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A MECHANISTIC APPROACH TO UNDERSTANDING THE COLOUR POLYMORPHISM IN BLACK SPARROWHAWKS (*ACCIPITER MELANOLEUCUS*)

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Declaration

I, Carina Nebel, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Abstract

Species that exhibit large variation in phenotypic traits, are commonly considered to have a stronger evolutionary potential. However, how they are capable to maintain polymorphisms remains a fundamental problem in evolutionary biology. Colour polymorphic species provide ideal study systems to explore the processes that lead to variation maintenance. About 3.5% of all bird species are colour polymorphic, but it is an especially common phenomenon in Accipitridae (22%), which indicates that it has an adaptive function in this bird group and makes them ideal model systems to study evolutionary processes.

The black sparrowhawk (*Accipiter melanoleucus*) occurs in two discrete colour variants: dark and light. The two morphs differ in the expression of white and black feathers on the breast, belly and underwing coverts. The morph has been associated with ambient light-dependent foraging success and activity behaviour: Dark morphs forage more and have higher foraging success under low light conditions whereas light morphs forage independently of light levels but are better foragers under bright light conditions. This is hypothesized to be due to a crypsis advantage for the morphs under these conditions. During the winter breeding period, the predominating low light conditions on the Cape Peninsula (Western Cape, South Africa) could create an advantage for dark morphs, which should result in their higher survival and higher breeding success. However, this is not the case and only when the two morphs come together to breed, there is a fitness difference: mixed-morph pairs (that consist of a dark and a light morph) have higher breeding success than like-morph pairs (that consist of the same morph) and offspring of mixed-morphs have higher survival rates. This higher success of mixed-morph pairs is hypothesized to be due to emergent pair-level properties with the two morphs being able to expand the hunting niche as a pair. This ‘complementarity hypothesis’ is based on previous research conducted on the study system.

The aim of this PhD was to explore the mechanistic background of colour polymorphism maintenance in the black sparrowhawk. (i) I performed an experiment in which I test whether there is a morph- and ambient light-dependent crypsis advantage in the black sparrowhawk. I measure the reaction time of feral pigeons towards a simulated hawk attack but did not find indication of such an effect as pigeons reacted the same towards the two morphs. In line with the complementarity hypothesis, (ii) I found that mixed-morph parents provide food more consistently to the nest than like-morph parents. This results in a more predictable food supply for nestlings and buffers against long periods of malnourishment. However, (iii) I was unable to determine the mechanistic link between food supply and higher survival: nestlings of mixed- and like-morph pairs had the same levels of innate immune function. Thus, an improved innate immune function in nestlings of mixed-morph pairs is unlikely associated with their higher survival rates. (iv) I performed individual-based model simulations which incorporate multiple key fitness parameters and found that complementarity – in combination with morph-dependent seasonality-associated fitness effects – explains the stable colour morph equilibrium in this population.

I conclude that emergent pair-level properties which arise due to the complementary nature of the two morphs play an important role in maintaining polymorphism in this species. Complementarity might not only be restricted to colour polymorphic species but could be present in other polymorphic traits that allow parents to behaviourally complement each other when raising their young.

Chapter 1

Introduction

Ever since the phrase "survival of the fittest" was coined by Darwin (1859), evolutionary biologists have sought to understand how this fitness has influenced selection of an individual, a population, or a species. Survival of the fittest implies that natural selection should lead to relatively monomorphic populations through selection of the variant that is best adapted to its environment. However, this is rarely the case and many species exhibit large phenotypic variation (polymorphism), which are generally associated with higher evolutionary potential as they often exploit wider realised niches and have larger distribution ranges (Delhey et al. 2013; Forsman 2016; Forsman and Wennersten 2016; Galeotti and Rubolini 2003; Skúlason and Smith 1995; Van Valen 1965), faster speciation (Cattin et al. 2016; Hugall and Stuart-Fox 2012; Jamie and Meier 2020) and have lower extinction risks (Delhey et al. 2013; Ducatez et al. 2017; Forsman 2016; Takahashi and Noriyuki 2019) than monomorphic relatives. In fact, there are several key selective processes that can allow polymorphism to be maintained within species or populations, three of them are: i) heterozygote advantage, ii) disruptive selection and iii) frequency-dependent selection. I will now introduce and describe these three mechanisms:

<u>Heterozygote advantage:</u> Natural selection is caused by the conditions individuals experience in the environment. Thus, if conditions are uniform, stabilizing selection should result in monomorphic traits. However, there is an exception: If stabilizing selection puts individuals carrying a heterozygous genotype at an advantage over those carrying a homozygous genotype, it allows polymorphism to be maintained (Fisher 1922; Gemmell and Slate 2006; Gray and McKinnon 2007; Sellis et al. 2011).

<u>Disruptive selection:</u> If conditions are spatially or temporarily heterogenous, different trait variants can be favoured under different conditions, which can also give rise to stable polymorphic equilibria. Such 'disruptive selection' describes changes in a population in which extreme variants for a trait are favoured over intermediate variants (Galeotti and Rubolini 2003; McKinnon and Pierotti 2010; Roulin 2004), with gene flow between cohorts of the two variants

slowing the speciation process. Disruptive selection can be caused by spatial conditions (expressed through the complexity of landscapes and habitats), but also temporarily varying conditions like seasonality (Tate et al. 2017), the circadian rhythm, lunar phases (San-Jose et al. 2019), or by varying weather conditions (Tate and Amar 2017; Tate et al. 2016; Gangoso et al. 2020).

Frequency-dependent selection: 'Frequency-dependent selection' is an evolutionary process whereby the fitness of a phenotype varies dependent on the phenotype composition within a population at that specific point in time (Bond 2007; Gray and McKinnon 2007; Roulin 2004). Under negative frequency-dependent selection, a rare trait is favoured, which can result in polymorphism maintenance (Gray and McKinnon 2007; Roulin 2004). Rare traits experience a fitness advantage which leads to a numerical increase, while previously common traits will decrease. Typically, an equilibrium is reached at which all traits experience similar fitness.

When studying evolutionary processes that result in polymorphism maintenance, it is important to understand the genetic architecture underlying the study trait as it will determine which evolutionary processes might be important. The evolutionary processes described above and throughout the thesis will only apply to traits that are genetically controlled by one gene (typical Mendelian inheritance, Mendel 1865), however, most heritable traits are polygenic, which means that they are controlled by more than one gene. Evolutionary mechanisms like heterozygote advantage or frequency-dependent selection cannot be major forces in maintaining polymorphisms in polygenic traits and, instead, mutations are more important (i.e. Bürger et al. 1989; Josephs et al. 2017).

Studying adaptive colour polymorphism in birds

Studying evolutionary processes is possible with all traits that express variability. The most convenient traits to study are those which are visually identifiable, for example colour of an organism's fur or plumage (Huxley 1955). Colouration is also a trait expected to be under high selective pressure as it directly influences how an individual is visually perceived in its natural wold, i.e. by determining its camouflage, concealment and predation risk (Cuthill 2019; Stevens and Merilaita 2011).

Colour polymorphism refers to the occurrence of at least two genetically determined phenotypes ('colour morphs' or 'morphs') in the same population that are expressed independently of sex (Ford 1945; Huxley 1955). Furthermore, individuals exhibiting different colour morphs can freely interbreed. Only about 3.5% of all bird species are colour polymorphic (Galeotti et al. 2003; Roulin 2004), however, it is relatively common in predatory birds, like hawks (Accipitridae), owls (Strigidae), falcons (Falconidae), herons (Ardeidae) or skuas (Stercorariidae) (Galeotti et al. 2003). Thus, colour polymorphism might have an adaptive function in these predatory groups, making them good model systems to study evolutionary processes responsible for the maintenance of polymorphism (Fowlie and Kruger 2003; Galeotti and Rubolini 2003; Hugall and Stuart-Fox 2012; Roulin and Wink 2004). As with other variable traits, two of the most common mechanisms proposed to explain maintenance of colour polymorphism are negative frequency dependence and disruptive selection. I will now discuss these two topics in more detail from the context of colour polymorphism with a focus on predatory birds.

Negative frequency-dependent selection in colour polymorphic species

Negative frequency-dependent selection has been proposed numerous times as a potential mechanism for the maintenance of colour polymorphism. This hypothesis is assumed to most commonly act via predator-prey systems. The key assumption behind this hypothesis is that animals generate search-images to detect prey or predators (i.e. Arcos 2007; Bond 2007; Karpestam et al. 2013; Punzalan et al. 2005; Rohwer 1983; Rohwer and Paulson 1987). These search-images are the product of previous experience, and thus they will be based on the most commonly encountered appearance of prey or predators. In such a system, a rarer morph can have a selective advantage, either by not being recognised as a predator by prey or as prey by predators. Due to a selective advantage of the rare morph over the common morph, the number of rare phenotypes will increase until a frequency-dependent equilibrium is reached at which all morphs experience the same selective pressure (Clarke 1962a; Clarke 1962b; Paulson 1973; Rohwer 1983; Rohwer and Paulson 1987).

Although this mechanism is credible, conditions in the natural world are more complex than expected under negative frequency-dependency: Most environments are not completely homogeneous and create different crypsis via background matching. Thus, the lack of exposure to the rare morph might not determine predation risk, but rather an individual's visibility within its environment. This can result in the opposite pattern being experienced (i.e. positive frequency-dependent selection), with rare phenotypes being more likely to be identified as prey rather than less likely (Rohwer 1983; Rutz 2012). This contrasting visibility of different morphs under different conditions is also fundamental for disruptive selection.

Disruptive selection in colour polymorphic species

By determining the visual appearance of an individual, colour morph can influence directly how well adapted a species is to its environment. The efficacy in camouflage of different morphs will diverge in different habitats and under different weather or light conditions and can thus directly determine an individual's behaviour (i.e. Ahnesjö and Forsman 2006; Allen et al. 2020; Chaniot 1970; Koskenpato et al. 2020; Marshall et al. 2015; Tate and Amar 2017; Tate et al. 2016; Vignieri et al. 2010). In predatory species that rely on their colouration to remain hidden from their prey, the interaction of the visual appearance of the predator with its environment may determine hunting behaviour (Green 2005; Preston 1980; San-Jose et al. 2019; Tate and Amar 2017; Tate et al. 2016). For example, Preston (1980) found that hunting perches differ between morphs of the red-tailed hawk (*Buteo jamaicensis*), with darker birds being more likely to perch in front of a more enclosed background than lighter coloured individuals, potentially due to a camouflage advantage of dark plumage in a dark environment. In reddish egrets (*Egretta rufescens*) which have a reddish-brown or an all-white colour morph, environment and visual appearance determine foraging tactics: Reddish-brown individuals show a more active hunting behaviour in shallow waters whereas white individuals are passive foragers in deeper waters (Green 2005). In the black sparrowhawk (*Accipiter melanoleucus*), activity patterns differ between morphs both temporally and spatially, with the darker morph hunting and providing more food under low light conditions and hunting in enclosed forests whereas light morph individuals forage more in open landscapes and are more likely to provision food to the nest under bright light conditions (Tate and Amar 2017; Tate et al. 2016).

Such partitioning in hunting behaviour can allow individuals of the same species to exploit different habitats (or times of day) with alternative food resources, and thereby allow the species to exploit a wider realised niche (Bolnick et al. 2002; Dreiss et al. 2012; Gross 1996; Koskenpato et al. 2020; Tate et al. 2016). Niche expansion leads to overall more diverse resources being available to individuals of the same species and could therefore relax intra-specific competition between individuals occupying the same area (Galeotti and Rubolini 2003; Skúlason and Smith 1995). These characteristics could be beneficial, making a species more able to respond successfully to environmental changes, to have larger distribution ranges and in turn, to reduce their extinction risk (Delhey et al. 2013; Ducatez et al. 2017; Forsman 2016; Takahashi and Noriyuki 2019).

The concept of a niche expansion does not have to be restricted to the population- or species-level but can create beneficial synergies for a pair that exhibits two different morphs when raising offspring. If pairs consisting of two different morphs produce more young or young of higher quality, this might be a viable mechanism explaining colour polymorphism maintenance in species.

Behavioural complementarity of mixed-morph parents

This less well known, and less explored theory, which has been recently proposed to potentially explain the maintenance of colour polymorphism, is the 'complementarity hypothesis' (Sumasgutner et al. 2016a; Tate et al. 2017). Many studies have shown that morphs can be associated with differences in reproductive success and offspring quality. For example, heavier spotted barn owl (*Tyto alba*) females raise higher quality offspring due to their higher resistance to parasites (Roulin and Altwegg 2007; Roulin et al. 2000; Roulin et al. 1998; Roulin et al. 2001). In this example, the unit of selection is on the individual-level. When a specific morph has an advantage, that morph is expected to dominate in the population and should ultimately go to fixation. Thus, an individual's morph determines its fitness. However, individual quality can also be influenced by early life conditions (Cam and Aubry 2011; Lindström 1999). Thus, under specific conditions, it might be possible to expand the unit of selection to the parental pair-level. Such an expansion can be found in altricial bird species with bi-parental care (Cockburn 2006; Kendeigh 1952).

Nestlings of altricial bird species are completely reliant on the resources provided by their parents and parental care can determine their offspring's long-term fitness via carry-over effects (Cam and Aubry 2011; Lindström 1999). Thus, a specific combination of parental phenotypic traits could create ideal early life conditions for the offspring (Cam and Aubry 2011; Cam et al. 2003) which could support larger brood sizes or young in better condition and thus, of higher fitness. Such emergent properties that come from different parental morph combinations (i.e., putting "mixed-morph parents" at an advantage) could allow the rarer morph, which may even have a selective disadvantage, to be maintained within a system. It is on this basis that the complementarity hypothesis is proposed to operate, and thereby maintain colour polymorphism in a population.

Complementarity might be prominent in colour polymorphic raptors as niche expansion is commonly hypothesized to be a main driver of maintaining colour polymorphism in species and populations (Galeotti and Rubolini 2003). If different colour morphs exploit different habitats or forage under different environmental conditions (Galeotti and Rubolini 2003; Passarotto et al. 2018; Tate and Amar 2017; Tate et al. 2016), it may allow a mixed-morph pair to expand their foraging niche and either provision the offspring with a higher diversity of prey species (either by exploiting different habitats through a spatial niche expansion or foraging during different times of the day through a temporal niche expansion) or provision food more predictably (by expanding foraging across temporally alternating conditions, Tate et al. 2017). Such behavioural complementarity could promote disassortative mating (meaning that the parents express different trait variants). Disassortative mating systems are a known mechanism to allow polymorphism to be maintained over time (Roulin and Bize 2007). By favouring pairs that express different phenotypes to produce offspring, this reinforces geneflow and thereby slows the speciation process, which actively maintains polymorphism in a population. However, complementarity does not necessarily have to be associated with spatial or temporal niche expansion that offspring of mixed-morph pairs benefit from but could be associated with any heritable behavioural trait variation. For example, in the white-throated sparrow (*Zonotrichia albicollis*) morphs are correlated with behaviour and alternative reproductive strategies and individuals mate disassortatively (Houtman and Falls 1994; Knapton et al. 1984; Knapton and Falls 1983; Kopachena and Falls 1993; Tuttle 2003). Disassortative mating might be driven by behavioural complementarity in this species but could also be explained by a genetic mechanism reducing the viability of homozygote white-striped individuals (Thorneycroft 1975). Complementarity of heritable behavioural traits in species exhibiting bi-parental care has large potential at maintaining variability in many species, however, it has never been explored before.

Support for the complementarity hypothesis can be found in the black sparrowhawk, where parental morph combination has been shown to influence the probability to breed successfully (Tate et al. 2017) and the quality of offspring (Sumasgutner et al. 2016a; Tate et al. 2017), with mixed-morphs pairs breeding more successfully and producing offspring with higher survival rates than like-morph pairs. The complementarity hypothesis could explain these fitness differences but there are other candidate mechanisms, i.e. heterozygote advantage as mixed-morph pairs might produce more heterozygous offspring (Mendel 1865) or the avoidance-image hypothesis, which is a similar mechanism to complementarity, but arises through predator-prey interactions (Rohwer 1983; Rohwer and Paulson 1987).

This large range of candidate mechanisms and a solid framework of previous research on the species makes the black sparrowhawk an ideal candidate to explore the likely mechanisms that maintain colour polymorphism; which is the principle topic of my thesis.

The study species – the black sparrowhawk

The black sparrowhawk is a large Southern African *Accipiter* species that occurs in two discrete adult plumage morphs: dark and light (Amar et al. 2013; Ferguson-Lees and Christie 2001; Thiollay 1994). The two morphs are easy to distinguish in the field: light morphs exhibit a white breast, belly and underwing coverts whereas dark morphs are predominantly melanistic with a variably sized white throat and white speckles on the breast (Figure 1.1). Although the two colour morphs can vary in the extent of their white and black colouration, the morphs were found to be discrete (*c.f.* continuous) and there is no gradual transition in plumage morph, unlike some other raptors, i.e. the common buzzard (*Buteo buteo*, Kappers et al. 2017) or the red-tailed hawk (Preston 1980). Colour ringing and long-term monitoring have revealed that the morph is stable throughout an individual's life and does not change with age once they are in adult plumage (Amar et al. 2013). Beside the adult colour polymorphism, black sparrowhawks also exhibit a juvenile plumage that is typical for all *Accipiter* hawks, and ranges from pale to rufous colouration with no apparent link to adult plumage (Amar et al. 2013; Thiollay 1994).

Figure 1.1. The two black sparrowhawk morphs and their difference in plumage. The light morph (top) is predominantly white on the breast, belly and the underwing coverts. The dark morph (bottom) melanistic with a variable white throat and speckles on the breast. Photo: E. Rodseth (Black Sparrowhawk Project, 2017).

The species inhabits all types of forests, mature woodland and dense riverine growth and occurs from tropical lowlands to high montane patches of sub-Saharan Africa (Thiollay 1994) and due to its extremely large distribution is listed as Least Concerned (LC, BirdLife International 2021). It can be difficult to find due to its elusive nature during non-breeding but benefits from forest plantations within urban areas where it can reach high breeding densities (Martin et al. 2014a).

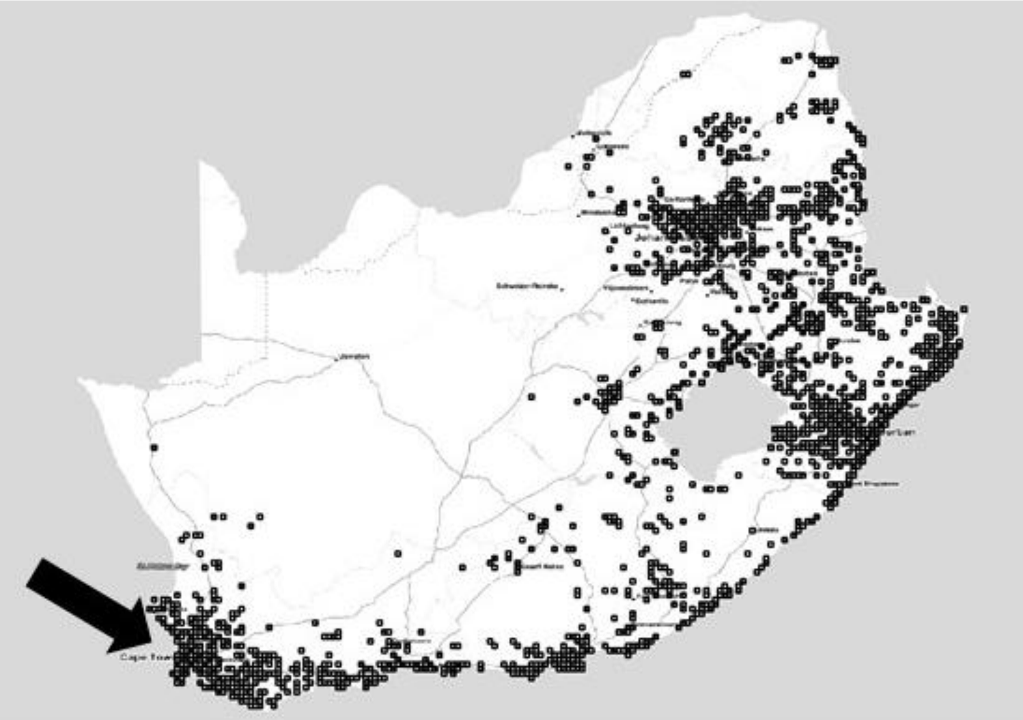


Figure 1.2. Extant distribution of the black sparrowhawk (*Accipiter melanoleucus*) in South Africa (white, grey area sea and neighbouring countries). Distribution mapped based on sighting data obtained from the Southern African Bird Atlas Project 2 (SABAP2, http://sabap2.adu.org.za/). Black squares are pentads with recorded presence of black sparrowhawks. Black arrow indicates the location of the study area: the Cape Peninsula (Western Cape, South Africa).

Following a range expansion from the east of South Africa, black sparrowhawks recently colonised the Cape Peninsula (Western Cape, South Africa, Figure 1.2) with the first breeding attempt recorded in 1993 (Curtis et al. 2007; Oettlé 1994). Initially, the population grew rapidly after colonization, but is currently stable at about 40 breeding pairs (Figure 1.3, Amar et al. 2013; Martin et al. 2014b). Black sparrowhawks mainly nest in alien pine (*Pinus* ssp.) and eucalyptus (*Eucalyptus* ssp.) but also occasionally in native Afromontane forest (Hockey et al. 2005). Use of alien tree plantations for nesting and the high abundance of prey, in the form of pigeons and doves that inhabit urban and suburban landscapes in the metropolitan area of Cape Town (Suri et al. 2017), might explain the successful colonisation of the study area. A temporal expansion of the breeding season to approximately nine months (March – November) and spatial overlaps with neighbouring territories likely allow such a high breeding density (Martin et al. 2014b; Sumasgutner et al. 2016b).

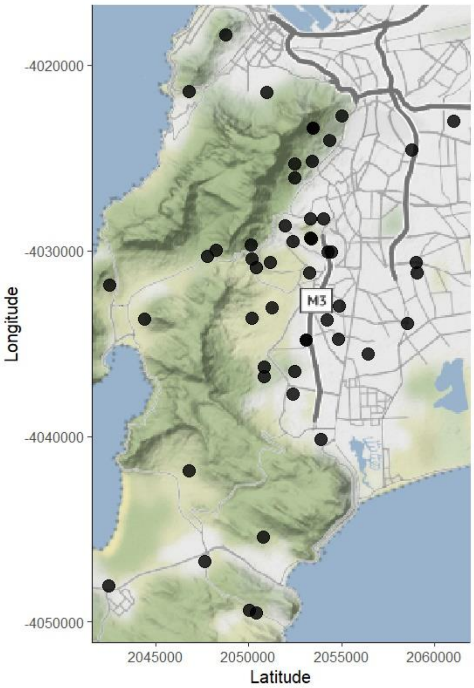
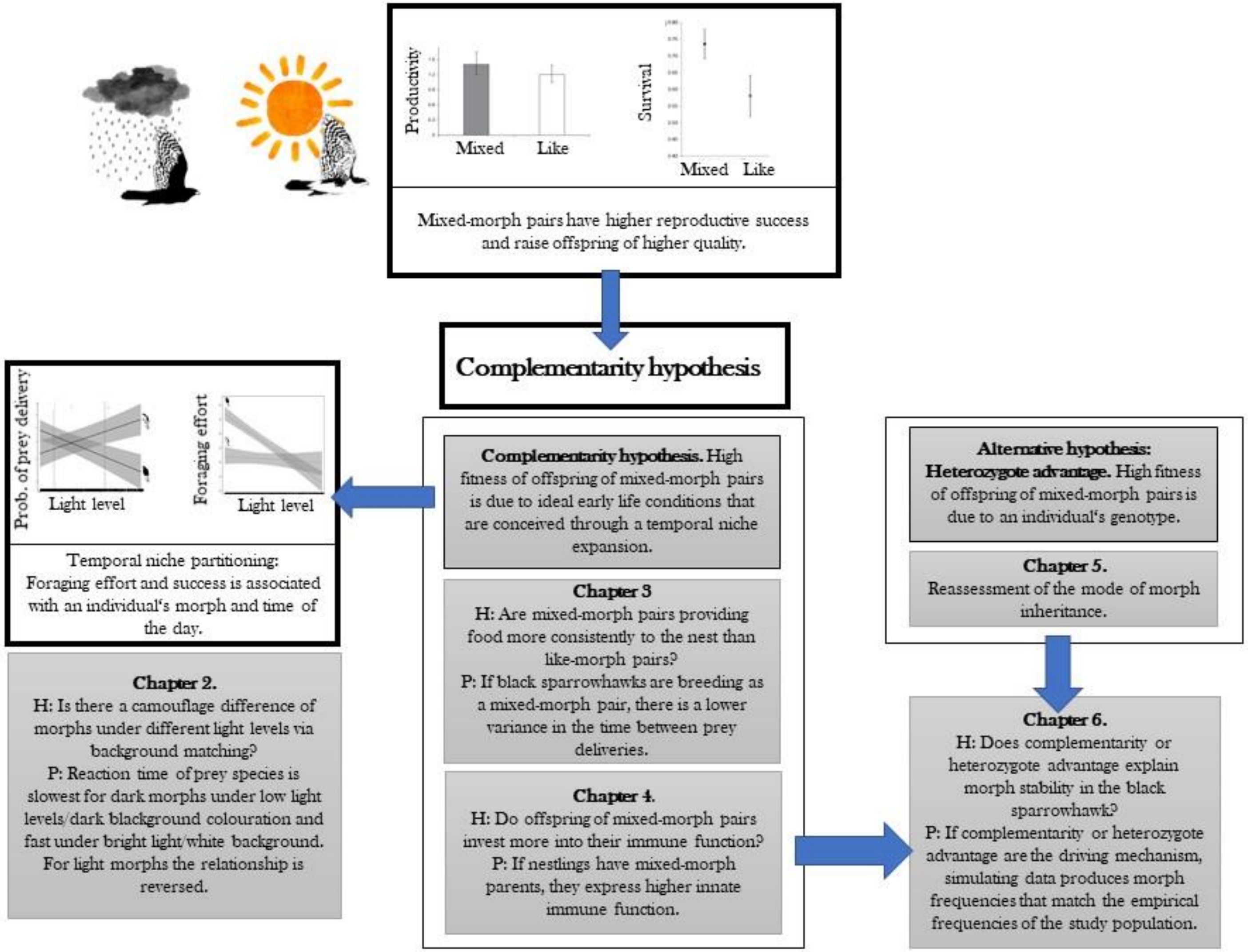


Figure 1.3. Study area on the Cape Peninsula (34°00' S, 18°26' E). The black dots indicate black sparrowhawk nest sites that were active between 2015 and 2019. Most of them are concentrated in the greenspace along Table Mountain, but black sparrowhawks also occupy territories in the suburban landscape of Cape Town.

On the Cape Peninsula, black sparrowhawks encounter different breeding conditions than in the rest of their distributional range in South Africa (Figure 1.2). The species is a winter breeder, a season characterized by sunny and dry weather in eastern South Africa (part of the historic distribution, Figure 1.2), but overcast and wet weather in the Western Cape (Amar et al. 2014). The morph distribution follows the clinal variation of the winter rainfall regime (Amar et al. 2014) and the intensity of solar radiation (Tate et al. 2016). Light morphs are more frequent in the eastern and northern parts of the species' distribution range (Ferguson-Lees and Christie 2001; Kemp and Kemp 1998; Steyn 1982) whereas dark morphs numerically dominate in the west. This high frequency has remained constant at 68% to 81% (mean = 75%) in the study area (Amar et al. 2013) and the numerical dominance of dark morphs is unlikely to be due to a founder effect (Tate 2017).



Mixed-morph pairs have higher reproductive success and raise offspring of higher quality.

Complementarity hypothesis

Complementarity hypothesis. High fitness of offspring of mixed-morph pairs is due to ideal early life conditions that are conceived through a temporal niche expansion.

Temporal niche partitioning: Foraging effort and success is associated with an individual's morph and time of the day.

Chapter 2.
H: Is there a camouflage difference of morphs under different light levels via background matching?
P: Reaction time of prey species is slowest for dark morphs under low light levels/dark blackground colouration and fast under bright light/white background. For light morphs the relationship is reversed.

Chapter 3
H: Are mixed-morph pairs providing food more consistently to the nest than like-morph pairs?
P: If black sparrowhawks are breeding as a mixed-morph pair, there is a lower variance in the time between prey deliveries.

Chapter 4.
H: Do offspring of mixed-morph pairs invest more into their immune function?
P: If nestlings have mixed-morph parents, they express higher innate immune function.

Alternative hypothesis: Heterozygote advantage. High fitness of offspring of mixed-morph pairs is due to an individual's genotype.

Chapter 5.
Reassessment of the mode of morph inheritance.

Chapter 6.
H: Does complementarity or heterozygote advantage explain morph stability in the black sparrowhawk?
P: If complementarity or heterozygote advantage are the driving mechanism, simulating data produces morph frequencies that match the empirical frequencies of the study population.

Figure 1.4. Flowchart explaining the previous key research findings that set the framework for this PhD and the resulting research questions. Hypothesis (H) and predictions (P) given.

Previous key findings and thesis overview

The population of black sparrowhawks on the Cape Peninsula has been closely monitored since 2000 and previous research has used mainly correlative analyses to identify potential mechanisms that result in colour polymorphism maintenance. To advance in our understanding of colour polymorphism maintenance in this population of black sparrowhawks, I use a mechanistic approach to explain the observed correlations (Figure 1.4):

Chapter 2: Foraging effort in the black sparrowhawk is associated with a temporal component. Dark morphs forage more and are more likely to bring food to the nest during the early and late hours of the day (when it is darker), whereas light morphs forage independently of time of the day but bring more food to the nest during midday (when light is brighter, Tate and Amar 2017; Tate et al. 2016). This is hypothesized to be due to the two morphs having different hunting success associated with ambient light conditions, probably due to improved background crypsis with dark morphs being better foragers under low light levels (or with a dark background) and light morphs under bright light levels. In this chapter, I set up an experiment to test whether avian prey (feral pigeons) vary in their reaction time when exposed to an attack of a taxidermy mounted black sparrowhawk (of both morphs) under varying conditions. I predict that due to a difference in camouflage that the prey's reaction time is delayed when the attacking hawk is a dark morph under low light conditions (or in front of a dark background) or a light morph under bright light conditions (or in front of a white background). A slower reaction time would lead to higher catch rates and improved foraging success that is morph- and ambient light-dependent under natural conditions.

Chapter 3: Although the high number of dark morph black sparrowhawks in the study area (Amar et al. 2013) imply a fitness advantage, dark morphs do not have higher fitness compared to light morphs (Tate et al. 2017). Only when a pair comes together to breed, their parental morph combination influences reproductive success and offspring quality: Pairs consisting of contrasting morphs (a light and a dark morph, termed "mixed-morph") have a higher chance to breed successfully than pairs consisting of the same morph ("like-morph"). The two individual morphs show strong indications to forage during different times of the day (Tate and Amar 2017; Tate et al. 2016) and as a result, mixed-morph pairs might behaviourally complement each other when foraging by expanding the (temporal) foraging niche within a pair. This is the basis for the complementarity hypothesis proposed by Tate et al. (2017). Alternatively, mixed-morph pairs might deliver more food to the nest under the avoidance-image hypothesis

(Rohwer 1983; Rohwer and Paulson 1987). Under both hypotheses, differences in reproductive success and offspring quality are due to a difference in prey delivery behaviour of the two morph combinations. Under the complementarity hypothesis, the benefit for offspring of mixed-morph pairs is assumed to be through receiving prey more consistently, whereas under the avoidance-image hypothesis, benefits would arise through receiving overall more prey.

Chapter 4: Previous research has shown that nestlings produced by mixed-morph pairs not only have higher survival rates than offspring of like-morph pairs (Sumasgutner et al. 2016a), they also have a significantly lower body mass index at nestling age (Tate et al. 2017). This is a surprising results, as lower body mass is generally considered as a sign of lower quality (i.e. Barrett and Runde 1980; Christe et al. 1998; Cleasby et al. 2010; Losdat et al. 2013; Morosinotto et al. 2020). One explanation for this discrepancy is that offspring of mixed-morph pairs are investing more resources in their innate immune function instead of somatic growth. In this scenario, nestlings of mixed-morph pairs would be able to fight off pathogens more successfully, thus lowering their mortality rates. In this chapter, I explore the link between differences in food supply (Chapter 2) and survival rates of nestlings of different parental morph combinations (Sumasgutner et al. 2016a). I combine a correlative study of innate immune function and an experimental immune challenge to identify the potential trade-off between somatic growth and physiological development. I predict offspring of mixed-morph pairs to show reduced levels of inflammation and express higher innate immune function. This would provide an explanation for the higher survival of offspring of mixed-morph pairs: Not physical development would be indicative of survival, but a nestling's development of its innate immune system.

Chapter 5 and Chapter 6: It is unclear whether the demographic variables that we observe in this system, especially those related to the complementarity hypothesis (Tate et al. 2017; Sumasgutner et al. 2016a), can explain the observed morph frequencies in this population. This can be achieved by using a simulation approach. I first re-evaluate the mode of morph inheritance which is an integral part of the simulation (Chapter 5). Then, I construct an individual-based model (Chapter 6) which is parameterized using empiric demographic values. I predict that while seasonality-associated conditions explain the numerical predominance of dark morphs, only the complementary nature of mixed-morph pairs or heterozygote advantage allow light morphs to persist over time. I simulate morph frequencies and compare the results to empirical data to determine if the complementarity hypothesis or heterozygote advantage are potential driving mechanisms of polymorphism maintenance in the system.

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Chapter 2

Response time of an avian prey to a simulated hawk attack is slower in darker conditions, but is independent of hawk colour morph

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Abstract

To avoid predation, many species rely on vision to detect predators and initiate an escape response. The ability to detect predators may be lower in darker light conditions or with darker backgrounds. For birds, however, this has never been experimentally tested. We test the hypothesis that the response time of avian prey (feral pigeon *Columba livia f. domestica*) to a simulated hawk attack (taxidermy mounted colour polymorphic black sparrowhawk *Accipiter melanoleucus*) will differ depending on light levels or background colour. We predict that response will be slower under darker conditions, which would translate into higher predation risk. The speed of response of prey in relation to light level or background colour may also interact with the colour of the predator, and this idea underpins a key hypothesis proposed for the maintenance of different colour morphs in polymorphic raptors. We therefore test whether speed of reaction is influenced by the morph of the hawk (dark/light) in combination with light conditions (dull/bright), or background colours (black/white). We predict slowest responses to morphs under conditions that less contrast with the plumage of the hawk (i.e. light morph under bright light or white background). In support of our first hypothesis, pigeons reacted slower under duller light and with a black background. However, we found no support for the second hypothesis, with response times observed between the hawk morphs being irrespective of light levels or background colour. Our findings experimentally confirm that birds detect avian predators less efficiently under darker conditions. These conditions, for example, might occur during early mornings or in dense forests, which could lead to changes in anti-predator behaviours. However, our results provide no support that different morphs may be maintained in a population due to differential selective advantages linked to improved hunting efficiencies in different conditions due to crypsis.

Introduction

Predation is a strong evolutionary force that will shape prey behaviour and consequently population dynamics (Caro 2005; Lima 1998; Lima and Dill 1990). The spatial and temporal distribution of prey in the landscape may be a function of both the distribution of resource and of predators (Brown and Kotler 2004; Cresswell 2008; Kotler et al. 2010; Laundré et al. 2001; Laundré et al. 2010). A prey's ability to detect a predator and decide on an appropriate response are important factors that will influence predation risk (Lima 1998; Ydemberg and Dill 1986). Many diurnal species rely primarily on vision to detect predators. This may be compromised by environmental factors, such as lower ambient light levels and the nature of the background cover (Bonter et al. 2013; Cerri 1983; Cooper 2000; Fitzpatrick 1997; Kacelnik 1979; Lima 1988; Lima and Dill 1990). Birds as such vision-dependent species are known to reduce activity in conditions where they are less able to detect predators (Carr and Lima 2014; Cerri 1983; Fernández-Juricic et al. 2012; Lima 1988; Rand et al. 1997).

Some habitats might also offer greater concealment for predators of varying phenotypes. Differences in concealment and detectability due to crypsis have been hypothesised for the maintenance of colour polymorphism in raptors (Galeotti and Rubolini 2003; Galeotti et al. 2003; Passarotto et al. 2018). Barn owl females (*Tyto alba*) show different habitat use, with reddish females occupying territories with less wooded areas compared to white females (Dreiss et al. 2012) and foraging success being dependant on moonlight (San-Jose et al. 2019). In the tawny owl (*Strix aluco*) rufous birds occupied more wooded territories than grey birds (Galeotti and Sacchi 2003), a pattern that might be driven by crypsis advantages for the different morphs. The hypothesis for a selective advantage of certain morphs under different environmental conditions has recently received some empirical support in the colour polymorphic black sparrowhawk (*Accipiter melanoleucus,* Lei et al. 2013; Tate et al. 2016). One of these studies found that nestling prey provisioning rates by the different morphs were dependent on light levels, i.e. light morphs provisioned more in brighter, and dark morphs provisioned more in lower (hereafter 'dull' or 'duller') light conditions (Tate et al. 2016). The mechanism behind this finding was also hypothesised to be linked to improved crypsis of the two morphs under different environmental conditions, resulting in higher hunting success and thus higher provisioning rates (Tate et al. 2016). However, other data from the black sparrowhawk study system provided less support for this idea, whereby dark morph black sparrowhawks forage more under dull light condition while light morphs did not show a light-dependent activity pattern (Tate and Amar 2017). This suggests that a dark morph bird's higher provisioning under dull light conditions might simply reflect increased foraging effort during these conditions, rather than any improvement in foraging success. However, it does also suggest that light morphs may be more successful at hunting in brighter conditions. The mechanism for any such cryptic advantage for light morph individuals under bright light conditions remains as yet untested.

In this study, we experimentally test the hypothesis that reaction time of an avian prey (feral pigeon, *Columba livia f. domestica*) to a simulated attacking avian predator (a taxidermy mounted black sparrowhawk) will differ depending on light conditions or background colouration, with the prediction being that speed of response will be slower under dull light conditions or with a darker background. Additionally, we test the hypothesis that response time will differ depending on the morph of the attacking hawk in relation to either light condition or background colour, with our prediction being that response time will be slowest where the hawk's plumage contrasts less with light levels or background colour (i.e. towards a light morph under bright light conditions or with a white background).

Material and Methods

The experimental trials were carried out in April and May ('control-hawk' and 'light level-morph' experiments), and June 2018 ('background-morph' experiment, a fifth trial was used for the 'light level-morph' experiment). In total, we caught 185 feral pigeons (hereafter: 'pigeons') on the Cape Peninsula, South Africa, which were then kept overnight at the experimental site in individual 50 x 30 x 30 cm cages. While in our care (for a maximum of 24 h), pigeons had access to water *ad libitum*, but were only fed during the experimental trials the next day.

We used four male black sparrowhawks (hereafter: 'hawk'), with two replicates per morph, which were taxidermy mounted in a flight position (Figure S2.1). They had the same wingspan (67 – 72 cm), body length (45 – 48 cm) and weight (250 g). The black sparrowhawks were previously collected during the monitoring of the study population on the Cape Peninsula, South Africa, with causes of death being unknown in all cases. Black sparrowhawks regularly prey on feral pigeons in our study area and analysis of prey remains have shown that about 28% of all prey items recorded are feral pigeons (Suri et al. 2017).

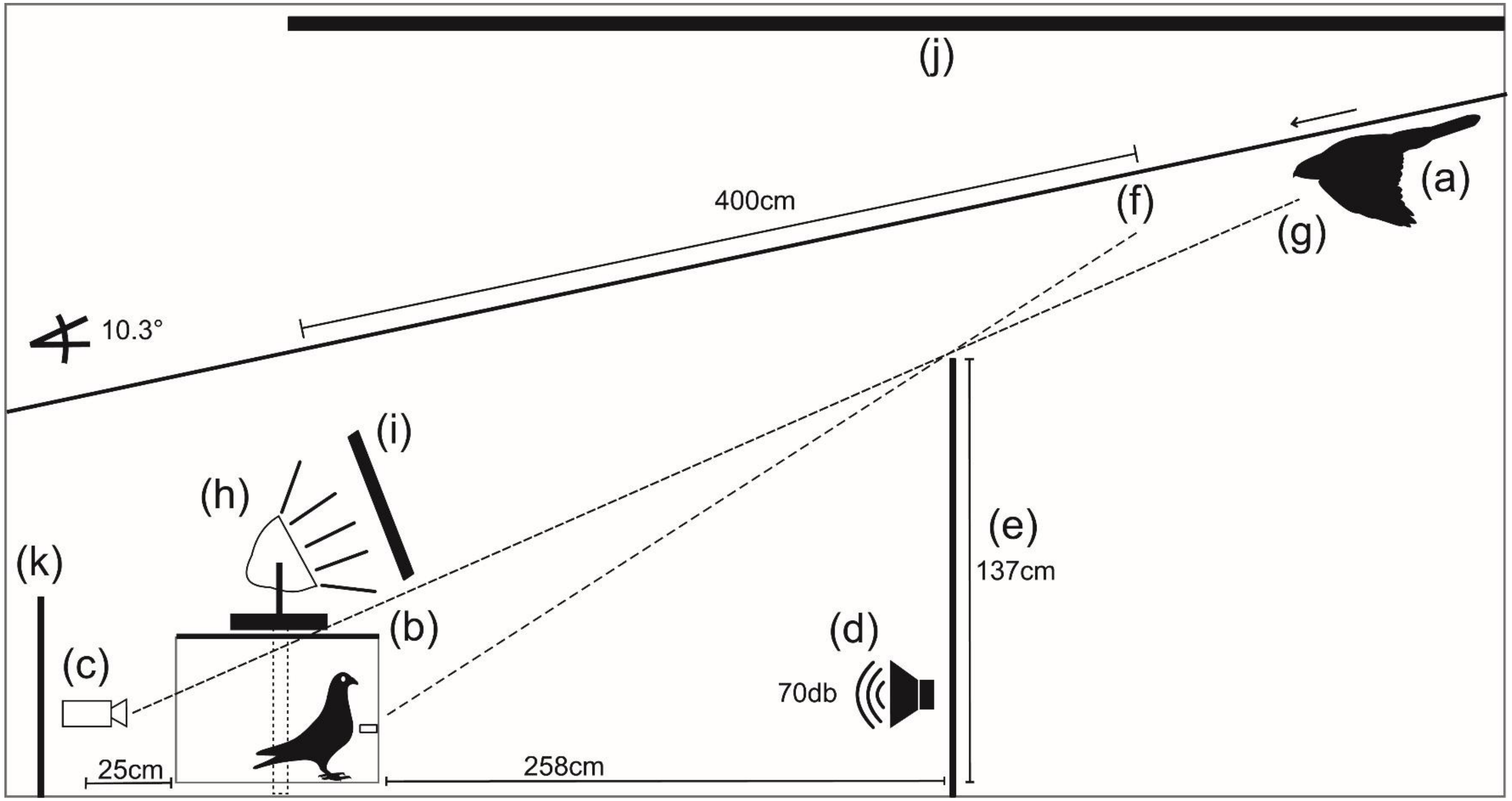

Figure 2.1. The experimental set-up. The hawk (a) was first visible to the pigeon (b) at point (f) and to the camera (c) at point (g). First visibility was standardized by a blind (e). Lamps (h) were dimmed with a black fabric for dull light conditions (i). Background colour was changed by spanning a black fabric above the set-up or using the white wall (j). We played white noise (d) and a second blind (k) was installed to block out the hawk to the pigeon after the attack.

To confirm that within our experimental set-up pigeons were able to recognize a predatory attack as such and did not only respond to a moving object per se, we used a control which was a squared fabric bag (Figure S2.2).

The experimental set-up was similar to other research (Cresswell et al. 2009; Cresswell et al. 2003; Whittingham et al. 2004) (Figure 2.1) and consisted of a hawk “flying” down a 12 m long and 10.3° angled line. A 137 x 120 m blind standardized the point of first visibility to the pigeon. The cage, containing a single pigeon, was placed in a fixed location, one meter beneath the line. Food was provided from a feeder at the front of the cage thereby standardising the pigeon’s position during each trial.

The experiment was set up indoors (room: 15 x 4 x 2.9 m) with a light source consisting of four lamps (Lightstar, Professional Lighting), angled to shed light on the on-coming hawk (Figure 2.1). The bulbs used were tungsten halogen incandescent lamps (Osram, 800 W, 240 V) that produce a continuous spectrum of light, including near UV light (zeiss-campus.magnet.fsu.edu). Our choice of positioning and angling of the lights was taken for several reasons: (1) to ensure that the lights did not result in direct glare to the pigeon that would have hindered its visual capabilities in detecting the hawk, (2) to ensure that the ventral area of the hawk was illuminated in a manner that made it most easily to distinguish the two morphs, which differ in colouration on their throat, breast, belly and underwing coverts (Amar et al. 2013). Alternatively placing the light behind the attacking hawk resulted in a backlit hawk silhouette that would have likely reduced the ability for the pigeon to differentiate between the two morphs. (3) Positioning the light on either or each side of the experimental set-up resulted in the moving hawk casting a shadow of the hawk onto the wall that was visible to the pigeon before the actual hawk. Currently, almost nothing is known how avian predators approach prey in relation to the sun, but based on the aforementioned considerations, we chose the present set-up as the most reasonable design to explore our hypotheses.

A high-speed camera (XDV 4K camera, 90 fps) was mounted in a fixed position to record time of first visibility of the hawk and response of the pigeon. Food for the pigeon (a seed mixture for wild garden birds) was placed in a specific food hopper at the front of the cage. After initiating feeding, we waited one minute before releasing the hawk, to prevent the pigeon from associating food with an attack. In case of an unsuccessful trial (i.e. the pigeon would not feed), we aborted the trial after 10 min and would repeat after a minimum 20 min break. 20 min was also the minimum time between every trial. Pigeons were exposed to a maximum of five trials, all conducted on the same day.

We created two light treatments: ‘bright light’, using four lamps on highest intensity (2182, SD = 65 lx); and ‘dull light’ with two dimmed lamps (112, SD = 12 lx). In comparison to a real-life situation, 112 lx would be comparable to the light during a very dark overcast day, i.e. as it is encountered in a thick forest or during the early or late hours of the day. The bright light situation, whilst considerably brighter, is comparable to an overcast day during noon (Table S2.1). This maximum light intensity was limited by the luminance output of our lamps and the heat production. Our dullest light intensity was limited by the capabilities of our camera to record interpretable images. Lux levels were measured at the blind with a digital multimeter (MS8229, Mastech). The same device was used to measure temperature (°C) at the pigeon cage after every trial. Between the first visibility of the hawk and when it passed over the pigeon, the mount covered a distance of four meters. We played white noise throughout the trials to block out any sound of the moving hawk mount (70 db white noise, measured at the pigeon cage).

The ‘light level-morph’ experiment consisted of a complete crossed design with four treatments (dark morph-dull light, dark morph-bright light, light morph-dull light, light morph-bright light) in randomised order. In all these trials the background behind the hawk was the white colour of the ceiling. Throughout these trials, we also randomly substituted the hawk for the control to confirm that pigeons recognized the hawk as a predator (by reacting quicker; ‘control-hawk’ experiment).

The ‘background-hawk’ experiment consisted of a complete crossed design (dark morph-black background, dark morph-white background, light morph-black background, light morph-white background). Background colour was changed by spanning a black fabric across the ceiling (Figure 2.1). These experiments were all done under dull light conditions, as we did not have the capacity to conduct these treatments under both dull and bright light levels. No control object was used in this second setup, but we added a random hawk-morph under bright light to increase the sample size of the light level-morph experiment and to have five trials per pigeon. We confirmed that the different hawk morphs were contrasting with the background colour by calculating the contrast ratio (relative luminance, for details see Table S2.2 and Figure S2.3 and Figure S2.4).

For each trial, we use the high-speed video to measure the duration from the moment the hawk came into view until two different responses by the pigeon: (a) detection time, the initiation of a head movement to face the attacking hawk; and (b) reaction time, the initiation of a physical escape response (in most cases, the pigeon would visibly tense and lower its body or escape into the back of the cage, Figure S2.5). These measurements represent different cognitive processes: detection time is the detection of an on-coming object, whereas the reaction time will incorporate the detection, the perception of a predatory attack and the decision to respond. We were unable to measure reaction time in 9.5% of all trials, because the pigeon was already looking directly in the direction of the on-coming hawk. Additionally, we removed trials from the experiment where no measurable escape reaction was initiated (8.8% of all trials, because the pigeon did not show a measurable reaction). Time stamps were recorded on the high-speed video in slow motion with the free software MPC-HC 1.7.13. Additionally, we measured the speed of the hawk and control by dividing the distance from the point of view to a fixed-point towards the end of the line by time (m/s).

Statistical Analysis

We fitted three different linear mixed models (LMM) using the 'lme4' package (v. 1.1-17; Bates 2010) in the software R, version 1.1.442 (R Core Team 2013). Our response variable was either (a) detection or (b) reaction time (both log-transformed), with the random term "pigeon ID" fitted to control for lack of independence among trials conducted on the same pigeon. In all models, we tested for an influence of i) speed of the hawk or control, ii) pigeon's head position (head down at the feeder or looking up), iii) temperature (°C) , iv) hawk replicate (1 – 4), v) daytime (hour) vi) number of trial (1 – 5) and vii) number of experimental set-up (1 – 3; the experiment was set-up in total three times). Final co-variates were chosen by stepwise backwards elimination, until only terms that were significant at $P < 0.1$ level remained.

Model 1 ('control-hawk' experiment) tested whether response times differed between a hawk and a control; the key explanatory variable was mount type (hawk or control). Model 2 ('light level-morph' experiment) explored whether response times changed under different light conditions, and whether this varied depending on the hawk-morph. In this model, we used all data with a hawk and a white background. Our key explanatory variables were light level (dull or bright light), hawk-morph (dark or light morph) and their interaction. Model 3 ('background-hawk' experiment) explored whether response times differed between differently coloured backgrounds, and whether this varied depending on the hawk-morph. Our key variables were background colour (white or black background), hawk-morph (dark or light morph) and their interaction. We then used the lsmeans function from the 'lsmeans' package (Lenth and Hervé 2015) to extract mean estimates (and 95% CI), plots were generated using 'ggplot2' (Wickham et al. 2019).

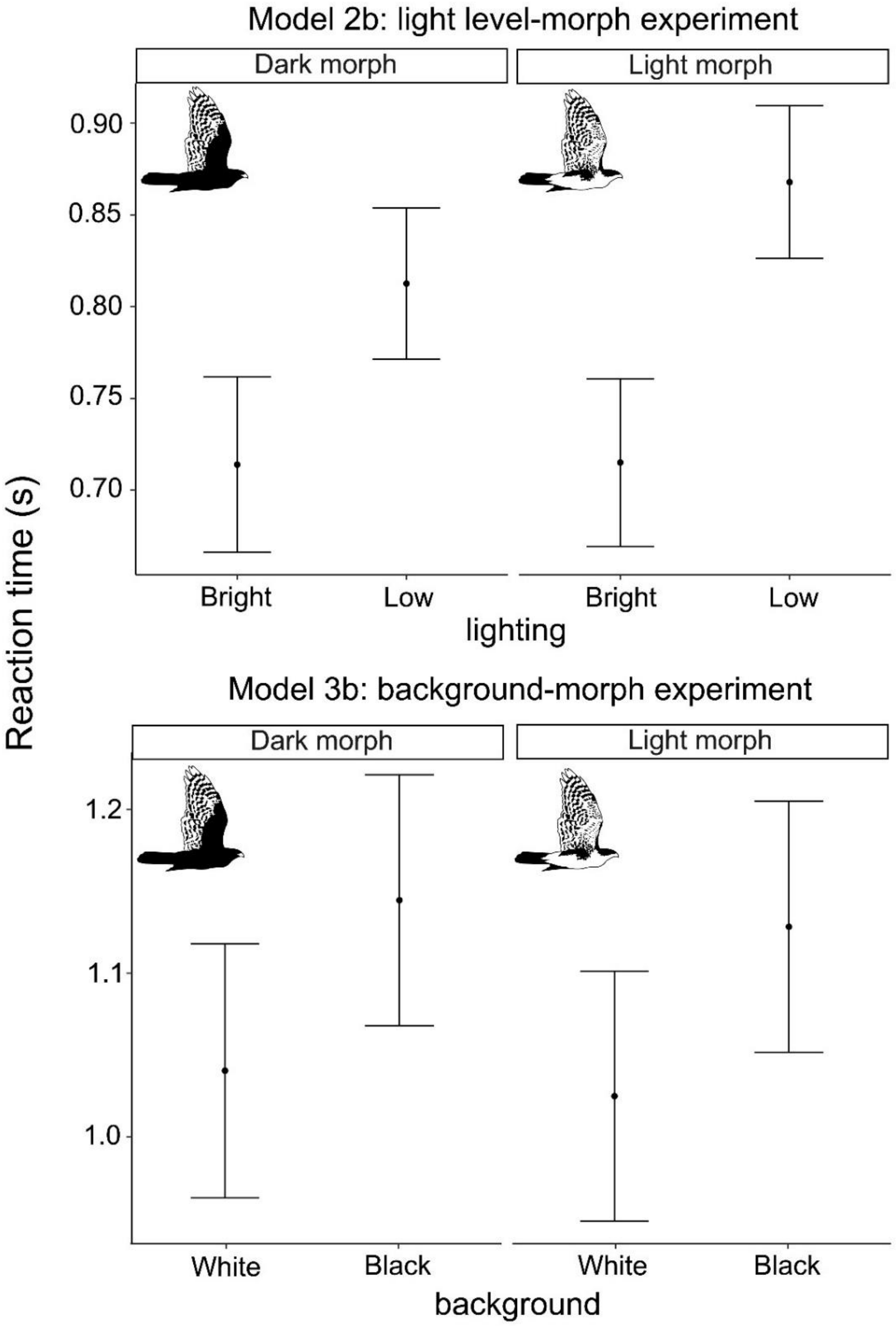

Figure 2.2. Reaction time (s) of pigeons to simulated attacks of mounts depending on light (Model 2b) or background (Model 3b). Figure based on fitted values of LMMs with 95% CIs.

Results

Pigeons detected and reacted slower to the control than to a hawk (in Model 1a and 1b, Figure S2.6), suggesting that they recognised the hawk as a predatory threat. They detected the approaching hawk and initiated an escape response later under dull light conditions and with a black background (Table 2.1 and 2.2; Figure 2.2). However, we found no significant interaction between hawk-morph and either light level or background colour (Table 2.1, Figure 2.2).

Pigeons detected and reacted significantly faster if they were looking up. Furthermore, we found a significant influence of the experimental set-up, a co-variate that was correlated with the speed of the approaching hawk. Pigeons reacted faster if the hawk was approaching with higher speed (Table 2.1). Temperature had a significant effect on the reaction time in the 'control-hawk' and 'light level-hawk' models with pigeons reacting faster if it was warmer. It was not significantly different in detection time (Table 2.1). Control variables time of the day, number of times the pigeon had seen the hawk or control ('number of trial') or the hawk replicate did not have a significant influence on detection or reaction time.

Discussion

Previous studies have shown that birds avoid foraging in lower light conditions (i.e. dusk and dawn), suggesting that this is a strategy to reduce predation risk (Cerri 1983; Kacelnik 1979; Lima 1988; Lima and Dill 1990). Our study provides experimental support for the hypothesis that response time will be slower under duller light conditions, thus resulting in higher predation risk in nature and providing an explanation why birds minimise foraging activity during early daylight hours (Kacelnik 1979; Lima 1988). Similarly, we found that response times were slower with a black background; further nurturing the hypothesis that birds are exposed to a higher predation risk in darker environments. Little research has been conducted on the influence of background colouration on speed of predator detection; so far, the focus has mainly been from the perspective of the predator. For example, background complexity is an important factor contributing to an increased foraging effort (Dimitrova and Merilaita 2009; Dimitrova and Merilaita 2011; Dimitrova et al. 2009; Kjernsmo and Merilaita 2012). In natural settings, the complex interplay of the light environment, background colouration, appearance and visual acuity will determine detection time and predation risk (Dimitrova and Merilaita 2014; Fernández-Juricic et al. 2012; Götmark and Unger 1994; Karpestam et al. 2013; Rojas et al. 2014; Seymoure et al. 2017) and ultimately shape anti-predator behaviours with light levels and background colours being a strong selective agent in birds.

If hunting success was closely correlated to predator detection speed by prey, then our results suggests that both morphs should catch disproportionally more prey in darker light conditions, which is not the case (Tate et al. 2016). Speed of predator detection might not be the only factor responsible for the probability of successful hunting and avian predators' low visual acuity under darker conditions might likewise limit their own foraging success (Potier et al. 2018).

Contrary to our predictions, we found similar response times towards the two black sparrowhawk morphs irrespective of light levels or background colour. The contrasting provisioning rates in relation to light levels in the two colour morphs were proposed to be due to improved crypsis (Tate et al. 2016). However, tracking data already cast doubt on this idea, showing that the two morphs expressed different foraging activities with dark morphs foraging more under darker conditions and light morphs equally over all light levels (Tate and Amar 2017). Thus, higher provisioning rates of dark morph individuals might simply reflect higher foraging effort and not a crypsis advantage under darker conditions. Increased foraging effort could also reflect a compensation for the hawk's own low visual acuity under darker conditions. For light morph hawks, a higher provisioning rate in brighter conditions (Tate et al. 2016) was not related to increased foraging effort (Tate and Amar 2017), although our results suggest it is not explained by improved hunting success through better crypsis either, since reaction time did not differ between morphs and light levels. Thus, the mechanism for the selective advantage of the two morphs remains unidentified. Physiological adaptation to different light or temperature conditions may be a reasonable, alternative explanation (Galván et al. 2018; Wilson 2013).

We did not find support that predator detectability could be a driver for the evolution of colour polymorphism in raptors (Galeotti and Rubolini 2003; Galeotti et al. 2003; Passarotto et al. 2018; Tate and Amar 2017; Tate et al. 2016). However, there are caveats to our experiment: crypsis works best if animals are motionless (Haller et al. 2014; Ioannou and Krause 2009). Thus, the crypsis advantage might only come into place when the hawk is perched (as suggested in a correlative study, Preston 1980), which our experiment would not reveal. Furthermore, we chose the position of the light source in a way that the morphs could be easily distinguished by the pigeon; however, predators might use different approach strategies in relation to the position of the sun (i.e. great-white sharks *Carcharodon carcharias,* Huveneers et al. 2015).

Nothing is known about morph-specific hunting techniques in the colour polymorphic black sparrowhawk. Lastly, the light we used is artificial and the interaction of prey's vision and environmental spectral reflectance under natural conditions (Lind et al. 2014; Tedore and Nilsson 2019) might be the crucial factor in the detection of different morphs in different habitats.

Despite finding for the black sparrowhawk that crypsis was neither morph- nor environmental-dependent, we still recommend similar experiments to be carried out in other study systems of colour polymorphic raptors to identify the drivers of adaptive colour polymorphism. For example, for both barn and tawny owls, a difference in crypsis under varying environmental conditions has recently been experimentally confirmed (San-Jose et al. 2019; Koskenpato et al. 2020). Thus, future experiments will likely produce promising results in other colour polymorphic raptors.

Conclusion

Our experiments show an effect of environmental conditions (light and background colour) on the response times of pigeons, thus providing support that birds experience higher predation risk in darker settings and environments. We did not, however, find support for morph-dependent detectability under different visual conditions.

Table 2.1. Results of Model 1, 2 and 3. Effect sizes of type of trial (either towards a hawk mount/control), morph of the hawk (light/dark), light (dull/bright), background (black/white) and an interaction between light/background with the hawk-morph, and co-variates speed (of hawk or control) or experiment set-up ID (experiment ID), head position of pigeon (down at the feeder or looking up) and temperature on (a) detection and (b) reaction time of pigeons. Sample size (n) for each model given. The LMER was fitted with a log-function. The key variables for each model are indicated in bold.

Model 1 – *control-hawk experiment*									
response variable:	reference category		(a) detection time, n = 451				(b) reaction time, n = 407		
fixed-effects		estimate	SE	χ2	P	estimate	SE	χ2	P
model type	**hawk**	**-0.060**	**0.013**	**22.33**	**<0.001**	**-0.141**	**0.018**	**61.24**	**<0.001**
pigeon head position	looking up	-0.185	0.011	300.15	<0.001	-0.170	0.014	152.14	<0.001
light	Low	0.039	0.008	22.40	<0.001	0.055	0.011	25.77	<0.001
experiment ID		0.044	0.023	3.73	0.053	0.145	0.031	22.51	<0.001
temperature						0.009	0.004	5.16	0.023
Intercept		0.272	0.047	33.00	<0.001	-0.108	0.097	1.23	0.267
Model 2 – *light level-hawk experiment*									
response variable:	reference category		(a) detection time, n = 598				(b) reaction time, n = 554		
fixed-effects		estimate	SE	χ2	P	estimate	SE	χ2	P
light	**dull**	**0.025**	**0.010**	**5.76**	**0.016**	**0.045**	**0.013**	**12.24**	**<0.001**
hawk-morph	**light**	**-0.001**	**0.011**	**0.01**	**0.960**	**-0.002**	**0.013**	**0.01**	**0.911**
pigeon head position	looking up	-0.171	0.010	321.47	<0.001	-0.168	0.012	192.91	<0.001
experiment ID		0.051	0.008	41.59	<0.001	0.117	0.011	120.03	<0.001
temperature						0.008	0.003	6.58	0.01
light*hawk-morph	**light morph*dull light**	**0.021**	**0.014**	**2.05**	**0.152**	**-0.175**	**0.070**	**1.24**	**0.265**
Intercept		0.196	0.020	96.00	<0.001	-0.175	0.070	6.29	0.012
Model 3 – background-hawk experiment									
response variable:	reference category		(a) detection time, n = 271				(b) reaction time, n = 254		
fixed-effects		estimate	SE	χ2	P	estimate	SE	χ2	P
background	**white**	**-0.030**	**0.014**	**4.84**	**0.028**	**-0.009****	**0.011****	**8.51****	**0.004****
hawk-morph	**light**	**0.012**	**0.014**	**0.68**	**0.412**	**-0.005**	**0.016**	**0.07**	**0.769**
pigeon head position		-0.127	0.015	74.58	<0.001	-0.085	0.018	22.78	<0.001
speed of the hawk		-0.078	0.039	4.12	0.042				

background*hawk-morph	**light morph*white background**	**0.001**	**0.019**	**0.00**	**0.998**	**-0.009**	**0.022**	**0.17**	**0.684**
Intercept		0.558	0.078	50.70	<0.001	0.407	0.012	1063.60	<0.001

** without interaction term

Table 2.2. Least square means of response times towards a control/hawk and under varying conditions. Displayed are lsmeans ± SE of key explanatory variables of main results, the list of other co-variates considered can be found Table 2.1.

	(a) detection time (s)		**(b) reaction time (s)**	
	lsmeans ± SE	**P**	**lsmeans ± SE**	**P**
Model 1: ***control-hawk*** **experiment**				
control	0.53 ± 0.03	**<0.001**	0.82 ± 0.04	**<0.001**
trial (hawk mount)	0.38 ± 0.02		0.54 ± 0.01	
Model 2: ***light level-hawk*** **experiment**				
dull light	0.47 ± 0.02	**0.016**	0.66 ± 0.02	**<0.001**
bright light	0.38 ± 0.02		0.56 ± 0.01	
Model 3: ***background-hawk*** **experiment**				
black background	0.66 ± 0.03	**0.028**	0.99 ± 0.03	**0.004****
white background	0.56 ± 0.03		0.90 ± 0.03	

** without interaction term

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MPC-HC 1.7.13 can be obtained here https://mpc-hc.org/ (13.2.2019)

Supplementary Material

Figure S2.1. One of the four black sparrowhawk mounts (a light morph) at the starting point of the line. All four mounts were in a flight/attack position and had similar dimensions.

Figure S2.2. The two control objects flown instead of hawk mounts to test whether pigeons differentiated a control object from a predator attack. We used two differently coloured controls, the pink fabric bag (left) and the blue fabric bag (right).

Table S2.1. Illuminance levels (lx) measured in natural settings as part of this study. Ambient light levels were measured with a luxmeter (MS8229, Mastech). Respective date is given. "Type of environment" relates to the type of canopy (i.e. no tree canopy indicates an open environment with no trees providing shade, light forest a more open canopy and strong forest a more closed canopy). The "weather condition" briefly describes the weather when the measurement was taken and "time" the time of the day. "Lx" gives the illuminance measurements obtained; a range indicates that multiple measurements under slightly varying conditions were taken.

date	type of environment	weather condition	time	lx
24.4.2018	no tree canopy	no overcast, direct sunlight	10:00 am	Higher than 40 000
24.4.2018	light forest canopy	no overcast	10:00 am	2000 – 5000
26.4.2018	open, no tree canopy	overcast, rain	10:00 am	3000
26.4.2018	light forest canopy	overcast, heavy rain	5:00 pm	300
28.6.2018	open, no tree canopy	overcast, no rain	4:00 pm	2000 – 2500
28.6.2018	strong forest canopy	overcast, no rain	4:00 pm	125 - 250
28.6.2018	light forest canopy	overcast, no rain	4:00 pm	100 – 1300
28.6.2018	open, no tree canopy	overcast, no rain	6:00 pm	180
28.6.2018	strong forest canopy	overcast, no rain	6:00 pm	12
28.6.2018	light forest canopy	overcast, no rain	6:00 pm	25
29.6.2018	open, no tree canopy	overcast, no rain	7:30 am	6
29.6.2018	open, no tree canopy	overcast, no rain	8:00 am	100
29.6.2018	open, no tree canopy	overcast, no rain	8:30 am	400

Contrast ratio between hawk morphs and backgrounds

We used the online tool based on the Web Content Accessibility Guidelines 2.0 (see contrast-ratio and WCAG10) to calculate the contrast ratio between the background and hawk colour in the RGB colour space, defined as the relative luminance (L). The highest ratio is obtained by plain black and plain white (L = 22) whereas a minimum score is reached by the same colours (L = 1). A high ratio therefore implies high colour contrast (and good visibility) whereas a low ratio indicates low colour contrast (better crypsis).

In our background experiment, we encounter two different light-background conditions: (1) dull light – white background and (2) dull light – black background. In the light-change experiment, we encounter two different light-background conditions; we used (1) bright light – white background and (2) dull light – white background.

First, we evaluated the consistency in space and time of the background colouration. It showed a high consistency where the hawk first came into view, therefore we chose one pixel of the background colouration at the beginning of every trial video as the background colour.

Second, the contrast of the hawk against the background was measured at four fixed points: first, the breast (one pixel at the front, one in the back) and, second, the underwing coverts (left and right side). These four points are representing areas of high plumage colouration differences between the morphs. Here we expect to see the differences of the contrast ratio between morphs to show a difference with light and dark morphs having a high contrast ratio against a black or white background, respectively, and low contrast values where the colour of the hawk matches the colour of the background.

Table S2.2. The mean contrast ratio (relative luminance, L) and its standard deviation of the two black sparrowhawk morphs (dark or light) against the background colouration under varying conditions during this experiment. For the 'light-hawk' experiment, attacks were simulated either under bright light – white background or dull light – white background. In the 'background-hawk' experiment, attacks were simulated either under dull light – white background or dull light – black background.

	morph	
conditions	**light**	**dark**
bright light – white background	1.35 (SD = 0.33)	7.56 (SD = 2.16)
dull light – white background	1.55 (SD = 0.34)	2.8 (SD = 0.09)
dull light – black background	5.49 (SD = 2.22)	1.20 (SD = 0.10)

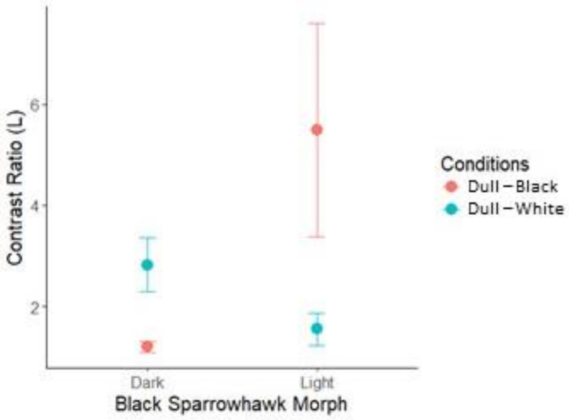

Figure S2.3. Contrast ratio of the ventral colouration of the light and dark black sparrowhawk morph against the background colour in the background-hawk experiment. The conditions are dull – black: dull light – black background and dull – white: dull light and white background. solid circles depict the contrast ratio (L) mean, error bars depict standard deviation. Mean and standard deviation were calculated based on four ventral point measurements per hawk (in total 20 measurements, ten per morph, four per hawk replicate).

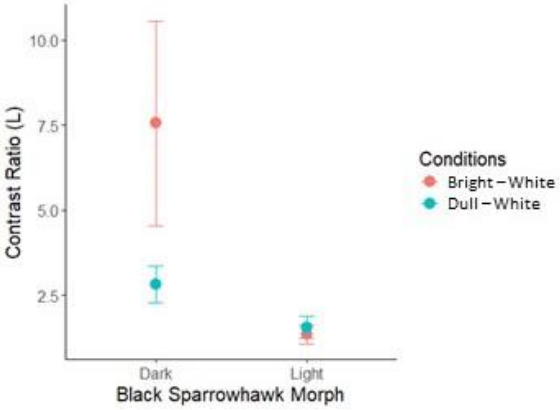

Figure S2.4. Contrast ratio of the ventral colouration of the light and dark black sparrowhawk morph against the background colour in the light-change experiment. The conditions are bright – white: bright light – white background and dull – white: dull light and white background. Solid circles depict the contrast ratio (L) mean, error bars depict standard deviation. Mean and standard deviation were calculated based on four ventral point measurements per hawk (in total 20 measurements, ten per morph, four per hawk replicate).

The result of the contrast ratio shows a high consistency between trials for the two morphs. These results validate our methodology to measure contrast in the RGB colour space.

The ventral side shows varying contrast ratios, depending on the background colour and the morph of the attacking hawk. The light morph shows a high contrast ratio when attacking in front of a black background, similarly we obtained high contrast ratio for a dark morph attacking in front of a white background. Low contrast ratio values were recorded for the dark morph attacking in front of a black background and for a light morph attacking in front of a white background (Table S2.2, Figure S2.3).

No such effect was found for the light-change experiment – where the background colour stayed the same and only the light condition was altered. The contrast ratio measurements show that the contrast is very high for the dark morph under bright light levels but evens out and becomes more similar to the contrast ratios of the light morph when the light level is decreased. No large drop of the contrast ratio is observed for the light morph, likely because the background colour and the colour of the hawk mount were similarly affected by a change of light conditions (Table S2.2, Figure S2.4).

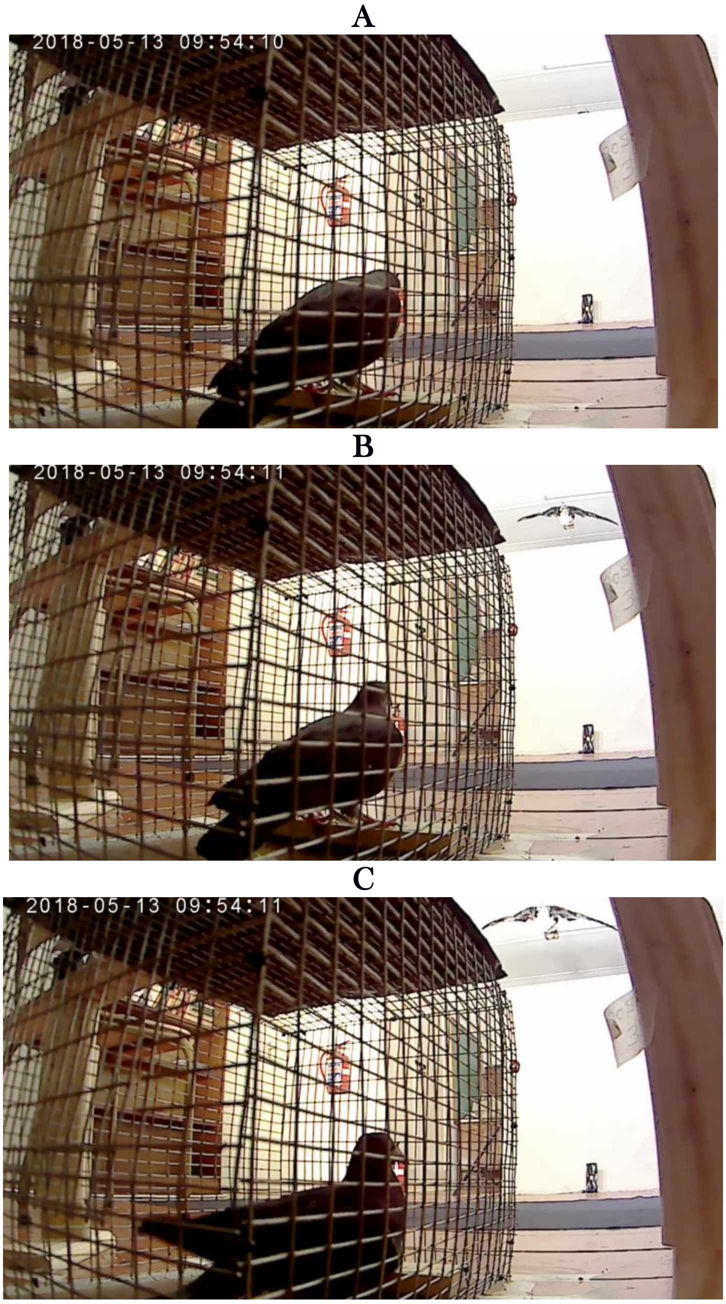

Figure S2.5. Example of a trial (with a white background, bright light levels and a light morph black sparrowhawk) with the three measuring points: In A, the pigeon is seen feeding during the trial and the hawk is barely visible over the blind. B shows the detection of the hawk by the pigeon (by raising its head from the feeder) and C is the initiation of an escape response (flight by foot into the back of the cage).

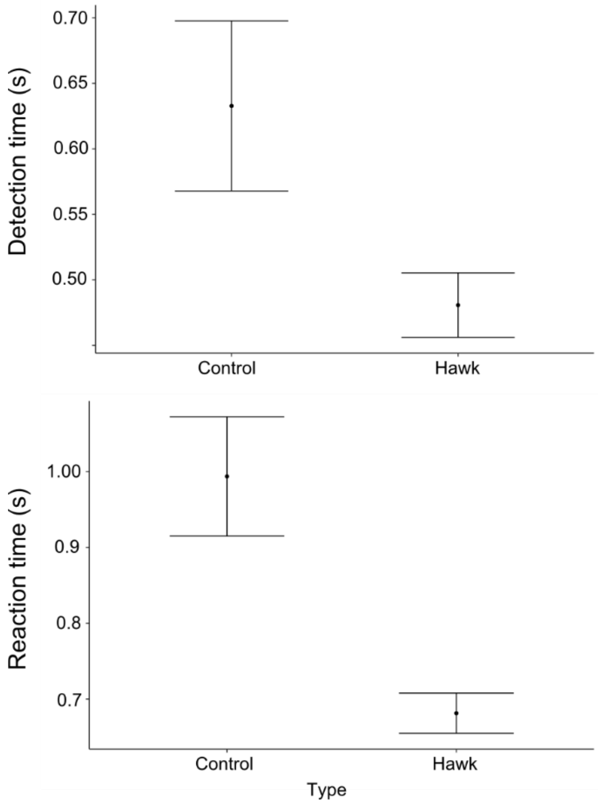

Figure S2.6. (a) Detection (top) and (b) reaction time (bottom) of pigeons to controls and hawks (attacks of black sparrowhawk mounts). Figure based on fitted values of LMMs with 95% CIs.

References

Online tool contrast-ratio: www.contrast-ratio.com (accessed 28.6.2019, 14:00)

WCAG10: Web Content Accessibility Guidelines 1.0 available at www.w3.org/TR/WAI-WEBCONTENT/ (accessed 28.6.2019, 14:00)

Chapter 3.

Contrasting parental colour morphs increase regularity of prey deliveries in an African raptor

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Abstract

Disassortative mating in colour polymorphic raptors is a proposed mechanism for the maintenance of colour polymorphism in populations. Selection for such a mating system may occur if there are fitness advantages of mating with a contrasting morph. In the black sparrowhawk (*Accipiter melanoleucus*), mixed-morph pairs may have a selective advantage, since they produce offspring which have higher survival rates. Two hypotheses, which may explain the mechanism, are the avoidance-image and complementarity hypotheses: The first suggests that within a predator's territory, prey develop a search-image for the more commonly encountered parental morph, for example, the male morph during incubation and brooding. Females of a contrasting morph to their partner would then have higher capture rates once they commence hunting in the later nestling phase. Thus, the avoidance-image hypothesis predicts higher provisioning rates for mixed-morph pairs. Alternatively, the complementarity hypothesis posits that different colour morphs exploit different environmental conditions, allowing mixed-morph pairs to hunt under a wider range of conditions and predicts that food is delivered more consistently. We test these hypotheses using nest cameras to record prey delivery rates during the late nestling phase when both parents are foraging. We found support for the complementarity hypothesis, with mixed-morph pairs delivering food more consistently, but not at a higher rate. This higher consistency in prey deliveries may explain the improved survival of the offspring of mixed-morph pairs and could therefore play a role in maintaining the stability of colour polymorphism in this system.

Introduction

Phenotypic variation has long been recognized for its importance in species' evolution (Darwin 1859). When this variability is expressed as two or more genetically determined colour variants that occur in the same population, which freely interbreed, it is called "colour polymorphism" (Huxley 1955). The presence of multiple morphs in the same population has challenged evolutionary ecologists for decades (Huxley 1955; Mayr 1942; Darwin 1859) since natural selection would be expected to favour the fittest morph and eradicate the less fit colour variants from a population (Darwin 1859), unless there is a selective advantage for all morphs under specific conditions that allows multiple colour morphs to co-exist (Huxley 1955; Antoniazza et al. 2010).

Disassortative mating has been proposed as a viable mechanism for the maintenance of colour polymorphism in birds of prey (Rohwer and Paulson 1987; Galeotti et al. 2003; Roulin 2004; Houtman and Falls 1994). One way such a mating system could develop is if pairing with a differently coloured partner provides a fitness advantage. Two key hypotheses have been proposed to explain why mixed-morph pairs may have such a selective advantage. The avoidance-image hypothesis was introduced by Rohwer and Paulson (1987) and builds on the foundations of the apostatic selection hypothesis (Clarke 1969; Paulson 1973; Payne 1967; Clarke 1962b). It states that a fitness advantage for rare morphs will arise, because prey will be more attuned to recognize the more common predator morph. As a result, prey will be easier to catch by the rarer predator morph. Rarer morphs would then be expected to increase in number due to this advantage until a frequency-dependent equilibrium is reached (Clarke 1962a; Clarke 1969; Paulson 1973; Payne 1967). Rarity in the apostatic selection hypothesis operates at the population level, whereby prey familiarize with the most frequently occurring predator morph at the population level. The avoidance-image hypothesis invokes the same mechanism as the apostatic selection hypothesis but at the scale of a pair's home range. Thus, the hypothesis proposes that within a mixed-morph pair's territory, prey would develop a search-image for the male's morph, as they would be more frequently encountered during the incubation and early nestling stage – when the female is not hunting (Sonerud et al. 2014). Mixed-morph pairs would then have a foraging advantage during the late nestling stage, when the female also starts hunting for prey, because prey within the territory would have less of a search images for the female's morph (Rohwer and Paulson 1987). As far as we are aware, no empirical test of this hypothesis has ever been conducted.

The complementarity hypothesis proposes a different mechanism to explain the higher reproductive success of mixed-morph pairs (Tate et al. 2017). It is based on the idea that different morphs complement each other because each morphs has a foraging advantage in different environmental conditions which may exist within the same area or during the same period of time. These conditions could relate to different levels of crypsis of the different colour morphs to their prey (Green 2005; Preston 1980; Passarotto et al. 2018; Tate and Amar 2017; Tate et al. 2016). For example, dark morph red-tailed hawks (*Buteo jamaicensis*) use perches in more densely structured environments compared to lighter coloured hawks, potentially due to the difference in their detectability by prey against different backgrounds (Preston 1980). In reddish egrets (*Egretta rufescens*), rufous individuals are more active foragers in shallow waters, whereas white birds hunt in a passive sit-and-wait fashion in deeper waters (Green 2005). Thus, a combination of water depth, plumage colouration and hunting mode may interact and determine individual behaviour in this species. In the red-tailed hawk and the reddish egret, colour polymorphism likely leads to a spatial difference in foraging success. A temporal foraging difference driven by ambient moon-light conditions is found in the nocturnal barn owl (*Tyto alba*): individuals with a white plumage have a foraging advantage under full moon whilst reddish owls experience a disadvantage (San-Jose et al. 2019). Light variability in the habitat has been linked to the expression of multiple colour morphs in owls (Passarotto et al. 2018) and a temporal partitioning of foraging activity might allow species to reduce intraspecific competition (Carothers and Jaksić 1984).

Recent research of the colour polymorphic black sparrowhawk (*Accipiter melanoleucus*) found that pairs of a dark and a light morph (mixed-morph pairs) had higher reproductive success than pairs of the same morph (like-morph pairs; Tate et al. 2017), and that their offspring had higher survival rates (Sumasgutner et al. 2016). The avoidance-image hypothesis and the complementarity hypothesis both offer potential explanations for the higher survival of the offspring (Sumasgutner et al. 2016) from mixed-morph pairs of the black sparrowhawk. The species shows morph specific behavioural differences which fit the assumptions of the complementarity hypothesis. First, different morphs have differential prey delivery rates depending on ambient light levels, with light morphs provisioning more prey during bright light conditions, whereas dark morphs provision more prey during low light conditions (Tate et al. 2016). In the course of the day, mixed-morphs pairs may experience conditions which alternately favour one morph over the other and thereby lead to more consistent prey provisioning compared to like-morph pairs. The 'avoidance-image' hypothesis is reliant on several key conditions described by Rohwer and Paulson (1987): prey must visually recognize the morphs, there must be a high likelihood for prey to experience attacks and escape to learn from them, and prey must be intelligent enough to remember the image of the attacking predator. Our black sparrowhawk system meets these key conditions; the two morphs are strikingly different visually, success rates of *Accipiter* raptors are relatively low (e.g. 16% for northern goshawks *Accipiter gentilis;* Rutz, 2006), and their avian prey – primarily Columbids, Suri et al., 2017 – are intelligent vertebrates capable of memorizing predator appearance and shapes Cook et al. 2005; von Fersen and Delius 1989).

In this study, we use prey deliveries to the nest to test the two competing hypotheses proposed to explain the higher reproductive success and higher offspring survival for mixed-morph pairs. The avoidance-image hypothesis predicts that prey delivery rates to the nest would be higher for mixed-morph pairs compared to like-morph pairs. Under this hypothesis the benefit for nestlings of mixed-morph pairs would come through receiving overall more prey. In contrast, the complementarity hypothesis predicts more consistent (not necessarily more frequent) prey delivery rates, in which case the variance in the time gaps between prey deliveries should be lower for mixed-morph pairs. A larger variance, on the contrary, would indicate that time gaps can be both short and long, suggesting that prey deliveries concentrate under specific conditions – as expected for like-morph pairs. Under the complementarity hypothesis, the benefit for nestlings of mixed-morph pairs is assumed to accrue through receiving prey more consistently.

Material and Methods

Study species and study area

Adult black sparrowhawks occur in two genetically distinct morphs, light and dark (Amar et al. 2013). The light morph ('L') is more common in most of its distribution range; however, on the Cape Peninsula (Western Cape, South Africa), about 80% of individuals are dark ('D') (Amar et al. 2014). The parental morph combination (hereafter 'parental morph combination' or 'pair morph') can consist of either the same morphs (D♂D♀ and L♂L♀; 'like-morph' pair) or contrasting morphs (D♂L♀ and L♂D♀; 'mixed-morph' pair). The black sparrowhawk population on the Cape Peninsula (34°00'S, 18°26'E), has been studied since 2001 (see Amar et al. 2013; Martin et al. 2014), with data for the present study being collected between 2012 and 2018. Territories were monitored each breeding season from March to November.

Parental morphs were determined from visits to territories and further confirmed from nest camera footage. Sexes are relatively easy to distinguish in the field by size with females being about 30% larger than males (Hockey et al. 2005; Ferguson-Lees and Christie 2001; Thiollay 1994). Broods were aged either by the hatching date as seen on camera; or back dated based on the state of development during ringing using reference photographs of known aged black sparrowhawk nestlings.

Black sparrowhawks display asymmetrical parental care, typical of most raptors (Sonerud et al. 2014). During the nestling period, female brooding progressively decreases as nestlings age, allowing her to hunt and provision more prey (Katzenberger et al. 2015). Thus, any expected benefit of mixed parental morphs is only predicted to occur once nestlings are old enough to thermoregulate (Newton 2010). More specifically, when nestlings are 14 days old, female brooding is reduced by approximately 50% (Katzenberg et al. 2015). Coinciding with this time, nestlings experience an increase of nutritional needs in the northern goshawk (Kenward 2012), a close relative of similar size (Kunz et al. 2019), thus 14 days of age is a critical time of a developing nestling's life and the role the mother plays in contributing food might be crucial for a nestlings' survival. In order to capture the later nestling rearing period when both parents are predicted to contribute by hunting, we examine provision rates when offspring were between 15 days to 42 days old (when the nestlings start branching and are fed outside the nest).

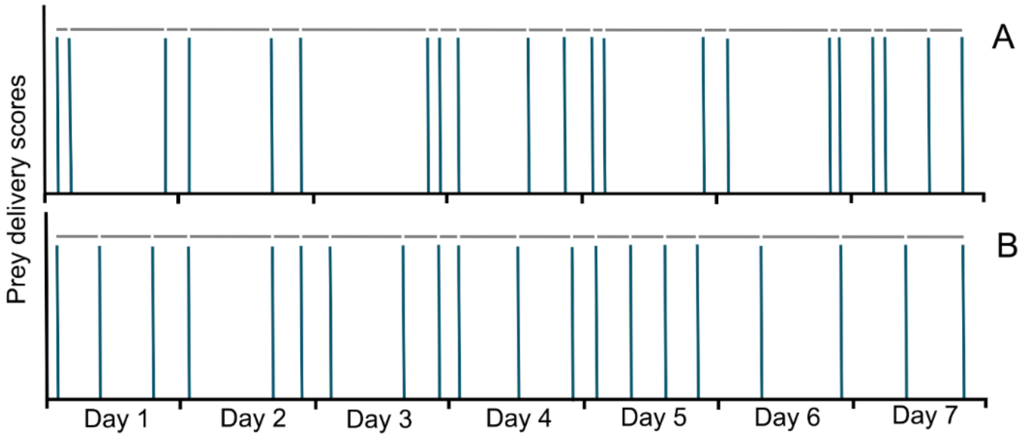

Figure 3.1. Theoretical illustration of how the variance of time gaps can differ depending on the timing of prey deliveries, irrespective of prey delivery rates. In both cases, 21 prey items per week are delivered. Vertical lines depict single prey delivery events, their spacing over time (grey horizontal lines) results in a large variance (A) and small variance (B) of time gaps. Time gaps are illustrated as horizontal grey lines above the gaps between prey delivery scores. This illustration is not based on actual prey delivery data.

Nest cameras and data processing

We used remote cameras at 34 nest sites (18 like-morph pairs, 16 mixed-morph pairs) to record prey deliveries to the nest in 2012, 2013, 2017 and 2018. Four different camera types were used: Ltl Acorn 6210MM, Ltl Acorn 5310A (Zuhai Ltl Acorn Electronics Co., Ltd, Zuhai City) and Bushnell E3 (Bushnell Co., Ltd, Overland Park, KS, USA) and set to take photos in a time interval between one and five minutes. A fourth camera model developed by the Royal Society for the Protection of Birds (Bolton et al. 2007) was activated by a motion trigger (for details on territory location and camera settings, see Table S3.1). Previous comparisons between time lapse and continuous recording showed that cameras set up with time lapse photos successfully recorded all prey deliveries (Tate et al. 2016). Cameras were mounted on a branch around one meter away from the nest, in a way that minimized the likelihood that adults or nestling would obscure the delivered prey items (McPherson et al. 2016; Reif and Tornberg 2006). All cameras started recording an hour before sunrise and stopped an hour after sunset. Only full days of recording were used and resulted in a total footage of 570 full days of footage showing 1608 prey deliveries.

We derived two different measures to quantify prey deliveries: First the number of prey items delivered per hour, and second, the time between two prey deliveries (in hours) to derive a ‘time gap’ variable based on two consecutive feeding events. For the ‘time gap’ variable we calculated this measure both including overnight time gaps and excluding these overnight time gaps. Black sparrowhawks on the Cape Peninsula has an extended breeding period lasting nine months (Martin et al. 2014), and dark morph males breed earlier in the season than light morph males (Tate et al. 2017). This might potentially introduce additional variation in the day length during which prey can be delivered, and, vice versa, in the length of night when prey would not be delivered. Although overnight time gaps reflect natural events where nestlings are without food, they are in other respects different from time gaps during the day, since energetic expenditure is much lower (Weathers and Sullivan 1991) and feeding is unlikely to happen during the night (Kenward 2012). We therefore calculated time gaps based on the amount of daylight only, by removing overnight time gaps. For instance, a 24-hour time gap between two prey deliveries with a 10-hour overnight time period, translates into a 14-hour time gap. Our main measure used in our analysis was the variance in these time gaps (excluding night gaps), hereafter termed ‘variance’. However, we additionally calculated the variance including overnight gaps (always explicitly referred to as ‘variance including night gaps’) to evaluate the strength and sensitivity of any results to this aspect. These two measurements (i.e. variance with and without night gaps) were significantly correlated (repeated measures correlation = 0.67, $P < 0.001$, $N = 98$, Bakdash and Marusich (2017), see Figure S3.1), suggesting that removing night gaps did not result in a profound alternation of the data. Variance of time gaps can differ at the same prey delivery rate, with more clustered prey deliveries resulting in a larger variance of time gaps (Figure 3.1A) in comparison to a low variance of time gaps with prey deliveries being evenly spread out (Figure 3.1B).

We summarized data on delivery rates on a weekly basis, to quantify the total number of feeding events and the variance in the mean time gaps for each week. As black sparrowhawks frequently deliver only one or two prey items to the nest during one day and at least three prey items are needed to calculate the variance of 'time gaps', summarizing the data on a weekly basis allowed us to use all the available data. The 570 full days of footage resulted in 98 weeks of data (52 from like-morph and 46 from mixed-morph pairs (see Table S3.1). The prey delivery rate was obtained by dividing the total number of feeding events per week by the total amount of daylight hours and thus results in a rate of prey deliveries per hour.

Statistical analyses

We used linear mixed models (LMM) fitted with the package 'lme4' (v. 1.1-17; Bates et al. 2015) within the R environment (version 3.4.0, R Core Team, 2016) to explore whether parental morph combinations were associated with differences in either prey delivery rates or variance in time gaps for each week. In the first model exploring the hourly delivery rates, we used the 'rate' (items/daylight hour) as a response variable. In the second model, we explored the differences in the weekly variance in time gaps between feeding events (including and excluding night gaps) as the response variable. Both the variance variables were square root transformed to ensure normality for use in LMM. Not all weeks consisted of a full seven days of camera footage. To account for our measurements being less well estimated in weeks with fewer days, we weighted our analysis by using the 'weights' option in the model and fitting as a weighted term the square root of the number of observation days for each week (1 – 7). We used the square root of observational days since the robustness of the estimates is unlikely to continue to increase in a linear manner with increasing number of observational days. In all models, we controlled for brood size (continuous variable: 1 – 3) and nestling age (continuous variable: 3 – 6 weeks) as covariates since these aspects could potentially influence prey deliveries. Furthermore, for the additional model exploring the variance of time gaps 'including night gaps' we also added the mean amount of daily daylight hours as a covariate, to control for the amount of time available for prey deliveries. Year and a unique identifier for each nest were fitted as a nested random term to control for the repeated observations of the same nest over different weeks and also for observations on the same territory between years. Additionally, we fitted the interaction of parental morph combination and nestling age to explore differences in prey delivery rates and variance in time gaps when nestlings grew, and their energetic demand increased. In one case, we observed a brood size reduction during the observational period from three to two nestlings (territory number “12” in 2013, D♂L♀, nestlings were 19 days old). In this case, the brood size changed from three (at nestling age week 3) to two (at nestling age week 4) in the analyses. No information is available for the cause of death of this nestling.

The explanatory variable of greatest relevance to our hypotheses in each model was the parental morph combination (mixed-morph or like-morph pairs). In subsequent examination, we undertook some further analysis to explore whether any association between the pair-morph combinations was also linked to the sex of the different morphs. For this additional analysis we used the same models as above, but instead of the 2-level pair morph combination, we fitted a 4-level pair morph combination which incorporated the sex of the different morph. We assessed the significance of explanatory terms using their partial (Type III) significance values (likelihood-ratio χ^2 tests). We then used the lsmeans function from the ‘lsmeans’ package (Lenth and Hervé 2015) to determine pairwise contrasts and to obtain mean estimates (and 95% CI) for each category.

Results

Prey delivery rates and variance in relation to parental morph combinations

On average, pairs provided 0.26 (SD = 0.09) prey items per daylight hour, equivalent to approximately one prey item every four hours of daylight. We found no difference in the overall hourly delivery rate between mixed-morph and like-morph pairs (Table 3.1, Figure 3.2A). Delivery rate was significantly negatively associated with nestling age, with less prey items delivered to older nestlings, but did not differ between morphs (P = 0.22) and was unrelated to brood size (Table 3.1, Figure 3.3). Irrespective of the parental morph combination, black sparrowhawks showed two distinct peaks in prey deliveries, with the first peak occurring with sunrise and the second peak occurring around an hour before sunset (Figure 3.4).
However, in contrast to delivery rates, we found significant differences in the variance of time gaps between the parental morph combinations, with more consistency in time gaps for mixed-

morph pairs (Table 3.1, Figure 3.2B). Variance was also significantly associated with nestling age and brood size, with greater variance for older nestlings and greater variance in smaller brood sizes (Table 3.1, Figure 3.3). Although variance in time gaps increased when nestlings grew older, this did not differ between the parental morph combination (P = 0.37). We confirmed that the key result was not simply attributable to differences in the night length, by reusing variance measures, which included overnight time gaps. These analyses produced materially similar results with mixed-morph pairs having less variance in the timing of their prey deliveries (Estimate: -0.68, SE = 0.41, $\chi 2$ = 2.79, P = 0.10, Table S3.2).

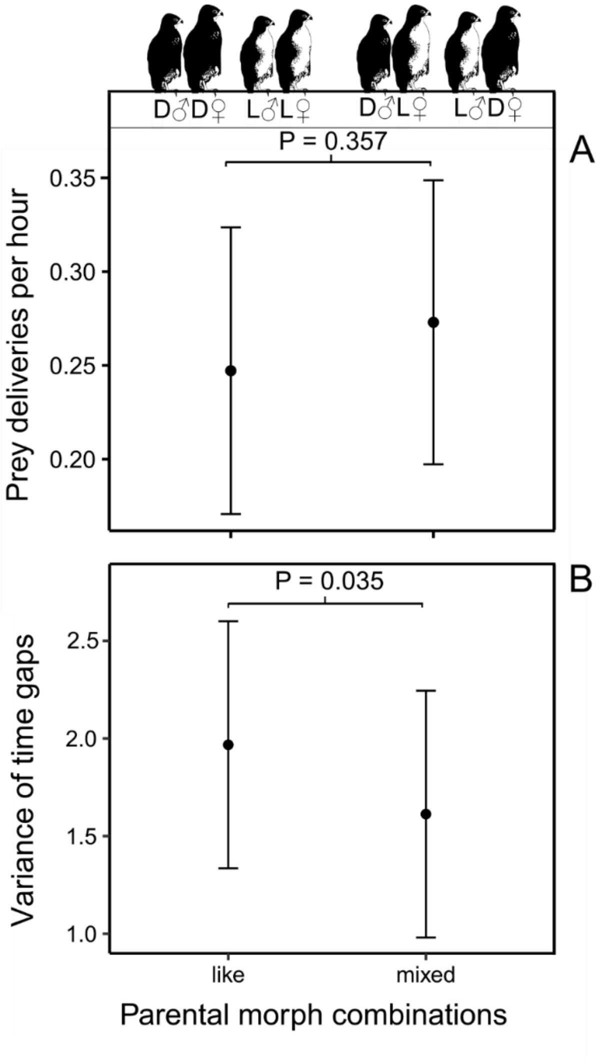

Figure 3.2. (A) Prey delivery rate per hour and (B) variance of time gaps (excluding overnight gaps) for the parental morph consistency: 'like'-morph and 'mixed'-morph. Like-morph pairs consist of a pair that exhibits the same colour morph (both light or dark) whereas mixed-morph pairs have contrasting morphs. Data are the least squared means ± 95% CI taken for the linear mixed model output.

Table 3.1. (A) Factors influencing prey delivery rates per hour and (B) Factors influencing the variance of time gaps (excluding overnight gaps) between feeding events. Morph consistency (either mixed- or like-morph) is the key explanatory variable. Output of linear mixed-model with the covariates ‘age of the nestlings’ (in weeks, 3 – 6) and ‘brood size’ (1 – 3). The ‘variance of time between feeding events’ model was weighted by the square root number of observation days (1 – 7). Significant variables are indicated in bold; total sample size was 98 weeks of data.

A. Prey delivery rate per hour					
fixed effect	**reference category**	**estimate (± SE)**	**χ2**	**df**	**P**
morph consistency	mixed	0.023 (± 0.025)	0.847	1	0.357
age of the nestlings	**-**	**-0.034 (± 0.009)**	**13.241**	**1**	**< 0.001**
brood size	-	-0.001 (± 0.017)	0.001	1	0.975
Intercept	**-**	**0.268 (± 0.045)**	**36.026**	**1**	**< 0.001**
B. Variance of time between feeding events (time gaps)					
morph consistency	**mixed**	**-0.442 (± 0.209)**	**4.462**	**1**	**0.035**
age of the nestlings	**-**	**0.361 (± 0.133)**	**7.346**	**1**	**< 0.001**
brood size	-	-0.230 (± 0.158)	2.120	1	0.145
Intercept	**-**	**3.168 (± 0.418)**	**57.310**	**1**	**< 0.001**

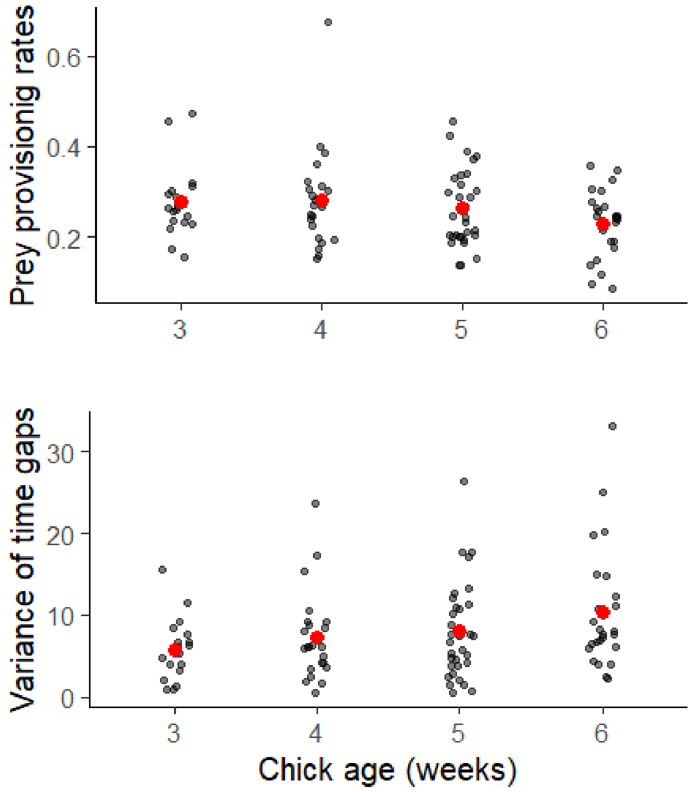

Figure 3.3. Change of prey delivery rates (top) and the variance in time gaps (excluding overnight time, bottom) in relation to nestling age (in weeks). Recorded chick age in this study ranges from three weeks to six weeks and interaction between pair morphs (like- or mixed-morph) was not statistically significant (prey provisioning rate: χ2 = 1.53, P = 0.22; variance in time gaps: χ2 = 0.82, P = 0.37). Dots present raw data values, red dot mean value.

The 4-level factor pair morph combination did not show a significant difference for either hourly prey delivery rate (χ2 = 1.96, P = 0.58, Figure S3.2, Table S3.3) nor the variance of time gaps (χ2 = 4.98, P = 0.17, Figure S3.3, Table S3.4). However, the results were consistent with our previous 2-level analysis (i.e. like- vs mixed-morph pairs) whereby the variance was lower for the D♂L♀ and L♂D♀ pairs as compared to the L♂L♀ and D♂D♀ pairs (Figure S3.3, Table S3.4). When performing the same analysis on the variance including time gaps, we found the trend effect between like-morph and mixed-morph to be driven by the difference between D♂D♀ and D♂L♀ pairs (Estimate: 0.65, SE = 0.41, P = 0.08, Figure S3.4, Table S3.5).

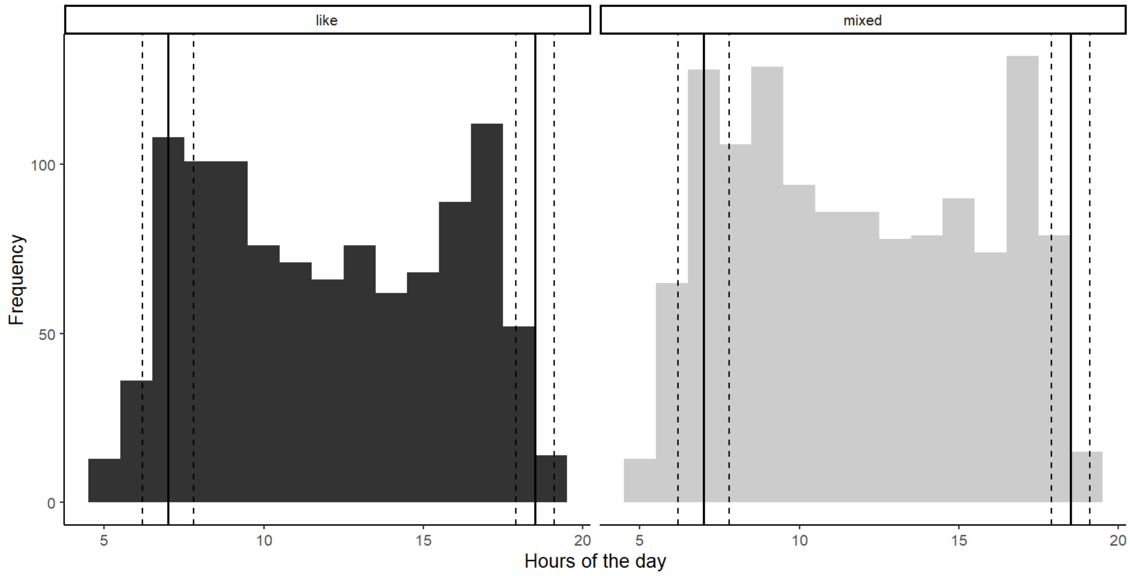

Figure 3.4. Frequency and timing of prey deliveries of 'like-' and 'mixed-morph' black sparrowhawk pairs over the course of the day. Black vertical lines indicate mean time of sunrise and sunset with dashed vertical lines indicating standard deviations of sunrise/sunset. Prey provisioning by both parental morph combinations showed peaks at sunrise and just before sunset.

Discussion

We found lower variance in time gaps of prey deliveries for mixed-morph pairs, providing support for the complementarity hypothesis proposed to explain the higher reproductive success for mixed-morph pairs (Tate et al. 2017) and the subsequent higher survival rates of their offspring (Sumasgutner et al. 2016). The complementarity hypothesis predicts that a mixed-morph pair is better able to exploit a wider range of environmental conditions for successful foraging and thereby provide prey at more regular intervals. In this system, the different environmental conditions which the two morphs may be able to exploit are thought to relate to varying ambient light conditions (Tate et al. 2016; Tate and Amar 2017). Although the natural daily rhythmic changes in light conditions are predictable, weather conditions also influence light levels, but in a less predictable manner. A mixed-morph pairs' ability to exploit a wider range of conditions than a like-morph pair could buffer against longer periods of similar conditions, i.e. during which foraging conditions are sub-optimal for one morph type, and temporal partitioning of foraging activity of a pair might also reduce the overall competition at foraging sites within the territory (Carothers and Jaksić 1984). Thus, nestlings of mixed-morph pairs might overall be less likely to experience undernourishment.

It is likely that a more consistent and predictable food supply will confer additional advantages for nestlings of mixed-morph pairs. For example, lower energy expenditure due to a decrease in begging (Moreno-Rueda and Redondo 2012) and a reduction of sibling competition or aggression during feeding (Ricklefs 1982; Bortolotti 1986). Additional indications that more regular feeding provides an advantage to growing nestlings, comes from differences in growth rates between hand-reared captive nestlings in comparison to nestlings raised by their parents (i.e. Wolf and Kamphues 2003; Bird and Laguë 1982; Whitmore and Marzluff 1998), a pattern which might be due to a difference in feeding frequency. In a cross-species comparison of hand-reared corvids, Whitmore and Marzluff (1998) found that higher feeding frequency allowed some, but not all study species, to increase their total food intake in comparison to conspecifics that were fed at a lower frequency. They also expressed less fault bars in their feathers, an indication of malnutrition or stress (Jovani and Rohwer 2017) and had a lower chance to survive after being released to the wild (Whitmore and Marzluff 1998). Unfortunately, long-term effects of feeding intervals are rarely evaluated in wild species and we have limited existing knowledge of whether a higher consistency in food provision, as seen in this study, could explain some of the fitness benefits known for mixed-morphs pairs in our study system.

In a somewhat contrasting result, Tate et al. (2017) found that the body mass index of black sparrowhawk nestlings from mixed-morph pairs was significantly lower than nestlings of like-morph pairs. Generally, a low body mass index is associated with lower quality in nestlings (i.e. Barrett and Runde 1980; Lima 1986; Christe et al. 1998; Cleasby et al. 2010; Magrath 1991), but for some animals, infrequent feedings of larger meals can encourage an increase of fat deposits where excess energy is stored (Cohn 1963; Yu et al. 1990). Thus, a more predictable food supply as seen in mixed-morph pairs could result in a lower body mass index, whereas its unpredictability could result in a higher body mass index. Consequently, the more constant rate of food supplied by mixed-morph black sparrowhawk pairs may allow the nestlings to invest more into body and feather growth or physiological and immunological functions, than just muscle or fat. However, some bird species can also loose body mass when facing an unpredictable food supply (i.e. Acquarone et al. 2002; Cucco et al. 2002; Boon et al. 1999; Jönsson et al. 1999; Fokidis et al. 2012; Granadeiro et al. 1998). A more detailed examination of nestling's growth rates, their physiological and immunological functions and post-fledging dependency period, are necessary to identify the detailed mechanisms involved in a selective advantage for offspring of mixed-morph pairs in our study system.

The results from our study did not support the avoidance-image hypothesis, although its key conditions are fulfilled in our study system. To our knowledge, our findings represent the first empirical test of this hypotheses at outlined by Rohwer and Paulson (1987). Rohwer and Paulson (1987), however, did lay out some other pre-condition, with one being the most noteworthy and it relates to the ability of prey to remember predator morphs. Feral pigeons are capable of remembering a large numbers of images (Cook et al. 2005) and, most importantly, they are also capable of recalling them over long periods of time (von Fersen and Delius 1989). Hence, the absence of an incubating and brooding female in the territory for approximately two months (Katzenberger et al. 2015), is unlikely to result in a foraging advantage under the avoidance-image hypothesis.

A possible limitation of our study is the incomplete knowledge on how much females are actually contributing to the hunting during the nestling rearing period. Although nest camera footage show that females reduce their brooding effort as nestlings age (Katzenberger et al. 2015), this gives little indication if the female actually goes off hunting. GPS tracking data suggests that male foraging effort declines during the fledgling period (Tate and Amar 2017), which could indicate that hunting is shared with the female. In the northern goshawk, the female contributed between 9 to 29% of all prey items caught (Schnell 1958; Good et al. 2001; Kenward 2012), whereas in other populations, the female does not hunt at all (Brown and Brown 1979; Hockey et al. 2005). Similar observations were made in Eurasian sparrowhawks (*Accipiter nisus*, Newton 1978; Eldegard et al. 2003). This indicates a large difference in female hunting contributions during the nestling phase that might be driven by environmental conditions (Newton 1986), by individual variation or physical condition (Eldegard et al. 2003; Kelly and Kennedy 1993) or to compensate unsuccessful foraging trips of the male (Simmons

1986; Newton 1986). Unfortunately, our camera footage does not provide information on which sex caught the prey item, because food is usually transferred from the male to the female before being delivered to the nest. Ideally, future research will include the radio-tracking of both parents to explore how the two sexes forage in combination with each other.

Average prey delivery rates recorded in our study were similar to those reported for other *Accipiter* species of similar size (e.g. northern goshawk: 0.33 – 0.40 prey items per hour, Rogers et al. 2006) and *Accipiter* species living in sympatry with the black sparrowhawk (red-breasted sparrowhawk *Accipiter rufiventris*: 0.45, Simmons 1986). Furthermore, our results revealed interesting diel patterns: prey deliveries peak in the early morning and again around an hour just before sunset and was seen for both like- and mixed-morph pairs. This pattern might reflect the predominance of dark morphs in the population (Amar et al. 2013) which are known to provision food under darker conditions (Tate el al. 2016). This pattern might also reflect a higher foraging success that might occur at this time of day due to typical *Accipiter* activity patterns (see i.e. Rutz 2006; studying urban northern goshawks) or due to prey being less well able to detect predators in lower light levels (Nebel et al. 2019, Chapter 2).

We found that as nestlings aged, prey delivery became less frequent and the variance in time gaps increased. A similar higher prey delivery rate for younger nestlings was observed in northern goshawks (Rogers et al. 2006) and other *Accipiter* species like the Eurasian sparrowhawk (Eldegard et al. 2003; Frumkin 1994), brown goshawk (*Accipiter fasciatus*, Aumann 2016) and red-breasted sparrowhawk (Simmons 1986). Possibly, this higher prey provisioning does not reflect higher foraging success when nestlings are young. Younger nestlings have small crops and thus females may therefore cache leftover food and delivery repeatedly from one item (Siewert 1933; Schnell 1958; McDonald 2004). Using nest cameras, it is difficult to distinguish between new, but partly consumed, and previously cached prey items as sparrowhawks decapitate and pluck prey before bringing them to their nest (Kenward, 2012).

Conclusion

Our study provides one explanation on how colour polymorphism can be maintained within a population by a foraging advantage of a pair consisting of two contrasting morphs. The complementarity hypothesis provides an explanation why mixed-morph pairs raise offspring that have better survival rates. The complementarity hypothesis suggests that mixed-morph pairing should have a selective advantage and thus should favour disassortative mating in polymorphic raptors. However, within our system no evidence has been detected for disassortative mating and pairs appear to choose partners independent of their own and their partner's colour morph (Tate et al. 2017). This could be due to a very young population inhabiting the Cape Peninsula, since it was only colonized in the last 30 years (Oettlé 1994; Curtis et al. 2007). Thus, the selective pressure for disassortative mating patterns might still take some time to become established. Future research, using integrated modelling of the different fitness parameters of the different morph combinations and their offspring, may reveal the explanation for the numerical dominance of the dark morph black sparrowhawks in this population and specifically whether the fitness advantages of mixed-morph pairs could be sufficient to maintain colour polymorphism in this system.

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Supplementary Material part 3.1

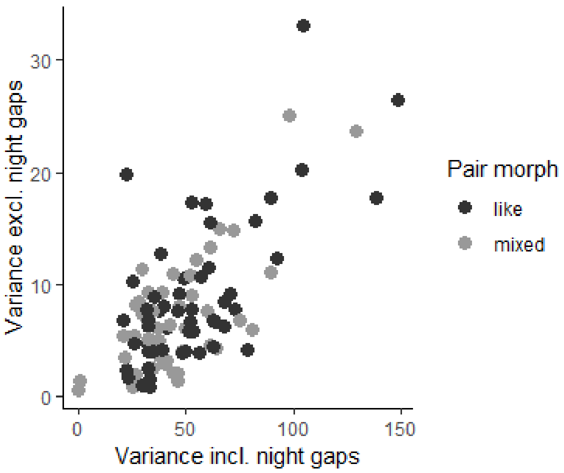

Figure S3.1. Relation of variance of time gaps including night gaps (x-axis) and variance of time gaps excluding overnight gaps (y-axis). One dot represents one week of observational data of chicks being 2 – 6 weeks old, the colours indicate whether it was collected from a like-morph (light grey) or mixed-morph pair (dark grey).

Table S3.1. List of territories with installed nest cameras. 'Territory' is a unique nest identifier where the nest camera was installed, 'year' indicates when the footage was collected. Latitude and longitude coordinates indicate the approximate nest location. We differentiate between the following recording types: motion triggered and time lapse between one to five minutes. The morphs are listed as male and female morph (light or dark) and in their pair morph consistency (like- or mixed-morph pair). The month the eggs were laid was backdated from the age of the chicks at ringing. The recorded age of the chicks on camera in the 'chick ages recorded' column is given in days. The brood size ranges between 1 (one chick) and 3 (three chicks).

nr	territory	year	latitude	longitude	recording type	female morph[1]	male morph[2]	pair morph	lay month	chick ages recorded	brood size
1	nest 1	2012	-34.00	18.45	motion triggered	dark	light	mixed	May	15-42	1
2	nest 2	2012	-34.15	18.42	time lapse 4 min	dark	light	mixed	Jul	27-42	1
3	nest 3	2012	-34.00	18.45	motion triggered	dark	dark	like	Apr	26-42	2
4	nest 4	2012	-33.96	18.45	motion triggered	dark	dark	like	May	17-38	3
5	nest 5	2012	-33.97	18.45	time lapse 5 min	dark	dark	like	Jun	22-31	2
6	nest 6	2012	-33.95	18.46	motion triggered	dark	dark	like	Jul	15-41	2
7	nest 7	2012	-34.01	18.35	time lapse 3 min	dark	light	mixed	Sep	21-37	3
8	nest 8	2012	-34.06	18.42	time lapse 3 min	light	light	like	Oct	15-42	2
9	nest 9	2013	-34.01	18.44	time lapse 2 min	dark	light	mixed	Aug	15-42	3
10	nest 10	2013	-34.10	18.39	time lapse 2 min	light	dark	mixed	Jun	15-23	2
11	nest 3	2013	-34.00	18.45	time lapse 2 min	dark	dark	like	Mar	15-38	1
12	nest 11	2013	-34.14	18.34	time lapse 2 min	light	light	like	Sep	15-40	1
13	nest 12	2013	-34.06	18.44	time lapse 2 min	light	dark	mixed	Aug	15-38	3/2
14	nest 13	2013	-34.01	18.43	time lapse 2 min	light	dark	mixed	Jul	15-40	2
15	nest 14	2013	-34.07	18.44	time lapse 2 min	light	dark	mixed	Jul	15-42	2
16	nest 15	2017	-34.02	18.45	time lapse 2 min	dark	light	mixed	May	22-35	1
17	nest 16	2017	-33.96	18.51	time lapse 2 min	light	light	like	May	15-41	3
18	nest 1	2017	-34.00	18.45	time lapse 2 min	dark	light	mixed	Apr	15-35	1
19	nest 17	2017	-34.01	18.42	time lapse 2 min	dark	dark	like	May	15-42	2
20	nest 18	2017	-33.98	18.44	time lapse 2 min	dark	light	mixed	May	15-41	2
21	nest 4	2017	-33.96	18.45	time lapse 2 min	dark	dark	like	Jun	16-41	2
22	nest 19	2017	-34.04	18.44	time lapse 2 min	light	light	like	May	31-42	3
23	nest 9	2018	-34.00	18.44	time lapse 2 min	dark	light	mixed	May	32	2
24	nest 10	2018	-34.10	18.39	time lapse 1 min	light	dark	mixed	May	20-27	2

25	nest 20	2018	-33.95	18.42	time lapse 1 min	dark	dark	like	Jun	29-30	2
26	nest 18	2018	-33.98	18.44	time lapse 1 min	dark	light	mixed	Apr	28-34	2
27	nest 12	2018	-34.06	18.44	time lapse 1 min	dark	dark	like	May	33-42	2
28	nest 13	2018	-34.01	18.43	time lapse 1 min	light	dark	mixed	Apr	29-34	2
29	nest 21	2018	-34.04	18.45	time lapse 1 min	dark	dark	like	May	32-42	1
30	nest 4	2018	-33.96	18.45	time lapse 2 min	dark	dark	like	Jun	31-42	2
31	nest 19	2018	-34.04	18.44	motion triggered	light	light	like	Jun	33-42	1
32	nest 22	2018	-34.00	18.43	time lapse 1 min	dark	dark	like	May	33-39	2
33	nest 23	2018	-34.13	18.39	time lapse 1 min	dark	dark	like	May	33-42	2
34	nest 24	2018	-34.08	18.45	time lapse 2 min	dark	light	mixed	Jun	29-42	3

[1] Observed morph of the female confirmed by camera footage of prey delivery

[2] Observed morph of the male confirmed by footage of the nest camera or by field observations (courtship, mating or prey deliveries to the female)

Table S3.2. Output from a linear mixed-model exploring the factors influencing the variance of the mean of food delivery time gaps (including time overnight). The key explanatory variable is the morph consistency (like-morph or mixed-morph). The covariates were age of the nestlings (in weeks, 3 – 6), brood size (1 – 3) and length of the day (in hours). The model was weighted by the square root number of observation days (1 – 7). Significant results are indicated in bold; sample size was 98.

Variance of time between feeding events (time gaps, including overnight gaps)						
fixed effect	reference category	estimate	SE	χ2	df	P
morph consistency	mixed	-0.683	0.414	2.719	1	0.099
age of the nestlings	-	0.303	0.246	1.519	1	0.218
brood size	-	-0.499	0.319	2.441	1	0.118
daylight hours	-	0.024	0.211	0.013	1	0.909
Intercept	**-**	**8.085**	**0.757**	**113.808**	**1**	**< 0.001**

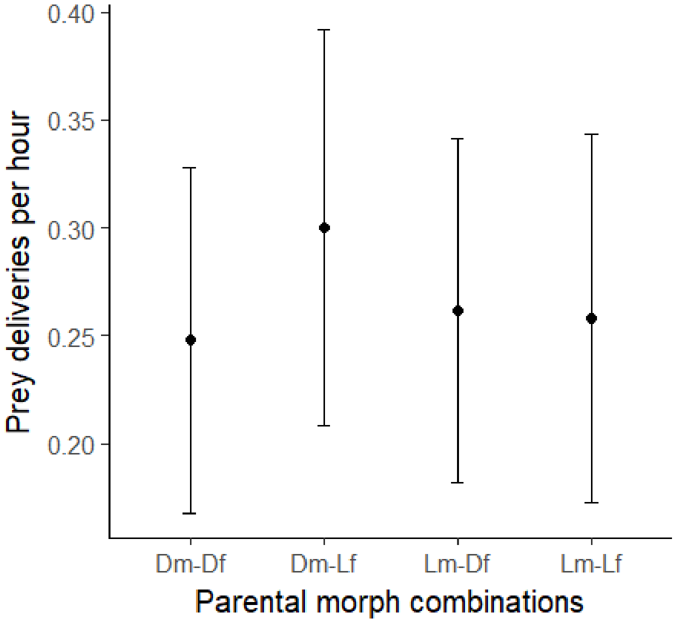

Figure S3.2. Prey delivery rate per hour for four parental morph combinations, dark morph male and female (D♂D♀, Dm-Df), dark morph male and light morph female (D♂L♀, Dm-Lf), light morph male and dark morph female (L♂D♀, Dm-Lf) and light morph male and female (L♂L♀, Lm-Lf). Least squared mean ± 95% CI.

Table S3.3. Pairwise differences of prey delivery rate per hour between the four pair morph combinations based on post-hoc contrasts derived with lsmeans (± SE, top-right). P-values (bottom-left) were adjusted based on the tukey method for comparing a family of four estimates.

pair morph combination	**like-morph**		**mixed-morph**	
	D♂D♀	**L♂L♀**	**D♂L♀**	**L♂D♀**
D♂D♀	-	-0.017 (± 0.038)	-0.056 (± 0.043)	-0.016 (± 0.030)
L♂L♀	0.970	-	0.039 (± 0.048)	0.001 (± 0.039)
D♂L♀	0.561	0.850	-	0.040 (± 0.044)
L♂D♀	0.950	1.000	0.804	-

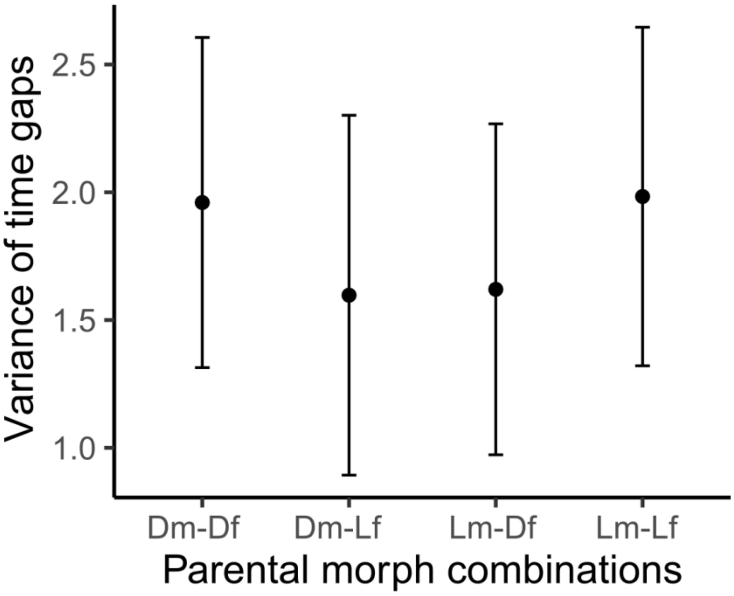

Figure S3.3. Variance of the mean time gaps between prey deliveries in relation of the four pair morph combinations, dark morph male and female (D♂D♀, Dm-Df), dark morph male and light morph female (D♂L♀, Dm-Lf), light morph male and dark morph female (L♂D♀, Lm-Df) and light morph male and female (L♂L♀, Lm-Lf). Least squared mean ± 95% CI.

Table S3.4. Pairwise differences of variance of time gaps of prey provisioning events between the four pair morph combinations based on post-hoc contrasts derived with lsmeans (± SE, top-right). P-values (bottom-left) were adjusted based on the tukey method for comparing a family of four estimates.

pair morph combination	**like-morph**		**mixed-morph**	
	D♂D♀	**L♂L♀**	**D♂L♀**	**L♂D♀**
D♂D♀	-	0.115 (± 0.322)	0.670 (± 0.372)	0.406 (± 0.265)
L♂L♀	0.984	-	-0.555 (± 0.414)	-0.290 (± 0.332)
D♂L♀	0.281	0.539	-	-0.264 (± 0.384)
L♂D♀	0.423	0.817	0.902	-

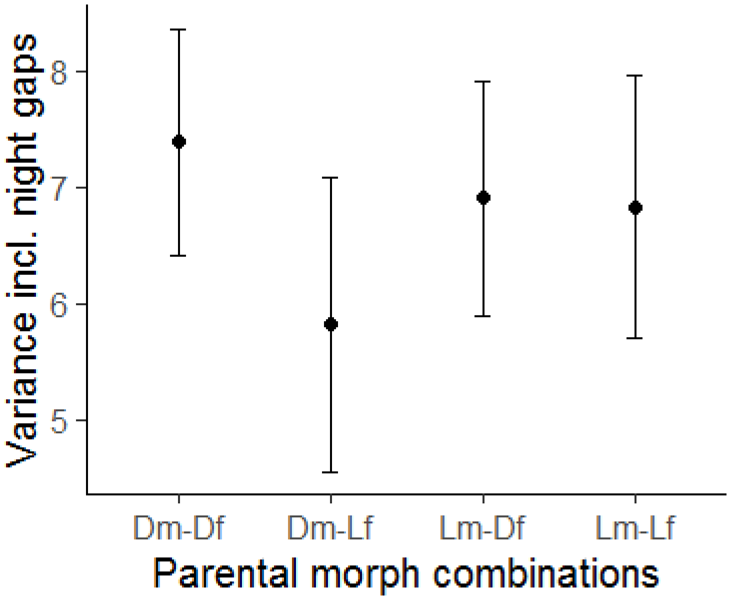

Figure S3.4. Variance of the mean time gaps including night gaps in relation of the four pair morph combinations, dark morph male and female (D♂D♀, Dm-Df), dark morph male and light morph female (D♂L♀, Dm-Lf), light morph male and dark morph female (L♂D♀, Lm-Df) and light morph male and female (L♂L♀, Lm-Lf). Least squared mean ± 95% CI.

Table S3.5. Pairwise differences of variance of time gaps including night gaps between the four pair morph combinations based on post-hoc contrasts derived with lsmeans (± SE, top-right). P-values (bottom-left) were adjusted based on the tukey method for comparing a family of four estimates.

pair morph combination	like-morph		mixed-morph	
	D♂D♀	**L♂L♀**	**D♂L♀**	**L♂D♀**
D♂D♀	-	0.649 (± 0.594)	1.629 (± 0.664)	0.558 (± 0.483)
L♂L♀	0.695	-	-0.980 (± 0.756)	-0.091 (± 0.602)
D♂L♀	0.075	0.568	-	-1.071 (± 0.693)
L♂D♀	0.657	0.999	0.416	-

Chapter 4.

Skinny but stronger? Disentangling immunological and physical investment strategies during the nestling phase of a colour polymorphic African raptor.

A modified version of this chapter has been submitted as:
Nebel C, Amar A, Hegemann A, Isaksson C., Sumasgutner P. Skinny but stronger? Disentangling immunological and physical investment strategies during the nestling phase of a colour polymorphic African raptor.

Abstract

Conditions experienced during early life can have long-term individual consequences by influencing dispersal, survival, recruitment and productivity. Resource allocation during development can have carry-over effects onto these key parameters and is directly determined by the quality of parental care. In the black sparrowhawk (*Accipiter melanoleucus*), a colour polymorphic raptor, parental morphs influence nestling somatic growth and survival, with pairs consisting of different colour morphs ('mixed-morph pairs') producing offspring with a lower body mass index, but higher apparent survival rate. Resource allocation theory could explain this relationship, with nestlings of mixed-morph pairs trading off a better innate immune function against somatic growth. Hence, we predict that mixed-morph pairs' offspring exhibit better immune function compared to like-morph offspring. We quantified several innate immune parameters of nestlings (hemagglutination, hemolysis, bacteria-killing and haptoglobin concentration) and triggered an immune response by injecting lipopolysaccharides. Although we found that nestlings with lower body mass index had higher survival rates, we found no support for the proposed hypothesis: neither immune function nor immune response of nestlings was associated with parental morph combination. These results suggest that these immune parameters are unlikely involved in providing a selective advantage for the different colour morphs' offspring, and thus immunity does not appear to be traded off against a great allocation of resources to somatic growth. Alternative hypotheses explaining the mechanism of a low nestling body mass index leading to subsequent higher survival could be related to the post-fledgling dependency period or differences in dispersal patterns for the offspring from different morph combinations.

Introduction

Individual fitness can be determined by conditions experienced during early-life stages. These "silver spoon effects" have been found in a wide range of animal taxa (Cam and Aubry 2011; Lindström 1999) and can impact different demographic parameters throughout life, such as dispersal (Briga et al. 2017; Cam et al. 2003; Stamps 2006; Tilgar et al. 2010), reproductive performance (Cooper and Kruuk 2018; Descamps et al. 2008; Song et al. 2019; Tilgar et al. 2010; Van De Pol et al. 2006), survival (Descamps et al. 2008; Van De Pol et al. 2006) and senescence (Cooper and Kruuk 2018; Murgatroyd et al. 2018). The mechanisms underlying such long lasting effects are less understood, but range from an improved ability to cope with stressors (Dmitriew and Rowe 2007) or competitors (Hopwood et al. 2014; Royle et al. 2005) to stronger immunocompetence (Mugabo et al. 2010). The variability of such early-life experiences are mainly associated with environmental factors (Cam and Aubry 2011; Lindström 1999) and parental care, which, in turn, might be influenced by parental quality (Cam et al. 2003; Vitikainen et al. 2019), parental phenotype (Chakarov et al. 2008; Emaresi et al. 2014; Grunst et al. 2018; Morosinotto et al. 2020; Nebel et al. 2020; Roulin 2004; Sumasgutner et al. 2016; Chapter 2) or parents' experiences (Rödel et al. 2009).

Nestlings of altricial bird species are completely reliant on the resources provided by their parents (Clutton-Brock 1991; Cockburn 2006). When these resources are limited, individuals must allocate them amongst competing functions, such as somatic growth or the development of the immune system (Aastrup and Hegemann 2020; Norris and Evans 2000; van der Most et al. 2011). These trade-offs will have profound impacts on an individual's fitness and are thus likely to be under strong selective pressure. Understanding an individual's development and which specific traits translate into long-term fitness effects is key to unravelling the ecological and evolutionary processes (Cam and Aubry 2011) that can shape an individual's life history.

In species with biparental care, males and females might have different strategies when allocating resources to their offspring. Likewise, parental investment might also vary between individuals (Järvistö et al. 2015; Limbourg et al. 2013; Ratikainen and Kokko 2010) which can result in offspring differing in quality. Within colour polymorphic species, morphs may display different parental strategies (Emaresi et al. 2014; Pryke and Griffith 2009). For example, different colour morphs in white-throated sparrows (*Zonotrichia albicollis*) show differential investment in parental care (Grunst et al. 2018; Knapton and Falls 1983; Kopachena and Falls 1993). Thus, the young of different parental morph combinations may be exposed to different early-life experience and this may have carry-over effects on their survival and other fitness related traits.

The black sparrowhawk (*Accipiter melanoleucus*) is a colour polymorphic raptor that occurs as light or dark morph adults (Amar et al. 2013). Although there is no indications of morph-dependant mating patterns (Tate et al. 2017), the combination of morphs within a pair (consisting of contrasting morphs, termed 'mixed-morph' or consisting of the same morph, termed 'like-morph' pairs) have been shown to affect reproductive performance: Mixed-morph pairs have a higher probability of breeding successfully (Tate et al. 2017). Offspring from mixed-morph pairs also have been found to be associated with a lower body mass index (Tate et al. 2017), but higher subsequent survival (Sumasgutner et al. 2016). Although survival and nestling body mass index have not yet been mechanistically linked in this species, this runs counter to the usual assumption that nestlings that fledge with a higher body mass index will have higher survival or recruitment rates (i.e. Christe et al. 1998; Cleasby et al. 2010; Losdat et al. 2013; Morosinotto et al. 2020; Ringsby et al. 1998; Tinbergen and Boerlijst 1990; Vermeulen et al. 2016b).

Individual quality can also be assessed by measures other than body mass index, for example by examining parameters linked to the expression of the immune system (Bowers et al. 2014; Norris and Evans 2000; Vennum et al. 2019). The innate immune system is the first line of defence against pathogens without need of prior exposure (Calder and Sonnenfeld 2017). Due to its importance in maintaining an individual's health, it plays a crucial role in survival (Hegemann et al. 2015; Hegemann et al. 2013b; Wilcoxen et al. 2010), especially in young that still develop immune function (Aastrup and Hegemann 2020; Apanius 1998; Klasing and Leshchinksy 1999), but it is a costly trait to invest in (Bonneaud et al. 2003; Costantini and Moller 2009). To quantify the innate immune system of black sparrowhawk nestlings, we use a combined approach; whereby we measure several components of baseline innate immune function and perform an experimental endotoxin immune challenge to examine immune response. The measured baseline innate immune parameters are the complement system, natural antibodies, microbiocidal activity, which play an important role in host defense against infectious agents (Matson et al. 2005; Müller-Eberhard 1988), and haptoglobin, which is an acute-phase protein (Cray et al. 2009; Dobryszycka 1997; Matson et al. 2012) that is released by the liver during infections, and its concentration is usually negatively correlated with an individual's body mass index (Hegemann et al. 2012). For the innate immune challenge, we use lipopolysaccharides (LPS) that naturally occur on the outer surface membrane of bacteria and mimic a bacterial infection when injected (Alexander and Rietschel 2016). All components of this study focus on the innate immune function, which is better developed than the cellular or adaptive immune function in young birds (Aastrup and Hegemann 2020; Apanius 1998; Klasing and Leshchinksy 1999). A multicomponent approach has advantages over single-assay studies at capturing immune function. Baseline innate immune function and its response are differently regulated and their combination allows a more comprehensive view (Hegemann et al. 2013a; Hegemann et al. 2012; Vermeulen et al. 2016a; Vinterstare et al. 2019).

According to alternative resource allocation theory, individuals must allocate resources to competing functions (Norris and Evans 2000; van der Most et al. 2011). Offspring from mixed-morph pairs might trade-off investment in immune function at the expense of somatic growth, whereas nestlings of like-morph pairs might invest more in somatic growth and less in immune function and this may explain why mixed-morph offspring have on average a lower body mass index. This 'alternative resource allocation' hypothesis could also explain why mixed-morph offspring have better survival rates as their higher investment in immune function could provide them with a selective advantage against pathogens (Lei et al. 2013; Suri et al. 2017) and higher immune function is often associated with higher survival (Hegemann et al. 2015; Hegemann et al. 2013b; Wilcoxen et al. 2010). Differences in resource allocation could be caused by parental morph-dependant prey delivery behaviour. Whilst black sparrowhawk parental morph combinations do not influence overall amount of prey delivered to nestlings, it can influence consistency in food delivery, with more regular feeding patterns observed for mixed-morph pairs (Nebel et al. 2020; Chapter 2). These differences may promote differential physical and physiological investment between the offspring of mixed- and like-morph pairs.

In this study, we use three datasets to explore alternative resource allocation between somatic growth, innate immune function and survival by offspring, by using mark-recapture ('apparent survival') data from individuals encountered between 2001 and 2019, correlative innate immune data collected between 2015 and 2019 and experimentally challenged immune response data collected in 2018 and 2019. Due to their elusive nature during non-breeding, survival of black sparrowhawks is usually assessed with recruitment into the breeding population which happens at approximately 2.8 years of age (Sumasgutner et al. 2016). As a result, we assess nestling innate immune function and subsequent survival rates indirectly. Under the 'alternative resource allocation' hypothesis, we would predict that (i) immune function of nestlings of mixed-morph pairs is higher than those of like-morph pairs, (ii) birds with higher immune function will have a leaner body (low body mass index); and (iii) nestlings with a lean body have higher apparent survival rates. In more detail, we predict nestlings of mixed-morph pairs (known to have a lower average body mass index, Tate et al. 2017) to exhibit a stronger complement system, natural antibodies and microbiocidal activity than like-morph chicks, because we predict they allocate more resources into their immunological development than somatic growth. Furthermore, we predict that nestlings of mixed-morph pairs show lower haptoglobin concentrations in comparison to those of like-morph pairs. Such a result would indicate that nestlings of mixed-morph pairs invest more into the development of innate immune function and are thus able to fight pathogens more effectively, which should result in low infection and inflammation rates. Lastly, we predict nestlings of mixed-morph pairs to show a stronger response to the mimicked bacterial infection, which under attack by a real pathogen would mean a faster clearance of the pathogen.

Material and Methods

Study system and productivity data

The black sparrowhawk study population is located on the Cape Peninsula (34°00' S, 18°26' E), Western Cape, South Africa. Each year, 11 to 45 (mean 32) black sparrowhawk territories show breeding activity. Territories were checked from April until October, initially monthly, until breeding activity was recorded and from then on weekly. During visits, the sex and morphs of the territorial pair were identified. Sexes can be easily distinguished in the field with males being considerably smaller than females (Christie and Ferguson-Lees 2010; Hockey et al. 2005).

Nestling ringing and body condition measurement

Black sparrowhawk nestlings were fitted with a metal ring supplied by the South African Bird Ringing Unit (SAFRING, Paijmans et al. 2019; Rose et al. 2019) and a unique colour ring combination at the age of 20 – 35 days. Systematic ringing of black sparrowhawk nestlings started in 2006, but four individuals were ringed at nestling age in 2001 and 2003 and were included in our survival analysis. Subsequent re-sightings of these marked birds were recorded annually between 2001 and 2019 and allowed survival estimates to be obtained. Nestlings were aged by comparing their plumage development with reference photos of chicks of known age (Katzenberger et al. 2015). During ringing, nestlings were weighted using a scale (to the nearest 1 g) and their tarsus length was measured using a calliper (to the nearest 0.1 mm). To derive the body mass index, we extracted the residuals of a linear regression between body mass and tarsus length, controlling for sex.

Blood samples and immune challenge

When nestlings were ringed, we also took a blood sample. Blood samples were taken within 20 min of removing them from the nest, well within the time period where no handling effect is expected on the nestling's immune function (Buehler et al. 2008; Zylberberg 2015). A blood sample was taken from the brachial vein for the baseline immune analysis from 2015 to 2019. In 2018 and 2019, an immune challenge was performed in addition to the baseline sampling. At these nests, after taking the first blood sample for the baseline immune analysis, the immune challenge was carried out: nestlings were injected with a 1 mg/kg LPS solution (in phosphate-buffered saline, PBS) subcutaneously on the breast. A second blood sample ('post-exposure') to quantify the response to the endotoxin was taken between 16 to 18 h (mean = 17.17 h, SD = 0.48 h) after the immune challenge. This post-exposure sample was used to determine the change in haptoglobin concentration from the baseline value (see details further below). The field procedure in 2018 and 2019 was always the same: nestlings were injected with LPS in the afternoon (between 15:00 and 17:33), immediately returned to their nest and a second blood sample was taken the next morning (between 08:26 and 11:11). The total blood volume taken per nestling was max. 1 ml in total (approximately 500 µl per bleeding), which is well below the safety limit of 1% of body mass (650-980 g; would in theory allow 6-10 ml, which translates into < 10% of the animal's blood volume). Directly after blood collection, red blood cells and plasma were separated by centrifuging in the field (10,000 rpm/20 min) and frozen in liquid nitrogen immediately afterwards. All samples were stored at -80 °C until processing and randomized before laboratory work began.

Innate immune assay protocols and lab procedures

We measured three different components of the baseline innate immune system: The complement system and natural antibodies were assessed via the hemolysis (HL) and hemagglutination (HA) assay (Matson et al. 2005). They were determined as the lysis and agglutination titre of 12.5 µl foreign red blood cells (rabbit blood) in a serial dilution of 12.5 µl black sparrowhawk plasma in 12.5 µl PBS. Titre scoring was performed visually after 20 min (hemagglutination) and 90 min (hemolysis) of incubation in a 37 °C distilled water bath and wells counted that showed signs of hemagglutination or hemolysis. Titres were scored twice, both times randomized and blind to sample identity (ID). In case the difference between both scores was larger than one (measured in wells), the titre was scored a third time. The mean of two scores or median of three was then used for the statistical analysis. On each plate, the serial dilution of two chicken (*Gallus gallus domesticus*) plasma samples was used as a control and produced an inter-assay coefficient of variance (CV) of 16.3% (HA) and 10.6% (HL).

The bacteria-killng (BK) assay was carried out following the methods of French and Neuman-Lee (2012), but using 2/3rd of the reagents and measuring final bacteria growth at 600 nm (Eikenaar and Hegemann 2016). The initial bacteria concentration was 10^5 *Escherichia coli*/ml and bacteria volume 3.5 µl per well. Plasma volume was 4.5 µl and 8 µl PBS per well. On each plate, a positive (not containing any plasma) and a negative control (not containing any *E. coli* or plasma) were run in quadruplicates. Before incubation, background absorption was measured at 600 nm. Samples were incubated for 12 h at 37 °C before the final absorption reading at 600 nm was done. The bacteria-killing capacity was quantified as the bacteria growth in plasma after 12 h (in %) subtracted by the background absorption in relation to a bacteria positive control that grew on the same plate. Samples were run in triplicates with an intra-assay coefficient of 7.92%.

As a third baseline parameter, we measured the haptoglobin concentration of plasma: the baseline concentration and the post-exposure concentration to assess the response of the innate immune system to the LPS injection. We used a commercially available colorimetric assay kit (TP801; Tri-Delta Diagnostics, Maynooth, County Kildare, Ireland) following the manufacturer's instructions. Haptoglobin concentration was measured at a wavelength of 650 nm. We did an additional reading at 450 nm that was performed directly before adding the final reagent, allowing us to control for plasma redness (Matson et al. 2012). A standard provided by the manufacturer was added on every plate and produced an inter-assay CV of 5.44%.

The sample sizes varied between assays because the amount of plasma varied between individuals (Table 3.1). All innate immune parameters ('hemolysis', 'hemagglutination', 'bacteria-killing', 'baseline haptoglobin', 'haptoglobin response') and the co-variate 'plasma redness' (450 nm haptoglobin measurement) were standardized per year by using the scale function of 'base' R, which allowed us to remove any between-year variation that might be caused by differences during transport (samples from 2016 and 2017 thawed during the shipment to the lab, but see Hegemann et al. 2017), time in storage (2016 samples were stored a year longer before being analysed than samples from other years (but see Hegemann et al.

2017) or processing. Lab work was done in four batches corresponding to collection years: batch 1 (2015), batch 2 (2016, 2017), batch 3 (2018), batch 4 (2019).

Statistical analysis

We used R version 3.5 (R Core Team 2019) to fit linear mixed models (LMM) using the 'lmerTest' package (Kuznetsova et al. 2017). First, we explored relationships between innate immune function and parental morph combination (hereafter either 'parental morph combination' or 'pair morph'), which was fitted as a two-level factor (mixed- or like-morph). The 'baseline immune' response variables analysed were 'hemolysis', 'hemagglutination', 'bacteria-killing' and 'baseline haptoglobin'. Multiple covariates were added to control for their potential influence on baseline innate immune function: nestling's 'sex' (factor variable), to account for sex-specific variation in immune function (McCurdy et al. 1998) and 'age of the nestlings' (continuous variable: 20 – 35, in days), to control for the transitional changes of immune function during development. A nest-specific covariate of 'brood size' (continuous variable: 1 – 3) was included, which controlled for variation in sibling competition experienced (Parejo et al. 2007). As breeding performance in this species is influenced by timing of breeding (Rose et al. 2017; Tate et al. 2017), we included the calendar week when ringing occurred (1 being the first week of the year, continuous variable: 22 – 46). Lastly, we controlled for 'time of the day' (continuous variable: 7 – 17, in hours) to account for diurnal patterns in immune function (Kanikowska et al. 2005). The bacteria-killing score and baseline haptoglobin concentration were log-transformed to improve normality. For the baseline haptoglobin model we fitted a reading at 450 nm wavelength to control for the redness of the plasma. In all the 'baseline immune' models, territory ID was included as a random term, but was removed from the final 'hemolysis' model as it explained 0% of variance.

We explored the change of haptoglobin concentration after the injection of LPS. Within this 'innate immune response' linear model, we fitted the 'haptoglobin response' (post-haptoglobin subtracted by the baseline concentration) as the response variable. We controlled for 'sex' (factor variable), 'age' of the nestling (continuous variable: 20 – 32, in days), 'brood size' (continuous variable: 1 – 3), and 'seasonality' (continuous variable, week number of ringing: 23 – 43). In addition, we included the haptoglobin concentration reading (650 nm) from the baseline sample to control for the initial haptoglobin concentration and a reading of the baseline sample at 450 nm to control for 'plasma redness' (Matson et al. 2012). In the 'innate immune response' models, we initially added territory ID as a random term, but as it explained 0% of variance, we removed it from the final model.

Linked to the predictions from our alternative resource allocation hypotheses, we directly explored whether there was an association between somatic growth (body mass index) and innate immune function, irrespective of parental morph combination. These analyses used the same models as previously described (both the three 'baseline innate immune' models and the 'innate immune response' model), however, we replaced the 'pair morph' explanatory variable with our continuous 'body mass index' measure.

All continuous covariates that were used in LMMs, were scaled beforehand (standardized to mean = 0 and SD = 1) in order to bring the variables to comparable dimensions to facilitate interpretation of effect sizes.

Apparent annual survival in relation to body mass index

We used the packages RMark (Laake 2013) and software MARK (White and Burnham 1999) to estimate means (and standard errors) of apparent survival (Φ) and re-sighting probability (ρ) of individuals that were ringed as nestlings. We used Cormack-Jolly-Seber (CJS) models with body mass index as a covariate. For this analysis, birds were placed into three groups according to their body mass index, with group 1 ('lean') being up to the 15th percentile (from -153.35 to -54.12, N = 66), group 2 ('normal') between the 15th and 85th percentile (from -54.12 to 51.59, N = 306) and group 3 ('heavy') being above the 85th percentile (51.59 – 195.12, N = 66). Using the 15% and 85% percentile represents an accurate representation of the body mass index data structure (Figure S3.1). A goodness-of-fit test using RELEASE (test2 & test3, Burnham 1987) programs was performed to ensure that data met the homogeneity assumption: $\chi^2_{\text{df}=73} = 133.411$; $\hat{c} = 1.82$. Due to the goodness-of-fit indicating overdispersion, standard errors were corrected for $\hat{c}$. Post-hoc comparison based on estimates and standard errors from extracted coefficients of survival were performed using the CONTRAST package (Sauer and Byron 1989).

Results

Baseline immune function in relation to pair morph

We found no difference in any of the baseline innate immune parameters in relation to parental pair morph combination (Table 4.1, Figure 4.1, Table S4.1 – S4.8 for full model outputs). Variation in baseline innate immune function was, however, associated with some of our other covariates: Female nestlings had a significantly stronger bacteria-killing capacity than male nestlings (Estimate: -0.064, SE = 0.024, $N_{df=6,161}$ = 168, χ^2 = 7.94, P = 0.007, Table S4.7, Table S4.8). Haptoglobin levels varied with season, with nestlings hatched earlier having higher haptoglobin levels than later hatched nestlings (Estimate: -0.022, SE = 0.011, $N_{df=7,169}$ = 177, χ^2 = 4.03, P = 0.045, Table S4.5, Table S4.6). Haptoglobin levels also varied with brood size, with nestlings in larger broods having higher haptoglobin concentrations ($N_{df=7,169}$ = 177, χ^2 = 4.02, P = 0.045; Hp concentration of single chick broods = 0.178, SE = 0.063, broods with two chicks = 0.174, SE = 0.035, broods with three chicks = 0.125, SE = 0.016).

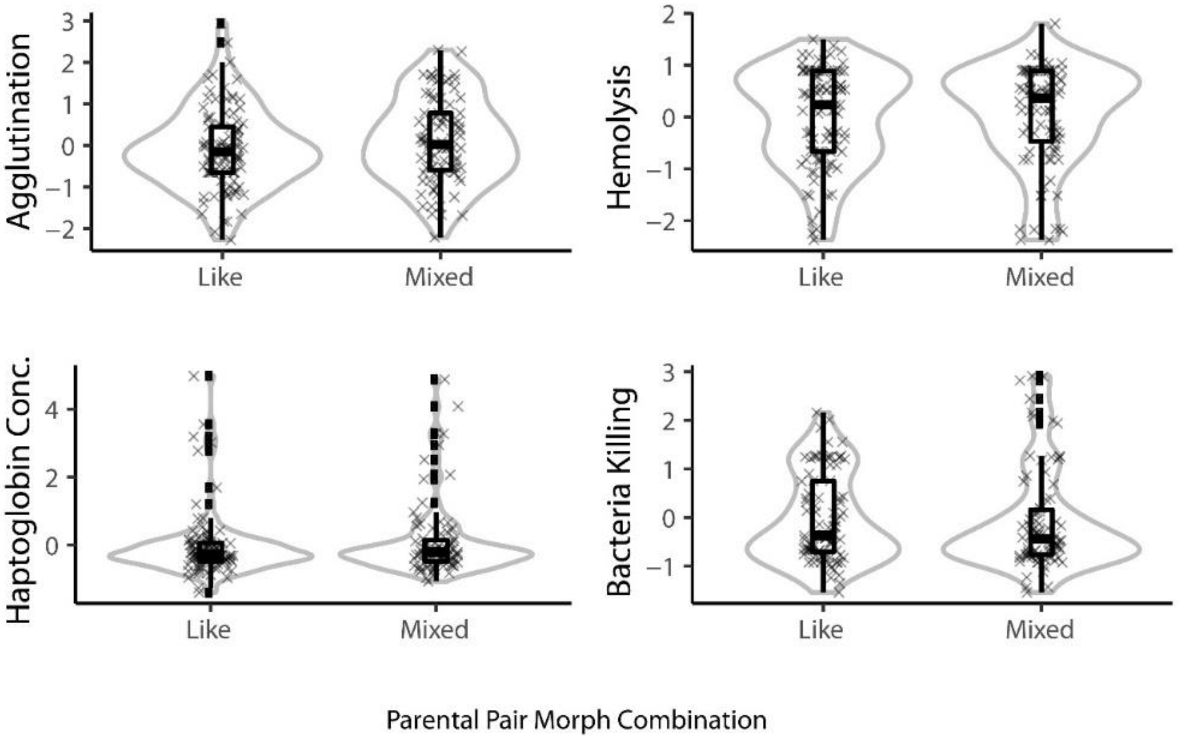

Figure 4.1. Baseline innate immune parameters (scaled values and centred to the mean of the year) in relation to parental pair morph combination (‘like-morph’: dark male and dark female D♂D♀ and light male and light female L♂L♀; or ‘mixed-morph’: dark male and light female D♂L♀ and light male and dark female L♂D♀). Boxplots are based on standardized raw data (individual data values shown as cross marks), outliers shown as black points, grey outlined violin plot gives an estimate of the distribution of data.

Innate immune response in relation to pair morph

The post-exposure haptoglobin levels of nestlings injected with LPS were higher than their baseline pre-exposure the haptoglobin levels (Estimate: -0.912, SE = 0.166, $N_{df=2,46}$ = 49, χ^2 = 23.71, P < 0.001; baseline Hp conc.: 0.28, SD = 0.48, post-exposure Hp conc.: 0.63, SD = 0.50, however, the haptoglobin response did not differ between parental morph combinations ($\chi^2 = 0.27$, P = 0.610, Figure 4.2), nor with any of our other covariates (see Table S4.9 and S4.10 for full model outputs).

Relationship between nestling body mass index and immune function.

We found no negative relationship between any baseline innate immune parameter or the innate immune response in relation to the nestling body mass index (Table 4.1). Instead, for the hemagglutination titre, we found a marginally significant positive association with body mass index, albeit effect sizes were small (Estimate: 0.003, SE = 0.002, $N_{df=6,172} = 179$, $\chi^2 = 3.94$, P = 0.049, Figure 4.3); indicating higher hemagglutination levels for nestlings in better condition.

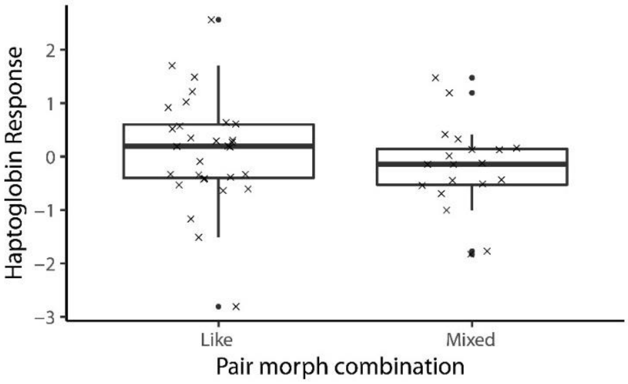

Figure 4.2. Haptoglobin response (scaled values and centred to the mean of the year) in relation to parental pair morph combination ('like-morph': dark male and dark female D♂D♀ and light male and light female L♂L♀; or 'mixed-morph': dark male and light female D♂L♀ and light male and dark female L♂D♀). The haptoglobin response variable is the difference in haptoglobin concentration between the baseline haptoglobin and the concentration measured after the injection of lipopolysaccharides (LPS). Cross marks are scaled raw values, black dots outliers. All values scaled and centred to the mean of the collection year to remove between-year variation.

Apparent survival in relation to pair morph

Although apparent survival (Φ) displayed a negative relationship with increasing body mass index (Figure 4.4), variation around these estimates were relatively large, and our CONTRAST analysis indicated that the difference between these groups were not statistically significant; contrast between lean and heavy nestlings, $\chi^2 = 1.27$, N = 132, P = 0.260) after correcting for overdispersion.

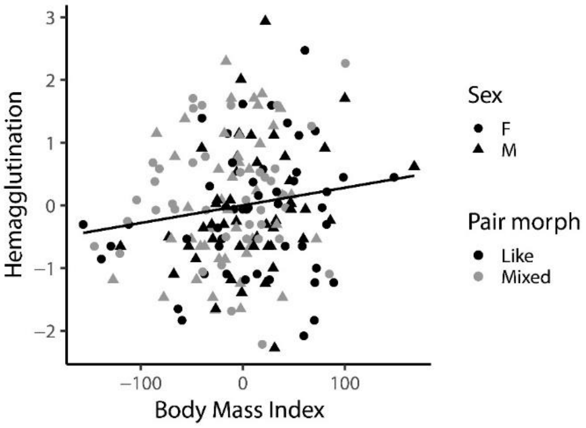

Figure 4.3. Relationship between hemagglutination titre score and body mass index. Each symbol represents one observation (nestling). Colour represent parental pair morph combination (pair morph): black symbols are offspring produced by like-morphs, grey symbols offspring of mixed-morph parents. Circles are females, triangles males. Trend line is the linear regression of hemagglutination and the body mass index (y = 0.002x + 0.006, R^2 = 0.019), grey shaded areas the 95% confidence intervals. The hemagglutination titre values were scaled and centred to the mean of the year.

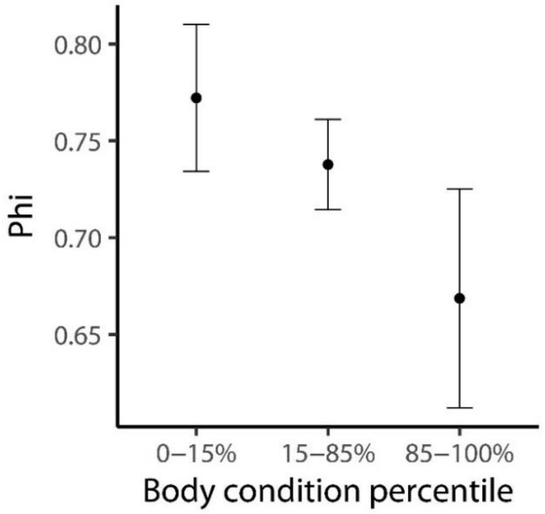

Figure 4.4. Apparent survival (Φ) estimates and standard error of nestling black sparrowhawks in relation to body mass index percentile groups (group 1, 0 – 15%: 0.77, SE = 0.04; group 2, 15 – 50%: 0.74, SE = 0.02; group 3, 85 – 100%: 0.67, SE = 0.06). Resightings data was collected between 2001 and 2019 (N = 438).

Discussion

We found almost no support for our alternative resource allocation hypotheses proposed to explain why black sparrowhawk offspring from mixed morph parents produced nestlings with a low body mass index but with higher apparent survival rates. We found no support for our first prediction, that innate immune function of nestlings of mixed-morph pairs is higher than those of like-morph pairs. Furthermore, we found no support of our second prediction, that birds with higher immune function will have a leaner body (i.e. low body mass index); only one immune parameter was associated with nestling body mass index, and this relationship ran counter to our predictions, with higher hemagglutination levels for nestlings with a heavier body. Our third prediction was that nestlings with a lean body would have higher apparent survival rates. Although there was an indication for higher survival rates for nestlings with the lowest body mass index compared to nestlings with the highest body mass index, these differences were not significant.

Table 4.1. Model outputs of linear mixed models for the key explanatory variables of the 'baseline innate immune models' (response variables: hemagglutination, hemolysis, bacteria-killing capacity and baseline haptoglobin) and 'innate immune response model' (response variable: haptoglobin response, quantified as the difference between baseline haptoglobin concentration and haptoglobin concentration post-injection of lipopolysaccharides). Key explanatory variables are parental 'pair morph' (factor in two levels: 'like-morph': dark male and dark female D♂D♀ and light male and light female L♂L♀; or 'mixed-morph': dark male and light female D♂L♀ and light male and dark female L♂D♀) or the 'body mass index'. All 'baseline innate immune models' were fitted with individual 'sex' (male or female), 'age' (numeric, in days: 20 – 35), 'brood size' (numeric, 1 – 3), 'seasonality' (numeric, week number: 22 – 46), and 'time of the day' when sample was obtained (7 – 17, in hour) as additional co-variates and nest ID as a random factor. Bacteria-killing and baseline haptoglobin concentration were log-transformed, the baseline haptoglobin model included an additional co-variate: a reading at 450 nm to control for plasma redness. The 'innate immune response model' was fitted with two baseline readings (650 nm and 450 nm) to control for initial haptoglobin concentration and plasma redness, 'sex', 'age' (numeric, week number: 22 – 35), 'brood size', 'seasonality' (numeric, week number: 23 – 43), as co-variates and nest ID as a random factor. All response variables were scaled and centred to the mean of the year, whereas all continuous variables were scaled. Study period (in years), sample size (n, for mixed- and like-morph), numerator and denominatior degrees of freedom (ndf and ddf) given in respective columns. See Tables S3.1 – S3.9 for full model outputs.

		n		**model output 'pair morph' explanatory variable**						**model output 'body mass index' explanatory variable**					
measurement	**study period**	**like**	**mixed**	**estimate**	**SE**	**χ2**	**ndf**	**ddf**	**P**	**estimate**	**SE**	**χ2**	**ndf**	**ddf**	**P**
Baseline innate immune models															
hemagglutination	2015 – 2019	98	81	0.247	0.172	2.07	1	172	0.156	**0.003**	**0.002**	**3.94**	**1**	**172**	**0.047**
hemolysis	2015 – 2019	98	81	0.030	0.147	0.04	1	172	0.837	0.002	0.001	1.34	1	172	0.225
baseline haptoglobin	2015 – 2019	99	78	0.012	0.021	0.32	1	169	0.574	-0.001	0.001	0.24	1	169	0.624
bacteria killing	2015 – 2019	91	77	0.008	0.030	0.08	1	161	0.783	0.001	0.001	0.90	1	161	0.344
Innate immune response model															
haptoglobin response	2018 – 2019	30	19	-0.158	0.307	0.27	1	41	0.607	-0.001	0.003	0.16	1	41	0.694

Contrary to our prediction, black sparrowhawks in good physical condition also showed the best natural antibody titre (agglutination). In general, this finding is in line with observations in other species which have found associations between a high body mass index and strong immune-competence (Alonso-Alvarez and Tella 2001; Christe et al. 1998; Merino et al. 1999). However, in the present study system, in which nestlings with a low body mass index show the highest survival rates, the association of a high body mass index and high innate immune function challenges the original idea that a strong innate immune system is linked to higher survival rates. Natural antibodies do not need prior exposure to antigens and play an important role in facilitating pathogen recognition and clearance of apoptotic cells (Boes 2000; Grönwall et al. 2012; Ochsenbein and Zinkernagel 2000), especially in young that still do not have a well-developed adaptive immune system (Boes 2000). If resources are limited, animals are known to redistribute their investment from energetically expensive immune functions to cheaper ones, like natural antibodies (Klasing 2004; Martin et al. 2008; Matson et al. 2005; Vinterstare et al. 2019). This could be the case for black sparrowhawks, whereby nestlings that invest more into somatic growth, may down-regulate expensive and upregulate cheap immune parameters. However, we did not find any indication of a down-regulation of expensive innate immune function, i.e. bacteria-killing capacity and complement system (Matson et al. 2005; Vinterstare et al. 2019), which does not provide support for this hypothesis of redistribution among innate immune functions. Unfortunately, without a direct assessment of nestling immune function and subsequent survival rates, which isn't possible with our current data sets, we cannot fully disentangle the relationship of somatic growth, natural antibodies and survival.

The apparent lack of a trade-off between innate immunological and physical development could be due to multiple reasons: First, individual variation can make it difficult to unravel trade-offs, especially when working with field data (Glazier 1999; Van Noordwijk and de Jong 1986). Second, the innate immune system is complex and consists of many components and physiological relationships that can interact with one another. Although we used multiple parameters to gain a more complete picture, our chosen parameters might not be suitable to reveal differences in resource allocation. Third, differences in the innate immune function might only become visible in a later development stage, i.e. during the post-fledging dependency period. Fourth, differences might be only prevalent in the adaptive or cellular immune system. Another explanation for the higher apparent survival of mixed morph pairs, may be linked to emigration. Sumasgutner et al. (2016) found that offspring of mixed-morph pairs had higher apparent survival, but such survival estimates may be determined by a combination of survival and emigration out of the study area (Newton et al. 2016). Thus, higher apparent survival for chicks produced by mixed-morph pairs could also emerge if offspring from like-morph pairs were more likely to disperse or if dispersal was positively associated with the body mass index. Support for a body condition-dependant juvenile dispersal in *Accipiter* hawks can be found in northern goshawks (*Accipiter gentilis*, Kennedy and Ward 2003) and Eurasian kestrels (*Falco tinnunculus*, Terraube et al. 2015). However, such a pattern is not present in all such studies (Delgado et al. 2009; Rosenfield et al. 2016). Unfortunately, with only a handful of re-sightings of colour-ringed individuals from outside our intensely monitored study population, exploring this possibility is unfeasible at present.

Our study has revealed sex-specific differences in the innate immune system with female nestlings showing stronger bacteria-killing capacities. Such sex-specific differences have previously been reported in other species, and have been linked to sex hormones (Klein 2004; Klein and Flanagan 2016). These relationships might also explain why male animals are prone to larger parasite loads than females (Alexander and Stimson 1988; Zuk 1990; Zuk and McKean 1996). In support of this, adult male black sparrowhawks show higher *Haemoproteus* blood parasite infection intensity than adult females (Lei et al. 2013), suggesting that sex-specific differences of nestling immune function continue into adulthood.

Nestlings of broods with three chicks had high levels of haptoglobin concentration, indicating increased levels of inflammation. This might conform with the ‘tasty chick’ hypothesis (Christe et al. 1998; Roulin et al. 2003) which posits that one sibling (usually the weakest) might be an increased target for parasites and thus show elevated levels of inflammation. Such a pattern is linked to hatching asynchrony, which also occurs in black sparrowhawks, where nestlings usual hatching at one to three day intervals (Christie and Ferguson-Lees 2010; Hockey et al. 2005). However, it is difficult to determine hatching rank based on size and plumage development in black sparrowhawks, which shows strong sexual size dimorphism, thus such a hypothesis is difficult to test without individual marking at hatching.

The acute-phase protein haptoglobin showed a seasonal effect: Nestlings that were ringed at the beginning of the season showed higher concentrations which declined during the course of the season. This seasonal pattern might be due to higher pathogen pressure during the colder and wetter period at the beginning of the breeding season (Lei et al. 2013; Martin et al. 2014; Suri et al. 2017), even though early months are characterized by higher breeding success in the population on the Cape Peninsula (Rose et al. 2017; Tate et al. 2017). High haptoglobin concentrations during the wet season with potentially higher pathogen pressure may indicate that nestlings rely on high baseline values which enable a faster response to infection (Horrocks et al. 2012; Horrocks et al. 2015). High breeding success during the early months of the breeding season could then be a combination of earlier breeders being of higher individual quality and/or occupying higher quality territories (e.g. sequential settlement, Sergio et al. 2007). Low haptoglobin concentrations towards the end of the breeding season could accordingly reflect lower pathogen pressure (Horrocks et al. 2012; Horrocks et al. 2015).

Conclusion

A low body mass index of nestlings of mixed-morph pairs, but higher apparent survival rates of these offspring suggests that nestlings are following different investment strategies and trade-off physical development for other aspects of growth, i.e. the innate immune system. However, our study did not reveal such an association. Nestlings of mixed-morph pairs showed the same baseline innate immune levels and innate immune response as compared to nestlings of like-morph pairs. Although the innate immune system is usually a good predictor of survival, our findings imply that differences in the innate immune system are unrelated to differences in survival in our study system. Furthermore, contradicting to our prediction, nestlings with a high body mass index also showed the highest natural antibody titre. This finding indicates potential contrasts in up- and downregulation of different immune parameters, although we found no evidence of such a redistribution taking place. Although we did not find support for any of our predictions, our study is the first to present data on the innate immune system of nestlings of a colour polymorphic *Accipiter* hawk. Future studies should focus on the post-fledgling and dispersal behaviour of black sparrowhawk juveniles which might be shaped by parental morphs and could explain the observed life-history traits.

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Supplementary Material

Full model output hemolysis

Table S4.1. Model output of a linear model exploring the hemolysis score (standardized per year) in relation to parental pair morph combination (either mixed-morph or like-morph, mixed morph as the reference here). Covariates fitted in the model were nestling age (in weeks), brood size, sex (male as reference), season (in weeks) and time of the day. Random effect (territory ID) was removed from the original linear mixed model as it explained 0% of the variance. All continuous variables were scaled. Sample size is 179.

variable	estimate	SE	$\chi2$	ndf	ddf	P
pair morph	0.030	0.147	0.04	1	172	0.837
nestling age (in days)	0.018	0.073	0.06	1		0.802
brood size	0.019	0.071	0.06	1		0.792
sex	-0.081	0.143	0.29	1		0.577
season (week number)	0.021	0.076	0.07	1		0.781
time of the day (hour)	0.038	0.073	0.25	1		0.603
Intercept	0.043	0.121	0.11	1		0.724

Table S4.2. Model output of a linear mixed model exploring the hemolysis score (standardized per year) in relation to individual body condition. Covariates fitted in the model were nestling age (in weeks), brood size, sex (male as reference), season (in weeks) and time of the day. Radom effect (territory ID) was removed from the original linear mixed model as it explained 0% of the variance. All continuous variables were scaled. Sample size is 179.

variable	estimate	SE	$\chi2$	ndf	ddf	P
body mass index	0.002	0.001	1.34	1	172	0.225
nestling age (in days)	-0.003	0.075	0.01	1		0.969
brood size	0.030	0.071	0.16	1		0.674
sex	-0.090	0.145	0.35	1		0.537
season (week number)	0.021	0.074	0.07	1		0.780
time of the day (hour)	0.029	0.073	0.14	1		0.694
Intercept	0.053	0.106	0.36	1		0.548

Full model output hemagglutination

Table S4.3. Model output of a linear mixed model exploring the hemagglutination score (standardized per year) in relation to parental pair morph combination (either mixed-morph or like-morph, mixed morph as the reference here). Covariates fitted in the model were nestling age (in weeks), brood size, sex (male as reference), season (in weeks) and time of the day. Random effect was territory ID. All continuous variables were scaled. Sample size is 179.

variable	estimate	SE	$\chi 2$	ndf	ddf	P
pair morph	0.247	0.172	2.07	1	172	0.156
nestling age (in days)	-0.055	0.077	0.50	1		0.480
brood size	0.002	0.008	0.01	1		0.983
sex	0.001	0.151	0.01	1		0.996
season (week number)	0.004	0.084	0.02	1		0.961
time of the day (hour)	0.080	0.077	1.05	1		0.306
Intercept	-0.139	0.139	0.67	1		0.416

Table S4.4. Model output of a linear mixed model exploring the hemagglutination score (standardized per year) in relation to individual body condition. Covariates fitted in the model were nestling age (in weeks), brood size, sex (male as reference), season (in weeks) and time of the day. Random effect was territory ID. All continuous variables were scaled. Statistically significant variable is indicated in bold. Sample size is 179.

variable	estimate	SE	$\chi 2$	ndf	ddf	P
body mass index	**0.003**	**0.002**	**3.94**	**1**	**172**	**0.047**
nestling age (in days)	-0.087	0.079	1.21	1		0.271
brood size	0.019	0.078	0.06	1		0.811
sex	-0.023	0.151	0.02	1		0.879
season (week number)	-0.011	0.083	0.02	1		0.898
time of the day (hour)	0.061	0.078	0.62	1		0.430
Intercept	0.011	0.114	0.01	1		0.925

Full model output baseline haptoglobin

Table S4.5. Model output of a linear mixed model exploring the baseline haptoglobin concentration (standardized per year) in relation to parental pair morph combination (either mixed- or like-morph, mixed morph as the reference here). Covariates fitted in the model were nestling age (in weeks), brood size, sex (male as reference), season (in weeks), time of the day (in hours) and a reading at 450 nm to control for plasma redness. Random effect was territory ID. The response variable haptoglobin was log-transformed, all continuous variables were scaled. Statistically significant variables indicated in bold. Sample size is 177.

variable	estimate	SE	χ2	ndf	ddf	P
pair morph	0.012	0.021	0.32	1	169	0.574
nestling age (in days)	0.008	0.010	0.52	1		0.470
brood size	**0.020**	**0.010**	**4.02**	**1**		**0.047**
sex	-0.032	0.020	2.52	1		0.115
season (week number)	**-0.022**	**0.011**	**4.03**	**1**		**0.047**
time of the day (hour)	0.017	0.010	2.70	1		0.102
450nm	-0.006	0.013	0.20	1		0.654
Intercept	**0.372**	**0.017**	**457.04**	**1**		**<0.001**

Table S4.6. Model output of a linear mixed model exploring the baseline haptoglobin concentration (standardized per year) in relation to individual body condition. Covariates fitted in the model were nestling age (in weeks), brood size, sex (male as reference), season (in weeks), time of the day (in hours) and a reading at 450 nm to control for plasma redness. Random effect was territory ID. The response variable haptoglobin was log-transformed, all continuous variables were scaled. Statistically significant variables indicated in bold. Sample size is 177.

variable	estimate	SE	χ2	ndf	ddf	P
body mass index	-0.001	0.001	0.24	1	169	0.624
nestling age (in days)	0.009	0.011	0.68	1		0.408
brood size	0.020	0.010	3.80	1		0.051
sex	-0.031	0.020	2.36	1		0.124
season (week number)	**-0.023**	**0.011**	**4.70**	**1**		**0.030**
time of the day (hour)	0.017	0.013	2.75	1		0.097
450nm	-0.005	0.013	0.14	1		0.709
Intercept	**0.377**	**0.015**	**652.50**	**1**		**<0.001**

Full model output bacteria-killing

Table S4.7. Model output of a linear mixed model exploring the bacteria-killing score (standardized per year) in relation to parental pair morph combination (either mixed- or like-morph). Covariates fitted in the model were nestling age (in weeks), brood size, sex (male as reference), season (in weeks) and time of the day. Random effect was territory ID. All continuous variables were scaled. Statistically significant variables indicated in bold. Sample size is 168.

variable	estimate	SE	χ2	ndf	ddf	P
pair morph	0.008	0.030	0.08	1	161	0.783
nestling age (in days)	0.016	0.012	1.74	1		0.187
brood size	0.015	0.013	1.49	1		0.225
sex	**-0.064**	**0.024**	**7.40**	**1**		**0.007**
season (week number)	0.002	0.014	0.03	1		0.858
time of the day (hour)	-0.001	0.012	0.01	1		0.939
Intercept	**0.398**	**0.024**	**270.63**	**1**		**<0.001**

Table S4.8. Model output of a linear mixed model exploring the bacteria-killing score (standardized per year) in relation to individual body condition. Covariates fitted in the model were nestling age (in weeks), brood size, sex (male as reference), season (in weeks) and time of the day (in hours). Random effect was territory ID. All continuous variables were scaled. Statistically significant variables indicated in bold. Sample size is 168.

variable	estimate	SE	χ2	ndf	ddf	P
body mass index	0.001	0.001	0.89	1	161	0.344
nestling age (in days)	0.014	0.013	1.22	1		0.270
brood size	0.017	0.013	1.71	1		0.190
sex	**-0.064**	**0.024**	**7.94**	**1**		**0.005**
season (week number)	0.004	0.014	0.07	1		0.791
time of the day (hour)	-0.002	0.012	0.03	1		0.866
Intercept	**0.403**	**0.020**	**410.34**	**1**		**<0.001**

Full model output lipopolysaccharide challenge

Table S4.9. Model output of a linear model exploring the haptoglobin response in a lipopolysaccharide immune challenge in relation to parental pair morph combination (either mixed- or like-morph). Response variable was the delta value (post-LPS concentration subtracted by the baseline concentration). Covariates fitted in the model were nestling age (in weeks), brood size, sex (male as reference), season (in weeks), the baseline haptoglobin concentration and a reading at 450 nm of the baseline plasma sample to control for plasma redness. Random effect was territory ID but explained 0% variance and was thus emitted from the model. All continuous variables were scaled. Statistically significant variables indicated in bold. Sample size is 49.

variable	estimate	SE	χ2	ndf	ddf	P
pair morph	-0.158	0.307	0.27	1	41	0.275
nestling age (in days)	0.086	0.154	0.31	1		0.480
brood size	-0.007	0.163	0.01	1		0.968
sex	-0.494	0.310	2.54	1		0.119
season (week number)	-0.161	0.163	0.97	1		0.331
baseline haptoglobin conc.	-0.184	0.149	1.51	1		0.225
450 nm baseline	0.047	0.146	0.10	1		0.750
Intercept	0.255	0.231	1.22	1		0.275

Table S4.10. Model output of a linear model exploring the haptoglobin response in a lipopolysaccharide immune challenge in relation to individual body condition. Response variable was the delta value (post-LPS concentration subtracted by the baseline concentration). Covariates fitted in the model were nestling age (in weeks), brood size, sex (male as reference), season (in weeks), the baseline haptoglobin concentration and a reading at 450 nm of the baseline plasma sample to control for plasma redness. Random effect was territory ID but explained 0% variance and was thus emitted from the model. All continuous variables were scaled. Statistically significant variables indicated in bold. Sample size is 49.

variable	estimate	SE	χ2	ndf	ddf	P
body mass index	-0.001	0.003	0.16	1	41	0.695
nestling age (in days)	0.057	0.058	0.98	1		0.328
brood size	0.042	0.060	0.07	1		0.491
sex	-0.092	0.116	0.09	1		0.430
season (week number)	-0.034	0.061	0.04	1		0.583
baseline haptoglobin conc.	-0.088	0.059	0.32	1		0.143
450 nm baseline	0.008	0.056	0.02	1		0.887
Intercept	**0.415**	**0.080**	**26.96**	**1**		**<0.001**

Body condition residuals and body condition percentiles used for survival analysis

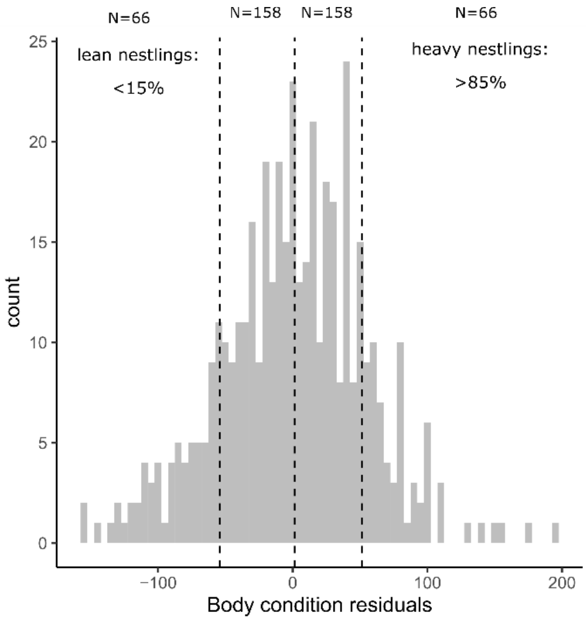

Figure S4.1. Histogram of the black sparrowhawk nestling body mass index residuals. Dashed vertical lines correspond, from left to right, to the 15% percentile boundary, the 0% boundary, 85% boundary of the body condition percentiles used for the MARK mark-recapture survival analysis. Individuals on the left size of the histogram are very lean (low body mass index) whereas chicks on the very right are heavy (high body mass index). The sample sizes for each body mass index percentile are depicted above the histogram.

Chapter 5.

Multigenerational pedigree analysis of wild individually marked black sparrowhawks suggests that dark plumage is a dominant autosomal trait

A modified version of this chapter has been submitted as:
Nebel C, Sumasgutner P, Rodseth E, Ingle RA, Childs DZ, Curtis-Scott O, Amar A. Multigenerational pedigree analysis of wild individually marked black sparrowhawks suggests that dark plumage is a dominant autosomal trait.

Abstract

The black sparrowhawk (*Accipiter melanoleucus*) is a colour polymorphic sub-Saharan raptor with adults occurring in two discrete colour morphs: dark and light. It has previously been suggested that plumage colouration is determined by a one-locus two-allele system with the light allele being dominant over the dark allele. Here, we revisit that assumption with an extended dataset of 129 individuals and pedigree information from 75 individuals spanning five generations. We test the observed offspring phenotypic ratio against the expected ratio under Hardy-Weinberg equilibrium and find significant deviations from the expected values. Contrary to the previous assumption, our data indicates that the dark allele is in fact dominant over the light allele. Similarly, the multigenerational pedigrees obtained are incompatible with a one-locus two-allele system where the light allele is dominant but are consistent with a scenario where the dark allele is dominant instead. However, without knowledge of the underlying molecular basis of plumage polymorphism, uncertainty remains, and the intra-morph variation observed suggests that modifier genes or environmental factors may also be involved. Our study provides a foundation for future research on the adaptive function of colour polymorphism in the species, but also highlights the need for caution when drawing conclusions about the mode of inheritance in wild animal populations in the absence of genetic data, especially when one colour variant is numerically much rarer than the other.

Introduction

Colour polymorphic species are those which express at least two different heritable morphs ('phenotypes') in the same population, which are independent of sex and can freely interbreed (Huxley 1955). They represent ideal models to explore evolutionary processes as the colouration of an individual is relatively easy to distinguish, even in a natural setting. This permits non-invasive studies of polymorphism maintenance in wild animal populations (Gray and McKinnon 2007; McKinnon and Pierotti 2010; Roulin 2004).

Colour polymorphism is relatively rare in birds with only 3.5% being colour polymorphic (Roulin 2004). However, it is much more common in predatory species like owls (Strigidae), hawks (Accipitridae), falcons (Falconidae) or skuas (Stercorariidae) (Roulin 2004; Fowlie and Kruger 2003; Galeotti et al. 2003). Differences in plumage colouration in birds are often associated with differences in melanin pigmentation (Roulin 2004; Galeotti et al. 2003) and morphs can either manifest as two or more discrete phenotypes, or show continuous variation. Raptors showing two discrete colour phenotypes include Eleonora's falcons (*Falco eleonorae*, Gangoso et al. 2011), ferruginous hawks (*Buteo regalis*, Schmutz and Schmutz 1981), little eagles (*Hieraaetus morphinoides*, Larkin and Debus 2020), booted eagles (*Hieraaetus pennatus*, Martínez et al. 2016; Bosch et al. 2019) and black sparrowhawks (*Accipiter melanoleucus*, Amar et al. 2013). Incomplete dominance of one allele, meaning that heterozygotes show intermediate plumage types, can be found in Swainson's hawks (*Buteo swainsoni*, Briggs et al. 2010). Continuous variation is often inherited in a complex fashion, i.e. oligogenic (gyrfalcon *Falco rusticolus*, Chang et al. 2010) or by a quantitative polygenic trait (i.e. common buzzard *Buteo buteo*, Kappers et al. 2018).

The adaptive mechanisms enabling colour polymorphism in the black sparrowhawk have been studied extensively in South Africa (i.e. McCarren et al. 2020; Sumasgutner et al. 2018; Sumasgutner et al. 2016; Tate et al. 2017; Amar et al. 2014). Adults of the species display in two discrete colour morphs: a dark morph which is predominantly black on the throat, breast, belly and underwing coverts and a light morph, which is white on these body parts. There is considerable within morph variation, for example dark morphs range from completely melanistic individuals to individuals with large white throat patches and white speckles on the breast. Similarly, light morphs display varying amounts of dark flecks on their belly and underwing coverts (Figure S5.1 and S5.2, Amar et al. 2013). However, the overall percentage of white plumage on the ventral side of these birds shows a clear bimodal distribution, with no intermediate phenotypes, supporting the broad classification of this species into two discrete morphs (Amar et al. 2013).

In our study population on the Cape Peninsula (Western Cape, South Africa), the dark morph is numerically dominant, with a frequency of 76% (Amar et al. 2013). Previous research has suggested that the mode of inheritance for plumage morph is autosomal with a simple one-locus two-allele system, where the light allele is dominant over the dark allele (Amar et al. 2013). However, this was based on an analysis of only 33 offspring produced from parents of known morph (Amar et al. 2013): while 13 dark x dark matings yielded only dark progeny, the single light x light pairing in this study yielded a dark offspring, which would only be possible in a one-locus two-allele system if the light allele were dominant. However, it subsequently came to light that this was a case of double brooding (Curtis et al. 2005) with two different males on this territory and that the father of the dark offspring was in fact a dark morph. In conclusion, it appears that the evidence for the dark morph allele being recessive is relatively weak.

In the present study, using a larger data set and a more comprehensive analysis, we revisit the idea that the allele responsible for the dark phenotype is recessive and test this hypothesis by comparing observed offspring phenotypes (with known parental phenotypes) to the expected phenotype ratios. We also construct pedigrees obtained from wild black sparrowhawks that span across multiple generations to support our conclusions. We build on the previously collected data presented in Amar et al. (2013) by adding new data, almost quadrupling the total number of observations.

Material and Methods

Study population and data collection

The black sparrowhawk study population is located on the Cape Peninsula (S 34°00’, E 18°26’), Western Cape, South Africa, and data was collected from 2001 to 2019. Territories were visited monthly until breeding activity was recorded and then weekly until the nestlings reached ringing age (details in Martin et al. 2014). During nest visits the plumage morph of the male and female were identified. Females are considerably larger than males, allowing sex to be distinguished in the field (Hockey et al. 2005; Thiollay 1994). On average 74% (ranging from 71 – 80% since 2007) of the breeding individuals per annum were ringed, allowing determination of parenthood in the resulting progeny. In their first year of life, black sparrowhawks have mottled brown and rufous juvenile plumage (Hockey et al. 2005; Thiollay 1994); thus, the adult plumage morph can only be determined once the nestlings are sighted again as adults, with the transitional moult taking place at 12 – 18 months of age (Hartley 1976). Nestlings were ringed 20 – 35 days after hatching with unique colour combinations, and 129 were subsequently resighted as adults, allowing determination of adult plumage morph.

Exploring inheritance patterns under different dominance relationships

Following Amar et al. (2013), we initially assume the dark allele to be recessive and the light allele to be dominant. Thus, the dark morph would be homozygous ('ll') and the light morph either heterozygous ('Ll') or homozygous ('LL'). Alternatively, we assume that the dark allele is dominant and the light allele recessive, which means that dark morphs would either be heterozygous ('Dd') or homozygous ('DD') and the light morph homozygous ('dd'). Assuming a one-locus, two-allele system with complete dominance, mating between recessive morphs will always yield only recessive morph progeny, whereas pairs consisting of a light and a dark morph bird ('mixed-morph pair') will produce either 100% heterozygous dominant morph progeny (if the dominant morph parent is homozygous) or heterozygous dominant and recessive morph progeny at an expected ratio of 1:1 (if the dominant morph parent is heterozygous). Pairings between two birds of the dominant morph will yield all dominant morph homozygous progeny (if both parents are homozygous), dominant morph homozygous and dominant morph heterozygous progeny at an expected ratio of 1:1 (if one parent is homozygous and the other heterozygous) or dominant morph homozygous, dominant morph heterozygous and recessive morph progeny at an expected ratio of 1:2:1 respectively (if both parents are heterozygous).

Statistical analysis

First, we explore whether there is any indication that plumage morph is a Z-linked trait (males being ZZ, females ZW). If plumage morph is a Z-linked trait we would expect to see a difference in morph frequency between the two sexes, while no such difference would be evident if it is an autosomal linked trait, we explore this question fitting the count of male morphs against female morphs in a 2 x 2 contingency table and using χ^2 goodness of fit analysis. For our other analyses, as we do not know the number of heterozygotes in our population, we assume that the population is in Hardy-Weinberg equilibrium and use the proportion of the known genotype in the entire population (homozygous recessive individuals, 76% when assuming dark to be recessive, 24% when assuming light to be recessive) to estimate the proportions of the other genotypes by using the Hardy-Weinberg formula:

$p^2 + 2pq + q^2 = 1$ with p being the dominant homozygote, $2pq$ the heterozygote and q the recessive homozygote frequency (Hardy 1908; Weinberg 1908). Hardy-Weinberg conditions are tied to five key assumptions – no gene flow, no selective pressure on the study genes, random mating, no mutation and infinite population sizes (Hardy 1908; Weinberg 1908). Indications are strong that there is no direct selective pressure on the morph (study gene) nor morph-dependent mate choice in the study population (Tate et al. 2017), which means that Hardy-Weinberg is partly met. Although most natural systems are violating one if not all Hardy-Weinberg assumptions, it nevertheless provides approximate conditions in absence of true genotype frequency information (Table S5.1). Based on this, and assuming that the dark allele is recessive, we expect 6.9% of all light morphs to be homozygous (if the dark allele is recessive) or 34.2% of all dark morphs to be homozygous (if the dark allele is dominant). That means, on the population level, we expect light x light pairings to produce 21.7% and mixed-morph pairs 46.6% dark offspring (assuming dark to be recessive, Table S5.2). Alternatively, assuming that the dark allele is dominant, dark x dark pairings should produce 10.8% and mixed-morph pairs 32.9% light offspring (Table S5.3). We use these expected offspring phenotype ratios and test them against the observed values using χ^2 goodness of fit tests. We only perform χ^2 goodness of fit tests on progeny of mixed-morph pairs as a large sample size was available. We did not remove non-independent observations of multiple offspring from the same parents, which may inflate χ^2 values. All analyses were carried out in R version 3.6.2 (R Core Team 2019).

Table 5.1. Observed offspring phenotypes in relation to parental phenotypes. Male and female parental phenotypes are 'dark' (dark phenotype) or 'light' (light phenotype). Sample size (n) and respective observed offspring phenotype count numbers are provided. Beside the raw data of offspring phenotypes, percentage values are given in brackets. Proportion (%) of expected dark morph offspring when assuming the dark allele to be recessive or dominant and under the assumption that the study population is under Hardy-Weinberg are given.

parental phenotype			obs. offspring phenotype		exp. dark morph offspring	
male	female	n	dark	light	recessive	dominant
dark	dark	55	53 (96.4)	2 (3.6)	100.0	89.2
light	light	8	0 (0.0)	8 (100.0)	21.7	0.0
light	dark	31	26 (83.9)	5 (16.1)	46.6	67.1
dark	light	35	26 (74.3)	9 (25.7)	46.6	67.1
mixed		66	52 (78.8)	14 (21.2)	46.6	67.1

Pedigree construction

We had pedigree data for 75 individuals spanning multiple generations. The informal assessment of multigenerational inheritance can help identifying the mode of inheritance (see i.e. captive-bred colour polymorphic gyrfalcon pedigree, Chang et al. 2010) and can further aid in determining whether a trait is autosomal or sex (Z)-linked. For example, in the case of a single Z-linked locus, hemizygous females with the dominant allele mated to homozygous recessive males can only produce male offspring with the dominant phenotype and female offspring with the recessive phenotype. Pedigrees were prepared using the 'kinship2' package in R (Sinnwell et al. 2014) to assess the inheritance of the phenotype from generation to generation. They are examined for any violations of inheritance and used to visually reinforce any conclusions from our other analysis.

Results

In total, we resighted 105 dark and 24 light morphs with known parental morph combinations in our population. If plumage morph were determined by a single Z-linked gene this would be expected to lead to unequal morph frequencies in the population between the two sexes due to hemizygosity for Z-linked loci in females. We did not see any statistically significant difference in morph frequency between male and females (dark males: 57, dark females: 48, light males: 16, light males: 8; $\chi 2 = 0.77$, df = 1, P = 0.381). Similarly, our pedigrees were not consistent with Z-linked inheritance, irrespective of whether the dark or light allele is assumed to be dominant. For example, assuming light to be dominant, mixed-morph pairs where the female is light and male dark should give rise to dark females and light males only. Yet the mating between 798784 (light female) and 6H03235 (dark male) gave rise to two dark females, and that between the same female and another dark male (687856) to two light males (Pedigree 2, Figure 5.2). Similarly, assuming the dark allele is dominant, mixed-morph pairs where the female is dark and male light should give rise to light females and dark males only. Again, this does not hold; for example, the mating between 796777 (dark female) and ATM1 (an unringed light male, but identifiable by unique plumage characteristics) giving rise to a dark female and two light males, and that between an unringed dark female and 6H03251 (light male) to two dark females (Pedigree 1, Figure 5.2). Overall, our data are thus more consistent with an autosomal mode of inheritance.

We then explored our data assuming the dark allele to be recessive. For this analysis we found statistically significant deviations from the progeny frequencies predicted if the population were in Hardy-Weinberg equilibrium. Pairs consisting of light and dark morph birds produced more dark morph offspring than expected ($\chi2 = 27.52$, df = 1, $P < 0.001$, Table 5.1). Additionally, we found two light offspring produced by dark x dark pairings, which is not possible if the dark allele were recessive (Figure 5.2). In contrast, assuming the dark allele to be dominant explained 100% of all observations in the population with no violations of Mendelian inheritance (Figure 5.2). The observed offspring phenotype ratios also significantly differed from the expected, but with a much smaller χ^2 and larger P-value ($\chi2 = 4.07$, df = 1, $P = 0.044$). Assuming the dark allele to be dominant, the allele frequency calculated is 0.51 for the dark allele under Hardy-Weinberg conditions.

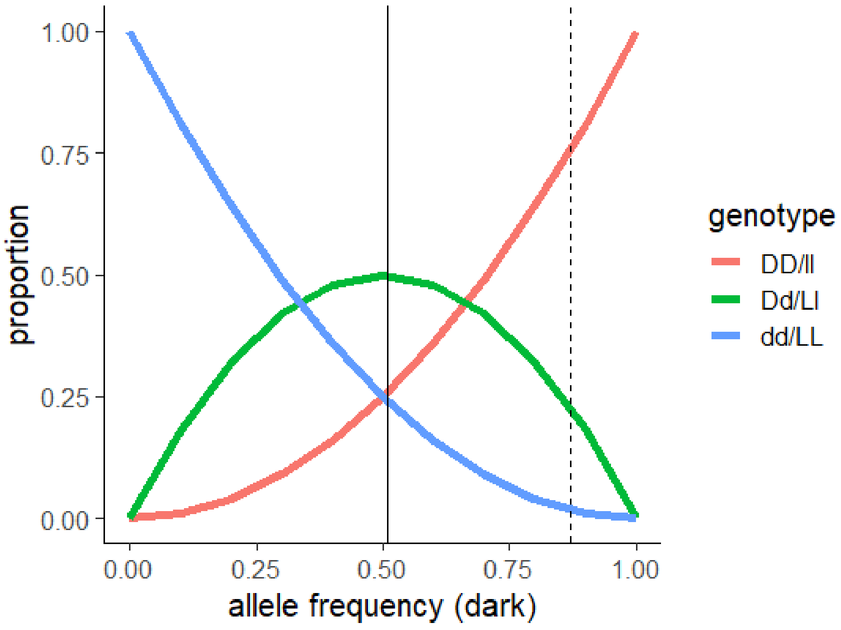

Figure 5.1. Allele frequencies under Hardy-Weinberg conditions. According to Hardy-Weinberg, the frequency of alleles is predictable in absence of selective forces, genetic drift effects or gene flow. Different colours translate to different genotype proportions (assuming the dark allele to be recessive: 'LL', 'Ll' and 'll' and assuming the dark allele to be dominant: 'DD', 'Dd', 'dd'). The vertical lines indicate the proportion of the alleles in the population under Hardy-Weinberg. Dashed vertical line is the proportion assuming the dark allele to be recessive with 87% dark alleles and 13% light alleles, following Amar et al. 2013). The solid vertical line assumes that the dark allele is dominant. Under Hardy-Weinberg conditions, 0.24 light phenotypes predict 0.26 homozygous dark phenotypes and 0.50 heterozygous dark phenotypes in the population, which means that the alleles are present at an almost equal ratio: 0.51 dark alleles and 0.49 light alleles. This also translates in about 0.66 heterozygote dark phenotypes of all dark phenotypes.

The inheritance of plumage morph in birds over five generations (Figure 5.2) is entirely consistent with a single autosomal gene mode of inheritance where the dark allele is completely dominant over light. For example, consider the light individual 6H03251 (Figure 5.2, Pedigree 1) with two dark parents. In this case, the light allele would be passed on from a light grandfather (681800) via the dark heterozygote (687870). Notable also are 798784 (Pedigree 2, Figure 5.2) and ATM1 (Pedigree 1, Figure 5.2). Both have a light phenotype and bred with two different dark mates. The female 798784 produced only dark progeny with 6H03235 (most likely a homozygote 'DD') but only light morph progeny with 687856 (a heterozygote 'Dd'). The light male ATM1 produced a mix of dark and light progeny with a dark female (798777, a heterozygote 'Dd') but only dark offspring with a different dark female (798799, most likely a homozygote 'DD'). Lastly, the two light like-pairs shown in the pedigree (798775 x 6H03270 and 798775 x 6H05512) produced only light offspring, albeit sample size is small.

Discussion

In contrast to Amar et al. (2013) which suggested that the dark allele was recessive, we instead now conclude that the dark allele is most likely dominant. We also conclude, similarly to Amar et al. (2013) that the mode of inheritance is autosomal. This dominance relationship is similar to two other raptor species, the Eleonora's falcon (Gangoso et al. 2011) and the ferruginous hawk (Schmutz and Schmutz 1981). In both species, colour polymorphism is expressed in two discrete colour morphs with the dark allele being dominant.

A key issue relating to the morph inheritance is the large within-morph variability seen in the species. Although the two morphs can be considered as discrete, and the ratio of black to white colouration follows a bimodal distribution (Amar et al. 2013), there is considerable variability within the two morphs (Figure S5.1 and S5.2). The observed within-morph variability could be due to expressivity (the degree to which a given genotype is phenotypically expressed, Abolins-Abols et al. 2018; Li et al. 2012; Xu et al. 2013) resulting from the influence of modifier genes on the final phenotype (as in Chang et al. 2010; Kappers et al. 2018) and/or environmental factors (Rosenfeld 2010). Based on our results, it appears as if the discrete morphs may be determined by a one-locus two-allele system with the dark allele being dominant. However, it is important to note that the molecular basis of plumage polymorphism in this species is not yet understood. Identification of the gene(s) involved will shed light on the within morph variation observed in the black sparrowhawk.

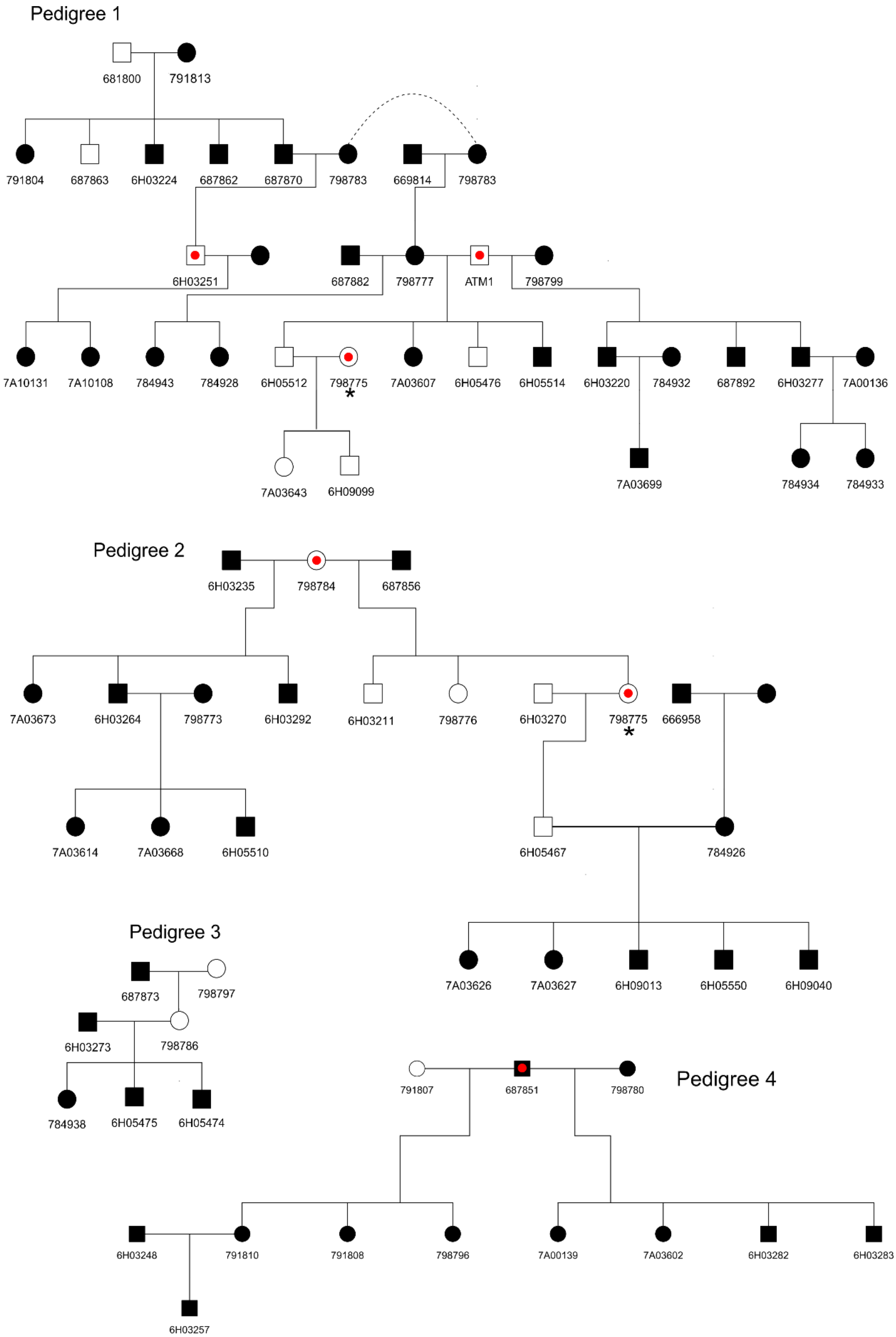

Figure 5.2. Four pedigrees of wild black sparrowhawks. Squares are males, circles females. Solid black colour indicates a dark phenotype, white colour light phenotype. Red dots indicate individuals of interest and are explained in the text. Dashed lines between individuals or symbols are used to indicate duplicates and connections between pedigrees. ID (SAFRING number) written below individual symbols. If an individual is unringed, space beneath the symbol is empty.

Whether the dark allele is dominant, or recessive has profound implications for the allele frequencies in the population. Our study population is numerically dominated by dark phenotypes, assuming the dark allele to be recessive, results in high proportion of the dark allele in our population (0.87 dark allele, Amar et al. 2013), whereas our current interpretation would lead to a much lower allele frequency (0.51 dark allele). In this case, the two alleles are thus present in similar proportions, but the light phenotype appears much rarer than the dark phenotype.

In addition, our current interpretation challenges previous conclusions about this study population. For example, we know that mixed-morph pairs produce higher quality offspring, based on apparent survival rates (Sumasgutner et al. 2016). However, previous work concluded that heterozygote advantage (Fisher 1922; Gemmell and Slate 2006; Gray and McKinnon 2007; Hedrick 2012) was unlikely to be the mechanism behind this finding because the proportions of heterozygote progeny were not thought to be very different between those of like-morph pairs and those from mixed-morph pairs, when assuming the population to be under Hardy-Weinberg (Tate et al. 2017). However, those previous assumptions no longer hold, and if the dark allele is dominant, mixed-morph pairs are expected to produce overall more heterozygote offspring than any other parental morph combination (mixed-morphs between 50 - 100%, assuming Hardy-Weinberg conditions: 66%; dark like-morphs between 0 – 50%, assuming Hardy-Weinberg conditions: 33%). Therefore, this makes heterozygote advantage a plausible mechanism: It results from heterozygote individuals having a fitness advantage over homozygotes.

Usually, heterozygote advantage arises through incomplete dominance in heterozygotes (Fisher 1922). Colour polymorphism in grey wolves (*Canis lupus*) in Yellowstone national park is thought to be maintained by heterozygote advantage. Although coat colouration cannot be visually differentiated between heterozygotes and homozygotes, heterozygous black individuals have higher fitness than homozygous black individuals (Coulson et al. 2011; Hedrick et al. 2014). Although the exact mechanism behind this is unknown, this asymmetric fitness advantage for black coat colouration is believed to drive colour polymorphism maintenance in this population (Hedrick et al. 2014) and similar mechanisms might be in effect in black sparrowhawks.

A weakness of observational inheritance data is that parentage can only be confirmed with certainty with genetic data. However, extra pair paternity is a rare phenomenon in socially monogamous raptors with proportions ranging from 1.3% in the closely related northern goshawk (*Accipiter gentilis*, Gavin et al. 1998; Kunz et al. 2019; Rutz 2005) to 0% in merlin (*Falco columbarius*, Warkentin et al. 1994) to 3 – 11.2% in American kestrels (*Falco sparverius*, Villarroel et al. 1998). In Cooper's hawks (*Accipiter cooperii*), extra pair paternity can be considered as high as 19.3% of all nestlings (Rosenfield et al. 2015), but such high levels of extra pair paternity seem to be an exception. Thus, potential errors linked to extra-pair paternity should be minor and not compromise the interpretation of our results.

Conclusion

Our study emphasizes that considerable caution must be applied when using small sample sizes to explore inheritance patterns, especially when one phenotype is much rarer than the other. In the case of the black sparrowhawk, due to a combination of small sample size of light x light pairs and one observation being wrongly assigned to a parental morph, the dominance relationship was wrongly interpreted. Analysis of our larger dataset suggests that an autosomal mode of inheritance with the dark allele dominant over light is a more likely model for the genetic basis of plumage polymorphism in this species.

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Supplementary Material part 5.1

Table S5.1. Expected allele frequencies under the assumption that the dark allele is recessive or dominant and under Hardy-Weinberg conditions. The Hardy-Weinberg formula is $p^2 + 2pq + q^2 = 1$ with p^2 being the proportion of dominant homozygotes, $2pq$ is the proportion of heterozygotes f and $q^2$the proportion of recessive homozygotes. The respective calculated frequencies are given in the column 'frequencies'. Assuming the dark allele to be recessive, 6.85% of all light morphs would be homozygous, whereas assuming the dark allele to be dominant, 34.24% of all dark morphs are homozygous. Hardy-Weinberg conditions – no gene flow, no mutations, no selection on the target gene, random mating, infinite population size – are unlikely to be met in most natural conditions, thus, these expected frequencies must be taken with caution.

allele	dominant allele	frequencies
q^2	light	0.76
$2pq$	light	0.22
p^2	light	0.02
q^2	dark	0.24
$2pq$	dark	0.50
p^2	dark	0.26

Table S5.2. Expected progeny frequencies of different parental genotype and morph combinations assuming the dark allele to be recessive, under Hardy-Weinberg conditions (Hardy 1908; Weinberg 1908) and following Mendelian inheritance (Mendel 1865). Genotypes are either 'LL' or 'Ll' (light phenotypes, 6.85% homozygote) or 'll' (dark phenotype). Frequencies were calculated based on the proportion of the known genotype in the entire population, which is the homozygous recessive 'll'. Column 'pair genotype' lists all potential genotype combinations, 'mating frequency' the expected proportion of pairs under Hardy-Weinberg conditions. 'Progeny frequency' follows Mendelian inheritance and takes pair frequency into account. 'Sum' lists the frequency of specific mating combinations and the column dark morph progeny the expected number of dark morph progeny for specific pair morph combinations. Numbers were rounded and shown to three decimal places.

pair genotype	mating frequency	progeny frequency			sum	dark morph progeny (%)
		LL	Ll	ll		
LL x LL	<0.001	<0.001	0	0		
LL x Ll	0.007	0.004	0.004	0		
Ll x Ll	0.050	0.012	0.025	0.012		
light x light pairs					0.058	21.7
LL x ll	0.025	0	0.025	0		
Ll x ll	0.340	0	0.170	0.170		
mixed-pairs					0.365	46.6
ll x ll	0.578			0.578		100
dark x dark pairs						

Table S5.3. Expected progeny frequencies of different parental genotype and morph combinations assuming the dark allele to be dominant, under Hardy-Weinberg conditions (Hardy 1908; Weinberg 1908) and following Mendelian inheritance (Mendel 1865). Genotypes are either 'DD' or 'Dd' (dark phenotypes, 34.24% homozygote) or 'dd' (light phenotype). Frequencies were calculated based on the proportion of the known genotype in the entire population, which is the homozygous recessive 'dd'. Column 'pair genotype' lists all potential genotype combinations, 'mating frequency' the expected proportion of pairs under Hardy-Weinberg conditions. 'Progeny frequency' follows Mendelian inheritance and takes pair frequency into account. 'Sum' lists the frequency of specific mating combinations and the column light morph progeny the expected number of light morph progeny for specific pair morph combinations. Numbers were rounded and shown to three decimal places.

pair genotype	**mating frequency**	**progeny frequency**			**sum**	**light morph progeny (%)**
		DD	**Dd**	**dd**		
DD x DD	0.068	0.068	0	0		
DD x Dd	0.260	0.130	0.130	0		
Dd x Dd	0.250	0.062	0.125	0.062		
dark x dark pairs					0.58	10.8
DD x dd	0.125	0	0.125	0		
Dd x dd	0.240	0	0.120	0.120		
mixed-pairs					0.36	32.9
dd x dd	0.058	0	0	0.058		
light x light pairs						

Figure S5.1. Within-morph variability expressed in the black sparrowhawk. All individuals shown here are considered dark morphs following Amar et al. 2013. The two top rows show the expression of plumage variability on the throat, breast and belly, the two bottom rows show expression of plumage variability on the underwing coverts. Variability is expressed as varying amount of black and white feathers, i.e. as a white throat or white speckles on the breast. In some cases, black sparrowhawks are almost completely melanistic on these body parts (top left image).

Figure S5.2. Within-morph variability expressed in the black sparrowhawk. All individuals shown here are considered light morphs following Amar et al. 2013. The top row shows the expression of black and white plumage colouration on the throat, breast and belly, the three bottom rows show variability on the underwing coverts.

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Chapter 6.

Heterozygote advantage via pair complementarity and seasonality best explains colour polymorphism maintenance in an African raptor

Abstract.

Variability in different phenotypes is associated with high evolutionary potential of populations and species. Thus, it is important to understand how polymorphic traits persist. One hypothesis to explain how different morphs are maintained is heterozygote advantage, which benefits heterozygous individuals over both homozygous variants. Another proposed hypothesis is the complementarity hypothesis, whereby individuals raised by different parental phenotypes (mixed-morph pair) have a selective advantage over individuals raised by the same parental phenotype. In this study, we explore which mechanism might explain the maintenance of colour polymorphism in a population of black sparrowhawks (*Accipiter melanoleucus*) with an individual-based model. The species occurs as a dark and light colour variant, with dark morphs being numerically dominant on the Cape Peninsula, South Africa. The two morphs forage during different times of the day, which means that they can temporarily expand their hunting niche within a pair and potentially raise fitter offspring when they breed as a mixed-morph pair. At the same time, they would also produce more heterozygous offspring. Mixed-morph pairs have nestlings with higher survival rates, due to either heterozygote advantage or the complementary nature of the two morphs. Furthermore, seasonality-associated effects benefit dark morph males in this system. Simulations show that both heterozygote advantage and complementarity can produce evolutionary stable polymorphisms. By adding morph-dependent seasonality effects, we explain the numerical predominance of dark morphs in the study population. Complementarity, in combination with seasonality effects, indirectly causes heterozygote advantage with heterozygous males having the highest lifetime reproductive success. Our simulations indicate that the observed morph frequencies in the black sparrowhawk are likely due to combined effects of complementarity mechanisms and seasonality-associated selective agents.

Introduction

How several morpho-types can co-exist when natural selection is assumed to favour the fittest is a long standing question (Darwin 1859; Huxley 1955). In the absence of mechanisms stabilising such morpho-types all species would be monomorphic, expressing no variability in their phenotypes. In reality, natural populations show the opposite pattern, and species that display higher genetic or phenotypic variability are generally associated with higher evolutionary potential, lower extinction risk (Cattin et al. 2016; Ducatez et al. 2017; Forsman 2016; Forsman and Wennersten 2016; Takahashi and Noriyuki 2019), faster speciation processes (Hugall and Stuart-Fox 2012) and larger distributions with wider niches (Galeotti and Rubolini 2003; Takahashi and Noriyuki 2019). Thus, high variability is beneficial for a species and several mechanisms could explain how polymorphism may be maintained.

In this study we outline two specific hypotheses proposed to explain the maintenance of colour polymorphism. Firstly, heterozygote advantage, which operates in diploid and polyploid organisms (Fisher 1922), whereby heterozygotes have a fitness advantage over homozygotes, thus promoting a stable polymorphism. Empirical examples are commonly associated with traits found in domestic animals (see i.e. Adalsteinsson 1980; Fujii et al. 1991; Gemmell and Slate 2006; Hillbertz et al. 2007; Karlsson et al. 2007; Mosher et al. 2007), but also in wild populations by providing resistance to diseases or pesticides (Allison 1954; Gemmell and Slate 2006; Hedrick 2012; Rost et al. 2004), or by being associated with colour polymorphism (Coulson et al. 2011; Hedrick 2012; Hedrick et al. 2014; Kellenberger et al. 2019; Pruvost et al. 2011). Secondly, pair complementarity, whereby an individual's fitness advantages can arise through ideal early life conditions via carry-over effects (Cam and Aubry 2011; Lindström 1999) when raised by parents that exhibit different phenotypes that behaviourally complement each other (Sumasgutner et al. 2016; Tate et al. 2017). Early life conditions are mainly influenced by the environment (Cam and Aubry 2011), but in altricial species which exhibit biparental care (Clutton-Brock 1991; Cockburn 2006; Sonerud et al. 2014), the quality of parental behaviour can determine the quality of offspring (Cam et al. 2003; Vitikainen et al. 2019). Under the complementarity hypothesis, parents that exhibit different heritable phenotypes ("mixed variants") to each other, may behaviourally complement each other when raising young and thus determine their offspring's long-term fitness (Sumasgutner et al. 2016; Tate et al. 2017). Such differences might be due to different parental investment strategies (Grunst et al. 2018; Järvistö et al. 2015; Limbourg et al. 2013; Ratikainen and Kokko 2010), antipredator tactics (Morales et al. 1989; Rastogi et al. 2006), foraging behaviour (Both et al. 2005; Dingemanse et al. 2004; García-Navas and Sanz 2011; Lewis et al. 2002) or phenotype colouration that is often associated with aforementioned behavioural differences (i.e. Ducrest et al. 2008; Galeotti et al. 2003; Knapton and Falls 1983; McKinnon and Pierotti 2010; Pryke and Griffith 2009b; Roulin 2004; Tuttle 2003).

A population in which either mechanisms could theoretically operate, is the black sparrowhawk (*Accipiter melanoleucus*), a sub-Saharan colour polymorphic raptor inhabiting the Cape Peninsula (Western Cape, South Africa). It occurs in two distinct colour variants, a dark and a light adult morph (Amar et al. 2013; Hockey et al. 2005), which are inherited by a one-locus two-allele system with the allele coding for the dark allele most likely being dominant (light phenotype: 'dd' genotype; dark phenotype: 'Dd' or 'DD', Nebel et al. under review-b). The morph frequency has been stable at 75.6% dark morphs in the study population (Amar et al. 2013).

Previous research suggests that there is little variation in the vital rates of individuals depending on their phenotype, however, there are fitness differences depending on the parental morph combination, with mixed-morph pairs (consisting of two differently coloured parents) having higher breeding success (Tate et al. 2017) than like-morph pairs (pairs consisting of the same colour morph), and their offspring having higher apparent survival rates (Sumasgutner et al. 2016). The reason for this is unknown. It could be due to heterozygote advantage with mixed-morph pairs producing more heterozygous offspring ('Dd') than any other parental morph combination (Chapter 5; Nebel et al. under review-b) or it could be due to the complementary nature of the two morphs when foraging: Hunting behaviour and foraging success differ temporarily for the different morphs (Tate et al. 2017; Tate and Amar 2017) and might allow mixed-morph pairs to effectively expand their foraging niche (Chapter 2; Chapter 3; Nebel et al. 2020; Nebel et al. 2019). This emergent property found in mixed-morph pairs, may improve offspring quality and thus long-term fitness (Chapter 5; Nebel et al. under review-a; Sumasgutner et al. 2016).

Independently of the fitness benefits for offspring of mixed-morph pairs, there is a strong morph-dependent seasonality component with early breeders being more productive than later ones (Martin et al. 2014b; Rose et al. 2017; Tate et al. 2017). Over the nine-month egg-laying season (Martin et al. 2014b), environmental conditions vary, with early conditions seeming to favour breeding dark morphs when hunting and later conditions favouring light morphs

(Sumasgutner et al. 2016; Tate et al. 2017). In combination with the well pronounced roles during the breeding season, with the male providing food to both female and young, while the female is mainly incubating and brooding (Newton 1978), this favourable conditions specifically act on males which is reflected in a morph-dependent timing of breeding: Dark morph males nest earlier than light morph males (Tate et al. 2017). Additionally, nestlings hatched early in the season have a higher probability of recruiting into the breeding population when their father is a dark morph whereas the probability of recruitment is higher for later hatched nestlings only if their father is a light morph (Sumasgutner et al. 2016). This morph-dependent seasonality-associated effect introduces an additional component which might drive morph frequencies in the population.

In this study, we explore whether the two mechanisms, heterozygote advantage and complementarity, can explain the maintenance of colour polymorphism in this population by building an individual-based model. First, we explore heterozygote advantage and complementarity in isolation of morph-dependent seasonality effects, how they might influence genotype frequencies and how the two scenarios might differ in terms of long-term genotype frequencies. Second, we explore how morph-dependent seasonality effects influence genotype frequencies in conjunction with complementarity and heterozygote advantage. By an informal comparison of the simulated data with the empirical morph frequency, we evaluate which one of the two mechanisms, heterozygote advantage or complementarity, could best explain the frequency seen in the population. We predict that while morph-dependent seasonality is driving the numerical predominance of dark phenotypes in the population by facilitating dark morphs to breeding during the prime early breeding conditions, heterozygote advantage or complementarity allow light morphs to persist over time. Lastly, if complementarity is capable to produce a stable morph equilibrium, it might be indirectly associated with heterozygote advantage. To explore whether complementarity alone or its combination with morph-dependent seasonality-associated fitness cause heterozygote advantage, we extract genotype-specific fitness parameters from the simulated data. We predict that if complementarity alone is sufficient to induce heterozygote advantage, heterozygous females ('Dd') should have highest lifetime reproductive success as they are unaffected by seasonality. Contrary, if only integrating complementarity and seasonality induce heterozygote advantage, it will manifest itself in males, which are known to be strongly affected by seasonality (Tate et al. 2017).

Material and Methods

Model structure

We construct an individual-based model with the purpose to understand how morph-dependent seasonality-associated (hereafter 'seasonality-associated fitness') and genotype-specific parameters determine morph frequencies over time. Model description follows the standard Overview Design concepts and Details format (Grimm et al. 2006).

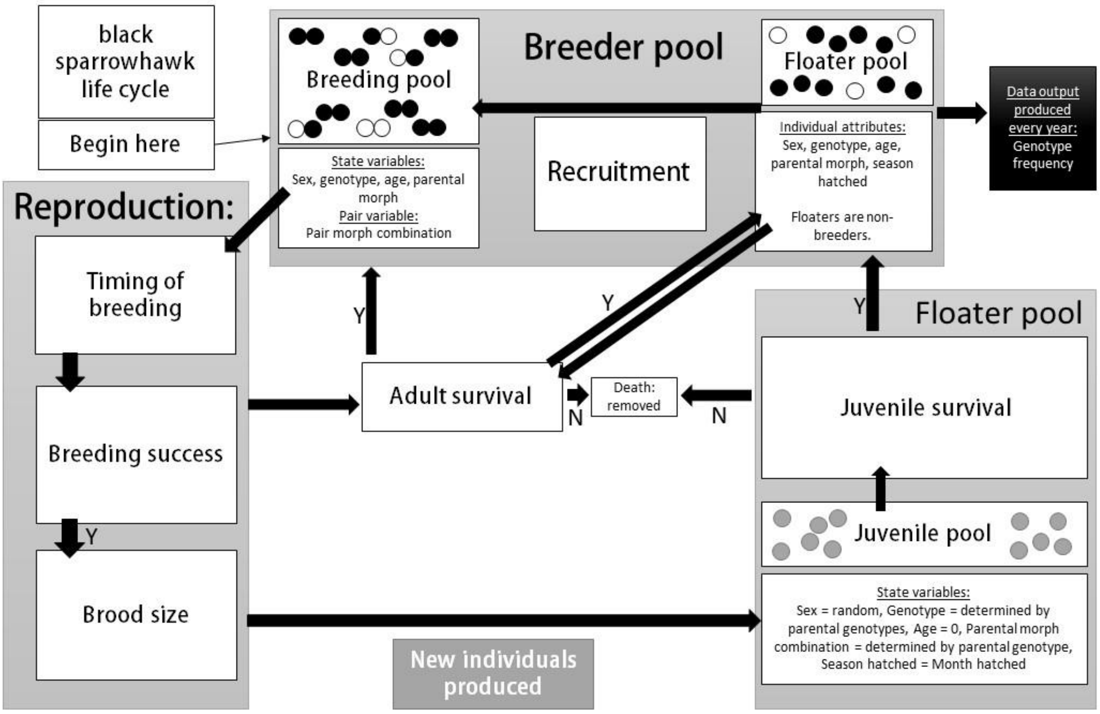

Figure 6.1. Individual-based lifecycle used for this model. The population consists of 50 breeding pairs. Timing of breeding is determined either dependent (seasonality runs: 'complementarity + seasonality', 'heterozygote advantage + seasonality') or independent of the morph of the breeding male ('complementarity' and 'heterozygote advantage' runs). In the latter models, breeding success is only determined by timing of breeding. Nestlings that are produced are moved to the juvenile/floater pool. Survival of adults and non-breeders is determined by parental morph combination ('complementarity hypothesis' runs) or heterozygote advantage ('heterozygote advantage' runs). Death of individuals produces vacant breeding slots and are filled by individuals from the juvenile/floater pool (recruitment). Recruitment is determined by either a lottery model or by seasonality and the morph of an individual's father ('seasonality' runs). Individuals that are hatched early, have a higher chance to be recruited if they have a dark morph father and late individuals have a higher chance to be recruited if they have a light morph father. Letter 'Y' indicates a positive outcome (successful breeding attempt or survival) whereas the letter 'N' indicates a negative outcome (mortality).

The model structure distinguishes between breeding pairs that consist of a male and a female individual ('breeder pool') and individual non-breeders ('floater pool', consisting of strict non-breeders < 1 years old and potential breeders that > 1 years). Breeding individuals are characterized by the following state variables: age, sex, genotype (heterozygous individuals: 'Dd', homozygous individuals: 'DD', 'dd') and parental morph combination ('mixed-morph' or 'like-morph'). Individual non-breeders are characterized by the state variables: age, genotype, parental morph combination, morph of their father and month hatched, which is the parental month of breeding in the year when born.

The model proceeds in annual time steps. Within each simulated year (lifecycle, Figure 6.1) four modules are processed in the breeder pool: Timing of breeding, breeding success, brood size, and survival of breeding individuals. Two modules are then processed in the floater pool: survival and recruitment from the floater into the breeding pool. At the end of each simulated year, young produced by the breeders were added to the non-breeder pool and the whole population aged by one year.

Depending on the assumptions of the model variant, a pair's month of egg-laying (months 3 – 11) was either determined independent or dependent on the morph of a pair's male. Reproduction consisted of breeding success (binomial, successful or not successful) and by brood size (1 – 3). First breeding success was determined, then brood size for every successfully reproducing pair. Breeding success was always determined by a pair's timing of breeding with early breeders having a higher chance to reproduce successfully. Only pairs with both male and female being older than a year could reproduce, which reflects age at maturity (Sumasgutner et al. 2019; Sumasgutner et al. 2016). Breeding pairs inherit genotype to their offspring by following Mendelian inheritance with the dark allele being dominant over the light allele (Mendel 1865; Chapter 5; Nebel et al. under review-b). Thus, successfully breeding pairs were determining genotype and morph frequencies found in the population in subsequent years.

An individual's baseline survival rate was determined by age, but survival was also influenced by either an individual's genotype or parental morph combination, depending on the model assumption. Individuals which do not survive were discarded. Death by breeders produced vacancies, which were then filled by individuals from the floater pool. Individuals were assigned to their partner independently of morph as mate choice is absent in this population (Tate et al. 2017), and divorce only occurred when one partner died. Although divorce occurs in the black sparrowhawk, it does so at low rates (Martin et al. 2014a) and thus is unlikely to play a role in maintaining colour polymorphism in the species (Tate et al. 2017). Finally, all chicks produced by breeding pairs were added to the floater pool.

Model assumptions related to our research questions

The two research questions were explored in two different scenarios: (1) Under complementarity (consisting of the 'complementarity' and 'complementarity + seasonality' runs), an individual's survival was determined by its parental morph combination, whereas under heterozygote advantage (consisting of the 'heterozygote advantage' and 'heterozygote advantage + seasonality' runs), individual survival was determined directly by an individual's genotype.

i) To answer the first research question, we perform a sensitivity analysis of survival with the aim to explore how complementarity and heterozygote advantage translate into long-term genotype frequencies in absence of seasonality-associated fitness ('complementarity' and 'heterozygote advantage' runs). Absence of seasonality-associated fitness means that timing of breeding is not determined of the breeding male's morph, breeding success was determined by timing of breeding, survival was determined by parental morph combination ('complementarity' run) or genotype ('heterozygote advantage' run) and recruitment was not determined by the father's morph and seasonality (meaning by a lottery model).

ii) To answer the second research question, how complementarity and heterozygote advantage behave in conjunction with seasonality-associated fitness and which one explains the observed morph frequencies best, we incorporated seasonality-associated fitness into the model ('seasonality' runs: 'heterozygote advantage + seasonality' and 'complementarity + seasonality'). For this, we assumed that timing of breeding was determined by the morph of the male with dark morphs breeding earlier than light morphs and the recruitment probability being determined by the father's timing of breeding in the year when born and the morph of the father.

Parameterization of the model

Timing of breeding: All probabilities determining timing of breeding are listed in Table S6.1 (Tate et al. 2017). *Breeding success*: The probability of a pair breeding successfully (P_{suc}) was determined by the equation

$$P_{suc} = -0.066x + 1.25 \tag{1}$$

with *x* being the month of a pair's breeding in the respective simulated year (ranging between 3 – 11). Eq. (1) states that early breeders have higher breeding success than those later in the season (Tate et al. 2017). *Brood size*: How many nestlings (1 – 3) a pair produces was determined by the probabilities: 0.3 (one), 0.5 (two) and 0.2 (three chicks). Probabilities were extracted from published data (Tate et al. 2017). *Baseline survival rate*: Baseline age-structured survival probabilities (P_{surv} or $P_{\text{surv baseline}}$) were $P_{\text{surv 1}}$ = 0.56, $P_{\text{surv 2}}$ = 0.74, $P_{\text{surv 3}}$ = 0.75, $P_{\text{surv 4}}$= 0.86. $P_{\text{surv 4}}$ was applied for all individuals of age 4 or older. The baseline age-structured survival probabilities were obtained specifically for this study (see supplementary material part 1 and 2). *Recruitment*: An individual's probability to be recruited into the breeding population was either determined independent ('complementarity' and heterozygote advantage' runs) or dependent on the morph of the father and seasonality ('complementarity + seasonality' and 'heterozygote advantage + seasonality' run). If recruitment was determined independently, it was a lottery model, meaning that every individual had the same chance to be recruited into the breeder population. If recruitment was determined dependent on the morph of the father and seasonality, individuals that were born earlier in the season had a higher chance to be recruited if their father was a dark morph. The reverse was the case for individuals of light morph fathers. All probabilities of recruitment can be found in Table S6.2 (probabilities extracted from models used in Sumasgutner et al. 2016).

Simulation experiments

To perform a sensitivity analysis, we designed a gradient of survival probabilities. This allowed us to explore how varying strengths in complementarity and heterozygote advantage survival translates into long-term genotype frequencies in the population. The gradient of survival probabilities consisted of a total of 100 parameters, split into two sets containing 50 parameters each. Survival probability differences (Δ_{surv}) between homozygous and heterozygous individuals or offspring of like-morph or mixed-morph pairs were pronounced in the first three years of an individual's life (for details see supplementary material part 6.1 and 6.2). First, homozygotes or offspring of like-morph pairs had an increased survival probability ($P_{\text{surv+ i}}$) following the equation

$$P_{surv+\ age\ i} = 0.0007x + P_{surv\ baseline\ age\ i} \tag{2}$$

with *x* being the parameter number (1 – 50) and *i* the individual's age in the respective simulation year (age 1 – 3, the baseline parameter was used for older individuals). Heterozygotes or offspring of mixed-morph pairs had baseline survival probabilities, which produced a $\Delta_{\text{surv}} < 0$. Then, Eq. (2) was used to calculate $P_{\text{surv+ i}}$ for heterozygotes or offspring of mixed-morph pairs. For these parameters, homozygotes or offspring of like-morph pairs had baseline survival probabilities, which produced a $\Delta_{surv} > 0$. The survival gradient produced a Δ_{surv} with a range from -0.13 to 0.13. The empiric Δ_{surv} is 0.09 (SD = 0.05) and favours the heterozygous genotype or offspring of mixed-morph pairs.

For initialization, the model consisted of a stable number of breeders ('breeder pool', 50 males and 50 females) and an initial number of 100 non-breeders ('floater pool' or 'juvenile pool') from which recruitment took place. The size of the individual-based model breeder pool is very similar to our study population (Martin et al. 2014a). In both pools, initial genotype frequency was 0.5 homozygote dark morphs ('DD'), 0.2 heterozygote dark morphs ('Dd') and 0.3 homozygote light morphs ('dd'). These values were chosen arbitrarily with a mix of all genotypes that do not reflect expected proportions (Chapter 5; Nebel et al. under review-b). Initial parental morph combination was determined based on an individual's genotype and initial genotype frequencies. Initialization was the same for all runs.

Every run was completed after 5 000 years to ensure that morph frequencies had either reached fixation or a stable equilibrium, but a burn-in of 3 000 years was discarded as the dynamics were slow to reach a stable equilibrium. We performed 10 repeats and calculated the mean genotype frequencies for each year.

Informal model comparison of morph frequency behaviour

To compare the simulated data in representative units that reflect empirical observations (2001 – 2010, Amar et al. 2013), we summarized the yearly model means into 10-year means. We carried out an informal comparison of the simulated morph frequencies to the 95% CI of observed morph frequencies (mean dark morphs = 75.6%, CI = 72.9 – 78.3) in relation to the empirical parameters. The individual-based model was coded in R (version 3.5, R Core Team 2019). Computations were performed using facilities provided by the University of Cape Town's ICTS High Performance Computing team: hpc.uct.ac.za.

Genotype-specific LRS, breeding time and mean reproductive success

We used one of the ten individual runs of the 'complementarity + seasonality' fitted with the empiric survival rate ($\Delta_{surv} = 0.09$) to explore genotype-specific fitness. From the simulated data, we extracted genotype-specific lifetime reproductive success ('LRS'), time an individual spent in the breeder pool ('breeding time') and the mean number of nestlings produced per year. Breeding time is influenced by an individual's survival rate, whereas the number of nestlings produced is determined by seasonality and is dependent on the morph of the male of a pair. LRS is the sum of chicks an individual produced in its lifetime and is thus the combined effect of breeding time and reproductive success. We calculated the mean individual LRS, breeding time and number of nestlings its standard errors for males and females. All plots were generated using the package 'ggplot2' (Wickham et al. 2019).

Results

Model confirmatory results

When favouring offspring of like-morph pairs ('complementarity' runs), the stimulated population reaches fixation of 'DD', which likely happens due to the numerical dominance of homozygote dark morphs at initialization (Figure 6.2, Figure 6.3, $\Delta_{surv} < 0$). If the model is parameterized to favour both homozygote variants, summarized over all repetitions, both 'dd' and 'DD' remain at equal proportions in the population (Figure 6.2, $\Delta_{surv} < 0$). This means that each homozygote genotype has the same chance to come to fixation. Neutral selective forces (Figure 6.2, Figure 6.3, $\Delta_{surv} \sim 0$) allowed polymorphism to be maintained over considerable time spans just by chance, but usually lead to random fixation of one genotype in the long-term. These results show that our individual-based model is capable to produce predictable results.

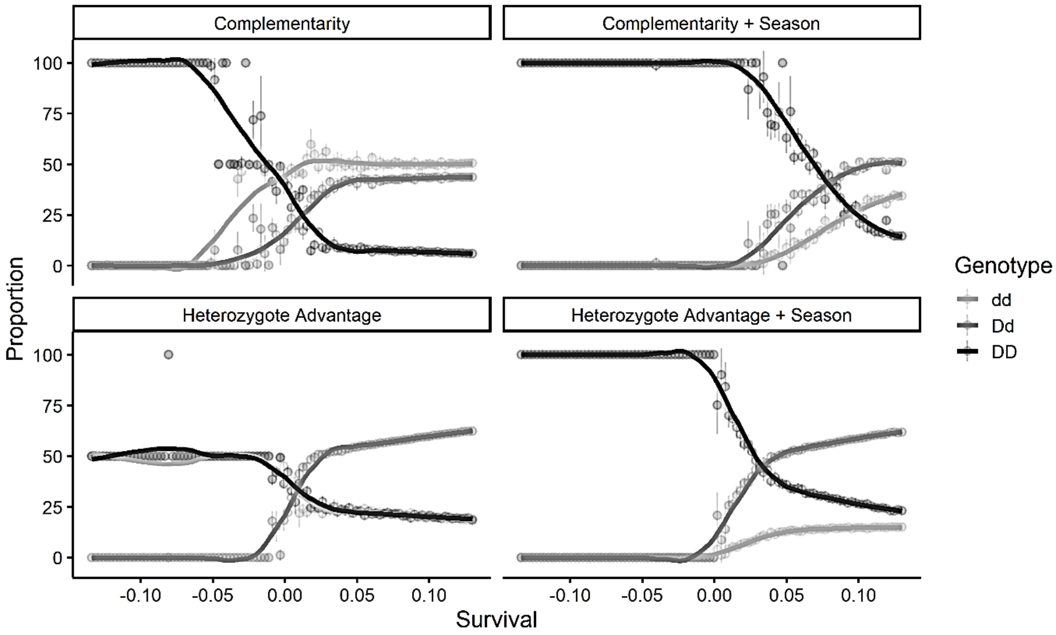

Figure 6.2. Mean genotype frequencies along the survival gradient which either favour homozygotes ('heterozygote advantage', $\Delta_{\text{surv}} < 0$) or offspring of like-morphs ('complementarity', $\Delta_{\text{surv}} < 0$) or heterozygous offspring ('heterozygote advantage', $\Delta_{\text{surv}} > 0$) or offspring of mixed-morphs ('complementarity', $\Delta_{\text{surv}} > 0$). Dots represent accumulated 10-year means. Lines smoothed are conditional means. The 'complementarity' and 'heterozygote advantage' scenarios are simulated in absence of morph-dependent seasonality effects, whereas the 'complementarity + season' and the 'heterozygote advantage + season' scenarios are simulated in presence of seasonality effects: timing of breeding is determined by the morph of the father and recruitment is determined by the morph of the father and timing of breeding of the parents.

Simulated morph frequency outputs

The 'heterozygote advantage' run produced a population in which half the individuals are heterozygotes ('Dd': 53.5%, SD = 9.6) with homozygous genotypes present at roughly a quarter of the population each ('dd': 23.0%, SD = 5.3; 'DD': 23.4%, SD = 5.1; $\Delta_{\text{surv}} > 0$). This translates into 77.0% (SD = 5.3) dark morph individuals. The mean frequency of heterozygotes is positively related to the selective benefit for 'Dd' (increase from 18.4% to 62.4%, Figure 6.2). In contrast, if survival is determined by parental morph combination and offspring of mixed-morph pairs have higher survival ('complementarity' run, $\Delta_{\text{surv}} > 0$), the model produced equal numbers of dark and light morphs (ranging from Δ_{surv} 0.02: dark morphs: 51.4%, SD = 3.7, light morphs: 48.6%, SD = 3.7, 'Dd': 34.8%, SD = 2.8, 'DD': 16.6%, SD = 1.6, ranging to Δ_{surv} 0.13 dark morphs: 49.5%, SD = 2.2, light morphs: 50.5%, SD = 2.2, 'Dd': 43.6%, SD = 1.7, 'DD': 5.9%, SD = 0.9, Figure 6.2). Furthermore, adding seasonality-associated fitness parameter increased the number of dark morphs over time. It did so for both the complementarity and the heterozygote advantage runs. In the 'complementarity + seasonality' scenario, a small range (Δ_{surv} 0.08 – 0.10) produced matches within the 95% CI (mean dark morphs = 75.6%, CI = 72.9 – 78.3) expected morph frequencies (ranging from Δ_{surv} 0.08: 'dd': 18.0%, SD = 2.3, 'Dd': 37.3%, SD = 2.2, 'DD': 44.7%, SD = 3.1, to Δ_{surv} 0.10: 'dd': 28.0%, SD = 2.6, 'Dd': 51.1%, SD = 1.4, 'DD': 21.0%, SD = 2.6). This range coincides with the empirical survival rate (Δ_{surv} = 0.09, SD = 0.05). In contrast, the 'heterozygote advantage + seasonality' scenario did not produce any matches within the 95% CI (Figure 6.3, ranging from Δ_{surv} 0.02; dark morphs: 92.8%, SD = 1.7, 'DD': 55.9%, SD = 4.7, 'Dd': 36.9%, SD = 3.4, 'dd': 7.2%, SD = 1.7; to Δ_{surv} 0.13; dark morphs: 84.9%, SD = 1.41, light morphs: 15.1%, SD = 1.41, 'Dd': 61.8%, SD = 1.31, 'DD': 23.1%, SD = 1.85) and overestimated the number of expected dark morphs by an average of 13% (percentage averaged over Δ_{surv} 0.08 – 0.10).

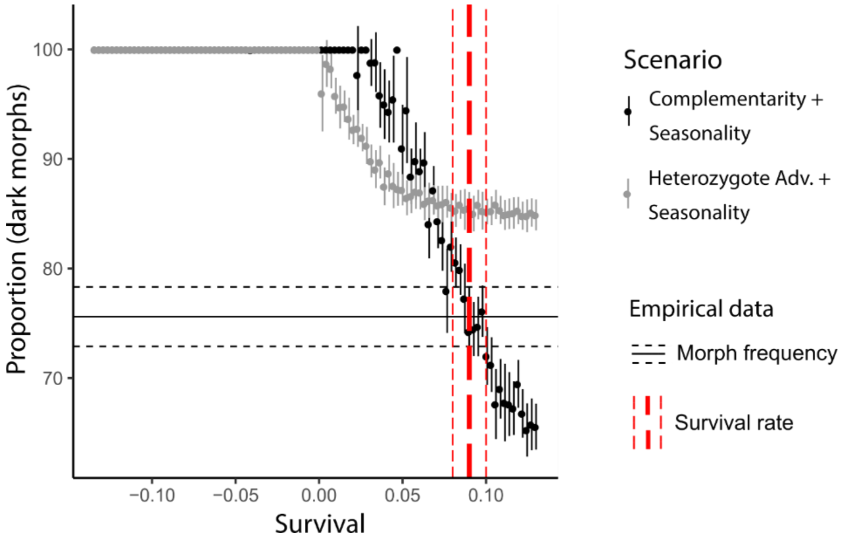

Figure 6.3. Simulated morph frequency (only dark morphs shown, summarized 'DD' and 'Dd' genotypes) along the survival gradient under the two competing scenarios ('complementarity' and 'heterozygote advantage'), which were performed in combination with seasonality effects. The survival gradient is the cumulative difference at age 1 – 3 between offspring of like-morphs ($\Delta_{\text{surv}} < 0$) or mixed-morphs ($\Delta_{\text{surv}} > 0$, 'complementarity + season') or homozygotes ($\Delta_{\text{surv}} < 0$) or heterozygotes ($\Delta_{\text{surv}} > 0$, 'heterozygote advantage + season'). The horizontal lines depict the mean morph frequency of dark individuals (solid) and the 95% CI (dashed). The vertical red lines are the mean difference in the empirical survival estimate between offspring of mixed-morph and like-morph parents ($\Delta_{\text{surv}} = 0.09$) and the standard error. Where the empirical data intercepts, we predict morph frequencies of the most likely scenario to match.

Genotype-specific LRS, breeding time and mean nestlings produced per year

Combining complementarity and morph-dependent seasonality effects resulted in different lifetime fitness in males and females: female LRS reflected the time an individual spent in the breeder pool, with genotypes 'Dd' and 'dd' spending most time in the breeder pool and having highest LRS (LRS: ‘DD’: 8.87, SE = 0.11; ‘Dd’: 9.20, SE = 0.07, ‘dd’: 9.18, SE = 0.09; breeding time: ‘DD’: 7.08, SE = 0.08, ‘Dd’: 7.28, SE = 0.05, ‘dd’: 7.28, SE = 0.07). Reproductive success was the same across all genotypes in females (nestlings per year: ‘DD’: 1.25, SE = 0.01, ‘Dd’: 1.26, SE = 0.01, ‘dd’: 1.26, SE = 0.01, Figure 4). In males, heterozygote ‘Dd’ spend 7.32 (SE = 0.08) years in the breeder pool, produced 1.28 (SE = 0.01) nestlings per year and 9.39 (SE = 0.07) offspring in their lifetime. Homozygote ‘DD’ spend 7.02 (SE = 0.08) years in the breeder pool and produced 1.28 (SE = 0.01) nestlings on average, which resulted in LRS of 8.97 (SE = 0.01). Homozygote ‘dd’ spend 7.22 (SE = 0.07) years in the breeder pool and produced 1.21 (SE = 0.01) nestlings on average, with LRS of 8.72 (SE = 0.07). Heterozygote males have the highest LRS in comparison to the two homozygous variants, whereas homozygous light morphs and heterozygotes have the highest LRS in females.

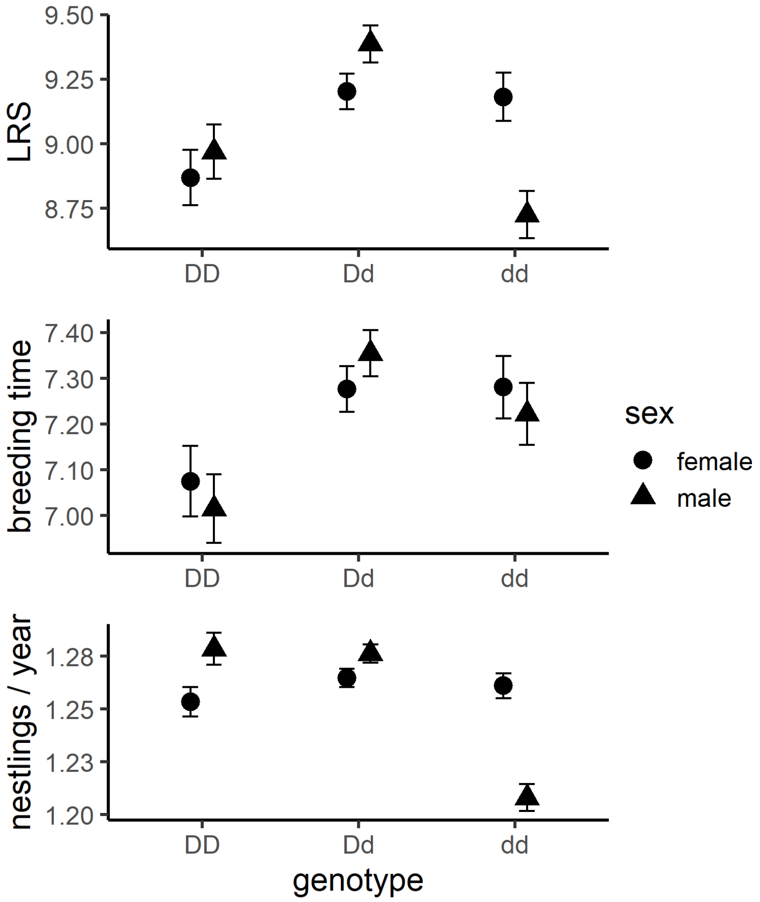

Figure 6.4. Genotype-specific lifetime reproductive success (‘LRS’), time an individual spends in the breeding pool (‘breeding time’) and average number of nestlings per year (‘nestlings / year’) produced by integrating complementarity and morph-dependent seasonality-associated fitness effects. Mean LRS incorporates the time an individual spends in the breeder pool (due to complementarity effect) and breeding success (reflected by nestlings per year, which is morph-dependent in males). Shapes depict female (dot) and male (triangle). Genotypes are either dark morphs (‘DD’, ‘Dd’) or light morphs (‘dd’). Error bars are standard error.

Discussion

Our age-structured individual-based model revealed that both heterozygote advantage, which is associated with a direct fitness benefit for heterozygote individuals, and the complementarity hypothesis, which is associated with a fitness advantage for offspring produced by mixed-morph pairs, are viable mechanisms allowing polymorphism to be maintained over time. As predicted, seasonality effects explained the high number of dark morphs in the study system (Martin et al. 2014b; Rose et al. 2017; Sumasgutner et al. 2016; Tate et al. 2017), whereas both complementarity and heterozygote advantage allowed light morphs to persist in the population. However, the complementarity model, in combination with morph-dependent seasonality, explained the observed morph frequency best. This combination with seasonality indirectly caused heterozygote advantage in males: Males with a heterozygous genotype have the highest LRS. This can be explained by the combination of genotype inheritance, complementarity and seasonality effects. Complementarity determines the breeding time and advantages both 'Dd' and 'dd', but disadvantages homozygous dark morphs 'DD'. This is due to 'DD' only being produced by dark like-morph parents, whereas heterozygous dark morphs 'Dd' and homozygous light morphs 'dd' can be produced by both like- and mixed-morph pairs (Nebel et al. under review-b). Seasonality-associated effects benefit dark morph ('Dd' and 'DD'), but disadvantage light morph males ('dd'). While the 'Dd' genotype is advantaged by both complementarity and seasonality, 'DD' and 'dd' are disadvantaged by complementarity and seasonality, respectively. This combination results in the high LRS of heterozygous males. In contrast, seasonality-associated fitness effects are absent in females, thus, female LRS is only influenced by survival, which reflects only effects caused by parental morph combination (complementarity).

Complementarity is a viable mechanism which might be present in many systems that express heritable behavioural traits. It is an emergent property, which comes about for species showing biparental care, when parents which express two different variants of a trait raise fitter offspring than parents that express the same variants. For complementarity to operate, the two trait variants must be genetically compatible (Pryke and Griffith 2009a). Another important prerequisite of complementarity is that conditions must allow multiple trait variants to co-exist. As a result, complementarity, which acts on the pair-level, is always associated with mechanisms acting on the individual-level as well, i.e. disruptive selection or neutral selection (Roulin 2004). In the black sparrowhawk, the two morphs forage under different environmental conditions and complementarity is hypothesized to arise when the two morphs provide food as a pair to their young (Chapter 3; Nebel et al. 2020; Sumasgutner et al. 2016; Tate et al. 2017), although the precise mechanism resulting in a fitness advantage for the offspring is not yet fully understood (Chapter 2; Chapter 3; Chapter 4; Nebel et al. 2020; Nebel et al. 2019).

It is hypothesized that niche expansion is a main driver for colour polymorphism maintenance in raptorial species (Galeotti and Rubolini 2003; Passarotto et al. 2018). Thus, selective mechanism related to the complementarity hypothesis might be a common phenomenon. However, there is no such indication in the published literature. For example, common buzzards (*Buteo buteo*) have higher reproductive success when mating assortatively than disassortatively (Kappers et al. 2020; Krüger et al. 2001). Other species however do not show differential reproductive success dependant on parental morph combination (Gangoso and Figuerola 2019; Johnson and Burnham 2013; Morosinotto et al. 2020).

Complementarity mechanisms do not have to be restricted to colour polymorphic species or avian systems. Study systems which might yield mechanisms related to the complementarity hypothesis stems for instance from personality studies (Schuett et al. 2010). Disassortative mating of individuals that express different extremes of a behavioural traits could hypothetically lead to a reproductive advantage (Both et al. 2005; Gabriel and Black 2012; Schuett et al. 2010). However, assortative mating appears to be more common than disassortative mating in respect to behaviour (reviewed by Chira 2014; Schuett et al. 2010), in birds this might be driven by matching personalities improving pair bonds (Gabriel and Black 2012; Spoon et al. 2006). Additionally, assortative mating pairs often have higher reproductive success and are thus adaptive (Collins et al. 2019; Rangassamy et al. 2015; Schuett et al. 2010), further weakening the assumption that mechanisms associated with complementarity might be widespread among species that exhibit biparental care.

Our simulations show that while complementarity produces stable 50:50 ratios of the two morphs, only the combination of morph-dependent seasonality and complementarity explains observed morph frequencies. This highlights the importance of seasonality-associated fitness effects in this system. However, morph frequencies are only explained in a narrow range. This indicates that colour morph frequency will change depending on which mechanism (complementarity or morph-dependent seasonality), outbalances the other. Where morph-dependent seasonality effects that benefit dark morphs override complementarity effects, the population would be predicted to see an increase in dark morphs, whereas if complementarity outbalances seasonality, light morphs would be expected to increase.

Conclusion

Our individual-based model simulations show that complementarity, in combination with seasonality, is indirectly associated with heterozygote advantage and produced highest LRS for heterozygous males. No such effect could be found in females. This mechanism could, theoretically, also be present in other systems that express heritable behavioural traits. Our empirical example also shows that it can be difficult to distinguish complementarity from heterozygote advantage, as they both show similar characteristics, such as higher fitness of offspring of parents exhibiting mixed-trait variants. Lastly, although our simulations show that complementarity and its indirect effects on genotype-specific fitness parameters is a capable mechanism, any mechanism that produces similar direct fitness effects as the combination of complementarity and seasonality could explain the stable morph equilibrium in combination with seasonality in the black sparrowhawk. Only by knowing genotype-specific empiric data, which are yet unavailable for this system, it will be possible to verify the results of this study.

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Supplementary Material part 6.1

For this study, we had to obtain age-structured and morph-dependent survival rates for the black sparrowhawk (*Accipiter melanoleucus*). This allowed us to determine whether the difference in survival was uniform across all age-classes between offspring of different parental morph combinations or whether the difference was restricted to certain age-classes.

Age-structured survival rates

Black sparrowhawk nestlings with known parental morph combinations were ringed on the Cape Peninsula (Western Cape, South Africa; 34°00'S, 18°26'E) between 2001 and 2015. To individually mark them, we used two metal colour rings and a metal SAFRING (Rose et al. 2019). The unique colour ring combination allowed us to subsequently identify black sparrowhawks in the field until 2019. We scored individual resightings for every year and age until an individual was failed to be recorded in the population. Individuals were considered as alive even if not seen alive in a specific year between ringing and year of last resighting. Survival was scored as 1 (which meant 'sighted alive'), but the final data point was always 0 (which could mean 'dead', 'dispersed' or 'not seen', which we cannot differentiate between based on our observational data).

Statistical analysis

We constructed a generalized linear mixed model of the binomial family in R (version 3.5, R Core Team 2019) to explore apparent survival rates in relation to parental morph combination and year. We fitted survival (binomial, either 0 or 1) as the response variable and parental morph combination (factor, either 'mixed-morph' - D♂L♀ and L♂D♀ - or 'like-morph' - L♂L♀ and D♂D♀) as the key explanatory variable and age (as a quadratic and linear, numeric 1 – 10) as a co-variate. The individual ID was fitted as a random term. We used the predict function to extract effect sizes and standard error.

Results and Discussion

In total, we ringed 458 black sparrowhawk nestlings with known parental morph combination (mixed-morph: N = 201; like-morph: N = 257) between 2001 and 2015. Of these nestlings, 141 were subsequently sighted again (mixed-morph: N = 70, like-morph: 71). The age-structured survival estimates obtained from this data were typical for most bird of prey (Newton et al. 2016), first low (age 1: 0.47, SD = 0.07, N = 458), which reflect high mortality during the first year of life (but also dispersal), then higher survival with increasing age (age 2: 0.66, SD = 0.07, N = 141; age 3: 0.75, SD = 0.05, N = 105; age 4: 0.82, SD = 0.04, N = 80; age 5: 0.82, SD = 0.04, N = 62) and finally showing a decrease again (age 6: 0.78, SD = 0.04, N = 48; age 7: 0.69, SD = 0.06, N = 32; age 8: 0.51, SD = 0.07, N = 22; age 9: 0.29 ± 0.06, N = 13; age 10: 0.13, SD = 0.03, N = 3, Figure S2), which potentially reflects senescence effects, but could also be due to low resighting probability caused by displacement from the breeding territory (Newton et al. 2016). For higher age-classes, i.e. above age 6, sample sizes become small (below 50) and estimates should thus be taken with caution.

When taking the parental morph combination into account, offspring of mixed-morph pairs showed overall higher survival rates than offspring of like-morph pairs (mixed-morph: 0.63 ± 0.16, N = 473; like-morph: 0.53 ± 0.16, N = 491).

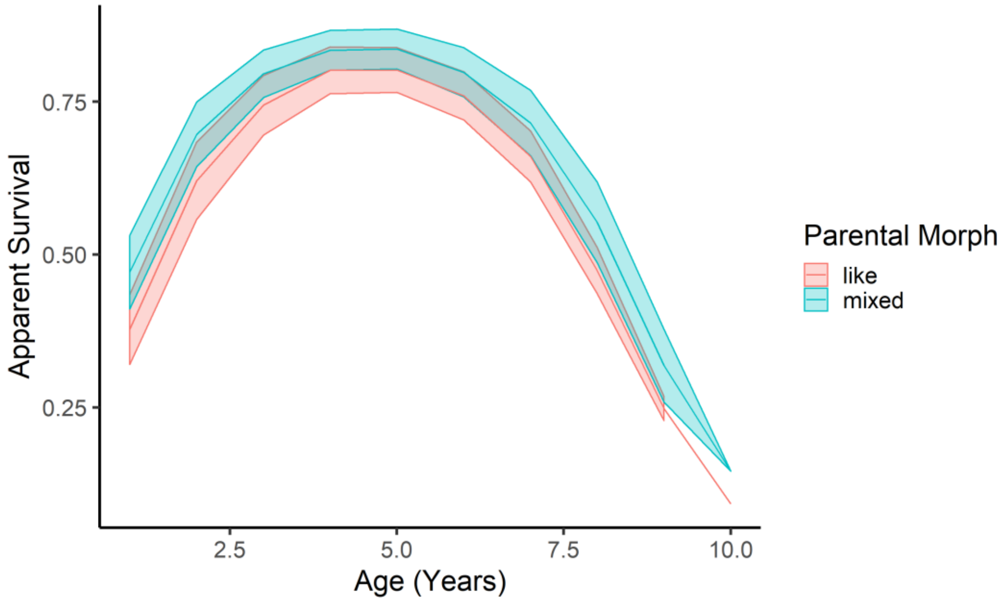

Figure S6.1. Apparent survival effect sizes which depend on age (numeric, 1 – 10) and parental morph combination (either 'mixed-morph' when parents exhibit different parental morphs; or 'like-morph' when parents exhibit the same parental morph). Solid blue centre line depicts the mean effect size for offspring of mixed-morphs and shaded blue its standard deviation, solid pink centre line depicts the mean effect size for offspring of like-morphs and shaded pink its standard deviation.

Differences in apparent survival are largest in the first three age cohorts (the mean difference between like- and mixed-morphs over these age cohorts is 0.09, SE = 0.05), although the errors are overlapping (Figure S6.1). At age 4, the mean effect sizes lie within error of the other parental morph cohort. Although differences at higher age classes (age 7 – 10) appear to be larger again, this might be due to smaller sample sizes and not due to a real effect, although only with additional long-term data such effects will be able to be explored in the future.

Supplementary Material part 6.2

Parameters for the age-structured individual-based model

The difference in survival between offspring of mixed- and like-morph pairs appears to be most prominent in the first three years of an individual's life (Figure S6.1). Thus, we distinguish between four different age-specific survival rates: age class 1, age class 2, age class 3 and age class 4. Age class 4 was applied to all individuals that were 4 years or older. No senescence effects were considered as these were impossible to parameterize based on the empiric data available. Apparent survival parameters obtained from empiric data have one key issue: they are a combination of actual mortality and dispersal from the study site (Newton et al. 2016). This results in much lower apparent survival rates than known from a closely related species, i.e. the northern goshawk (*Accipiter gentilis*, Wiens et al. 2006). This is most prominent in an individual's first year of life, when dispersal from the natal site occurs (Kenward 2012).

This resulted in a problem for the individual-based model simulation: The survival parameters for age class 1 (mean = 0.47) and age class 2 (mean = 0.66) did not produce enough viable runs, resulting in extinction of the simulated population. Thus, we performed a sensitivity analysis in which we increased the empiric survival rates for age 1 and age 2 to find the point at which they produce satisfyingly (100% of all runs being successful).

The obtained values were 0.56 (mean for age class 1) and 0.74 (mean for age class 2). This produced the following baseline survival parameters for the model: mean = 0.56 (age 1), mean = 0.74 (age 2), mean = 0.75 (age class 3) and mean = 0.86 (age class 4 and older). The survival parameter used for the age class 4 and older is more in line with the literature on black sparrowhawks (Martin et al. 2014) than what was estimated in this study.

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Supplementary Material part 6.3

Table S6.1. Probability of timing of breeding dependent on male-morph (either ‘dark’ or ‘light’). Timing of breeding is determined by the month of egg-laying (March – November). Egg-laying was determined by field observations either by the timing of first observation of incubation behaviour or by back-dating the month of egg-laying based on nestling age at ringing. Probabilities were obtained from Tate et al. (2017).

	male morph		random
month	**dark**	**light**	**-**
March	0.02	0	0.01
April	0.14	0.06	0.10
May	0.14	0.14	0.14
June	0.13	0.04	0.09
July	0.12	0.18	0.15
August	0.20	0.25	0.23
September	0.20	0.22	0.21
October	0.05	0.11	0.08
November	0.01	0	0.01

Table S6.2. Probability values to be recruited from the floater pool into the breeder pool which is dependent on an individual’s morph of the father (light or dark morph) and timing of breeding (March – November). Probabilities were obtained from the raw data presented in Sumasgutner et al. (2016).

month	probability	
	dark	**light**
March	0.579	0.001
April	0.460	0.003
May	0.346	0.008
June	0.247	0.021
July	0.169	0.058
August	0.112	0.147
September	0.073	0.327
October	0.047	0.579
November	0.029	0.795

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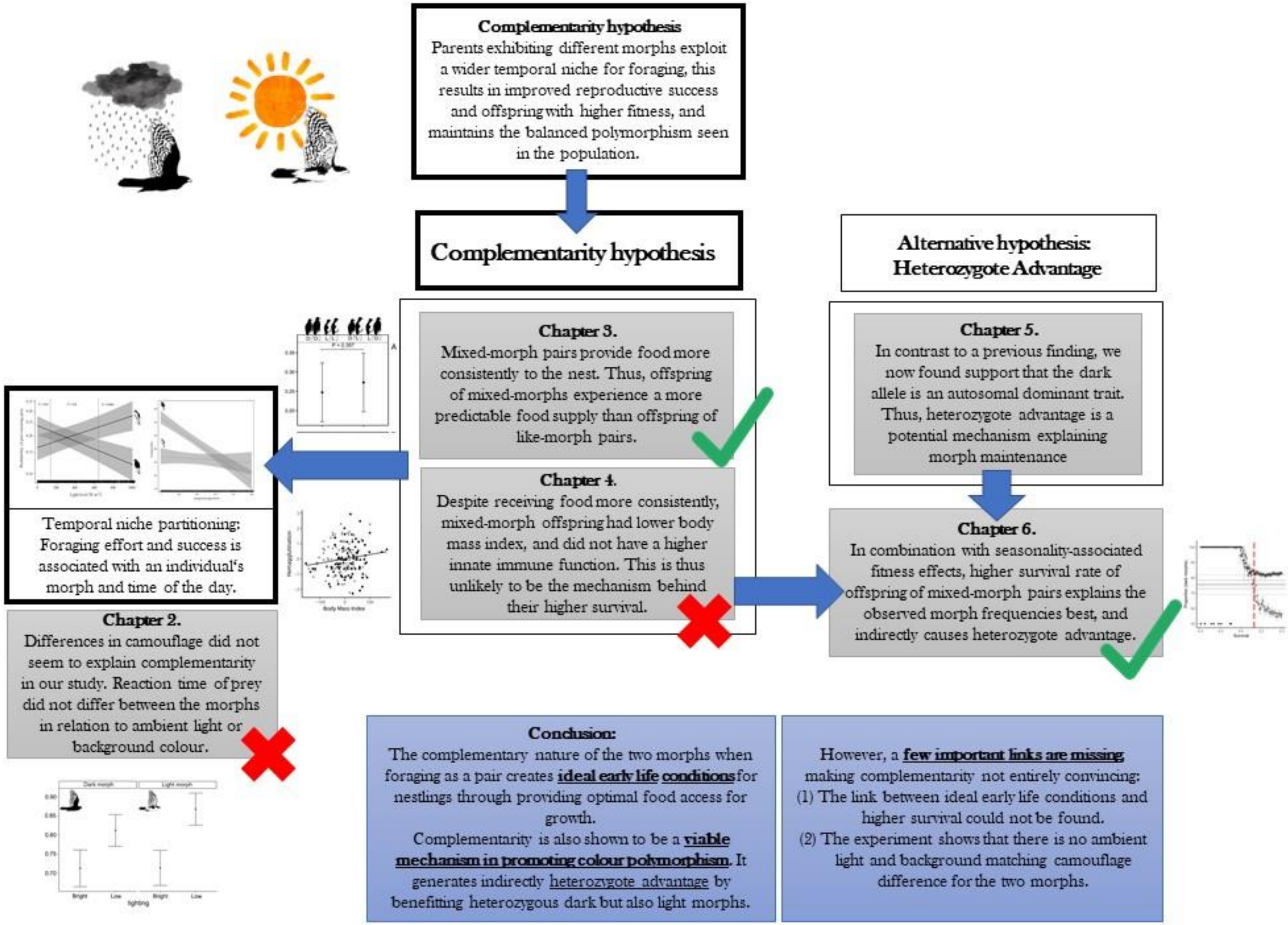
Chapter 7.

Discussion

The goal of this PhD thesis was to explore the potential mechanism that may be operating to allow colour polymorphism to be maintained within a species, focusing on a population of black sparrowhawks on the Cape Peninsula in South Africa. In this study system, there are indications that the complementarity hypothesis might be driving the maintenance of colour polymorphism.

Dark and light morphs are associated with contrasting foraging activity and hunting success across varying ambient light levels (Tate and Amar 2017; Tate et al. 2016). This results in a temporal partitioning of foraging activity of the two morphs. Due to this, mixed-morph pairs are hypothesized to complement their foraging niche in time, which allows them to exploit a wider range of resources. This results in better breeding success (Tate et al. 2017) and in offspring that have higher survival rates (Sumasgutner et al. 2016), which enhances admixture and gene flow, slows speciation and buffers against the loss of diversity in this population.

In this thesis, I have tested various mechanistic explanations related to the complementarity hypothesis and potential alternatives to explain the persistence of the two morphs in this population (Figure 7.1). Here, I will summarize the main results from my thesis and discuss them in a broader context, followed by a discussion on what future research should now be undertaken to better understand the maintenance of colour polymorphism.



Complementarity hypothesis
Parents exhibiting different morphs exploit a wider temporal niche for foraging, this results in improved reproductive success and offspring with higher fitness, and maintains the balanced polymorphism seen in the population.

Complementarity hypothesis

Chapter 3.
Mixed-morph pairs provide food more consistently to the nest. Thus, offspring of mixed-morphs experience a more predictable food supply than offspring of like-morph pairs. ✓

Chapter 4.
Despite receiving food more consistently, mixed-morph offspring had lower body mass index, and did not have a higher innate immune function. This is thus unlikely to be the mechanism behind their higher survival. ✗

Temporal niche partitioning: Foraging effort and success is associated with an individual's morph and time of the day.

Chapter 2.
Differences in camouflage did not seem to explain complementarity in our study. Reaction time of prey did not differ between the morphs in relation to ambient light or background colour. ✗

Alternative hypothesis: Heterozygote Advantage

Chapter 5.
In contrast to a previous finding, we now found support that the dark allele is an autosomal dominant trait. Thus, heterozygote advantage is a potential mechanism explaining morph maintenance

Chapter 6.
In combination with seasonality-associated fitness effects, higher survival rate of offspring of mixed-morph pairs explains the observed morph frequencies best, and indirectly causes heterozygote advantage. ✓

Conclusion:
The complementary nature of the two morphs when foraging as a pair creates **ideal early life conditions** for nestlings through providing optimal food access for growth.
Complementarity is also shown to be a **viable mechanism in promoting colour polymorphism**. It generates indirectly heterozygote advantage by benefitting heterozygous dark but also light morphs.

However, a **few important links are missing** making complementarity not entirely convincing:
(1) The link between ideal early life conditions and higher survival could not be found.
(2) The experiment shows that there is no ambient light and background matching camouflage difference for the two morphs.

Figure 7.1. The following infographic represents a summary of the overall structure and key findings of this study.

Summary key findings

The key finding in this study system that ambient light levels create contrasting activity behaviour and foraging success for the two morphs (Tate and Amar 2017; Tate et al. 2016), concluded that these patterns were most likely due to a difference in camouflage via background matching. In **Chapter 2**, I tested this assumption experimentally. This experiment measured the reaction time of feral pigeons (a main prey species in the population, Suri et al. 2017) towards a simulated attack of black sparrowhawks (of different morphs) attacking under varying light conditions, and also with different backgrounds. Differences in reaction time should reflect differences in camouflage and lead to different catch rates in natural settings. However, the findings from this experiment did not support my predictions with pigeons reacting consistently slower in lower ambient light levels but irrespective of hawk morph. Thus, the reaction time of pigeons was slower in darker conditions but was the same for both morphs under all light conditions and backgrounds.

In **Chapter 1**, I outline the idea of the complementarity hypothesis. I predicted that due to temporal niche expansion (“complementarity”) in mixed-morph pairs in comparison to like-morph pairs, mixed-morph pairs provide food more consistently to the nest. In **Chapter 3, I** explore this prediction by analysing nest camera footage to record timing and frequency of prey deliveries. I found support for this prediction: Mixed-morph pairs provide food more consistently to the nest, backing up the idea of the complementarity hypothesis. However, the biggest uncertainty of this finding remains: how does such consistent (or more predictable) food supply translate into a selective advantage for offspring of mixed-morph pairs?

Nestlings of mixed-morph pairs receive food more regularly (Chapter 3), but have a lower body mass index (Tate et al. 2017) and higher apparent survival (Sumasgutner et al. 2016). This is in contrast to what might be expected as low body mass in nestlings is commonly associated with lower fitness (i.e. Christe et al. 1998; Cleasby et al. 2010; Losdat et al. 2013; Morosinotto et al. 2020; Ringsby et al. 1998; Tinbergen and Boerlijst 1990; Vermeulen et al. 2016).
One explanation for this relationship is that nestlings of mixed-morph pairs are trading their somatic growth against physiological development. In **Chapter 4**, I explored this idea by measuring one aspect of physiological development – innate immune function – which is the first line of an animal’s defence against pathogens. Innate immune function is especially important in nestlings since they do not have a well-developed acquired immune system (Apanius 1998; Klasing and Leshchinksy 1999). Higher investment in the innate immune

system in early life could lead to higher subsequent survival rates due to their effective defences against pathogens. I expected offspring of mixed-morph pairs having higher innate immune parameters than those of like-morph pairs. In contrast to these predictions, however, I found that all nestlings had similar innate immune levels – both, in baseline parameters but also after an experimental immune challenge - irrespective of the parental morph combination. Thus, this aspect of my PhD research did not reveal the mechanism of why the offspring of mixed-morph pairs may have higher survival rates.

On top of the higher survival rate of offspring of mixed-morph pairs, there is a morph-dependent seasonality component in this system with dark morph males breeding earlier than light morph males and early breeders having higher breeding success than later pairs (Martin et al. 2014; Rose et al. 2017; Tate et al. 2017). Despite the considerable amount of research on morph-dependent demographic parameters in the black sparrowhawk in Cape Town (i.e. Sumasgutner et al. 2018; Sumasgutner et al. 2016; Tate et al. 2017), it remains unclear whether differences between the two morphs can explain the persistence of stable colour polymorphism and the numerical dominance of dark morphs in this population. In **Chapter 6**, I incorporated all demographic differences into an individual-based model simulation to explore whether their combination could explain the morph patterns seen in this population. Through this integrated model approach, I found support for the complementarity hypothesis, whereby complementarity acts as a buffer against the loss of variability and ultimately allows light morphs to persist over time. Furthermore, this individual-based model simulation also shows that complementarity is associated with heterozygote advantage (**Chapter 5**) and benefits dark heterozygotes in males and heterozygotes and light homozygotes in females but puts dark homozygotes at a disadvantage.

To summarise, I found support for the complementarity hypothesis (Chapter 3, Chapter 6) but at the same time, I did not find support for several mechanisms which could underly this hypothesis (Chapter 2, Chapter 4). Thus, the complementarity hypothesis is not entirely convincing and future research needs to focus on alternative explanations that could result in colour polymorphism maintenance in the black sparrowhawk. In the following paragraphs, I will discuss the potential of complementarity for polymorphism maintenance in more detail.

Could complementarity play a role in maintaining colour polymorphism in other species?

In my study system, I have found some support that the complementarity hypothesis may be important in maintaining colour polymorphism; here, I explore the evidence for whether it could also be operating in other systems. I do so by assessing differences in reproductive success or offspring quality in disassortative and assortative pairs, and also by exploring mate choice strategies. Disassortative mating or higher reproductive success and higher offspring quality in disassortative pairs in comparison to assortative pairs could indicate support for complementarity operating.

In theory, a temporal or spatial foraging niche partitioning could be widespread among birds of prey, which are often colour polymorphic (Fowlie and Kruger 2003; Galeotti and Rubolini 2003; Galeotti et al. 2003; Roulin 2004; Roulin and Wink 2004). If they are colour polymorphic, they exploit larger niches than monomorphic species (i.e. Galeotti and Rubolini 2003; Passarotto et al. 2018; Preston 1980). This might make it more likely that niche complementarity is present in these bird groups when they are mating disassortatively. However, there does not appear to be evidence to support this. For example, in the common buzzard (*Buteo buteo*), morphs show preference to mate assortatively and assortative pairs have higher reproductive success than disassortative ones (Kappers et al. 2020; Krüger et al. 2001). In the northern fulmar (*Fulmarus glacialiscode*) which is a predator and a scavenger, a similar pattern was found with assortative pairs having higher reproductive success than disassortative pairs (Hatch 1991). Likewise, no difference in reproductive success was found dependant on parental morph combination in colour polymorphic Eleonora's falcons (*Falco eleonorae*, Gangoso and Figuerola 2019), gyrfalcons (*Falco rusticolus*, Johnson and Burnham 2013) or tawny owls (*Strix aluco*, Morosinotto et al. 2020).

Different colour morphs are commonly associated with different behavioural strategies (McKinnon and Pierotti 2010), which might allow a complementarity mechanism to manifest without the need to any foraging niche complementarity in mixed-morph pairs to occur. Disassortative behavioural trait variants might be more compatible than others when parents raise offspring together and might create beneficial synergies, resulting in higher breeding success in dissimilar pairs. In white-throated sparrows (*Zonotrichia albicollis*) and the Gouldian finches (*Erythrura gouldiae*), different phenotypes are associated with different parental investment strategies, for example different aggression levels, and habitat choice (Knapton and Falls 1983; Kopachena and Falls 1993; Pryke and Griffith 2009b; Williams et al. 2012) or alternative reproductive strategies (Tuttle 2003). In the white-throated sparrow, the morphs preferably mate disassortatively (Knapton et al. 1984) and although this might be a strong indication of a complementarity mechanism being in place, it is unclear what is driving this mating preference (Tuttle 2003). Contrary to that, in Gouldian finches, mixed-morph pairs have low reproductive success due to genetic incompatibility (Pryke and Griffith 2009a). Thus, although they might behaviourally complement each other (Pryke and Griffith 2009b), genetic incompatibility is overruling such beneficial effects.

Complementarity could also, theoretically operate independently of colour polymorphism and could be present in any other system that expresses heritable behavioural trait variation, e.g. personality (Chira 2014; Réale et al. 2010; Réale et al. 2007; Schuett et al. 2010; van Oers et al. 2005). However, currently there is also little support for complementarity for this aspect either. For example, generally it appears that birds prefer partners that are behaviourally more similar to themselves (Schuett et al. 2010). Disassortative mating is rare and tends to be associated with low reproductive success in birds (Both et al. 2005; Collins et al. 2019; Dingemanse et al. 2004; Gabriel and Black 2012; Schuett et al. 2010) and mammals (Rangassamy et al. 2015). This might be due to better behavioural compatibility of individuals with the same personality, which leads to a stronger pair bond (Black and Hulme 1996) and improves successful offspring rearing (Ihle et al. 2015; Spoon et al. 2006). Thus, it also appears, complementarity is unlikely to be a common mechanism in maintaining variation in heritable personality traits.

Although I demonstrated in a theoretical modelling approach that complementarity is a viable mechanism capable to maintain polymorphisms that are controlled by a simple Mendelian inheritance pattern, it is questionable whether complementarity could be a viable mechanism in polygenic traits as well. This will be a future question that needs to be addressed to expand the complementarity hypothesis as a mechanism that is also applicable to a wider range of systems.

Overall, evidence from the published literature body suggests that selective mechanisms associated with the complementarity hypothesis are not common and it is unclear whether selective mechanisms associated with complementarity are broadly applicable to other species or whether it is a rare phenomenon.

Using mechanistic approaches to explain function of adaptive colour polymorphism

In this PhD thesis, I have applied a mechanistic approach to explore the patterns of colour polymorphism seen in the black sparrowhawk study system. Historically, most research on colour polymorphisms has tended to focus on describing correlations between colour morph and other characteristics, whereas recent approaches are increasingly focussing on explaining these observed relationships, thus adding mechanistic understanding (Ducrest et al. 2008; McKinnon and Pierotti 2010; Roulin and Ducrest 2011). For example, in the tawny owl, the adaptive mechanism of grey plumage colouration arises through better camouflage in snowy landscapes (Koskenpato et al. 2020) and a structural change in feather morphology that leads to better insulation (Koskenpato et al. 2016). Due to this advantage, grey morphs occur in populations that experience cold and snowy winter weather (Karell et al. 2011). In barn owls (*Tyto alba*), differences in reproductive performance are due to foraging success that contrasts for the morphs during moonlit nights (San-Jose et al. 2019). The white morph induces longer freezing times in its rodent prey during strong moonlight, which results in higher catch rates (San-Jose et al. 2019). In Eleonora's falcon, male morphs display different nesting strategies with pale morphs nesting in colonies and dark morphs being more territorial, which is driven by differences in morph-dependent social behaviour (Gangoso et al. 2015). This results in different breeding success rates, but only in years of low food supply. Then, cannibalism in colony nesting pale birds is more common, reducing their breeding success whereas dark birds are not as strongly affected (Gangoso and Figuerola 2019, Gangoso et al. 2020). In the pygmy grasshopper (*Tetrix undulata*) black morphs have different temperature preferences, likely due to better solar absorption caused by their melanistic colouration, thus the heterogeneity of the habitat allows colour polymorphism maintenance in this species (Ahnesjö and Forsman 2006). Only such mechanistic approaches are ultimately capable to explain the adaptive function of colour polymorphism and future research on colour polymorphic species should focus on such (McKinnon and Pierotti 2010).

Using integrated modelling approaches to understand how selective mechanisms interact

From research that has been undertaken on multiple systems, it is clear that there are often different and potential counter acting pressures operating on different morphs or different demographic parameters. Thus, using demographic modelling and simulations, as I have attempted to do so in this thesis, can help integrate these different components to better understand how selective mechanisms may act individually or in combination to maintain such variation. Thus, such studies are needed to fully understand how colour polymorphism is maintained. Similar approaches have been used to explain colour polymorphism maintenance in the Gouldian finch (Kokko et al. 2014) and interaction of predator diet wariness and the maintenance of prey morphs (Franks and Oxford 2011). In this thesis, the insights gained from the modelling chapter suggests that such an approach could be valuably applied to other systems to better understand the various factors involved in a more integrated manner.

Future research

Whilst the research I have conducted has advanced our understanding to some degree, it has also highlighted some of the major gaps that need to be addressed to have a better mechanistic understanding on the process involved in maintaining polymorphism in the black sparrowhawk study system. Here, I outline these components:

1) In the black sparrowhawk, the two morphs forage during different times of the day and their foraging success is correlated with ambient light levels. Crypsis was hypothesized to be the mechanism responsible for this. However, my experiment (Chapter 2) did not support this, although it did suffer from several limitations. To be sure that morphs do not have cryptic advantage in different light conditions or dependant on background, this experiment should be repeated, but under natural conditions and by incorporating other factors, i.e. the effect of dappling light (Cuthill et al. 2019; Matchette et al. 2018; 2019) on motion detection of the two morphs. Such an experiment, conducted under natural conditions, could give deeper insight into morph- and ambient light-dependent or background matching crypsis, or else it could confirm that a different mechanism is likely involved in the key finding that different morphs have a different foraging advantage across different ambient light levels.
2) There is a strong need to identify the underlying genetic framework determining colour polymorphism in the species. Understanding this aspect will allow future studies to incorporate genotype-associated fitness parameters and validate the results from our individual-based model simulation. Furthermore, it might also reveal other mechanistic relationships.
3) Given the higher reproductive success and improved offspring survival, it is surprising that black sparrowhawks do not mate disassortatively with respect to their morph (Tate et al. 2017). A reinspection of the now larger data set should evaluate whether mate choice changes over time or if individuals choose partners based on their previous experience after divorce. A learning effect would result in disassortative mating patterns

only manifesting themselves in individuals that had multiple partners throughout their life. Given the fitness advantage of disassortative mating, we might expect that such a mating system could still evolve over time in this relatively young population inhabiting the Cape Peninsula. Identifying it in this population would greatly strengthen the idea of the complementarity hypothesis.

Conclusion

The research output generated from this PhD shows that the complementarity hypothesis remains a viable candidate in explaining the maintenance of colour polymorphism in the black sparrowhawk (Chapter 3, Chapter 6). However, simultaneously, my research provided little evidence for some of the main mechanism which have been proposed to operate; for example mechanism driving the difference in temporal partition of food deliveries (Chapter 2, Tate et al. 2016; Tate and Amar, 2017) or the differences in the immune function for mixed morph offspring (Chapter 4). Although complementarity could be also be a common mechanism across other systems it has rarely been explored, and where it has been support is generally lacking. It is clear that there still remains important knowledge gaps that need to be addressed before we have confidence that this is the mechanism underpinning the persistence of colour polymorphism in this system.

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Ethic statement

This PhD was conducted under CapeNature (Permit no. 0056-AAA041-00171, 0037-AAA041-00134, 0056-AAA041-00099, 0056-AAA007-00105, CN44-30-4175, CN18-31-224) and SanPark Permits (CRC/2015/009–2012, CRC/2017-2018/009–2012/V2, CRC/2020-2021/009–2012/V2) and was approved by the UCT's ethics committee SFAEC (Permit numbers: 2012/V37/AA, 2016/v11/AA, 2018/v2/AA, 2018/v5/AA, 2020/v2/AA/A1).

Data accessibility

Published datasets are available as electronic material on UCT's ZivaHub. Data of unpublished manuscripts will be accessible after publication.

Chapter 2: Nebel C, Sumasgutner P, Pajot A, Amar A. 2019. Response times of an avian prey to a simulated hawk attack is slower in darker conditions but independent of hawk colour morph: Data. doi: 10.25375/uct.8332436; modified chapter published in Royal Society Open Science.

Chapter 3: Nebel C, Sumasgutner P, McPherson SC, Tate GJ, Amar A. 2020. Contrasting parental colour-morphs increase regularity of prey deliveries in an African raptor: Data. doi: 10.25375/uct.11347475.v1; modified chapter published in Behavioral Ecology.

Chapter 4: Nebel C, Amar A, Hegemann A, Isaksson C, Sumasgutner P. Skinny but stronger? Disentangling immunological and physical investment strategies during the nestling phase of a colour-polymorphic raptor: Data. doi: 10.25375/uct.12780803; modified chapter submitted.

Chapter 5: Nebel C, Sumasgutner P, Rodseth E, Ingle R, Childs DZ, Amar A. Multigenerational pedigree analysis of wild individually marked black sparrowhawks suggests that dark plumage colouration is a dominant autosomal trait: Data. doi: 10.25375/uct.13574228; modified chapter submitted.

Photo Credits

Introduction: Dr. Shane McPherson gives instructions on how to hold a black sparrowhawk nestling during my first ringing. Copyright © 2017 Dr Petra Sumasgutner

Chapter 2: A black sparrowhawk nestling during ringing procedure at Kliproad Cemetery. Copyright © 2017 Mayur Prag

Chapter 3: You should not pick favorites, but this male black sparrowhawk is definitely mine. He is a very successful dad and raised young every year during my PhD studies.
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Chapter 4: The female nesting in Bel Ombre is a bit of a superstar among birders and photographers. Over the years, she has raised many chicks and my pedigree analysis revealed that her progeny lives all across the study population. Copyright © 2020 Marlene Hofmeyr

Chapter 5: The black sparrowhawk pair in Tierboskloof mating. Unfortunately, this pair would frequently fail at nesting due to Egyptian geese usurpation. In 2020, the little patch of trees, including the nesting tree, was cut down and the female dispersed to a different territory. There, she successfully raised one young. Copyright © 2019 Colin Brown

Chapter 6: Portrait of a black sparrowhawk nestling during ringing at Imhoff's Farm. Copyright © 2017 Dr Petra Sumasgutner

Discussion: Black sparrowhawks exhibit considerable within-morph variation. There are very light dark morphs (melanistic with a lot of white speckles and a large white throat) and very dark light morphs. Although the two morphs are considered as distinct, there are abnormalities like this female that is nesting in Glencairn. Copyright © 2020 Carina Nebel