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

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Abstract

The black sparrowhawk (*Accipiter melanoleucus*) is a color-polymorphic sub-Saharan raptor, with adults occurring in two discrete color morphs: dark and light. It has previously been suggested that plumage coloration 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 130 individuals and pedigree information from 75 individuals spanning five generations. We test the observed offspring phenotypic ratio against the expected ratio under the Hardy–Weinberg equilibrium and find significant deviations from the expected values. Contrary to the previous assumption, our data indicate 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 not only provides a foundation for future research on the adaptive function of color 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 color variant is numerically much rarer than the other.

Introduction

Color-polymorphic species are those which occur in at least two different heritable morphs (or ‘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 coloration 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 (Roulin, 2004; Gray & McKinnon, 2007; McKinnon & Pierotti, 2010).

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

The adaptive mechanisms enabling and maintaining color polymorphism in the black sparrowhawk have been studied extensively in South Africa (e.g. Sumasgutner *et al*., 2018; McCarren *et al*., 2020; Nebel *et al*., 2020). Adults of the species occur in two discrete color 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 however 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 (Figs S1 & S2, Amar *et al*., 2013). However, the overall percentage of white plumage on the ventral side of these birds shows a clear bimodal distribution, 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); whereas 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 evidence for the dark morph allele being recessive is relatively weak.

In the present study, using a larger dataset and a more comprehensive analysis, we revisit the idea that the allele responsible for the dark morph is recessive and test this hypothesis by comparing observed offspring morphs (with known parental morphs) with the expected ratios under the Hardy–Weinberg equilibrium. We also construct pedigrees obtained from wild black sparrowhawks that span 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.

Materials 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 were collected from 2001 to 2020. 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 was identified. Females are considerably larger than males, allowing sex to be distinguished in the field (Thiollay, 1994; Hockey *et al*., 2005). On average, 74% (ranging from 71 to 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 (Thiollay, 1994; Hockey *et al*., 2005); 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 color combinations, and 130 were subsequently re-sighted as adults, allowing for determination of adult plumage morph.

Exploring inheritance patterns under different dominance relationships

Following Amar *et al*., (2013) and 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-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 sex (Z)-linked trait (males being ZZ and females ZW). If plumage morph is a Z-linked trait, we would expect to see a difference in the morph frequency between the two sexes, although no such difference would be evident if it is an autosomal trait. We explore this question by fitting the count of male morphs against female morphs in a 2 x 2 contingency table and using χ^2 goodness of fit tests.

For our other analyses, as we do not know the number of heterozygotes in our population, we assume that the population is in a Hardy–Weinberg equilibrium and use the proportion of the known genotype in the parent generation that produced the observed offspring of known morph (homozygous recessive individuals: 77% when assuming dark to be recessive, 23% when assuming light to be recessive) to estimate the proportions of the other genotypes. To obtain expected frequencies, we use 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). Although most natural systems violate one if not all Hardy–Weinberg assumptions, it nevertheless provides approximate genotypic frequencies in the absence of true frequency information (Fig. 1; Table S1). We use these expected offspring morph 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-pairs as a large sample size was available. Note that 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).

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, e.g. captive-bred color-polymorphic gyrfalcon pedigree, Chang *et al.*, 2010) and can further aid in determining whether a trait is autosomal or 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 morph and female offspring with the recessive morph. Pedigrees were prepared using the 'kinship2' package in R (Sinnwell, Therneau & Schaid, 2014) to assess the inheritance of the morph from generation to generation. They were examined for any violations of inheritance and used to visually reinforce any conclusions from our other analysis.

Results

In total, we re-sighted 105 dark and 25 light morphs with known parental morph combinations in our population. These birds were produced from 97 reproductive events of 68 different breeding pairs (note, however, as not all observed breeding individuals were individually marked, this number might be an underestimate of the true number of breeding pairs).

If plumage morphs 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 the morph frequency between males and females (dark males: 57, dark females: 48, light males: 16, light females: 9; $\chi^2 = 0.43$, d.f. = 1, $P = 0.512$). 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-pairs where the female is light and male dark should give rise to dark females and light males only. Yet mating between a light female (798784) and a dark male (6H03235) gave rise to two dark females, and mating between the same female and another dark male (687856) gave rise to two light males (Fig. 2 - Pedigree 2). Similarly, assuming the dark allele to be dominant, mixed-pairs where the female is dark and male light should give rise to light females and dark males only. This does not hold; for example, mating between a dark female (796777) and an unringed male (ATM1, an unringed light male, but identifiable by unique plumage characteristics) gave rise to a dark female and two light males, and mating between an unringed dark female and a light male (6H03251) gave rise to two dark females (Fig. 2 - Pedigree 1). Overall, our data are thus more consistent with an autosomal mode of inheritance.

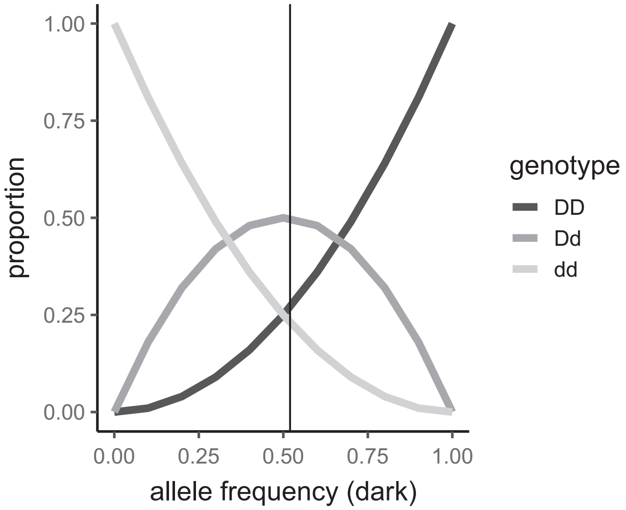

Figure 1 Allele frequencies under Hardy–Weinberg conditions. According to the Hardy–Weinberg equilibrium, the frequency of alleles is predictable in the absence of selective forces, genetic drift effects or gene flow. Different colors translate to different genotype proportions, assuming the dark allele to be dominant: 'DD', 'Dd' and 'dd'. The vertical line indicates the proportion of the alleles in the population under the Hardy–Weinberg condition. Under Hardy–Weinberg conditions, 0.23 light phenotypes predict 0.27 homozygous dark phenotypes and 0.50 heterozygous dark phenotypes in the population, which means that the alleles are present at an almost equal ratio: 0.52 dark alleles and 0.48 light alleles. This means that 66% of all dark phenotypes are heterozygotes.

We observed three light offspring produced from dark x dark pairings, which is not possible if the dark allele were recessive, and light morph pairs only produced light morph progeny (Table 1, Fig. 2). Thus, assuming the dark allele to be dominant, under the Hardy–Weinberg equilibrium, mixed-pairs should yield 67.6% dark offspring (Table S3), and the observed percentage of dark progeny (78.8%) from such matings was not significantly different from this expected value ($\chi^2 = 3.77$, d.f. = 1, $P = 0.052$). The allele frequency calculated for the dark allele under Hardy–Weinberg conditions is 0.52, which translates into an expected number of 23.0% (dd), 49.9% (Dd) and 27.0% (DD) (Fig 1).

Furthermore, the inheritance of plumage morph in birds over five generations (Fig. 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 (Fig. 2 - Pedigree 1) with two dark

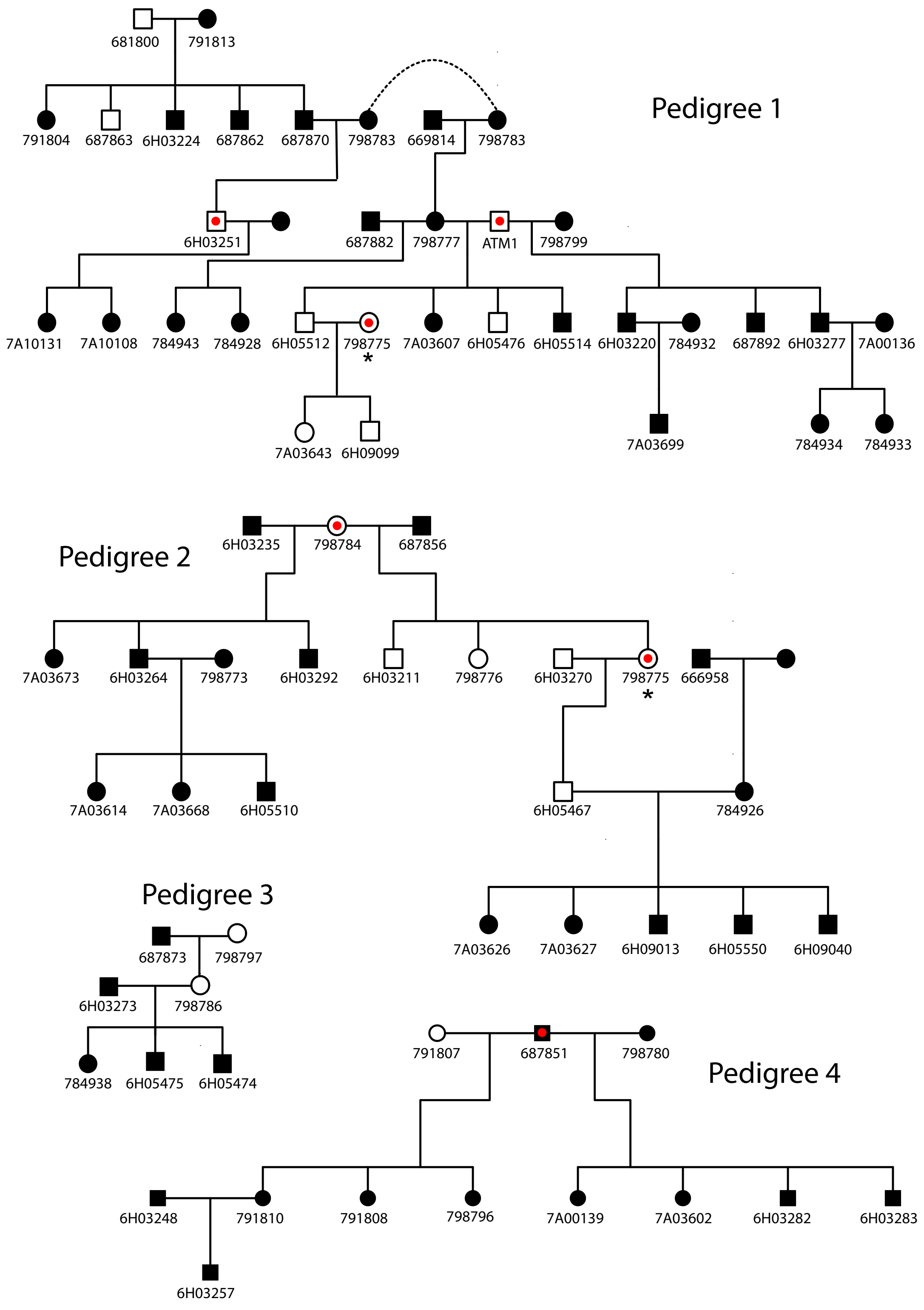
Pedigree 1
681800
791813
791804
687863
6H03224
687862
687870
798783
669814
798783
6H03251
687882
798777
ATM1
798799
7A10131
7A10108
784943
784928
6H05512
798775
*
7A03607
6H05476
6H05514
6H03220
784932
687892
6H03277
7A00136
7A03643
6H09099
7A03699
784934
784933
Pedigree 2
6H03235
798784
687856
7A03673
6H03264
798773
6H03292
6H03211
798776
6H03270
798775
*
666958
7A03614
7A03668
6H05510
6H05467
784926
7A03626
7A03627
6H09013
6H05550
6H09040
Pedigree 3
687873
798797
6H03273
798786
784938
6H05475
6H05474
791807
687851
798780
Pedigree 4
6H03248
791810
791808
798796
7A00139
7A03602
6H03282
6H03283
6H03257

Figure 2 Four pedigrees of wild black sparrowhawks. Squares are males and circles females. Solid black color indicates a dark phenotype and white color a light phenotype. 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. IDs (SAFRING number) are written below individual symbols. If an individual is unringed, the space beneath the symbol is empty. To improve readability of the figure, a digital version can be found in appendix S2.

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 (Fig. 2 - Pedigree 2) and ATM1 (Fig. 2 - Pedigree 1). Both are light morphs and bred with two different dark mates. The female 798784 produced only dark progeny with 6H03235 (likely a homozygote ‘DD’) and 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’) and 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.

Discussion

In contrast to Amar *et al*., (2013) which suggested that the dark allele was recessive, we instead conclude that the dark allele is most likely dominant. We also conclude, in line with Amar *et al*., (2013), that the mode of inheritance is autosomal. This dominance relationship is similar to two other raptor species, Eleonora’s falcon (Gangoso *et al*., 2011) and the ferruginous hawk (Schmutz & Schmutz, 1981).

A key issue relating to the morph inheritance is the large within-morph variability seen in the species that follows a bimodal distribution (Amar *et al*., 2013, Fig. S1 and S2). The observed within-morph variability could be due to expressivity (the degree to which a given genotype is phenotypically expressed, Li *et al*., 2012; Xu, Zhang & Pang, 2013; Abolins-Abols *et al*., 2018), resulting from the influence of modifier genes on the final morph (as in Chang *et al*., 2010; Kappers *et al*., 2018) and/or environmental factors (Rosenfeld, 2010). However, it is important to note that the molecular basis of plumage polymorphism in this species is not yet understood, and the identification of the gene(s) involved will shed light on the observed within-morph variation.

Our study population is numerically dominated by dark morphs, and assuming the dark allele to be dominant, our current interpretation would lead to a much lower frequency for the dark allele (0.52) than 0.87 obtained by Amar *et al*., 2013, who assumed the dark allele to be recessive. In this case, the two alleles are thus present in similar proportions, but the light morph is much rarer than the dark morph as it only appears in homozygous recessive individuals.

The borderline significant deviation ($P = 0.052$) in progeny morph ratios when assuming the dark allele to be dominant might indicate violation of Hardy–Weinberg conditions. There are strong indications, thus far, that there is no morph-dependent mate choice in the study population (Tate *et al*., 2017), which means that Hardy–Weinberg condition is partly met. However, there are a few violations: the population is finite and has overlapping generations. Furthermore, we assume considerable levels of connectivity and admixture with neighbouring populations. The result could also imply selective pressure with conditions favouring the survival of one genotype or morph, that is of dark morphs in a primarily rainy and overcast environment during breeding (see Tate *et al*., 2016 and Tate & Amar, 2017, but also Tate *et al*., 2017) or physiological benefits (e.g. Galván *et al*., 2010). Skewed offspring phenotype ratios could also be due to non-random allele transmission (see Bosch *et al*., 2019) or simply caused by pseudo-replication (with 130 offspring being produced by only about 68 breeding pairs, which can inflate χ^2 values). Violation of Hardy–Weinberg conditions are not very surprising, given that natural animal populations never fulfil all five key assumptions.

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 & Slate, 2006; Gray & McKinnon, 2007; Hedrick, 2012) was unlikely to be the mechanism

Table 1 Observed offspring morph in relation to parental morphs

Parental morph					Obs. offspring morph		Exp. dark morph offspring	
Male	Female	*N*	Breeding pairs	Repr. events	Dark	Light	Recessive	Dominant
Dark	Dark	56	36	44	53 (94.4)	3 (5.4)	100.0	89.5
Light	Light	8	5	6	0 (0.0)	8 (100.0)	21.8	0.0
Light	Dark	31	10	21	26 (83.9)	5 (16.1)	46.7	67.6
Dark	Light	35	17	26	26 (74.3)	9 (25.7)	46.7	67.6
Mixed		66	27	47	52 (78.8)	14 (21.2)	46.7	67.6

Male and female parental morphs are ‘dark’ (dark morph) or ‘light’ (light morph). The number of offspring morph with known parental morphs (*N*) and respective observed offspring morph count numbers are provided. Beside the raw data of offspring morph, 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 the Hardy–Weinberg condition are given in the ’Exp. dark morph offspring’ column. The number of breeding pairs (‘Breeding pairs’) and number of reproductive events (‘Repr. events’) that produced the number of offspring (*N*) are given in the respective columns.

behind this finding because the proportions of heterozygous 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 conditions (Tate *et al.*, 2017). However, those previous assumptions no longer hold, and if the dark allele is dominant, mixed-pairs are expected to produce overall more heterozygous offspring than any other parental morph combination (mixed matings between 50 and 100%, assuming Hardy–Weinberg conditions: 66%; dark–dark matings between 0 and 50%, assuming Hardy–Weinberg conditions: 33%). Therefore, this makes heterozygote advantage (heterozygous morph genotype, not necessarily high overall genomic heterozygosity), a plausible mechanism (see, e.g. Krüger *et al.*, 2001 and also Boerner *et al.*, 2013). Alternatively, high reproductive success and high offspring fitness of mixed-pairs could be due to niche complementarity (Tate *et al.*, 2016), which means that the two morphs complement each other when foraging (Nebel *et al.*, 2020). This emergent property, which is only present in mixed-pairs, could result in higher reproductive success and higher fitness of their offspring (but see Nebel *et al.*, 2021a).

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, Rutz, 2005, Kunz *et al.*, 2019) to 0% in merlin (*Falco columbarius*, Warkentin *et al.*, 1994) to 3–11.2% in American kestrels (*Falco sparverius*, Villarroel, Bird & Kuhnlein, 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 morph 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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Ethical statement

This study was conducted under CapeNature (Permit no. 0056-AAA041-00099, 0056-AAA007-00105, CN44-30-4175, CN41-59-13515) and SanPark Permits (CRC/2015/009—2012, CRC/2017-2018/009—2012/V2, CRC/2020-2021/009--2012/V2) was approved by the UCT's SFAEC (Permit numbers: 2012/V37/AA, 2016/v11/AA, 2018/v5/AA, 2020/V2/AA/A1).

Data availability statement

The inheritance dataset has been made available on UCT's online repository ZivaHub and published by Nebel *et al.* (2021b).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. **Table S1**. Expected allele frequencies under the assumption that the dark allele is recessive or dominant and under Hardy-Weinberg conditions.

Table S2. 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).

Table S3. 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).

Figure S1. 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 (top left image).

Figure S2. 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 plumage variability on the throat, breast and belly, the three bottom rows show expression of plumage variability on the underwing coverts. Variability is expressed as varying amount of black and white feathers, i.e. on the underwing coverts or in the ‘armpits’.

Appendix S2. Online high resolution version of the four pedigrees of wild black sparrowhawks. Squares are males and circles females. Solid black color indicates a dark phenotype and white color a light phenotype. Dots indicate individuals of interest and are explained in the text of the manuscript. Dashed lines between individuals or symbols are used to indicate duplicates and connections between pedigrees. ID (SAFRING number) is written below individual symbol. If an individual is unringed, the space beneath the symbol is empty.