Genetic color polymorphism is associated with avian malarial infections

Laura Gangoso[1]*, Rafael Gutiérrez-López[1], Josué Martínez-de la Puente[1], Jordi Figuerola[1]

[1] *Department of Wetland Ecology, Estación Biológica de Doñana, CSIC, C/Américo Vespucio, s/n, E-41092, Seville, Spain.*

[*]Corresponding author: laurag@ebd.csic.es

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Abstract

Individual genetic diversity is predicted to influence host-parasite interactions. Together with the genes directly associated with immune responses, variation in genes regulating vertebrate melanin-based pigmentation may play an important role in these interactions, mainly through the pleiotropic effects that affect color-specific physiology, behavior, and immunity. Here we test the hypothesis that the prevalence of avian malarial parasites differs between phenotypes in a raptor species in which the genetic basis of color polymorphism and its pleiotropic effects over immune functions are known. We found that dark morphs had a higher prevalence of *Plasmodium* parasites than pale ones but detected no such association for *Haemoproteus*. This pattern may be associated to unequal exposure to vectors or, as suggested by our circumstantial evidence, to a differential ability to mount an immune response against blood parasites.

Keywords: color-polymorphism, haematozoa, host-parasite interactions, *Plasmodium*

Background

Understanding the role of individual genetic diversity in resistance to infectious diseases is crucial for forecasting evolutionary responses and long-term conservation of host populations [1,2]. In birds, genetic color polymorphism – defined as a highly heritable variation in expressed plumage coloration that is independent of age and sex – is often associated with variation in life-history traits, including physiology, behavior, and immunity [3,4]. These associations may result from pleiotropic effects of genes regulating melanogenesis, such as the melanocortin-1-receptor (*Mc1r*). For example, pharmacological research has shown that melanocortin receptors and their ligands are key regulators of immune functions. *Mc1r* is constitutively expressed on

monocytes/macrophages, but also on dendritic cells and lymphocytes with antigen-presenting and cytotoxic functions. The activation and binding of the peptide alpha-melanocyte stimulating hormone (α-MSH) to its receptor MC1R in non-melanocytic immune cells modulates both the innate and the acquired immune responses, with overall anti-inflammatory and, apparently, immunosuppressive effects [5]. On the other hand, it has been proposed that the phagocytic function of melanocytes could confer higher protection from pathogens to more melanized individuals [6].

Parasites of the genera *Plasmodium, Haemoproteus,* and *Leucocytozoon* are all pathogenic to some degree, yet *Plasmodium* is considered as the most virulent one [7]. Parasite lineages exhibit antigenic differences that will influence the effectiveness of the bird immune system. Consequently, virulence strongly depends on the interplay between specific lineages and the ability of the avian host to cope with the parasite infection [8]. In birds that survive infection, the initial acute phase, when severest fitness consequences generally occur, is followed by a rapid decline in parasitaemia to chronic levels with less fitness consequences for the bearer [7,8]. Immune response to malarial infection is mainly cell-mediated through the lymphoid-macrophage system, while antibodies play an important supportive role [8]. Although the precise mechanism is unclear, a number of studies have proposed that the adaptive function of melanin-based color polymorphism is associated with parasite resistance and could cause differences in vector-borne parasite loads between morphs [e.g. 9,10].

Eleonora's falcon (*Falco eleonorae*) is a migratory raptor that breeds throughout the Mediterranean basin and winters in Madagascar. It occurs in two distinct melanin-based color morphs owing to variation in the *Mc1r* gene [11]. Although the relationship between coloration and blood parasite infection in this species is unknown, both inflammatory and humoral immune responses are lower in dark than in pale nestlings

[5,11]. Therefore, in light of the link between *Mc1r*-genotypes and both arms of the immune system, we hypothesize that the two morphs will differ in parasite prevalence because dark morphs are less able to cope with parasite infections (genetic link hypothesis). Alternatively, parasite prevalence could differ due to morph-specific exposure to vectors, either if both morphs exploit different habitats with different vector abundances or if both morphs are differently appealing to vectors, thus creating unequal infection probabilities (exposure hypothesis).

Methods

Sampling was conducted in July–October in 2006–2014 on Alegranza islet (Canary Islands; 1050 ha, 289 m above sea level). Adult Eleonora's falcons were captured every year (mean = 23.22 individuals, range= 5-47) and their color morph was determined visually [11]. All birds were weighed and their wing length measured. Blood samples were preserved in absolute ethanol and stored at -20°C until molecular analysis were performed. All birds were marked with numbered rings and released after manipulation.

DNA extraction and blood parasite determinations

We analyzed 209 blood samples from 183 individuals: 151 pale morphs (91 females and 60 males) and 32 dark morphs (22 females and 10 males). The remaining 26 samples belonged to 19 individuals recaptured in successive years. Genomic DNA was used to determine the prevalence of *Plasmodium, Haemoproteus,* and *Leucocytozoon* parasites following [12] (see electronic supplementary material, ESM).

Statistical analyses

The probability of different morphs being infected by blood parasites was assessed using generalized linear mixed models (GLMMs) with binomial error and logit link function in R v3.0.2 [13] using the dataset available in [14]. To prevent pseudoreplication, we used a random subsampling (1,000 iterations) of the 19 resampled birds for each parasite genus (see ESM). The infection by *Plasmodium* and *Haemoproteus*, respectively, was defined as a binary variable (0/1) and used as the response variable. The morph type, sex (only for *Plasmodium*), and their interaction were included as fixed factors. We also included a body-condition index as a covariate, estimated for each sex separately as the standardized residuals of a linear regression of body mass on wing length. Year was included as a random term. We did not perform a third model for *Leucocytozoon* because only one individual was found to be infected by this parasite. Parameter estimates and standard errors from the resulting GLMMs were averaged and the range of *p*-values and percentage of models where each term was statistically significant calculated.

Results

Overall, the prevalence of blood parasites was 12.9% (Figure 1, table S1 in ESM for details on parasite lineages, [14]). Of the 19 resampled individuals, 13 (10 pale females and 3 dark females) were never infected. However, one and two pale females became infected by *Plasmodium* and *Haemoproteus*, respectively, between the first and second sampling period. By contrast, three pale females, two infected by *Haemoproteus* and one by *Plasmodium*, were found to be uninfected one year later.

Dark falcons had a greater *Plasmodium* prevalence than pale ones (mean estimate = 2.50 ± 0.01 s.e. *p* range = 0.001 - 0.01, Figure 1). Sex was not significant (mean estimate = 0.75 ± 0.01 s.e. *p* range = 0.59-0.99), whereas body condition (mean estimate

= 0.011 ± 0.001 s.e. *p* range = 0.02-0.59) and the interaction between sex and morph (mean estimate = -2.20 ± 0.012 s.e. p range = 0.03-0.22) were significant only in 6.5% and 16.6% of the models, respectively. There was no significant relationship between the probability of infection by *Haemoproteus* and any explanatory variable (in all cases *p* range = 0.30-0.99).

Discussion

We found that dark falcons had a higher prevalence of *Plasmodium,* the commonest parasite genus, than pale ones but found no significant relationship for *Haemoproteus*. Different factors such as differential exposure to vectors, the differing virulence of parasite genus/lineages, and/or the host's capacity to fight infections may influence this result. In support of the first possibility, [9] found that rufous-morph tawny owls (*Strix aluco*) hosted higher total blood parasite burdens than grey morphs owing to both greater exposure to vectors and greater susceptibility to parasites. In feral pigeons (*Columba livia*), alternative morphs were distributed non-randomly across an urban gradient and had different parasite risks [15]. However, the different Eleonora's falcon morphs inhabit small islands sympatrically during the breeding season and local transmission of blood parasites at breeding grounds is absent, due to the lack of suitable vectors [16]. Therefore, differences in the exposure to vectors must occur during migration and/or in their wintering quarters, where insect vectors abound [17] and the parasite transmission is likely to be higher.

The most prevalent *Plasmodium* LK6 is thought to be transmitted by *Culex pipiens*, while P_ACCTAC01 is transmitted by *Coquillettidia aurites* (see ESM, table S1) and these are common mosquitoes in Africa and Madagascar [17]. The lineage LK6 was recently isolated from passerines from Macaronesian archipelagoes, the Iberian

126 Peninsula and Morocco (ESM, table S1). Although the transmission areas remain
127 unclear, it has been proposed that migratory birds such as Eleonora's falcon could
128 spread blood parasites to resident birds on the main islands, where insect vectors are
129 present [16]. It has been suggested that darker colors are more attractive to mosquitoes
130 than light colors and so entirely dark plumages could increase host-vector contact rates.
131 However, feeding preferences of these mosquitoes with regard to color attractiveness
132 are unknown. In addition, data on GPS-tagged falcons do not indicate the existence of
133 morph-specific habitat exploitation during winter (authors' unpublished).
134 This suggests that the difference in prevalence between morphs is unlikely to be
135 due to morph-specific exposure to vectors but probably results from differential abilities
136 to mount an immune response. Pale falcons could be more susceptible to *Plasmodium*
137 infection than dark ones and their lower prevalence could in turn be the reflection of
138 greater mortality. No study has addressed the effects of LK6 on host survival (ESM,
139 table S1). However, we cannot rule out a selective disappearance of pale morphs due to
140 a higher mortality during the acute phase of infection. Dark Eleonora's falcons have
141 poorer immune responses than pale ones from the nestling stage onwards [5,11]. It is
142 thus likely that dark falcons have lower immune capacities in adulthood since this
143 negative relationship is due to their *Mc1r*-derived genotype and not to the environment
144 [5]. The fact that three infections found in pale females became undetectable in
145 successive years partially supports the idea of greater immune competence in pale
146 falcons for fighting infections. Nonetheless, the effects of infection can greatly depend
147 on the parasite load. Previous studies addressing the relationship between plumage
148 coloration and blood parasites have found differences in infection intensity rather than
149 in prevalence, thereby suggesting that differences are due to resistance to parasites
150 rather than exposure to vectors [6,10,18]. However, we estimated prevalence rather than

infection intensity because birds were caught during a relatively long period (from arrival at breeding grounds to fledglings' emancipation) and across years. Infection intensity may vary greatly along and between breeding seasons [19], thus making between-individuals comparisons difficult to interpret. Further experimental approaches would be needed to clarify the relationship between color polymorphism, blood parasite intensity and immune competence.

In conclusion, our results are in accordance with the genetic link hypothesis, yet we cannot completely rule out the exposure hypothesis and both mechanisms could contribute to the skewed prevalence of *Plasmodium* to the dark morph. To our knowledge, this is the first study addressing the relationship between color polymorphism and parasite prevalence in which both the gene responsible for color polymorphism and its pleiotropic effects on immune functions are known, which thus enabled us to infer potential mechanisms underlying this covariation.

Ethics

All experimental procedures were approved by the CSIC ethics committee and Animal Health authorities as per Spanish law (Nº: 534/2014).

Data accessibility

The R code used in this article has been uploaded as part of the ESM. Data are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.tk41q

Authors' contributions

LG, JMP, and JF designed the study; LG collected field data, and RGL and JMP performed the molecular laboratory work. LG analyzed the data and wrote the paper

with input from all other authors, who gave their final approval for publication. All authors agree to be held accountable for the content therein.

Competing interests

We have no competing interests.

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257 **Figure 1**. Prevalence (number of infected/total * 100) of the two blood parasite genera
258 infecting adult Eleonora's falcons of pale (white) and dark (grey) morph.
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