Journal of Ornithology (2026) 167:777–787
https://doi.org/10.1007/s10336-025-02356-3

ORIGINAL ARTICLE

Plumage colour of Blue-capped Manakin males predicts female visitation rates but does not signal avian haemosporidian infection

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Received: 13 July 2025 / Revised: 14 November 2025 / Accepted: 18 December 2025 / Published online: 13 January 2026

Abstract

Colourful plumages may bear high production and maintenance costs. The parasite hypothesis posits that visual signals work as honest indicators of heritable resistance to parasites. However, the value of structural and melanin-based colours as honest signals is still an open question awaiting evidence, especially in lekking species, for which sexual selection is supposedly more intense than in socially monogamous species. In this context, we sampled 24 adult male and 26 adult female Blue-capped Manakins (*Lepidothrix coronata*) from a population in central Amazonia to test the hypothesis that (i) plumage colour of male Blue-capped Manakins influences the attraction of female individuals; and (ii) the occurrence of haemosporidian parasites is associated with the plumage colouration patterns of Blue-capped Manakin. We estimated the frequency of female visits at individual perches of Blue-capped Manakin males through behavioural observations, measured colour spectra from eight plumage patches of male and female individuals, and examined whether these individuals were infected with haemosporidian parasites using molecular screening techniques. We found that variation in melanin-based pigment and structural colours amongst male Blue-capped Manakin was associated with female visits. However, variation in these colours did not correspond to the occurrence of haemosporidian parasites. In contrast to previous studies, we suggest that the structural and melanin-based colouration of male and female Blue-capped Manakin may not be costly enough to be used as an honest signal of susceptibility to haemosporidian parasites.

Keywords Birds · Parasites · Signal efficacy · Signal content · Handicap principle · Central Amazonia

Zusammenfassung

Die Gefiederfarbe von Blauscheitelpipramännchen erlaubt Vorhersagen zur Besuchshäufigkeit von Weibchen, sagt aber nichts über eine Infektion mit Hämosporidien aus

Bunte Federn können in Entstehung und Unterhalt größere Kosten verursachen. Die Parasitenhypothese besagt, dass optische Merkmale als verlässliche Hinweise auf eine vererbbare Resistenz gegen Parasiten dienen. Aber der Wert struktureller und melaninbasierter Farben als verlässliche Indikatoren ist nach wie vor ungeklärt und muss noch bewiesen werden, vor allem für Arten mit Balzritualen, bei denen die sexuelle Selektion vermutlich eine größere Rolle als bei monogamen Arten spielt. Hierzu untersuchten wir 24 adulte Männchen und 26 adulte Weibchen des Blauscheitelpipras (*Lepidothrix coronata*) aus

Communicated by V. Palinauskas.

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einer Population im zentralen Amazonasgebiet, um zu überprüfen, ob (i) es einen Zusammenhang zwischen der Gefiederfarbe männlicher Blauscheitelpipras und ihrer Attraktivität für Weibchen und (ii) einen Zusammenhang zwischen dem Auftreten von Hämosporidien-Parasiten und den Mustern der Gefiederfarben der Männchen gibt. Anhand von Beobachtungen bestimmten wir die 61 Häufigkeit der Besuche von Weibchen an einzelnen Sitzplätzen von Blauscheitelpipramännchen, maßen die Farbspektren von acht Farbfeldern im Gefieder von männlichen und weiblichen Einzeltieren und bestimmten mithilfe molekularer Screening-Techniken, ob diese Tiere mit Hämosporidien-Parasiten infiziert waren. Wir fanden einen Zusammenhang zwischen unterschiedlichen melaninbasierten Pigment- und Farbstrukturen männlicher Blauscheitelpipras und den Besuchen von Weibchen, nicht aber mit dem Auftreten von Hämosporidien-Parasiten. Im Gegensatz zu früheren Untersuchungen vermuten wir, dass die strukturelle und melaninbasierte Färbung des Gefieders männlicher und weiblicher Blauscheitelpipras nicht kostspielig genug ist, um als verlässlicher Indikator für eine Anfälligkeit für Hämosporidien-Parasiten zu dienen.

Introduction

Visual signals are amongst the most important traits used in communication both amongst and within species (Zahavi 1975; Hamilton and Zuk 1982). In birds, plumage colour is a particularly prominent visual signal, and its efficiency depends on the quality of the signal itself, the sensory systems of receivers and environmental conditions (Bradbury and Vehrencamp 2011). These factors are, in turn, shaped by natural, sexual or social selective pressures (Endler and Mclellan 1988; Endler 1992; Endler and Basolo 1998). Therefore, by studying variation in signalling traits—such as plumage colours in birds—their effects on receivers, and their relationship with the biotic environment, the role of these traits as indicators of phenotypic quality in sexual selection contexts can be better understood (Endler and Mclellan 1988; Hill and McGraw 2006a, b).

For almost a century, multiple hypotheses of sexual selection by female choice have attempted to explain the evolution of visual signals, especially in polygynous clades, in which sexual selection is expected to be strongest (e.g. good genes, Fisher's runaway selection, sensory drive; reviews in Andersson (1994); Prum (1997); Price (2008)). The handicap hypothesis proposed by Zahavi (1975) suggests that in polygamous species, in which males contribute nothing beyond their sperm, females are expected to select males based on genetic benefits to the offspring. Genetic benefits of males (i.e. good genes) are expected to be honestly communicated to females though sexual signals such as cost-added signals (i.e. signals whose cost is greater than that required to transmit the information; Zahavi (1975)).

A variant of the handicap hypothesis was later proposed by Hamilton and Zuk (1982). This hypothesis posits that secondary sexual characteristics work as an honest indicator of heritable resistance to parasites and pathogens (McGraw and Hill 2000; Hill et al. 2005). Selected partners should be the healthiest, because only the least parasitized individuals could allocate energy to invest in the production of costly conspicuous secondary sexual characters (Hamilton and Zuk 1982).

Although these classic examples are biassed towards males, there is growing evidence that coloration signals in females can also play a key role in the context of sexual selection (Claire et al. 2020; Doutrelant et al. 2020). For example, in a Spanish Eurasian Blue Tit (*Cyanistes caeruleus*) population, females infected by multiple genera of avian malaria parasites were paler on carotenoid-based plumage colour (del Cerro et al. 2010) and the opposite was reported in a population of Eurasian Blue Tits which had higher levels of brightness and UV chroma in infected females (Janas et al. 2018). However, how females signal individual quality and what phenotypic traits are involved in the wild are still poorly understood.

Amongst the mechanisms of colour production, melanin-based and structural colours are traditionally considered to provide less honest information than carotenoid-based colouration (Badyaev and Hill 2000; McGraw and Hill 2000). However, studies have shown that darker eumelanic colouration is often associated with higher immune capacity and parasite resistance (Jacquin et al. 2011), and that structural and melanin-based colour can signal the intensity of infection from blood parasites (Doucet and Montgomerie 2003). Although haemosporidian parasites are unlikely to directly disrupt the nanostructures that produce structural colours or the biochemical pathways responsible for melanin synthesis, they may indirectly influence both types of plumage coloration through physiological stress and energetic constraints during moult and feather maintenance. Energetic and oxidative costs of infection may affect feather growth and structural integrity, altering reflectance properties (Peters et al. 2004). Likewise, melanin-based coloration is synthesised endogenously from amino acid precursors rather than obtained from the diet varies with oxidative stress and physiological condition (Galván and Alonso-Alvarez 2009). These indirect mechanisms could link haemosporidian infection to both structural and melanin-based colours, thereby supporting the exploration of how parasite-mediated physiological stress may influence plumage traits involved

in sexual selection. Therefore, further studies will provide a better understanding of the link between parasite infections and the evolution of colouration in birds, especially on the structural and melanin-based colours (review in Côte et al. 2018).

Manakins (i.e. Aves: Pipridae) are amongst the most conspicuous bird families with respect to bright plumage colour and elaborate displays. Manakins are also known to host haemosporidian parasites, and amongst avian hosts, the Blue-capped Manakin (Aves: Pipridae: *Lepidothrix coronata*; Fig. S1) is one of the species with the highest diversity and prevalence of *Haemoproteus* and *Plasmodium* lineages. (Fecchio et al. 2017). A previous study using data from the same population of Blue-capped Manakins shows that individuals infected with these hemoparasites (*Plasmodium* and *Haemoproteus* genera) exhibit significantly lower behavioural activity during the breeding season. Nevertheless, infected individuals were no less attractive to females than uninfected individuals suggesting that females did not use behavioural traits alone to select mates (Bosholn et al. 2016). However, whether blood parasites may influence male plumage colour in Blue-capped Manakin is still unknown. Hence, studying the influence of structural and melanin-based plumage colour of male Blue-capped Manakin on behavioural responses of females (e.g. female visitation rates) and the association of this visual signal to haemosporidian parasites may help to explain which evolutionary mechanisms are associated with phenotypic diversification mediated by sexual selection in manakins and other avian taxa.

In this context, we sampled adult males and females from a population of Blue-capped Manakin, an abundant Amazonian bird, to understand the evolutionary role of male plumage colouration as a visual signal preferred by females and its relation to haemosporidian infection. Considering that sexual selection predicts the evolution of more conspicuous visual signals (Endler 1992; Endler and Basolo 1998), and that male manakins use plumage colouration as a visual signal of intersexual communication (Stein and Uy 2005; Kirwan and Green 2012), we tested the hypothesis that (i) more colourful plumages positively attract more females. Thus, we expected that male Blue-capped Manakin individuals with more conspicuous plumage colour will receive more female visitation than males with less conspicuous plumage colour. Furthermore, considering that visual signals may provide honest information on the phenotypic and/or genetic quality of individuals (Zahavi 1975), such as their resistance to pathogens (Hamilton and Zuk 1982b; Doucet and Montgomerie 2003), we tested the hypotheses that: (ii) the occurrence of haemosporidian parasites is negatively associated with plumage colour and that individuals infected with haemosporidian parasites will have less conspicuous plumage than uninfected Blue-capped Manakin males and females.

Material and methods

Study species

Within the family Pipridae, the Blue-capped Manakin (Fig. S1) is one of the most geographically variable species in plumage pattern (Hellmayr 1929; Snow 2004; Kirwan and Green 2012; Teófilo et al. 2018). Males of this species have marked sexual dimorphism, in which females are green and adult males may have black, green or black with green plumage with a bright blue crown, depending on the subspecies/distribution (Snow 2004; Ryder and Durães 2005; review in Anciaes et al. 2009). Male *L. coronata arimensis*, the subspecies endemic to our study site, are mostly blackish (melanin-based colour; Teófilo et al. 2018; review by Kirwan and Green 2012) (Fig. S1). Nevertheless, besides the blackish plumage, definitive plumage males can be differentiated from those in pre-definitive plumages by their complete bright violet blue crown, which is produced by structural colour mechanisms.

The Blue-capped Manakin is a Neotropical endemic bird species known for its polygynous lekking mating system, in which adult males display their conspicuous plumage through complex nuptial performances to attract females; in such systems, males gather in communal display areas known as leks (Sick 1967; Bradbury and Gibson 1983; Prum 1997). In the polygynous lekking mating system, females receive no resources, except sperm, and are solely responsible for nest construction and parental care (Andersson 1994). Blue-capped Manakin adult males engage in displays on either solitary or exploded leks, with up to seven individuals, including juvenile males (Prum 1994; Anciães et al. 2009; Durães 2009; Bosholn et al. 2016). During the breeding season, besides performing acrobatic displays, adult males vocalise from display perches as part of their courtship behaviour (Durães 2009; Bosholn et al. 2016). However, females visit leks only occasionally (Durães 2009; Bosholn et al. 2016). Females and young males have similar green plumage, and one of the main ways to distinguish them is through vocal behaviour, as young males vocalise more frequently than females (Durães 2009). The reproductive season of Blue-capped Manakin is characterised by increased frequency of displays and acoustic activity of adult males (review by Kirwan and Green 2012; Bosholn et al. 2016).

Field sampling and behavioural sampling

We conducted fieldwork at a Biodiversity Research Programme (PPBio) site located approximately 100 km from Manaus, Amazonas, Brazil, within the Purus–Madeira interfluvium, south of the Amazon River (03°40′S;

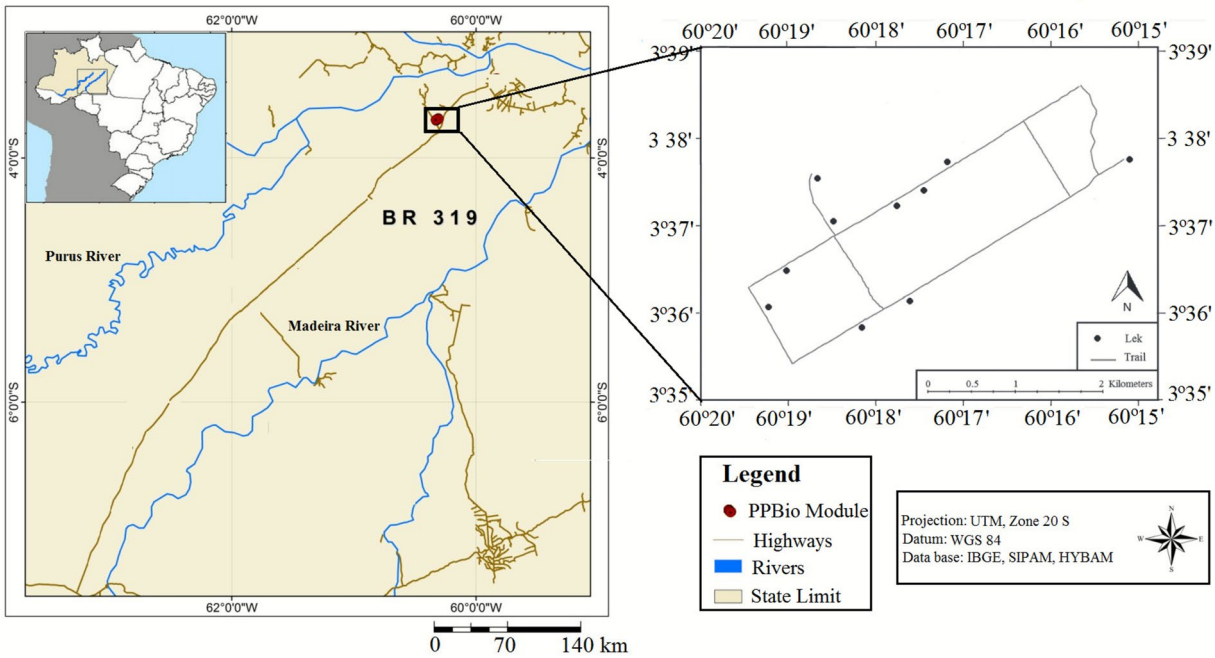

Fig. 1 Map of the study area in the Purus–Madeira interfluvium (PPBio plot, red dot in the left panel) and schematic figure of the trail system (adapted from Bosholn et al. 2016) which shows sampled leks in the research plot 'Manaquiri', Careiro-Castanho municipality, Amazonas state, Brazil (right panel)

60°16′–60°20′W; Fig. 1). We recorded the behavioural activity at all leks between October and November 2013 as well as between June and July 2014. We sampled one population of Blue-capped Manakins and located ten leks through acoustic and visual censuses of male individuals along the main trails and within the forest interior. We defined lekking behaviour as the persistence of individual males performing typical courtship displays on a given perch for at least three consecutive days (Durães 2009). After locating individual perches, we used approximately 15 mist nets to capture birds (12 m × 2.5 m, Ecotone) for three consecutive days at each lek. We sampled six leks in 2013, and four leks in 2014. We opened nets from 7:00 a.m. to 4:00 p.m. and banded captured individuals with a unique combination of colour bands on the right tarsus and one metallic band, each with a unique number combination, on the left tarsus (Licences from Instituto Chico Mendes de Conservação da Biodiversidade [ICMBIO]—licence number 5540–1/ 2013 to M.A., and licences 41,069–1/2013; 53,130 1/2015 to M.B.—and Centro Nacional de Pesquisa para Conservação das Aves Silvestres [CEMAVE]—licence number 3767 to M.B.). We collected approximately 50 µL of blood from each captured individual via brachial vein puncture using a disposable hypodermic needle and heparinized capillary tubes for use in molecular sexing and parasite analysis. We stored the blood in microtubes with 1 mL of 95% ethanol, and in the laboratory, we stored these microtubes in a freezer at − 20 C until DNA extraction.

We sexed all captured individuals in the field according to the plumage colour and also confirmed the sex identification through molecular sexing. For each sample, we carried out polymerase chain reaction (PCR) amplification in a total volume of 12.5 µL. The reaction mixture contained 2.9 µl H2O Milli-Q; 2.5 µl KCl; 1.25 µl MgCl2 (25 µM); 1.2ul of dNTP (10 µM); 0.15 µl of Taq polymerase; 2 µl Primer P2 (2 µM), 1 µl Primer MP (2 µM), and 1 µl Primer NP (2 µM). A volume of 0.5 µL of DNA from each individual extract was used for amplification. For thermocycling conditions, we set the initial denaturation step at 68 °C for 1 min, followed by 35 cycles at 93 °C for 10 s, 52 °C for 35 s, and 68 °C for 30 s. A final run at 68 °C for 7 min completed the programme. We separated PCR products using electrophoresis for 45 min at 90 V in a 1% agarose gel in 1 × TBE 0.5X and visualised the resultant bands under UV light. In this case, the males exhibit a single band, whereas females exhibit two bands.

We collected approximately 5 feathers from each colour patch (crown, breast, belly, back, rump, greater wing coverts) from 24 male and 26 female individuals captured in the field. For the flight feathers (remiges and rectrices), we collected only two to three feathers per individual, as these are larger and sufficient for accurate reflectance measurements. We carefully placed these feather samples into opaque paper envelopes to minimise colour degradation until the analysis of spectra data was conducted in the lab.

Two observers conducted behavioural observations on display perches during the mornings (07:00 am – 12:00 pm) and afternoons (1:00–4:00 pm) through continuous focal sampling with 5-min intervals (Altmann 1974). In total, we observed 24 adult males, each for approximately 3 days, totalling 24 h of observation. Each observer observed males from approximately five metres away. During the observations, we recorded 70 visits of banded and unbanded females. In some cases, the same female visited different males. We followed the observation protocol of Bosholn et al. (2016), which included frequency of observed female visits for each focal male during each observation interval. We classified visitation events as those in which both banded and non-banded females visited the display perches. Therefore, any event in which a non-banded, green-feathered individual visited a perch occupied by an adult male and remained in front of it without emitting vocalizations, we considered that individual a female. Only female visitation to adult males was analysed to avoid bias in the results. Frequency of female visits was calculated using the following index:

$$\frac{\text{Freg of females}}{\text{Observation interval}} = \frac{n_t}{\sum n_{intind}}$$

where 'n_t' denotes the total number of female visits observed at an individual perch, and "$\sum n_{intind}$" denotes the total number of observation intervals at focal perches.

Spectrophotometry and colour analysis

We mounted feathers black velvet to avoid background noise and placed one feather on top of another to simulate the original plumage pattern found on live birds, following Vaqueiro-Alba et al. (2016). We collected spectral data using an Ocean Optics USB-2000 UV–VIS spectrophotometer connected to a cosine-corrected probe (R400-2 UV–VIS) and a deuterium-halogen light source. We acquired spectral data from 24 adult males and 26 adult females captured in the study area using *SpectraSuite* software. Depending on the quality of the spectral readings observed in the *SpectraSuite* plots, we mounted between five and ten feathers per sample. We measured the colour spectra of eight plumage patches: tail, wing coverts, crown, breast, back, remiges, rump, and belly. To ensure consistency and minimise observer bias, all measurements were taken by the same observer (FT). For each plumage patch, we calculated the average of ten readings from the same area, with an integration time of 20 ms and zero boxcar correction. We analysed spectra between 350 and 700 nm corresponding to the spectral range of maximum visual sensitivity of the family Pipridae (Ödeen and Håstad 2013).

We performed all analyses in *R* version 3.6.1 (R Core Team 2019) and used the *pavo 2* package (Maia et al. 2013) to import, visualise and process spectral data. Specifically we used *getspec()*to read spectral files and *procspec()* to remove electrical noise from the spectrometer output. We obtained colour purity metrics with the same package following Stoddard and Prum (2008): (i) maximum chroma, corresponding to the maximum chroma of a pure hue, and (ii) achieved chroma, corresponding to the proportion of maximum possible chroma for a given hue (Fig. S2).

We estimated variation in plumage colour using the avian visual model constrained by receptor noise (Vorobyev and Osorio 1998; Vorobyev et al. 2001) and computed visual chromatic coordinates (x, y, z) following Delhey et al. (2015), representing the position of each reflectance spectrum in the avian visual space. The model incorporated cone sensitivities, noise-to-signal ratios, irradiance spectrum (forest shade), and oil droplet and ocular medium absorbance (Vorobyev et al. 2001; Delhey et al. 2015) with a V-type visual system consistent with Pipridae visual sensitivity (Ödeen and Håstad 2013).

We reduced the dimensionality of the chromatic coordinate data by conducting a principal component analysis (PCA) using the *rda()* function from the *vegan* package (Oksanen et al. 2022), based on covariance matrices for each sex. We retained the first two principal components (explaining 75–95% of cumulative colour variation across datasets) as dependent variables in subsequent statistical analyses and visualised data using *ggplot2* (Wickham 2016).

DNA extraction, parasite detection and sequencing

We extracted DNA using the Qiagen DNeasy 96 blood and tissue kit (Qiagen, Valencia, CA), following the Qiagen tissue protocol for blood stored in 95% ethanol. We screened hosts for infections of the haemosporidian genera *Haemoproteus*, *Leucocytozoon* and *Plasmodium* and identified cytochrome-b (cyt-b) lineages of parasites from positive samples using a nested PCR-based detection and sequencing approach that targeted a 478 base pair cyt-b barcoding fragment from the haemosporidian mitochondrial genome. Protocols detailing reactions, reagents and cycling conditions can be found in Hellgren et al. (2004). In the first nested PCR, we used HAEMNFI and HAEMR2 primers. In the second PCR, we used HAEMF and HAEMN2 primers. We included positive controls, typically a sample that was a known positive in previous projects that always amplified, in all PCR runs. Due to the high sensitivity of nested PCR, we also included negative controls (H_2O) to check for possible contamination, although none was found in any PCR run of the study samples. We then ran all products from PCR amplifications on a 1% agarose gels with 10,000 × SYBR

Safe DNA gel stain (Life Technologies, Carlsbad, CA) and visualised the presence or absence of a band under UV light.

We purified positive PCR products using ExoSAP-IT (Affymetrix, Santa Clara, CA), sequenced them using the BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA), purified cycle sequencing reaction products using ethanol precipitation with resuspension in 10 µl of dH_2O and then ran these purified sequencing products on an ABI 3100 DNA sequencer (Applied Biosystems, Foster City, CA). We visualised, assembled and reconciled forward and reverse sequences using Geneious (v8.1.8; http://www.geneious.com/; Kearse et al. 2012) and aligned consensus sequences using BioEdit v7.2.0 (Hall 1999). As evidence indicates that haemosporidian lineages differing by one cyt-b nucleotide may be reproductively isolated entities (Bensch et al. 2004), we followed the standard practice of treating each unique cyt-b sequence as a unique parasite lineage. We scored chromatograms that showed the presence of multiple infections as co-infections and separated these co-infections into haplotypes using the programme phase 2.1.1 (Stephens et al. 2001; Stephens and Donnelly 2003) following the protocol of Harrigan et al. (2014). We verified sequence identities with a local BLAST against the MalAvi database (Bensch et al. 2009). We named new lineages after the host of origin following the standard protocol (Bensch et al. 2009), which can be found in Bosholn et al. (2020). We deposited all sequences in MalAvi and GenBank (accession numbers MK029822–MK029864).

Statistical analysis

Relationship between female visitation and plumage colour.

To test whether plumage colour of male Blue-capped Manakin individuals is related to female visitation, we used generalised linear models (GLM, family = Poisson) with frequency of female visits as the response variable and colour variables (x, y and z coordinates) as the predictor variables. To test whether female visitation rate is greater in males with the most conspicuous plumage, we conducted a Pearson correlation (cross-correlation method; Ranta et al. 1998) between the scores of the two first $PCA_{x,y,z}$ axes with saturation metrics (maximum chroma: maximum chroma of a given hue; and achieved chroma: the proportion of maximum chroma of a given hue) for each plumage patch separately. As the data were normally distributed, we applied a parametric correlation test. We detected significant cross-correlations for each plumage patch analysed (Table 1).

We performed all statistical analyses in *R* version 3.0.3 (R Core Team 2019) and used the *stats* package (*glm()* function) for GLM fitting, (*cor.test()* function) for Pearson correlation analyses and *ggplot2* for data visualisation (Wickham 2016).

Relationship between haemosporidian parasites and plumage colour.

Table 1 Results of the cross-correlations of Blue-capped Manakin males for each plumage patch analysed. Maximum chroma = the maximum chroma of a pure hue; achieved chroma = the proportion of maximum possible chroma for its hue (Stoddard and Prum 2008). Significance level: $*p < 0.05$, $**p < 0.01$

Plumage patch	PCs	Maximum chroma	Achieved chroma
Back	$PC1_{x,y,z}$	0.84**	0.59**
Back	$PC2_{x,y,z}$	–0.44*	0.64**
Breast	$PC1_{x,y,z}$	0.70**	0.98**
Breast	$PC2_{x,y,z}$	–0.03	0.11
Belly	$PC1_{x,y,z}$	0.67**	0.58**
Belly	$PC2_{x,y,z}$	–0.16	–0.62**
Crown	$PC1_{x,y,z}$	0.65**	–0.71**
Crown	$PC2_{x,y,z}$	–0.37	–0.57**
Remige	$PC1_{x,y,z}$	0.32	0.76**
Remige	$PC2_{x,y,z}$	0.76**	–0.45*
Rump	$PC1_{x,y,z}$	0.83**	0.20
Rump	$PC2_{x,y,z}$	–0.32	–0.03
Tail	$PC1_{x,y,z}$	0.84**	0.50**
Tail	$PC2_{x,y,z}$	0.43*	–0.53**
Wing covert	$PC1_{x,y,z}$	0.63**	0.23
Wing covert	$PC2_{x,y,z}$	–0.35	–0.66**

To test whether the plumage colour of the Blue-capped Manakin indicates the occurrence of haemosporidian parasites, we estimated the significance of the chromatic variation per plumage area between infected and uninfected individuals and each sex separately using a one-way multivariate analysis of variance (MANOVA). We included the PCAx,y,z axes described above as dependent variables and the occurrence of avian malaria as the independent variable.

To evaluate whether infected and uninfected individuals are perceptually discriminable in the avian colour space, we ran a bootstrap analysis as implemented by Maia and White (2018). With this method, new samples are produced through re-sampling (with replacement) of individuals of each group, from which geometric means and their distances are calculated. This procedure generates a distribution of mean distances that is used to estimate a confidence interval. We considered chromatic variation amongst individuals as significant when 1) at least one plumage patch was statistically different between infected and uninfected groups from MANOVA test, 2) when this difference was above the 1 JND threshold of colour discrimination and 3) where there was no overlap between the confidence intervals of the infected and uninfected data (Maia and White 2018).

We performed all analyses in *R* version 3.6.1 (R Core Team 2019), used the *stats* package for MANOVA (*manova()* function), the *boot* package for the bootstrap analysis (*boot()* function) and *ggplot2* For data visualisation of chromatic differences (Wickham 2016).

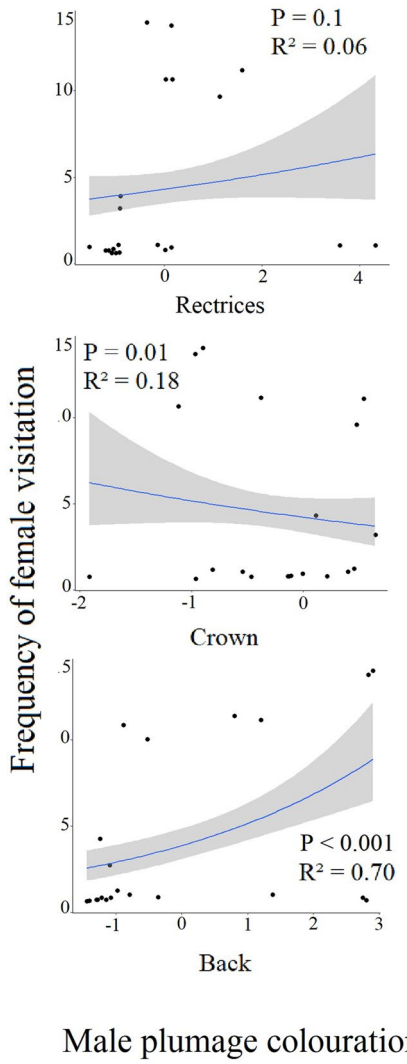

Fig. 2 Relationship between frequency of female visitation and plumage colour of male Blue-capped Manakin (PC1 scores of chromatic variables x, y, z)

Table 2 Relationship of the male Blue-capped Manakin plumage colour and frequency of female visits to the leks. *df* = degrees of freedom; statistical *z* value = test statistic from the model; r^2 = coefficient of determination. Significance level: **p* < 0.05, ***p* < 0.01

Plumage patch	*df*	Statistical *z* value	r^2	*p* value
Wing coverts	20	9.86	0.25	< 0.01**
Breast	20	12.17	0.34	< 0.01**
Back	20	11.68	0.70	< 0.01**
Remige	20	12.59	0.42	< 0.01**
Rump	20	11.64	0.57	< 0.01**
Belly	20	9.86	0.31	< 0.05*
Crown	20	12.96	0.18	< 0.01**
Tail	20	13.76	0.06	> 0.05

Results

Male Blue-capped Manakin plumage colour was associated with the frequency of female visits to the leks (Fig. 2, Table 2). Plumage colour of wing coverts, breast, back, remiges, rump and belly were positively related to the frequency of female visitation. However, crown colour was negatively related to frequency of female visitation ($r^2 = 0.18$, $p = 0.01$), and back colour showed a significant positive relationship ($r^2 = 0.7$, $p < 0.001$). The frequency of female visits to leks was not related to the plumage colour of the tail, and the null model better explained the pattern of female visits than colour pattern for this plumage area (Fig. 2, Table 2).

We detected haemosporidian parasites in $^{12}/_{24}$ male and $^{12}/_{26}$ female manakins corresponding to a prevalence of 50% and 46%, respectively (Table 3). Our analysis of cyt-b mitochondrial gene sequences revealed the presence of nine haemosporidian lineages: five lineages of *Plasmodium*, three lineages of *Haemoproteus* and one lineage of *Leucocytozoon*.

Considering coinfections, *Plasmodium* was the most prevalent genus (47%), followed by *Haemoproteus* (4%) and *Leucocytozoon* (2%). We found coinfections in only five individuals: two with *Plasmodium* and *Leucocytozoon* lineages (TACRUB01 and SETAUD30), two with different *Haemoproteus* lineages (COLTAL01 and COLPAS04), and one with *Haemoproteus* and *Leucocytozoon* lineages (LEPCOR08 and SETAUD30) (Bosholn et al. 2020).

Presence of haemosporidian parasites was not correlated with plumage colour of either adult male or female Blue-capped Manakin (Table 4).

Discussion

In a number of Manakin species, as well as many other avian taxa, male individuals possess bright or gaudy plumage colouration. This is largely due to sexual selection (Price 2008; Kirwan and Green 2012). In this study, structural and melanin-based plumage coloration of Blue-capped Manakin males was generally positively associated with female visitation rates at leks, particularly in plumage regions such as wing coverts, breast, back, remiges, rump and belly, consistent with previous findings that plumage coloration can function as a sexual signal in manakins (McDonald 1989; Prum 1994). These results suggest that more conspicuous coloration in these areas may serve as an attractive visual signal to females, potentially reflecting aspects of male quality or condition (Doucet and Montgomerie 2003; McGraw 2006). In contrast, crown colour was negatively associated with female visitation frequency. This negative association

Table 3 Haemosporidian lineages found in 49 individual Blue-capped Manakins in a central Amazonian population. The asterisk (*) indicates a male individual for which sequencing was unsuccessful and the lineage could not be identified

Sex	Parasite genus	Lineage	*n*° of haplotypes	GenBank accession numbers
Male *	*Plasmodium*	LEPCOR04	4	MK029827–37
		LEPCOR10	1	MK029840
		TACRUB01	4	MK029841-62
	Plasmodium–Leucocytozoon	TACRUB01–LEPCOR08	1	MK029841-62; MK029838
	Haemoproteus–Haemoproteus	COLTAL01–COLPAS04	1	MK029822-4; MK0298825-6
Female	*Plasmodium*	LEPCOR04	3	MK029827-37
	Haemoproteus	TACRUB01	2	MK029841-62
		TUMIG03	3	MK029863-4
	Leucocytozoon	COLPAS04	1	MK029822-4
		LEPCOR08	2	MK029838
	Plasmodium – Leucocytozoon	LEPCOR10 – LEPCOR09	1	MK029840; MK029839

Table 4 Relationship of the occurrence of haemosporidian parasites on plumage colour of Blue-capped Manakin. *df* degrees of freedom, statistical *z* value = test statistic from the model; r^2 = coefficient of determination. Significance level: $*p < 0.05$, $**p < 0.01$

Sex	Plumage patch	*df*	*F* value	*p* value
Male	Tail	3	0.90	0.45
	Wing covert	3	0.82	0.49
	Crown	3	0.15	0.92
	Back	3	0.38	0.76
	Throat	3	0.29	0.82
	Breast	3	1.52	0.23
	Remige	3	1.35	0.28
	Rump	3	1.09	0.37
	Belly	3	1.37	0.27
Female	Tail	3	1.63	0.20
	Wing covert	3	0.57	0.63
	Crown	3	0.98	0.42
	Back	3	1.46	0.25
	Throat	3	1.23	0.32
	Breast	3	1.66	0.20
	Remige	3	0.97	0.42
	Rump	3	0.97	0.42
	Belly	3	1.06	0.38

should be interpreted cautiously, as the relatively low variance explained and the modest sample size may contribute to this pattern (Nakagawa and Cuthill 2007). Thus, the observed relationship might not reflect a biologically meaningful signal. Overall, these findings indicate that female Blue-capped Manakins respond differently to variation in plumage colour across body regions, emphasising the complex role of multiple ornamental traits in sexual selection (Prum 1994; Doucet and Montgomerie 2003). Understanding the physiological and evolutionary mechanisms underlying these patterns may further clarify how sexual selection shapes plumage diversity in this species.

Here, we demonstrated that the structural and melanin-based plumage coloration of Blue-capped Manakin males is related to female visitation rate, although it may not serve as an honest signal of quality in reference to haemosporidian parasite infections. In contrast to previous studies (Hill et al. 2005; Badás et al. 2018), we suggest that the structural and melanin-based colour of Blue-capped Manakin males might not be costly enough to be used as an honest signal of susceptibility to haemosporidian parasites. However, this colouration may honestly predict other aspects of male quality not assessed in this study. Bosholn et al. (2016) showed that male displays in this species can be used as a cue for infection by haemosporidian parasites, suggesting that behavioural displays may signal one condition that structural and melanin-based colouration does not. For instance, data from the same population of manakins show that body plumage coloration, including structural and melanin-based components, is not correlated with display frequency in male Blue-capped Manakins (Bosholn et al., unpublished work). Perhaps female mate choice relies on multiple signals that relate with multiple components of the individual condition and quality.

The lack of correspondence between previous results indicates decreased male mating behaviour in leks with higher prevalence of malaria infection (Bosholn et al. 2016), and our results may be due to at least three other non-mutually exclusive reasons: First, male individuals may carry over pigments in their plumage from the previous moulting season, such that plumage colour may reflect their condition at the time of moult rather than their current infection status (e.g. Delhey et al. 2010; Lumpkin et al. 2014). Second, infections by haemosporidian parasites take between 7 and 15 days to affect the host (Valkiūnas 2005). Thus, it is possible that these parasites first affect more labile characteristics

such as singing and behaviour of the individuals prior to affecting morphological characters. In Blue-capped Manakins, individuals may have only one complete moult per year (Ryder and Durães 2005), which occurs at the end of the breeding season, as reported for manakins in general. The physiological processes that govern feather pigmentation and moult timing are usually coincident. Therefore, recently infected host individuals may still exhibit their previous season's plumage, and changes in plumage colour would only be observed in the medium-to-long term. Third, only male individuals in the lek who survived acute infection may have been captured. These males may, therefore, have chronic infections, in which individuals remain infected but with low parasite intensity, and thus these hosts seem unaffected by the symptoms of avian malaria, even during the breeding season (Valkiūnas 2005). Thus, individuals with low level of infection could invest in feather pigmentation, such as structural mechanisms and melanin, which are synthetized endogenously (Borovansky et al. 2011).

In summary, this study demonstrates that the conspicuousness of structural and melanin-based plumage colours of male Blue-capped Manakins predict female visitation rates. However, avian malaria parasite occurrence is not related to male structural or melanin-based colours, suggesting that these visual signals do not reliably reflect the individual's ability to resist infection by avian malaria parasites. Therefore, we suggest that colours of the Blue-capped Manakin may reflect aspects of male quality not directly assessed in this study, or that other sexual selection models (e.g. sensory drive or Fisher's runaway sexual selection) may explain the evolution of plumage colouration in this species. Future studies could investigate other sexual selection mechanisms that drive the evolution of plumage coloration in this and related species.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10336-025-02356-3.

Acknowledgements The authors thank M. Tolentino, N. Soto, M. Maximiano, G. Lima, P. Diniz, G. Christo, R. Gonçalves, P.A.C.L. Pequeno, and J. Ferreira (Zelão) for field assistance and the Brazilian Biodiversity Research Programme (PPBio) for logistical support. The authors thank ICMBio and CEMAVE–SNA for providing banding permits and metal bands.

Funding The field data collection was based upon research supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (UNIVERSAL number 471092/2012–6 Faixa C. to M. Anciães), by Association of Field Ornithologists, and by Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM) (PRONEX—proj. 003/2009, number 653/2009 to M. Anciães and A. Lima). Haemosporidian laboratory work was funded by NSF DEB-1503804 to J. Weckstein. During the project, F. Teófilo received a fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and CNPq. M.B. received a fellowship from FAPEAM and CNPq. E.V. received a fellowship from CNPq. A. Fecchio is supported by São Paulo Research Foundation (FAPESP), process number 2025/09699–4.

Data availability We deposited all sequences in MalAvi and GenBank (accession numbers MK029822–MK029864).

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval Licences were permitted from Instituto Chico Mendes de Conservação da Biodiversidade [ICMBIO]—licence number 5540–1/ 2013 to M.A., and licences 41069–1/2013; 53130 1/2015 to M.B. and Centro Nacional de Pesquisa para Conservação das Aves Silvestres [CEMAVE]—licence number 3767 to M.B.

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