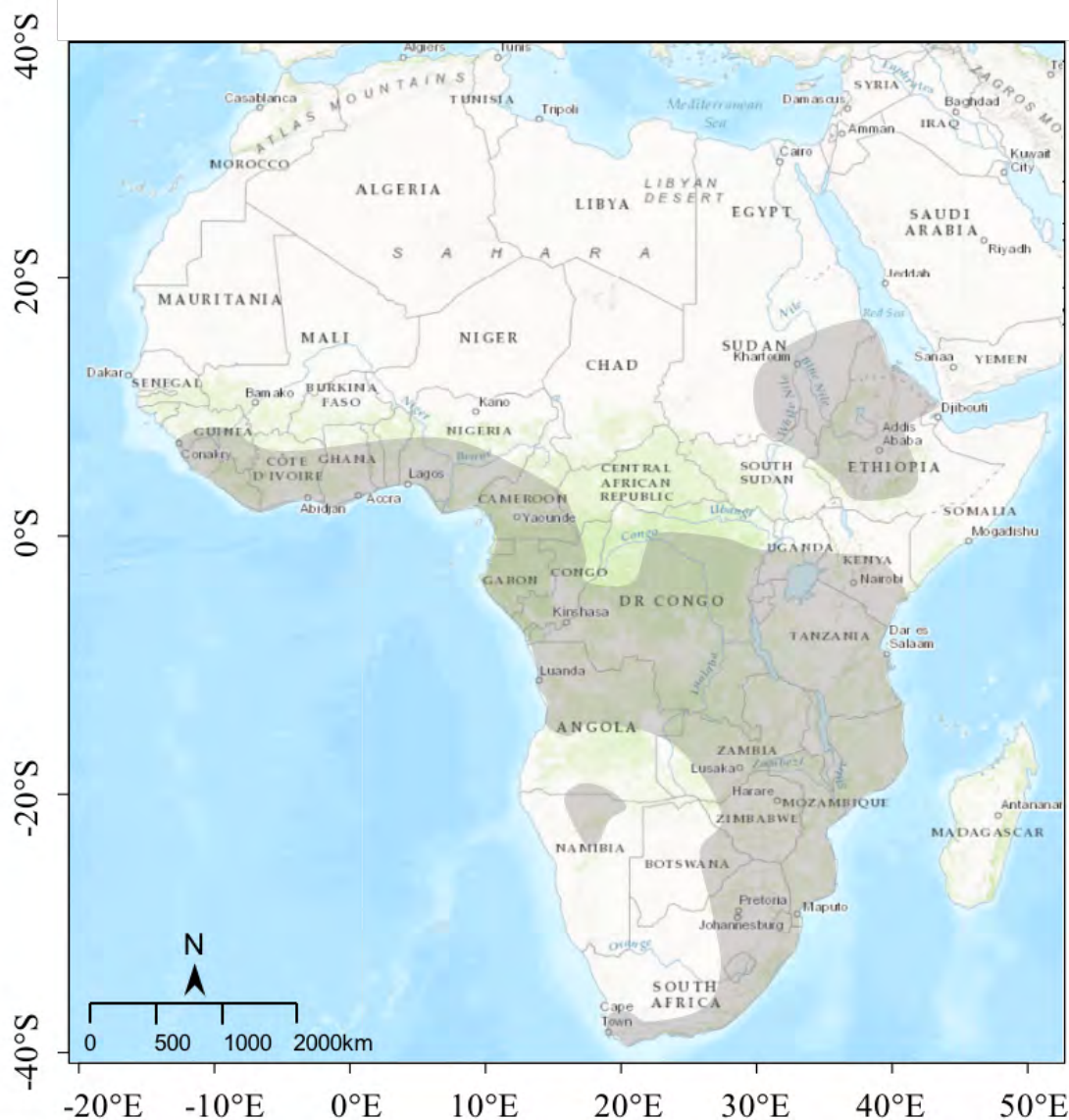


Figure 2. Global distribution of the Black Sparrowhawk *Accipiter melanoleucus* (www.birdlife.org)

Within South Africa, the species has undergone a fairly rapid southward and westward range expansion over the last two decades (~1990s to 2016) and now breeds throughout much of the Western Cape where it has recently successfully colonized the Cape Peninsula, with the first breeding attempt recorded here in 1993 (Oetlé, 1994; Curtis *et al.*, 2007). The species is highly productive in this region with the Peninsula population growing considerably over the last decade through both internal recruitment and immigration (Amar *et al.*, 2013; Martin *et al.*, 2014). Highlighting their success this newly colonised area currently supports over 50 breeding pairs (Fig. 4; Katzenberger *et al.*, 2015; Sumasgutner *et al.*, 2016) with the first recorded cases of multiple-brooding in the species (Curtis *et al.*, 2005). While their success in numbers and range expansion may be linked with climate change (as argued for a variety of South African bird species Hockey *et al.*, 2011b; Cunningham *et al.*, 2015) abundant prey resources (*Columba livia*, *C. guinea* and *Streptopelia semitorquata*; Malan & Robinson, 1999; Baigrie, 2013) and the availability of nesting sites found within this human-modified environment certainly contribute to the species' success in the region (Curtis *et al.*, 2005; Gil & Brumm, 2014; Møller *et al.*, 2015). Further, large stands of exotic Pine (*Pinus spp.*) and Eucalyptus (*Eucalyptus spp.*) plantations throughout South Africa is thought to be a key factor facilitating their range expansion, providing ideal and preferred nesting sites (Malan & Robinson, 2001; Hockey *et al.*, 2011a). Despite adapting well to eucalyptus and other exotic plantations in southern Africa, the species is suspected to be decreasing across its range due to habitat degradation and loss and over-use of pesticides (Ferguson-Lees & Christie, 2001).

The recent range expansion of the black sparrowhawk has also brought the species into a novel climatic regime (Fig. 3; Linder *et al.*, 2010; Martin *et al.*, 2014). As winter breeders (Hockey *et al.*, 2005; Martin *et al.*, 2014), black sparrowhawks now experience two vastly contrasting climates in South Africa; dry winters in their historical range in the north and east, and wet winters in the Mediterranean climate of the recently colonised Western Cape region. Conditions on the Cape Peninsula particularly reflect these differences with the area receiving exceptionally high amounts of winter rainfall due to its exposure to southern ocean weather systems and orographic effects of the local topography dominated by Table Mountain (Martin *et al.*, 2014). Within South Africa, black sparrowhawks display clinal variation in their morph frequencies, with the frequency of dark morphs declining from > 75 % in the far south

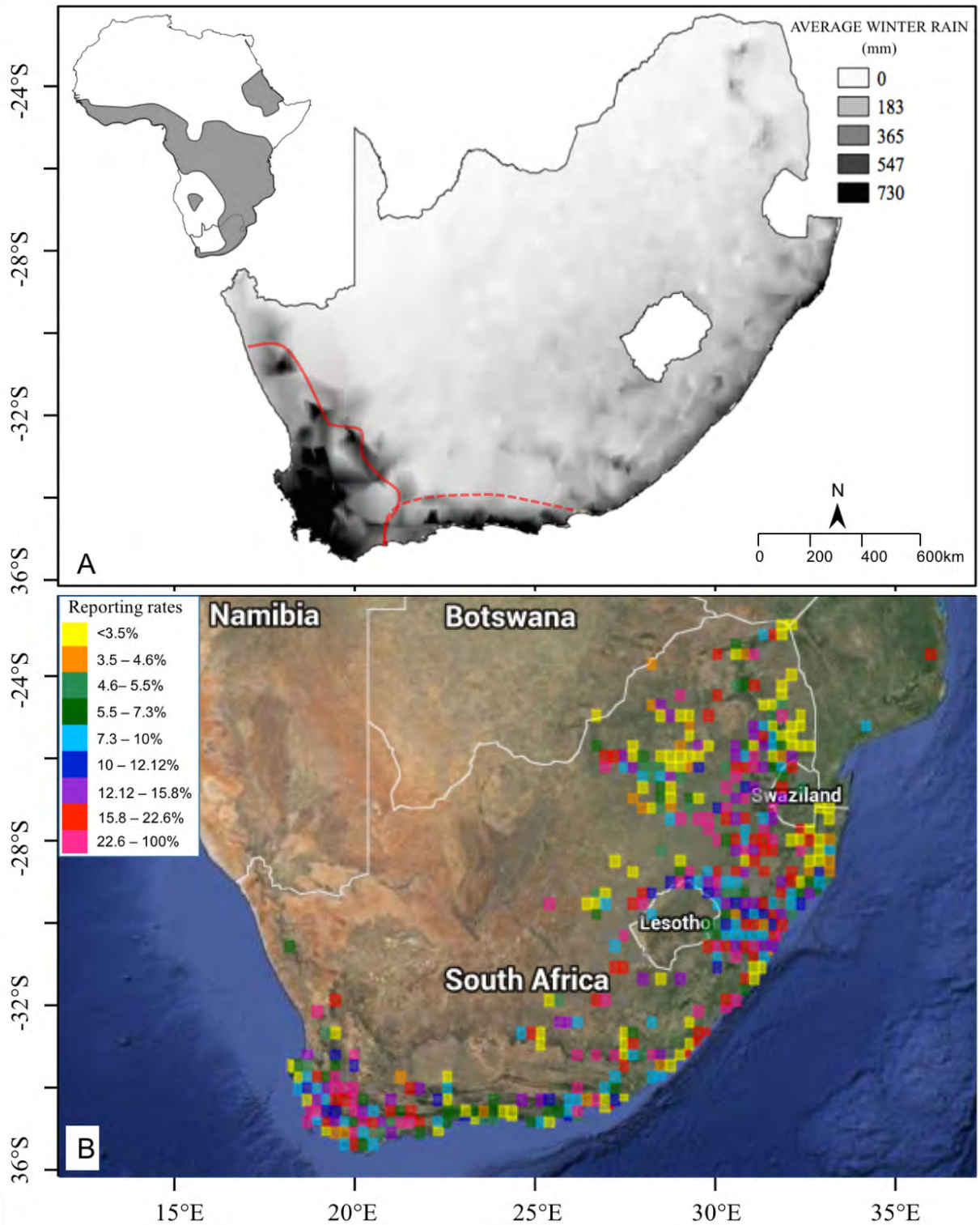


Figure 3a. Shaded regions represent the average annual winter rainfall in mm (April–October) during the Black Sparrowhawk breeding season across South Africa. Lines indicate the approximate positions of the different rainfall zones of South Africa: Solid line indicates the winter rainfall zone; dashed line indicates all year rainfall zone; and the rest of South Africa receives summer rainfall. **3b.** South African distribution of the Black Sparrowhawk (South African Bird Atlas Project SABAP2).

west on the Cape Peninsula, Western Cape, to < 20 % in the northeast of the country (Amar *et al.*, 2014). This variation correlates with temperature and rainfall during the winter breeding period (April-October; Martin *et al.* 2014), with dark morphs predominating in areas with higher rainfall (Amar *et al.*, 2014).

A long-term research database reveals that the ratio of dark and white morph birds has remained relatively constant on the Cape Peninsula over the last decade (Amar *et al.*, 2013). This population is also highly unusual, as the morph frequencies are reversed compared to populations in the rest of the species traditional north eastern range and, as far as it is understood, throughout the entire African distribution (Ferguson-Lees & Christie, 2001).

Two main hypotheses have been developed for the high frequency of dark morph birds in the Cape Peninsula population (Tate *et al.* 2016; Amar *et al.* 2013); (i) a non-adaptive hypothesis argues that the variation in morph proportions may be evolutionarily neutral and simply due to a chance founder effect and maintained via strong genetic drift. Under this scenario a larger proportion of dark birds may have colonised the region and the initial morph frequencies are now maintained through neutral processes. If this was the case, following a founding event on the Cape Peninsula, the presence of the alleles coding for the usually rare dark morph, and thus allelic frequencies, may have rapidly deviated from source populations (Sanchez-Guillen *et al.*, 2011; Hugall & Stuart-Fox, 2012). As an alternative (ii) an adaptive hypothesis proposes that, irrespective of the founding morph ratios, dark morphs have a selective advantage in this newly colonised environment with its novel winter rainfall regime.

RESEARCH AIMS AND STUDY AREA

This study investigates plumage polymorphism in the black sparrowhawk focusing on the population from the Cape Peninsula. The main aims of this research were to determine the (i) ecological and evolutionary mechanisms that influence the maintenance of colour polymorphism in the species and (ii) to establish explanations for the unusually high proportion of dark morphs on the Cape Peninsula. The study area encompasses a variety of natural and human modified (urbanised) environments (Fig. 4),

which include the slopes of Table Mountain and adjacent sand flats, with altitude ranging from sea level to ~400m. The area is characterised by a mosaic of urban gardens and green belts, golf courses, schools, small pockets of indigenous Afromontane forest, botanical gardens, fynbos shrubland and alien pine (*Pinus* spp.) and eucalyptus (*Eucalyptus* spp.) plantations along the eastern slopes of Table Mountain (Curtis *et al.*, 2005).

Using a range of ecological and evolutionary approaches I explored both neutral and adaptationist explanations for the high frequency of dark morphs in my study population. Data from the mitochondrial control region was used to examine the distribution of genetic diversity in several geographic populations of black sparrowhawks across South Africa, allowing the exploration of trait divergence under neutrality. Using a phylogeographic framework, genetic variation was used to (i) quantify the extent to which population structure and gene flow may influence the observed pattern of colour morphs in the focal study population on the Cape Peninsula, and (ii) explore how selection and gene flow may interact to explain the patterns of morph frequencies in my study system. I was specifically interested in answering the following questions: (1) Is there evidence for a founder effect in the study population of black sparrowhawks? (2) Have the proportions of morphs diverged in the presence of strong gene flow? And if so, what are the likely selective forces and adaptive responses?

To explore an explanation based on local selection and an adaptive response I used a variety of ecological approaches to examine whether local conditions favour dark morph individuals on the Cape Peninsula and thereby account for the significant divergence in morph frequencies in this newly colonized environment. To do this I investigated various components of behaviour; including foraging success (prey provisioning) as well as foraging behaviour and habitat preference together with key demographic fitness parameters (reproduction and survival) between the two morphs in the Cape Peninsula population, to answer the following questions: (1) What adaptive value does plumage colour have in the black sparrowhawk? (2) What local environmental conditions influence the frequency of

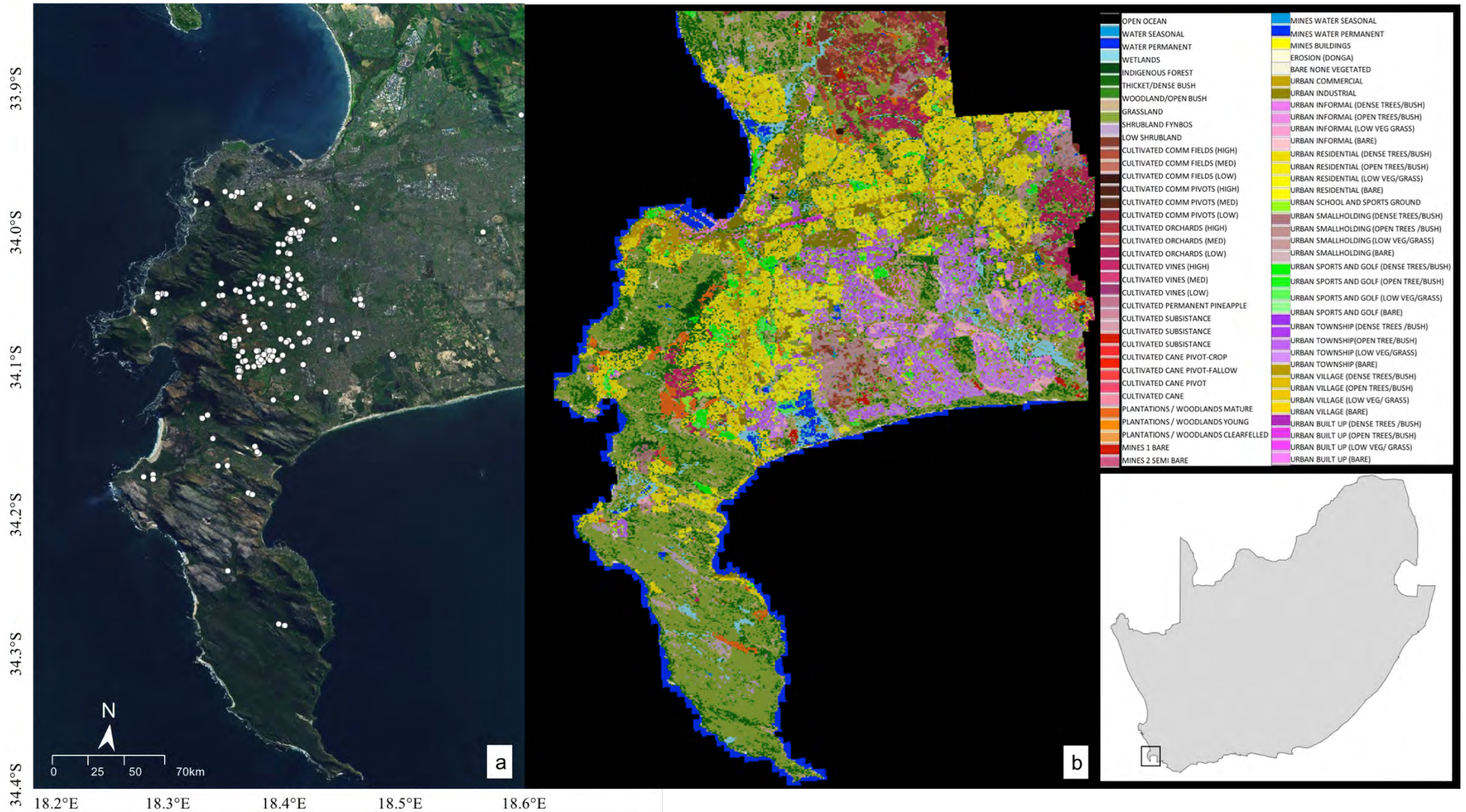


Figure 4a. The study site on the Cape Peninsula, South Africa, showing active nesting sites (white dots) used in this study from 2012-2015, **4b.** as well as the different land-cover classes on the Peninsula (SANBI).

dark morphs? (3) And lastly, what are the local fitness consequences of being a dark morph individual on the Peninsula?

THESIS OVERVIEW

This thesis is presented as a series of stand-alone chapters formatted to facilitate publication (Chapters 2–5). As such, there is some inevitable repetition of information in the introduction, methods and discussion sections of these chapters. Redundancies and inconsistencies may occur between chapters. Within these data chapters I use the term “we” – since there were several authors on each paper, although in all cases I am the principle lead author. In the appendix I have also included additional co-authored publications that are informed by my research.

CHAPTER 2 quantifies the extent to which population genetic structure and gene flow contribute to the high frequency of dark morphs on the Peninsula and explores how selection and gene flow may interact to characterise the patterns of morph frequencies in my study system. This is tested using a phylogeographic framework and data from the mitochondrial (mtDNA) control region, to examine the distribution of genetic diversity in several geographic populations of black sparrowhawks across South Africa.

CHAPTER 3 examines the potential adaptive functions plumage colour may serve in the black sparrowhawk on the Cape Peninsula and what local environmental forces may be driving and maintaining the high proportion of dark morphs in my study population. It also investigates if these environmental conditions may be driving the variation in morph frequencies throughout the species range. To test this I investigated the foraging success (measured by prey delivery rates to nests) between black sparrowhawk morphs across variable ambient light conditions on the Cape Peninsula. Using data on the morph frequencies throughout South Africa I also explore whether the clinal variation in morph ratios observed in this species across its' South African range may be explained by varying light levels experienced during the winter breeding season.

CHAPTER 4 uses tracking data from GPS tagged males to examine whether foraging activity and foraging effort differs between the morphs depending on light conditions on the Peninsula and so builds on Chapter 3. Chapter 4 also assesses whether habitat preference (specifically related to the light conditions inside each habitat) differs between morphs.

CHAPTER 5 explores the local fitness consequences of being dark on the Peninsula and examines key demographic fitness parameters (survival and reproductive performance) between morphs which may explain how polymorphism is maintained in the study population. Using individually colour marked adults I examine whether there is evidence for differential survival between the morphs. And using long-term breeding data from the study population I compared reproductive performance between the morphs, either in isolation or in combination with their partner morph (i.e. mixed or like-pairs), which also allowed tests for assortative or disassortative mating patterns in the study population.

CHAPTER 6 presents a synthesis of the findings and evaluates their relevance to evolutionary thinking broadly and specifically to adaptive trait evolution and discusses the maintenance of colour polymorphism in animals.

ETHICS

All research was approved by the University of Cape Town's Science Faculty Animal Ethics Committee (Permit number 2012/V37/AA). The methods were carried out in accordance with the Ethics Committee's relevant guidelines and regulations.

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CHAPTER 2

TRAIT DIVERGENCE IN THE PRESENCE OF GENE FLOW?

A MITOCHONDRIAL PERSPECTIVE ON TRAIT VARIATION AND COLONISATION OF THE CAPE PENINSULA BY THE BLACK SPARROWHAWK *ACCIPITER MELANOLEUCUS*.



Sparrowhawk sampling trip, August 2014. Releasing a healthy white morph female trapped amongst the sugar cane fields in Scottburgh, Kwazulu Natal. This individual formed part of my analysis looking at gene flow and the population genetic structure of Black Sparrowhawks across their South African distribution. Photo: Gareth Tate

ABSTRACT

Ecological regimes and thus local selective pressures frequently differ across heterogeneous environments and can drive adaptive trait divergence between populations. Geographic variation in colour morph ratios occurs frequently in polymorphic species and is often considered an ideal model system to examine the interplay of gene flow and local selection in populations. Adaptive explanations for such complex patterns of colour variation invoke natural selection for improved crypsis via background matching, under disruptive selection. Gene flow can also have profound effects on the genetic diversity of a population and neutral evolutionary processes can influence trait (i.e. colour) divergence between populations. In this study we quantify the extent to which population genetic structure and gene flow contribute to the unusually high frequency of dark morph black sparrowhawks on the recently colonised Cape Peninsula, South Africa, compared to the the rest of species historical range where the white morph predominates. We explore how selection and gene flow may interact to characterise the patterns of morph frequencies in the species. This is tested using a phylogeographic framework and data from the mitochondrial control region to examine the distribution of genetic diversity in several geographic populations of black sparrowhawks across South Africa. Our analysis revealed a very low genetic differentiation between our sample sites which suggests that substantial gene flow occurs among populations. The persistence of the pronounced colour differentiation despite this strong homogenising gene flow is consistent with the hypothesis that selection, and thus local adaptation, is the primary force maintaining colour variation among black sparrowhawk populations. Therefore, the high proportion of dark morphs in our study population is most likely the result of local adaptation to prevailing environmental conditions in the newly colonised region.

Keywords:

Raptor, population structure, founder effect, neutral evolutionary processes, local adaptation, geographic variation, mtDNA, gene-flow, selection, colour polymorphism, adaptive traits.

INTRODUCTION

Evolutionary biologists have long been fascinated by the processes that drive and maintain phenotypic variation within, as well as among species (Darwin, 1859; Ford, 1945). One of the most taxonomically diverse, easily measurable, and well documented forms of phenotypic variation in nature is variation in animal body colour (Hingston, 1933; Endler, 1977; McLean & Stuart-Fox, 2014). Body colouration is predicted to be under strong selective pressure (Bortolotti, 2006; Roulin *et al.*, 2009; Hubbard *et al.*, 2010) and has been shown to have many functions, including a role in thermoregulation (Stuart-Fox & Moussalli, 2009; Dreiss *et al.*, 2015), intraspecific communication (Endler, 1990) and sexual selection (Roulin & Bize, 2007; Boerner & Kruger, 2008; Pryke, 2010), as well as providing protection against the harmful effects of UV-radiation (McGraw, 2006; Dubey & Roulin, 2014) and pathogens (Roulin *et al.*, 2001; Lei *et al.*, 2013). Numerous studies argue that enhanced crypsis achieved via background matching with local environments is an equally important function of colouration in animals (Endler, 1978; Lythgoe, 1979; Merilaita, 2001; Galeotti *et al.*, 2003; Stuart-Fox *et al.*, 2004; Bortolotti, 2006); minimizing detection by visual predators (Endler, 1984; Rudh *et al.*, 2007; Troscianko *et al.*, 2016), and reducing predator conspicuousness to prey (Preston, 1980; Götmark, 1987; Rohwer, 1990; Dreiss *et al.*, 2012; Tate *et al.*, 2016).

The degree of colour variation within populations varies geographically in many species (Gould & Johnston, 1972; Endler, 1977; Merilaita, 2001; Calsbeek *et al.*, 2009). This variation is generally assumed to be adaptive (Cott, 1940) and because environments are rarely constant either spatially or temporally adaptive traits in populations are predicted to be closely linked to the prevailing environmental conditions (Darwin, 1859; Antoniazza *et al.*, 2010; Emaresi *et al.*, 2014). Thus, fitness of individuals are likely to change across heterogeneous landscapes (Haldane, 1948) and this can lead to genetic differentiation among geographically separated populations (Endler, 1977).

Numerous colour polymorphic species, where multiple colour variants or morphs coexist in a breeding population irrespective of age or sex (Ford, 1945; Huxley, 1955), display geographic colour variation (Gould & Johnston, 1972); whereby, across a species' geographic distribution, populations may differ in the number, type and frequency of coexisting morphs. At least 20 % of polymorphic bird species exhibit clinal variation in morph frequencies (Galeotti *et al.*, 2003). For example, the bananquit *Coereba flaveola* shows morph ratio clines across the island of Grenada, where the frequency of dark morphs is strongly correlated with altitude and annual rainfall (Wunderle, 1981), while variation in the tawny owl *Strix aluco* across its European range varies in a mosaic pattern rather than a clinal one (Galeotti & Sacchi, 2003). Researchers suggest that the observed geographical patterns of variation in morph frequencies of many species are associated with underlying environmental gradients (James, 1991; McLean & Stuart-Fox, 2014) and are thus most likely the result of local selective pressures (Antoniazza *et al.*, 2010; Dreiss *et al.*, 2012). These include climate (Wunderle, 1981; Amar *et al.*, 2014; Tate *et al.*, 2016) and predator or prey abundance (Merilaita, 2001; Roulin, 2004a) and often occur in combination with some degree of frequency-dependent selection (Galeotti *et al.*, 2003; Roulin, 2004b; Calsbeek *et al.*, 2009). Habitat structure, vegetation cover (Preston, 1980; Rohwer, 1990; Dreiss *et al.*, 2012) and substrate colour (Merilaita, 2001; Stuart-Fox *et al.*, 2004; Alström *et al.*, 2013; Mclean *et al.*, 2014) have also been invoked to drive changes in the proportion of morphs across a species range.

Geographic variation in traits may also be influenced by random processes (Rudh *et al.*, 2007; Calsbeek *et al.*, 2009). Neutral evolutionary processes such as gene flow (Antonovics & Bradshaw, 1978; Slatkin, 1987; King & Lawson, 1995; Räsänen & Hendry, 2008) and genetic drift (Slatkin, 1973; Endler, 1977; Odendaal *et al.*, 2014) as well as past demographic events such as population bottlenecks (Keller *et al.*, 2001) and founder events (Luikart *et al.*, 1998; Tarr *et al.*, 1998; Sonsthagen *et al.*, 2012) can exert a significant influence on genetic diversity and thus contribute to geographic variation in traits such as colouration (Reillo & Wise, 1988; Antoniazza *et al.*, 2010). Founder events generally reduce genetic variation (Wright, 1932; Luikart *et al.*, 1998) and in newly colonised areas the presence of rare alleles and thereby allelic frequencies may rapidly deviate from source populations via genetic drift (Sanchez-Guillen *et al.*, 2011; Hugall & Stuart-Fox, 2012). Genetic diversity in newly founded populations

strongly depends on the number of initial colonisers and the duration of the colonisation process; for example, whether a single colonising event occurred or whether the establishment of a new population involved repeated recruitment from a single or multiple source populations (Slatkin, 1987; Shephard *et al.*, 2005). Genetic diversity can also be influenced by behaviour (Sonsthagen *et al.*, 2012); species that display strong dispersal and colonisation capabilities may recover genetic diversity to founded populations through subsequent immigration (Keller *et al.*, 2001). Extensive gene flow may promote adaptation in some populations by making genetic novelties available throughout the species range (Slatkin, 1987; Merilaita, 2001), however, it can also restrict adaptive trait variation (such as adaptive colour variation) by homogenising gene pools that would otherwise diverge in response to selection under different ecological regimes (Antonovics & Bradshaw, 1978; Slatkin, 1987; Räsänen & Hendry, 2008).

In concert, adaptation to local selective pressures and gene flow between populations can maintain genetic variation in populations (King & Lawson, 1995; Merilaita, 2001). Thus, studying the relationship between gene flow and trait divergence is central to a better understanding of the processes leading to local adaptation (Antonovics & Bradshaw, 1978; Bridle & Vines, 2006) and adaptive traits such as colour are clearly a valuable model (McLean & Stuart-Fox, 2014). In particular, when colour polymorphism associated with crypsis is considered, selection will likely be associated with local conditions; where optimal colouration will be determined by the visual characteristics of the local environment (Endler, 1978; Merilaita *et al.*, 1999; Merilaita, 2001).

The black sparrowhawk *Accipiter melanoleucus* is a broadly distributed polymorphic raptor, occurring throughout much of the forested regions of sub-Saharan Africa (Ferguson-Lees & Christie, 2001). The species exhibits a discrete colour polymorphism with adults occurring as either dark or white morphs (Hockey *et al.*, 2005; Amar *et al.*, 2013). Within South Africa, black sparrowhawks have expanded their range over the last few decades into the extreme south west of the country (Hockey *et al.*, 2005; Martin *et al.*, 2014, Fig. 2). And within this region, the species has recently successfully colonised the Cape Peninsula, with the first breeding attempt recorded in 1993 (Oetlé, 1994; Curtis *et al.*, 2007). This

expansion brings the species into contact with a novel climatic regime (Hockey *et al.*, 2011). As winter breeders black sparrowhawks now experience two different rainfall regimes across their range; dry winters in their traditional range in the north and east, and wet winters in the Mediterranean climate of the recently colonised Western Cape region (Fig. 1; Linder *et al.*, 2010). Across South Africa, black sparrowhawks display clinal variation in their morph frequencies, with the frequency of dark morphs increasing from < 20% in the historical northeast of the country, to >75% in the far south west on the Cape Peninsula, Western Cape (Fig. 2a). Recent research reveals that the proportion of dark morphs correlates most closely with breeding season light levels (Tate *et al.*, 2016), driven by the difference in rainfall patterns across the species range (Amar *et al.*, 2014). As a result, the recently colonised Cape Peninsula population contains an unusually high frequency of dark morph birds and the morph ratios in this population have remained stable for over a decade (Amar *et al.*, 2014).

Two contrasting hypotheses have been proposed for the high frequency of dark morph birds in the Cape Peninsula population (Tate *et al.* 2016; Amar *et al.* 2013); (1) that colour variation is non-adaptive and is simply due to a chance founder effect and strong genetic drift and (2) that irrespective of the founding morph ratios, dark morphs have a selective advantage in this newly colonised environment with its novel winter rainfall regime. In this study we use data from the mitochondrial control region to explore the distribution of genetic diversity in several geographic populations of black sparrowhawks across South Africa and, using a phylogeographic framework, quantify the extent to which population genetic structure and gene flow may contribute to the observed pattern of colour morphs in our focal study population on the Cape Peninsula. We were specifically interested in answering the following two questions: i) is there evidence of a genetic founder effect in our study population of black sparrowhawks? and ii) have the relative frequencies of the two colour morphs diverged in the presence of gene flow?

MATERIALS AND METHODS

Study Area and Sample Collection

Blood samples were collected from 115 black sparrowhawks throughout their South African distribution, detailed in Table 1 and Figure 1. Six populations were sampled representing several biomes in the region; these include fynbos and forest in the south and Western Cape, and grassland, savannah and Indian Ocean coastal belt biomes in the north and eastern areas of the species' range (Rutherford et al., 2006). Across South Africa the distribution of the black sparrowhawk is characterised by two distinct environmental gradients that influence regional rainfall patterns; (i) a latitudinal gradient of increasing aridity occurs northwards, and (ii) a shift in rainfall seasonality from summer rainfall at the species' north eastern range, to a predominantly Mediterranean winter rainfall regime in the recently colonised south-western extent (Fig. 1; Linder et al., 2010; Odendaal et al., 2014).

Black sparrowhawks were trapped on their territories using bal-chatri traps baited with live pigeons *Columba livia* (following Berger and Mueller, 1959), or sampled as chicks at the nest. We also sampled birds from falconers (n=5) and rehabilitation centres (n=11); for these samples only birds whose exact origins were known were used. Blood was collected from the brachial vein (Johnson, 1981) using 0.5ml insulin syringes (BD Micro-Fine™) and stored in 1 ml of 96% ethanol at room temperature. A total of 67 of these samples were collected from five regions, representing a maximum distance of ~1800km, from Mpumalanga, Gauteng, Kwazulu-Natal, Eastern Cape and coastal south Western Cape during the 2014 breeding season (April- October), while the remaining samples were all collected from the Cape Peninsula during the breeding periods of 2012-2015 (Table 1). The minimum distance and the mean distance between any two populations were 326 km and 986 km (SD: 575 km) respectively.

Table 1. Sampling details for blood samples taken from Black Sparrowhawk individuals throughout their South African distribution.

Sampling location	Co-ordinates	Sample size			Morphs			Distance from Cape Peninsula (km)	Individuals sampled
		N	M	F	D	W	U		
Mpumalanga (MP)	S25.52 E30.04	14	8	6	3	11	0	1734	All adults
Gauteng (GP)	S25.62, E28.19	11	5	6	1	10	0	1530	All adults
Kwazulu-Natal (KZN)	S29.86, E30.83	15	11	4	1	9	4	1397	11 adults, 4 juveniles
Eastern Cape (EC)	S33.95, E25.56	16	7	9	5	7	4	832	12 adults, 2 juveniles, 3 chicks
Coastal south-Western Cape (WC)	S33.96, E23.56	9	0	9	6	1	2	498	8 adults, 1 juvenile.
Cape Peninsula (CP)	S34.08, E18.45	50	17	33	29	4	16	0	36 adults, 14 chicks.

*N-sample size, M-male, F-female; W-White morph, D-dark morph, U-unknown morph (juvenile/chick)

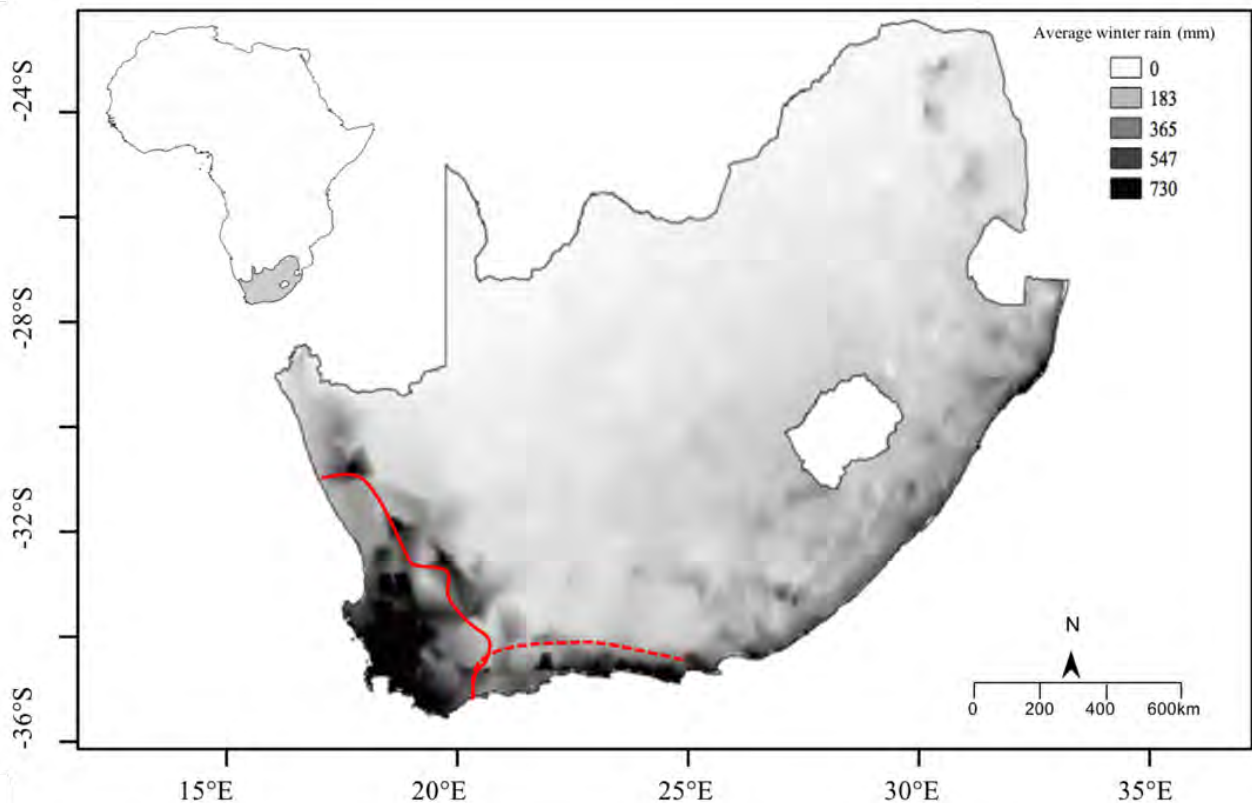


Figure 1. Map indicating the rainfall regimes throughout the Black Sparrowhawks range in South Africa. Shaded regions represent the average annual winter rainfall in mm (April–October) during the black sparrowhawk breeding season across South Africa. Red lines indicate the approximate positions of the different rainfall zones of South Africa: Solid line indicates the winter rainfall zone; dashed line indicates all year rainfall zone; and the rest of South Africa receives summer rainfall.

DNA Extraction, PCR and mtDNA Sequencing

Genomic DNA was extracted from all individuals. Extractions were performed either using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's protocols or by a standard salt extraction (Aljanabi et al., 1997). All extractions were diluted 1/100 in 1X Tris-EDTA (TE) buffer (pH 8.0) and stored at 4°C until use. Data from other raptor species suggest that the black sparrowhawk mitochondrial DNA (mtDNA) genome is likely organized in the "novel" arrangement described in Mindell *et al.* (1998). We therefore used primers developed by Sonsthagen *et al.*, (2004) for northern goshawks *Accipiter gentilis* to amplify the ~600bp of the mtDNA control region, corresponding to domain I of the avian mitochondrial control region (Baker & Marshall, 1997). Primer sequences were L16064 (5'-TTGGTCTTGTAACCAAAGA-3') and H15426 (5'-CACCAAAGAGCAAGTTGTGC-3') as reported in Sonsthagen *et al.*, (2004).

Polymerase chain reaction (PCR) was performed in 20µl reaction volumes. Each reaction contained 0.5pmole/µl forward and reverse primers 1.5mM MgCl and and DreamTAQ™ DNA polymerase (Thermo Fisher Scientific). PCR conditions included an initial denaturation step at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 secs, primer annealing at 54°C for 45 secs, and primer extension at 72°C for 45 secs. A final step at 72°C for 7 min was used to complete primer extension. PCR products were checked on 1% agarose gel, cleaned through Microcon-PCR (Millipore Corp.) and sequenced in one direction using the forward primer H15426 on an ABI 3730 (Applied Biosystems) automated sequencer using dye-terminator chemistry (BigDye kit 3.1, Applied Biosystems). Chromatograms were edited using the program BioEdit™ 7.2.5 (Hall, 1999), aligned in CLUSTAL X (Thompson *et al.*, 1997) and checked manually. The identity of all sequences was checked using the BLASTn search tool in the NCBI nucleotide database on GenBank. The presence of nuclear copies of mitochondrial genes (numts) was assessed by amplifying the target gene region using DNA extracted from blood and muscle tissue from a number of natural mortality individuals (n=5) to ensure recovery of the same DNA sequence irrespective of tissue source. Because avian red blood cells are nucleated there is increased potential for nuclear sequences to be amplified instead of or in addition to the target mtDNA gene region (Sorenson & Quinn, 1998; Bensasson *et al.*, 2001)

Spatial Population Structure and Patterns of Gene Flow

In order to examine whether neutral evolutionary processes such as a founder effect may have influenced the high frequency of dark morphs in the Cape Peninsula population we (i) determined the degree to which genetic variation is spatially structured in the black sparrowhawk across South Africa, specifically between the Cape Peninsula and surrounding populations, and (ii) quantified historic patterns of gene flow among the sampled populations.

We explored the evolutionary relationships among mtDNA haplotypes using standard genetic diversity statistics (number of segregating sites S , average and population haplotype diversity Hd , and nucleotide diversity π) estimated in DNASP v. 5.0 (Rozas & Rozas 1999). To visualise relationships among mtDNA haplotypes across our sample populations, we constructed a unrooted Neighbour-net network in SplitsTree4 v4.14.2 (Huson & Bryant, 2006), using uncorrected 'p' distances. Network analysis allows reticulations among branches and is thus highly suitable for analysing evolutionary relationships among populations (Cassens *et al.*, 2003; Huson & Bryant, 2006; Odendaal *et al.*, 2014).

We used a number of statistical approaches to determine the degree to which genetic variation is structured in the black sparrowhawk. We examined the degree to which genetic variation is spatially clustered using the Bayesian framework of BAPS v6.0 (Cheng *et al.*, 2013). To test for the presence of discrete evolutionary lineages we estimated the most probable number of genetic clusters (K) among populations using a spatially explicit Bayesian clustering mixture model in BAPS. We performed spatial clustering for groups with K ranging from 2-6. The analysis was repeated ten times for each maximum K and the log marginal likelihood value for each genetic partition was evaluated. We also used Analysis of Molecular Variance (AMOVA) calculated in GenAlEx v6.5 (Peakall & Smouse, 2012) to quantify the partitioning of genetic variation within and among the regions sampled. The significance of variance components and Φ_{ST} (a measure of population genetic differentiation analogous to the fixation index F_{ST} ; Wright, 1965) were estimated with 999 random permutations.

To obtain estimates of recent historic gene flow among populations we used a maximum likelihood method based on the coalescent implemented in MIGRATE-N v3.3.2 (Beerli, 2009). MIGRATE-N uses an equilibrium model that estimates migration rates averaged across the coalescent history and simultaneously estimates Θ , the effective population size scaled by mutation rate where $\Theta = N_e\mu$, together with pairwise migration rates summarised as $M = m/\mu$, where m is the effective immigration rate per generation between populations (Beerli & Felsenstein, 1999). Based on current ringing and tracking data (G. Tate unpubl.), we estimated Θ and M using a custom designed migration matrix where migration was only allowed between neighbouring populations and where multiple unique haplotypes were shared (Table 2; MIGRATE-N). Starting values for all parameter estimates were initially obtained using F_{ST} (Beerli & Felsenstein, 1999) and the following search parameters were used: 10 short chains with 500 000 gene trees sampled and 5000 trees recorded; three long chains with 50 million sampled trees of which 50 000 were recorded. The first 10 000 trees were discarded as burn-in and a static heating scheme with six temperatures and a swapping interval of one was used (Odendaal *et al.*, 2014). The results were averaged over five replicate runs.

Table 2. Upper and lower likelihood percentiles of M , the number of immigrants per generation scaled by mutation rate, calculated in Migrate-N (Beerli, 2009)

Source population	Receiving population	Lower percentile (0.05)	Upper percentile (0.95)
CP	WC	2.64	175.35
WC	CP	517.56	2308.12
WC	EC	51.29	318.74
EC	WC	497.12	2124.35
EC	CP	0.058	127.46
EC	KZN	89.34	407.51
KZN	EC	661.27	2494.89
GP	KZN	35.67	143.95
MP	KZN	978.79	4197.28
KZN	MP	412.76	2049.20
MP	GP	1359.62	5513.78
GP	MP	357.49	1487.29

Comparing Colour Trait Divergence and Genetic Distance

Physical isolation as well as varying levels of genetic connectivity can strongly influence both trait and neutral genetic divergence among populations (Rudh *et al.*, 2007; Odendaal *et al.*, 2014). To explore the relationship between genetic structure, morph colour divergence and geographic distance we tested

for Isolation by Distance (IBD; Slatkin, 1993; Wright, 1943) using Mantel (Mantel, 1967) and partial Mantel tests. Matrix correlations were calculated in GenAlex v6.5 (Peakall & Smouse, 2012) with 999 random permutations. The three matrices analysed were pairwise geographic distances (km) calculated as straight line distances from geographic coordinates (mid point of our sampling area) using QGIS (QGIS Development Team, 2015), genetic distance using Slatkin's linearized Φ_{ST} (Slatkin, 1993), and as a measure of trait divergence across the sampling range we calculated the differences in the proportion of dark morphs among populations. The partial Mantel test determined whether differences in colour morph frequencies were associated with genetic distance while controlling for the effect of geographic distance (isolation by adaptation).

RESULTS

A total of 23 unique mtDNA d-loop haplotypes (Appendix; Table 1) were identified from 115 individuals sampled across the distributional range of the black sparrowhawk in South Africa (Fig 2; Table 1). Estimates of nucleotide diversity ranged from 0.0016 (GP) to 0.032 (MP) (Table 3) while haplotype diversity ranged from 0.46 (GP) to 0.91 (EC) (Table 3). The highest number of unique ($n=11$) was found in the Cape Peninsula population (CP), with the lowest in WC and KZN (both $n=0$). All populations shared at least one haplotype with their nearest neighbours, and in some cases multiple haplotypes were shared between distant populations (i.e. haplotype 2, 6 & 7; Fig. 2b, Table 4). Samples used to test for the possible presence of aberrant d-loop numts recovered the same sequences for both the tissue and blood derived DNA samples.

Table 3. Summary of genetic diversity statistics, indicating sample size for each locality (N), total number of unique haplotypes, haplotype diversity (Hd) and nucleotide diversity (π).

Sample Site	N	Haplotypes	Hd	π
All	115	23	0.67	0.0015
Cape Peninsula	50	15	0.55	0.0066
Coastal south-Western Cape	9	7	0.91	0.0046
Eastern Cape	16	8	0.87	0.0074
KwaZulu Natal	15	4	0.46	0.0016
Gauteng	11	4	0.67	0.0023
Mpumalanga	14	6	0.71	0.032

Population Genetic Structure of the Black Sparrowhawk Across South Africa

The neighbour-net network (Fig. 3a) revealed numerous reticulations between haplotypes with no clear geographically defined clades. Our analysis quantifying the level of population genetic structure across South Africa indicated a spatially broad degree of genetic structuring between populations, with Bayesian clustering identifying two spatially defined genetic groups (Fig. 3b); these comprise MP, GP, KZN and EC in the traditional north eastern range of the black sparrowhawk and WC and CP in the southern and south-western regions. Our results, however, reveal no unique clustering between these populations. Examining for evidence of population genetic differentiation, our investigation of hierarchical population genetic structure revealed no significant partitioning of genetic variation within or among the sampled populations $\Phi_{ST}=0.082$, with most variation found within populations (4.4), compared to the variation between populations (0.4) ($P=0.001$).

Spatial Population Genetic Structure and Patterns of Gene Flow

Estimates of historical migration rates (M) revealed considerable gene flow between neighbouring populations (Fig. 3b), with a general pattern of gene flow along the eastern coast of South Africa from the historical north-eastern regions. There is also evidence for low-levels of gene flow across longer distances from EC to CP (740 km straight line distance) and GP and KZN (482 km straight line distance). Although there doesn't appear to be a dominant source population, relative migration rates are highest from and among populations in the north eastern regions, i.e. MP and KZN (Fig. 3b), with a southward pattern of gene flow into the EC, WC and CP regions.

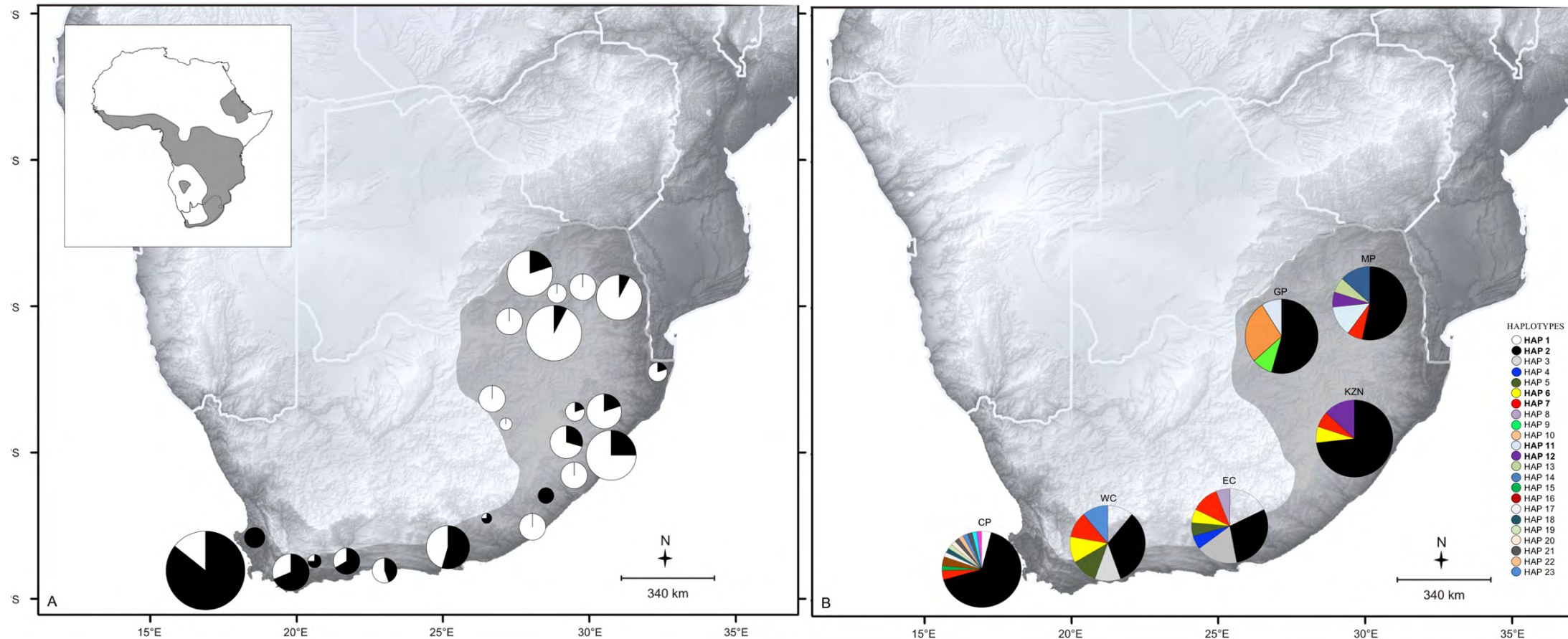


Figure 2a. Map indicating the distribution of Black Sparrowhawk morphs across South Africa (adapted from Tate *et al.*, 2016); Each cluster represents the ratios of dark and white morph birds (pie graphs: shaded region = dark morphs, white region = white morphs). The sample size for each cluster is represented by the relative size of each pie diagram (i.e. largest pie $n = 80$, smallest pie $n = 1$). **2b.** The distribution of 23 unique haplotypes across the six populations of Black Sparrowhawks sampled in this study throughout RSA. Key to acronyms given in Table 1. Pies show the relative frequency of haplotypes found in each region. Haplotypes highlighted in bold (in figure legend) indicate shared haplotypes across sample sites (see Table 4 for more information on private and shared haplotypes). Shaded area indicates Black Sparrowhawk distribution throughout South Africa (RSA).

Table 4. Number (*and frequency*) of the 23 mtDNA control-region haplotypes found in 115 Black Sparrowhawks across South Africa, as well as nucleotide (π) and haplotype (Hd) diversity. Also shown are the total number of private and shared haplotypes. Shared haplotypes indicated in bold.

Haplotype	Freq.	Sampling (<i>frequencies</i>)					
		MP	GP	KZN	EC	WC	CP
Hap1	6				3(0.2)	1(0.1)	2(0.04)
Hap2	67	7(0.5)	6(0.5)	11(0.7)	4(0.3)	2(0.3)	37(0.7)
Hap3	4				3(0.2)	1(0.1)	
Hap4	1				1(0.06)		
Hap5	2				1(0.06)	1(0.1)	
Hap6	3			1(0.06)	1(0.06)	1(0.1)	
Hap7	7	1(0.06)		1(0.06)	2(0.1)	1(0.1)	2(0.04)
Hap8	1				1(0.06)		
Hap9	1		1(0.09)				
Hap10	3		3(0.3)				
Hap11	3	2(0.1)	1(0.09)				
Hap12	3	1(0.06)		2(0.2)			
Hap13	1	1(0.06)					
Hap14	2	2(0.1)					
Hap15	1						1(0.2)
Hap16	2						2(0.04)
Hap17	1						1(0.2)
Hap18	1						1(0.2)
Hap19	1						1(0.2)
Hap20	1						1(0.2)
Hap21	1						1(0.2)
Hap22	1						1(0.2)
Hap23	2					1(0.1)	1(0.2)
Total	115	14	11	15	16	9	50
#Haplotypes	23	6	4	4	8	7	12
#Private haplotypes		2	2	0	2	0	11
#Shared haplotypes		4	2	4	6	4	4
Hd	0.67	0.71	0.67	0.46	0.87	0.91	0.55
π	0.0015	0.032	0.0023	0.0016	0.0074	0.0046	0.0065

Influence of Genetic Distance and Geographic Isolation

The role of geographic distance in contributing to the distribution of genetic variation and trait variation in the study populations differed considerably (Fig. 4a & 4b respectively). Geographic distance doesn't contribute to the variation in genetic distance in black sparrowhawk populations across South Africa (Mantel Test: $R^2 = 0.0015$, $P > 0.05$) but does contribute to the pattern of variation in morph colour (Mantel Test: $R^2 = 0.36$, $P < 0.01$) across the study area. The relationship between genetic distance and differences in the proportion of dark morphs among populations was negligible (Fig. 4c) and remained consistent when controlling for the effect of geographic distance (Partial Mantel Test: $R^2 = 0.04$, $P < 0.001$).

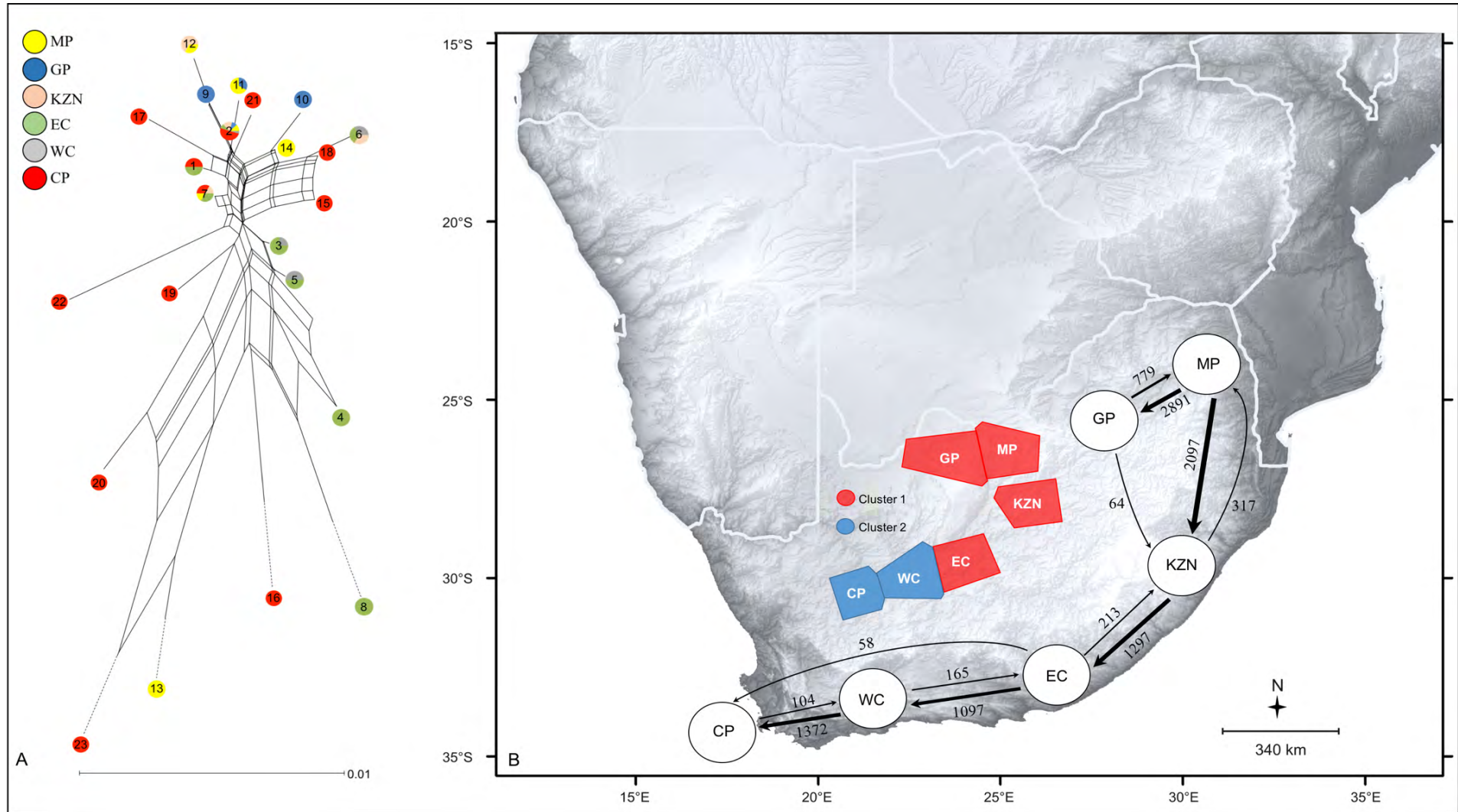


Figure 3a. Neighbour-Net network based on corrected p -distances of the 23 unique haplotypes isolated in this study. Each circle represents a unique haplotype coloured according to the population in which it occurs. Pie graphs indicate where haplotypes are shared across several populations. The network indicates no distinct geographic clustering in haplotypes across sampling sites. **3b.** Estimated migration patterns among populations of black sparrowhawks throughout South Africa. Relative arrow thickness indicates the migration rates; values M = number of immigrants per generation scaled by mutation rate. The 95% confidence intervals are provided in: Table 2. Inset: Voronoi tessellation showing the assignment of populations to two spatially defined genetic clusters. A key to acronyms used are given in Table 1.

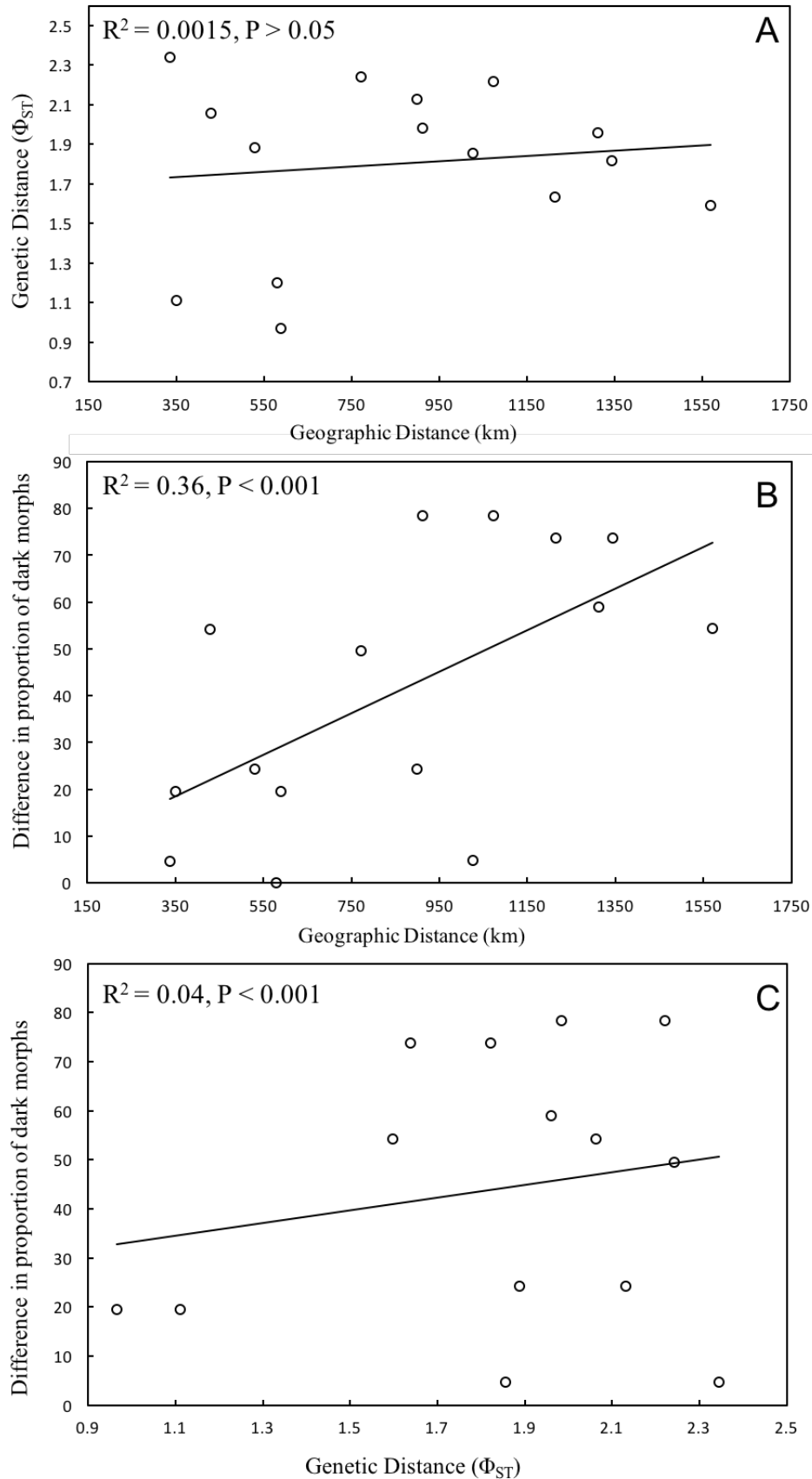


Figure 4. Analysis of isolation by distance using pairwise geographic distance versus **a:** genetic distance, and **b:** differences in the proportion of dark morphs among populations; and **c:** genetic distance versus differences in the proportion of dark morphs among populations.

DISCUSSION

The Role of Gene Flow in Determining Spatial Patterns in Adaptive Colouration

Our results reveal minimal evidence for fine-scale geographic structuring in black sparrowhawk mitochondrial lineages; rather they clearly reflect the recent colonisation of the Western Cape region of South Africa by multiple mitochondrial lineages originating from across the local range of the species. Bayesian clustering analysis used to quantify the extent of structuring across South Africa identified two spatially defined mitochondrial groups; with one cluster of populations in the traditional north and eastern ranges (EC, KZN, GP & MP), and a second cluster comprising populations from the newly colonised southern and south-western regions (WC & CP) (Fig. 3b). Examining the population genetic structure further, our investigation revealed no significant partitioning or differentiation of genetic variation within or among populations. Although mitochondrial gene flow is bi-directional between most of the populations we sampled, we found highest migration rates between populations in the north and eastern parts of the country and from these areas into the south and newly colonised region of the Western Cape (Fig. 3b). We also found evidence for long distance gene flow, although only at very low levels. Once again, this may be the result of low frequency dispersal over longer distances possibly from stable, well established source populations. The low levels of genetic differentiation at the mtDNA control region marker supports a scenario of substantial gene flow among black sparrowhawk populations throughout South Africa.

Relatively high levels of genetic connectivity occur among the sampled populations, with multiple shared haplotypes between neighbouring populations and several haplotypes shared between geographically distant populations (Fig. 2b, Table 4). These may reflect recent long-distance dispersal events or the retention of common ancestral polymorphisms (Odendaal *et al.*, 2014). In the historic heartland of the species distribution i.e. Mpumalanga and Kwazulu-Natal (Allan, 1997; Malan & Robinson, 2001; Hockey *et al.*, 2005), we found both high haplotype diversity and nucleotide diversity, suggesting long term stability within populations (Zink, 1994; Roques & Negro, 2005). In areas where

the species has recently colonised i.e. the coastal south Western Cape and specifically the Cape Peninsula population (Oettlé, 1994; Tarr *et al.*, 1998; Malan & Robinson, 2001; Curtis *et al.*, 2005), haplotype diversity was also relatively high, but accompanied by relatively low nucleotide diversity (Table 4). This combination can be characteristic of a recently founded population (Crochet, 2000; Sonsthagen *et al.*, 2004; de Volo *et al.*, 2013), and suggests that while black sparrowhawks are indeed fairly new colonists to the region, they currently comprise a combination of both unrelated haplotypes together with a number of closely related haplotypes.

A number of studies report similar patterns of gene flow in species displaying strong divergence in colour between populations across their geographic distribution (Coyne & Orr, 2004; Merilaita, 2001; Crispo *et al.*, 2006; Antoniazza *et al.*, 2010). For example, the European barn owl *Tyto alba* also displays significant morph colour differentiation throughout its range, varying continuously from white in south-western Europe to reddish-brown in north-eastern Europe. Nevertheless Antoniazza *et al.* (2010) found high levels of gene flow across the species range and suggest that the persistence of the pronounced colour differentiation in the presence of substantial gene flow provides support for the hypothesis that local adaptation is the primary force maintaining geographic colour variation in the species. Subsequent work on the barn owl has also revealed morph-specific matching with local habitat, particularly in females, which appears to contribute to the distribution and maintenance of colour variation in the species (Dreiss *et al.*, 2012). Similar patterns of evidence for local trait adaptation have also been reported in reptiles (*Nerodia sipedon*; King and Lawson, 1995) and amphibians (*Dendrobates pumilio* Rudh *et al.*, 2007) and extend beyond vertebrates (Merilaita, 2001; Cristinae, 2014). For instance, in the colour polymorphic isopod *Idotea baltica*, Merilaita (2001) found that morph frequencies varied substantially among populations across his sampling sites and suggested that locally varying selection for cryptic colouration contributes to the maintenance of colour polymorphism in the species in the face of strong gene flow. Although many studies suggest that natural selection for cryptic colouration against local habitat background is the primary force driving variation in a species' colour across heterogeneous environments (Merilaita *et al.*, 1999; Merilaita, 2001; Stuart-Fox *et al.*, 2004; Rudh *et al.*, 2007), local selection can be counteracted by gene flow (Slatkin, 1973, 1987; Merilaita,

2001; Rudh *et al.*, 2007). Odendaal *et al.*, (2014) recently demonstrated how an adaptive trait can diverge in the presence of substantial gene flow. In the Cape horseshoe bat *Rhinolophus capensis* significant sensory trait differentiation (i.e. echolocation frequencies) occurs between neighbouring populations despite extensive historical gene flow, suggesting strong local selection for optimizing echolocation frequencies to different environments across their range in South Africa. The authors propose that differences in habitat structure between biomes (from cluttered to open vegetation), drive the divergence in adaptive echolocation frequency in their study species.

The Adaptive Significance of Plumage Colour

Galeotti *et al.* (2003) argue that colour polymorphism in birds probably evolved via disruptive selection under selective regimes linked to individual detectability under variable light conditions. Ambient light is likely a major selective force (Galeotti & Rubolini, 2004) for a number of reasons; light condition varies considerably between habitats as well as across different climatic conditions (Martin, 1990) and strongly affects the ability of prey to visually detect certain colours and patterns in predators, or conversely, for predators to detect certain colours and patterns in prey (Endler, 1991; Green & Leberg, 2005; Bortolotti, 2006; Bond, 2007). The discrete ventral dark and white plumage displayed in the different morphs of the black sparrowhawk (Taylor & Louette, 2009; Amar *et al.*, 2013) may therefore be beneficial under different ambient light conditions throughout their range. For example, dark morphs are predicted to be more concealed in low light conditions via background matching (i.e. on cloudier days, during dusk/dawn, or in closed habitats) and in these environments, appear to accrue a foraging advantage (Preston, 1980; Rohwer, 1990; Tate *et al.*, 2016). Conversely, white morphs may be more cryptic in brighter conditions and forage more successfully as a result in these conditions (i.e. on sunny days, in the middle of the day, or in open habitats) (Galeotti *et al.*, 2003; Roulin, 2004b; Roulin & Wink, 2004). This may also explain why Rohwer, (1990) and Preston, (1980) found that the different morphs in the Pacific reef heron *Egretta sacra* and the red-tailed hawk *Buteo jamaicensis* respectively, appeared to select habitat cover that best conceal them from their prey.

In this paper, we were specifically interested in testing whether a founder effect could explain the unusually high proportion of dark morphs in our study population. Although our results do support reports that the black sparrowhawk is indeed a recent colonist to the Cape Peninsula region, we did not find strong evidence to support a founder effect as the most likely explanation for the morph frequencies observed. Rather in the presence of strong historical, homogenizing gene flow, the persistence of the pronounced colour differentiation in the Cape Peninsula population of black sparrowhawks contributes to a growing body of evidence that variation in plumage colour is unlikely to be due to neutral evolutionary processes alone (Slatkin, 1973; Antoniazza *et al.*, 2010). Our results are therefore more consistent with the hypothesis that selection, and thus local adaptation, is the primary force influencing the unusually high frequency of dark morphs in our study population (Amar *et al.*, 2014).

Gene Flow and Selection in the Structuring of Morphs Across South Africa

Given the levels of gene flow among our sampled populations, if the patterns of plumage colouration are maintained purely by neutral processes in the black sparrowhawk we would expect a more random distribution in morph frequencies across South African populations. We would also expect the frequency of morphs in populations to better reflect genetic connectivity and founder events; but we don't see this. Instead, the most recently founded populations of black sparrowhawk in the Western Cape are characterised by relatively high levels of genetic variation that suggest colonising lineages came from populations both near and far. A final and very interesting finding of our study is the apparent uncoupling of spatial variation in neutral mtDNA and the proportion of dark morph birds (Fig. 4) across the study area. While genetic variation in the black sparrowhawk is not shaped by a pattern of isolation by distance, the geographic distribution of differences in the proportion of dark morphs is positively correlated with geographic distance despite evidence for gene flow across the sampled range of the study. Clearly geographic distance is not a significant barrier to gene flow in this species and we argue that the geographic distribution of dark morph birds most likely reflects the underlying environmental cline in light conditions related to rainfall seasonality across South Africa, favouring dark morph individuals in the more recently colonised areas of the Western Cape.

Conclusion

Our study reveals that significant plumage trait differentiation in black sparrowhawks of the Western Cape has proceeded despite substantial gene flow and provides supporting evidence for a strong role for local selection in the maintenance of colour polymorphism and the structuring of morphs in the black sparrowhawk. Our results highlight the importance of population level analyses to understand the complex interplay between selection and gene flow in the evolution of adaptive traits. Studying gene flow and local adaptation in geographically variable species, such as the black sparrowhawk, can reveal the degree to which evolutionary processes often act in concert on populations and to what extent their relative influence may vary across heterogeneous landscapes.

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CHAPTER 3

DIFFERENTIAL FORAGING SUCCESS ACROSS A LIGHT LEVEL SPECTRUM EXPLAINS THE MAINTENANCE AND SPATIAL STRUCTURE OF COLOUR MORPHS IN A POLYMORPHIC BIRD.



Chart farm female, August 2013, fiercely guards her two chicks as our intrepid climber, Mark Cowen, attempts to collect the youngsters for colour ringing. Note the camera installed above the nest, which was instrumental in recording prey provisioning rates used in this chapter. Photo: Mark Cowen

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ABSTRACT

Detectability of different colour morphs under varying light conditions has been proposed as an important driver in the maintenance of colour polymorphism via disruptive selection. To date, no studies have tested whether different morphs have selective advantages under differing light conditions. We tested this hypothesis in the black sparrowhawk, a polymorphic raptor exhibiting a discrete white and dark morph, and found that prey provisioning rates differ between the morphs depending on light condition. Dark morphs delivered more prey in lower light conditions, while white morphs provided more prey in brighter conditions. We found support for the role of breeding season light level in explaining the clinal pattern of variation in morph ratio across the species range throughout South Africa. Our results provide the first empirical evidence supporting the hypothesis that polymorphism in a species, and the spatial structuring of morphs across its distribution, may be driven by differential selective advantage, via improved crypsis, under varying light conditions.

Keywords:

Accipiter melanoleucus, ambient light, background matching, black sparrowhawk, colour polymorphism, crypsis, disruptive selection, predator–prey interaction, raptor.

INTRODUCTION

Persistent colour polymorphism is the occurrence of two or more phenotypically distinct, genetically based forms or morphs within a natural breeding population (Huxley, 1955b). Although classified as generally rare, colour polymorphism is phylogenetically widespread (Hugall & Stuart-Fox, 2012) and has long fascinated evolutionary biologists (Darwin, 1859; Ford, 1945; Huxley, 1955). And yet, the ecological and evolutionary mechanisms maintaining most colour polymorphisms in natural populations remain unclear. The relative fitness advantage of maintaining morphs within species has been explored extensively within the context of sexual selection (Roulin & Bize, 2007); but arguably an area deserving of more attention in this field is that the presence of colour polymorphism indicates the use of alternative strategies by species, where equivalent morph fitness may be achieved in heterogeneous environments under scenarios of frequency-dependent (Allen, 1988), balancing (Losey *et al.*, 1997) and disruptive selection (Roulin & Wink, 2004). Thus the coexistence of multiple morphs, and the maintenance of a permanent polymorphism within a population, suggests a selective trade-off between alternative morphs, all enjoying some advantage, but commonly also incurring some cost (Fisher 1930).

Colour polymorphism is widely considered to serve an adaptive function (Götmark, 1987; Galeotti *et al.*, 2003; Roulin, 2004b) with different colour morphs frequently associated with a variety of behavioural, physiological and life-history traits (Galeotti *et al.*, 2003; Roulin, 2004b; Chakarov *et al.*, 2008). For example, morphs may differ in prey foraging and provisioning (Roulin & Wink, 2004), in mate preference (Roulin, 2004b; Hurtado-Gonzales *et al.*, 2014), in their ability to resist bacterial feather degradation (Burt & Ichida, 2004) and parasites (Chakarov *et al.*, 2008, 2015), in the degree of oxidative stress and cell damage caused by UV radiation (Galván *et al.*, 2010; Roulin, 2014), and in their ability to thermoregulate (Roulin, 2014; Dreiss *et al.*, 2015). Additionally, many studies provide evidence suggesting that different morphs may be better adapted to certain environmental conditions,

such as prevailing weather (Karell *et al.*, 2011), habitat type (Dreiss *et al.*, 2012) and amount of vegetation cover (Rohwer, 1990). Therefore, in the same species, the proportions of different morphs may differ from one area to another depending on the relative predominance of key environmental conditions. Together, these adaptations may explain why polymorphic species commonly show clinal variation in their morph frequencies, with morph ratios frequently varying along environmental gradients (Gloger, 1833; Galeotti *et al.*, 2003; Burt & Ichida, 2004; Antoniazza *et al.*, 2010; Amar *et al.*, 2014).

In many species dominant morphs closely match their local substrate; this may act to minimize detection by visual predators, or to reduce predator conspicuousness to their prey (Dreiss *et al.*, 2012). In the case of some polymorphic ectotherms, the frequency of morphs often vary across different thermal environments (Rosenblum *et al.*, 2004). Such local selective advantage may lead to disruptive selection (Mather, 1955b), with extreme phenotypes favoured under different prevailing conditions (Rueffler *et al.*, 2006). Disruptive selection has been invoked as the focal hypothesis proposed for the evolution and maintenance of polymorphism across a variety of species (Rueffler *et al.*, 2006; Antoniazza *et al.*, 2010), including insects (de Jong & Brakefield, 1998), reptiles (Alsteadt *et al.*, 2006), mammals (Rounds, 1987) and birds (Galeotti *et al.*, 2003; Antoniazza *et al.*, 2010; Amar *et al.*, 2014).

In birds, colour polymorphism has been extensively studied, although it is relatively rare (c. 3.5% of all bird species) (Galeotti *et al.*, 2003; Roulin, 2004b; Rueffler *et al.*, 2006). In their review, Galeotti *et al.* (2003) concluded that colour polymorphism probably evolved under selective pressures linked to individual detectability in variable light conditions and argue that it is most likely maintained by disruptive selection. Ambient light conditions are likely to be strong selective agents, since light intensity within an environment during the course of the day, between habitats or in different weather conditions can vary dramatically. For example, shading from a tree canopy or increased cloudiness can give rise to variation in ambient light levels on the order of 300 billion-fold (Martin 1990). The light regime experienced by animals also strongly depends on seasons and latitude (Martin 1990). Differences in light conditions therefore offer a potential mechanism under which disruptive selection

could operate, favouring the maintenance of two or more colour morphs within a species range (Galeotti *et al.*, 2003). Central to this argument is the idea that different morphs enjoy selective advantages under differing light levels (Galeotti *et al.*, 2003; Roulin & Wink, 2004). For example, because light variation affects the ability of prey to visually detect predators (Green & Leberg, 2005), dark morphs may benefit from crypsis in low light conditions i.e. on cloudier days, during dusk/dawn, or in closed habitats, and may accrue a foraging advantage via background matching (Preston, 1980; Rohwer, 1990). Conversely, light morphs may forage more successfully in brighter conditions, benefitting from improved crypsis against a lighter background i.e. on sunny days, in the middle of the day, or in open habitats (Galeotti *et al.*, 2003; Roulin, 2004b; Roulin & Wink, 2004). Whilst these ideas are highly credible, no empirical study has yet explicitly tested these underlying assumptions.

In birds, colour polymorphism has evolved most frequently in raptorial species (Fowlie & Kruger, 2003; Roulin & Wink, 2004), suggesting that predator-prey relationships may be a key component in the evolution and maintenance of polymorphism (Galeotti *et al.*, 2003; Roulin & Wink, 2004). In diurnal raptors polymorphism is particularly common in the genus *Accipiter* (Fowlie & Kruger, 2003), where almost a quarter of the species are polymorphic (Ferguson-Lees & Christie, 2001). Many *Accipiters* are specialist predators of birds (Fowlie & Kruger, 2003). Given the excellent visual acuity of birds (Jacobs, 1992), their memory capacity (Clayton, 1998) and ability to detect and evade their predators (Roulin & Wink, 2004), the relatively high frequency of polymorphism within *Accipiters* may reflect selection for reduced detection under variable environmental conditions; more cryptic morphs may therefore be favoured through improved foraging success in environments with greater background matching. The black sparrowhawk *Accipiter melanoleucus* is one such polymorphic *Accipiter*. The species is broadly distributed, occurring throughout most of the forested areas of sub-Saharan Africa (Ferguson-Lees & Christie, 2001), and exhibits discrete polymorphism with adults occurring as either dark or white morphs (Amar *et al.*, 2013). Within South Africa, black sparrowhawks have undergone a range expansion over the last few decades (Hockey *et al.*, 2005; Martin *et al.*, 2014), bringing the species into a novel climatic regime (Martin *et al.*, 2014). As winter breeders, black sparrowhawks now experience two contrasting climatic regimes in South Africa; dry winters in their historical range in the north and

east and wet winters in the Mediterranean climate of the recently colonised Western Cape region. Conditions on the Cape Peninsula particularly reflect these differences with the area receiving exceptionally high amounts of winter rainfall due to its exposure to southern ocean weather systems and orographic effects of the local topography dominated by Table Mountain (Martin *et al.*, 2014). Within South Africa, black sparrowhawks display clinal variation in their morph frequencies, with the frequency of dark morphs declining from > 75 % in the far south west on the Cape Peninsula, Western Cape; Fig. 1, to < 20 % in the northeast of the country (Amar *et al.*, 2014). This variation correlates with temperature and rainfall during the winter breeding period (April-October; Martin *et al.* 2014), with dark morphs predominating in areas with higher rainfall (Amar *et al.*, 2014).

In this study, we undertake the first explicit test of the hypothesis proposed by Galeotti *et al.* (2003), which we term the ‘light level-detectability hypothesis’; that different morphs have a selective advantage due to their detectability by prey under varying light levels. Using at nest video footage, we compare prey provisioning rates of dark and white male black sparrowhawks under different light conditions. If crypsis via background matching is important in prey capture we predict a negative relationship between provisioning rates and increasing light levels for dark morphs, and a positive relationship for white morphs. Secondly, we explore whether the clinal variation in morph ratios observed in this species across its’ South African range might be explained by varying light levels experienced during the winter breeding season. Such variation in light levels is predicted due to the different rainfall regimes experienced by the species through its range (Martin *et al.*, 2014). Given the link between the light environment and colour signals in birds, we argue that the visual environment is a dominant driver favouring colour polymorphism in black sparrowhawks.

MATERIALS AND METHODS

Timing of Prey Provisioning

Our study uses the closely monitored urban black sparrowhawk population on the Cape Peninsula (34° 00’S, 18°26’E) in the Western Cape, South Africa, which has been monitored since 2000 (Amar *et al.*

2013; Martin *et al.* 2014 for more detail of the study site). We collected data on prey provisioning from 17 different pairs of black sparrowhawks on the Cape Peninsula in the 2012 and 2013 breeding seasons (Table 1).

Table 1. Details for the 17 Black Sparrowhawk nests monitored on the Cape Peninsula, Western Cape, South Africa; colour morph, geographical coordinates, timeframe recorded, chick ages monitored (days) and brood size.

Territory	Male morph	Latitude (°S)	Longitude (°E)	Start date	End date	Brood size	Chick age
Chart Farm	White	34.004	18.445	28/06/2012	18/08/2012	2	0-21
Newlands	Dark	33.965	18.454	01/08/2012	30/08/2012	2	0-21
Rhodes Mem	Dark	33.946	18.46	14/09/2012	11/10/2012	3	0-21
Stone Church	White	34.055	18.424	10/11/2012	20/12/2012	2	0-21
Alphen trail	White	34.901	180388	24/07/2013	20/11/2013	2	0-21
Audreys nest	White	34.005	18.388	28/06/2013	09/08/2013	3	0-21
Glen Dirk	Dark	33.9959	18.445	10/04/2013	09/06/2013	2	0-21
Imhoff	White	34.141	18.34	30/09/2013	06/12/2103	2	0-21
Tokai Forest	Dark	34.054	18.44	05/09/2013	07/11/2013	1	0-21
Marlenes nest	Dark	34.013	18.425	22/07/2013	22/09/2013	3	0-21
Naked Lady	Dark	34.043	18.405	01/11/2013	16/12/2013	2	0-11
Newlands 1	Dark	33.966	18.445	29/08/2012	30/10/2012	2	14-21
Oakley avenue	Dark	34.065	18.44	06/08/2013	22/09/2013	2	0-21
Ryteplaats	White	34.009	18.356	05/11/2012	21/12/2012	2	18-21
Newlands 4	Dark	33.978	18.437	09/08/2012	14/08/2012	1	20-21
Tokai	Dark	34.6649	18.408	22/05/2013	22/07/2013	2	0-6
Zonnestraal	Dark	34.012	18.456	24/09/2013	21/10/2013	3	0-19

We monitored the timing of prey provisioning to the active nests using remote cameras located c. 1.5-2 m above the nest. Provisioning rates were used as a reflection of foraging success, an assumption which has been previously confirmed for at least one raptor (Korpimaeki *et al.*, 1994). Black sparrowhawks have a relatively modest diet, with 80% of their prey consisting of three similar-sized avian species (*Streptopelia semitorquata*, *Columba livia*, *Columba guinea*). In our study population no differences in the relative abundances of prey species has been detected between morphs (unpublished data), thus it is unlikely that there is any substantial trade-off in prey size-delivery frequency. Camera units were set to record on a daily duty cycle from one hour before sunrise until one hour after sunset. Two camera types were used: 1) Solar powered remote trail cameras (Ltl Acorn Model: Ltl-6210MC), set on a timer, recording images at three minute intervals. 2) Continually recording, motion triggered cameras, developed by the Royal Society for the Protection of Birds. Previous research has shown these

two approaches produced near identical results in nest behaviour measurements (Katzenberger *et al.*, 2015).

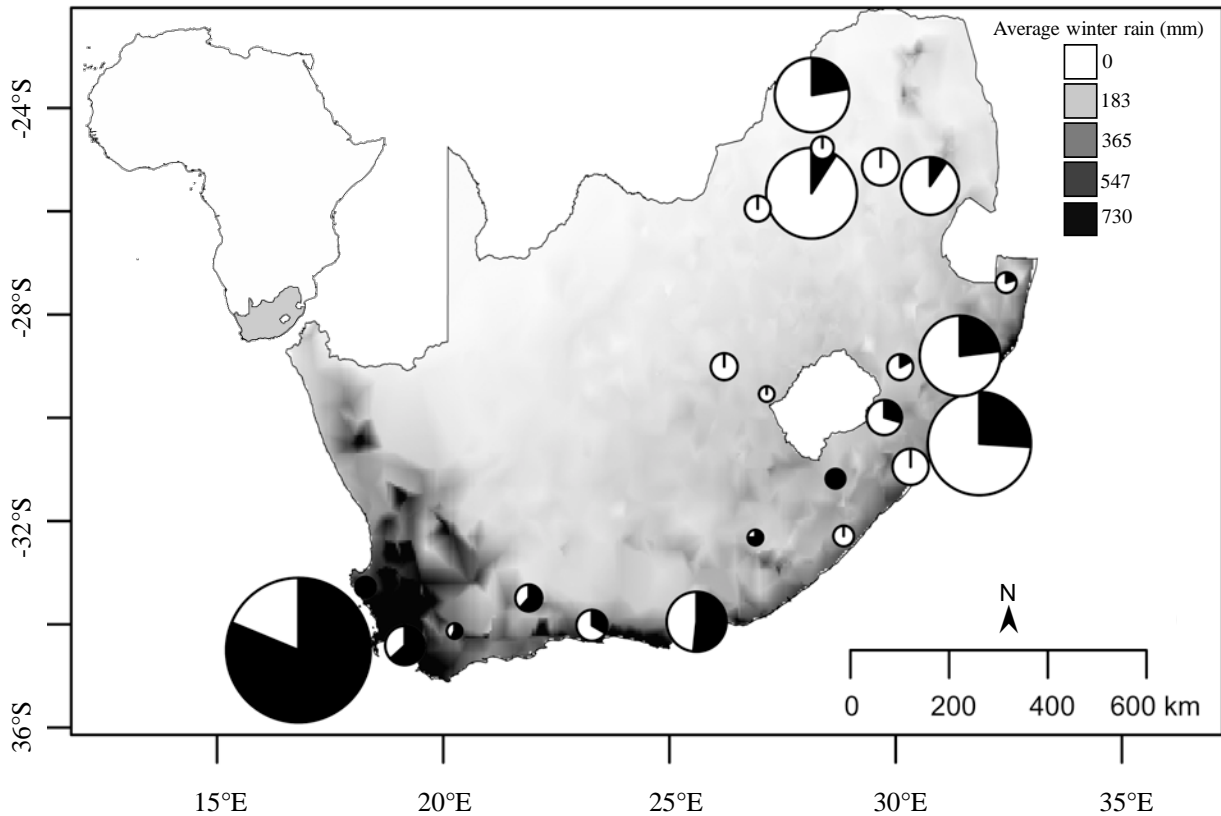


Figure 1. Location of the 25 sampling clusters distributed throughout South Africa. Each cluster represents the ratios of dark and white morph birds (pie graphs: shaded region = dark morphs, white region = white morphs). The sample size for each cluster is represented by the relative size of each pie diagram (i.e. largest pie $n = 80$, smallest pie $n = 1$). Average breeding season light level was matched with each cluster using an interpolated irradiance map of South Africa. Shaded regions represent the average annual winter rainfall in mm (April–October) during the Black Sparrowhawk breeding season across South Africa.

Images from the nest cameras were analysed manually and the hour of each prey delivery event was recorded. Males provide almost all of the food during the early nestling stage, while the females spend most of the time brooding the chicks (Katzenberger *et al.*, 2015). Thus, to ensure we could attribute provisioning to a specific morph (the male morph) we used the first 21 days of the nestling stage. Chick age was determined either from the date of hatching recorded from the camera footage or, for older chicks where hatch date was unknown, by comparing the relative plumage development and chick size to a reference collection of photographs of known age nestlings (Katzenberger *et al.*, 2015).

Ambient Light and Weather Data

For all monitored nests we matched each hour of provisioning data with the average ambient light that fell during the hour. For this we used average hourly irradiance i.e. solar radiation in W/m^2 (see Hurtado-Gonzales *et al.* (2014)), hereafter referred to as light level, from a nearby South African Environmental Observation Network (SAEON) weather station (33.952° S, 18.459° E), positioned out in an open field. This station was located on average 9.5 km (range: 0.4 – 22.9 km) from the studied nests and was also used to collect information on average rainfall (mm) and temperature (C°) for each hour.

Morph Frequency Data

To explore variation in black sparrowhawk morph ratios throughout South Africa (total study $n=439$) we collated all available data of known morph ratios of breeding black sparrowhawks from Amar *et al.* (2014). We also added additional data collected from field sampling of birds throughout their South African distribution ($n=175$). Most of these birds were viewed at or close to their nest sites ($n=140$), although some individuals were recorded incidentally in the field ($n=35$). Following Amar *et al.* (2014), we grouped individual birds into logical spatial clusters (Fig. 1; Table 2), such that birds within a cluster were nearer to each other than to points within other clusters. These samples covered the majority of the distributional range of breeding black sparrowhawks in South Africa.

To test whether the morph ratios across South Africa were related to local ambient light levels during the breeding season, we also obtained light levels at a national scale (South Africa). For each cluster location (where we had information on morph ratios) we collated the average ambient light level during the winter breeding period (April-October; Martin *et al.* 2014) using an interpolated map of irradiance (W/m^2) throughout South Africa. These data were obtained using the Joint Research Centre's (JRC) portal at <http://re.jrc.ec.europa.eu/pvgis/apps4/pvest.php?lang=en&map=africa>. For each cluster we also obtained rainfall and temperature data during the same period matching each cluster to the nearest weather station listed in the Climate Information Platform (CIP) (<http://cip.csag.uct.ac.za/webclient2/datasets/south-africa-cmip5/>), following the same approach as Amar *et al.* (2014).

Table 2. Geographical location of all clusters (n=25) throughout South Africa showing the proportion of morphs and the percentage of dark morphs found at each cluster site.

Cluster	Province	Latitude(°S)	Longitude (°E)	No. dark birds	No. light birds	Total birds	% Dark
LP Grp 1	Limpopo	28.0	24.3	8	28	36	22.2
MP Grp1	Mpumalanga	30.7	25.5	3	25	28	10.7
MP Grp 2	Mpumalanga	29.6	25.1	0	18	18	0
KZN Grp 1	Kwazulu Natal	30.7	29.0	9	30	39	23.1
KZN Grp 2	Kwazulu Natal	30.1	29.0	1	5	6	16.6
KZN Grp 3	Kwazulu Natal	31.1	30.0	13	37	50	26
KZN Grp 4	Kwazulu Natal	29.7	29.9	5	12	17	29.4
KZN Grp 5	Kwazulu Natal	30.3	30.9	0	2	2	0
KZN North	Kwazulu Natal	32.4	27.4	1	4	5	20
FS Grp 1	Free State	27.1	29.5	0	2	2	0
FS Grp 2	Free State	26.2	29.0	0	8	8	0
GP Grp 1	Gauteng	27.9	26.7	0	2	2	0
GP Grp 2	Gauteng	28.4	24.9	0	5	5	0
GP Grp 3	Gauteng	28.1	25.6	4	40	44	9.1
GP Grp 4	Gauteng	27.2	25.9	0	4	4	0
EC Grp 1	Eastern Cape	28.9	32.1	0	2	2	0
EC Grp 2	Eastern Cape	32.1	31.1	3	0	3	100
EC Grp 3	Eastern Cape	25.1	33.9	15	14	29	51.7
EC Grp 4	Eastern Cape	26.9	32.3	3	1	4	75
WC Grp 1	Western Cape	18.4	33.9	65	15	80	81.2
WC Grp 2	Western Cape	19.1	34.4	12	7	19	63.1
WC Grp 3	Western Cape	20.2	34.1	4	3	7	57.1
WC Grp 4	Western Cape	21.9	33.5	8	5	13	61.5
WC Grp 5	Western Cape	18.3	33.3	1	0	1	100
WC Grp 6	Western Cape	23.3	34.0	5	10	15	66.7
Total				165	274	439	37.6

STATISTICAL ANALYSES

Relationship Between Provisioning Rate, Light Levels and Male Morph

We tested the relationship between male morph and provisioning rate at varying light levels using a Generalised Linear Mixed Model (GLMM) fitted within R, version 3.0.3 (R Core Team 2013) using the “lme4” package (v. 1.1-5) (Bates & Maechler, 2014). The response variable was hourly prey delivery fitted as a binary measure (1=prey delivered, 0=no prey delivered) using a binomial distribution and a logit link function, and we included ‘nest’ as a random factor to control for the lack of independence between the data collected from the same nest. Although multiple prey items were occasionally delivered in the same hour, this was relatively rare (n=18/1384 prey delivery events; 1.3%

of 1406 hours with prey delivery), and treating the data as binary therefore helped fit a sensible distribution to the data with little loss of variation. Explanatory variables in the model were male morph (dark or white), hourly light levels and the interaction between these two terms.

Whilst our main interest in this study was the relationship with light levels, we recognised this variable would be closely correlated with other weather variables such as rainfall, temperature and time of day. Thus, any relationship between light levels and foraging success found in this study could simply be due a relationship with these other terms rather than light levels themselves. We therefore also included an additional analysis using the same model as described above, but substituted hourly average rainfall, hourly average temperature or time of day (and the quadratic term) to establish whether these other variables provided a better fit to the data than light levels (Table 3). We then compared the models using their corrected Akaike Information Criterion scores (AICc), which is the AIC corrected for a small sample size. Lastly, to explicitly test the light conditions under which foraging success between the morphs differs significantly, we ran the same GLMM model using a moving subset of light level values.

Morph Ratios and Light Levels Across South Africa

To assess whether the morph ratios across South Africa correlate with local light levels, we used a two-vector response variable, including both the number of dark and white birds present in each cluster, fitted with a binomial distribution in a Generalized Linear model (GLM). This approach automatically accounts for the different sample size of the clusters by weighting each sample according to the total number of birds in each cluster (Amar *et al.*, 2014). Average level of light experienced in each cluster over the breeding season was then fitted as the explanatory variable.

Again, to establish whether any relationship at this landscape scale was robust, and not simply due to correlations with other environmental variables, we ran additional models including those terms that had previously been identified by Amar *et al.* (2014) as the key explanatory variable for the distribution of morphs across South Africa. Thus, we ran the same model but substituted light levels with average

breeding rainfall, average breeding temperature and altitude. We then assessed whether light levels, rather than any of these other three variables, had the most explanatory power by comparing AICc values for the four models.

RESULTS

From the 17 territories (six white morph, 11 dark morph) monitored in this study, we collected a total of 9518 hours of camera footage during the early nestling stage (i.e. ≤ 21 days old) of which 1406 hours included a provisioning event (15%). (Table 1).

We found no difference in the overall provisioning rates between the two male morphs ($\chi^2_{1} = 1.19$, $P=0.27$), however there was a significant effect of light level ($\chi^2_{1} = 16.42$, $P<0.001$) and most importantly, we found a highly significant interaction between male morph type and light level ($\chi^2_{1} = 62.15$, $P<0.0001$). There was a clear contrasting relationship between the two morphs in their probability of prey delivery in relation to light levels; prey delivery rates by white morph males increased with higher light levels, whereas for dark morph males, prey delivery rates decrease as light level increased (Fig. 2).

The moving window analysis showed that foraging success was significantly better for dark morphs when light levels were $<130 \text{ W/m}^2$ ($\chi^2_{1} = 4.52$, $P=0.03$), whereas white morphs performed significantly better at light levels $>650 \text{ W/m}^2$ ($\chi^2_{1} = 3.55$, $P=0.04$). Between $130\text{-}650 \text{ W/m}^2$ there was no significant difference in foraging success rates between the morphs ($\chi^2_{1} = 2.81$, $P=0.1$) (Fig. 3).

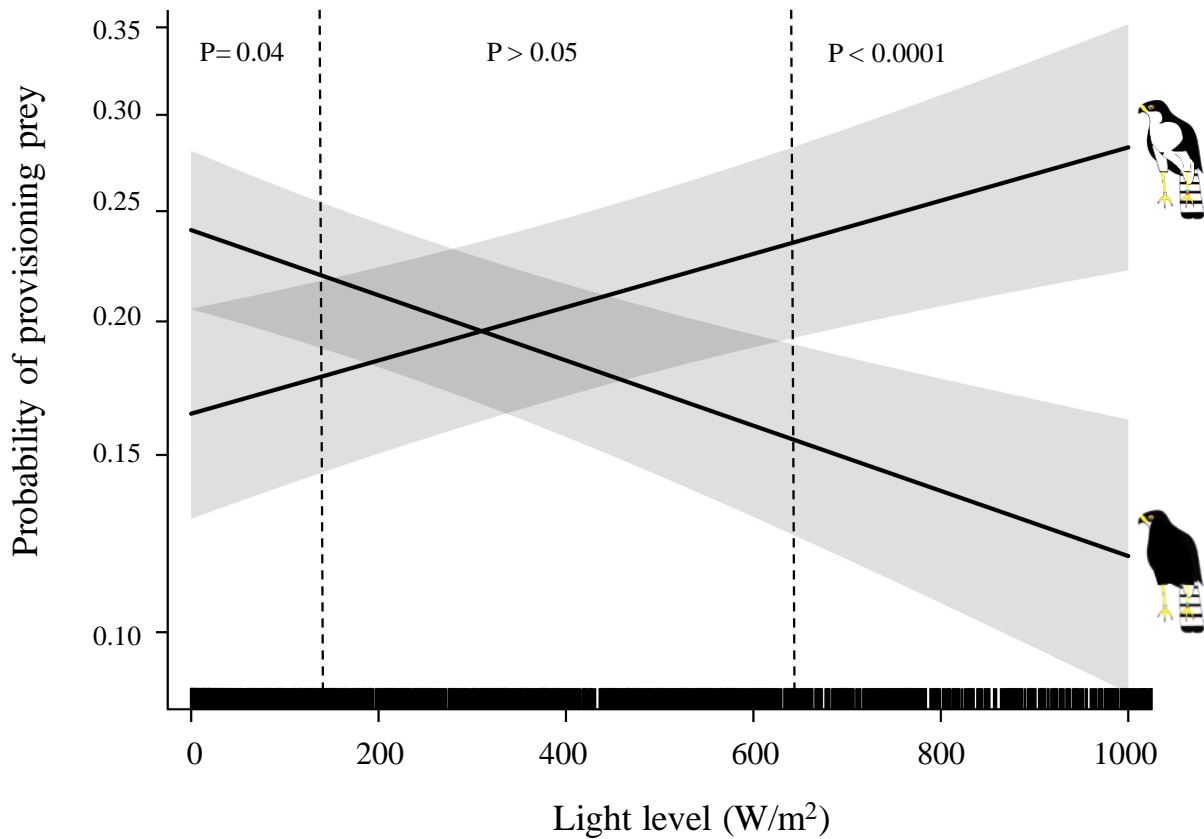


Figure 2. Relationship between male morph foraging success (probability of provisioning) at various ambient light levels [irradiance (W/m^2)] throughout the early nestling stages of the breeding season on the Cape Peninsula. Subsets of the data, where significantly higher prey delivery rates by either dark morphs or white morphs, are also indicated by the vertical dotted lines. Below $130 \text{ W}/\text{m}^2$, dark morph males had a higher delivery rate, whereas at $\geq 650 \text{ W}/\text{m}^2$, white morphs delivery rates were significantly higher. The black horizontal bar on the x-axis represents the sample size and spread of provisioning data across the light spectrum.

Our models including light levels, and their interaction with male morph, were better at explaining delivery rates than either temperature, rainfall levels or time of day (Table 3). Although the models including time of day (and its quadratic term) and light levels had similar AICc scores, when both terms were combined in the same model we found that the interaction between light levels and morph remained very highly significant ($P < 0.0001$). This result was obtained even after controlling for the significant quadratic influence of time of day (Table 4), indicating that light levels were important at influencing prey provisioning rates in the two morphs independent of the time of day.

Morph Ratios and Light Levels Across South Africa

Across the species South African distribution, we found a highly significant relationship between the proportion of morphs and light levels, ($\chi^2_1 = 97.81$ $P < 0.0001$, $R^2 = 0.85$), with more dark morph birds found in clusters exposed to lower winter light levels (≤ 650 W/m^2) (Fig. 4).

Table 3. Model selection based on corrected Akaike Information Criterion scores (AICc). Results show the top ranked model (with Delta AICc < 2 , shown in bold) included the interaction between male morph and light level and held 100 % of the weight. The analysis revealed that light levels, and the interaction with male morph, was the better explanatory variable when compared with either temperature or rainfall levels, indicating that bird provisioning rates were most likely a mechanism of this variable than other correlated variables. AICc weight (W_i).

Model	K	AICc	Delta AICc	W_i	Cum. Wt
Male morph + light levels + Male morph*Light level	5	7679.4	0.00	0.83	0.83
Male morph*Time+ Male morph*Time ²	7	7682.9	3.18	0.17	1
Male morph + Temperature + Male morph*Temperature	5	7726.06	46.3	0	1
Male morph + Rain + Male morph*Rain	5	7746.3	66.5	0	1

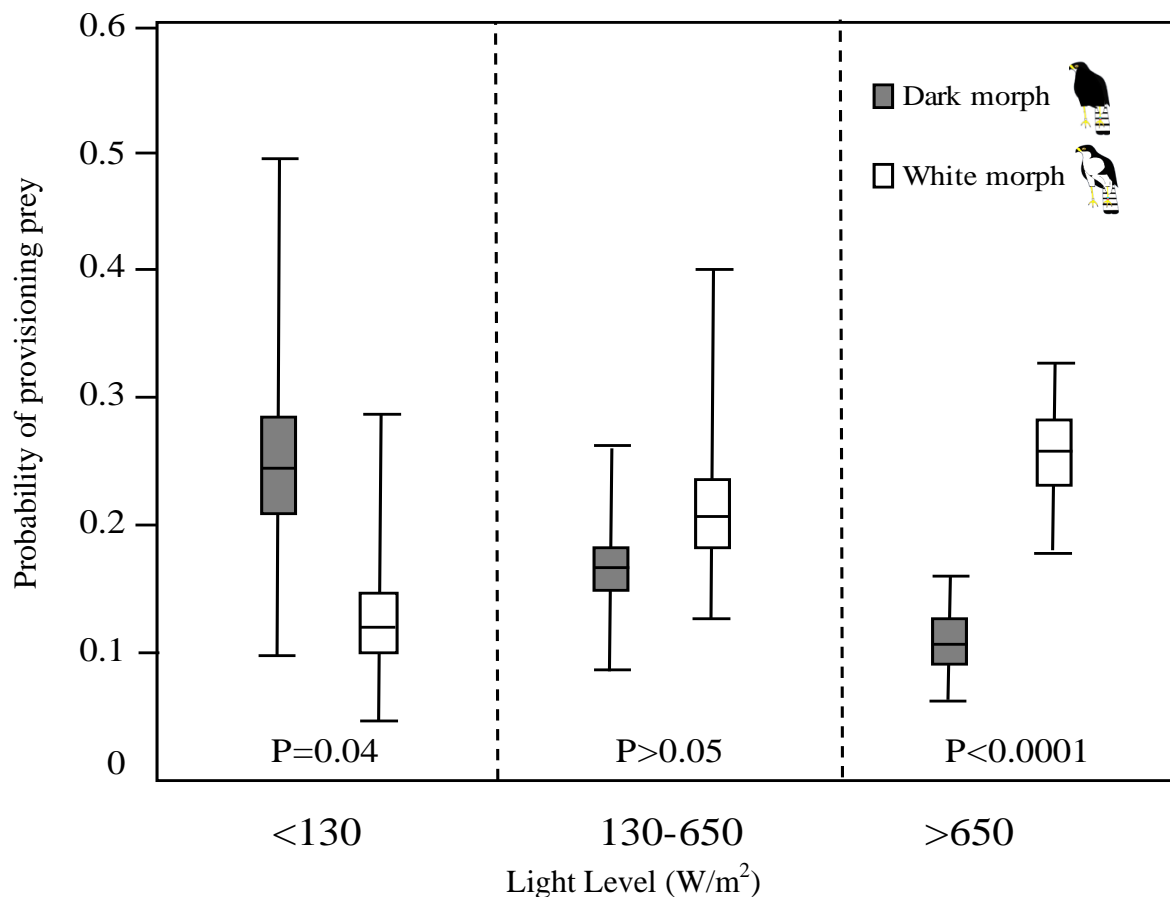


Figure 3. Probability estimates of male Black Sparrowhawks provisioning prey at various light level subsets (irradiance W/m^2). At low light levels (< 130 W/m^2), dark morph males have significantly higher probability of provisioning prey, whereas at higher light levels (≥ 650 W/m^2), white morph males have significantly higher provisioning. Between 130 and 650 W/m^2 , there is no significant difference in prey provisioning between morphs. Error bars indicate upper and lower 95% confidence intervals.

Table 4. Analysis of deviance table (Type III Wald χ^2 tests) examining for the effect of light and light*morph after controlling for the significant quadratic influence of time of day. When both terms were combined in the same model we found that the interaction between light levels and morph remained very highly significant ($P < 0.0001$), indicating that light levels (in bold) are important at influencing prey provisioning rates in the two morphs, independent of the time of day.

	Intercept	df	χ^2	P
Male morph		1	0.3	0.6
Light Level		1	65.3	P<0.0001
Time		1	8.2	0.004
Time ²		1	41.2	P<0.0001
Male morph*Time		1	6.9	0.008
Male morph*Time ²		1	0.002	0.96
Male morph* Light level		1	45.49	P<0.0001

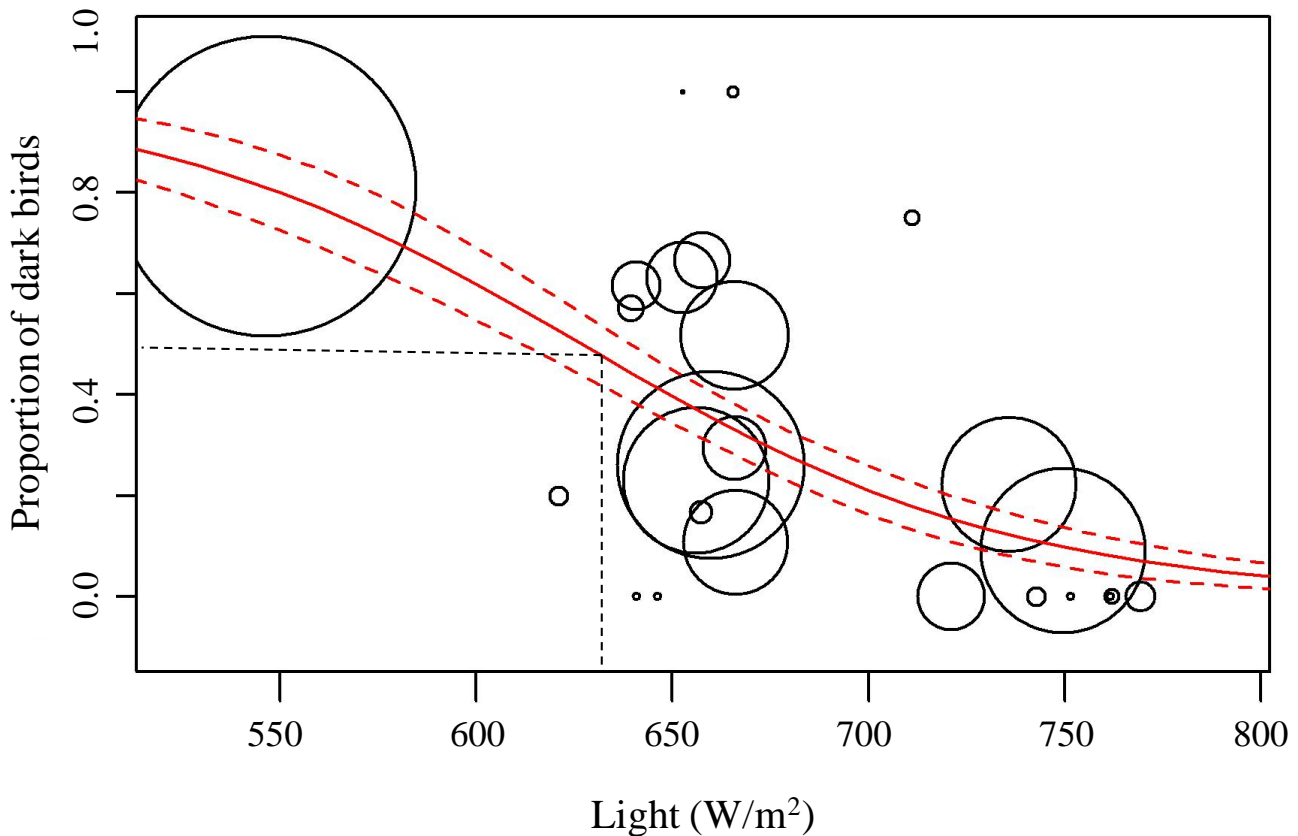


Figure 4. Relationship between the proportion of dark morph Black Sparrowhawks in the population and the ambient light level [irradiance (W/m²)] environment across South Africa (measured as the total average solar radiation during the breeding period, April–October). Fitted lines are taken from the parameter estimates (with 95% confidence intervals) generated by a GLM ($R^2 = 0.85$). Size of the points reflects the sample size of each cluster ($n = 25$) of birds. The vertical dotted line represents the region of light (~ 640 W/m²) where there is a reversal in morph ratios, from dark morph dominated clusters to white morph dominated clusters. The horizontal dotted line indicates the area where populations are 50% dark, 50% white.

The inflection point of this relationship occurs around $640\text{W}/\text{m}^2$; at this level of light there is a 1:1 ratio of dark: white morphs. This switch in morph ratio corresponds very closely with the light conditions under which white morphs have significantly higher foraging success than dark morphs. In comparison to other key environmental variables previously found to be important predictors of the spatial structure of morphs (breeding rainfall, breeding temperature and altitude) we found the best support for the relationship between morph and light levels (Table 5).

Table 5. Model selection based on corrected Akaike Information Criterion scores (AICc). Light level measured as solar radiation (W/m^2) over the breeding season (April - October) measured from sampling clusters across South Africa. Breeding Rain=average rainfall during the breeding season, Breeding Temp=average temperature during the breeding season and Altitude, in metres above sea level. K denotes the number of parameters (K), change in AICc relative to the highest ranked model (ΔAICc), AICc weight (W_i). Results show the top ranked model (with Delta AICc <2, shown in bold) included the term light level and held 99% of the weight.

Model	K	AICc	Delta AICc	W_i	Cum. Wt
Light level	2	115.8	0.00	0.99	0.99
Breeding Rain	2	126.4	10.5	0.01	1
Breeding Temp	2	138.9	23.09	0	1
Altitude	2	197.9	82.07	0	1

DISCUSSION

We found that ambient light level strongly influences the prey provisioning rate of different coloured morphs in a polymorphic species, the black sparrowhawk. Our results provide the first empirical support for the ‘light level-detectability hypothesis’ as hypothesised by Galeotti *et al.* (2003) for the evolution and maintenance of plumage colour polymorphism in birds. Here we propose that a possible mechanism maintaining the presence of two colour morphs across this species’ range is the degree of crypsis afforded to hunting individuals under variable light conditions. Our findings provide important supporting evidence that predator-prey interactions are a major component in the maintenance of polymorphisms in natural populations and that visual detection by prey could be a key selective agent for colouration in a predatory hawk.

Foraging Success is Enhanced According to Background Matching of Morphs

The detectability of animal colour and pattern depends on its degree of contrast against the background and, accordingly, is strongly influenced by both the ambient light available in a habitat (Endler, 1987) and animal visual systems (Bond, 2007; Hurtado-Gonzales *et al.*, 2014). Under certain environmental conditions some colours are more difficult to detect, providing animals with improved crypsis (Bond, 2007). We propose that increased crypsis in black sparrowhawks is the result of enhanced background matching, whereby dark morphs are less easily detected by their avian prey against a darker background, such as against cloudier skies or in darker closed vegetation. Conversely, white morphs are less detectable when attacking in environments that are strongly illuminated by the sun, i.e. against a bright clearer sky or in sparse, open vegetation (Rohwer, 1990; Merilaita & Lind, 2005; Bond, 2007).

Our analysis revealed that dark morph black sparrowhawks had significantly higher prey provisioning rates at low light (<130 W/m²) than white morphs, which in turn had higher provisioning rates in brighter conditions (>650 W/m²). At intermediate light levels, no differences were apparent. This result offers some explanation for the numerical dominance of dark morphs on the Cape Peninsula, where >75% of birds are dark morphs (Amar *et al.*, 2014). Here, for 48 % of daylight hours during the breeding season, the average light level fell below the 130 W/m² level, and under these conditions dark morphs should be favoured. Light levels favouring the white morph on the Peninsula during the breeding season occurred for only 13% of the time (Fig. 5).

Our explicit test of differential morph foraging success at varying levels of light is further supported by a number of more anecdotal studies. For example, Rohwer (1990) found that on one island occupied by the pacific reef herons *Egretta sacra* where foraging opportunities occurred exclusively in shaded habitats, only dark morph birds were present, and suggested this was most likely because dark morphs would be more cryptic to their aquatic prey against a dark background. Similarly, Preston (1980) observed that light morph red-tailed hawks *Buteo jamaicensis* occupy more open perches when hunting, whereas dark morphs were found to use perches characterized by dense cover; suggesting an adaptive morph-habitat association, with morphs selecting perches that best conceal them from their prey.

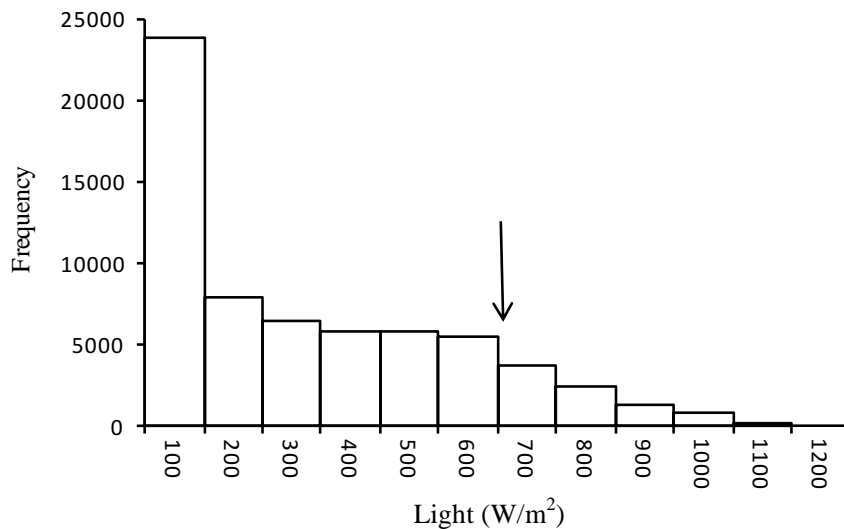


Figure 5. Results show that ambient light conditions are lower than 650 W/m^2 87 % of the time during the breeding season on the Cape Peninsula. This suggests that the threshold light level, indicated by the black arrow, at which it becomes disadvantageous to being dark in terms of foraging success, does not occur during the breeding season on the Peninsula; therefore the trade-off to be dark is not compromised by too much light. Low light levels that favour dark morphs ($0 - 130 \text{ W/m}^2$) occurs 48 % of the breeding season, whereas high light levels that favour white morphs ($>650 \text{ W/m}^2$) only occur 13 % of the time.

In many avian predators such as hawks (Falconiformes) and skuas (Charadriiformes), colour polymorphism is primarily expressed ventrally, the side most apparent to potential prey below them (Galeotti *et al.*, 2003). Black sparrowhawks, too, display ventral plumage polymorphism (Amar *et al.*, 2013). Ventral colouration is considered to decrease the conspicuousness of a predator against its background (Bortolotti, 2006) which, in combination with ambient light condition (Endler, 1987), appears to influence the ability of prey to visually detect predators (Green & Leberg, 2005). This suggests that ventral plumage may play an important role in foraging success. Avian predators often prey upon intelligent, sharp-sighted prey such as mammals and birds, thus ventral colour may be important in a predator's detectability and recognition from prey species below, which will likely directly influence the success of an attack (Merilaita & Lind, 2005). Although not strictly related to polymorphism, the idea that ventral colour may influence prey capture rates also has some experimental support. Götmark (1987) dyed the white underparts of gulls black and found a significant reduction in the prey capture rates for these experimentally altered birds. Furthermore, using dark and light painted models of gulls, Phillips (1962) found that dark models elicited a stronger, quicker escape response in prey than white models, and that white models were able to approach closer to prey before it fled.

Light as a Selection Agent in the Distribution of Bird Colour Polymorphisms

Clinal variation of colour morphs has been reported in a high proportion of polymorphic species, from insects (de Jong & Brakefield, 1998), to reptiles (Alsteadt *et al.*, 2006), mammals (Rounds, 1987) and birds (Galeotti *et al.*, 2003; Antoniazza *et al.*, 2010; Amar *et al.*, 2014). Although the exact mechanism(s) driving these clines have rarely been established, and may differ from one biological system to the next, our results demonstrate that variation in the light environment across a geographical range of a polymorphic species, may have a stronger influence on the distribution of morphs across the landscape than previously recognized.

A possible selective advantage for dark morph black sparrowhawks at low light levels is reflected in our countrywide analysis. Across the species range in South Africa, we found that the proportion of dark morphs within populations was closely correlated with local light levels experienced during the breeding season. Furthermore, the inflection point at which the proportion of morphs shifted from a predominance of dark morphs to that in favour of white morphs also corresponded almost precisely with the point at which our analysis suggested that foraging success was higher for white morphs (i.e. 650 W/m²). This landscape scale analysis provides significant support suggesting that the spatial structuring of morphs is strongly influenced by this variable. Previous studies found that species with the largest distributional ranges were most likely to show polymorphism (Forsman & Hagman, 2009; Hugall & Stuart-Fox, 2012). These findings provide support for our hypothesis, since species with large distributions are more likely to exist over multiple light conditions, either through their occurrence in areas with variable climatic regimes (as in this study) or across contrasting habitat types, and are thus more likely candidates for trait evolution by disruptive selection (Galeotti *et al.*, 2003; Rueffler *et al.*, 2006). Many other factors however are also likely to co-vary with latitude in addition to light. We also attempted to explore the key variables that might be confounded (i.e. rainfall, temperature, elevation) and found that light level was consistently better at explaining variation in morph frequency than these other terms. Thus we acknowledge that there may be additional variables that we have not been able to test which may also co-vary with light levels (i.e. habitat) and this should be considered when interpreting these results.

Under disruptive selection, variable light condition is likely to be one of the strongest selective mechanisms maintaining colour polymorphism in birds (Galeotti *et al.*, 2003) and may explain the clinal variation commonly exhibited in many other polymorphic species across their distributions. Amongst birds, Galeotti *et al.* (2003) found that of 67 polymorphic species investigated, 78% showed some degree of a geographic cline in their distribution, with 18% living along a climatic gradient of dry to wet and 4% occurring along a clear habitat cline, either from an open to closed or dark to light vegetation background. Importantly, all of these clines are characterised by variation in ambient light conditions. Interestingly, most species that displayed clinal variation along a geographical or environmental gradient had significantly more dark morphs in habitats characterised by low light conditions. Once again, this may be due to improved crypsis in darker individuals under low light conditions, and our study provides the first empirical evidence supporting this theory.

Future Research

While the findings in our study relate to foraging success under variable light conditions over the course of a day, and specifically between days with different weather conditions, foraging success may also be affected by variation in light condition caused by differences in habitat type (i.e. open vs closed), and in the case of nocturnal species, may be influenced by moonlit and non-moonlit nights. Further, differential foraging success may not be due to variable capture success or crypsis under varying light conditions, but rather related to inherent behavioural differences between morphs in their coping strategies under variable light conditions. Although we focus on the detectability of a predator in a single system, the argument we present is also applicable to predator avoidance by prey, or indeed any visually guided interaction between individuals and/or species, for example mobbing, competition or detection by vectors or parasites (Chakarov *et al.*, 2008). One of the key results from Galeotti *et al.*'s (2003) analysis that informs the variable light level-detectability hypothesis is that colour polymorphisms are maximally expressed in species showing a daily activity rhythm extending from day into the evening/dusk, and living in both open and closed habitats. Although highly credible, universal application of the light level-detectability hypothesis to other polymorphic species and

systems remains untested. To further assess how detectability differs between the two morphs in our study system, it will be valuable to experimentally examine the behavioural response and reaction times of prey species to taxidermied mounts of the different morphs under varying light conditions. Additionally, it would be important to examine whether the two morphs utilise different habitat types. For example, whether dark morphs hunt more frequently in low light habitats i.e. under closed vegetation, versus whether white morphs prefer to hunt in brighter, open habitats.

Conclusion

Our study presents compelling evidence that a significant fitness advantage under local light conditions may be important in maintaining a higher than expected proportion of dark morphs in a study population of black sparrowhawks. This advantage is also reflected in the frequency of morphs across the highly seasonal landscape of South Africa, where differential hunting success during the breeding period may promote the maintenance of two colour morphs in varying proportions. These findings support the hypothesis that colour polymorphism in the black sparrowhawk is most likely maintained through possible fitness advantages under different light conditions.

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CHAPTER 4

MMORPH SPECIFIC FORAGING BEHAVIOR BY A POLYMORPHIC RAPTOR UNDER VARIABLE LIGHT CONDITIONS.



Kommetjie, Cape Town, September 2013! Imhoff male being fitted with a solar powered GPS logger. These loggers were developed by ECOTONE© and weigh around 14 grams, which makes up only ~3% of the bird's total body weight. This individual paired up and bred with another white morph female and in 2014 nested in an old fish eagle's nest. Black Sparrowhawks have been known to breed with the same partner for up to ten years on the same territory, although extra pair copulation has been observed in the study population. Photo: Gareth Tate

A modified version of this chapter is currently under review in Scientific Reports:

Tate, G.J. & Amar, A. In Review. Morph specific foraging behavior by a polymorphic raptor under variable light conditions. *Scientific Reports*.

ABSTRACT

Colour polymorphism may be maintained within a population by disruptive-selection. One hypothesis proposes that different morphs are adapted to different ambient light conditions, with lighter morphs having a selective advantage in bright conditions and darker morphs having advantages in darker conditions. The mechanism for this advantage is proposed to be through enhanced crypsis via background-matching. In this study, we explore this hypothesis in a polymorphic raptor that exhibits a discrete dark and white-morph, the black sparrowhawk. We use GPS-tracking data to contrast the foraging behaviour and habitat selection of black sparrowhawks morphs. As predicted we found that ambient light-levels influenced the foraging behaviour in different ways for the two morphs; Dark-morphs showed a decrease in foraging with increasing light-levels; whereas no relationship was found for white-morphs. Furthermore, we found differential degrees of habitat selection, with more enclosed habitats being selected by dark-morphs compared to white-morphs. These findings provide some support for the hypothesis that different morphs may be better adapted to foraging under different light-conditions, which might play a role in maintaining colour polymorphism in this species. Our results may also help explain why dark-morphs predominate in this study region which, unlike the rest of the country, experiences high rainfall and thus lower light-levels during the breeding period. This study suggests that avian morphs may allocate or partition their foraging activity by weather conditions or habitat in such a way that they maximise their concealment from their prey.

Keywords:

Plumage colour, polymorphism, habitat preference, foraging behaviour, weather, raptor, activity, effort, vegetation cover.

INTRODUCTION

Animal colouration has been widely recognised to play an important role in a variety of ecological processes, from camouflage (crypsis) (Troscianko *et al.*, 2016) to intraspecific communication (Endler, 1990) and mate choice (Bortolotti *et al.*, 2008); all of which are likely to be under considerable selective pressure from both natural and sexual selection (Bortolotti, 2006). Thus, the colour of an individual animal, or a species, is thought to be shaped by complex evolutionary processes that drive and maintain phenotypic variation and genetic diversity in nature (Ford, 1945).

The plumage colours and patterns that bird species display have fascinated evolutionary biologists and ornithologists for decades, although general explanations for them still often remain elusive (Brooke, 2010). For some bird species, colouration and patterning in their plumage is likely to play a vital role in camouflage and crypsis via background matching (Bortolotti, 2006; Troscianko *et al.*, 2016). According to this principle, the more similar to the visual background the colours and geometry of patterns of an individual are, the more concealed it should be (Endler, 1990). This can be fundamental either for prey to avoid detection by predators (Stuart-Fox *et al.*, 2004; Troscianko *et al.*, 2016) or equally so, for predators to avoid detection by their prey (Götmark, 1987; Rohwer, 1990). For example, in nightjars (Caprimulgiformes), the colour and plumage pattern of incubating females closely matches their surrounds, reducing their detection from visual predators, and improving their overall survival (Troscianko *et al.*, 2016).

Given the likely adaptive value of colour (Cott, 1940), it is therefore logical to assume the same processes may be operating on colour polymorphic species, where multiple genetically based colour morphs occur within the same age and sex class of a breeding population (Huxley, 1955). Consequently, evolutionary biologists have frequently used colour polymorphic species as model systems for the study of evolutionary processes such as natural and sexual selection (McLean & Stuart-Fox, 2014) and

specifically, to test how colour variants (i.e. multiple morphs) are maintained in species in the face of natural selection and genetic drift (Darwin, 1859; Johnson *et al.*, 2012; Roulin & Ducrest, 2013). In theory, the principles of natural selection predict that the fittest morph should be selected and that morphs with lower fitness should be eliminated from a population (Darwin, 1859). Therefore, it has been proposed that for multiple morphs to coexist, and for polymorphism to be maintained within a species, morphs must have equal fitness over time (Ford, 1945), whereby, a selective trade-off exists between alternative morphs, both enjoying some advantages, but frequently also incurring some costs (Fisher, 1930; Forsman *et al.*, 2008).

Colour polymorphism occurs in 3.5% of bird species globally (Galeotti *et al.*, 2003), although there is considerable variation between different groups. It is particularly common amongst raptors (owls and hawks), where 30% are polymorphic (Galeotti *et al.*, 2003). Numerous studies have suggested that different morphs may represent alternative strategies adapted to certain prevailing environmental conditions (Antoniazza *et al.*, 2010). Disruptive selection, in which selection favours contrasting phenotypes under different environmental conditions, has been invoked as one of the main mechanisms maintaining colour polymorphism (Galeotti *et al.*, 2003).

As ambient light condition strongly influences the level of crypsis in certain colours (Endler & Théry, 1996; Théry, 2006) and also affects the ability of prey to visually detect predators, or for predators to detect prey (Stuart-Fox *et al.*, 2004), light could play an important role in driving disruptive selection in polymorphic species. Indeed, based on their review and analysis, Galeotti *et al.* (2003) concluded that colour polymorphism in birds probably evolved under selective pressures linked to individual detectability under variable light conditions, and argued that it is most likely maintained by disruptive selection. In predator-prey systems, dark and light plumage may therefore be beneficial under alternating light conditions. For example, darker morphs may be less detectable in low light conditions (i.e. on cloudier days, during dusk/dawn, or in closed habitats), and in these conditions darker predators may accrue a foraging advantage via background matching (Preston, 1980; Rohwer, 1990). Conversely, lighter morphs may be less detectable in brighter conditions and may forage more successfully as a

result under these conditions (i.e. on sunny days, in the middle of the day, or in open habitats) (Galeotti *et al.*, 2003).

Galeotti *et al.*'s (2003) hypothesis has recently received some empirical support; Tate *et al.* (2016) found that prey delivery rates for black sparrowhawks *Accipiter melanoleucus* were significantly higher for dark morph individuals during low light conditions, whereas the opposite relationship was found for white morphs, which appeared to have an advantage in brighter conditions. These findings suggest that light conditions interact with morph colour to improve foraging success and supports the hypothesis that the two morphs may be better adapted to foraging under different light conditions (see also Sumasgutner *et al.* 2016). Within South Africa, black sparrowhawks display clinal variation in morph ratios, with the frequency of dark morphs declining from > 75 % in the southwest to < 20 % in the northeast of the country (Amar *et al.*, 2014; Tate *et al.*, 2016). This variation is most closely correlated with ambient light levels during the winter period, with higher proportions of dark morphs being associated with darker winters (Tate *et al.*, 2016) and correspondingly with higher winter rainfall conditions (Amar *et al.*, 2014). Within our study population, on the newly colonised Cape Peninsula (Oetlé, 1994), where rainfall during the winter breeding period is particularly high (Martin *et al.*, 2014a), the dark morph phase predominates (Amar *et al.*, 2013, 2014; Tate *et al.*, 2016).

In this paper, we further explore the hypothesis tested by Tate *et al.* (2016) using the same study system. In their study, Tate *et al.* (2016) examined provisioning rates as a surrogate for foraging success in relation to ambient light levels. In this study, we explore the issue more directly using GPS tagged male black sparrowhawks, to investigate whether activity and foraging effort differs between the morphs depending on light conditions. Black sparrowhawks typically attack their avian prey from above via short ambush flights (Hockey *et al.* 2005) and appear to accrue foraging benefits/advantages in ambient light conditions (habitat/time of day/rainy vs cloudy days) that best conceal them against the background from their prey (Tate *et al.* 2016). If foraging success is influenced by light levels differently for the two morphs in the manner expected, we predict that dark morphs will forage more during lower light conditions, when they would be expected to be most successful, whereas white morph

birds will show the opposite relationship. Furthermore, we also test whether habitat selection varies between the morphs. Here we predict that dark morph birds will show a greater preference for more enclosed habitats (which should represent darker habitats) than white morph birds, which might show a greater preference for more open habitats.

MATERIALS AND METHODS

We examined morph specific foraging behaviour and habitat use in a newly colonised black sparrowhawk population on the Cape Peninsula (34 ° 00'S, 18°26'E) Western Cape, South Africa. The study area encompasses a variety of natural and human modified (urbanised) environments which include the slopes of the Cape Peninsula Mountain chain and adjacent sand flats, with altitude ranges from sea level to about 400m. The region features a mosaic of urban gardens and green belts, golf courses, schools, small pockets of indigenous afro-montane forest, botanical gardens, fynbos shrubland and alien pine (*Pinus* spp.) and eucalyptus (*Eucalyptus* spp.) plantations-particularly along the eastern slopes (Curtis *et al.*, 2005).

Tracking Data

Six adult male black sparrowhawks (3 white and 3 dark morphs) were trapped on active territories, using a *bal-chaltri* trap baited with live white pigeons *Columba livia* (Berger & Mueller, 1959) and fitted with GPS loggers using a back pack harness made of 7mm teflon ribbon (Bally Ribbon Mills, Bally, PA, USA). All information on the tracking data obtained for each individual is provided in Table 1. We focused on males as they undertake the majority of the provisioning during the breeding season, while females incubate and brood chicks (Katzenberger *et al.*, 2015). Tags were solar powered EP3.5 'Harier' loggers manufactured by ECOTONE (Sopot, Poland). GPS loggers weighed around 14 grams, which is 2.6% of the average male weight (~540g; Hockey *et al.* 2005), which falls within the ethically approved weight for loggers (i.e. <5% of the species total weight; Wilson & McMahon, 2006). Our sample size was limited by the fact that male black sparrowhawks are difficult to trap, are present at the nest less frequently than females and by the expensive costs of the tags themselves. Additionally, white

morph males are particularly rare in our population. All loggers were set to record GPS fixes (accurate to within 1-15m) every fifteen minutes on a daily cycle from 4am to 9pm (SAST). Fixes recorded on the loggers were downloaded via Very High Frequency (VHF) transmission once a month using a portable base station and directional antenna. Downloads could occur when the base station was within *c.* 4km of the tagged bird.

Weather Data

For all monitored individuals, we matched each hour of GPS data with the average ambient light that fell during the hour, from a nearby South African Environmental Observation Network (SAEON) weather station (33.952° S, 18.459° E), positioned in an open field, on average 12.2 ± 21 km from the territories of our tagged birds. For ambient light, we used average hourly irradiance i.e. solar radiation in W/m^2 , hereafter referred to as light level (Tate *et al.*, 2016). We also gathered information from the same weather station on hourly rainfall (mm) and temperature (°C) which we also linked to the tracking information.

Habitat Data

To examine whether there were any differences in habitat preference between the morphs, a Geographic Information System (GIS) was used to explore the underlying habitat for each GPS fix. For habitat, we collated data for the Cape Peninsula using a national landcover dataset for South Africa, developed by the South African National Biodiversity Institute in 2013/14 (SANBI; http://bgis.sanbi.org/DEA_Landcover/project.asp). The national landcover dataset is based on 30 x 30-meter raster cells and has been derived from multi-seasonal Landsat 8 imagery, using operationally proven, semi-automated modelling procedures developed specifically for the creation of this dataset, based on repeatable and standardised modelling routines (Thompson, 1999). The dataset has been created by GEOTERRAIMAGE (GTI) (Thompson, 1999) and has a total of 72 landcover classes. However, for our habitat preference models, we grouped the SANBI landcover data into the following six categories, ‘cultivated land, open, urban open, urban cover, plantations and closed vegetation’.

For a separate analysis, we collected data on the percentage of tree canopy cover on the Cape Peninsula, using the Hansen global tree canopy cover layer (Hansen *et al.*, 2013). This data set displays tree canopy cover, defined as canopy closure for all vegetation over five meters in height, over all global landcover at 30 × 30-meter resolution, encoded as a percentage per output grid cell, in the range 0–100%.

Table 1. GPS tracking details for the six Black Sparrowhawk males on the Cape Peninsula over 2012-2015. We tagged three dark morph males and three white morph males with solar powered loggers. The table shows different breeding stages recorded for each individual. Individual Multiple Convex Polygons (MCP) and 10% Kernel Density Estimates (KDE) were calculated using GPS data from actively breeding individuals. We also report the breeding activity recorded for each individual and provide information on the dates of the breeding stages recorded and the stage which breeding reached for each breeding attempt.

Individual hawk	Morph	Date tagged	Total number of fixes obtained	Breeding activity record	Number of fixes actively breeding	MCP size (km ²)	Core territory size (10% KDE) (km ²)	Total days tagged actively breeding
Zonnestraal	Dark	4/09/2012	23125	2012/10-Incubation 2013/09- Nestlings 2014/08-Preplay 2015/08-Preplay	6739	16.29	4.61	240
Spillhaus	Dark	24/10/2014	21894	2014/12- Fledglings 2015/11- Fledglings	4872	49.37	0.800	228
Tokai Arboretum	Dark	23/04/2013	15501	2013/04-Nestlings 2014/05-Incubation 2015/04-Preplay	4464	66.40	0.081	161
Stone Church	White	9/09/2013	17023	2013/10-Nestlings 2014/07-Incubation	4686	25.88	0.062	177
Tokai Picnic	White	25/09/2014	6211	2014/10-Incubation 2015/09- Fledglings	1928	59.43	1.23	64
Imhoff	White	17/082013	9388	2013/12- Fledglings 2014/06-Preplay 2015/11- Fledglings	5678	39.03	0.018	97

The habitat in some territories has been dynamic over the duration of this study, with some clearing of alien plantations since 2012, coinciding with when our first individual was GPS tagged. Therefore to create an up-to-date dynamic layer of landcover and tree cover on the Cape Peninsula, we projected our habitat layers onto a high resolution satellite areal image of the study area (provided by the ESRI World imagery base map; http://server.arcgisonline.com/arcgis/rest/services/ESRI_Imagery_World_2D/), and manually updated areas where and when trees had been clear-felled. This was processed in the program QGIS (QGIS Development Team, 2015).

We projected all GPS fixes onto the high resolution landcover and tree canopy cover layers for the study area. We used QGIS to process this information and to extract tree canopy cover data for each GPS fix, and we used the statistical software R, version 3.2.2 © (R Development Core Team, 2015) with the package “raster” to extract data from the SANBI landcover dataset.

STATISTICAL ANALYSIS

For all spatial analyses, GPS fixes were projected to the UTM coordinate system (WGS 1984 UTM Zone 35S) (Martin *et al.*, 2009) and we only used GPS data from actively breeding individuals i.e. during their prelay, incubation, nestling and fledgling stages (Table 1). For breeding data, territories were monitored approximately monthly for signs of occupation and then more frequently (approximately every 2 weeks) once breeding was detected. Black sparrowhawks are territorial tree nesters, usually nesting in large *Eucalyptus* and *Pinus* tree species (Hockey *et al.*, 2005) and establish territories well before eggs are laid. The prelay period was classified as the stages leading up to breeding; when birds were courting, mating, nest building and females started going into egg-laying lethargy (Newton, 1979), a stage where we observed males start to provision their partners with the majority of their food. Incubation period included egg laying (usually clutches of 1-3) until chicks hatched; incubation lasts 37-38 days (Curtis *et al.*, 2005). Nestling period is 40–47 days and the post-fledging period can exceed 80 days (Curtis *et al.*, 2005). Duration of breeding periods differed in length between different pairs, particularly during prelay stages when males had to secure territories and provision their mates. Locational fixes were sufficiently regular and recorded in enough volume to be assumed to approximate the activity of the birds to which they were attached (Whitfield *et al.*, 2015).

Foraging Activity and Effort Between Morphs in Relation to Light Levels

We examined morph ‘foraging activity’ in relation to weather condition, specifically light level. We defined individuals as actively foraging, when the birds were outside of their core nesting territory. To define this area, we used the core utilization distribution (UD) (i.e. 10% kernel density estimate (KDE)) of each GPS tagged individuals, estimated by means of a kernel density approach in R using the package

‘adehabitatHR’ classes and methods for home range estimation with the package “rgdal”. The KDE was derived by fitting contour lines (i.e. isopleths) based on the volume of the curve under the UD which defined the core polygon, whose area was then calculated (Fig. 1, Table 1; Fieberg 2007). To estimate the 10% KDE for each individual, we only used GPS data from actively breeding individuals.

We assume that when birds were outside of their core nesting territories, they were actively foraging, although we recognise that they may have also been engaging other activities such as territorial defense, or avoiding begging females and chicks. For each individual in each hour, we then calculated the total number of fixes where the bird was actively foraging (i.e. outside of core nesting area) and not actively foraging (i.e. inside the core nesting area). This measure of foraging activity was then used as a two-vector response variable, fitted with a binomial distribution. This approach also therefore accounted for the slight variation in the number of total fixes received per hour. Data were analysed using Generalized Linear Mixed Models (GLMM), with individual bird fitted as a random factor to account for the lack of independence of the repeated hourly observations from the same tracked bird. Light condition experienced in each hour was fitted as an explanatory variable, together with morph type and the interaction between light levels and morph (Table 2).

This was therefore a similar analytical approach as that taken by Tate *et al.* (2016) to examine for differential provisioning rates between morphs in relation to light levels. As foraging activity is likely to vary between breeding stages, (i.e. because food requirements vary between different stages of the breeding cycle), we also included ‘breeding stage’ as an additional explanatory variable, to control for potential differences in foraging activity between prelay, incubation, nestling and fledgling stages (Table 2).

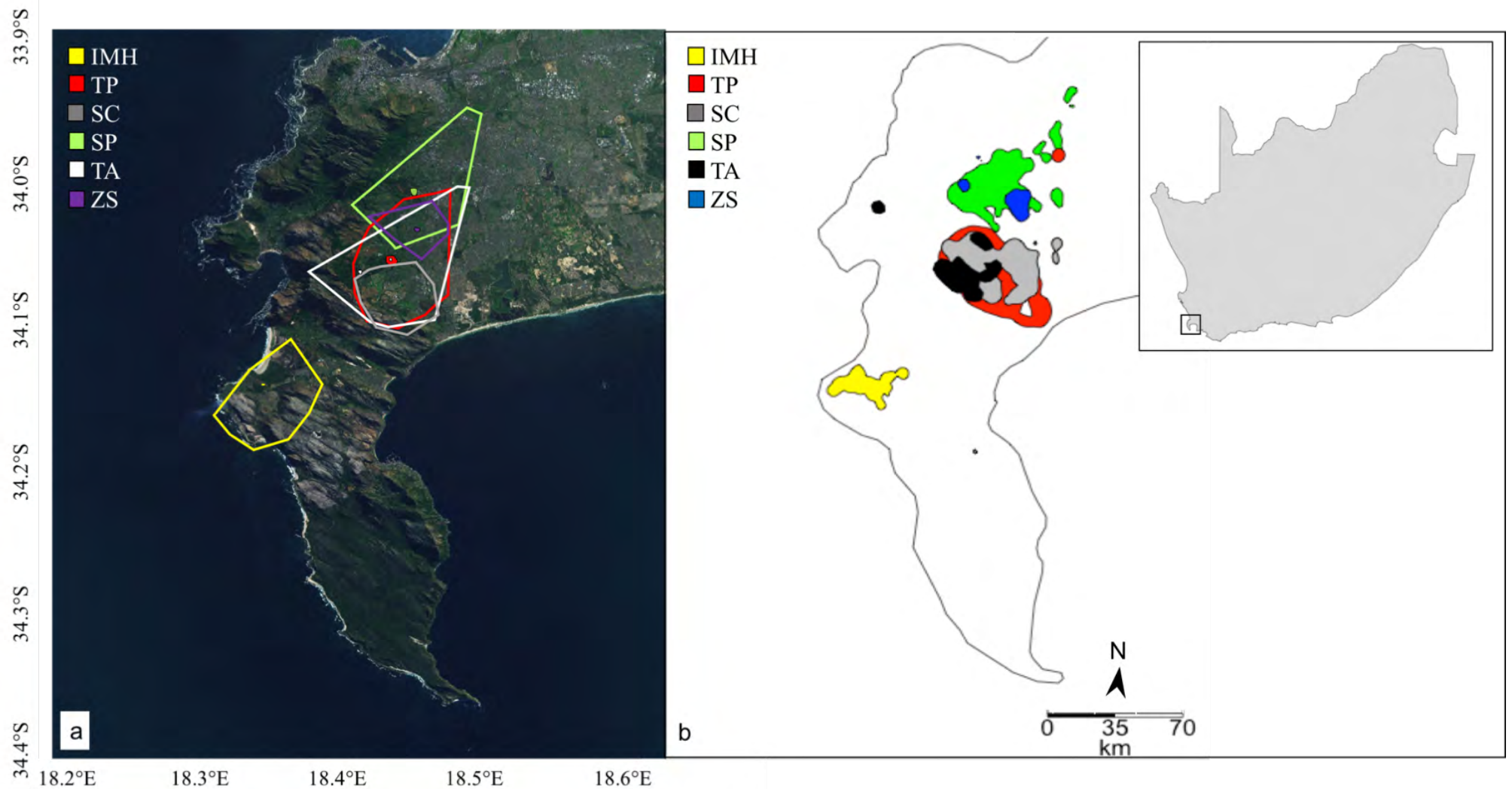


Figure 1a. Territory locations, Multiple Convex Polygons (MCP), territory cores (10%KDE) and **1b.** home ranges of our six GPS tagged male Black Sparrowhawks on the Cape Peninsula, South Africa from 2012-2015. Satellite image: ©2015 Google, DigitalGlobe. Maps generated in R, version 3.2.2 © <https://www.r-project.org/> (R Development Core Team 2015).

We also used a secondary measure of activity – ‘foraging effort’. For this we calculated foraging distance, which was the average hourly distance travelled. Although this measure is likely to be correlated with our other foraging measure (foraging activity), we thought it sensible to additionally include this variable, since any similar relationships with light levels would provide reassurance that our analyses were indeed capturing foraging behavior and not simply avoidance of the core nesting area (i.e. when males avoid begging females and young). Greater distances travelled outside of the core nesting area would thus confirm that the birds are indeed foraging or active, although we acknowledge they could still have been engaging in other activities besides foraging, such as territorial defense.

Distance travelled by actively breeding adults has been invoked as a good measure of foraging effort in a variety of bird species (Stauss *et al.*, 2005). During the active breeding period we calculated the distance between each GPS fix using the package ‘geosphere’ in R. This was used to calculate the average hourly distance travelled by each individual, hereafter referred to as foraging effort. This foraging effort was used as the response variable and was analyzed in relation to light levels using the identical approach as for foraging activity, except that GLMMs were fitted using a Gaussian distribution (Table 2).

Table 2. Summary of our main GLMM analyses examining morph specific foraging activity and foraging effort across the ambient light spectrum, as well as level of habitat selection, using six habitat categories (HAB) and percentage tree canopy closure (TREE). Explanatory variables were M=morph type (dark or white) L=light level (W/m^2) and breeding stage (BS). Additional analyses (in parentheses) were also undertaken for foraging activity and effort where light level was replaced with either temperature (Te), rainfall (R) or time (and time²) (Ti).

Analysis	Family	Random term	Response variables	Explanatory variables
Foraging activity	Binomial	Individual	binary response variable: #fixes actively foraging/ #fixes not foraging	$M + L + BS + M*L$ (Te,R,Ti)
Foraging effort	Gaussian	Individual	Average hourly distance travelled	$M + L + BS + M*L$ (Te,R,Ti)
Use by Habitat	Binomial	Individual	binary response variable: presences/ pseudo-absences	$M + HAB + M*HAB$
Use by Canopy cover	Binomial	Individual	binary response variable: presences/ pseudo-absences	$M + TREE + M*TREE$

Whilst our main interests in these analyses were the relationship with light levels, we recognised this variable would be closely correlated with other weather variables such as rainfall, temperature and time of day. Thus, any relationship between light levels and foraging activity or effort could simply be due to a relationship with these other terms rather than light levels themselves. We therefore also included an additional analysis using the same model as described above, but substituted average hourly rainfall, average hourly temperature or time of day (and the quadratic term), and their interaction with morph (Table 3), to explore whether these other variables provided a better fit to the data than light levels. We then compared these models using their corrected Akaike Information Criterion scores (AICc), which is the AIC corrected for a small sample size, with the AICc scores for light level analyses (Table 3 & 4).

Table 3. Models explaining variation in foraging activity (presence or absence from the core nesting area (10% kernel estimate)). Model selection based on Akaike Information Criterion scores (AIC). Results show the top ranked model included light level (measured as solar radiation (W/m^2)) and held 100 % of the weight. The analysis revealed that light level was the better explanatory variable when compared with either temperature, rainfall or time of day. AICc weight (W_i). Rain=average hourly rainfall, Temperature =average hourly temperature and Time= time (and its quadratic term). K denotes the number of parameters (K), change in AIC relative to the highest ranked model (ΔAIC), AIC weight (W_i). Morph=M, Light=L, Breeding stage=BS, Time=Ti, Temperature=Te, Rain=R.

Model	K	AIC	ΔAIC	W_i	Cum. Wt
M+ L + BS + M* L	8	24653.02	0.00	1	1
M + (Ti+Ti ²) + BS + M*(Ti+Ti ²)	10	24824.1	171.08	0	1
M + Te + BS + M*Te	8	24843.15	190.12	0	1
M + R + BS + M*R	8	24978.04	325.02	0	1

Table 4. Models explaining variation in foraging effort (Average hourly distance travelled by individuals outside of the core nesting area (10% kernel estimate)). Model selection based on corrected Akaike Information Criterion scores (AIC). Light level measured as average hourly solar radiation (W/m^2). Rain=average hourly rainfall, Temperature =average hourly temperature and Time= time (and its quadratic term). K denotes the number of parameters (K), change in AIC relative to the highest ranked model (ΔAIC), AIC weight (W_i). Results show the top ranked model (with $\Delta AIC < 2$, shown in bold) included the term light level and held 100% of the weight. The following results reveal that light was the best predictor for foraging effort. Morph=M, Light=L, Breeding stage=BS, Time=Ti, Temperature=Te, Rain=R.

Model	K	AIC	ΔAIC	W_i	Cum. Wt
M+ L + BS + M* L	9	131271.6	0.00	1	1
M + Te + BS + M*Te	9	131442.1	170.46	0	1
M + R + BS + M*R	9	131573.1	301.46	0	1
M + (Ti+Ti ²) + BS + M*(Ti+Ti ²)	11	132306.1	1034.53	0	1

Habitat Preference Between the Morphs

To explore habitat selection, we compared fixes of each bird with a series of random pseudo-absence points (i.e. points from the area that could have been visited by the observed individuals, but were apparently not (Whitfield *et al.*, 2015) generated within each individual's home range generated from all fixes gathered during the breeding season. Thus random fixes were generated within the area of the Minimum Convex Polygon (MCP), an area which encompassed 100% of all GPS fixes obtained for each individual (Fig. 1). Each individual's MCP was estimated in R using the package 'adehabitatHR' classes and methods for home range estimation v.0.4.10 (Calenge, 2006) with the package 'rgdal v.0.9-1 (Bivand *et al.*, 2014) to process the spatial data. Three times as many pseudo-absence data points were chosen as observed points in the presented model (Aarts *et al.*, 2008; Beyer *et al.*, 2010). For our habitat preference analysis, to ensure habitat use was not just a reflection of the habitat around the nest site, we only used data outside of their core nesting areas (i.e. outside of the 10% KDE) for both our bird GPS fixes and our random points.

In our analyses, we used the presences (GPS fixes =1) or pseudo-absences (random points=0) as a binary response variable, analysed within a GLMM with a binomial error structure. Explanatory variables in the model were habitat category (the six categories), male morph (dark or white) and the interaction between these two terms (which was our main variable of interest). Individuals were again treated as random effects to account for the lack of independence of points from the same bird.

Lastly, we ran a further habitat selection analysis exploring specifically if individuals showed stronger selection for denser tree canopy cover within their territories, depending on morph. For this analysis we again used a GLMM with the presences (GPS fixes =1) or pseudo-absences (random points=0) as a binary response variable, but with tree canopy closure (i.e. percentage of vegetation canopy cover), male morph (dark or white) and the interaction between these two terms as the explanatory variables. Individual was again treated as a random term in the model. To explicitly test where significant differences in canopy cover selection occurred between morphs, we ran the same GLMM model using a moving subset of tree canopy cover (Tate *et al.*, 2016).

RESULTS

Foraging Activity and Effort in Relation to Light Levels

During the active breeding season, we found that overall foraging activity (i.e. fixes away from core nesting territories) was greater in dark morphs (mean \pm SE = 0.84 ± 0.17) than white morphs (mean \pm SE = 0.68 ± 0.16) ($\chi^2_1 = 16.5$, $P < 0.0001$). There were also significant differences in foraging activity across the four different breeding stages ($\chi^2_1 = 55.60$, $P < 0.0001$; Fig. 2), with birds spending greater periods of time away from the nests during prelay, nestling and fledgling stages compared to the incubation period.

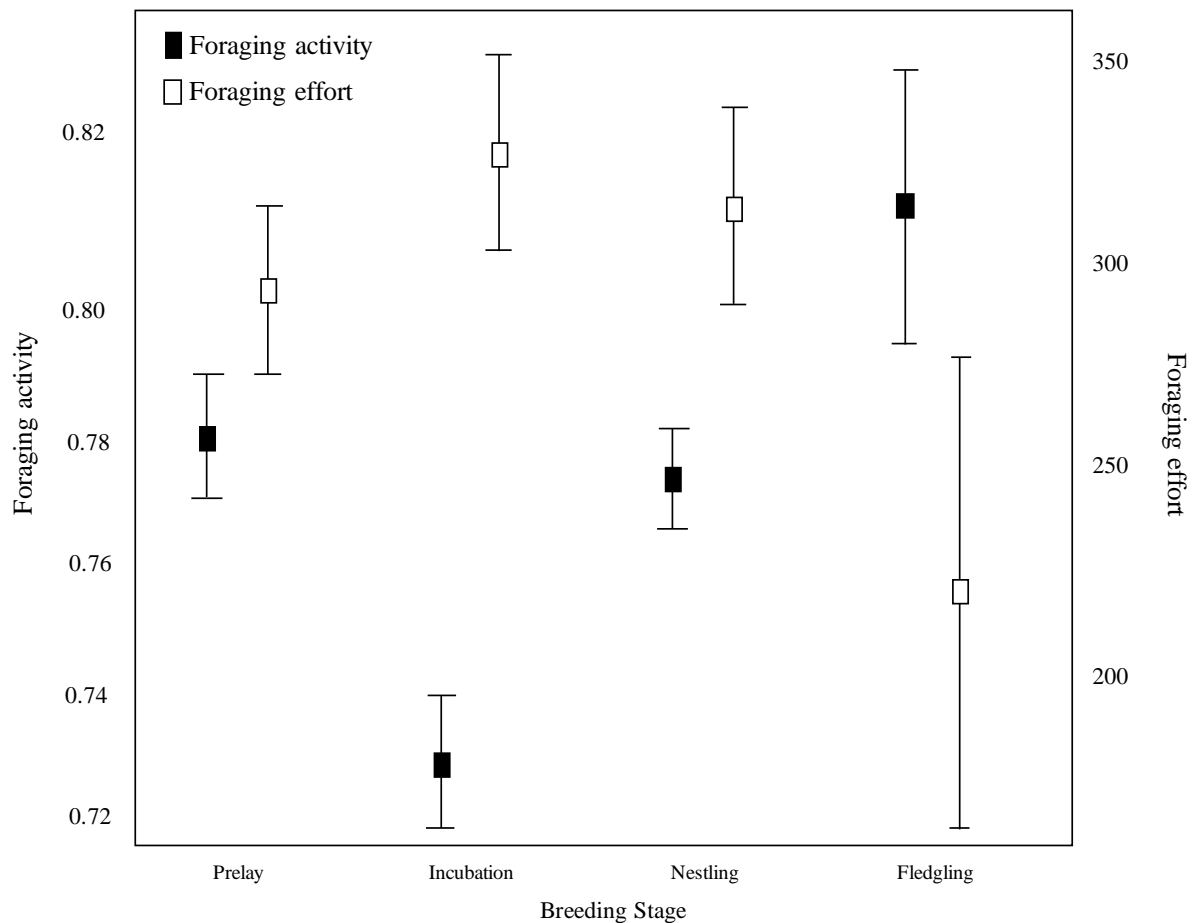


Figure 2. Black Sparrowhawk foraging activity (measured as the probability of individuals being away from their core nesting territories) and foraging effort (measured as the average distance travelled (m)) across the various breeding stages on the Cape Peninsula.

After controlling for breeding stage, there was a significant interaction between morph and light levels ($\chi^2_1=18.2$, $P=0.01$); for white morph birds, foraging activity was not influenced by light conditions ($\chi^2_1=8.7$, $P=0.8$; Fig. 3), whereas activity levels for dark morphs declined substantially and significantly as light levels increased ($\chi^2_1=4.8$, $P<0.0001$; Fig. 3). Examining this relationship in more detail, below around 800 W/m^2 dark morphs remain more active than white morphs, whereas in conditions brighter than this there was little difference in foraging activity between the two morphs, as can be seen from the overlapping confidence limits (Fig. 3). Our model including light levels and morphs better explained variation in foraging activity than the other models which substituted other weather variables (hourly rainfall or temperature) or time for day (and its quadratic term) (Table 3).

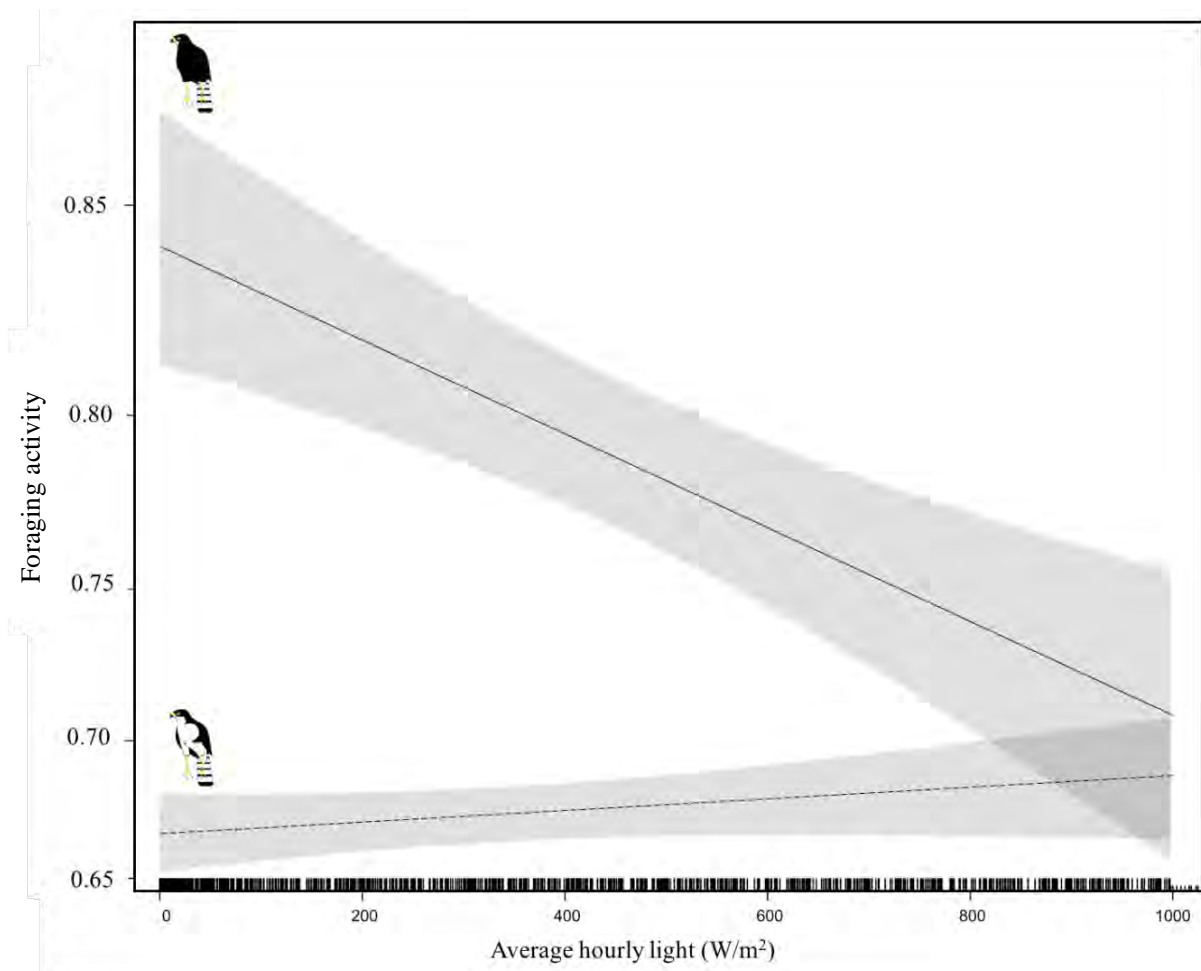


Figure 3. Foraging activity (measured as presence or absence from the core nesting area (10% kernel estimate) of black sparrowhawks on the Cape Peninsula across the ambient light spectrum with 95% confidence intervals. During the breeding season, we found a significant interaction between light level (W/m^2) and morph ($\chi^2_1=18.2$, $P=0.01$); Controlling for breeding stage, for white morph birds, foraging activity increased slightly as conditions became brighter ($P=0.8$), whereas activity levels for dark morphs declined substantially and significantly as light levels increased ($P<0.0001$). Examining this relationship in more detail, dark morphs remain more active below around 800 W/m^2 , in conditions brighter than this there was little difference in activity levels away from the nest between the two morphs, as can be seen from the overlapping confidence limits.

We found a very similar relationship for our secondary foraging measure – foraging effort, which was the average hourly distance travelled. For this term no overall differences were noted between the morphs ($\chi^2_1 = 1.027$, $P=0.31$). Foraging effort did however differ between breeding stages ($\chi^2_1 = 32.40$, $P=0.004$) with greatest foraging effort during incubation and nestling stages and lowest during prelay and fledgling stages (Fig. 1). After controlling for breeding stage, we again found a significant interaction between light levels and morph ($\chi^2_1 = 28.66$, $P=0.002$; Fig. 4). The interaction was similar in nature to our previous result for foraging activity, with foraging effort remaining similar across light levels for white morphs ($P=0.9$), but decreasing significantly as conditions became brighter for dark morphs ($P<0.0001$). Again we found that our models including light levels and morph better explained variation in foraging effort than models including the other weather terms or time (and its quadratic term) (Table 4).

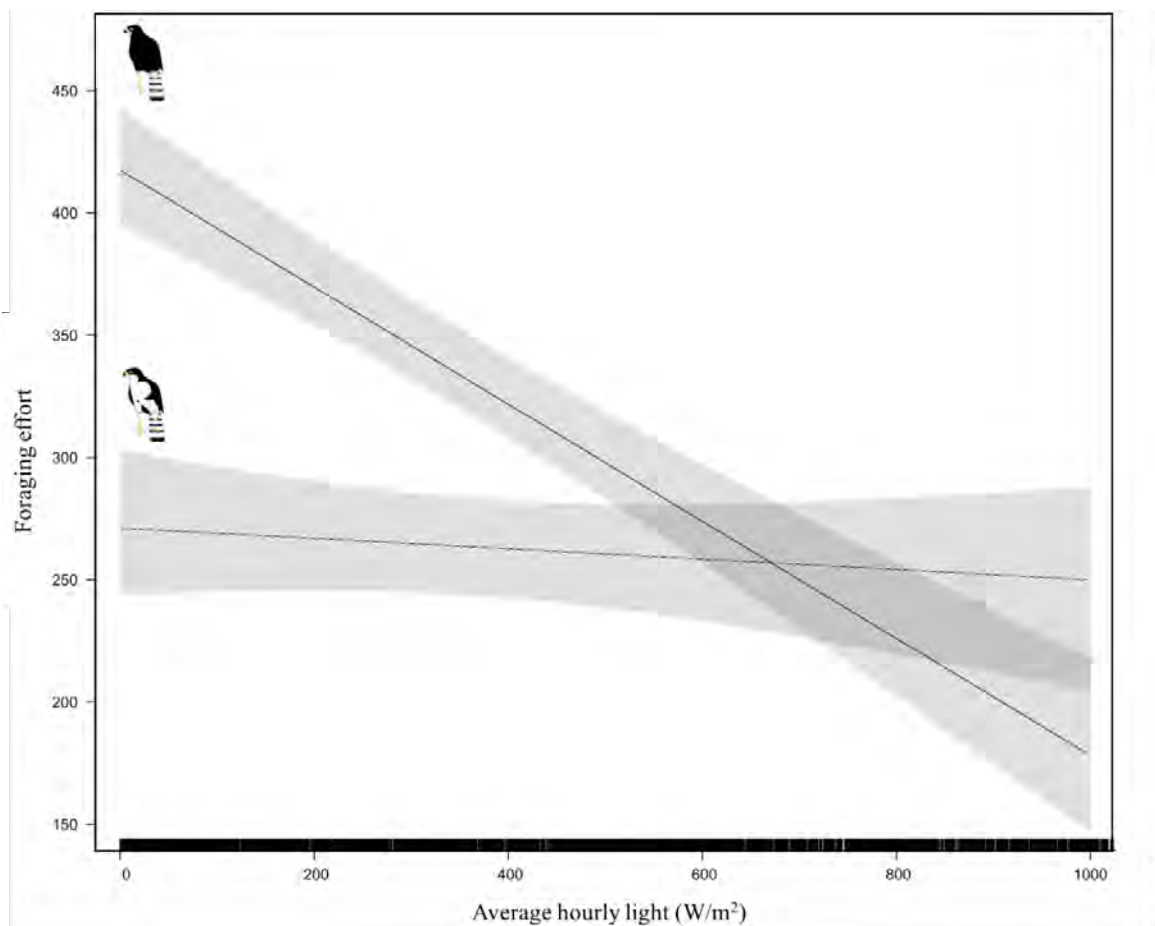


Figure 4. Foraging effort, measured as the average hourly distance travelled (meters) by individuals outside of the core nesting area (10% kernel estimate) with 95% confidence intervals. We detected a significant interaction between light levels and morph, with foraging effort decreasing as conditions became brighter for dark morphs. The same trend was apparent white morphs, however, there was no significant difference in foraging effort across the light spectrum.

Morph Habitat Preference

Across our six habitat types, we detected differences in the degree of habitat selection between white and dark morph black sparrowhawks that were close to significant ($\chi^2_1 = 3.10$, $P=0.05$; Fig. 5). Our post-hoc analysis, however, revealed significant differences between the two morphs in their level of habitat selection for the two most enclosed habitat types; with dark morphs showing significantly greater selection for the more enclosed ‘alien plantations’ (Lsmeans pairwise test, $P=0.0005$) and ‘closed habitat’ (Lsmeans pairwise test, $P<0.001$) than white morphs.

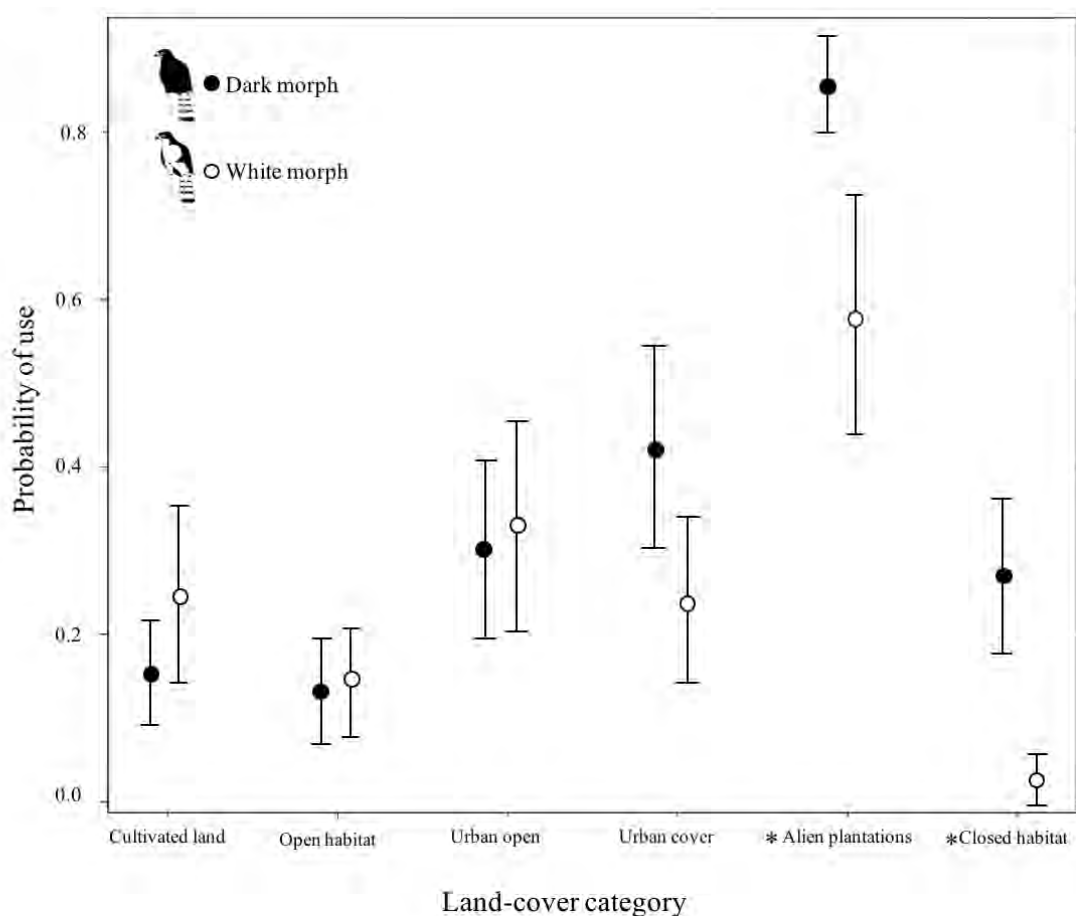


Figure 5. Morph specific habitat selection on the Cape Peninsula. The two morphs differed in their level of habitat selection; with white morphs tending to show greater selection for more open habitats of ‘urban open’ (Lsmeans pairwise test, $P=0.99$), ‘cultivated land’ (Lsmeans pairwise test, $P=0.8$) and ‘open habitat’ (Lsmeans pairwise test, $P=1.0$) than dark morphs. Conversely, dark morphs showed significantly greater selection for the more closed ‘alien plantations’ (Lsmeans pairwise test, $P=0.0005$) and ‘closed habitat’ (Lsmeans pairwise test, $P<0.001$) than white morphs. Morphs had a similar selection for the habitat ‘urban cover’ (Lsmeans pairwise test, $P=0.2$).

Lastly, exploring habitat use in relation to the percentage of tree canopy cover/closure between the morphs, we found a highly significant interaction between male morph type and the percentage of tree

cover ($\chi^2_1 = 87.33$, $P < 0.001$); Analysing this further using a moving window analysis, we found that dark morphs displayed a significantly higher probability of using habitats with denser tree canopy cover (67-100% canopy cover) compared to white morphs ($\chi^2_1 = 11.71$, $P = 0.0006$) (Fig. 6). However, no significant differences in selection were found for low (0-33%) ($\chi^2_1 = 3.13$, $P = 0.07$) or medium (34-66%) canopy cover ($\chi^2_1 = 0.27$, $P = 0.60$).

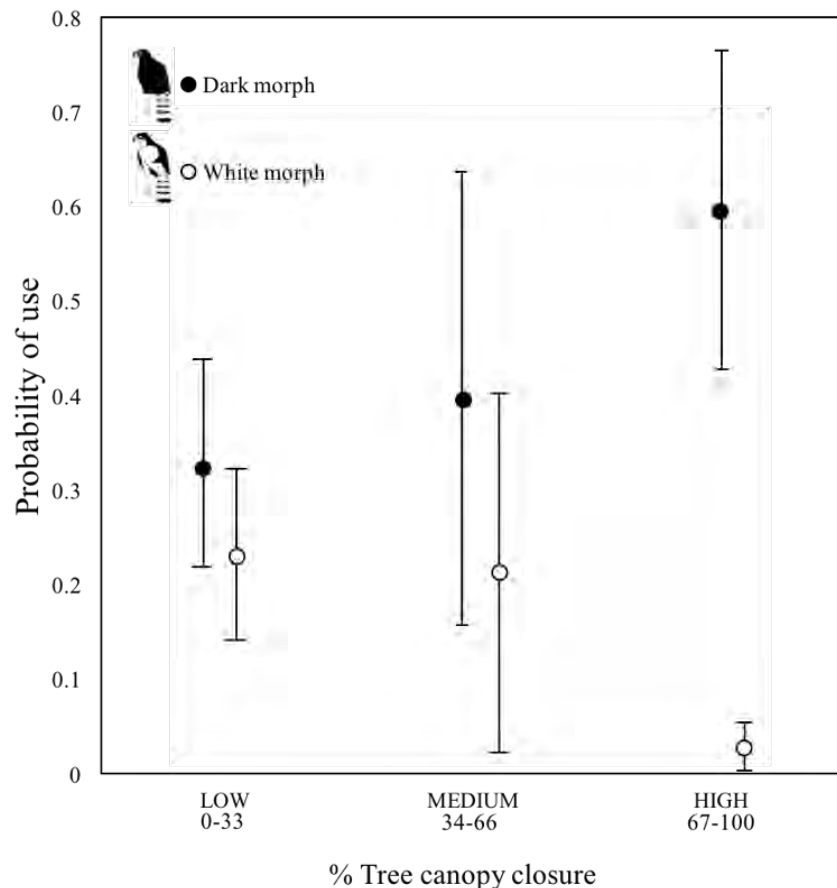


Figure 6. Probability of use (\pm SE) of areas with different canopy cover levels (percentage of canopy closure) by male black sparrowhawk morphs on the Cape Peninsula. Dark morph males had a significantly higher probability of using high tree cover (67-100% canopy cover) compared to white morphs ($\chi^2_1 = 11.71$, $P = 0.0006$). No significant differences in selection were found for low (0-33%) ($\chi^2_1 = 3.13$, $P = 0.07$) or medium (34-66%) canopy cover ($\chi^2_1 = 0.27$, $P = 0.60$).

DISCUSSION

Our tracking data enabled us to compare foraging activity, foraging effort and habitat selection between the two morphs in our study population of black sparrowhawks. Although our sample size was small, with only three birds of each morph tracked, our results did provide support for the hypothesis that the

two morphs are adapted for foraging in different light environments. We found support for both our predictions; dark morphs displayed greater foraging activity and effort in lower light conditions and showed a stronger degree of selection for closed habitats with greater vegetation cover. In contrast, white morphs displayed greater foraging activity and effort in brighter conditions and showed a stronger degree of selection for open habitat types. The fact that we found support for these predictions with our relatively small sample size suggests the nature of these relationships are pervasive among individuals of the two morphs.

In this study, our assumptions are that morphs would invest most time into foraging activities during the light conditions or within the habitats, that will maximize their foraging success, as would be expected under optimal foraging theory (Charnov, 1976). Our results are generally consistent with the findings from Tate *et al.* (2016), which found that prey provisioning rates to nests are associated with light levels differently between the morphs in the same study population. These results, from provisioning rates to nests, are unlikely to have been confounded by differential prey sizes for two reasons; i) because within our study area >80% of prey items consist of 4 similar sized dove species (*Streptopelia semitorquata*, *Columba livia*, *Columba guinea*, *Streptopelia capicola*) and ii) we have found no differences in diet composition between morphs (unpublished data). Thus, from our results, it appears that dark morphs make trips away from the nest site more often, and hunt with greater effort under light conditions where they may benefit from improved crypsis. As light levels increase, foraging presumably become less profitable for this morph and so they become less active and thus cover smaller distances on these trips. The results from this study, in combination with those from Tate *et al.* (2016), may provide further information on how light levels may influence foraging success of the two morphs. For example, white morphs appear to spend the same amount of effort foraging irrespective of the light levels; however, they provide more prey during brighter conditions. The most likely explanation for this is that this morph has higher capture rates during brighter conditions, presumably due to their improved crypsis (Tate *et al.*, 2016). However, in contrast, the results do not necessarily support the conclusions of Tate *et al.* (2016) for dark morphs. For dark morphs, both foraging effort and provision rates were influenced in a similar manner by light conditions, with both measures increasing by around

50% over the light spectrum. This result is therefore suggestive that prey capture rate for dark morphs does not vary in relation to light levels (*cf.* white morphs), as was previously suspected, but rather that it is proportional to the amount of time spent hunting. Unfortunately, it is very difficult to measure hunting success directly. However, with the novel use of accelerometers in tags, this may soon be possible and could usefully be applied to understand the relationships between hunting and light levels in these different morphs

In our analysis, we found that local light conditions in the study area were the most important predictor of morph foraging activity and effort, which has important implications for understanding how polymorphism is maintained in our study system. The ambient light intensity within an environment during the course of the day, between habitats or in different weather conditions (*i.e.* cloud cover) can vary considerably (Martin, 1990; Bortolotti, 2006; Théry, 2006) and is likely to be a strong selective agent (Galeotti *et al.*, 2003; Galeotti & Rubolini, 2004). In many predator-prey systems, individuals may therefore gain improved crypsis, and thus enjoy selective advantages such as enhanced hunting success, under certain ambient light conditions (Galeotti *et al.*, 2003).

Background matching is strongly enhanced by the amount of ambient light in an environment (Endler, 1990; Théry, 2006), whereby darker colours are harder to visually detect in low light conditions (Rohwer, 1990), and lighter colours are more cryptic in brighter conditions (Götmark, 1987; Théry, 2006). Ambient light is significantly different between open and closed habitats, with shading from vegetation strongly reducing light levels (Théry, 2006) (*i.e.* in the order of 300 billion fold; Martin 1990). Thus, the interplay of background colour, coupled with light condition, may have substantial influence on an individual's detectability or level of crypsis. Background matching with surrounding habitat, and light condition below vegetation canopy, may therefore represent an important factor in the maintenance of colour morphs in predator-prey systems, and may explain why polymorphism is most common in species living in both open and closed habitats (Galeotti *et al.*, 2003) and across spatially variable environments or backgrounds (Galeotti & Rubolini, 2004; Bortolotti, 2006).

Several studies have documented differential habitat selection across morphs, often within the same population (Wunderle, 1981). This habitat preference has frequently been linked with foraging benefits accrued for different morph types in certain habitats (Roulin, 2004). For, example, in the red-tailed hawk *Buteo jamaicensis*, light morph birds were observed to occupy more open perch sites when hunting, compared to dark morphs (Preston, 1980). Rohwer (1990) found that the two morphs of the Pacific reef heron *Egretta sacra* used different hunting techniques and utilized different habitat types within the same locality, with dark morphs foraging almost exclusively under dense canopy cover, while light morphs foraged more often in brighter, open habitats. A study done on the tawny owl (*Strix aluco*) also provides data supporting that dark (rufous) morph owls inhabit more wooded and dense-covered territories than light (grey) morphs (Galeotti & Sacchi 2003). The observed morph habitat preferences found in our study also supports an adaptive morph-habitat association. Our findings further reinforce the conclusions from other studies and support the idea that black sparrowhawk morphs forage during conditions, and within habitats (related to the light conditions in a specific habitat), that are likely to enhance their crypsis from their avian prey.

Numerous studies have shown that body colouration may be associated with environmental factors that are not related to crypsis, hunting or predation. These include correlated physiological effects which may lead to geographically related advantages of different morphs, such as thermoregulation (Bortolotti, 2006), optimal heat or water balance, or improved protection against UV-radiation and feather abrasion (Niecke *et al.*, 1999). For example, in some polymorphic reptiles, dorsal body colour was found to be strongly correlated with the thermal environment, thus, serving a thermoregulatory purpose (Rosenblum *et al.*, 2004). In the bananquit *Coereba flaveola*, dark morphs were found to prefer shaded, wetter habitats over the brighter yellow morphs in the absence of predation and thus have no purpose for enhanced crypsis (Wunderle, 1981). Therefore, activity and behaviour in some species may not be due to variable capture success or crypsis under varying light conditions, but rather related to intrinsic behavioural differences between morphs in their coping strategies under variable light conditions.

Although these physiological correlates are, in theory, credible explanations for the presence of morphs in some environments, our results strongly suggest that light levels explained variation in foraging activity and effort in the black sparrowhawk better than other weather conditions or the time of day. Our findings are further reinforced by the fact that black sparrowhawk morphs display ventral plumage polymorphism, a side most obvious to their avian prey below. This plumage pattern conforms to the classic example of countershading proposed under Thayer's law (Thayer, 1909; Cott, 1940) and is a common pattern seen in predators of many taxa, particularly those which attack their prey from above (see Götmark's (1987) experiment dyeing the underparts of black-headed gulls, *Larus ridibundus*). This fits with the mode of attack of black sparrowhawks, which mainly attack via short ambush flights when their bird prey is on the ground (Hockey *et al.*, 2005). Ventral colouration is therefore considered to have an important role in decreasing the conspicuousness of predators against their background (Thayer, 1909; Götmark, 1987; Bortolotti, 2006).

Our findings also offer an explanation for the numerical dominance of dark morphs on the Cape Peninsula, where > 75 % of birds are dark morphs (Amar *et al.*, 2014). The recent range expansion in black sparrowhawks has brought the species into contact with a novel climatic regime (Martin *et al.*, 2014a), particularly during the species winter breeding season. Throughout South Africa, the species distribution is now characterized by two contrasting climatic windows, with dry winters in their historical range in the north and east, and wet winters in the recently colonized Western Cape region (Martin *et al.*, 2014b). The Cape Peninsula receives particularly high levels of rainfall as a result of its exposure to southern oceanic fronts and the orographic effects of Table Mountain (Martin *et al.*, 2014a). Therefore, local light conditions are particularly low during the wet breeding season and, under these conditions, dark morphs have been shown to have a potential advantage over white morphs via improved foraging success (Tate *et al.*, 2016). These local breeding conditions may therefore be important in maintaining a higher than expected proportion of dark morphs in our study population of black sparrowhawks. For example, Tate *et al.* (2016) showed that for the majority of the winter breeding period, light levels are below that where white and dark morphs show similar foraging behaviours. This is further reinforced by recent findings by Sumasgutner *et al.* (2016), which revealed that recruitment

of chicks strongly depended on their father morph in associated with the timing of breeding. Significantly higher recruitment rates were reported for nests with dark morph males breeding earlier on in the season (when light conditions are generally lower), whereas higher recruitment rates were documented in nests with white morph males breeding later in the season (when conditions became brighter).

Interestingly, although winter breeding conditions (i.e. the predominantly low light conditions) on the Peninsula appear to favour dark morphs in terms of their hunting success, this is not being translated into overall advantages in other fitness related traits, such as survival and breeding performance. In fact, recent work conducted on the same study population by Tate *et al.* (in press) has revealed that survival and productivity does not differ between morphs at the individual level, however, their study did show that the morph combination of breeding adult pairs influenced productivity significantly, with mixed-morph pairs producing more offspring (around a quarter more) per year than pairs consisting of the same morph. The authors propose that the higher productivity of mixed-pairs may be the result of the complementary nature of care provided by the different morphs, with differential foraging success between black sparrowhawk morphs under varying light conditions (Tate *et al.*, 2016) allowing mixed-pairs to expand their foraging niche (i.e. across the full light spectrum (low and bright light conditions)). The results from this study also suggest this may also extend, to some degree, to different habitats types and specifically, vegetation cover. Thus mixed pairs will be able to exploit a wider range of habitat types, than like-pairs. Subsequently, emergent pair-level properties may play an important role in promoting and maintaining polymorphism in the species.

In this study, we have assumed that when birds are outside of their core nesting territories, they are actively foraging, however, we recognise that they may have also been engaging other activities such as territorial behaviour, or avoiding begging females and chicks (although the inclusion of foraging effort in our separate analysis should account to a degree for this potential bias). Numerous studies on polymorphic bird species have documented differences in aggression across morphs (Boerner & Kruger, 2008), with melanistic morphs frequently displaying higher levels of aggression, and lower

sensitivities to stress, compared to lighter morphs (Boerner & Kruger, 2008; Roulin & Ducrest, 2013). Therefore, rather than engaging in foraging activities, dark morph black sparrowhawks may be making more regular trips away from their nest sites as they are defending their breeding territories more actively and aggressively, and therefore appear more active than white morphs. Extra pair copulation (EPC) has also been observed in our study population (Martin *et al.*, 2014b), and it may be that dark morphs spend more time away from their nests actively pursuing and securing additional mates compared to white morphs, a trait that has been witnessed in other polymorphic bird species (Roulin *et al.*, 2004). Whilst this might explain the higher overall levels of activity seen for the dark morphs, it does not explain the contrasting relationships in activity in relation to light levels seen for the different morphs in this study.

The current study represents the first attempt to explore whether foraging behaviour by different morph types is influenced differently by ambient light conditions. Our findings suggest that the two colour morphs in the black sparrowhawk have differential foraging activity and foraging effort across variable light conditions. Additionally, we found morph differences in habitat and cover preference-likely related to the light conditions provided by these habitats. This appears to be linked with the conditions under which they have improved crypsis and thus optimum foraging success (Tate *et al.*, 2016). Our study suggests that a significant foraging advantage under local light and habitat conditions may be important in maintaining a higher than expected proportion of dark morphs in the Cape Peninsula population of black sparrowhawks. As breeding light conditions throughout South Africa are likely to be highly variable as a result of the different climatic regimes that occur across the black sparrowhawks distribution, our study provides important supporting evidence for the role of breeding season light levels in spatial structuring (i.e. clinal variation; Amar *et al.* (2014)) of morphs across the landscape of South Africa, as established by Tate *et al.* (2016). Interestingly, many *Accipiters* share a similar type of colour polymorphism to that shown by the black sparrowhawk. It would therefore be particularly interesting to repeat these analyses for other polymorphic *Accipiter* species, to see whether similar relationships hold and therefore whether generalizations can be drawn across the polymorphic *Accipiters*.

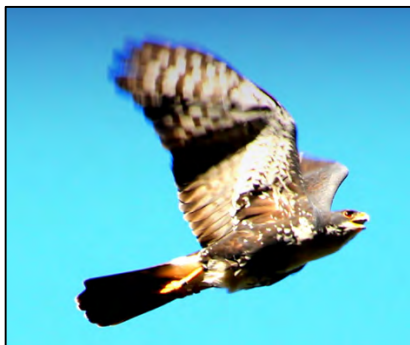
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CHAPTER 5

PAIR COMPLEMENTARITY INFLUENCES REPRODUCTIVE OUTPUT IN THE POLYMORPHIC BLACK SPARROWHAWK *ACCIPITER MELANOLEUCUS*.



A dark morph female stands guard over her nestlings, Cape Town, July, 2015. With abundant prey resources and nesting sites, Black Sparrowhawks are particularly well adapted to living in urban environments. Throughout South Africa, the species now nests almost exclusively in the forks of large introduced Eucalyptus and Pine trees.

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ABSTRACT

How multiple morphs are maintained within populations of colour polymorphic bird species remains a challenging question in evolutionary ecology. In some systems, differential productivity or survival between morphs are thought to play a role. Here we examine key demographic parameters between the two discrete adult morphs that characterise the polymorphic black sparrowhawk *Accipiter melanoleucus*. Using long-term breeding and survival data from a population on the Cape Peninsula, South Africa, we test for differences in reproductive performance between white and dark morphs, both in isolation and in combination with their partner morph and adult survival between morphs. We found that neither morph had a specific advantage in terms of productivity or survival. Despite this lack of difference between the individual morphs, we did however find that morph combination of adult pairs influenced productivity significantly, with mixed-pairs producing more offspring per year than pairs consisting of the same morph. The body condition of the offspring showed the opposite relationship, with nestlings of mixed-pairs having lower body condition than nestlings of like-pairs. While our results suggest an advantage of mating with the opposite morph, there was no evidence for disassortative mating; instead breeding pair morph combinations were random with respect to the background frequencies of the two morphs. Higher productivity of mixed-pairs may be the result of the complementary nature of care provided by the different morphs. We propose that differential foraging success between black sparrowhawk morphs under varying light conditions allows mixed-pairs to expand their foraging niche. We conclude that emergent pair-level properties may play an important role in promoting and maintaining polymorphism and may be important for other bird species which display bi-parental care.

Keywords:

Adult survival, breeding success, colour polymorphism, niche expansion, productivity, raptor, reproductive performance.

INTRODUCTION

The extent of heritable phenotypic variation present in a population is fundamental for its evolution, as it governs a population's ability to respond and adapt to environmental change (Dobzhansky 1951, Van Valen 1965, Forsman *et al.* 2015). Greater phenotypic variation has therefore been proposed as beneficial for both population stability and abundance (Forsman *et al.* 2008). For example, colour polymorphic species, where multiple phenotypically distinct colour variants occur within the same population irrespective of age or sex (Huxley 1955), are predicted to be less vulnerable to environmental change and experience lower extinction risks and range contractions compared to species with monomorphic phenotypes (Forsman & Hagman 2009; Roulin *et al.* 2011; Amar *et al.* 2014; Roulin 2014a).

Colour polymorphic species have long fascinated evolutionary biologists (Huxley 1955, Roulin 2004, Hill & McGraw 2006), and since they are visually recognisable, they have been considered as ideal model systems to study some of the major unanswered questions in evolutionary biology. Namely, how variation in fitness-related traits persists within populations (Fisher 1930) and more fundamentally, how colour polymorphisms are maintained in species despite natural selection and genetic drift (Darwin 1859, Krüger & Lindstrom 2001, Briggs *et al.* 2011b). Part of this interest centres upon the question of whether different morphs also vary in aspects other than their physical appearance, such as physiology or behaviour.

Although phylogenetically widespread (Hugall & Stuart-Fox 2012), colour polymorphism is rare in birds, involving around 3.5% species globally (Galeotti *et al.* 2003). However, amongst Strigidae (owls) and Accipitridae (hawks), it occurs in about 38.5% and 22.1% of the species respectively (Galeotti *et al.* 2003), which makes the study of plumage colour polymorphism of great interest in avian biology, specifically in raptors (Holderby *et al.* 2012). Colour polymorphism is widely considered to serve an

adaptive function (Galeotti et al. 2003, Roulin 2004, Roulin & Ducrest 2013) and has also been found to influence various key life history and fitness related traits, including survival and reproduction (reviewed in Roulin 2004). Roulin's (2004) review found differences in survival rates in four of the six species (67%) studied, and several other studies have subsequently found differences in survival rates between morphs (i.e. in tawny owls *Strix aluco* Brommer et al. 2005; Karell et al. 2011; Emaresi et al. 2014). Amongst diurnal raptors however, very few studies have examined survival rates across morphs. For example Krüger et al. (2001) found differences in survival rates between common buzzard *Buteo buteo* morphs. Using more robust methods, Jonker et al. (2013) found similar trends in the same buzzard population, but these differences in survival rates were only weakly supported. Interestingly, Briggs et al. (2011a) found only weak support for differential survival across morphs in the Swainson's hawk *Buteo swainsoni*.

Differences in reproductive performance between colour morphs co-existing in the same population potentially reflect an interaction between phenotypes and the environment, and frequently appear to be linked to the nesting or foraging ecology of certain morphs in specific habitats (Holderby et al. 2012). Roulin's (2004) review highlighted multiple differences in reproductive performance between morphs, with 9 out of 11 species (82%) analysed displaying statistical differences in productivity between morphs. For example amongst diurnal raptors, Johnson & Burnham's (2013) study on gyrfalcons *Falco rusticolus* in Greenland, found that white morph individuals have earlier clutches and produce more offspring compared to darker grey and silver morphs. Krüger & Lindstrom (2001) found that intermediate morphs of common buzzards display a higher lifetime reproductive success compared to light and dark conspecifics. In Swainson's hawks, however, Briggs et al. (2011b) found no differences in productivity or life-time reproductive success among morphs.

In theory, morph co-existence could stem from balancing selection between survival and reproduction (Stearns 1992, Briggs et al. 2011b), where one morph accrues an advantage through higher reproductive success but suffers from higher mortality, whereas the other morph may have lower reproductive success but higher long-term survival rates. It has frequently been proposed that different phenotypes

represent alternative strategies adapted to different prevailing environmental conditions (Roulin 2004, Ducrest *et al.* 2008, Gasparini *et al.* 2009, Antoniazza *et al.* 2010). This appears to influence the frequency and stability of morphs within a population (Roulin 2004, Ducrest *et al.* 2008, Gasparini *et al.* 2009). Differences in key demographic parameters such as survival and reproduction might explain the underlying mechanisms which maintain or segregate morphs in populations and across species distributions, and may also explain why one morph often numerically dominates over another in certain environments (Fisher 1930, McLean & Stuart-Fox 2014).

Although numerous studies have examined various morph-specific demographic traits (Roulin 2004), very few have explored these traits in relation to the combination of morphs within a breeding pair. The morph combination could have impacts on fitness related traits (i.e. productivity) for a variety of reasons. Different parental morph combinations will influence the genetic make-up of the offspring (i.e. heterozygote advantage; Krüger *et al.* 2001) or for species exhibiting bi-parental care, different parental morphs may provide complementary or divergent resources to their offspring (Selander 1966, Newton 1979, Nodeland 2013). In one of the few studies that explored this issue previously, Kristensen *et al.* (2014) found a selective advantage in mixed-morph common guillemot *Uria aalge* pairs, with like non-bridled and like bridled pairs producing smaller chicks with smaller tarsi compared to mixed-pair compositions. Importantly, the authors detected no differences between the two morphs in reproductive parameters without considering the morph of the partner, suggesting that reproductive decisions appear to not only depend on the morph but also on the tactic of the partner. Hatch (1991) found that reproductive success in Northern fulmars *Fulmarus glacialis* was influenced by the combination of the morph pair, with mixed-pairs exhibiting lower reproductive success than like-pairs. Although Johnson & Burnham's (2013) study on gyrfalcons did not look at productivity of pairs based on their morph consistency, they did find that pairs including a white male bred earlier and had higher productivity (larger clutches and more fledged offspring) than darker morphs. Consistently, Krüger *et al.* (2001) found like-pairings in common buzzards produced broods of offspring with lower fitness compared to mixed-pairings and pair combinations involving exclusively extreme morphs (i.e., light and dark) achieved lower breeding success compared with pair combinations involving one intermediate partner.

The black sparrowhawk *Accipiter melanoleucus* is a polymorphic raptor that employs bi-parental care, a universal attribute amongst the Accipitridae (Hockey *et al.* 2005). The species exhibits discrete polymorphism, with adults occurring as either dark or white morphs (Amar *et al.* 2013). The black sparrowhawk is broadly distributed throughout the forested regions of sub-Saharan Africa (Ferguson-Lees & Christie 2001) and within South Africa, has expanded its range over the last few decades into the Western Cape (Hockey *et al.* 2005, Martin *et al.* 2014a). Here black sparrowhawks have recently colonised the Cape Peninsula, with the first breeding attempt recorded in 1993 (Curtis, Hockey, & Koeslag, 2007; Oetlé, 1994). The white morph is most common across most of the species' South African range, except in the south-west, where the dark morph predominates numerically. Our study population on the Cape Peninsula contains >75% dark morphs in the breeding population, with these morph ratios remaining stable for over a decade (Amar *et al.* 2013, 2014).

In this study we use long-term breeding and survival data (2000-2014) from the population of black sparrowhawks on the Cape Peninsula, South Africa, to compare whether reproductive performance between the morphs differ, either in isolation or in combination with their partner morph (i.e. mixed or like-pairs, and the sex-morph specific combinations). We also test for potential differences in body condition of the chicks, to account for the offspring size-number trade-off hypothesis (see Gyllenberg *et al.* 2011). We then test for assortative or disassortative mating patterns in our study population (Galeotti *et al.* 2003, Galeotti & Rubolini 2004), which might be predicted if either like or mixed-pairs have higher productivity. Lastly, using individually colour marked adults, we examine whether there is evidence for differential survival between the morphs.

MATERIALS AND METHODS

DATA COLLECTION

Breeding and Colour Ringing Data

We monitored the black sparrowhawk population on the Cape Peninsula (34°00'S, 18°26'E), Western Cape, South Africa, between 2000-2014 (see Amar *et al.* 2013 and Martin *et al.* 2014a for more detail of the study site). During this time, the study population has grown from around 10 pairs to 50 (Martin *et al.* 2014b). Fieldwork was conducted during the breeding season (March to November; Martin *et al.* 2014b) each year. Territories were monitored approximately monthly for signs of occupation and then more frequently (approximately every week) once breeding was detected. Productivity (number of young produced per year, 0-4 chicks), reproductive success (whether a breeding attempt failed or not, binomial 0/1) and brood size (number of 1-3 chicks fledging from each successful brood) were recorded for each pair per breeding season. Where possible, we assigned the timing of breeding to the nearest month (hereafter 'lay month') based on the incubation behaviour of females (Martin *et al.* 2014a) or back dated based on the relative age/development of the chicks when ringed. We achieved this by comparing the plumage and development (i.e. chick size) to a reference collection of photographs of known age nestlings via video monitoring (Katzenberger *et al.* 2015). We also identified the morph (dark or light) and sex of each parent attending a nest. The species is easy to sex, with males weighing around 30% less than the females (Ferguson-Lees & Christie 2001, Hockey *et al.* 2005). To derive a nestlings' body condition index, we used residuals from a linear regression of body mass and tarsus length, corrected for variation explained by sex (females are heavier than males, two-way ANOVA); method described by (Roulin *et al.* 2007).

To examine survival rates in relation to morph type, we captured as many birds as possible and fitted them with unique colour ring combinations (SAFRING and two metal colour rings). Birds were trapped predominantly on territories using bal-chatri traps baited with live pigeons *Columba livia* (Berger & Mueller 1959). Additionally, in the study population, systematic chick ringing of 3-5 week-old nestlings

commenced from 2006. Many of the birds ringed as chicks entered the breeding population during the course of the study. By the end of 2013, 69% of the adults in our population were uniquely colour ringed (Sumasgutner *et al.* 2016). Resightings of individually marked black sparrowhawks were recorded annually since 2001, via reporting and photographs taken by researchers, volunteers and by members of the public (Martin *et al.* 2014a). In total 14 years of resighting data were used in our survival analysis.

STATISTICAL ANALYSIS

Breeding Parameters

To examine breeding performance in relation to morph type and the morph pair combination (i.e. mixed or like-pairs), we fitted Generalised Linear Mixed Models (GLMM) using the statistical software R, version 3.03 © (R Core Team 2014) and the “lme4” package (Bates & Maechler 2014). We fitted ‘year’ and ‘territory’ as random terms to control for the lack of independence of data taken from the same territory over multiple years and from multiple territories within the same year. We analysed 1) productivity and 2) brood size (of successful nests) with a Poisson error structure and a logit link function, 3) reproductive success with a binomial error structure and logit link function. In all these models, we additionally fitted the lay month as co-variable to account for potential variation along the breeding period. We then analysed 4) lay month and 5) body condition of the chicks with a Gaussian distribution and an identity link function using a linear fixed-effects model (lmer function). For body condition we also controlled for the timing of breeding as well as brood size and fitted the nest ID as additional random term (together with year and territory) to account for the lack of independence of measurements taken from the same brood. The statistical significance of the differences between the four level sex-morph pair combinations were tested with post-hoc pairwise comparisons using the “lsmeans” package (Lenth 2015) using the model estimates. For binomial models (Table 1), we present the results using F statistics, whereas for all other models we use the chi-square values from the GLMM ANOVA output.

Lastly, to examine mate selection and evidence for either assortative or disassortative mating, we compared the observed frequency of mixed-pairs each year with the frequency expected, given the rates

Table 1. Summary of reproductive performance in Black Sparrowhawks on the Cape Peninsula. We examined productivity (0-4 chicks per year), reproductive success (successful/unsuccessful), brood size (1-3 chicks fledged per successful brood) and lay month in isolation or in combination with their partner morph (i.e. mixed or like-pairs, and the sex-morph specific combinations). We found significantly higher productivity in mixed-pairs compared to like-pairs. Lay month only differed significantly between male morphs, with dark morphs found to breed earlier than white morphs. Significant P values in ***bold** (P<0.05).

Breeding parameter	Family	Sex	P value	Morph	Estimate	SE	DF	Morph pair	P Value	Estimate	SE	DF	Pair combo	P value	Estimate	SE	DF
Productivity	Poisson	Male	0.8	Dark	1.19	0.07	1	Mixed	*0.04	1.45	0.40	1	d♂-d♀	0.44	0.95	0.10	3
				Light	1.44	0.14	1						d♂-w♀		1.15	0.13	3
		Female	0.1	Dark	1.25	0.09	1	Like		1.15	0.30	1	w♂-d♀		1.02	0.16	3
				Light	1.48	0.12	1						w♂-w♀		0.97	0.34	3
Reproductive success	Binomial	Male	0.7	Dark	0.32	0.14	1	Mixed	0.23	0.62	0.20	1	d♂-d♀	0.38	0.55	0.16	3
				Light	0.43	0.25	1						d♂-w♀		0.66	0.23	3
		Female	0.09	Dark	0.23	0.14	1	Like		0.57	0.15	1	w♂-d♀		0.58	0.28	3
				Light	0.63	0.21	1						w♂-w♀		0.67	0.47	3
Brood size	Poisson	Male	0.7	Dark	1.86	0.14	1	Mixed	0.78	1.88	0.14	1	d♂-d♀	0.80	1.86	0.13	3
				Light	1.79	0.05	1						d♂-w♀		1.86	0.09	3
		Female	0.7	Dark	1.86	0.18	1	Like		1.82	0.19	1	w♂-d♀		1.89	0.11	3
				Light	1.79	0.26	1						w♂-w♀		1.52	0.17	3
Lay month	Gaussian	Male	0.06	Dark	6.8	0.18	1	Mixed	0.57	7.00	0.22	1	d♂-d♀	*0.05	6.80	0.20	3
				Light	7.5	0.3	1						d♂-w♀		6.70	0.26	3
		Female	0.6	Dark	6.95	0.19	1	Like		6.85	0.19	1	w♂-d♀		7.51	0.31	3
				Light	6.85	0.24	1						w♂-w♀		7.49	0.44	3
Nestling Body Condition	Gaussian	Male	* 0.03	Dark	15.09	9.96	1	Mixed	*0.010	2.15	11.57	1	d♂-d♀	*0.04	22.66	11.61	3
				Light	-6.36	12.28	1						d♂-w♀		7.70	12.35	3
		Female	0.5	Dark	11.95	10.43	1	Like		22.36	11.67	1	w♂-d♀		-7.46	12.59	3
				Light	6.36	11.46	1						w♂-w♀		8.19	20.33	3

of occurrence of the different male and female morphs in the population in each year. The expected rates of occurrence of each pair combination were calculated using the following equation:

$$\text{expected rate} = \frac{\text{number of males of morph } X}{\text{total number of pairs}} * \text{number of females of morph } X$$

We calculated the expected number for each male/female dark/light pair combination (i.e. four possible pairings), and summed the total of mixed-pairs (i.e. dark male*light female or light male*dark female). We then compared our expected number of mixed-pairs in each year with that observed, and tested for a departure from random mating using a Chi-squared test. All results are presented as means \pm SE.

Factors Influencing Local Survival Rates

The RMark package (Laake *et al.* 2013) was used to build all candidate survival models; results were exported to Program MARK (White and Burnham 1999) to examine differences in apparent survival (Φ) and resighting probability (ρ) of morphs and sexes. We used Cormack-Jolly-Seber (CJS) models with morph, sex and year as covariates. A list of candidate models with all possible predictor combinations was defined (total of 132 combinations of 14 years, four covariates and the interaction term between morph, sex and year). Fully time dependent models (i.e. year for both Φ and ρ), and models that assumed constant Φ and ρ , were excluded from the candidate set prior to analyses as these models were not separately identifiable, contained confounded parameters and potentially over-fitted the data. This approach left us with a total of 74 model combinations (Burnham *et al.* 1987, White & Burnham 1999, Whitfield *et al.* 2004), from which MARK ranked the models according to their AIC_c (Akaike's Information Criterion corrected for small sample size) and model weight ω_i (Burnham & Anderson 2002). A goodness of fit test using the program U-CARE (Choquet *et al.* 2009) was performed to ensure that data met the homogeneity assumption: ($\chi^2_4=5.24$; $\hat{c}=1.01$). A table of best candidate models with $AIC_c \leq 4.0$ is presented in Table 2. All models with $AIC_c \leq 4.0$ were extracted and consequently used for model averaging. To examine whether differences in adult survival and resighting probability were significant between morphs and the morph-sex categories, we ran a pairwise post-hoc contrast test using the program CONTRAST (Sauer & Williams 1989).

Table 2: Model selection results showing the top 8 models of apparent survival (Φ) and resighting probability (ρ) in Black Sparrowhawks on the Cape Peninsula, with $\Delta\text{AICc} < 4.0$. Model averaged results (from the models with $\Delta\text{AICc} < 4.0$) revealed that overall survival did not differ greatly between morphs or sexes. K number of parameters in the model, ω_i model weight (based on the entire list of candidate models). All top candidate models included time dependency (variable ‘year’) suggesting that time had high explanatory power in apparent survival in this population.

Model	ΔAICc	ω_i	K	Deviance
1. $\Phi(\text{year}), \rho(\text{morph} + \text{sex})$	0	0.2	15	517.05
2. $\Phi(\text{year} + \text{sex}), \rho(\text{morph} + \text{sex})$	0.8	0.1	16	515.80
3. $\Phi(\text{year} + \text{morph}), \rho(\text{morph} + \text{sex})$	2	0.07	16	517.00
4. $\Phi(\text{year}), \rho(\text{morph}*\text{sex} + \text{morph} + \text{sex})$	2.1	0.06	16	517.04
5. $\Phi(\text{year} + \text{morph} + \text{sex}), \rho(\text{morph} + \text{sex})$	2.9	0.04	17	515.80
6. $\Phi(\text{year} + \text{sex}), \rho(\text{morph}*\text{sex} + \text{morph} + \text{sex})$	3	0.04	17	515.70
7. $\Phi(\text{year}), \rho(\text{sex})$	4	0.02	14	523.20
8. $\Phi(\text{year} + \text{sex}), \rho(\text{morph})$	4	0.02	15	521.12

Φ =apparent survival probability, ρ =resighting probability, morph=adult morphs (dark and light), *= interaction term.

RESULTS

In total, we had 501 breeding records for pairs with known morphs, from 71 active breeding territories across 15 years (2000-2014). From these 501 breeding attempts, 302 were like-pairings (60%) whereas 199 were mixed-pairings (40%; 81 dark σ^* white f and 118 white σ^* dark f). Because of the numerical dominance of dark morphs in the population for over a decade (i.e. 2001 -2013; Amar et al. 2013), the 302 like-pairs consisted of 276 dark σ^* dark f pairings and only 26 white σ^* white f pairings. We had detailed measurements (weight [g] and tarsus length [mm]) of 268 chicks from 142 successful broods that were considered for the analyses of body condition.

Morph Specific Breeding Parameters

For both sexes, we detected no significant difference between morphs in their productivity (males: $\chi^2_1=0.02$, $P=0.8$, females: $\chi^2_1=2.3$, $P=0.1$), reproductive success (males: $F_1=0.15$, $P=0.7$, females: $F_1=2.9$, $P=0.09$) or brood size of successful nests (males: $\chi^2_1=0.1$, $P=0.7$, females: $\chi^2_1=0.2$, $P=0.7$). For

both productivity and reproductive success, there was a tendency for white morph females to have higher productivity and higher reproductive success (Table 1). When analysing the body condition of the nestlings we found dark morph fathers produced chicks with higher body condition than white morph fathers, independent of the timing of breeding or brood size ($\chi^2_1=4.85$, $P=0.03$). We found no effect of the mother's morph on the body condition of the chicks ($\chi^2_1=0.38$, $P=0.50$).

Based on the month of egg-laying, we found significant differences in timing of breeding between male morphs ($\chi^2_1=7.5$, $P=0.006$), with white morph males breeding approximately a month later in the season compared to dark morph males (dark morphs lay month=July (6.8 ± 0.18), white morphs lay month=August (7.5 ± 0.3 ; Fig. 1). However, no such differences in lay month were found between female morphs ($\chi^2_1=0.17$, $P=0.6$). Given the significant difference in the timing of breeding for males, we repeated the analyses on breeding parameters to control for any potential effect of lay month, but still found no statistically significant differences in any of the breeding parameters for either sex ($P>0.05$); productivity (males: $\chi^2_1=0.23$, $P=0.63$, females: $\chi^2_1=0.05$, $P=0.81$), reproductive success (males: $F_1=0.15$, $P=0.7$, females: $F_1=2.92$, $P=0.80$) or brood size of successful nests (males: $\chi^2_1=0.14$, $P=0.7$, females: $\chi^2_1=0.003$, $P=0.95$).

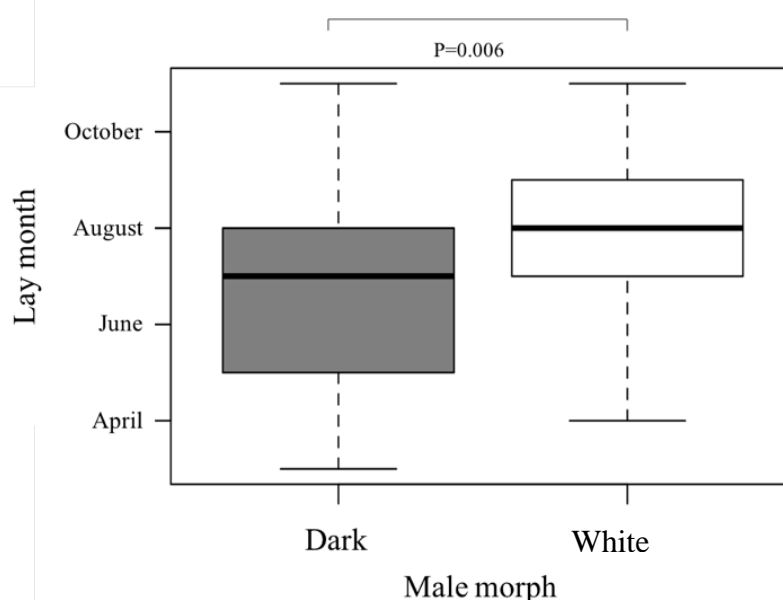


Figure 1. Lay months with respect to male morph (d=dark morph, l=white morph) of Black Sparrowhawks from active pairs monitored on the Cape Peninsula between 2001 and 2014. Plotted are raw data and not effect sizes, box and whisker plots showing median, 25th and 75th percentile and outliers. Estimates for mean lay months were produced from a Generalized Mixed Model with year and territory fitted as random terms in the model (see Table 1 for details on statistical values).

Influence of Morph Pair on Breeding Parameters

Mixed-morph pairs had significantly higher productivity than like-pairs, with mixed-pairs producing around 25% more chicks per year than like-pairs (mixed: 1.45 ± 0.42 , like: 1.15 ± 0.35 ; $\chi^2_1=4.7$, $P=0.04$; Fig. 2).

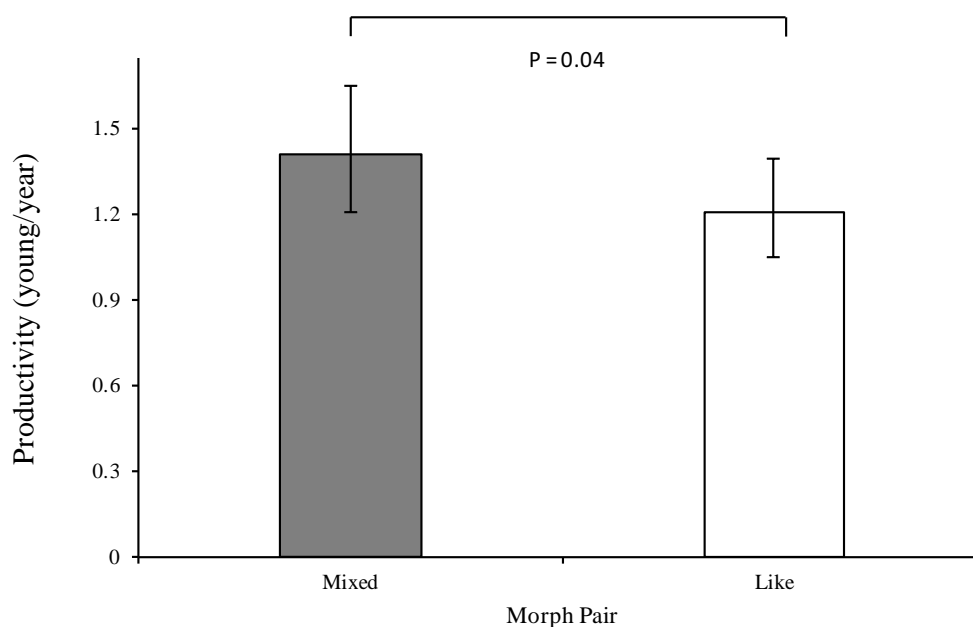


Figure 2. Productivity of mixed (dark morph mated with a white morph, irrespective of their sex) and like-pairs (where both birds are either dark or white morphs) monitored on the Cape Peninsula between 2001 and 2014. Plotted are raw data and not effect sizes; values given as mean \pm SE. Black numbers indicate sample size for each group. Averages are the mean modelled estimate produced from a Generalized Linear Mixed Model with year and territory fitted as random terms in the model (see Table 1 for details on statistical values).

Although mixed-pairs have slightly higher reproductive success ($F_1=1.5$, $P=0.23$) and larger brood sizes ($\chi^2_1=0.07$, $P=0.78$), we found no significant differences for these parameters. We detected no significant difference in the timing of breeding between mixed or like-pairs (lay month mixed-pairs= 7.0 ± 0.2 , like-pairs= 6.9 ± 0.2 ; $\chi^2_1=0.3$, $P=0.57$) suggesting that the differences in productivity were not simply due to differences in the timing of breeding. For nestlings' body condition we found the opposite patterns, with chicks of like-morph parents having a higher body condition than chicks of mixed-morph parents ($\chi^2_1=6.7$, $P=0.010$; Fig. 3).

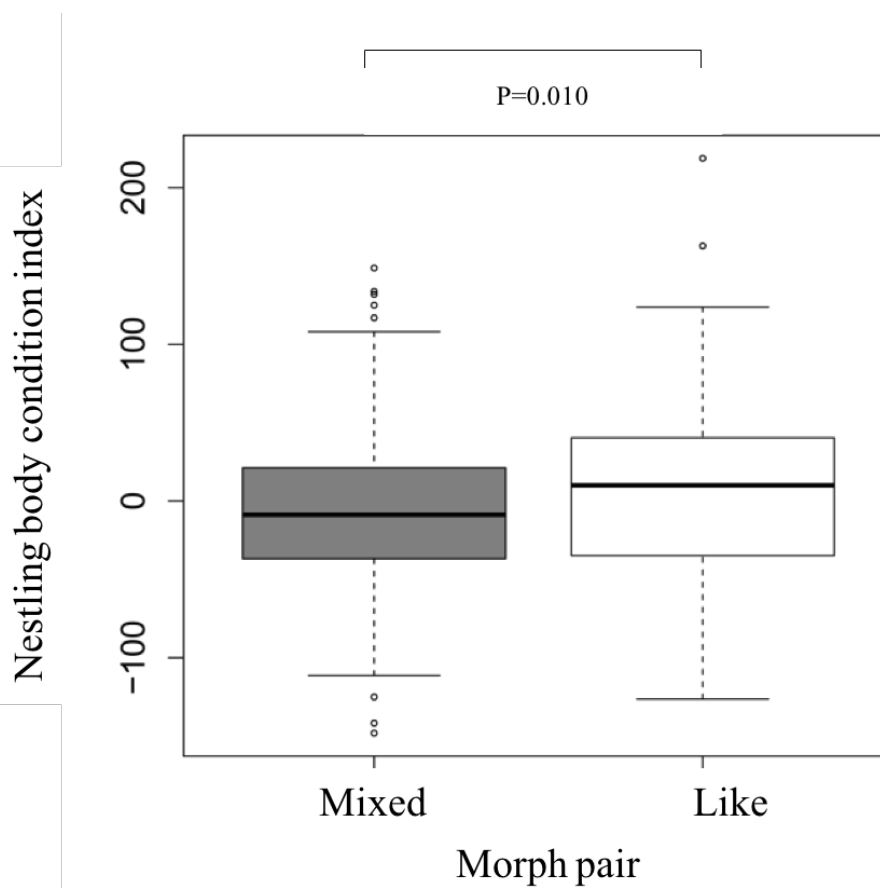


Figure 3. Body condition index (regression of weight [g] and tarsus length [mm] corrected by sex) of nestlings of mixed (dark morph adult mated with a white morph, irrespective of their sex) and like-pairs (where both parents are of either dark or white morphs) on the Cape Peninsula (2001-2014, n=268 chicks). Plotted are raw data (not effect sizes); box and whisker plots showing median, 25th and 75th percentile and outliers.

Splitting pairs into four categories according to the morph of each sex (4 combinations; i.e. $d^{\text{♂}}-d^{\text{♀}}$, $\text{white}^{\text{♂}}*\text{white}^{\text{♀}}$, $w^{\text{♂}}-d^{\text{♀}}$, $w^{\text{♂}}-w^{\text{♀}}$), we found no significant difference in any of the three reproductive parameters (productivity: $\chi^2_3=2.66$, $P=0.44$; reproductive success: $F_1=3.06$, $P=0.38$; brood size: $\chi^2_3=0.80$, $P=0.98$

see Table 1 for details). Despite this lack of significance, the two mixed-pair combinations (i.e. $d^{\text{♂}}-w^{\text{♀}}$, $w^{\text{♂}}-d^{\text{♀}}$) tended to have higher breeding performance (i.e. higher productivity) than the like-pairs (Fig. 3). However, none of the post-hoc contrasts between any of the pair combinations were significant (lsmeans: $\text{white}^{\text{♂}}*\text{white}^{\text{♀}}$ vs $d^{\text{♂}}-d^{\text{♀}}$, $P=0.37$; $d^{\text{♂}}-w^{\text{♀}}$ vs $l^{\text{♂}}-l^{\text{♀}}$, $P=0.88$; $l^{\text{♂}}-d^{\text{♀}}$ vs $d^{\text{♂}}-d^{\text{♀}}$, $P=0.94$; $l^{\text{♂}}-d^{\text{♀}}$ vs $l^{\text{♂}}-l^{\text{♀}}$, $P=0.99$, Fig. 4). For lay month, we found marginally non-significant differences between these four categories, with pairs including a dark morph male tending to lay earlier ($\chi^2_1=7.64$, $P=0.05$, Fig. 4, pairwise comparisons between all morph combinations: lsmeans $0.11 < P < 1.0$).

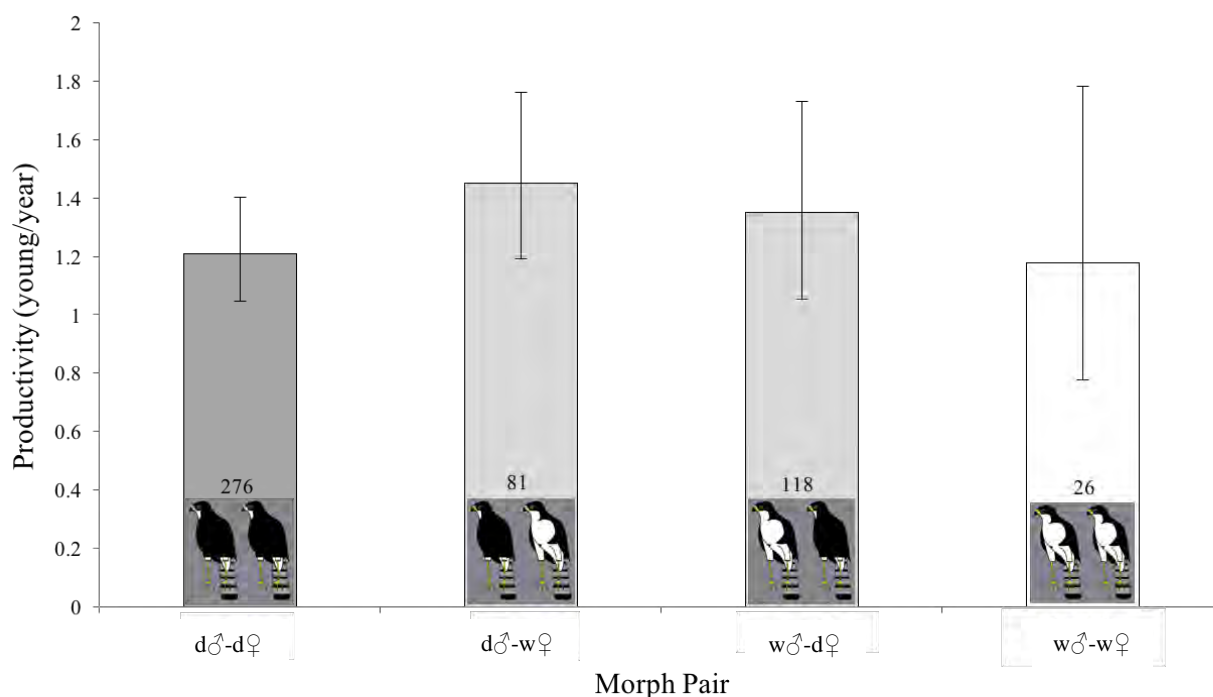


Figure 4. Productivity of the four morph pair combinations of Black Sparrowhawks from active pairs monitored on the Cape Peninsula between 2001 and 2014. Plotted are raw data and not effect sizes; values given as mean \pm SE. Numbers indicate sample size for each group. Averages are the mean modelled estimate produced from a Generalized Linear Mixed Model with year and territory fitted as random terms in the model. No significant differences were found see Table 1 for details on statistical values (see details in text).

For nestlings' body condition we found the opposite pattern, with chicks of d♂-d♀ and w♂-w♀-morph parents having higher body condition than chicks of the other morph combinations (Fig. 4, $\chi^2_1 = 8.28$, $P = 0.04$). Post-hoc comparisons revealed that this result was due to a significant difference between w♂-d♀ vs d♂-d♀ only (lsmeans: $P = 0.04$) whereby nestlings of d♂-d♀ parents had the highest body condition and nestlings of w♂-d♀ parents had the lowest body condition.

Mate Selection

We found no evidence for either assortative or disassortative mating. The number of like and mixed-pairs was similar to that predicted by random patterns of mating, given the frequency of the different male and female morphs in the population ($\chi^2_3 = 3.9$, $P = 0.72$; Fig. 5).

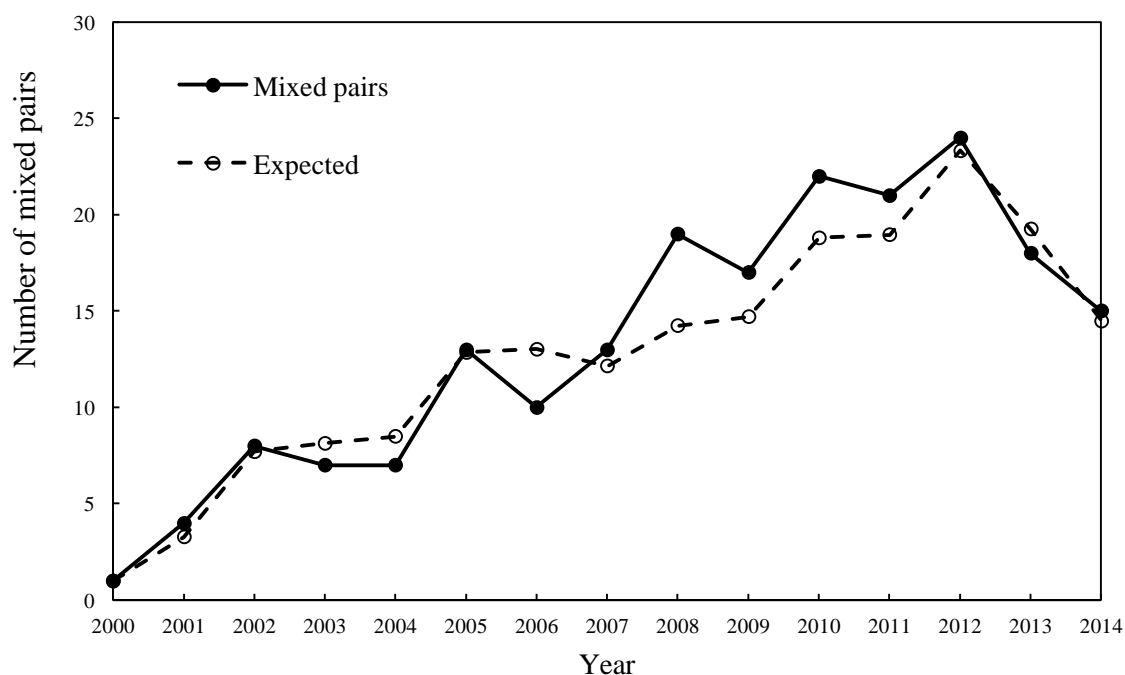


Figure 5. Number of mixed-pairings (dark male/light female or light female/dark male) between 2000 and 2014 observed in the Cape Peninsula population (dark line, solid circles) and expected (dashed lines and open circles) based on random pairing given the frequency of the different morphs (dark and light) using data only from pairs where both morphs had been identified in each year. There was no significant difference between the number of mixed-pairs observed and those expected ($\chi^2_1=3.9$, $P > 0.05$).

Adult Survival and Encounter Probability

Our survival analysis used the encounter histories of 150 known morph black sparrowhawks from 2001 to 2014. This included 73 females (58 dark / 15 white) and 77 males (65 dark / 12 white). We resighted an average of 42.8 ± 7.2 marked individuals per year, most of these records were on breeding territory.

Our models indicated greatest support for adult survival (Φ) dependent on time (i.e. year, Fig. 6), a variable which featured in all of our top eight models ($\Delta AIC_c \leq 4.0$; Table 2). Our analysis revealed that survival differed between years. Of the eight top models, sex featured in four and morph type featured in two. From our analysis we detected no significant difference in survival probability between morphs and sexes. From the model averaged results we found marginally higher survival in white morph females ($\Phi=0.87 \pm 0.05$) and dark morph females ($\Phi=0.86 \pm 0.06$), compared to white morph males ($\Phi=0.86 \pm 0.05$) and dark morph males ($\Phi=0.85 \pm 0.05$).

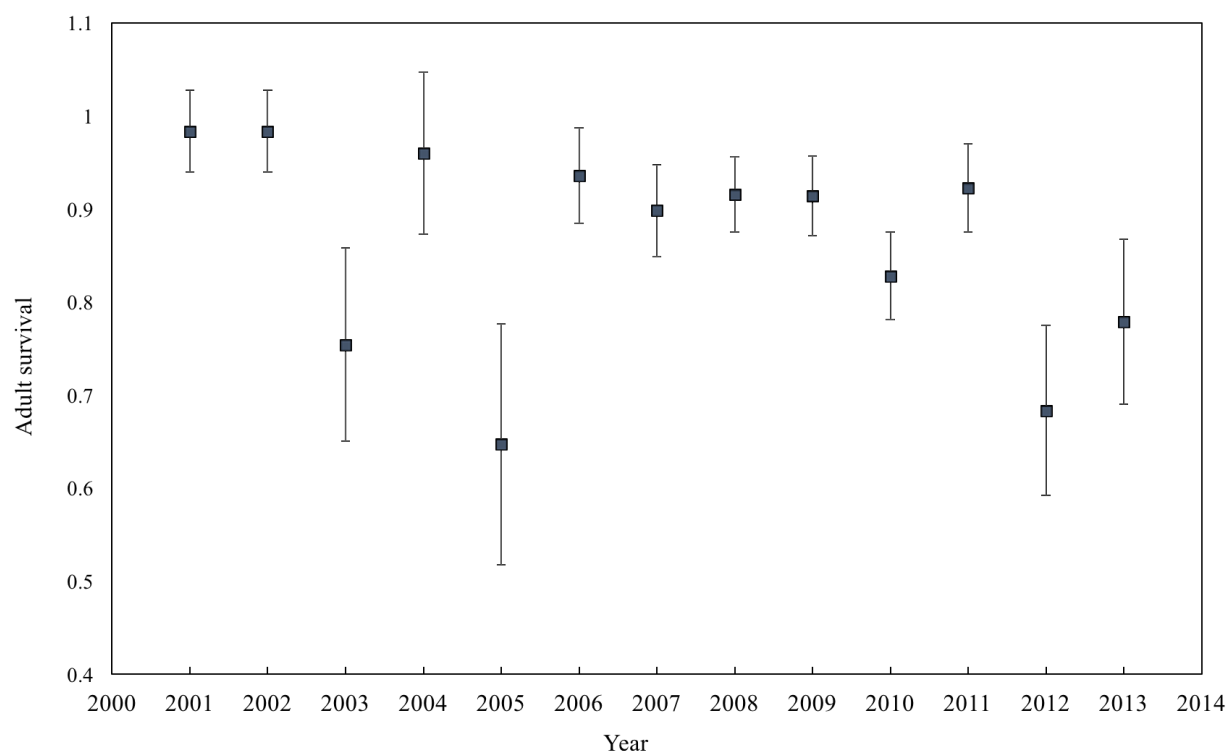


Figure 6. Model averaged estimates of Black Sparrowhawk survival on the Cape Peninsula over the 15 year course of our study (2001-2015). Our models indicated greatest support for adult survival (Φ) dependent on time (i.e. year), a variable which featured in all of our top eight models ($\Delta AIC_c < 4.0$; Table 1). We detected that survival in our study population differed between years.

Adult survival in this study are similar to Martin *et al.*'s 2014a previous survival estimates in the species, of around 0.89. The post-hoc test confirmed there was no significant difference in survival between dark and white morphs ($\chi^2_1=0.01$, $P=0.99$), between sexes ($\chi^2_1=0.09$, $P=0.87$), between light and dark males ($\chi^2_1=0.073$, $P=0.88$) or between light and dark females ($\chi^2_1=0.060$, $P=0.89$).

We found that resighting probability (ρ) was influenced by morph and sex, with both variables featuring in all top models. From the model averaged results, resighting probability was significantly different between morphs ($\chi^2_3=25.2$, $P=0.04$) with highest resighting probability in white morph females ($\rho=0.90\pm 0.03$) followed by white morph males ($\rho=0.84\pm 0.05$), dark morph females ($\rho=0.80\pm 0.03$) and lastly dark morph males ($\rho=0.69\pm 0.03$). Post-hoc contrasts revealed that resighting probability was significantly higher in light compared to dark morphs ($\chi^2_1=25.2$, $P=0.02$), higher in females compared to males ($\chi^2_1=25.2$, $P=0.03$) and higher in white morph males compared to dark morph males ($\chi^2_1=6.62$,

$P=0.01$). Resighting probability was however not significant between light and dark morph females ($\chi^2_1=2.94$, $P=0.08$).

DISCUSSION

Our results suggest that neither morph had a specific advantage in terms of reproductive performance or survival in our study population of black sparrowhawks. There does however appear to be an advantage of mating with the opposite morph, with mixed-pairs producing almost a quarter more offspring per year than pairs consisting of partners of the same morph. The body condition of the offspring showed the opposite relationship, with nestlings of mixed-pairs having lower body condition than nestlings of like-pairs. Despite this higher productivity for mixed-pairs, over the 15-year study period, we found no evidence for disassortative mating, with pairing in respect of morph appearing completely random.

Although we found earlier breeding in dark morph males, and earlier breeding is associated with higher productivity and survival in this population (Martin *et al.* 2014a), an individual's morph alone did not have an obvious influence on any of our measures of reproductive performance. Instead our results provide evidence that productivity in this population is driven by the interaction of the two morphs in a pair. This appears to be an example of an emergent pair level property in the species, which could be linked to the complementarity of a pair, either with respect to their genotypes or phenotypes in the novel colonised environment of the Cape Peninsula.

Morph Pair Breeding Performance - the Advantage of Mixed-morph Pairs in a Novel Environment

Heterozygote advantage, whereby individuals that are heterozygous for an allele encoding a trait have a fitness advantage compared with homozygotes (Gray & McKinnon 2007), is one of the simplest and well developed mechanisms proposed to explain the maintenance of colour polymorphism (Krüger & Lindstrom 2001, Briggs *et al.* 2011b, Boerner *et al.* 2013). Such a mechanism could be operating within our population, with heterozygote eggs and chicks being more viable (higher genetic variability, i.e.

individual quality; see heterozygosity–fitness correlations for example in David 1998; Chapman *et al.* 2009) and therefore having higher nestling survival rates (Foerster *et al.* 2003). This may explain why mixed-pairs have higher productivity than like-pairs, since they will produce more heterozygote offspring than dark*dark pairs or white*white pair combinations (Table 3).

Table 3: Table of all possible first generation offspring produced by mixed and like-pair Black Sparrowhawks breeding on the Cape Peninsula and the probability of the offspring being heterozygous. Probability of pairings were calculated using information from Amar *et al.* (2013); whereby 76 % of the population are homozygous dark morphs. 24 % are white morphs; the vast majority (92%) are heterozygous (Ld) while only 8% are homozygous (LL).

Morph pairing	Pairing	Parent alleles	Possible alleles	Probability of pairing	*Pr offspring heterozygous	Overall % offspring heterozygous
Dark-dark	Like	dd – dd	dd-dd	1	0	0
Dark-white	Mixed	dd - LL, Ld	dd-LL	0.08	0.08	54
			dd-Ld	0.9	0.46	
			LL-LL	0.006	0	
White - white	Like	LL, Ld - LL, Ld	LL-Ld	0.07	0.03	50
			Ld-Ld	0.85	0.42	
			Ld-LL	0.07	0.03	
White -dark	Mixed	LL, Ld – dd	LL-dd Ld-dd	0.08 0.9	0.08 0.46	54

*The probability of offspring being heterozygous is based on the product of the probability of their parental morphs pairing and the probability of their genotype occurring.

Within our population, however, 92% of white morphs are estimated to be heterozygotes (Amar *et al.* 2013), and thus we expect like-light pairs to produce only slightly less heterozygotes than mixed-pairs, with like-dark pairs producing the least (Table 3). Additionally, if heterozygote advantage influenced fitness we might expect to see lower productivity or survival in dark morphs, since dark individuals will be exclusively homozygous, which we did not observe. Thus, although we cannot rule this mechanism out entirely, it does seem unlikely that this is the explanation for the higher productivity observed for mixed-morph pairs.

An alternative explanation for the higher productivity of mixed-morph pairs may be that the two different morphs complement each other behaviourally, rather than genetically (i.e. heterozygote advantage). This may improve conditions experienced by the offspring of mixed-pairs. Thus, offspring may gain fitness benefits via the ecological complementarity of their parents which is driven by their different morphs. Similar suggestions have been made based on personality in birds (Dingemanse *et al.*

2004, Boerner & Kruger 2008) or sexual size dimorphism (Selander 1966, Newton 1979, Nodland 2013). Recent research on this population provides a potential mechanism for such complementarity: Tate *et al.* (2016) found that the two morphs have differential foraging success under differing light conditions, with white morphs foraging more successfully in brighter conditions and darker morphs foraging more successfully in low light conditions. Thus, it may be that within our population, mixed-morph pairs are able to exploit a wider range of resources than like-pairs, expanding their foraging niche when conditions vary in terms of brightness. Mixed-pairs therefore may have improved access to prey (i.e. more consistently), with the dark morph parent having higher foraging success during darker conditions (i.e. early or late in the day, or during cloudier skies, or in closed, denser habitats) and white morph birds being more successful in brighter conditions (i.e. middle of the day, during sunny days, or in more open habitat; see also (Preston 1980, Rohwer 1990, Théry 2006). Therefore, this may provide a selective fitness advantage for breeding in a mixed-pair, with the different morphs maintained in the same population through this benefit (Kristensen *et al.* 2014). Our findings are consistent with Sumasgutner *et al.* (2016), who found that survival of offspring from mixed-morph black sparrowhawk pairs was higher than those from like-pairs, which they also attributed to improved conditions experienced early in life due to the benefits of care provided by mixed-morph parents. We also found that body condition of nestlings was lower for mixed-pairs, which is in line with the offspring size-number trade-off (Gyllenberg *et al.* 2011). Thus mixed-morph and like-morph parents appear to deal differently with the trade-off number of offspring and offspring quality. It is not surprising that chicks in larger broods have lower body condition than chicks in smaller broods. However, within our population higher body condition at the time of chick-ringing does not translate into subsequent higher survival or recruitment rates (Sumasgutner *et al.* 2016); hence no long-term advantage is yet apparent. Interestingly, the higher body condition was found in chicks of d♂-d♀ parents and the lowest in w♂-d♀ parents, showing that dark morph fathers produce chicks with higher body condition than white morph fathers, and not the mother morph, underlining again the importance of the father morph.

Timing of Breeding

We did not find any significant differences in the timing of breeding between mixed and like-pairs, suggesting that earlier breeding is unlikely to explain the higher productivity recorded for mixed-pairs. However, at the individual level, we did find differences in lay months between male morphs, but not for females. Dark morph males bred significantly earlier in the season compared to white morph males, which might also be one explanation for the higher body condition of chicks raised by dark morph fathers. Controlling for these differences in lay months within the breeding performance models, morph remained non-significant for all three reproductive parameters measured. The differential foraging success between morphs in relation to light levels (Tate *et al.* 2016) might provide an explanation for the earlier breeding of dark morphs males. The earlier breeding period coincides with higher rainfall on the Peninsula (Martin *et al.* 2014a), and therefore a time when foraging birds would experience a cloudier, low light environment, which would favour foraging of dark morphs through improved background matching and crypsis (Rohwer 1990). In this regard it might be advantageous for dark morph males to breed under environmental conditions which favour their hunting strategy and/or success. Early breeding by dark morphs males may be advanced due to selection for earlier breeders, if timing of breeding is heritable and not sex linked, or it might be that females of dark morph males are receiving more food during the courtship feeding period due to their mates improved foraging success (Redpath *et al.* 2001). Hence, the female of a dark morph male would reach the necessary breeding condition earlier than the female of white morph male, independent of her own plumage colour. This hypothesis is supported by the findings of Sumasgutner *et al.* (2016), which showed that recruitment was higher for chicks that were raised by a dark morph male which bred early in the season, but that this relationship switched around later on in the season, when chicks from white morph fathers had higher recruitment probabilities. These relationships appeared to fit with the changes in local weather conditions that occur across the breeding season for our population.

Mate Choice

Mate choice has been proposed to play an important role in the promotion and maintenance of polymorphism (Both *et al.* 2005, Bortolotti *et al.* 2008). Higher productivity between similar phenotypes in polymorphic species is predicted to lead to selection for individuals that show assortative

mate choice (Gray & McKinnon 2007), whereas higher productivity between dissimilar phenotypes should lead to disassortative mating, potentially also indicating selection for outbred offspring (Tuttle 2003, Galeotti & Rubolini 2004). Given that productivity was higher for mixed-pairs in black sparrowhawks, we may have predicted to see evidence of disassortative mating. However, we found no supporting evidence, with mate choice apparently occurring at random. Maladaptive mate choice, where morphs choose to breed with less fit partners, has been recorded previously in common buzzards (Krüger *et al.* 2001). In Krüger *et al.*'s (2001) study, individuals chose mates matching their mother's morph, probably driven through imprinting on the maternal morph during the nestling period, and thus did not mate with morphs which would optimise productivity. It would therefore be interesting to see whether such mate choice is occurring in our study system (i.e. offspring mating with their maternal morph), and as our young colour ringed birds are recruited back into the population, it should be possible to undertake such an analysis in the near future. It is also important to note that in some species, mate-pairing may be influenced by the distribution of morphs across a species range i.e. non-random pairing can occur because of the non-random distribution of morphs across habitats (Gangoso *et al.* 2015). This does not, however, appear to be influencing the mate selection or morph pairing in our study population.

In the black sparrowhawk, inheritance of morph type appears to follow a simple Mendelian one-locus, two-allele system, whereby the light allele is dominant (Amar *et al.* 2013). Light birds are therefore homozygous or heterozygous (LL or Ld) and dark birds are always homozygous (dd). Therefore, the higher productivity from mixed-pairs will mean that there are more offspring from mixed-pairs than like-pairs. This result by itself could lead to polymorphism being maintained, as these morphs will give rise to the full range of offspring morphs, unlike like-pairs which will more often produce young of the same morph, in particular, homozygous dark morph pairs (Table 3). This combined with the higher recruitment of offspring from mixed-morph pairs (Sumasgutner *et al.* 2016), could therefore be the mechanism for balanced polymorphism in this species.

In the barn owl, Dreiss & Roulin (2014) found that incompatible partners (i.e. after a poor reproductive season) divorce and find new mates to restore reproductive success. Individuals appear to select compatible mates rather than a partner of higher quality, and by breeding more often together, faithful pairs improve coordination and subsequently obtain higher reproductive success after a divorce. In our study population of black sparrowhawks, although Martin *et al.* (2014b) did not look at divorce with respect to morph type, they did find improved breeding success in pairs that divorced following previous breeding failure. Therefore an alternative hypothesis for our findings is that mixed pairs are more compatible and thus more faithful, and improve coordination along the years, which is positively related to their reproductive success. Exploring divorce rates in relation to morph type in our population could therefore be especially revealing, and should be the focus of future research.

Morph Survival

When examining survival, we found no significant differences between the morphs of adult black sparrowhawks, with the average survival rates in our population similar to previous survival estimates in the species (i.e. 89%; Martin *et al.* 2014a). Given the numerical dominance of dark morphs in the population (Amar *et al.* 2013) we predicted higher survival in dark morphs, as the most common phenotype (and thus alleles) in a population is expected to better adapted to the local environment (Antoniazza *et al.* 2010, Briggs *et al.* 2011b). The underlying allele should therefore be under selection. However, adult survival does not appear to influence fitness at a significant level and thus is unlikely to be the main factor promoting the high proportion of dark morphs in our study population.

Briggs *et al.* (2011a) found similar weak indications of differential survival between their three morph classes in Swainson's hawks. They proposed that survival was not sufficient to drive fitness differences in their study population. However, they could not rule out the possibility that morphs are adapted to alternative environments as found in barn owls (*Tyto alba*; Antoniazza *et al.* 2010, Dreiss *et al.* 2012) and tawny owls (*Strix aluco*; Karell *et al.* 2011). Survival rates were higher for paler grey tawny owls than brown morphs during colder, snow-rich years, which appeared to drive the dominance of grey morphs over brown morphs. However, as winters became milder in recent years, survival differences

between the morphs became insignificant, and selection against the brown morph diminished. This suggests that alternating weather conditions, between years or over longer time periods, may favour certain black sparrowhawk morphs and potentially influence their survival on the Cape Peninsula. For example, Amar *et al.* (2014) found that the spatial pattern of dark morph frequencies across South Africa correlated strongly with rainfall. Therefore, in our study population, differences in environmental conditions, such as rainfall, could cause variation in selective pressure between the morphs over time, which could result in a lack of differences in fitness components across morph categories (see also Briggs *et al.* 2011a). This could potentially lower selection for an individual morph and thus allow them to co-exist.

Conclusion

Our study presents a novel case in a polymorphic raptor, whereby an emergent pair-level fitness benefit is accrued when two contrasting parent morphs breed together. In the black sparrowhawk, mixed-morph pairs appear to complement each other synergistically throughout the breeding period. The improved productivity in mixed-pairs is unlikely to be due to their offspring's genotype, as proposed by the heterozygote advantage hypothesis (Krüger *et al.* 2001, Briggs *et al.* 2011b, Jonker *et al.* 2014), but rather the result of improved conditions experienced on the nest. This appears to be influenced by the complementary nature of the parental care provided by their different morphs. In this recently colonised environment of the Cape Peninsula variations in ecological and behavioural traits of the different morphs may interact in a novel way to increase offspring fitness. In our system, we propose that differential foraging success between black sparrowhawk morphs under varying light conditions allows mixed-pairs to expand their foraging niche. This may be one of the mechanisms allowing them to accrue a fitness advantage via improved productivity. Importantly, this may provide an overarching explanation for the maintenance of colour polymorphism within our study species, and consequently, may apply in other polymorphic systems, particularly in species that rely on bi-parental care.

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CHAPTER 6

SYNTHESIS AND CONCLUSIONS



A defensive Black Sparrowhawk nestling, Cape Town, April 2015. Note how well lined the nest is with plant greenery. Black Sparrowhawks are known to line their nests with fresh fragrant leaves such as Pine, Camphor and Eucalyptus leaves, which have high concentrations of strong smelling phenolic lipids. It has been suggested that the fresh green plant material functions to repel insect pests and ectoparasites. Photo: Mark Cowen.

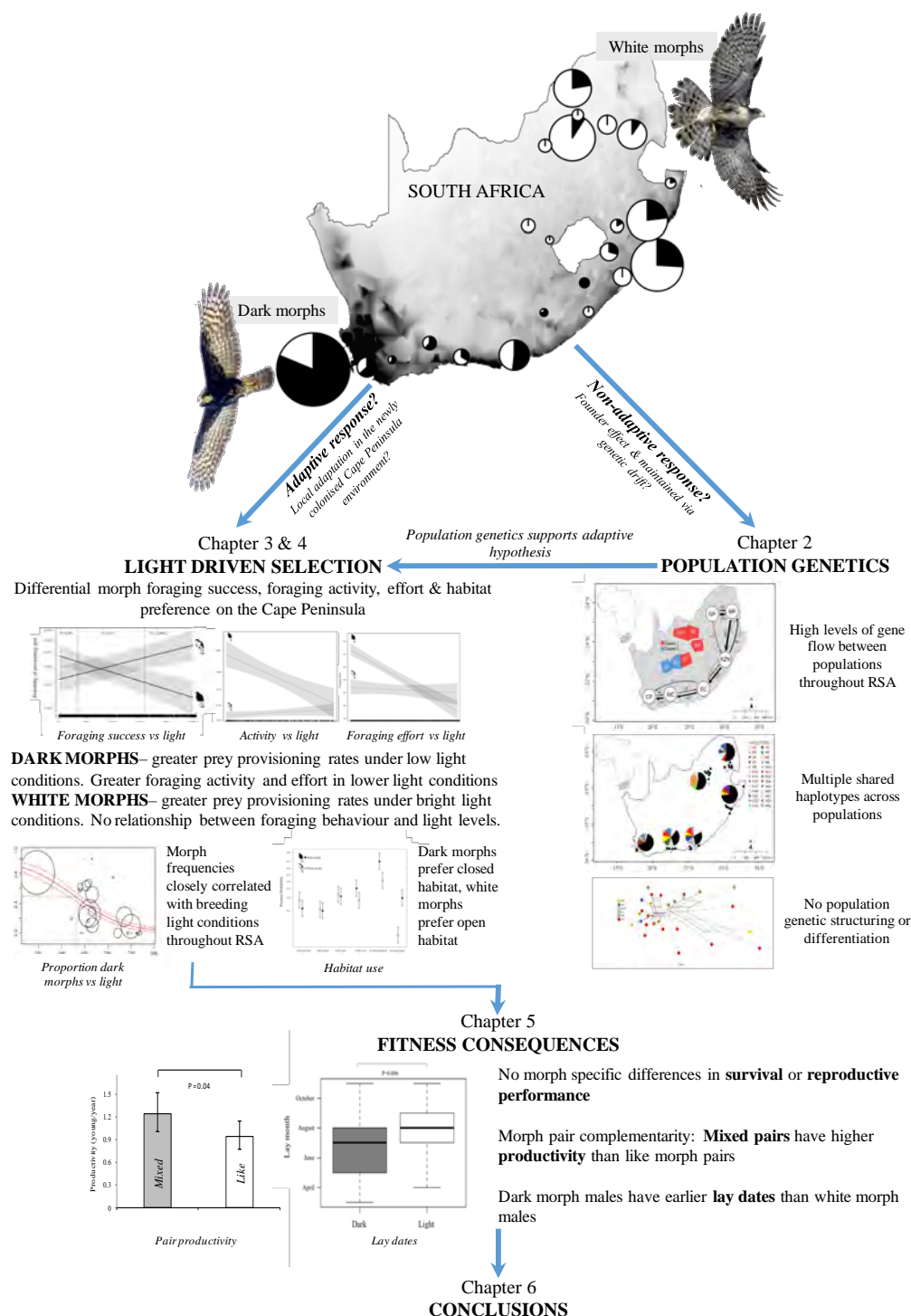
DISCUSSION

This study explores how polymorphism may be maintained within a species, specifically investigating why the proportions of different morphs may vary across a species' distribution. Focusing on a unique, newly colonised population of black sparrowhawks breeding on the Cape Peninsula, South Africa, I tested various explanations for the unusually high proportion of rare dark morph birds found here. I developed two overarching hypotheses: (1) that the high frequency of dark morphs may be evolutionarily neutral and simply due to a founder effect and maintained via genetic drift, and (2) that local selection favours the dark form in this newly colonised population, thereby providing dark morphs with an adaptive advantage under the novel local environmental conditions of the Western Cape.

Summary of Key Findings

The following infographic represents a summary of the overall structure and key findings of this study. DNA sequence data from the mitochondrial control region was used to examine the extent to which populations of black sparrowhawks are genetically structured throughout their distribution in South Africa and assess the levels of recent gene flow between the Cape Peninsula and surrounding populations (Chapter 2). No significant genetic structuring or spatial differentiation was found among populations of black sparrowhawks throughout South Africa. Rather, the data analysis supported high levels of gene flow between populations with multiple shared haplotypes throughout South Africa. Although most of the gene flow occurred between neighbouring populations, there is also clear evidence for long distance gene flow, albeit at low levels, across some of the more geographically distant populations. This is not unexpected in species such as birds, particularly raptors, that generally display a higher dispersal potential (Ward *et al.*, 1994; Crochet, 2000; Hull *et al.*, 2008).

The next component of my research was to investigate the possible adaptive functions that plumage polymorphism may serve in the black sparrowhawk. Chapters 3 and 4 detail the different approaches I



- High levels of gene flow supports the hypothesis that local adaptation is likely the most important ongoing driver of morph frequencies on the Cape Peninsula.
- Adaptive response = Light driven selection: Under disruptive selection, improved crypsis (via background matching) to prey in variable light conditions enhances morph foraging success.
- Dark morphs forage most actively with more effort under the light conditions which provide them with improved crypsis and optimal foraging success. White morphs show no significant foraging behaviour in response to light levels.
- Low breeding season light conditions favour the dark morph on the Cape Peninsula by providing them with a foraging advantage.
- Proportion of dark morphs closely correlated with breeding season light across RSA: more dark morphs in low light regions.
- Emergent pair level property: Differential morph foraging success and habitat preference allows mixed pairs to expand foraging niche and improve reproductive performance & may promote morph coexistence on the Cape Peninsula.

Infographic summarizing the investigation of plumage polymorphism in the Black Sparrowhawk, showing primary findings from each chapter, how they inform each chapter and the general conclusions of the study. 141

used to test how local environmental conditions may be operating to maintain the high proportion of dark morphs on the newly colonised Peninsula. Central to the hypothesis developed in the study was an important review paper by Galeotti *et al.* (2003) suggesting that colour polymorphism in birds most likely evolved under selective pressures linked to individual detectability in variable light conditions. The authors also argue that polymorphism across a range of species is most likely maintained by strong disruptive selection. In Chapter 3 I investigated whether variation in detectability of the two morphs under different light levels could be a selective agent involved in the maintenance of polymorphism in the Cape Peninsula's black sparrowhawk population. Foraging success was used as an estimate of local fitness and determined by measuring prey delivery rate to nests of the different morphs under various ambient light conditions. The hypothesis tested was that ambient light conditions, be it within different habitats, weather conditions or times of day, would offer the two morphs different levels of crypsis to their prey via background matching (as proposed by Galeotti *et al.*, 2003). The results revealed that local ambient light conditions strongly influence prey provisioning rates of the different coloured morphs; dark morphs deliver more prey during low light conditions, while the opposite relationship characterises white morphs, which appear to have a foraging advantage in brighter conditions.

Analyzing these findings more closely, I used tracking data in Chapter 4 to compare two measures of foraging behavior (foraging activity, foraging effort) and assessed habitat selection between the two morphs in my study population. The results provide additional compelling support that foraging by the two morphs is influenced by different light environments; dark morphs display both greater foraging activity and effort in lower light conditions and a stronger degree of selection for closed habitats with denser vegetation cover. In contrast, white morphs show no relationship with light levels and a weaker degree of selection for closed habitat types as compared with dark morphs.

To complement the findings of Chapter 3 and 4, I also investigated the fitness consequences (breeding or survival) of differential foraging success, foraging behaviour and habitat preference on the study population. Although dark morphs have numerically dominated the Cape Peninsula population for over a decade (Amar *et al.*, 2013), information on the overall fitness and success of the two morphs may

contribute to a more nuanced understanding of how both morphs persist within the study population. In Chapter 5 I therefore examined whether morph coexistence could stem from a degree of balancing selection between key demographic parameters, i.e. survival and reproduction (Stearns, 1992; Briggs *et al.*, 2011b).

Analysis of long-term adult survival for the two morphs collected over a 15-year period, as well as reproductive performance both in isolation and in combination with their breeding partner morph (i.e. mixed or like-pairs, and the sex-morph specific combinations) indicates that neither morph has had a specific advantage in terms of survival or breeding in the study population. There does, however, appear to be some advantage of mating with the opposite morph type; mixed-pairs produce almost a quarter more offspring per year than pairs consisting of partners of the same morph. And yet despite this higher productivity for mixed-pairs there is no evidence for disassortative mating, with pairing in respect of morph apparently random. The body condition of the offspring shows the opposite relationship, with nestlings of mixed-pairs having lower body condition than nestlings of like-pairs. Although I found earlier breeding in dark morph males, and earlier breeding is associated with higher productivity and survival in this population (Martin *et al.*, 2014), an individual's morph alone does not have an obvious influence on any of the measures of reproductive performance used in this study. Instead the results indicate that productivity in this population is strongly driven by an interaction between the morphs in a pair.

Assessing Gene Flow and Population Genetic Structure

The persistence of pronounced differentiation in morph frequencies in the Cape Peninsula population of black sparrowhawks, in the presence of substantial gene flow, provides important evidence that variation in morph frequencies is unlikely to be due to neutral evolutionary processes. These results provide valuable support for a working hypothesis that selection, and thus local adaptation, is the primary force maintaining the unusually high frequency of dark morphs in this population (Amar *et al.*, 2014).

In their study on the common barn owl, Antoniazza *et al.*, (2010) showed that substantial differentiation in morph colour can be maintained among populations in the presence of extensive gene flow. The barn owl varies continuously across their European range, from white in southwestern Europe to reddish-brown in northeastern Europe. Antoniazza *et al.*, (2010) found strong evidence for high levels of gene flow between populations, supporting the hypothesis that local adaptation is the most likely process maintaining color variation across European populations. Subsequent work on the species demonstrated that substantial background matching to the local habitat, particularly in females, provides morphs with a fitness benefit and appears to be a key mechanism maintaining colour variation in the barn owl (Dreiss *et al.*, 2012); whitish females produced more fledglings when breeding in wooded areas, whereas reddish females produced more fledglings when breeding in sites with more arable fields (Dreiss *et al.*, 2012). Chicks were also found to develop faster when their mothers bred in habitats that best matched their own coloration (Dreiss *et al.*, 2012). Similarly, habitat background is also implicated in explaining geographic variation in colour in the isopod *Idotea baltica*, where locally varying selection for cryptic colouration, in the presence of strong gene flow, contributes to the maintenance of colour polymorphism in the species (Merilaita, 2001).

Although not strictly related to polymorphism, Odendaal *et al.*, (2014) further demonstrate how adaptive traits can diverge in the face of strong gene flow and discuss how selection and gene flow interact to influence the rate and extent of adaptive trait evolution. In the Cape horseshoe bat *Rhinolophus capensis*, the authors found that significant sensory trait (i.e. echolocation frequency) differentiation occurred between populations in South Africa despite significant levels of historical gene flow. Interestingly, and much like the black sparrowhawk (Tate *et al.*, 2016) and the barn owl (Antoniazza *et al.*, 2010; Dreiss *et al.*, 2012), Cape horseshoe bats occur across extremely heterogeneous environments, experiencing locally variable ecological regimes. Odendaal *et al.*, (2014) propose that differences in habitat structure between biomes (from cluttered to open vegetation) drive divergence in adaptive echolocation frequency in their study species, arguing that selection for increased detection distance in relatively less cluttered habitats may have influenced the evolution of matched echolocation frequencies and habitats across different environments.

The Adaptive Value of Plumage Colouration

Data from this study provides convincing evidence that ambient light conditions interact with morph colour to improve foraging success in the black sparrowhawk, such that together the two morphs may be substantially better adapted to foraging under different light conditions. On the Cape Peninsula ambient light is better at explaining delivery rates than any other closely correlated weather variable (i.e. temperature, rainfall levels or time of day) and these findings are consistent with our hypothesis that the two colour morphs are maintained by a degree of disruptive selection.

Differential morph foraging success across the light spectrum is also reflected in the recent findings of Sumasgutner *et al.* (2016). Their study on the Cape Peninsula black sparrowhawks found an interaction between the timing of breeding and male morph in relation to the recruitment of offspring, whereby successful recruitment of offspring from dark morph fathers is more likely when fledging earlier in the season, at a time when ambient light conditions are particularly low. The opposite relationship was found for white morph fathers, with their offspring more likely to be recruited if fledged later in the breeding season when light levels increase on the Peninsula (also see Linder *et al.*, 2010). These findings can also be explained by more successful foraging by dark morph males during the early breeding period and thus the young of these dark morph fathers experience better care during the early part of the season. This in turn translates into better quality offspring who have a greater chance of recruiting into the population. Likewise, offspring from white morph fathers may receive better care during the latter part of the breeding season when light levels are higher and white morph male foraging is favoured.

Due to the high levels of rainfall in the region during their winter breeding season on the Cape Peninsula, black sparrowhawks experience particularly prolonged periods of low light conditions (Linder *et al.*, 2010; Martin *et al.*, 2014). The findings presented here provide important insight into how local light conditions may influence the higher than expected numbers of dark morphs via a significant fitness (foraging) advantage. This advantage is also reflected in the distribution of morphs across the highly seasonal landscape of South Africa (Rutherford *et al.*, 2006), where differential

hunting success during the breeding period may promote the maintenance of two colour morphs in varying proportions (Tate *et al.*, 2016). The data presented here strongly support a scenario where the two morphs are favoured due to the degree of crypsis afforded to hunting individuals under variable light conditions. This study provides the first empirical evidence supporting the hypothesis that polymorphism in a species, and the spatial-structuring of morphs across its distribution, may be driven by differential selective advantages via improved crypsis under varying light conditions, as proposed by Galeotti *et al.*, (2003).

Using data from GPS-tagged male black sparrowhawks Chapter 4 expanded on the hypothesis developed in Chapter 3 and investigated whether foraging behaviour differed between male morphs depending on ambient light conditions. The data provide conclusive evidence that provisioning rates in the two morphs are indeed influenced by light levels; dark morphs forage more intensively during lower light conditions (dawn/dusk or cloudy days), when they would be expected to be most successful, while white morph birds show the opposite relationship (Tate *et al.*, 2016). The role of habitat selection by the two morphs was also tested and found to vary significantly between the morphs; dark morphs show a greater preference for more enclosed habitats (which likely represent darker habitats) than white morph birds, which show a greater preference for more open habitats.

My results supported my predictions in Chapter 4, at least for dark morphs, and strongly suggested that dark morphs forage during conditions, and within habitats (related to the light conditions in a specific habitat), that are likely to enhance their crypsis from their avian prey, and thus are investing time into foraging activities during the conditions which will maximize their foraging success, as would be expected under the optimal foraging theory (Charnov, 1976; Stephens & Krebs, 1986). These results, however, do not necessarily support the conclusions of Chapter 3 for dark morphs; for dark morphs, both foraging effort and provision rates were influenced in a similar manner by light conditions, with both measures increasing by around 50% over the light spectrum (Chapter 3 & 4). This result is therefore suggestive that prey capture rate for dark morphs does not vary in relation to light levels as was previously suspected, but rather that it is proportional to the amount of time spent hunting. For

white morphs, no obvious pattern in terms of foraging effort or activity was found in relation to light levels – this lack of relationship is particularly interesting given that in Chapter 3, I had found an increase in provisioning rate with light levels for this morph likely. The most likely explanation for this is that the white morph has higher capture rates during brighter conditions, presumably due to their improved crypsis. Thus, from that result I conclude that white morphs are more successful at foraging in bright conditions. These findings provide important supporting evidence that predator-prey interactions may be major component in the maintenance of polymorphisms in natural populations. Visual detection by prey is therefore likely to be a key selective agent for colouration in polymorphic predatory hawks such as the black sparrowhawk.

This explicit test of differential morph foraging success and behaviour at varying levels of light, as well as morph specific habitat preference, is further supported by a number of more anecdotal studies. For example, Rohwer (1990) found that on one island occupied by the Pacific reef heron *Egretta sacra*, dark morphs forage almost exclusively on shaded streams under thick forest canopy, whereas white morphs frequent open shallow water. Rohwer (1990) suggested this was most likely because dark morphs would be more cryptic to their aquatic prey against a dark background whereas white morphs would be less conspicuous against a bright background. Similarly, Preston (1980) observed that light morph red-tailed hawks *Buteo jamaicensis* occupy more open perches when hunting, whereas dark morphs were found to use perches characterized by dense cover. Both of these studies suggest an adaptive morph–habitat association, with morphs selecting perches, or foraging sites, that best conceal them from their prey. Previous research on the tawny owl *Strix aluco* may also provide some insight as to how colour morphs are maintained within a population depending on the prevailing climate and habitat background and shows how certain environmental conditions may favour a particular morph; in their study, Karell *et al.*, (2011) demonstrated that during colder, snow-rich years, conditions appeared to favour pale morph tawny owls, which dominated over dark morphs in their study population. As winters grew milder in recent years, however, selection against the dark morph diminished, and the frequency of dark morphs were found to increase rapidly. Although not fully understood, this may be due to improved crypsis in morphs against the background, which like the black sparrowhawk, may

improve their foraging success and fitness. Pale morphs may be more concealed from their prey against the white snow, whereas dark morphs would be more cryptic against more exposed vegetation.

Local Fitness Consequences for Black Sparrowhawk Morphs

From Chapter 3, it appeared that light levels should favour dark morph foraging for longer periods of the year. Thus, I might expect this to translate into improved survival or breeding performance for dark morphs. This might also explain why dark morphs predominate in this population. I found no such evidence for either higher survival or breeding performance in relation to morph. Chapter 5 did however reveal a number of exciting results. Firstly, dark morphs males laid earlier than white morphs. This finding might be predicted given the findings from Sumasgutner *et al.*, (2016); that recruitment was higher for offspring of dark morphs produced earlier in the season. Secondly, body condition of nestlings was found to be lower for mixed-pairs. However, within the study population higher body condition at the time of chick-ringing does not translate into subsequent higher survival or recruitment rates (Sumasgutner *et al.*, 2016); hence no long-term advantage is yet apparent. Interestingly, the higher body condition was found in chicks of d♂-d♀ parents and the lowest in w♂-d♀ parents, showing that dark morph fathers produce chicks with higher body condition than white morph fathers, and not the mother morph. Highlighting again the importance of the father morph. Lastly, pairs consisting of mixed morphs had higher productivity than like-morph pairs. This higher productivity in mixed morph pairs is unlikely to be related to their offspring's genotype, as proposed by the heterozygote advantage hypothesis (Krüger *et al.*, 2001; Briggs *et al.*, 2011a; Jonker *et al.*, 2014), but rather the result of improved conditions experienced by the young on the nest. This appears to be driven by the synergistic, complementary nature of the parental care provided by the different morphs and is therefore an example of an emergent pair-level property in the black sparrowhawk. Similar suggestions have been made based on personality (i.e. aggression) in birds (Dingemanse *et al.*, 2004; Boerner & Kruger, 2008) or sexual size dimorphism (Selander, 1966; Reynolds, 1972; Newton, 1979; Nodeland, 2013).

In this recently colonised environment of the Cape Peninsula, variations in ecological and behavioural traits of the different morphs may interact in a novel way to increase offspring fitness. I propose that

differential foraging success and behaviour between black sparrowhawk morphs under varying light conditions (Chapter 3 & 4), as well as variable morph habitat preference (Chapter 4), allows mixed-pairs to expand their foraging niche; therefore, mixed-pairs are able to exploit a wider range of habitat (open and closed) as well as a broader window of light conditions (cloudy vs. non-cloudy or dawn & dusk vs. midday) in which to hunt. This may be one of the mechanisms allowing them to accrue a fitness advantage via improved productivity. Recent work done by Sumasgutner *et al.* (2016) on the same study population, further demonstrates the advantage of breeding in mixed pairs, with young produced by pairs of contrasting morphs displaying higher survival rates than young fledged from like-pairs. Importantly, this may provide an overarching explanation for the maintenance of colour polymorphism within my study species, and consequently, may equally apply in other polymorphic systems, specifically for species that rely on bi-parental care.

SHEDDING LIGHT ON COLOUR POLYMORPHISM

There exists a healthy debate amongst evolutionary biologists interested in the ecological basis of colour polymorphism and as to how natural selection shapes avian colouration (Bortolotti, 2006). This debate is fuelled by the strikingly diverse forms that plumage polymorphism takes in many species of bird, together with its uneven phylogenetic distribution among different avian groups (Owens, 2006). Among raptorial species, where there is a particularly high prevalence of colour polymorphism (Galeotti *et al.*, 2003; Galeotti & Rubolini, 2004), balancing apostatic selection, along with the “Avoidance Image Hypothesis” (Chapter 1), have regularly been proposed to explain plumage polymorphism (Rohwer & Paulson, 1987; Fowlie & Kruger, 2003; Bond, 2007; Paulson, 2011). A relatively recent surge in quantitative comparative research examining the ecological basis of colour polymorphism in birds (particularly hawks and owls) has however found no association between the incidence of polymorphism and foraging behaviour (Fowlie & Kruger, 2003; Galeotti *et al.*, 2003; Owens, 2006) and has instead steered the focus away from hypotheses centred on apostatic selection. In tackling all major avian groups Galeotti *et al.*'s (2003) review presented compelling evidence for the hypothesis that under disruptive selection, enhanced crypsis in variable light conditions is likely to be one of the

strongest selective mechanisms maintaining colour polymorphism in birds. Their findings are supported by the observation that polymorphism is particularly frequent in species (i) with a daily activity rhythm extending from day into the evening/dusk, and (ii) that live in variable light environments, be it within open or closed habitats, or across highly seasonal landscapes (Dale, 2006). Galeotti *et al.* (2003) also report that almost 80% of polymorphic species investigated show some degree of a geographic cline in their distribution, with 18% occurring along a climatic gradient of dry to wet, and 4% along a clear habitat cline, either from an open to closed or dark to light vegetation background. Importantly, all of these clines are characterised by differences in ambient light conditions. Additionally, most species that display clinal variation along a geographical or environmental gradient have significantly more dark morphs in habitats characterised by low light conditions. Once again, this may be due to improved crypsis in darker individuals under low light conditions, and Chapters 3 and 4 of my study now provide the first empirical evidence to support this theory. Differential crypsis in colour morphs under variable light conditions may also provide a central explanation for Gloger's climatic rule, whereby species tend to be darker in areas of higher humidity (which may represent darker environmental conditions with greater vegetation cover) and paler in drier climates (which may represent open, brighter conditions with lower vegetation cover) (Gloger, 1833; de Jong & Brakefield, 1998; Hogstad *et al.*, 2009; Kamilar & Bradley, 2011); it is worth noting that this would only be applicable in species where improved crypsis directly influences survival or other fitness-related traits (i.e. in predator - prey systems).

In support of Galeotti *et al.*'s (2003) ideas, a number of studies also demonstrate that polymorphic species frequently occupy larger distributional ranges (Forsman & Hagman, 2009; Hugall & Stuart-Fox, 2012) than monomorphic species, and are thereby more likely to occur over heterogeneous environments with variable light conditions. This could happen either through their occurrence in areas with multiple climatic regimes (as in Chapter 3) or across contrasting habitat types, and are thus more likely candidates for trait evolution by disruptive selection (Galeotti *et al.*, 2003; Rueffler *et al.*, 2006). This is clearly reflected in the black sparrowhawk whose range spans across much of sub-Saharan Africa (Fig. 2, Chapter 1; Ferguson-Lees & Christie 2001), occupying at least eight biomes and a range

of climatic regimes (Rutherford *et al.*, 2006), where they experience substantial variability in ambient light environments

Remaining Unseen; Predator - Prey Hide and Seek.

In many predator-prey systems individuals are locked into a constant battle to remain unseen (Endler, 1991; Edmunds & Dewhurst, 1994; Roulin & Wink, 2004; Stuart-Fox *et al.*, 2004; Stuart-Fox & Moussalli, 2009; Troscianko *et al.*, 2016). Numerous studies have discussed at length the importance of colouration in concealing/camouflaging prey from their predators (Dale, 2006; Owens, 2006; Stuart-Fox & Moussalli, 2009; Karpestam *et al.*, 2016; Troscianko *et al.*, 2016). A duality that is often overlooked, however, is that colouration (or any attribute for that matter) that provides crypsis and protects prey species, could similarly be used by a predator that seeks to capture it (Cott, 1940). This was highlighted in the work of Thayer (1909), which revealed that, like their prey, the colours and patterns in raptors were especially advantageous in concealing individuals from their prey against the habitat in which they foraged. Investigating the function of colour in predators such as hawks is extremely complex, not to mention challenging, especially when considering the ability to conceal themselves from prey (Bortolotti, 2006). This is, in part, due to the fact that predators often prey on a community of different species, many of which differ substantially in terms of their visual acuity and ability to detect and evade avian predators (Jacobs, 1992; Bortolotti, 2006). The development of new physiological models of avian vision will greatly enhance our ability to quantify crypsis from the perspective of avian prey (Stuart-Fox *et al.*, 2004; Owens, 2006) and is likely to be particularly useful in studies examining crypsis in specialist avian predators such as the black sparrowhawk (Simmons, 1986a; Hockey *et al.*, 2005; Baigrie, 2013).

An important aspect for understanding the role of colouration in the dynamics of predators and their prey is the cryptic behaviour of a species and the fine-tuned co-ordination between an individual and its environment (Bortolotti, 2006). Many studies have failed to document or consider the role of habitat and background choice, activity pattern or specific behaviour of predators (or prey) that reduce their visibility (Galeotti *et al.*, 2003; Bortolotti, 2006). This may be particularly relevant for understanding

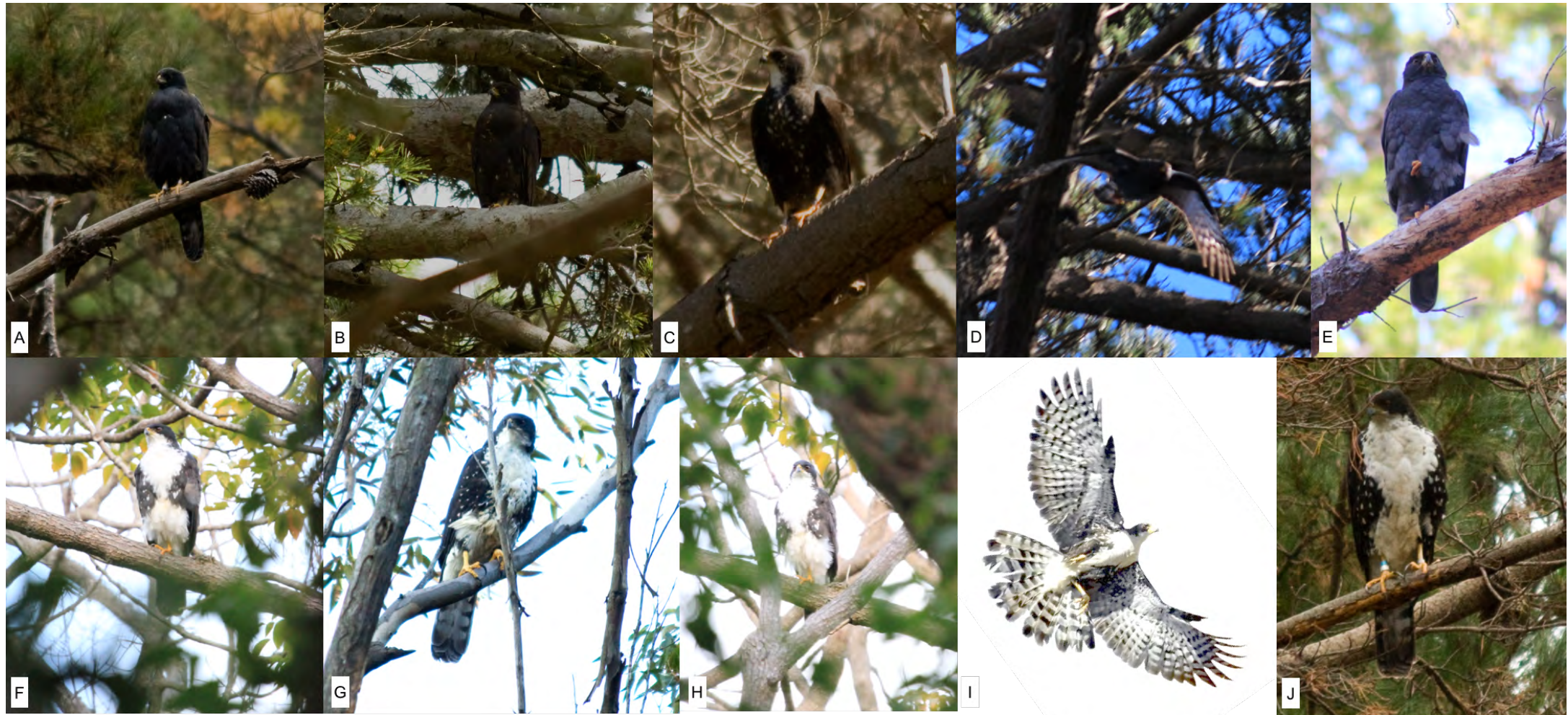
the mechanisms maintaining polymorphism in predatory species, whereby different morphs behave differently and gain improved crypsis through different hunting strategies or against habitat backgrounds of choice. Similarly to how a Eurasian bittern *Botaurus stellaris* evades detection by using its streaked cryptic plumage amongst emergent reed-vegetation, coupled with its “bill to the sky” behaviour when alarmed (Hockey *et al.*, 2005), different predatory morphs may be taking advantage of certain environmental conditions, habitats and behaviours to conceal their presence from ever alert prey. This idea is supported once again by the morph-specific habitat selection displayed in Pacific reef herons (Rohwer, 1990) and red-tailed hawks (Preston, 1980). Furthermore Rohwer (1990) also observed the morphs of the Pacific reef heron adopting different hunting behavioural strategies, with dark morphs pursuing prey more actively by running or walking, while white morphs sought prey more passively using a ‘flight, land and freeze’ hunting style. Indeed Green (2005) suggests that morphs alter their foraging tactics based on their degree of crypsis to prey. Black sparrowhawk morphs may therefore be optimising the match between plumage colour and their background, coupling this with certain behaviour, such as hunting under dark shaded forest canopy or in cloudy conditions in dark morphs, to gain improved access to their prey increasing their capture rates.

How is Concealment Achieved in Avian Predators?

Concealment of ventral plumage colouration has been shown to be fundamental in predators that attack from above (Götmark, 1987; Green & Leberg, 2005). How is concealment actually achieved by ventral plumage? Cott's (1940) remarkable book on adaptive colouration in animals discusses two modes of concealment that applies well to predators hunting from above and thus require crypsis from their prey below. These include the effects of countershading, whereby animals are shaded on their dorsal or ventral sides to counteract the natural effects of shade and light and become less visible (Thayer, 1896; Edmunds & Dewhurst, 1994; Wilkinson & Sherratt, 2008), and disruptive colouration, where colouring and patterns disrupt the shape, outline or form of an individual against the background (Cott, 1940). Countershading has been explored extensively in seabirds (Cairns, 1986; Götmark, 1987), and appears to explain why seabirds generally have relatively uniform colouration on their bodies which very effectively reflects the relative simplicity of their light environment (Bortolotti, 2006). It has also been

proposed that the white underparts of seabirds and other water birds provide them with crypsis against the bright sky (Green, 2005). Although not directly related to polymorphism, the idea that ventral colour may influence prey capture rates also has some experimental support; Götmark (1987) dyed the white underparts of gulls black and found a significant reduction in prey capture rates for experimentally altered birds. Disruptive colouration is particularly important in active animals that are in contact with backgrounds that vary in terms of colour, pattern and lighting (Bortolotti, 2006). Uniformly coloured individuals would be more visible against variable surroundings; therefore animals that live in dark environments tend to have white marks on dark bodies and black marks on white bodies in bright environments, to provide the most effective disruption (Cott, 1940).

Black sparrowhawk morphs appear to conform to both of these modes of concealment and my findings support the idea that different morphs have specific behaviours and strategies to facilitate/maximise their crypsis and thereby improve their foraging success (see Figure below). In this way the species is able to take advantage and utilize prevailing conditions and cover that the environment provides; dark morph individuals appear to have adapted to exploit the fleeting winter cloud cover that prevails on the Cape Peninsula during the breeding season, modifying their behaviour to forage more actively during conditions which maximize their crypsis from their avian prey (i.e. on dark cloudy days, and at dawn/dusk) and reduce their activity and effort as light conditions become brighter and foraging presumably becomes less profitable. Additionally, dark morphs use denser vegetation cover to their advantage, where their dark plumage under low light plays a key role in camouflaging them from their prey. As a result, dark morph individuals dominate the newly colonised Peninsula, where selection appears to favour the morph for longer periods of time, particularly during a critical stage of their life cycle - the breeding period. As for most *Accipiters* (Robert, 1966; Simmons, 1986b) this is a particularly demanding time for male black sparrowhawks, hunting to provision their partners, their chicks and themselves for a large proportion of the breeding period (which on average lasts around two months; i.e. during prelay, incubation and early nestling stages; Katzenberger *et al.*, 2015). Simultaneously, white morphs appear to have adapted to their local environment by adjusting their behaviour and hunt



The following photographs illustrate the role of ambient light condition, habitat choice and background matching in concealing the different black sparrowhawk morphs from their prey. The top row (A-D) demonstrates how dense background cover and low-lit habitat facilitates crypsis in the dark morph while perched and when in flight (D). The image on the top right (E) shows how the uniform ventral plumage colour of the dark morph stands out against a bright, variable background and are thus more easily detected. The bottom row (F-I) shows how the light dappled ventral plumage of the white morph is concealed against bright open habitat background both while perched and when in flight (I). The image on the bottom right (J) shows how white plumage stands out against a darker background characterised by denser cover. (Photographs A-C, G & J-Ann Koeslag, D, E, F, H, I-Gareth Tate).

more actively within more open habitats, and to some extent during brighter conditions, where their light underparts are likely to be less conspicuous to their prey below (Tate *et al.*, 2016). Although conditions on the Peninsula don't always favour the white morph during the breeding period, their improved reproductive performance when partnering with a dark morph may explain how the white morph persists within the study population (Chapter 5); other factors such as immigration from surrounding populations may also contribute to their numbers on the Peninsula (Newton, 1979, 2001). Furthermore, they also appear to have reproductive advantages when they breed later in the season, with higher recruitment rates for the offspring produced during later, brighter stages (Sumasgutner *et al.*, 2016).

The Versatility/Adaptability of Morph Plumage Colour Pattern in the Black Sparrowhawk

Throughout the black sparrowhawks' range, the white morph is likely to be more versatile in terms of its mode of concealment, as this plumage colour and pattern provides effective countershading against bright, open backgrounds, but also offers disruptive colouration (black specks on a white breast), which is vital for very active animals hunting against variable backgrounds (in terms of colour, light and pattern) (Cott, 1940; Endler & Théry, 1996). Therefore, relative to its ability to provide a degree of crypsis, the white morph's plumage can be thought of as a more "adaptable" plumage patterning. This may explain why the white morph predominates throughout the species range (Amar *et al.*, 2014; Tate *et al.*, 2016). The dark morph's more uniform underparts may render them more conspicuous to their prey against variable backgrounds. Thus, they may be more "specialised", or restricted in terms of their crypsis and foraging success, to hunt under particular light conditions or in certain habitats (i.e. under dense shaded cover). This may explain why dark morphs are generally classified as rare and may reveal why in the novel climate of the Cape Peninsula, where there is a complete change in the winter breeding climate, that a reversal in morph frequencies (~80% dark morphs) is observed in comparison to the species more historical South African range. Dark morphs may therefore be low-light hunting specialists. Although this is credible, no studies have effectively compared the different levels of concealment in the two morphs.

FUTURE RESEARCH

In order to better understand the dynamics of how polymorphism is shaped and maintained in black sparrowhawks in the study population, a fully integrated population model is required. This model would need to incorporate all the morph specific demographic data and local environmental (light) conditions experienced by individuals. We now have considerable data on the morph specific (and pair combination specific) demographic rates i.e. survival (Chapter 4; Sumasgutner *et al.*, (2016), productivity (Chapter 4) and recruitment (Sumasgutner *et al.*, 2016). Together these data could be used to construct scenarios of expected morph frequencies under varying conditions – and allow for assumptions for either open or closed populations. A model of this type could also be used to test morph viability in other populations given their different light levels – i.e. the seasonal effects as witnessed in our study, and thus explore how morphs are maintained throughout the species' distribution under different local conditions.

Whilst the results from this study (Chapter 3) clearly support differences in hunting success between the two morphs in different light levels, it would also be valuable to establish this experimentally using a system of model presentation to avian prey species. A relatively simple experimental method has already been established; using captive pigeons and measuring their behavioural response when presented with taxidermy mounts of attacking black sparrowhawk morphs launched at the pigeons in a cage using a line and pulley system. Mounts of the different black sparrowhawk morphs would be used with light levels or background colour and pattern varied from dark to light (to mimic different cloud and vegetation cover). One could then measure the response time of the pigeons to the attacking mounts against the different backgrounds/light conditions, similarly to experiments done by Whittingham *et al.*, (2004).

Non-Signaling Functions of Colouration

It also important to acknowledge an alternative explanation for the key findings presented here; that differential foraging success and behaviour of the two black sparrowhawk morphs may be related to inherent behavioural differences between morphs in their coping strategies under variable light conditions, rather than to variable capture success or crypsis under varying light conditions. Dark or white morphs may be adjusting to hunt during darker or brighter conditions (or habitats) for reasons other than improved foraging success. For example, different morphs may vary in their ability to thermoregulate during different times of the day and thus may display varying thermoregulatory strategies (Roulin, 2014; Dreiss *et al.*, 2015). This would require methods to test and compare other likely physiological features, such as thermoregulation in different morphs across varying light conditions.

Although this study is focused on the detectability of a predator in a single system, I believe the arguments presented here are applicable more generally to our better understanding of predator avoidance by prey, and indeed any visually guided interaction between individuals and/ or species where there is a potential ‘winner’ and ‘loser’ i.e. mobbing, inter and intraspecific competition or host detection by vectors or parasites (Chakarov *et al.*, 2008). Interestingly, working on the same study population, Lei *et al.* (2013) found higher infection intensity of the blood parasite *Haemoproteus nisi* in adult white morphs compared to dark morphs, suggesting increased resistance in dark morphs. This may be linked to the (possible) pleiotropic properties of the genes encoding melanin pigments (Ducrest *et al.*, 2008), which raise immune function and may be one of the mechanisms through which dark morph black sparrowhawks have a selective advantage in the study region (Lei *et al.*, 2013). It would therefore be interesting to test whether morphs vary in their selection by the vectors (i.e. by blood sucking flies and lice; Lei *et al.*, 2013).

Black Sparrowhawk Morphs throughout their African Range

Very little literature exists on the morph ratios across the rest of species sub-Saharan range; although it has been proposed that populations with a substantial proportion of rarer dark morphs are generally

restricted to specific regions of southern Africa, i.e. the Cape Peninsula, Western Cape, South Africa and a number of anecdotal reports exist identifying a population on the coast of Kenya supporting an unusually high proportion of dark morphs (Allan, 1997a; b; Kemp & Kemp, 1998; Hockey *et al.*, 2005). It would therefore be interesting to identify morph distributions across the rest of the species' African range and test the predictions developed here on other populations exposed to different climatic regimes and within different biomes. Gathering data on morph ratios could be carried out using the web application MORPHIC (Leighton *et al.*, 2016), which uses Google Images to extract data on geographical variation in phenotypic traits visible from photographs. This has already been successfully tested on web-based images of black sparrowhawks across their South African range (Leighton *et al.*, 2016).

CONCLUSIONS

This study reveals that the differentiation in morph frequencies that occurs in the Cape Peninsula population of the black sparrowhawk has proceeded in the presence of substantial gene flow, a result that is consistent with a significant role for local selection in the evolution of colour polymorphism in the black sparrowhawk. The substantial fitness advantage that dark morph individuals have in the study population provides support for the hypothesis that colour polymorphism in the black sparrowhawk is maintained through its adaptive interaction with different light conditions. In this study system, the differential foraging behaviour of black sparrowhawk morphs under varying light conditions, and under variable habitat selection, allows mixed-pairs to expand their foraging niche. This may be one of the mechanisms allowing individuals to accrue a fitness advantage via improved productivity and thereby provides an overarching explanation for the maintenance of colour polymorphism within the study population.

This study affords us exciting new insight into the role of animal colour in facilitating concealment of predators from their prey, contributing an important piece to the evolutionary puzzle of how animal colour is shaped by natural selection. The implications of the findings presented here extend far beyond

this study system; black sparrowhawks provide an excellent model system to better understand how colour polymorphism, and thus a variable phenotype, facilitates the successful colonisation of different environmental conditions, demonstrating why polymorphism is frequently associated with rapid phenotypic evolution and accelerated speciation rates (Forsman *et al.*, 2008; Hugall & Stuart-Fox, 2012; Wennersten & Forsman, 2012). In this way the black sparrowhawk, like other colour polymorphic species, may be better equipped to cope with future changes to their environment linked with light conditions, and possibly be less susceptible to climate change and range shifts (Roulin, 2014). The results presented here highlight the importance of population level analyses to better understand the complex interplay between selection and gene flow in the evolution of adaptive traits and bring to light a number of interesting avenues for future research into the evolution of the remarkable adaptive properties of colour in predator prey systems.

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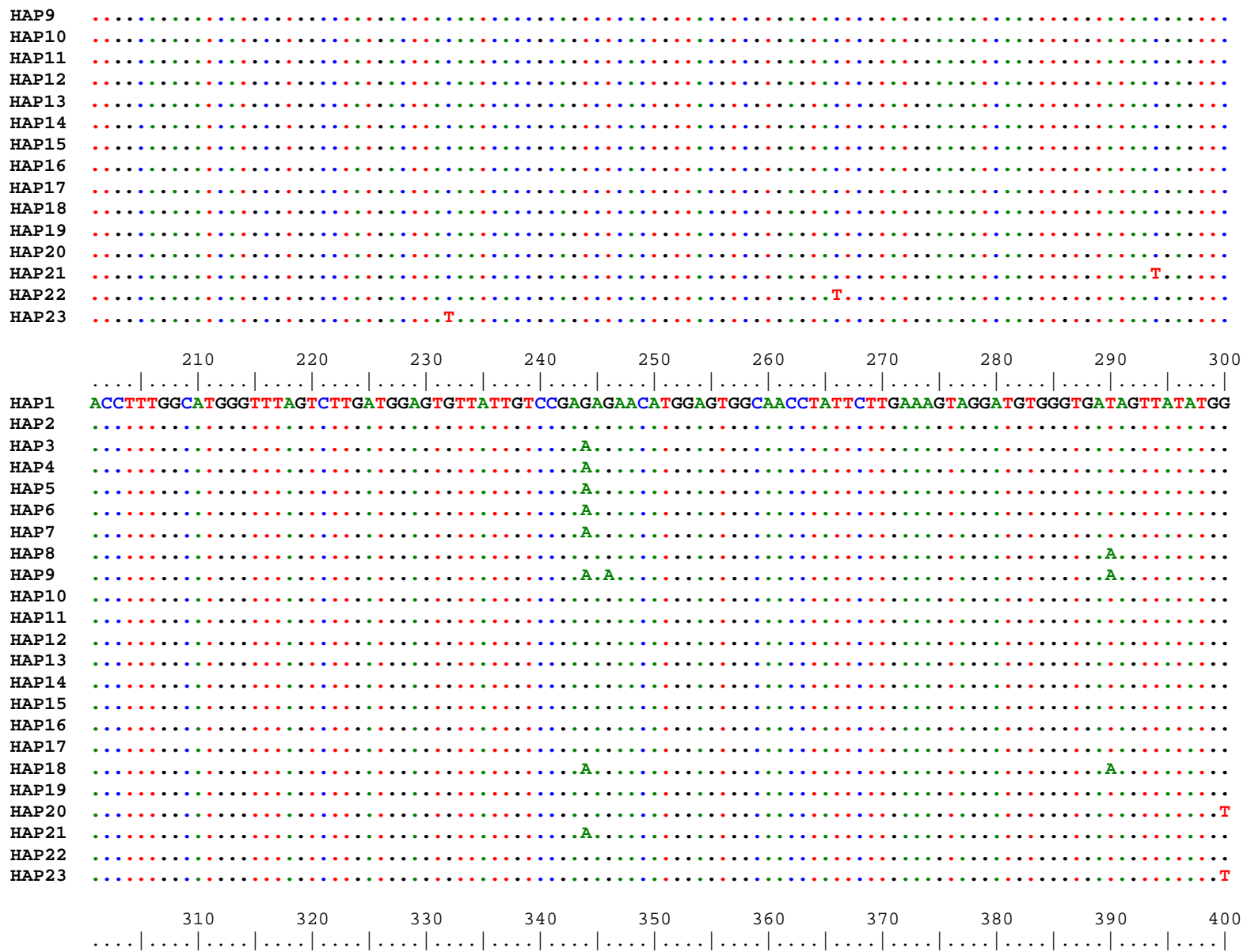
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APPENDIX

Table 1. 23 unique mtDNA d-loop haplotypes that were identified from 115 individuals sampled across the distributional range of the Black Sparrowhawk in South Africa.

	10	20	30	40	50	60	70	80	90	100
HAP1	ACGTGCACCGGGTTGATGATCTCTCTGGGACAGAACGATTAATAGATAAACCTGGCCGCTCTTGGTTAGCTAACGAGAGAGGTAATGGAAAGGCATGTTTG									
HAP2									
HAP3									
HAP4									
HAP5									
HAP6									
HAP7									
HAP8									
HAP9									
HAP10									
HAP11									
HAP12									
HAP13									
HAP14									
HAP15									
HAP16									
HAP17									
HAP18									
HAP19									
HAP20									
HAP21									
HAP22									
HAP23									

	110	120	130	140	150	160	170	180	190	200
HAP1	TTGGCAGAGATCATGCGTGGCCTATGACTTACAATCACCGCAGTCTCACTGTTACGTCGTGAGTACTCGTATGGAAGATCAAGTTGTGTATAACGAGTTC									
HAP2									
HAP3									
HAP4									
HAP5									
HAP6									
HAP7									
HAP8									




```

HAP19 .....G.....G.....T.....G.....
HAP20 .....G.....G.....T.....G.....
HAP21 .....G.....G.....T.....G.....
HAP22 .....G.....G.....T.....G.....
HAP23 .....G.....G.....T.....G.....

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          510      520      530
    ....|....|....|....|....|....|
HAP1  AGCCTTCATTCTTTGGTTACAAAACCAA
HAP2  .....G.....G.....
HAP3  .....CG.T.....A.....
HAP4  .....G.....G.....
HAP5  .....G.....G.....
HAP6  .....G.....G.....
HAP7  .....G.....
HAP8  .....G.....G.....
HAP9  .....G.....g.....
HAP10 .....G.....G.....
HAP11 .....G.....G.....
HAP12 .....G.....G.....
HAP13 .....G.....G.....
HAP14 .....
HAP15 .....T..T.....G.....
HAP16 .....G.....G.....
HAP17 .....G.....G.....
HAP18 .....G.....G.....
HAP19 .....g.....T..T.....
HAP20 .....G.....G.....
HAP21 .....G.....G.....
HAP22 .....G.....G.....
HAP23 .....G.....G.....

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