

Instituto Nacional de Pesquisas da Amazônia – INPA
Programa de Pós-Graduação em Ecologia

EVOLUÇÃO DA COLORAÇÃO DA PLUMAGEM E DIMORFISMO
SEXUAL EM PASSERIFORMES

Fernando Henrique Teófilo de Abreu

Manaus, Amazonas

Dezembro, 2019

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EVOLUÇÃO DA COLORAÇÃO DA PLUMAGEM E DIMORFISMO
SEXUAL EM PASSERIFORMES

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Tese apresentada ao Instituto Nacional de Pesquisas da Amazônia como parte dos requisitos para obtenção do título de Doutor em Biologia (Ecologia).

Manaus – Amazonas

Dezembro, 2019

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Bibliotecário responsável: Jorge Luiz Cativo Alauzo - CRB11/908

A162e Abreu, Fernando Henrique Teófilo de
Evolução da coloração da plumagem e dimorfismo
sexual em passeriformes / Fernando Henrique Teófilo
de Abreu; orientadora Marina Anciães ;
coorientador Rafael Maia . -- Manaus:[s.l], 2019.
96 f.

Tese (Doutorado - Programa de Pós Graduação em
Ecologia) -- Coordenação do Programa de Pós-Graduação,
INPA, 2019.

1. Seleção sexual. 2. Coloração. 3. Aves. I.
Anciães , Marina , orient. II. Maia , Rafael ,
coorient. III. Título.

CDD: 598

Sinopse: Estudou-se o papel da seleção natural e sexual na evolução da coloração da plumagem em Passeriformes. Para isto, coletaram-se dados espectrais de espécimes capturados em campo e de espécimes tombados em museus, dos quais foram relacionados a variáveis relacionadas a mecanismos de seleção natural, sexual e neutra.

Palavras-chave: parasitos, seleção sexual, seleção natural, modelo de visão das aves, dicromatismo sexual.

AGRADECIMENTOS

Agradeço ao INPA e ao seu Programa de Pós-graduação em Ecologia (PG-ECO). Desde o ano de 2012, esta tem sido minha segunda casa onde obtive grandes aprendizados e experiências. De um modo especial, agradeço às coordenadoras Albertina Lima e Camila Ribas pelas gestões, além de todos os professores e pesquisadores credenciados ou não ao INPA que contribuíram de alguma forma para minha formação acadêmica.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e ao CNPq pela concessão da bolsa de doutorado, e à Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM) pelo fomento financeiro durante minha passagem pelo doutorado.

À Association of Field Ornithologists pelo financiamento concedido ao meu projeto (Alexander Bergstrom Memorial Research Award) que contribuiu para a coleta de dados do segundo capítulo.

Ao Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) e ao Centro Nacional de Pesquisa para Conservação das Aves Silvestres (CEMAVE) por conceder as licenças e anilhas necessárias para realizar as expedições de campo.

Ao Programa de Pesquisa em Biodiversidade (PPBio), pelo apoio logístico, e aos moradores do “Ramal do 10” – Km 100 da BR 319, que gentilmente nos receberam durante as excursões de campo. Sou especialmente grato ao Sr. Davi e família por todo apoio oferecido durante a coleta de dados.

A todos que auxiliaram na coleta de dados na BR 319, módulo do Manaquiri (Gabriel Leite, Stephane Pereira, “Pinduca”, “Loro”, “Malagute”, Mariana Tolentino, Nayara, Mariane, Marina Maximiano e Pedro Pequeno). Agradeço por toda ajuda durante o trabalho de campo, e também pela amizade, convivência e parceria. Sem o auxílio dessas pessoas, as excursões de campo

não teriam sido realizadas.

À minha orientadora, Marina Anciães, pela colaboração e apoio incondicional; orientadora e exemplo profissional. Por não ter permitido que eu interrompesse o processo e pela confiança. Uma das pessoas mais humanas que já tive a oportunidade de conhecer. Agradeço também ao meu coorientador Rafael Maia, pela motivação e plena colaboração mesmo à distância.

Aos Drs. Sérgio Borges, Eduardo Barbosa, Igor Kaefer, Jansen Zuanon e Magali Henriques, que aceitaram compor minha banca de qualificação, pelas sugestões e análises significativas que cuidadosamente busquei atender durante todo o processo construtivo da tese. Agradeço especialmente ao Dr. Sérgio Borges que me acompanhou durante o estágio à docência na UFAM, momento de grande experiência e aprendizado.

Aos Drs. Sérgio Borges, Romina Batista, Renato Cintra, Fabricio Baccaro e Flávia Santana que compuseram a banca de defesa e ofereceram valiosas contribuições à versão final desta tese.

Às coleções Zoológicas do Museu Paraense Emílio Goeldi e Instituto Nacional de Pesquisas da Amazônia pela concessão de seu espaço e permissão de visitas para coleta de dados espectrais. Em especial aos curadores e gestores das coleções científicas Mario Cohn-haft, Marco Aurélio, Gisiane Lima, Alexandre Aleixo e Fátima Lima pela recepção, acolhimento e atenção disponibilizados gentilmente durante o desenho do projeto e coleta de dados.

Ao Drs. Alexandre Aleixo, Tomas Hrbek, Alan Fechio, Jason Weckstein pelas colaborações com dados e críticas construtivas ao projeto de doutorado.

Aos colegas do Laboratório de Biologia Evolutiva e Comportamento Animal (LABECA - INPA), pela colaboração acadêmica e espírito de equipe que fizeram minha passagem pelo doutorado tão útil quanto agradável; pelo auxílio direto ou indireto na execução deste trabalho e pela colaboração em trabalhos

paralelos à tese. Agradeço em especial à Mariana Tolentino e Mariane Bosholn, por lerem e revisarem meus manuscritos, por me aconselharem, por terem confiado em mim sempre e, mesmo à distância, serem meu pilar onde me apoiei para celebrar os bons momentos e superar os dias ruins.

À Sra. Clenilda Tolentino, pela hospitalidade e generosidade com que me acolheu em Belém durante minha visita ao Museu Paraense Emílio Goeldi.

Agradeço, por fim, à minha família, especialmente aos meus pais, por me terem ensinado a andar e a lutar. A meu pai (in memoriam), que onde quer que esteja nunca deixou de me amar, de me cuidar, nem de confiar em mim. À minha mãe, amor incondicional. Aquela que, muitas vezes, renunciou aos seus sonhos para que eu pudesse realizar o meu, partilho a alegria e a emoção deste momento. Às minhas irmãs Rosário e Sirliane que, mesmos distantes, estão sempre por perto. Pelas conversas, apoio e conselhos. Amo vocês.

“Nothing in Biology makes sense except
in the light of Evolution" (Theodosius Dobzhansky, 1973)

EVOLUÇÃO DA COLORAÇÃO DA PLUMAGEM E DIMORFISMO SEXUAL EM PASSERIFORMES

RESUMO

A coloração da plumagem das aves é um dos sinais visuais mais utilizados na comunicação entre espécies e coespecíficos e desempenha diversas funções de natureza adaptativa e reprodutiva. Nas aves, a diversidade de cores e mecanismos de produção de cor podem ser observados em ampla escala; desde entre diferentes espécies, entre populações de uma mesma espécie ou mesmo entre indivíduos de uma mesma população. Assim, algumas hipóteses baseadas em mecanismos de seleção natural e/ou sexual tem buscado explicar a evolução desses traços, em especial em clados poligínicos (e.g., indicador de qualidade, seleção runaway de Fisher, direção sensorial). Entretanto, pouco se sabe sobre a importância relativa destes mecanismos evolutivos na evolução da plumagem em diferentes escalas – em espécies e dentro de populações. Esta tese investigou estas questões usando espécies de passeriformes como objeto de estudo. Testou-se a hipótese de que o dicromatismo sexual seria relacionado à evolução da conspicuidade da plumagem de 26 espécies de aves monomórficas à visão humana, modelando a variação em cor para a visão das aves. Ainda, esta tese investigou o papel de parasitos na evolução da coloração da plumagem de uma espécie passeriforme, *Lepidothrix coronata*, e a relação da coloração da plumagem dos machos dessa espécie com a visita de fêmeas. No capítulo 1, mostro que o padrão de dicromatismo sexual é relacionado à conspicuidade da plumagem das espécies estudadas, indicando um papel importante da seleção sexual na evolução das diferenças cromáticas entre os sexos. No capítulo 2, revela-se o papel seletivo da seleção sexual na evolução da plumagem de *L. coronata*, de forma que a coloração conspícua da plumagem dos machos desta espécie atrai mais fêmeas embora não indique susceptibilidade a parasitos causadores da malária aviária. Assim, esta tese evidencia a evolução da plumagem em diferentes escalas de variação (entre sexos de uma mesma espécie e entre indivíduos de uma mesma população) e sugere que mecanismos de seleção sexual têm um papel importante na variação morfológica desses traços fenotípicos nestas espécies.

EVOLUTION OF THE PLUMAGE COLORATION AND SEXUAL DIMORPHISM IN PASSERINE BIRDS (AVES: PASSERIFORMES)

ABSTRACT

The plumage color is one of the most commonly visual cues used in communication contexts and can role adaptive and reproductive functions. In birds, color diversity and mechanisms of color production can be observed on a large scale; among species, among populations of the same species or even among individuals of the same population. Thus, hypotheses based on natural and/or sexual selection mechanisms have sought to explain the evolution of these traits, especially in polygynous clades (e.g., quality indicator, Fisher runaway selection and sensory direction). However, it is a little known on the relative importance of these evolutionary mechanisms in the evolution of plumage at different levels- in species and intrapopulation. This thesis investigated these issues using passerine species as focal species. The hypothesis was tested that sexual dichromatism was related to the evolution of plumage conspicuity of 26 monomorphic bird species to human vision, modeling the color variation for bird vision. Furthermore, this thesis investigated the role of parasites in the evolution of plumage staining of a passerine species, blue-crowned manakin (*Lepidothrix coronata*), and the relationship of male plumage color of this species with the frequency off emale visits. In chapter 1, it is shown that the pattern of sexual dichromatism is related to the plumage conspicuousness of the passerine species, indicating an important role of sexual selection in the evolution of color differences between the sexes. In Chapter 2, the selective role of sexual selection in the evolution of *L. coronata* plumage is revealed, so that the conspicuous plumage coloration of males of this species attracts more females although it does not indicate susceptibility to parasites causing avian malaria. Thus, this thesis highlights the evolution of plumage at different scales of variation (between sexes of the same species and between individuals of the same population) and suggests that mechanisms of sexual selection play an important role in the morphological variation of these phenotypic traits in these species.

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INTRODUÇÃO GERAL

A coloração da plumagem representa uma das características mais marcantes das aves e é também um dos sinais mais importantes na comunicação visual entre espécies. As aves estão entre as mais coloridas dentre os vertebrados terrestres com uma impressionante variação da plumagem representando quase todas as cores (Hill e McGraw 2006; Stoddard e Prum 2011). Pelo menos três principais mecanismos estão envolvidos na produção de cores em aves, sendo que dois deles envolvem pigmentação. Pigmentos carotenóides são os responsáveis pelas cores amarelo, laranja e vermelho enquanto pigmentos melânicos produzem cores mais escuras como marrom e preto (Prum et al. 1998, 1999, 2003). Por outro lado, as cores como azul, violeta e ultravioleta são produzidas através de um mecanismo meramente estrutural, onde a estrutura associada a esses pigmentos é responsável pela variação de cores observadas, não a concentração ou o tipo de pigmento (Prum et al. 1998, 1999, 2003). Assim, é possível observar uma extensa variação na coloração da plumagem das aves em escala interespecífica (eg. McNaught e Owens 2002; Heindl e Winkler 2003; Gomez e Théry 2004), intraespecífica (Anciães et al. 2009; Teófilo et al. 2018) ou até intrapopulacional (Lank e Dale 2001).

A coloração da plumagem pode exercer uma variedade de funções adaptativas associadas tanto a necessidades gerais de defesa (e.g. camuflagem, mimetismo e aposematismo; Ruxton et al. 2004; Prum 2014; Fowlie e Kruger 2003) quanto a necessidades mais específicas de sobrevivência. Nesse ponto, a heterogeneidade ambiental é um importante fator que atua como gerador de diversidade fenotípica (Price, 2008; Teófilo et al. 2018). De um modo geral, podemos afirmar que os fenótipos refletem a ecologia das espécies determinando, além do uso diferenciado do habitat, diferentes estratégias de exploração de recursos disponíveis no meio (MacArthur e Pianka 1966; Grant 1999). Partindo de uma escala macro-evolutiva para uma micro-evolutiva, é possível que a própria composição de espécies (i.e., predadores ou competidores) de uma comunidade afete as pressões adaptativas de populações e com isso seus fenótipos (Hoagstrom e Berry, 2008). Isso porque à medida que a composição de espécies varia, nichos ocupados por determinadas espécies também podem mudar e, com isso, variações fenotípicas podem ocorrer em resposta a tentativa de evitar possíveis predadores ou competidores (Goldberg e Lande 2006). Da mesma forma,

espécies podem ampliar sua área de forrageio na ausência de competidores, sofrendo assim, mudanças nos traços fenotípicos para se adaptar ao novo ambiente biótico disponível (Grant e Grant 1993, 1995, 2003). Deste modo, populações de uma mesma espécie que experimentam condições ambientais distintas podem sofrer pressões adaptativas de diferentes intensidades e levar à variação intraespecífica por processos adaptativos.

Além de funções adaptativas desempenhadas por caracteres de coloração, sinalizar informações importantes a receptores coespecíficos é outro papel importante dos caracteres visuais (Zahavi 1975; Hamilton e Zuk 1982; Rowe e Skelhorn 2004). Nas aves, plumagens coloridas são um dos exemplos mais clássicos de características sexuais secundárias, principalmente entre espécies com acentuado dimorfismo sexual, indicando forte atuação da seleção sexual nesta característica fenotípica (Darwin 1871; Henderson et al. 2013). Assim, grande discussão de como seleção natural e sexual tem modelado a coloração de aves ainda parecem sem consenso (Prum 2012) e estudos sobre diversidade de coloração oferecem grandes oportunidades para entender a origem e manutenção dessa diversidade (Hill e McGraw 2006 a,b).

Plumagens coloridas podem representar um alto custo para se produzir ou manter e algumas hipóteses baseadas em mecanismos de seleção sexual tem buscado explicar a evolução da conspicuidade nesses traços, em especial em clados poligínicos (e.g. indicador de qualidade, seleção runaway de Fisher, direção sensorial; revisão em Andersson 1994; Prum 1997; Price 2008). Mecanismos de seleção sexual variam com relação ao nível de influência que características do habitat podem ter na preferência de parceiros e assim sobre traços selecionados sexualmente. Mecanismos arbitrários, por exemplo, são conhecidos por não fornecer nenhum benefício e estão supostamente sob fraca influência do habitat. Fisher (1930) sugeriu que apenas a preferência de fêmeas em si poderia criar uma pressão seletiva para o surgimento e manutenção de caracteres mais exagerados em machos sem quaisquer benefícios além da possibilidade de transmitir características atraentes para a prole, sendo assim arbitrários quanto a importância de características do habitat (revisão em Prum 1997; Andersson 1994). Assim, altas frequências de traços exagerados de machos bem como da preferência de fêmeas por estes caracteres gerariam retroalimentação positiva com rápidas mudanças de traços sexuais secundários entre populações e assim entre espécies.

Contrariando o modelo de seleção sexual de Fisher, a hipótese do handicap proposta por Zahavi (1975) prevê que a preferência da fêmea por determinadas características conspicuas do macho não são arbitrárias, mas servem como indicadores de melhor qualidade genética individual (Zahavi 1975; Taylor e McGraw 2007). A evolução e manutenção de cores conspicuas dependeriam do balanço entre as exigências de escolha do parceiro e os custos de sobrevivência impostos pela seleção natural através de predação. O desenvolvimento de caracteres selecionados desta forma funcionaria como um marcador de qualidade efetiva, possibilitando a habilidade de um sexo detectar qualidade no sexo oposto. Assim o sexo que escolhe poderia ser informado sobre a qualidade de seu parceiro, enquanto o sexo selecionado poderia transmitir sinais sobre sua qualidade e provavelmente adquirir mais parceiros ou conseguir um parceiro melhor (Zahavi 1975; Taylor e McGraw 2007).

Uma variação da hipótese do handicap foi proposta por Hamilton e Zuk (1982) e defende que características sexuais secundárias servem como indicadores honestos de resistência herdável a parasitos e doenças (e.g., McGraw e Hill 2000; Massaro et al. 2003; Hill et al. 2005). Essa hipótese ficou conhecida como “hipótese dos parasitos” e apresenta duas previsões envolvendo a relação entre infecção por parasitos e características sexuais secundárias. Em escala interespecífica, espécies que foram mais expostas a parasitos durante sua evolução, apresentarão traços sexuais secundários mais desenvolvidos (e.g., plumagem mais colorida ou mais brilhante) dada a maior pressão seletiva a que foram expostas em relação a espécies sujeitas a menores taxas de parasitismo. Em escala interespecífica, espécies que foram mais expostas a parasitos durante sua evolução, apresentarão traços sexuais secundários mais desenvolvidos (e.g., plumagem mais colorida ou mais brilhante) dada a maior pressão seletiva a que foram expostas em relação a espécies sujeitas a menores taxas de parasitismo. Em escala intraespecífica, parceiros preferidos devem ser os menos parasitados pois apenas machos resistentes e com boas condições de saúde investiriam energia na produção de caracteres sexuais secundários mais desenvolvidos (Hamilton e Zuk 1982). Dessa forma, as fêmeas que escolhessem machos com ornamentos mais desenvolvidos receberiam benefícios para sua prole na forma de genes resistentes a parasitos.

Algumas hipóteses baseadas em seleção sexual prevêm uma associação mais direta entre a variação morfológica e o ambiente, de fato atribuindo importância da seleção natural na preferência sexual. A hipótese da direção sensorial (do inglês

“Sensory Drive”, Endler e McLellan 1988), por exemplo, postula que a produção e transmissão de sinais de comunicação de machos e os sistemas de percepção de sinais e preferências de fêmeas co-evoluem em resposta às características físicas do meio (Endler e McLellan 1988; Ryan 1990; 1992; Endler e Basolo 1998). A hipótese prevê ainda que divergências entre populações em caracteres selecionados socialmente podem se acumular rapidamente, após o surgimento ao acaso de novas variantes com efeitos favoráveis aos receptores sensoriais (Endler e Théry 1996). Desde que não haja fluxo gênico intenso, ou que haja variação em preferências inter-sexuais entre populações, tais mudanças podem promover a especiação por acelerar as divergências em caracteres selecionados sexualmente, auxiliando no isolamento reprodutivo ao diminuir a eficiência de comunicação entre co-específicos (Seenhausen et al. 2008). Deste modo, variações ambientais entre populações que se reflitam em alteração dos sinais visuais produzidos poderiam favorecer a divergência de fenótipos entre populações e, assim, entre espécies (Cummings 2007; Seenhausen et al 2008). Especificamente, uma das previsões da hipótese da direção sensorial é que diferentes espécies de aves apresentam diferentes cores simplesmente porque elas habitam diferentes ambientes de luz (eg. Endler 1993; Endler e Théry 1996; Andersson 2000; Gomez e Théry 2004; Heindl e Winkler 2003; Uy e Endler 2004; Anciães e Prum 2008). Assim, muitos autores propuseram hipóteses e previsões na qual determinadas cores da plumagem seriam mais apropriadas para comunicação visual em determinados ambientes de luz do que em outros por maximizar o contraste da plumagem contra a coloração (da luz ambiente) dos habitats (Endler 1993; Endler e Théry 1996; Gomez e Théry 2004; Heindl e Winkler 2003; Anciães e Prum 2008; Medeiros 2012). Por exemplo, Endler (1993) indicou que plumagens laranjas ou vermelhas seriam mais comuns em habitats fechados como florestas de terra firme, pois nestes ambientes, essas cores refletem principalmente comprimentos de onda mais longos e maximizariam o contraste contra a vegetação circundante (Endler 1993).

Esta tese investigou variação em coloração da plumagem em diferentes níveis (i.e., intra-específica e intra-populacional) usando espécies de passeriformes como objeto de estudo. Dentre as aves, os Passeriformes é um dos táxons que mais se destaca em termos de características da plumagem. Este grupo apresenta aproximadamente 6000 espécies (Snow 2004) que habitam os mais diferentes ambientes. A coloração da plumagem apresenta grande diversificação dentre as espécies de Passeriformes e

estudos indicam dicromatismo sexual críptico para a visão humana em muitas espécies monomórficas (Eaton et al. 2005, 2006). Dessa forma, estudos do padrão de cor da plumagem de espécies de Passeriformes, e os mecanismos envolvidos na diversificação dessas cores em espécies e dentro de espécies podem auxiliar na compreensão da história evolutiva desse grupo, além de permitir inferências sobre a contribuição de mecanismos seletivos e neutros no processo de diversificação fenotípica das espécies (Price 2008; Anciães et al. 2009).

Dentre os Passeriformes, o tangará-de-coroa-azul, *Lepidothrix coronata*, é uma das espécies da família com a mais ampla distribuição geográfica, além de apresentar um dos exemplos mais clássicos de variação geográfica em características da plumagem (Hellmayr 1929; Snow 2004; Cheviron et al. 2005; Kirwan e Green 2012; Teófilo et al. 2018). Os machos desta espécie possuem dimorfismo sexual acentuado, no qual as fêmeas são verdes e os machos adultos podem apresentar plumagem preta, verde ou preta com verde com uma coroa azul brilhante, dependendo da linhagem (Snow 2004; Ryder e Durães 2005; revisão em Anciães et al. 2009). Dado o considerável grau de diferenciação morfológica observada entre clados de *L. coronata*, é possível que existam ainda diferenças intra-populacionais da espécie em cor da plumagem, além de comportamentos exibidos e hierarquias sociais praticadas pelos machos. Machos de *L. coronata* podem contrair malária aviária e indivíduos infectados pelo agente causador da doença, o hemoparasito do gênero *Plasmodium*, exibem consideravelmente menos sinais comportamentais durante a estação reprodutiva (Bosholn 2015). Embora exibições comportamentais de machos de *L. coronata* possam ser consideradas sinais honestos do quão resistente à infecção da malária os indivíduos são, ainda não se sabe qual a influência destes hemoparasitos sobre o contraste de cor da plumagem, outra importante característica sexual fenotípica provavelmente selecionada por fêmeas de muitas espécies da família Pipridae (Prum 1997; Endler e Thery 1996; Stein e Uy 2004; Avilés e Parejo 2013). Assim, é possível que as condições de conspicuidade de cor exibidas na plumagem de machos possam apresentar algum indicativo para as fêmeas sobre sua qualidade genética relacionada à resistência imunológica aos hemoparasitos causadores da malária aviária e assim transmitirem esses genes à sua prole (Zahavi 1975; Hamilton e Zuk 1982).

Uma vez que a sinalização honesta deve indicar a capacidade de indivíduos enfrentarem ameaças como patógenos, espera-se que, em uma população, indivíduos

menos infectados apresentem maior qualidade fenotípica, expressa por exemplo pelo maior contraste de sua plumagem (Hamilton e Zuk 1982, Doucet 2002; Griggio et al. 2011). Sobretudo, é possível que as diferenças observadas em conspicuidade das plumagens de machos sejam explicadas por padrões de visitas e escolhas das fêmeas. Assim, estudar a influência de hemoparasitos na coloração da plumagem bem como as respostas comportamentais das fêmeas em diferentes populações da espécie frente a diferentes condições da plumagem apresentadas pelos machos permitirá testes de mecanismos microevolutivos associados à diversificação fenotípica da espécie, mediados por seleção sexual. Variações em coloração da plumagem poderiam levar a divergências genéticas populacionais por meio do isolamento reprodutivo entre populações, em situações de alopatria, parapatria ou simpatria (Price 2008) ou, alternativamente, diminuir a taxa de diferenciação entre populações através da manutenção de regimes de seleção intra- ou inter-sexual (e.g. hierarquias e preferências), em oposição a novidades fenotípicas (i.e. plumagens e comportamentos) que viessem a surgir entre populações (Stein e Uy 2006).

OBJETIVOS

Objetivo Geral

Estudar a evolução da coloração da plumagem em Passeriformes, investigando os mecanismos envolvidos no padrão de diversificação fenotípica em espécies e populações.

A fim de atender o objetivo geral do estudo, o presente trabalho foi dividido nos dois experimentos abaixo descritos:

Experimento 1: Evolution of conspicuous plumage predicts sexual dichromatism in passerine birds

Objetivos Específicos

Considerando as diferenças observadas no sistema visual das aves em relação à visão humana (Goldsmith, 1990; Losey et al. 1999), testamos a seguinte hipótese:

H1: A visão humana pode falhar em detectar dicromatismo sexual em aves.

Considerando que traços conspícuos são sexualmente selecionados e estão sob forte seleção direcional para maior elaboração (e.g., Darwin 1871, Kirkpatrick 1982, Andersson 1994, Martin e Badyaev 1996, Prum 2012), testamos a seguinte hipótese:

H2: O dicromatismo sexual está relacionado à conspicuidade da cor da plumagem.

Experimento 2: Plumage colour of male blue-crowned manakins predicts female visitation rates but not signal avian malária infection

Considerando que a coloração da plumagem é um importante sinal de comunicação intersexual, testaremos as hipóteses de que:

H1: A coloração da plumagem de machos da espécie *Lepidothrix coronata* está associada à frequência de visita de fêmeas desta espécie.

Considerando que a sinalização honesta deve indicar a capacidade de indivíduos de enfrentar ameaças como patógenos (Hamilton e Zuk 1982), testaremos a hipótese de que:

H2: A ocorrência de parasitos hemosporídeos afeta a coloração da plumagem de machos de uma população da espécie *L. coronata*.

CAPÍTULO 1

Evolution of conspicuous plumages predicts sexual dichromatism in passerine birds

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Em revisão em *Acta Amazonica*

ABSTRACT

Sexual dichromatism has been the subject of scientific studies for Centuries, but there are still divergent opinions about the relative importance of sexual and natural selection in the evolution of these colors. We estimated the sexual dichromatism of 26 passerine bird species that are monomorphic to the human eye to test for the roles of sexual and natural selection in species with potentially uncovering hidden evolutionary patterns of trait elaboration or crypsis. As such, we first test the prediction that the sampled species will be frequently sexually dichromatic from a bird visual perspective. Then, we tested the hypotheses that sexual or natural selection drives the evolution of sexual dichromatism in plumage colors within the sampled passerine bird species. For this, we measured the reflectance spectra of individual males and females and calculated the color distance for each pair of homologous patches between sexes. We evaluated the level of conspicuity of all plumage patches in units of just noticeable differences. Next, we run Phylogenetic Generalized Least Squares analysis using the chromatic contrast (ΔS) between the sexes of each species and plumage patches as response variables and the within-plumage contrast and the contrast of each patch against the background as predictor variables. We found sexual dichromatism in 83.4% of patches and in all species analyzed. Both variables related to plumage conspicuity were positively related to the level of sexual dichromatism in males and females. Since conspicuous traits are often sexually selected, we suggest that male and female colorful plumages are under directional selection for greater elaboration, favoring the evolution of sexual dichromatism.

Keywords: sexual dimorphism, coloration, conspicuity, visual models.

Evolução de plumagens conspicuas explicam dicromatismo sexual em

Passeriformes

RESUMO

Dicromatismo sexual tem sido objeto de estudos científicos há séculos, porém, opiniões divergentes com relação à importância relativa da seleção natural e sexual na evolução desses traços ainda permanecem sem consenso. Nós estudamos dicromatismo sexual de 26 espécies de Passeriformes que são monomórficas ao olho humano para testar as hipóteses de que (i) a visão humana falha em detectar dicromatismo sexual em Passeriformes e (ii) o dicromatismo sexual está relacionado à conspicuidade da plumagem das espécies estudadas. Para isso, nós medimos reflectância de indivíduos machos e fêmeas e calculamos a distância de cor para cada par de manchas da plumagem homólogas entre sexos. Nós avaliamos o nível de conspicuidade de todas as regiões da plumagem em unidades de *just noticeable differences*. Nós rodamos regressões filogenéticas usando contraste cromático entre os sexos de cada espécie e usamos cada mancha da plumagem como variável resposta e o contraste intra-plumagem e contraste de cada mancha contra o ambiente de fundo como variáveis independentes. Nós encontramos dicromatismo sexual em 83,4% das manchas analisadas e em todas as espécies amostradas. O contraste intra-plumagem e o contraste de cada mancha contra o ambiente de fundo foram positivamente relacionados ao nível de dicromatismo sexual. Uma vez que traços conspicuos são selecionados sexualmente, nós sugerimos que plumagens conspicuas estão sob seleção direcional para maior elaboração, favorecendo a evolução do dicromatismo sexual.

Keywords: dimorfismo sexual, coloração, conspicuidade, modelos visuais.

INTRODUCTION

The evolution of sexual dichromatism has been the subject of scientific studies for centuries, but divergent opinions remain (Badyaev and Hill 2003, Price and Eaton 2014, Dunn *et al.* 2015, Simpson *et al.* 2015). Darwin (1871) believed that sexual selection could drive the evolution of sexual dichromatism in polygamous species by favoring the evolution of conspicuous plumages, particularly in males (e.g. Darwin 1871, Andersson 1994, Martin and Badyaev 1996). Following Darwin, many studies have used sexual dichromatism as a proxy to estimate the magnitude of sexual selection and have associated the variation in sexual dichromatism with the diversity of mating systems (e.g. Gonzalez-Voyer *et al.* 2008, Cooney *et al.* 2017). Thus, classical hypotheses of sexual selection suggest that conspicuous plumages are the result of either arbitrary female choice (Fisher 1930, Kirkpatrick 1982, Prum 2012) or sensorial mechanisms (Endler 1992, Mutumi *et al.* 2016) or are indicators of good genes and immunity (Hamilton and Zuk 1982, Hill and McGraw 2006a), which might explain the extremely conspicuous colors exhibited by polygynous species (Kirkpatrick 1982, Prum 2012).

Alternatively, but not exclusively, Wallace (1889) suggested that sexual dichromatism is a consequence of natural selection for crypsis within females, since it provides camouflage during nest attendance (Bennett and Owens 2002, Badyaev and Hill 2003, Hofmann *et al.* 2008). As such, natural selection has been evoked as an important driver of the evolution of camouflaged plumages, especially in monogamous bird species and in the sex in charge of most of the parental care, which is usually the female (Wallace 1868, Soler and Moreno 2012, Simpson *et al.* 2015). Thus, environmental pressures may favor cryptic plumage patterns in both sexes or, in the

case of differential selective pressures operating between sexes, it may result in the evolution of sexual dichromatism through the gain of a drab plumage in either sex (Wallace 1868, Soler and Moreno 2012).

In a broader scope, drab morphological signals can also evolve due to social (non-sexual) selection pressures exerted in other competitive contexts (Crook 1972, West-Eberhard 1983, review in Tobias *et al.* 2012). For example, Simpson *et al.* (2015) suggested that loss of female conspicuousness in migratory species might be due to the relaxation of selection for female social signaling (e.g. for territorial and resource defense) at high latitudes. On the other hand, ecological processes may also drive the evolution of conspicuous traits which are often produced by sexual selection: handicap and costly signals (Lyon and Montgomerie 2012). In many species, females use conspicuous signals during competition for non-sexual resources to defend foraging territories (Murphy *et al.* 2009, Tobias *et al.* 2011, Tobias *et al.* 2012) or limited nesting sites (Heinsohn *et al.* 2005). In these cases, ecological factors mediate the evolution of visual traits (Hall and Magrath 2007) and model sexual dichromatism (West-Eberhard 1979, West-Eberhard 1983, Heinsohn *et al.* 2005, review in Tobias *et al.* 2012) independent of inter-sexual preferences.

Most of the documented cases of sexual dichromatism in birds are based on human vision, whose sensitivity ranges from wavelengths of 400 to 700 nm. However, color perception is a psychophysical process that involves the color produced by a signaler and how it is perceived by a viewer (Cuthill *et al.* 2017). In fact, animal groups exhibit a striking diversity of visual systems that allow them to perceive colors differently (Troscianko *et al.* 2017, Kelber *et al.* 2003, Cronin *et al.* 2014). The development of physiological visual models allowed the quantification of color

perception in the visual systems in diverse taxa, including wavelengths that are imperceptible to humans (Vorobyev and Osorio 1998, Stoddard and Prum 2008, Cuthill *et al.* 2017, Renoult *et al.* 2017). One of the first take-home messages of these studies was that color perception in humans should not be used as a proxy for color perception in other groups (Lythgoe 1979, Endler 1990, 1992, 1993, Bennett *et al.* 1994, Eaton 2005, 2006, Cuthill 2006, Håstad and Ödeen 2008, but see Armenta *et al.* 2008b, Seddon *et al.* 2010, Dale *et al.* 2015, Drury and Burroughs 2016, Bergeron and Fuller 2018). In sum, sexual dichromatism is widespread among species that appear monochromatic to us (Eaton 2005, 2006).

Passerine birds are highly visual animals (Bennett and Théry 2007) and are among the taxa with the most impressive diversity in plumage coloration, exhibiting a wide array of conspicuous and cryptic color patterns (Stoddard and Prum 2011) with different levels of sexual dichromatism across species (Eaton 2005, 2006). Passerine birds also exhibit great variation in mating systems, with some taxa chiefly consisting of polygamous species (e.g. Pipridae and Cotingidae), whereas others mostly include monogamous species (e.g. Tyrannidae and Dendrocolaptidae). This diversity makes Passeriformes ideal for studies aimed at testing hypotheses regarding the ecological and evolutionary basis of sexual dichromatism (Berv and Prum 2014, Teófilo *et al.* 2018).

In this context, we estimated sexual dichromatism from an avian perspective in 26 passerine bird species that are monochromatic to humans in order to evaluate the relative roles of sexual and natural selection in the evolution of sexual dichromatism in species with no obvious sexual dichromatism to the human vision, which may reveal hidden evolutionary patterns of trait elaboration or crypsis. Considering the differences observed in the visual system of birds in relation to that of humans (Goldsmith 1990,

Losey *et al.* 1999) and that human vision may often fail to detect sexual dichromatism in passerine birds (e.g. Eaton 2005, 2006), we expected that (i) the species selected for our study, which appear sexually monomorphic based on human vision, will frequently be sexually dichromatic from an avian visual perspective, e.g. using methods for the separation of color groups into the avian tetrahedral color space (Eaton 2005). Since conspicuous traits are thought to be sexually selected and are often under directional selection for greater elaboration in response to intrasexual or intersexual selection (e.g. Darwin 1871, Andersson 1994, Martin and Badyaev 1996, Kirkpatrick 1982, Prum 2012, but see Heinsohn *et al.* 2005, Murphy *et al.* 2009, Tobias *et al.* 2011, Tobias *et al.* 2012). We tested the hypothesis that sexual dichromatism would be associated with plumage conspicuousness. Thus, we expect (ii) a positive relationship between sexual dichromatism and male conspicuousness as result from sexual selection. Following Wallace (1889), who suggested that sexual dichromatism was a consequence of selection for crypsis of female plumages to reduce predation risk during nest attendance, we expect (iii) a negative relationship between sexual dichromatism and female conspicuousness as result from natural selection.

MATERIALS AND METHODS

Spectrophotometry and color analysis

We used museum skins of 26 passerine bird species that are monomorphic to the human eye, 10 of which belonged to families composed mostly of sexually dimorphic species, while 16 belonged to families of typically monomorphic species. All species were identified as monomorphic by independent observers and confirmed in the specialized literature (Sick 1997, del Hoyo *et al.* 2014). Species were chosen by

considering availability of specimens (at least five for each sex) in the bird collections from the Instituto Nacional de Pesquisas da Amazônia (INPA) and Museu Paraense Emilio Goeldi (MPEG). We used an Ocean Optics USB-2000 UV-VIS spectrophotometer connected to a cosine-corrected probe (R400-2 UV-VIS) and a deuterium-halogen light source to measure the color spectra of nine plumage patches from the study specimens located on the crown, neck, chest, belly, back, rump, remiges, greater wing coverts and rectrices. Specimens with plumage loss and skin damage had these patches excluded from analysis to avoid biased results (e.g., *Rhytipterna immunda*, *R. simplex*, *Phlegopsis nigromaculata* and *Xenops minutus*). Previous studies have reported that there is no significant effect of specimens age on plumage color (i.e. brightness, hue and saturation) and that museum specimens collected at least within the past 20 years resemble closely live birds in their feather colors (e.g. Armenta *et al.* 2008a) and look similar when viewed by birds (Martin *et al.* 2018). Even so, to control for possible differences in color due to museum specimen age (Doucet and Hill 2009), we used the most recently dead birds into the sample to quantify color. We measured between 5 and 10 specimens per sex for all sampled species. We used the average of 10 readings obtained on a given bird skin from the same area in a plumage patch, using an integration time of 20 milliseconds and a zero boxcar correction.

Visual sensitivity in birds falls into two main classes: violet sensitive (VS) and ultraviolet sensitive (UVS) (Cuthill 2000, Ödeen and Håstad 2011). For VS species, we analyzed the spectra between 350 and 700 nm and for UVS species, we analyzed the spectra between 300 and 700 nm corresponding to the range of maximum visual sensitivity associated with these species, respectively (Ödeen and Håstad 2011). We

visualized and manipulated spectral data and estimated color metrics based on visual models using PAVO (Maia *et al.* 2013) implemented in R (R Core Team 2018).

Sexual dichromatism estimation

We used a popular method of sexual dichromatism estimation following the protocol of Eaton (2005). To do so, we averaged the reflectance spectra of individual males and females for each homologous plumage patch and for each species studied. We then calculated the color distance for each pair of homologous patches of each sex using the Vorobyev–Osorio color discrimination model (Vorobyev and Osorio 1998). Eaton’s method considers a feather patch to be dichromatic when $\Delta S > 1.0$ just noticeable differences (jnd) and a species to be dichromatic if it possesses at least one dichromatic feather patch (Eaton 2005) (Table 1).

Estimation of plumage conspicuity

To investigate the effect of the plumage conspicuity of the visual signals produced by males to conspecifics and females to potential diurnal predators, we estimated plumage conspicuousness using the avian vision model limited by the receptor noise (Vorobyev and Osorio 1998, Vorobyev *et al.* 2001) of male and female individuals for each plumage body area and species. We model diurnal visual systems only, for simplicity, considering the potential diurnal avian predators, such as species within the Accipitridae and Falconidae families, besides a few owls (Strigidae), which are also V-type as most passerine species sampled here (17 out of the 26 species). Most differences between the visual system of raptors, diurnal owls and passerines derive

from variation in sensitivity peak of the VS cone receptors (Ödeen and Håstad 2003), and not from the relative proportions of receptors in the different channels (VS: SWS: MWS: LWS), whose noise are inversely proportional to their squared roots in the model (Vorobyev and Osorio 1998). Since we consider that when V-type bird vision is modeled, we obtain a conservative measure of dichromatism and conspicuity in comparison to UV-type, as the last provides higher jnd values than the former (due to larger wavelength range compared among spectra in UV-type vision, FHT personal observation).

We computed visual chromatic coordinates (xyz) for each reflectance spectrum. These coordinates map the position of individual colors in the visual space of birds and are calculated as just noticeable differences (jnd) (Cassey *et al.* 2009, Pike 2012) using supplementary material provided in Delhey *et al.* (2015). This model results from the conversion of the cone quantum catches from the 4 types of photoreceptor cones in the birds' retina, which are sensitive to the photons of longwave, medium-wave, shortwave and very shortwave light (Cuthill *et al.* 2006), into three chromatic coordinates (x, y and z) in the avian visual space. To run this model, we input information on (i) the cone sensitivities of the four single cones of birds, (ii) the noise-to-signal ratio (Weber fraction) of each single cone, (iii) the irradiance spectrum of the ambient light and (iv) the absorbance properties of the oil droplets and ocular medium in the birds' retina (Vorobyev *et al.* 2001, Delhey *et al.* 2015). The resulting model can be represented in a visual space wherein the chromatic information of each reflectance spectrum is defined by its x, y, z coordinates, and its Euclidean distances between points in the chromatic space correspond to just noticeable differences (Pike 2012, Delhey *et al.* 2015). We followed the protocol of Delhey *et al.* (2015) using a Weber fraction of 0.1 (Vorobyev

et al. 1998) and the following average cone proportions: VS = 0.38, S = 0.69, M = 1.13, L = 1.00 (Hart 2001). For these analyses, we used the forest shade spectrum irradiance for forest species and standard daylight (d65) for species from canopy, forest edges or open areas, where white light environments are predominant (Endler 1993). However, there is actually little contribution to visual signals arising from variation in light within terrestrial habitats (Cummings and Endler 2018).

We generated a span metric (average color span, or within-plumage contrast) by simply taking the Euclidean distances between the x, y, z coordinates of all the body patches of a sex and species and averaging them (Delhey *et al.* 2015). We evaluated the level of the conspicuity of all plumage patches by estimating the perceptual color contrast (ΔS) of each plumage patch against the background of the species habitat in units of jnd following Vorobyev and Osorio (1998) in PAVO (Maia *et al.* 2013). For forest species, we used a standard forest shade illuminance (Endler 1993), and for species from open habitats, we used an idealized illuminance with green foliage as the background. For a given patch, we first estimated pairwise color distances (ΔS) for each plumage area against the green background for forest species and a white background for open-habitat species. We then averaged this result to represent the average pairwise distance between any plumage area for each sex and species.

Relationship between sexual dichromatism and conspicuity of plumage color

To test whether the conspicuity of the plumage color of the sampled species predicts sexual dichromatism, we applied PGLS analysis (Garamszegi 2014), in order to control for species phylogenetic relationships that could render non-independence to

data. We used the phylogeny of birds extracted from the BirdTree database (<http://www.birdtree.org>) derived from the global avian phylogeny of Jetz *et al.* (2012).

We used the chromatic contrast (ΔS) between the sexes of each species and plumage patch as the response variable and the within-plumage contrast and contrast of each patch against the background as the predictor variables. We performed all statistical analyses using R 3.4.4 (R Core Team 2018).

RESULTS

We found biologically significant differences in color between males and females in all species (i.e. at least one dichromatic plumage patch) and in 83.4% of the patches analyzed. Even when the threshold for discrimination was doubled ($\Delta S = 2.0$ jnd), 18 of 26 species (69.23%) and 25.33% of the patches analyzed were still dichromatic to the avian vision system (Table 1).

The average color span in the species studied ranged from 3.29 jnd in males and 4.42 jnd in females of *S. turdina* due to their dark gray plumage to 29.29 jnd in males and 31.55 jnd in females of *T. chilensis* due to the high color contrast between their polychromatic plumage patches (Table 1). The chromatic contrast of individual patches against the background ranged from 4.30 jnd on the *N. chrysocephalum* breast to 16.52 jnd on the *G. mexicanus* crown. Finally, the chromatic contrasts of the overall plumage showed ΔS values ranging from 0.06 jnd (throat coloration of *C. flaveola*) to 9.25 jnd (wing coverts coloration of *C. cela*).

Both variables related to plumage conspicuity (within-plumage contrast and perceptual color contrast of each plumage patch against the background of the species

habitat) were positively related to level of sexual dichromatism for males (PGLS, F-statistic: 15.39, Degrees of freedom = 22, Adjusted R-squared: 0.38, $p < 0.001$; and PGLS, F-statistic: 13.52, Degrees of freedom = 22, Adjusted R-squared: 0.35, $p < 0.001$, respectively) and females (PGLS, F-statistic: 22.04, Degrees of freedom = 22, Adjusted R-squared: 0.47, $p < 0.001$; and F-statistic: 20.19, Degrees of freedom = 22, Adjusted R-squared: 0.45, $p < 0.001$, respectively) (Figure 1).

DISCUSSION

This study agrees with previous studies suggesting that human vision fails to discriminate color variation in birds (e.g. Eaton 2005, 2006). However, recent studies based on principal component analysis of spectral data concluded that human vision can detect much of the variation in coloration in the visible range (Bergeron and Fuller 2018) and can provide a valid proxy for avian perception of sexual dichromatism in antbirds (Thamnophilidae) (Seddon *et al.* 2010). Our results are only partially in agreement with these studies, since in all cases analyzed here, human vision failed to detect sexual dichromatism. As such, we reinforce the idea that color discrimination from the human perspective should be considered with caution.

Delhey and Peters (2008) have suggested that signaling colors are more variable than nonsignaling patches within the same species and that sexual dichromatism per se is not associated with increased variability in these traits. In this regard, conspicuous patches may play a role in signaling between conspecifics, unlike drab patches, which might have evolved for crypsis. Our results corroborate this idea, as we found that plumage conspicuity predicted sexual dichromatism. In general, most conspicuous

colors are produced by carotenoid pigments, which are often dependent upon the availability of food resources since birds cannot produce them (Hill *et al.* 2002, McGraw 2006a,b). Thus, conspicuous patches are costlier to maintain than less conspicuous patches (melanin pigments and structural colors), which should require lower levels of intraspecific variation and reduced strength of sexual selection to be maintained (Delhey *et al.* 2017). This may explain the greater number of observed cases of sexual dichromatism in conspicuous patches (i.e. the more variable colors between sexes than within them, suggesting diversifying selection) than in drab patches. Since conspicuous traits are sexually selected, it is expected that colorful plumages are often under directional selection for greater elaboration in response to male competition for access to mates (intrasexual selection) or mate choice (i.e. intersexual selection), favoring the evolution of conspicuous coloration (Andersson 1994, Delhey *et al.* 2008).

More recent studies that have addressed the relative importance of natural and sexual selection in modeling sexual dichromatism in birds have indicated that the latter leads to more accumulation of sexual differences in color, thus corroborating Darwin's theory (e.g. Matysioková *et al.* 2017). Nevertheless, other studies have suggested that sexual dichromatism may evolve via a mosaic of natural and sexual selection (Shultz and Burns 2017). Considering that sexual selection could explain the evolution of conspicuity, it may be important in modeling chromatic differences between the sexes, as shown here, where there is probably strong sexual selection to develop conspicuous plumage and a polygamous mating system (Sick 1997, Fraga 2011). Our findings support the observations of Dunn *et al.* (2015), who analyzed the color of 977 bird species separately for each sex in relation to indices of both natural and sexual selection and demonstrated that both mechanisms are important for the evolution of sexual

dichromatism and that sexual dichromatism in hue is strongest in polygynous and cooperatively breeding species. The fact that polygamous breeding species belonging to Pipridae and Cotingidae analyzed in this study were sexually dichromatic is consistent with the idea that mating behavior may have driven changes in sexual dichromatism in passerine birds towards signal efficiency (Doucet *et al.* 2007, Price and Eaton 2014, Berv and Prum 2015, Ribeiro *et al.* 2015).

It is important to highlight that our sampling procedure was designed to show whether species that are sexually monochromatic to human vision are sexually dichromatic to other birds. Nevertheless, it is interesting to evaluate the role of these evolutionary mechanisms in differences in colors which are hidden to us, which may be implicated in sexual dichromatism or not, by analyzing questions such as whether the level of sexual dichromatism is greater in more colorful species. Therefore, using the scale studied in this work, we demonstrated that in species that are monochromatic to our eyes, sexual dichromatism is positively related to plumage conspicuousness. Therefore, at this scale, sexual selection (e.g., colorful plumages) drives sexual dichromatism that is perceptible to birds, possibly due to sexual variation in color that remains fixed by the tested evolutionary mechanisms.

CONCLUSIONS

Our study demonstrates the importance of adequately estimating sexual dichromatism, which could provide novel insights into the relatively greater importance of sexual selection rather than natural selection in the evolution of plumage coloration. Because our visual system is very different from that of birds, methods based simply on

human vision can result in underestimation compared to the results of methods based on spectral data and those that use bird vision models. Therefore, we encourage researchers to use novel and more accurate frameworks available for color analysis throughout many other animal groups. Studies of the ecological drivers and evolutionary implications of sexual dichromatism in relation to the theories of natural and sexual selection will be greatly improved by considering such approaches.

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FIGURES

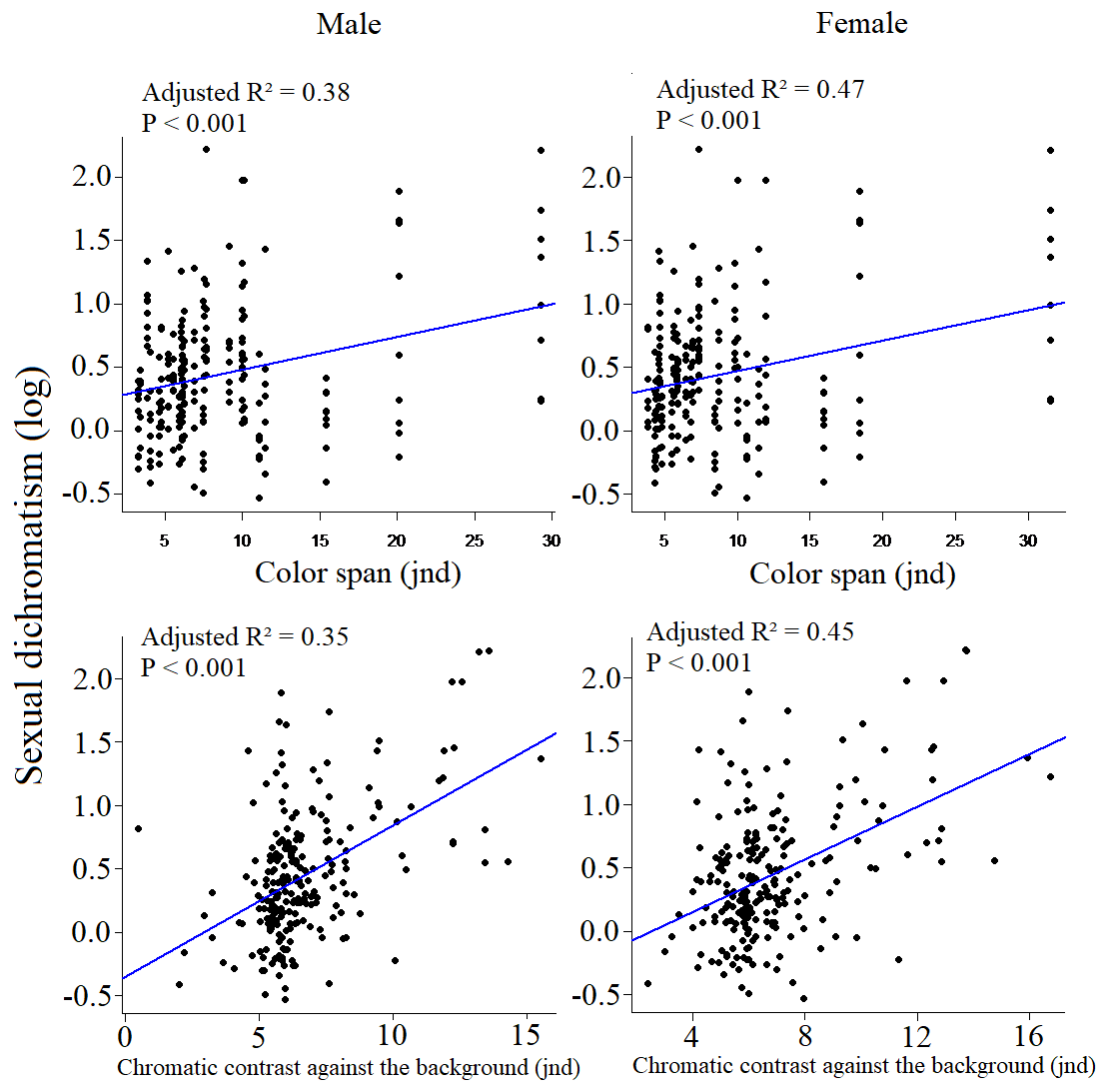


Figure 1 Relationship between sexual dichromatism of the studied taxa and conspicuity of feather color. Upper panels show the effect of color span (measuring within-plumage contrast) measured for male and female individuals (black dots) on sexual dichromatism (log-scaled), and bottom panels show chromatic contrasts against visual backgrounds for color patches from male and female plumages (black dots). Blue lines represent the best fit trend to the data. R-squared values were adjusted to phylogenetic signal across species in the PGLS models.

TABLES

Table 1: Average chromatic contrast (ΔS) between male and female individuals for all plumage patches and descriptors of visual color contrast for each species and sex sampled in the study. $n = 10$ (5 males, 5 females) for all calculations for each species.

Species	Patch	Sexual dichromatism	male		female	
			Color span	Chromatic contrast against the background	Color span	Chromatic contrast against the background
<i>Automolus infuscatus</i>	Back	1.50	6.12	5.84	6.44	4.14
	Belly	0.93	6.12	6.15	6.44	6.12
	Breast	1.16	6.12	6.34	6.44	6.10
	Crown	1.95	6.12	6.15	6.44	7.22
	Remige	1.12	6.12	7.75	6.44	7.77
	Rump	1.82	6.12	6.18	6.44	5.29
	Tail	1.75	6.12	8.21	6.44	8.74
	Throat	1.36	6.12	5.69	6.44	5.21
	Wing	1.30	6.12	6.46	6.44	6.94
<i>Cacicus cela</i>	Back	1.73	7.65	13.43	7.31	12.86
	Belly	2.25	7.65	13.43	7.31	12.86
	Breast	1.55	7.65	6.03	7.31	6.01
	Crown	1.94	7.65	6.31	7.31	6.28
	Remige	1.76	7.65	6.37	7.31	6.32
	Rump	2.61	7.65	5.92	7.31	5.96
	Tail	1.89	7.65	6.21	7.31	5.96
	Throat	3.20	7.65	5.95	7.31	5.98
	Wing	9.25	7.65	13.57	7.31	13.73
	Back	1.64	9.96	10.48	9.84	10.52

Species	Patch	Sexual dichromatism	male		female	
			Color span	Chromatic contrast against the background	Color span	Chromatic contrast against the background
<i>Cyphorhinus arada</i>	Belly	2.60	9.96	7.01	9.84	7.03
	Breast	3.74	9.96	5.84	9.84	5.36
	Crown	3.12	9.96	9.11	9.84	9.23
	Remige	1.74	9.96	6.24	9.84	6.27
	Rump	2.42	9.96	7.51	9.84	7.32
	Tail	1.82	9.96	6.60	7.31	7.24
	Throat	2.02	9.96	12.23	9.84	12.32
	Wing	1.84	9.96	6.18	9.84	6.12
<i>Coereba flaveola</i>	Back	1.07	11.46	5.61	11.48	4.36
	Belly	4.19	11.46	4.60	11.48	4.20
	Breast	4.17	11.46	11.91	11.48	12.52
	Crown	1.63	11.46	6.73	11.48	6.95
	Remige	1.32	11.46	5.66	11.48	5.42
	Rump	4.17	11.46	9.38	11.48	10.82
	Tail	1.45	11.46	6.56	11.48	6.90
	Throat	0.71	11.46	5.73	11.48	5.11
Wing	0.87	11.46	5.93	11.48	5.41	
<i>Cyanocorax violaceus</i>	Back	0.87	15.38	6.04	15.96	8.54
	Belly	1.17	15.38	8.07	15.96	6.93
	Breast	1.16	15.38	8.76	15.96	6.64
	Crown	1.51	15.38	6.06	15.96	6.23
	Remige	1.10	15.38	6.63	15.96	8.64
	Rump	1.35	15.38	8.53	15.96	6.37
	Tail	0.67	15.38	7.60	15.96	7.54
	Throat	1.36	15.38	8.24	15.96	8.88
Wing	1.05	15.38	6.89	15.96	6.84	
	Back	1.61	6.22	7.67	5.90	7.02

Species	Patch	Sexual dichromatism	male		female	
			Color span	Chromatic contrast against the background	Color span	Chromatic contrast against the background
<i>Dendrocincla merula</i>	Belly	1.42	6.22	7.74	5.90	6.55
	Breast	0.96	6.22	7.36	5.90	7.49
	Crown	1.74	6.22	14.28	5.90	14.76
	Remige	2.03	6.22	6.09	5.90	5.93
	Rump	1.70	6.22	7.71	5.90	8.24
	Tail	1.23	6.22	7.87	5.90	7.06
	Throat	2.58	6.22	6.39	5.90	5.59
	Wing	1.24	6.22	6.95	5.90	7.26
<i>Dendrocolaptes</i>	Back	1.38	7.54	7.06	7.32	7.39
	Belly	1.91	7.54	8.25	7.32	6.59
	Breast	2.66	7.54	6.96	7.32	6.16
	Crown	1.79	7.54	7.58	7.32	8.89
	Remige	3.47	7.54	7.24	7.32	9.81
	Rump	3.47	7.54	11.69	7.32	12.55
	Tail	2.04	7.54	8.03	7.32	9.88
	Throat	1.54	7.54	6.37	7.32	6.14
Wing	2.47	7.54	9.23	7.32	9.11	
<i>Formicarius colma</i>	Back	1.47	9.14	5.88	6.91	6.12
	Belly	1.25	9.14	6.74	6.91	6.23
	Breast	1.72	9.14	6.36	6.91	5.19
	Crown	4.29	9.14	12.28	6.91	12.57
	Remige	1.36	9.14	6.18	6.91	7.22
	Rump	2.03	9.14	5.76	6.91	6.14
	Tail	1.99	9.14	6.00	6.91	7.35
	Wing	1.92	9.14	6.13	6.91	6.12
	Back	1.66	6.90	6.54	8.71	6.70
	Belly	1.02	6.90	7.26	8.71	7.96

Species	Patch	Sexual dichromatism	male		female	
			Color span	Chromatic contrast against the background	Color span	Chromatic contrast against the background
<i>Gymnopathys leucaspis</i>	Breast	1.48	6.90	5.52	8.71	4.58
	Crown	3.60	6.90	7.00	8.71	6.62
	Remige	2.17	6.90	5.62	8.71	5.94
	Rump	2.04	6.90	7.51	8.71	7.52
	Tail	1.36	6.90	6.72	8.71	6.60
	Throat	0.64	6.90	5.96	8.71	5.74
	Wing	1.26	6.90	6.28	8.71	5.93
<i>Gymnomystax</i>	Back	1.06	20.12	5.48	18.4	5.86
	Belly	3.40	20.12	11.87	18.4	16.75
	Breast	5.27	20.12	5.75	18.4	5.77
	Crown	6.63	20.12	5.80	18.4	5.99
	Remige	1.82	20.12	5.78	18.4	5.66
	Rump	1.27	20.12	5.44	18.4	5.66
	Tail	0.81	20.12	5.58	18.4	5.56
	Throat	5.17	20.12	6.00	18.4	10.05
Wing	0.98	20.12	5.75	18.4	5.94	
<i>Gymnopathys rufigula</i>	Back	0.95	6.11	5.81	6.80	6.66
	Belly	1.94	6.11	5.95	6.80	4.24
	Breast	1.48	6.11	8.12	6.80	9.12
	Crown	1.50	6.11	6.84	6.80	6.92
	Remige	1.33	6.11	6.94	6.80	7.97
	Rump	1.65	6.11	7.00	6.80	10.34
	Tail	0.80	6.11	6.24	6.80	5.98
	Throat	2.39	6.11	10.13	6.80	10.62
Wing	1.07	6.11	6.33	6.80	6.81	
	Back	1.22	5.18	5.61	4.61	5.43
	Belly	1.01	5.18	5.40	4.61	5.44

Species	Patch	Sexual dichromatism	male		female	
			Color span	Chromatic contrast against the background	Color span	Chromatic contrast against the background
<i>Lipaugus vociferans</i>	Breast	1.76	5.18	5.40	4.61	6.01
	Crown	4.16	5.18	5.82	4.61	4.99
	Remige	1.21	5.18	5.60	4.61	6.02
	Rump	1.83	5.18	5.42	4.61	6.44
	Tail	1.52	5.18	5.88	4.61	5.97
	Throat	1.21	5.18	5.19	4.61	5.18
	Wing	1.69	5.18	5.94	4.61	5.01
<i>Neopelma</i>	Back	1.55	10.11	4.53	11.94	4.62
	Belly	1.07	10.11	4.36	11.94	5.18
	Breast	1.08	10.11	4.26	11.94	5.21
	Crown	7.27	10.11	12.20	11.94	12.92
	Remige	2.47	10.11	6.23	11.94	4.91
	Rump	1.21	10.11	4.98	11.94	4.45
	Tail	1.10	10.11	5.62	11.94	6.23
	Throat	1.76	10.11	4.86	11.94	5.17
Wing	3.23	10.11	5.27	11.94	5.23	
<i>Neopelma pallescens</i>	Back	0.74	7.46	5.11	8.43	7.00
	Belly	1.07	7.46	5.33	8.43	5.82
	Breast	0.61	7.46	5.23	8.43	5.97
	Crown	2.77	7.46	9.44	8.43	10.12
	Remige	0.83	7.46	4.75	8.43	4.29
	Rump	1.20	7.46	5.39	8.43	5.83
	Tail	1.13	7.46	5.81	8.43	5.78
	Throat	0.78	7.46	5.49	8.43	4.93
Wing	1.08	7.46	5.53	8.43	4.76	
	Back	0.82	11.08	5.85	10.65	7.10
	Belly	1.83	11.08	10.31	10.65	11.64

Species	Patch	Sexual dichromatism	male		female	
			Color span	Chromatic contrast against the background	Color span	Chromatic contrast against the background
<i>Pithys albifrons</i>	Breast	0.80	11.08	10.07	10.65	11.33
	Crown	0.80	11.08	5.84	10.65	5.93
	Remige	0.93	11.08	5.74	10.65	5.76
	Rump	0.96	11.08	8.25	10.65	9.10
	Tail	0.95	11.08	8.11	10.65	9.84
	Throat	1.24	11.08	5.47	10.65	5.87
	Wing	0.59	11.08	5.95	10.65	7.93
<i>Phlegopsis</i>	Back	0.88	5.89	5.88	4.81	5.99
	Belly	1.03	5.89	5.44	4.81	5.89
	Breast	1.21	5.89	5.80	4.81	5.99
	Crown	0.77	5.89	6.26	4.81	6.63
	Remige	1.30	5.89	6.69	4.81	7.21
	Rump	1.08	5.89	6.41	4.81	6.62
	Tail	1.31	5.89	7.16	4.81	5.86
Throat	1.12	5.89	5.46	4.81	4.76	
<i>Pitangus sulphuratus</i>	Back	1.27	10.01	5.99	10.04	5.75
	Belly	2.07	10.01	7.60	10.04	6.26
	Breast	1.65	10.01	8.24	10.04	4.92
	Crown	7.21	10.01	12.55	10.04	11.63
	Remige	2.08	10.01	5.85	10.04	5.90
	Rump	1.18	10.01	6.13	10.04	5.92
	Tail	1.50	10.01	5.81	10.04	6.25
	Throat	1.06	10.01	5.66	10.04	5.53
Wing	1.18	10.01	5.79	10.04	5.73	
	Back	1.55	5.53	5.88	5.83	5.72
	Belly	1.48	5.53	4.80	5.83	4.28
	Breast	0.86	5.53	5.02	5.83	5.04

Species	Patch	Sexual dichromatism	male		female	
			Color span	Chromatic contrast against the background	Color span	Chromatic contrast against the background
<i>Rhytipterna immunda</i>	Crown	1.51	5.53	5.26	5.83	6.16
	Remige	2.14	5.53	6.55	5.83	6.23
	Rump	1.37	5.53	5.59	5.83	5.46
	Tail	1.50	5.53	6.50	5.83	6.24
	Throat	1.76	5.53	5.67	5.83	4.96
	Wing	1.38	5.53	5.97	5.83	5.82
<i>Rhytipterna simplex</i>	Back	1.62	3.41	5.34	4.66	5.05
	Belly	1.11	3.41	5.31	4.66	5.95
	Breast	0.87	3.41	5.26	4.66	5.92
	Crown	1.44	3.41	5.26	4.66	5.21
	Tail	1.48	3.41	5.72	4.66	4.88
<i>Schiffornis turdina</i>	Back	0.82	3.29	5.15	4.42	5.22
	Belly	1.34	3.29	4.96	4.42	5.73
	Breast	1.38	3.29	5.49	4.42	6.63
	Crown	1.01	3.29	6.00	4.42	5.97
	Remige	1.48	3.29	6.48	4.42	6.86
	Rump	0.74	3.29	5.20	4.42	5.64
	Tail	0.81	3.29	5.74	4.42	5.82
	Throat	1.28	3.29	5.28	4.42	5.83
Wing	1.17	3.29	5.90	4.42	5.04	
<i>Turdus albicollis</i>	Back	0.77	4.63	6.33	5.52	6.12
	Belly	1.16	4.63	5.55	5.52	5.63
	Breast	1.79	4.63	5.47	5.52	4.82
	Crown	0.96	4.63	5.85	5.52	6.25
	Remige	0.83	4.63	5.74	5.52	5.20
	Rump	1.24	4.63	5.49	5.52	5.23
	Tail	0.77	4.63	5.92	5.52	5.41

Species	Patch	Sexual dichromatism	male		female	
			Color span	Chromatic contrast against the background	Color span	Chromatic contrast against the background
	Throat	1.03	4.63	5.41	5.52	3.99
	Wing	1.37	4.63	5.68	5.52	5.92
<i>Tangara chilensis</i>	Back	1.28	29.29	6.32	31.55	6.97
	Belly	2.70	29.29	9.46	31.55	9.23
	Breast	2.69	29.29	10.66	31.55	10.75
	Crown	4.54	29.29	9.47	31.55	9.32
	Remige	5.70	29.29	7.59	31.55	7.38
	Rump	9.20	29.29	13.20	31.55	13.76
	Tail	1.26	29.29	7.12	31.55	7.74
	Throat	2.05	29.29	12.24	31.55	12.75
	Wing	3.93	29.29	15.51	31.55	15.94
	<i>Thraupis episcopus</i>	Back	1.62	6.02	5.49	5.61
Belly		1.32	6.02	5.65	5.61	6.65
Breast		2.07	6.02	6.37	5.61	7.00
Crown		1.64	6.02	5.57	5.61	7.11
Remige		3.53	6.02	5.63	5.61	5.84
Rump		2.17	6.02	6.40	5.61	6.23
Tail		1.57	6.02	7.47	5.61	7.74
Throat		1.31	6.02	5.64	5.61	6.74
Wing	2.28	6.02	8.39	5.61	9.02	
<i>Thraupis palmarum</i>	Back	2.08	3.88	6.53	4.63	6.22
	Belly	2.76	3.88	4.78	4.63	4.15
	Breast	3.83	3.88	7.55	4.63	7.33
	Crown	2.53	3.88	7.30	4.63	6.83
	Remige	2.79	3.88	5.84	4.63	5.92
	Rump	2.90	3.88	7.59	4.63	7.13
	Tail	1.95	3.88	5.50	4.63	5.21

Species	Patch	Sexual dichromatism	male		female	
			Color span	Chromatic contrast against the background	Color span	Chromatic contrast against the background
	Throat	2.27	3.88	6.04	4.63	6.87
	Wing	1.29	3.88	5.12	4.63	6.43
<i>Tyranneutes</i>	Back	1.37	4.05	3.26	4.34	3.99
	Belly	1.14	4.05	2.94	4.34	3.50
	Breast	0.85	4.05	2.20	4.34	2.98
	Crown	1.86	4.05	5.63	4.34	5.04
	Remige	0.75	4.05	4.07	4.34	4.17
	Rump	0.79	4.05	3.66	4.34	4.66
	Tail	1.09	4.05	5.02	4.34	5.33
	Throat	0.66	4.05	2.01	4.34	2.40
	Wing	0.96	4.05	3.23	4.34	3.23
	<i>Xenops minutus</i>	Back	1.03	4.74	6.17	3.86
Belly		1.20	4.74	5.49	3.86	5.99
Breast		1.26	4.74	6.40	3.86	6.28
Crown		1.07	4.74	6.15	3.86	6.91
Remige		1.26	4.74	6.54	3.86	7.72
Rump		2.23	4.74	7.55	3.86	7.21
Tail		1.50	4.74	6.38	3.86	7.44
Wing		2.26	4.74	0.50	3.86	6.77

CAPÍTULO 2

Plumage colour of male blue-crowned manakins predicts female visitation rates but does not signal avian malaria infection

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Aceito em *Ecology and Evolution*

ABSTRACT

Colorful plumages may bear high production and maintenance costs. Parasite hypothesis posits that visual signals work as honest indicators of heritable resistance to parasites. However, the value of 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-crowned manakins from a population in central Amazonia to test the hypothesis that (i) plumage colour of male blue-crowned manakins influences the attraction of female individuals; and (ii) the occurrence of haemosporidian parasites is associated with the plumage colouration patterns of male blue-crowned manakins. Through behavioural observations, we estimated the frequency of female visits at individual perches of blue-crowned manakin males. We measured colour spectra from eight plumage patches of male 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 among male blue-crowned manakins predicts female visits. However, variation in these colors did not correspond to occurrence of haemosporidian parasites. In contrast to previous studies, we suggest that the structural and melanin-based colouration of male blue-crowned manakins may not be costly enough to be used as an honest signal of susceptibility to haemosporidian parasites.

Key words: signal efficacy, signal content, handicap, Neotropics, Central Amazonia.

INTRODUCTION

Visual signals are one of the most important characters used in communication among species or conspecifics (Zahavi 1975, Hamilton and Zuk 1982a, Rowe and Skelhorn 2004). Signal efficiency depends on the quality of the produced signals, the sensory systems of receivers and the environmental conditions (Andersson 2000, Bradbury and Vehrencamp 2011), which in turn are affected by natural or sexual selection (Endler and McLellan 1988, Ryan 1990, Endler 1992, 1993, Endler and Basolo 1998). Therefore, by studying variation among signals, their effects on receivers and their relationship with the biotic environment, we will better understand the evolution of sexual selection mechanisms (Endler and McLellan 1988, Hill and McGraw 2006).

Colorful plumages may bear high production and maintenance costs (McGraw et al. 2002, Huhta et al. 2003, Delhey et al. 2010). Among 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, McGraw et al., 2002, but see Veiga and Puerta, 1996). However, studies have shown that darker eumelanic colouration is often associated with higher immune capacity and parasite resistance (Roulin et al. 2001; Jacquin et al. 2011), and that structural and melanin-based colour can signal the intensity of infection from blood parasites (Doucet and Montgomerie 2003, but see Fitze and Richner 2002, Roulin et al. 2008). Therefore, further studies will provide a better understanding of the link between parasite infections and the evolution of colouration in birds.

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 Prum 1997, Andersson 1994, Price 2008). The handicap hypothesis

proposed by Zahavi (1975) suggests that in polygamous species, in which males contribute nothing beyond their sperm, females should 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 through sexual signals such as cost-added signals (i.e., signals whose cost is greater than that required to transmit the information). Thus, females that prefer mates with a handicap could be enhancing the likelihood of mating with a high-quality partner, and thus providing their offspring with good genes (Zahavi 1975, Taylor and McGraw 2007).

A variant of the handicap hypothesis was later proposed by Hamilton and Zuk (1982) and posits that secondary sexual characteristics works as an honest indicator of inheritable resistance to parasites and pathogens (McGraw and Hill 2000, Massaro et al. 2003, Hill et al. 2005). This hypothesis, known as the "parasite hypothesis", predicts an inverse relationship between infection by parasites and elaboration of secondary sexual traits within populations. 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). Multiple studies have indicated a negative relationship between the presence of haemosporidian parasites and conspicuous colours in birds (e.g., Doucet and Montgomerie 2003, Hill et al. 2005, Mougeot et al. 2009, del Cerro et al. 2010), although the effects of parasites on the structural and melanin-based colours remain poorly known (review in Côté et al. 2018).

Manakins (i.e., Aves: Pipridae) are among the conspicuous bird families with respect to bright plumage colour and elaborate displays. This Neotropical endemic bird clade contains approximately 50 species (Snow 2004) known for their polygynous lekking mating system, in which males exhibit their plumage during complex nuptial displays to attract females (Snow 1963, Sick 1967, Prum 1997). Females are hypothesized to receive no resources, except

sperm, and are solely responsible for nest construction and parental care (Bradbury and Gibson 1983, Andersson 1994). As such, arbitrary preferences (Fisher 1930) and sensory drive for coevolution of male visual signals and female visual perception (Endler and Mclellan 1988, Endler 1992, 1993, Endler and Basolo 1998, Ryan and Cummings 2013) have been proposed to explain the evolution of these and other lekking species (Prum 1997, 2012, Heindl and Winkler 2003).

Within the family Pipridae, the blue-crowned manakin, *Lepidothrix coronata*, (figure 1) is one of the most geographically variable species in plumage pattern (Hellmayr 1929, Snow 2004, Cheviron et al. 2005, Kirwan and Green 2012, Teófilo et al. 2018). Avian malaria and other hemosporidian parasites are common in blue-crowned manakins (Fecchio et al. 2017). One recent study showed that individuals infected with these hemoparasites (*Plasmodium* and *Haemoproteus* genera) exhibit significantly lower behavioral activity during the breeding season but, nevertheless, infected individuals were no less attractive to females than uninfected individuals suggesting that females did not use behavioral traits alone to select mates (Bosholn et al. 2016). However, it is still unknown whether blood parasites may influence male plumage colour in blue-crowned manakins, and likewise the roles of these parasites on the evolution of plumage colouration. Hence, studying the influence of structural colour of male blue-crowned manakins 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 male and female blue-crowned manakins from a population in Central Amazonia to understand the evolutionary role of male plumage colouration as a visual signal preferred by females. Considering that sexual selection predict the evolution of more conspicuous visual signals (Endler 1992, Endler and Basolo 1998,

Owings and Morton 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) plumage colour of blue-crowned manakin males influences the attraction of female individuals. Thus, we expect that male individuals with more conspicuous plumage colour will receive more female visitation than males with less conspicuous plumage colour. Furthermore, considering that visual signals provide honest information on the phenotypic and/or genetic quality of individuals (Zahavi 1975, Grafen 1990, Moller and Pomiankowski 1993, Johnstone 1998), such as their resistance to pathogens (Hamilton and Zuk 1982b, Doucet and Montgomerie 2003, Griggio et al. 2010, Galván et al. 2014), we tested the hypotheses that: (ii) the occurrence of haemosporidian parasites is associated with the plumage colouration patterns of blue-crowned manakin males; and we expect that individual males infected with haemosporidian parasites will have less conspicuous plumage than uninfected males. Alternatively, the null hypothesis, would predict that the occurrence of avian malaria is not associated with plumage colour in blue crowned manakin, indicating that other mechanisms than those related to indirect benefits (e.g., sensory drive or random preferences of females) may drive the evolution of plumage colour in this species.

MATERIALS AND METHODS

Field sampling and Behavioural sampling

We carried out fieldwork at a Biodiversity Research Program (PPBio) site located approximately 100 km from Manaus-AM, Brazil, within the Purus-Madeira interfluvium, south of the Amazon River (figure 2). We sampled one population of blue-crowned manakins and we located ten blue-crowned manakin leks through acoustic and visual censuses of male

individuals along the main trails and the forest interior to sample their behavioral activity at all leks during the 2013 and 2014 reproductive seasons (October and November - June and July, respectively). The reproductive season of *L. coronata* is characterized by increased frequency of displays and acoustic activity and also development of gonads (review by Kirwan and Green 2012, Bosholn et al. 2016). We located display perches (i.e., the location in the lek where adult males display to females) of adult males through continuous acoustic and visual census along the trails and interior of the forest. We considered lekking behaviour the persistence of individual males conducting 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 (12 x 2.5 m, Ecotone) for three consecutive days at each lek. Six leks were sampled in 2013, and four leks were sampled in 2014. Nets were open from 7a.m. to 4 p.m. We banded male individuals with color bands on the right tarsus and one metallic band on the left tarsus (Licenses from ICMBIO, Instituto Chico Mendes de Conservação da Biodiversidade and CEMAVE, Centro Nacional de Pesquisa para Conservação das Aves Silvestres, number 3767). We then collected approximately 50µL of blood sample from each individual through brachial vein puncture, using a disposable hypodermic needle and micro capillary tubes. We sexed individuals in the field according to the plumage colour and confirmed the sex identification through molecular sexing using the protocols of Ito et al. (2003). Male *L. coronata arimensis*, the subspecies endemic to our study site region, are mostly blackish (melanin-based colour) (Teófilo et al. 2018, review by Kirwan and Green 2012). Nevertheless, besides the blackish plumage, we can differentiate definitive plumage males from those in pre-definitive plumages by their complete bright violet blue crown, which is produced by structural colour mechanisms. We collected a minimum of 5 feathers from each colour patch from 24 male and 26 female individuals captured in the field for subsequent assessment in the lab: crown, breast, belly, back, rump, remiges, greater wing coverts and

rectrices. We carefully placed these feather samples into opaque paper envelopes to minimize color degradation until we conducted the analysis of spectra data in the lab.

We conducted behavioral observations on display perches during the mornings (07:00 – 12:00), and afternoons (13:00 – 16:00) through continuous focal sampling with five-minute intervals (Altmann 1974). 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 visiting events as those in which both banded and non-banded females, and both infected and uninfected females, visited the display perches. Females do not frequently vocalize when compared to young males (Durães 2009). Therefore, in every 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. We only analyzed female visitation to adult males to avoid bias in our results. We calculated frequency of female visits using the following index:

Frequency of females per observation interval (N_t of female visits observed at individual perch / $\sum N_{intind}$)

Where N_t denotes total number, and N_{intind} denotes total number of observation intervals to focal perches.

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. Parasite lineages from the genera *Haemoproteus*, *Leucocytozoon*, and *Plasmodium* were identified using PCR-based detection methods targeting a 477 base pair cytochrome-*b* (cyt-*b*) barcoding fragment from the haemosporidian mitochondrial genome. Protocols detailing reactions,

reagents, primer names, and cycling conditions can be found in (Hellgren et al. 2004). Positive controls were included in all PCR runs. Due to the high sensitivity of nested PCR, negative controls were also included in runs to check for possible contamination, although none was found in any PCR run of the study samples. Products from PCR amplifications were run on 1% agarose gels with 10,000x SYBR Safe DNA gel stain (Life Technologies, Carlsbad, CA) and visualized under UV light.

Positive PCR products were purified using ExoSAP-IT (Affymetrix, Santa Clara, CA) and sequenced using BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA). Cycle sequencing reaction products were purified using ethanol precipitation and were then re-suspended in 10 μ l of dH₂O, and run on an ABI 3100 DNA sequencer (Applied Biosystems, Foster City, CA). Forward and reverse sequences were visualized, assembled, and reconciled using Geneious (v8.1.8, Kearse et al. 2012). Consensus sequences were aligned 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 use the standard practice of referring to each unique *cyt-b* lineage as a unique parasite. Sequence identities were verified with a local BLAST against the MalAvi database (Bensch et al. 2009). New lineages were named after the host of origin following standard protocol (Bensch et al. 2009), can be found in Bosholn et. al. (2019), and were deposited in both MalAvi and GenBank.

Spectrophotometry and Colour analysis

For collecting spectra data from sampled feathers, we mounted the feathers on a piece of black velvet (to avoid background noise) one on top of another in an attempt to simulate the original pattern found on live birds, following the method detailed in Vaqueiro-Alba et al.

(2016). We used an Ocean Optics USB-2000 UV-VIS spectrophotometer connected to a cosine corrected probe (R400-2 UV-VIS), and a Deuterium-Halogen Light Source and used SpectraSuite software to process spectral data from the feathers of 24 adult males and 26 adult females captured at the study area. We measure the colour spectra of eight plumage patches from study specimens: crown, breast, belly, back, rump, remiges, greater wing coverts and rectrices. The lead author, FT, took all the measurements. We used the average of ten individual readings from the same area in a plumage patch, using an integration time of 20 milliseconds and zero boxcar correction. We analyzed spectra between 350-700 nm, corresponding to the spectrum of maximum visual sensitivity associated with the bird family Pipridae (Ödeen and Håstad 2013). We used the package PAVO (Maia et al. 2013) implemented in R (R Core Team 2019) to input, visualize and manipulate spectral data. Specifically, we used the `getspec` function to load spectral data files, and the `prospec` function to remove electrical noise arising from the spectrometer. We estimated the conspicuousness of colour pattern for each male individual sampled from the study population, using two metrics corresponding to saturation (or colour purity) for all plumage areas analyzed in package PAVO (Maia et al. 2013): Maximum chroma = the maximum chroma of a pure hue; Achieved chroma = the proportion of maximum possible chroma for its hue, both implemented by Stoddard and Prum (2008) (table S1).

Avian vision model

We estimated variation in plumage colour using the avian vision model limited by receptor noise (Vorobyev and Osorio 1998, Vorobyev et al. 2001). We assessed whether chromatic differences between infected and uninfected individuals reach the threshold of colour discrimination produced by 24 males and 26 female individuals for each plumage area. We computed visual chromatic coordinates (xyz) for each reflectance spectrum, which maps

position of individual colours in the birds' visual space using supplementary material provided in Delhey et al. (2015). These three chromatic coordinates result from the conversion of the four types of photoreceptor cones in the birds' retina which are sensitive to photons on long-wave, medium-wave, short-wave and very short-wavelengths of light (Hill and McGraw 2006a). To run this model, we input information on (i) the cone sensitivities of the four single avian cones, (ii) the noise-to-signal ratios of each single cone (iii) the irradiance spectrum of the ambient light and (iv) absorbance properties of the oil droplets and ocular medium in the birds' retina (Vorobyev et al. 2001, Delhey et al. 2015).

The resulting model is a visual space representation wherein the chromatic information of each reflectance spectrum is defined by its xyz coordinates, and its Euclidean distances between points in the chromatic space correspond to units of just perceptual differences (JNDs). Differences below one JND unit are not discernable to birds (Vorobyev and Osorio 1998, Cassey et al. 2009, Pike 2012). We followed the protocol in Delhey et al. (2015) using a Weber fraction of 0.1 (Vorobyev et al. 1998) and the following average cone proportions: VS = 0.38, S = 0.69, M = 1.13, L = 1.00 (Hart 2001). For these analyses, we used the forest shade spectrum, which corresponds to the irradiance light commonly found in forest habitats (Vorobyev et al. 1998). We used a V-type visual system since visual sensitivity associated with avian family Pipridae is typically sensitive to the violet region (Ödeen and Håstad 2013). We computed chromatic differences between parasitized and unparasitized individuals and their homologous plumage areas.

To reduce multidimensionality of the group of variables related to visual chromatic coordinates, we inserted these variables (x,y,z) from each plumage area into a Principal Component Analysis (PCA), for each sex. We used the first two axes of greatest variation in colour (which explained between 75% and 95% of the cumulative variation in the datasets for

each plumage patch and sex) as the dependent variable in the statistical analyses described below.

Relationship between female visitation and plumage colour

To test whether plumage colour of male blue-crowned manakin individuals is attractive to females, we built generalized 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 ran cross-correlations 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 used a Pearson correlation and the cross-correlation method (Ranta et al. 1998). We found significant cross-correlations for each plumage patch analyzed (Table 1). We performed all statistical analyses using R 3.0.3 (R Core Team, 2019).

Relationship between haemosporidian parasites and plumage colour

To test whether plumage colour of blue-crowned manakins indicates the occurrence of haemosporidian parasites, we estimated the significance of the chromatic variation between infected and uninfected individuals per plumage area and each sex separately in a one-way multiple analysis of variance (MANOVA). We included PCA_{x,y,z} axes described above as dependent variables and occurrence of avian malaria as the independent variable. This method considers a plumage area as dichromatic when $p < 0.05$.

To evaluate whether infected and uninfected individuals are perceptually discriminable in the avian colour space, we ran a bootstrap analysis 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, from which a confidence interval is estimated. We consider significance chromatic variation among individuals when they 1) possessed at least one feather patch 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).

RESULTS

We sampled a total of 50 blue-crowned manakin individuals, of which 24 were adult males, and 26 were adult females. We detected haemosporidian parasites in 12 males and 12 females corresponding to a prevalence of 50% and 46%, respectively. Partial sequencing of the *cyt-b* mitochondrial gene 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%). Coinfections were only found in 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. 2019). Presence of haemosporidian parasites was not correlated with plumage colour of either adult male or female blue-crowned manakins (table 2).

The male blue-crowned manakin plumage colour was associated to the frequency of female visits to the leks (fig. 2). Plumage colour of wing coverts (statistical z value = 9.86, degrees of freedom = 20, $R^2 = 0.25$, $p < 0.01$), breast (statistical z value = 12.17, degrees of freedom = 20, $R^2 = 0.34$, $p < 0.01$), back (statistical z value = 11.68, degrees of freedom = 20, $R^2 = 0.70$, $p < 0.01$), remiges (statistical z value = 12.59, degrees of freedom = 20, $R^2 = 0.42$, $p < 0.01$), rump (statistical z value = 11.64, degrees of freedom = 20, $R^2 = 0.57$, $p < 0.01$), and belly (statistical z value = 9.86, degrees of freedom = 20, $R^2 = 0.31$, $p < 0.05$) was positively related to frequency of female visitation. However, the plumage colour for crown was negatively related to frequency of female visitation (statistical z value = 12.96, degrees of freedom = 20, $R^2 = 0.18$, $p < 0.01$) (figure 3). The frequency of female visits to leks was not related to the plumage colour of tail and the null model better explained the pattern of female visits than colour pattern for this plumage area (statistical z value = 13.76, degrees of freedom = 20, $R^2 = 0.06$, $p > 0.05$) (figure 3).

DISCUSSION

In a number of manakin species as well as many other avian taxa, male individuals possess bright or gaudy plumage colouration due to sexual selection (Price 2008, Kraaijeveld et al. 2007, Kirwan and Green 2012). Here, we demonstrate that plumage colouration of blue-crowned manakin males is related to female visitation rate, although may not serve as an honest signal of quality in reference to haemosporidian parasite infections. In contrast to previous studies (Fitze and Richner 2002, Hill et al. 2005, Badás et al. 2018), we suggest that although the structural and melanin-based colour of blue-crowned manakin males might not be costly enough to be used as an honest signal of susceptibility to haemosporidian parasites, it 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 displays are likely more costly than plumage colours and, as such, may be a more reliable indicator of male quality. For instance, unpublished data from the same population of manakins shows that plumage colouration and display frequency of male blue-crowned manakins are not correlated (Bosholn et al. 2019 in prep.).

Higher frequency of female visits to males of more conspicuous plumages is also compatible with predictions of other mechanisms of sexual selection by female choice, such as those relying on sensory cues or arbitrary sexual selection in driving the evolution of conspicuous coloration in blue-crowned manakins. Under sensory drive, environmental characteristics influence the evolution of both the sensory system of females and signaling traits of males, thereby reducing costs associated with searching for mates and therefore susceptibility to predation, as demonstrated for some manakin species (Endler and Théry 1996, Heindl and Winkler 2003, Uy and Endler 2004), including the blue-crowned manakin. Fisher's (1930) runaway model assumes that selection exerted by arbitrary female choice governs the evolution of more exaggerated male traits, which is also consistent with our results and previous predictions for manakins (Prum 1997). Considering that uninfected males are also more active (Bosholn et al. 2016), we suggest that plumage colour attracts females, through enhanced conspicuousness, arbitrary choices or correlation with other quality indicators unmeasured by us, whereas male behaviour is used by females to obtain information about individual quality such as genes for resistance to avian malaria (e.g., Garamszegi 2005, Bosholn et al. 2016).

Our evidence that haemosporidian parasites is not correlated the plumage colour of *L. coronata* is nevertheless unexpected, considering the immunosuppressive costs of androgens hormones on melanic synthesis of plumage ornaments (Ros 1999, Martínez-Padilla 2006), and thus on susceptibility of individuals to diseases and parasites. However, melanin-based

colour and structural coloration is known to affect more ectoparasites than haemoparasites (Fitze and Richner 2002, but see Côte et al. 2018). The apparent lack of plumage colour change between parasitized and unparasitized individuals in our focal blue-crowned manakin population could be explained by co-infections with different lineages of haemosporidian parasites in the individuals sampled. We found 5 individuals infected with multiple lineages of haemosporidian parasites. Parasites of the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon* present different degrees of pathogenicity and, in general, *Plasmodium* is considered the most virulent (Van Riper et al. 1994). Consequently, even among parasitized individuals, differences in specific lineage and infection by multiples lineages may differentially influence the effectiveness of the bird immune system. That could explain conflicting results on the relationship between feather colouration and parasites (Badás et al. 2018).

Furthermore, plumage in blue-crowned manakin males is mainly black with a blue crown, which is the result of melanin deposition in the feathers (Hill and McGraw 2006a). Unlike carotenoids, melanin-based colouration is under strong genetic control, can be synthesized endogenously by the organism (Borovansky et al. 2011) and then is weakly sensitive to the environment conditions such as food stress (resources) (McGraw et al. 2002) or parasitism (Roulin and Dijkstra 2003, Bize et al. 2006), but see Veiga and Puerta (1996) and Fargallo et al. (2007). In fact, the majority of studies found an association between parasites and bird plumage colouration in birds with carotenoid-based plumage colour (Dunn et al. 2009, Trigo and Mota 2016). Fewer costs seem to be associated with production of melanin than carotenoid pigments, although the costs of structural and melanin-based colouration are debated and recent studies suggest that synthesizing and depositing melanin in feathers could be more costly than previously thought (Roulin 2016). Nevertheless, melanin might be strongly linked to male competition and dominance maintenance (Senar and

Camerino 1998) and this could explain, for instance, the link between male colour and female visitation rates in the blue-crowned manakin (McGraw and Hill 2000).

The lack of correspondence between previous results indicating 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 other three non-mutually exclusive reasons: First, male individuals may carryover pigments in their plumage from a season before the infection event, and thus plumage colour could be describing a previous health history rather than the current history. Second, infections by haemosporidian parasites take between seven and fifteen days to affect the host (Valkiūnas 2005). Thus, it is possible that this disease first affects more labile characteristics such as singing and behaviour of the individuals prior to affecting morphological characters. In fact, symptoms of parasites infections in general tend to affect the behavior of the hosts almost immediately. In manakins, individuals may have only one complete molt per year (Duval 2005) and the physiological processes that govern feather pigmentation and molt timing are usually coincident (Voitkevich 1966). Therefore, recently infected host individuals may still exhibit their previous seasons plumage and changes in plumage colour would only be observed in the medium-to-long term. Third, we may have only captured male individuals in the lek who survived acute infection. These males may therefore have chronic infections, in which infected individuals remain infected but with low parasite level and thus 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 melanin, which is synthesized endogenously (Borovansky et al. 2011).

In summary, this study demonstrates that the conspicuousness of structural and melanin-based plumage colours of male blue-crowned manakin predict female visitation rates. However, haemosporidian parasite occurrence is not related to male structural and

melanin-based plumages, suggesting that this visual signal is not an honest indicator of the resistance to haemosporidian parasites. Therefore, we suggested that colours of the blue-crowned manakins may predict other aspects of male quality that were not assessed in this study, or perhaps other sexual selection models (e.g., sensory drive or Fisher's runaway sexual selection) best explain the mechanisms that may drive the evolution of the plumage colouration in this species. Further studies are recommended to uncover whether pigment-based (melanin- or carotenoid-based) and structural signals relate to occurrence of single parasite species or coinfection by different parasite lineages in blue-crowned manakin, to further understand the association of the diversity of avian malaria parasites on melanin based and structural colours.

DECLARATIONS

Acknowledgements – We thank N.S., M.M., P.P. and M.T. for field assistance and the Brazilian Biodiversity Research Programme (PPBio) for logistical support. We thank ICMBio and CEMAVE – SNA for providing banding permits and metal bands.

FINANCIAL SUPPORT

The field data collection was based upon research supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (UNIVERSAL number 471092/2012-6 Faixa C to M.A.), and by Fundação de Amparo à Pesquisa do Estado do Amazonas (PRONEX – proj. 003/2009, number 653/2009 to M.A. and A.L.). Haemosporidian laboratory work was funded by NSF DEB-1503804 to J.D.W. F.H. received a fellowship from CAPES and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). A.F. was supported by a postdoctoral fellowship (PNPD scholarship) from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

DATA AVAILABILITY STATEMENT

All sequences are deposited in MalAvi and Genbank (accession numbers MK029822-MK029864) and can be found in Bosholn et al. 2019).

APPENDICES (SUPPORTING INFORMATION)

Additional appendices (supplementary material) may be found in the expanded online version of this article.

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FIGURES

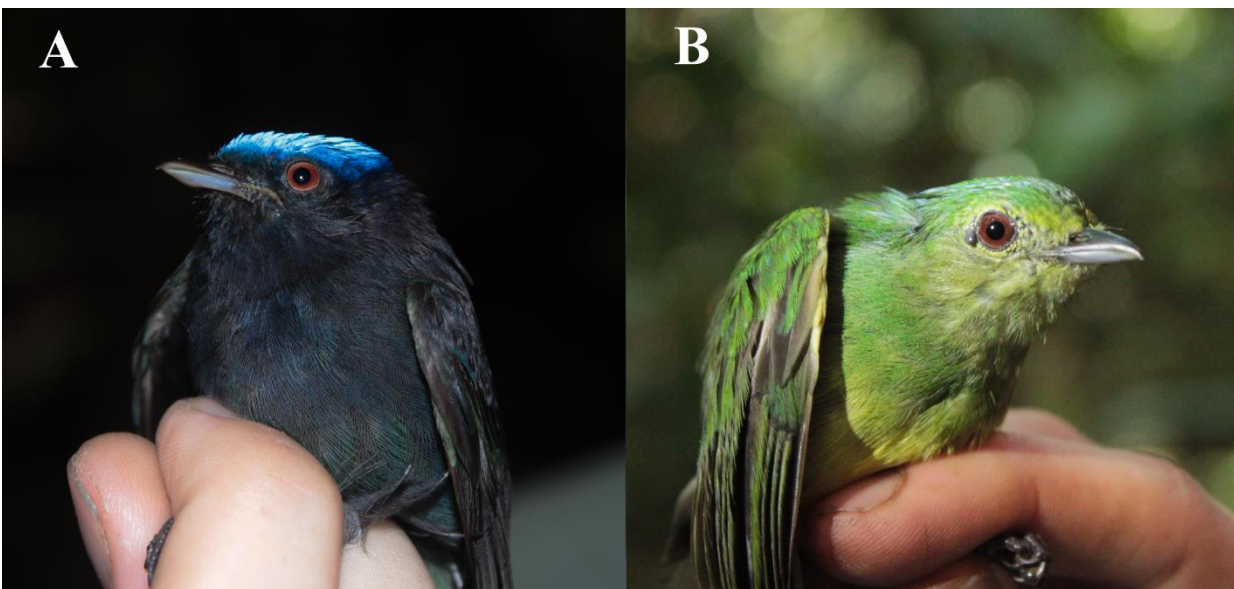


Figure 1: Blue-crowned manakin, *Lepidothrix coronata* (Aves: Pipridae). (A) Adult male individual. (B) Adult female individual. Pictures by Marina Maximiano.

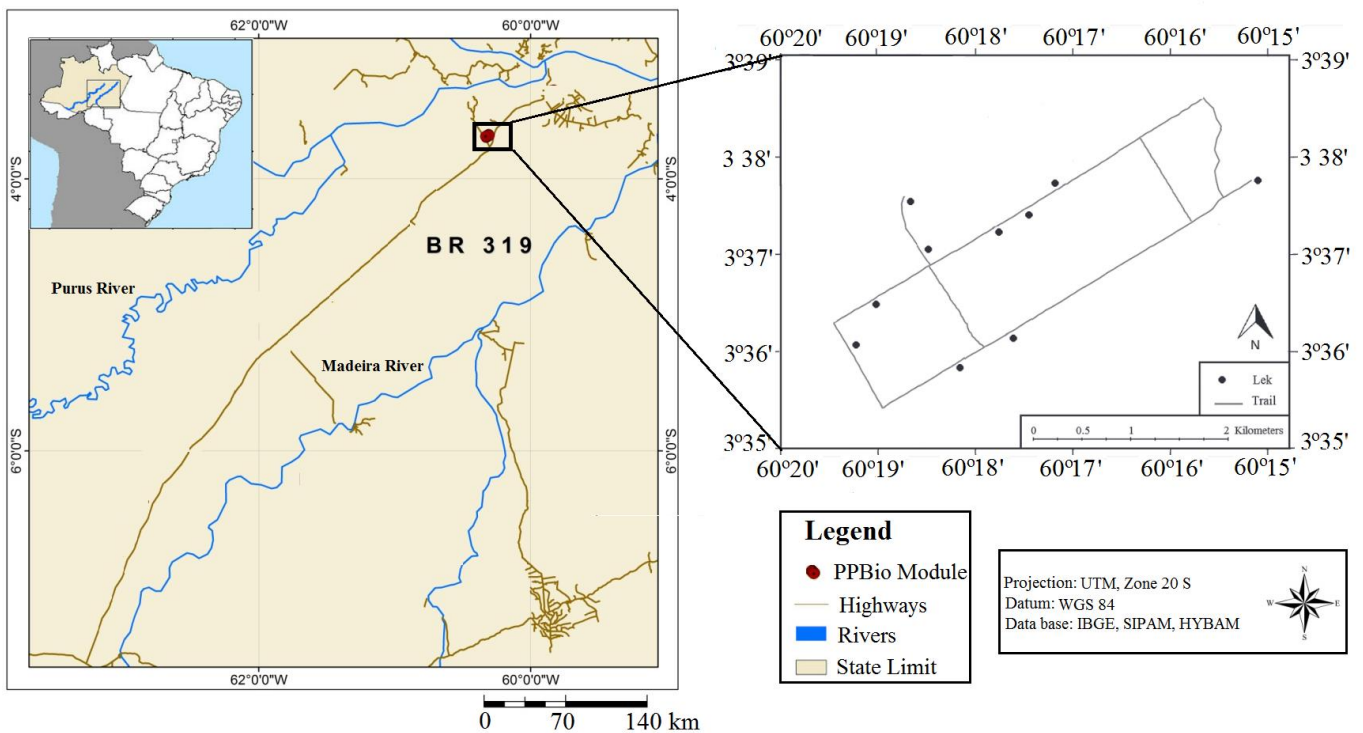


Figure 2: Map of the study area in the Purus–Madeira interfluvium (PPBio plot, red dot in the left panel) and schematic figure of the trail system (adapted from Bosholn et al. 2016) which shows sampled leks in the research plot ‘Manaquiri’, Careiro-Castanho municipality, Amazonas state, Brazil (right panel).

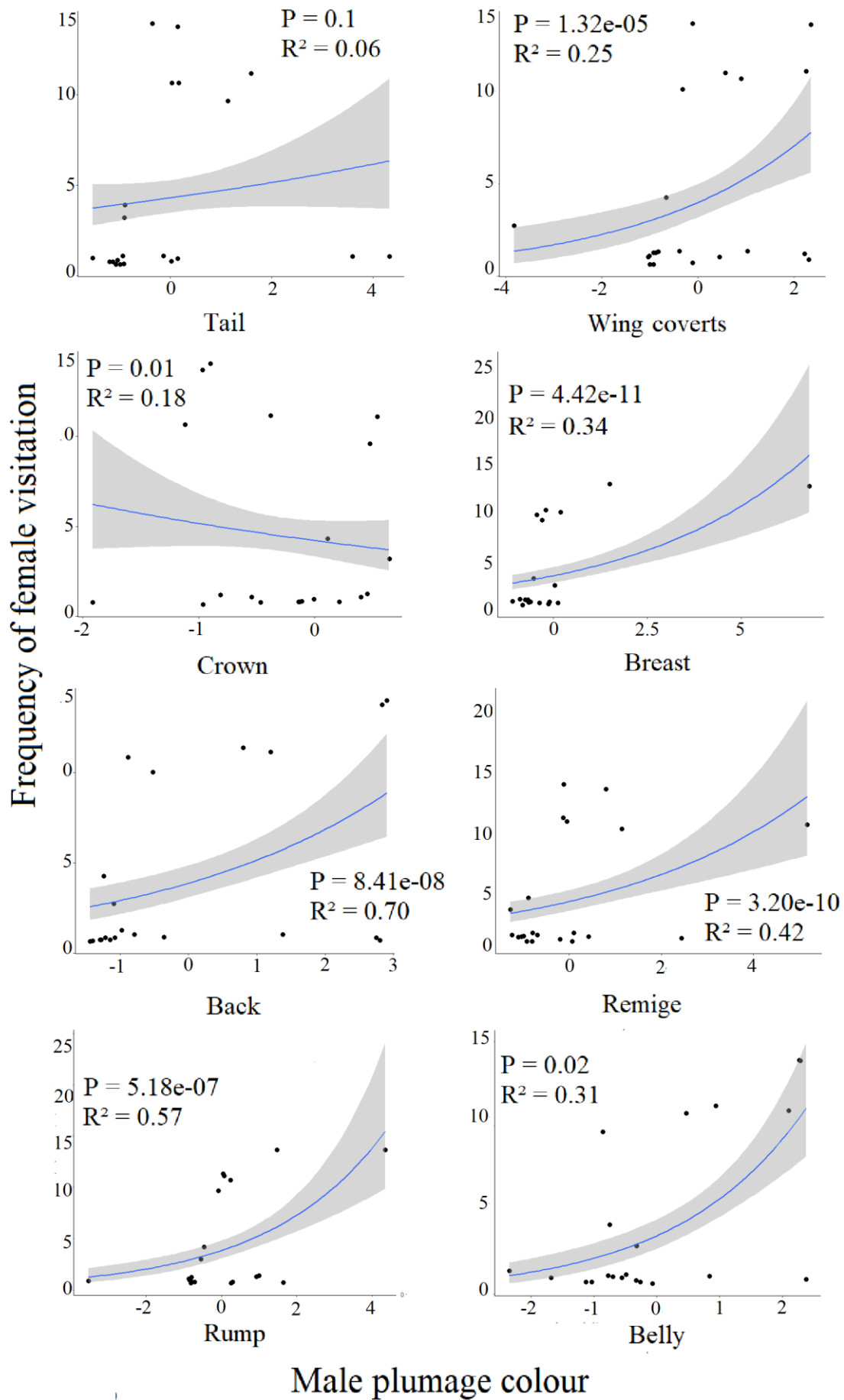


Figure 3: Relationship between frequency of female visitation and the explanatory variables included in the best models (PC1 scores of chromatic variables x,y,z).

TABLES

Table 1: Results of the cross-correlations of blue-crowned manakin males for each plumage patch analyzed. 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**

Table 2: Relationship of the occurrence of haemosporidian parasites on plumage colour of blue-crowned manakins.

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
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

Supporting Information

Plumage colour of male blue-crowned manakins predicts female visitation rates but does not signal avian malaria infection

TABLES

Table S1: Average estimations of conspicuousness (chroma data) of blue-crowned manakin males for each plumage patch analyzed. Maximum chroma = the maximum chroma of a pure hue; Achieved chroma = the proportion of maximum possible chroma for its hue (Stoddard and Prum 2008).

Plumage patch	Maximum chroma	Achieved chroma
Tail	0.32	0.03
Wing covert	0.45	0.59
crown	0.28	0.15
Back	0.44	0.57
breast	0.37	0.08
remige	0.37	0.36
rump	0.39	0.61
Belly	0.32	0.03

SÍNTESE

Nesta tese, investiguei variação em coloração em diferentes escalas (intersexual em espécies e intra-populacional em uma população do uirapuru-do-chapéu-azul, *Lepidothrix coronata* na Amazônia Central e os mecanismos envolvidos na diversificação desses fenótipos. No primeiro capítulo, as relações entre a intensidade de dicromatismo sexual em espécies sexualmente monocromáticas para a visão humana e a conspicuidade da coloração da plumagem foram averiguadas, controlando para as relações filogenéticas entre as espécies estudadas. No segundo e último capítulo, avaliou-se a associação entre a coloração da plumagem de machos de *L. coronata* e a frequência de fêmeas e também a relação entre a ocorrência de hemosporídeos e a coloração da plumagem em machos desta espécie.

No primeiro capítulo, foi observado dicromatismo sexual em todas as espécies estudadas com níveis de dicromatismo que variaram entre 1 e 9,25 jnd em 83,4% das manchas da plumagem. Foi observado ainda uma relação entre o dicromatismo sexual e a conspicuidade da plumagem (contraste intra-plumagem e contraste da plumagem em relação ao ambiente habitado por essas espécies). Ambos resultados evidenciam influência de seleção sexual na coloração da plumagem críptica para a visão humana.

No segundo e último capítulo foi observado que, dentre os 25 machos de uirapuru-do-chapéu-azul amostrados, 12 estavam infectados por protozoários hemosporídeos (prevalência = 50%). As linhagens mais comuns pertenciam ao gênero *Plasmodium* (prevalência ~ 47%), seguida do *Haemoproteus* (prevalência ~ 4%), e *Leucocytozoon* (prevalência ~ 2%), respectivamente. Observamos que a coloração da plumagem de machos de *L. coronata* previram a frequência de visita de fêmeas desta espécie indicando o papel da plumagem como sinal na atração de parceiros sexuais. Entretanto, nós também observamos que a coloração da plumagem de machos de *L. coronata* não foi associada com a ocorrência de hemosporídeos, o que sugere que esse sinal visual não seria um indicador honesto de infecção dos indivíduos por hemoparasitos causadores da malária aviária, embora possa sinalizar outros indicadores de qualidade não averiguados nessa tese.

As informações apresentadas na presente tese trazem aspectos inovadores sobre a associação entre a coloração da plumagem, ocorrência de parasitos hemosporídeos, e atributos relacionados a mecanismos seletivos geradores de diversidade fenotípica. Afinal, esse é o primeiro estudo a investigar esse tipo relação neste nível de dimorfismo sexual e ainda em uma espécie neotropical como modelo de estudo. Assim, esta tese evidencia a evolução da plumagem em diferentes escalas de variação (entre sexos de uma mesma espécie e entre

indivíduos de uma mesma população) e sugere que mecanismos de seleção sexual têm um papel importante na variação morfológica desses traços fenotípicos nestas espécies.

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