PROCEEDINGS B

royalsocietypublishing.org/journal/rspb

Research

Cite this article: Aguiar de Souza Penha V *et al*. 2022 Haemosporidian parasites and incubation period influence plumage coloration in tanagers (Passeriformes: Thraupidae). *Proc. R. Soc. B* **289**: 20221283. https://doi.org/10.1098/rspb.2022.1283

Received: 4 July 2022
Accepted: 27 October 2022

Subject Category:
Ecology

Subject Areas:
ecology

Keywords:
sexual dichromatism, sexual selection, female ornamentation, parasite prevalence, *Plasmodium*, *Parahaemoproteus*

Author for correspondence:
Victor Aguiar de Souza Penha
e-mail: victoraspenha@gmail.com

Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.6292019.

THE ROYAL SOCIETY PUBLISHING

Haemosporidian parasites and incubation period influence plumage coloration in tanagers (Passeriformes: Thraupidae)

Victor Aguiar de Souza Penha[1], Fabricius Maia Chaves Bicalho Domingos[2], Alan Fecchio[3], Jeffrey A. Bell[4], Jason D. Weckstein[5], Robert E. Ricklefs[6], Erika Martins Braga[7], Patrícia de Abreu Moreira[8], Letícia Soares[9], Steven Latta[10], Graziela Tolesano-Pascoli[11], Renata Duarte Alquezar[12], Kleber Del-Claro[13] and Lilian Tonelli Manica[2]

[1]Graduate Program in Ecology and Conservation, Federal University of Paraná, 81531-980, Curitiba, Paraná, Brazil
[2]Zoology Department, Federal University of Paraná, 81531-980, Curitiba, Paraná, Brazil
[3]Centro de Investigación Esquel de Montaña y Estepa Patagónica (CIEMEP), CONICET—Universidad Nacional de la Patagonia San Juan Bosco, U9200, Esquel, Chubut, Argentina
[4]Department of Biology, University of North Dakota, 58202-9019, Grand Forks, USA
[5]Academy of Natural Sciences of Drexel University and Department of Biodiversity, Earth, and Environmental Science, Drexel University, 19104, Philadelphia, PA, USA
[6]Department of Biology, University of Missouri–Saint Louis, Saint Louis, MO, USA
[7]Department of Parasitology, Federal University of Minas Gerais, 31270-901, Belo Horizonte, Minas Gerais, Brazil
[8]Federal University of Ouro Preto, 35400-000, Ouro Preto, Minas Gerais, Brazil
[9]Research Associate, National Aviary, Pittsburgh, PA, USA
[10]Conservation and Field Research, National Aviary, 15212, Pittsburgh, PA, USA
[11]Zoology Department, Institute of Biological Sciences, University of Brasilia, 70910-900, Brasilia, Distrito Federal, Brazil
[12]Animal Behavior Laboratory, Graduate Program in Ecology, University of Brasilia, 70910-900, Brasilia, Distrito Federal, Brazil
[13]Behavioral Ecology and Interactions Laboratory, Graduate Program in Ecology and Conservation of Natural Resources, Federal University of Uberlândia, 38405-240, Uberlândia, Minas Gerais, Brazil

VAdSP, 0000-0002-9036-3862; FMCBD, 0000-0003-2069-9317; AF, 0000-0002-7319-0234; JAB, 0000-0001-9146-4318; JDW, 0000-0001-7941-5724; RER, 0000-0001-7649-8800; EMB, 0000-0001-5550-7157; PdAM, 0000-0002-6020-449X; LS, 0000-0002-6933-8048; SL, 0000-0003-3789-9470; GT-P, 0000-0001-8219-191X; RDA, 0000-0001-8294-722X; KD-C, 0000-0001-8886-9568; LTM, 0000-0001-6005-7103

Birds are highly visually oriented and use plumage coloration as an important signalling trait in social communication. Hence, males and females may have different patterns of plumage coloration, a phenomenon known as sexual dichromatism. Because males tend to have more complex plumages, sexual dichromatism is usually attributed to female choice. However, plumage coloration is partly condition-dependent; therefore, other selective pressures affecting individuals' success may also drive the evolution of this trait. Here, we used tanagers as model organisms to study the relationships between dichromatism and plumage coloration complexity in tanagers with parasitism by haemosporidians, investment in reproduction and life-history traits. We screened blood samples from 2849 individual birds belonging to 52 tanager species to detect haemosporidian parasites. We used publicly available data for plumage coloration, bird phylogeny and life-history traits to run phylogenetic generalized least-square models of plumage dichromatism and complexity in male and female tanagers. We found that plumage dichromatism was more pronounced in bird species with a higher prevalence of haemosporidian parasites. Lastly, high plumage coloration complexity in female tanagers was associated with a longer incubation period. Our results indicate an association between haemosporidian parasites and plumage coloration suggesting that parasites impact mechanisms of sexual selection, increasing

differences between the sexes, and social (non-sexual) selection, driving females to develop more complex coloration.

1. Introduction

Plumage coloration is an important signalling trait in birds, because they are highly visually oriented organisms [1]. Males usually exhibit different patterns of plumage coloration in comparison to females [2], a phenomenon known as sexual dichromatism, indicating that sexual selection may be an important force generating coloration differences in bird species [3]. According to the female choice mechanism of sexual selection, high-quality males are better able to compete against other males and to attract mates [4]. In house finches (*Haemorhous mexicanus*), for example, more brightly coloured males initiated reproduction earlier and produced more offspring [5], thus impacting their reproductive success [6].

Sexual dichromatism may also evolve in response to the intensity of sexual selection, which may depend upon parameters such as body size and life-history traits. In birds, sexual dichromatism is inversely associated with body size, such that sexual selection appears to be stronger in smaller species [7]. This is the case in parrots, for example, and is likely due to shorter pair bond duration and increased mate turnover throughout their lifespan [8]. Therefore, larger species tend to be monochromatic, with both sexes displaying either conspicuous or dull coloration [7]. Life-history traits are important predictors of plumage coloration because they reflect patterns of survival and reproduction, and thus, individuals' ability to attract mates. For instance, investment in reproduction may influence plumage coloration in birds as species incubating for longer periods or incubating more eggs may face a trade-off between colour investment and reproductive output [9,10]. In *Carduelis* finches, a clade comprising 125 different species, melanin plumage complexity increases with decreasing clutch size and incubation period [11].

Perhaps one of the strongest hypotheses that may explain plumage coloration diversity in avian species is related to parasitism. In this case, plumage coloration is an honest signal because it is suggestive of genetic resistance to parasites and may signal increased reproductive ability [12]. Pigments, such as carotenoids, are also immune stimulators, meaning that overuse of carotenoids in plumage coloration may compromise other physiological functions associated with the immune system [13]. Therefore, carotenoid deposition may be an honest signal for parasite resistance (but not pigments producing structural coloration; see [14]). Intensely parasitized individuals usually have dull plumage because of (i) an energetic imbalance between investing in plumage coloration and mounting an immune response against parasites [15] or (ii) direct damage to feathers by parasites [16]. Also, parasites that do not directly reduce circulating carotenoids may depress the utilization of this pigment [15]. For example, plumage coloration saturation, brightness and carotenoid chroma were associated with haemosporidian parasite occurrence and prevalence in different passerine species, and differences between parasitized and non-parasitized individuals are greater in sexually dimorphic species [17–19]. Also, a positive association between infection by haemosporidian parasites and dichromatism was found in waterfowl [20], several suboscine passerines [21] and birds in general ([22]; but see [23]; [24,25]). These studies provide support for the hypothesis that species under stronger sexual selection are also more generally burdened by parasites. The authors argue that secondary traits involved in attracting mates may be energetically costly to individuals, and only those individual males that are parasite resistant may be more likely chosen by females [26]. Furthermore, genes related to immunity and feather pigmentation have been shown to be under similar selective pressures in birds, suggesting that resistance to parasites may be a key factor in female choice [27]. Haemosporidians (Order Haemosporida, genera *Plasmodium* and *Parahaemoproteus*) are vector-borne protozoans that infect avian blood cells and other tissues for reproduction. Since they are distributed worldwide and parasitize almost all avian families [28] with different degrees of dichromatism, these malarial pathogens provide an ideal study system to understand the relationships between avian host coloration and pathogen prevalence.

Tanagers (Passeriformes: Thraupidae) are songbirds with diverse life-history traits and elaborate secondary sexual characteristics, notably plumage coloration and song complexity. Within this large avian family, sexual traits have been the focus of important macroevolutionary studies (e.g. [29–31]). Since tanagers are hosts to several haemosporidian parasites [32–35], they provide an interesting opportunity for studying the relationship between plumage coloration and parasitism in birds. Also, dichromatism is widespread in the family Thraupidae, occurring at some level in most of the species [36], and both sexes have complex plumage coloration, although it is greater in males [30]. In a study of dichromatism, which analysed 351 species of tanagers, Shultz & Burns [30] found that dichromatism was more influenced by evolutionary changes in males than in females [30]. The importance of life-history traits on the evolution of plumage coloration in tanagers is also dependent on the light environment, with species showing brighter plumage in open rather than closed habitats [30]. The impact of haemosporidian infections on the dichromatism and coloration complexity in tanager species remains unknown. Since dichromatism is a good proxy for the strength of sexual selection, understanding the impact of parasitism on this trait will provide a better understanding of how parasites are involved in the decision-making process during mate choice. Here we aim to unravel this host–parasite relationship as well as to understand how avian life-history traits influence the evolution of plumage coloration in tanagers. Specifically, we tested whether dichromatism and plumage complexity were negatively related to haemosporidian parasite prevalence (proportion of infected individuals) and lineage richness (number of different parasite lineages, weighted by total number of screened individuals), species clutch size, incubation period and body length. We expect that highly parasitized species (higher prevalence and / or parasite lineage richness) would be more dichromatic, smaller in size and invest less in reproduction (lower clutch size and incubation period).

2. Material and methods

(a) Data collection

We screened 2849 individuals from 52 Thraupidae species collected between 2007 and 2018. Since we did not have data to

distinguish males from females and adults from juveniles for most individuals at capture, we did not have data on the differences in infections between males and females. Therefore, we used overall parasitism of each species and then relate those to coloration metrics from published data. Each species included in the study was represented by at least five captured individuals (sample size ranging from 5 to 591 individuals per species; electronic supplementary material, table S1). Samples included in the study came from eight countries and 92 locations, including Argentina [32,37], Brazil [19,34,35,38–41], Dominican Republic [42,43], Ecuador [44], Honduras, Mexico [33], Nicaragua and Peru [32]. We either banded all individuals and extracted a blood sample or collected blood samples from host specimens. We then screened these individual samples for the presence of haemosporidian parasites. All fieldwork followed each country's data collection laws, under specific licences (see ethics section at end of this paper). Some individual host specimens and their associated blood and/or tissue samples were collected and deposited in museum collections including Instituto Nacional de Pesquisas Amazônicas, Museu Paraense Emílio Goeldi, Field Museum of Natural History, Museo de Zoología Alfonso L. Herrera and the Academy of Natural Science of Drexel University.

(b) Haemosporidian lineage identification

We extracted DNA following the protocols described by [23,45], or by using the Qiagen DNeasy 96 Blood and Tissue kit (Qiagen, Valencia, CA). We screened DNA samples for the presence of *Parahaemoproteus* or *Plasmodium*. Specific molecular protocols can be found in [19,35,37,38,41–44,46]. Briefly, we amplified a standard barcoding region from the cytochrome *b* gene of haemosporidian parasites using nested PCR and then sanger-sequenced samples with positive amplifications. We used BIOEDIT v. 7.2.0 [47] to align sequences and conduct a local blast for comparison with the MalAvi database [48] to identify haemosporidian genetic lineages found in our samples. As our two different PCR protocols amplified two different regions of the cytochrome *b* gene, we compared longer mtDNA fragments [23,37,43] through a local BLAST on the MalAvi database [48]. We only categorized identities to the species level when if the sanger sequences were 100% identical to the MalAvi lineage from a given fragment, which was the case for all the lineages we found. To account for uneven sampling, we calculated 'lineage richness' of a host species as the total number of host lineages found each divided by the total number of screened individuals per host species. We accounted for a difference between haemosporidian parasite prevalence and the parasite lineage richness, in the sense that different lineages may differ in virulence within hosts [49], and that a greater parasite lineage richness may impose a greater burden to avian hosts [50–52]. Finally, we calculated haemosporidian (*Plasmodium* and *Parahaemoproteus*) parasite prevalence as the number of infected host individuals divided by the total number of screened individuals for every host species. Also, here we are treating *Parahaemoproteus* as a distinct genus from *Haemoproteus*, following recent discoveries and advancements in the haemosporidian parasite phylogeny [53–55].

(c) Host phylogeny and life-history traits

We used the tanager phylogeny from [56] and the *drop.tip* function from the *ape* package 5.0 [57] in R to prune the tree to the 52 species from our host screening database. We used the Handbook of the Birds of the World Alive (https://www.hbw.com; [58] to extract body length, incubation period (in days), and clutch size (number of laid eggs) for all tanager species.

(d) Museum data collection and plumage coloration data

To assess male and female plumage coloration complexity and dichromatism for the tanager species in our dataset we used data from Shultz & Burns [30], who generated data from museum specimens. Shultz & Burns [30] used spectrophotometric measurements to generate a reflectance tetrahedral colour space [59]. They also produced data on maximum, average and variance of colour span (the Euclidian distance among points inside the tetrahedron), colour volume (total volume from the polygon connecting all points in the tetrahedron), maximum, average and hue disparity (differences in angles from the vectors within the tetrahedron), average chroma (average distance between achromatic centre and a data point inside the tetrahedron for all members of a given species) and average brilliance (average reflectance). All of these variables were referred to as whole-plumage tetrahedral colour space (WPTCS) measurements. We used the average whole-plumage colour span as our dichromatism measurement (dichromatism from herein). Higher values of dichromatism (i.e. larger distances among points in the tetrahedron) mean that males and females have large differences in their plumage coloration patterns. We used Shultz & Burn's [30] PC1 axis from a principal component analysis including all WPTCS measurements for males and females as our plumage complexity measurement per sex (male and female plumage complexity from herein). Positive PC1 values (with reversed sign to facilitate interpretation) indicate higher values of all WPTCS measurements, suggesting more complex plumage, higher contrast among plumage sites, and larger regions of variation in WPTCS measurements [30]. In our dataset, dichromatism ranged from 0.016 to 0.406, whereas Male PC1 ranged from −6.368 (low complexity) to 5.982 (high complexity) and Female PC1 ranged from −4.506 (low complexity) to 6.574 (high complexity).

(e) Statistical analysis

We corrected the explanatory and response variables using the *normalize* function from *BBmisc* package v. 1.12 [60] in R, to normalize the distributions whenever necessary, and to scale all numeric variables using the *scale* function to keep all variables comparable. To test whether sexual dichromatism is related to haemosporidian parasitism and avian life-history traits, we built a phylogenetic generalized least-square (PGLS) model including dichromatism as response variable, and parasite lineage richness, haemosporidian parasite prevalence, clutch size, incubation period and body length as explanatory variables. We also built two separated PGLS models, one for each sex, to test the relationship between plumage complexity, parasitism (haemosporidian parasite prevalence and parasite lineage richness), clutch size, incubation period and cand body length.

We tested for the absence of multi-collinearity with the variance inflation factor (VIF), using the *VIF* function from the *regclass* package v. 1.6 [61] for all models. We used a conservative threshold of two for $\mathrm{GVIF}^{(1/(2*df)}$ to collinear predictors. We built both Ornstein–Uhlenbeck and Brownian Motion PGLS models and used Akaike information criterion values to test for model fit. We used an information-theoretic approach [62] to test the importance of the explanatory variables and the *dredge* function from the *MuMIn* package v. 1.46 [63] to generate all possible models with the explanatory variables. We used model averaging with the *model.avg* function from the *MuMIn* package to calculate the model-averaged estimates [64] whenever the best model had a weight less than 0.8. We selected the most important explanatory variables by assessing the estimate, conditional s.e. and 95% confidence interval (CI). All analysis were performed in R software version 2019 [65].

Table 1. Model-averaged estimates, s.e. and 95% confidence intervals of variables in the model using the dichromatism, female and male plumage complexities. Significant variables are marked with asterisks.

variables	estimate	s.e.	95% C.I.
dichromatism			
intercept[a]	0.33	0.09	0.13, 0.52*
haemosporidian parasite prevalence	0.07	0.02	0.01, 0.13*
lineage richness	−0.04	0.18	−0.40, 0.31
clutch size (three eggs)[a]	−0.08	0.06	−0.21, 0.04
cutch size (four eggs)[a]	−0.10	0.05	−0.22, 0.00
incubation period	−0.02	0.01	−0.04, −0.00*
body length	−0.03	0.03	−0.10, 0.02
male plumage complexity			
intercept[a]	0.45	0.08	0.28, 0.61*
haemosporidian parasite prevalence	0.06	0.03	−0.00, 0.13
lineage richness	0.13	0.24	−0.35, 0.61
clutch size (three eggs)[a]	−0.11	0.06	−0.24, 0.02
clutch size (four eggs)[a]	−0.14	0.12	−0.40, 0.11
incubation period	0.01	0.03	−0.05, 0.07
body length	−0.01	0.03	−0.08, 0.05
female plumage complexity			
intercept[a]	0.41	0.07	0.27, 0.55*
haemosporidian parasite prevalence	0.01	0.03	−0.04, 0.07
lineage richness	0.08	0.19	−0.38, 0.39
clutch size (three eggs)[a]	−0.02	0.06	−0.15, 0.10
clutch size (four eggs)[a]	−0.03	0.09	−0.23, 0.16
incubation period	0.06	0.02	0.01, 0.11*
body length	0.01	0.03	−0.05, 0.08

[a]Reference factor for clutch size was two eggs.

3. Results

In our sampled tanager species ($n = 52$), mean body size was 14.68 ± 2.94 cm, mode clutch size was two eggs ($n = 30$ species) and mean incubation period was 13.40 ± 1.06 days. We found 149 haemosporidian lineages, including 62 *Parahaemoproteus* and 87 *Plasmodium* lineages, in 1063 infected individual tanagers, which is 37% overall haemosporidian prevalence.

(a) Plumage dichromatism

We found that more dichromatic tanager species had higher haemosporidian parasite prevalence and shorter incubation periods (table 1; electronic supplementary material, table S2; figure 1). Parasite lineage richness, clutch size and body size were not significantly important variables in this model (table 1; electronic supplementary material, table S2).

(b) Male and female plumage complexities

The best models of female and male plumage coloration are included in electronic supplementary material, table S2. We found that more complex female plumages were associated with species having longer incubation periods (table 1; electronic supplementary material, table S2; figure 2). No variables explained male plumage complexity (table 1; electronic supplementary material, table S2).

4. Discussion

Here we provide results from a broad study of haemosporidian parasites from tanager species sampled from 92 locations throughout the family's geographical distribution. Using parasite prevalence and identified parasite lineages, we built phylogenetic models to test the relationship between parasite prevalence and lineage richness with plumage dichromatism and coloration complexity. Our main result suggests that parasite-mediated sexual selection may influence plumage dichromatism in tanagers. In brief, more dichromatic species had higher parasite prevalence and overall shorter incubation periods. We also found that highly complex female plumages were associated with longer incubation periods.

As dichromatism was positively related to higher haemosporidian parasite prevalence, in accordance with our predictions, it is suggestive that host species with a higher proportion of infected individuals appear to be under stronger sexual selection mediated by parasites [12]. These results may imply that individuals (usually males) that are able to

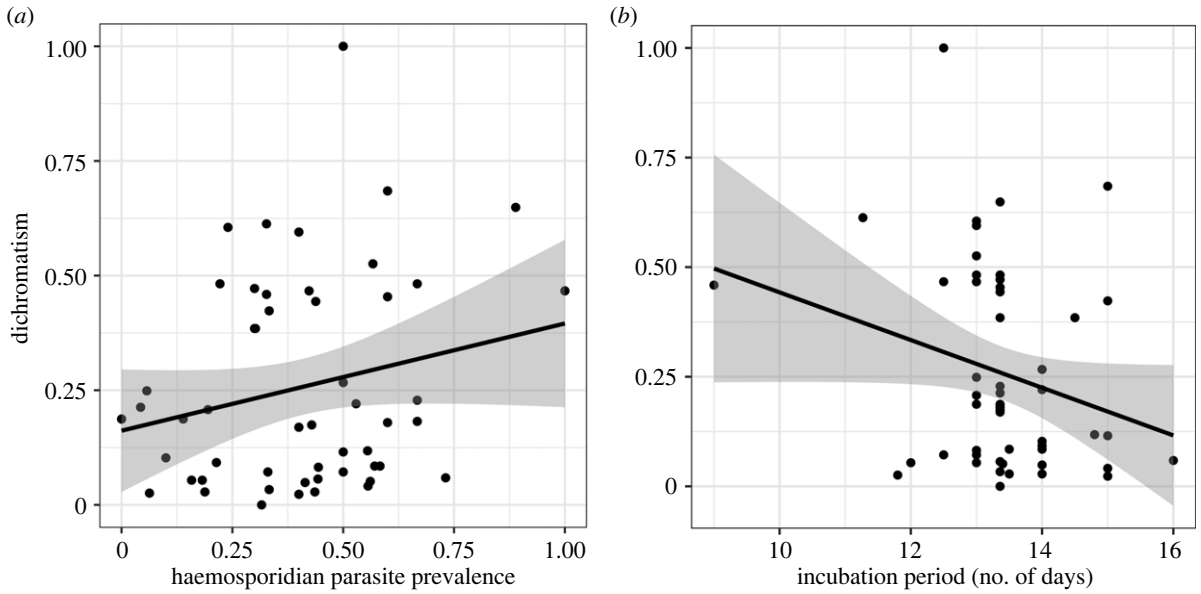

Figure 1. Dichromatism (average whole-plumage colour span) in relation to (*a*) haemosporidian parasite prevalence (number of infected individuals divided by the total number of screened individuals) and (*b*) incubation period.

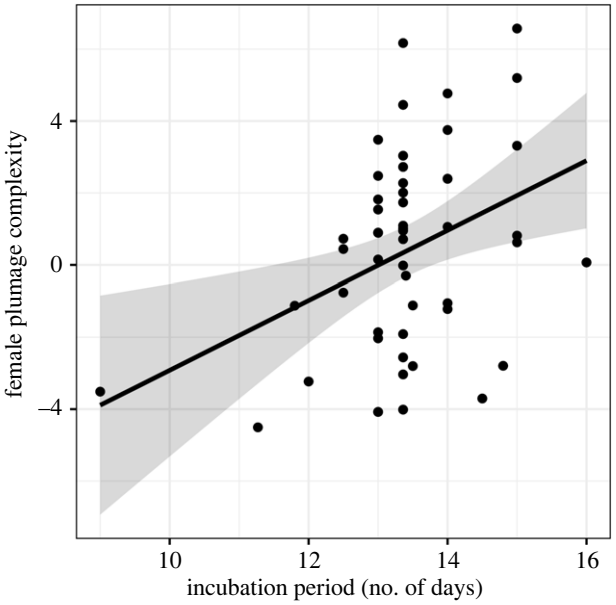

Figure 2. Female plumage coloration complexity (PC1 values from a principal component analysis of the WPTCS measurements) in relation to incubation period (number of days).

invest in plumage coloration without compromising other physiological functions, such as immunity [12], may be more likely chosen (usually by females) for mating, resulting in the evolution of more pronounced dichromatism in these species. Similar results have been found for rodents [66] and salamanders [67], suggesting that the relationship between dichromatism and parasites may be widespread in animals. Furthermore, Jaiswal *et al*. [27] found an evolutionary correlation between immune and feather pigmentation genes for 11 non-passerine species, which supports the Hamilton & Zuk [12] hypothesis of parasite-mediated sexual selection. Also, cell-mediated immunity from better supplemented females may pass onto nestlings, as stated by the transgenerational epigenetics hypothesis [68]. Thus, along with Jaiswal *et al*. [27] and Krüger *et al*. [68], our results indicate that female tanagers select highly conspicuous males as plumage serves as an honest signal for greater immune defenses against haemosporidian parasites, which may have important associations with improved immunity for nestlings. A focus for future studies is the relationship among gene expression, dichromatism and parasites in tanagers, which will help elucidate both the impacts of sexual selection on genes related to immunity and the overall relationship between immune function and feather pigmentation. Nevertheless, we found an association between sexual selection and infection by haemosporidian parasites. Hence, our results could potentially help in identifying disease reservoirs in the wild, since dichromatic species tend to be more parasitized than monochromatic species.

We also found that species incubating for a shorter period were more dichromatic. Our result suggests that individuals investing in plumage coloration may face a trade-off between ornamentation and incubation. Therefore, we propose two non-mutually exclusive hypothesis. (i) Natural selection may exceed sexual selection pressure in species with longer incubation periods due to increased risk of nest predation. Also, in these species, nest concealment and reduced nest attentiveness should reduce the likelihood of predator detection. In support of this hypothesis, Drury & Burroughs [69] found more evolutionary transitions from exposed to concealed nests in dichromatic icterids, compared to monomorphic species. However, support for this hypothesis is not universal with incubation influencing dichromatism in icterids [69] and, an independent association between incubation and dichromatism among 69 passerine species [70]. (ii) We also propose that individuals' large investments in plumage coloration may deplete resources for parental care, especially in systems under high sexual selective pressures [71]. It is already known that some species such as parrots (Order Psittaciformes) invest more in incubation length and have higher immunity [72], and thus these species may direct resources to nest concealment [73] and clutch size [74] in detriment to plumage coloration, as also demonstrated by our results. Nevertheless, we suggest that future studies explore the connections between nest shape, nest attentiveness, immunity, parasitism and dichromatism in birds, to better comprehend these relationships.

Finally, in addition to less dichromatism, we found that female but not male plumage coloration complexity was related to the length of the incubation period. This was contrary to our expectations, since we expected that females incubating for a longer period would have decreased resources available to produce more conspicuous coloration. Furthermore, females are solely responsible for incubation in the majority of passerine species, with some males also contributing to parental care [70,75]. However, our results are intriguing because they support the hypotheses that female traits may be under selective pressure due to social competition for non-sexual resources, such as territory, food or nesting sites [76]. For example, females with a larger white wing patch in pied flycatchers (*Ficedula hypoleuca*) [77] and with a more conspicuous rump in common kestrels (*Falco tinnunculus*) [9] had a higher competitive ability, compared to other females. Therefore, female tanagers with a higher competitive ability may be more likely to invest both in reproduction (longer incubation) and plumage coloration (higher plumage coloration complexity).

Tanagers with their diversity of life-history traits, and coloration are an ideal system for understanding the complex interplay between sexually selected traits and parasitism. Our results demonstrate that within such a diverse group, sexual selection has produced higher trait variability in those species under higher risk of infection. Additional work is warranted to determine whether these traits serve as honest signals of immune response to parasitism.

Ethics. All fieldwork followed each country's data collection laws, under specific licences, as follows. Ethics Committee in Animal Experimentation from Universidade Federal de Minas Gerais, Brazil: 254/2011; Universidade de Brasília, Brazil: 129022/2015; Instituto Chico Mendes de Conservação da Biodiversidade: 42578, 3964-7, 33206-1; Centro Nacional de Pesquisa e Conservação de Aves—CEMAVE: 3856, 3239. Our procedures followed American Ornithologist's Union and University Animal Care and Use Committee guidelines.

Data accessibility. Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.1g1jwsv0d [78].

Supplementary material is available online [79].

Authors' contributions. V.A.S.P.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing—original draft and writing—review and editing; F.M.C.B.D.: formal analysis, methodology, supervision and validation; A.F.: data curation, formal analysis, supervision, writing—original draft and writing—review and editing; J.A.B.: data curation, formal analysis, validation, writing—original draft and writing—review and editing; J.D.W.: data curation, formal analysis, validation, writing—original draft and writing—review and editing; R.E.R.: data curation, formal analysis, validation, writing—original draft and writing—review and editing; E.M.B.: data curation, formal analysis, validation, writing—original draft and writing—review and editing; P.A.M.: data curation, formal analysis, validation, writing—original draft and writing—review and editing; L.S.: data curation, formal analysis, validation, writing—original draft and writing—review and editing; S.L.: data curation, formal analysis, validation, writing—original draft and writing—review and editing; G.T.-P.: data curation, formal analysis and validation; R.D.A.: data curation, formal analysis and validation; K.D.-C.: data curation and validation; L.T.M.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing—original draft and writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. The authors declare no competing interests.

Funding. This study was funded in part by the U.S. National Science Foundation (DEB-1503804) to J.D.W. V.A.P. thanks Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the scholarship provided during the study. K.D.C. and E.M.B. thanks Conselho Nacional de Ciência e Tecnologia (CNPq). R.E.R. thanks U.S. National Science Foundation and the National Geographic Society.

Acknowledgements. We thank the Academy of Natural Sciences of Drexel University; Yale Peabody Museum of Natural History; Dr Regina Macedo from Universidade de Brasília; Dr Diego Gill from the Spanish National Research Council; and Daniela de Angeli Dutra for the help during lab analysis.

References

1. Espmark YO, Amundsen T, Rosenqvist G. 2000 *Animal signals: signalling and signal design in animal communication*. Trondheim, Norway: Tapir Academic Press.
2. Dale J, Dey CJ, Delhey K, Kempenaers B, Valcu M. 2015 The effects of life history and sexual selection on male and female plumage colouration. *Nature* **527**, 367–370. (doi:10.1038/nature15509)
3. Hill GE, McGraw KJ. 2006 *Bird coloration, volume 1: mechanisms and measurements*. Cambridge, MA: Harvard University Press.
4. Darwin C. 1871 *The descent of man, and selection in relation to sex*. London, UK: John Murray.
5. McGraw KJ, Stoehr AM, Nolan PM, Hill GE. 2001 Plumage redness predicts breeding onset and reproductive success in the House Finch: a validation of Darwin's theory. *J. Avian Biol.* **32**, 90–94. (doi:10.1034/j.1600-048X.2001.320114.x)
6. Siefferman L, Hill GE. 2005 Evidence for sexual selection on structural plumage coloration in female Eastern Bluebirds (*Sialia sialis*). *Evolution* **59**, 1819–1828.
7. Carballo L, Delhey K, Valcu M, Kempenaers B. 2020 Body size and climate as predictors of plumage colouration and sexual dichromatism in parrots. *J. Evol. Biol.* **33**, 1543–1557. (doi:10.1111/jeb.13690)
8. Toft CA, Wright TF. 2015 *Parrots of the wild: a natural history of the world's most captivating birds*. Berkeley, CA: University of California Press.
9. Morrison A, Flood NJ, Reudink MW. 2014 Reproductive correlates of plumage coloration of female Mountain Bluebirds. *J. Field Ornithol.* **85**, 168–179. (doi:10.1111/jofo.12058)
10. Hasegawa M, Arai E. 2016 Long incubation off-bouts of females paired with colorful males in Barn Swallows (*Hirundo rustica*). *Wilson J. Ornithol.* **128**, 86–96. (doi:10.1676/1559-4491-128.1.86)
11. Bókony V, Liker A. 2005 Melanin-based black plumage coloration is related to reproductive investment in cardueline finches. *Condor* **107**, 775–787. (doi:10.1093/condor/107.4.775)
12. Hamilton WD, Zuk M. 1982 Heritable true fitness and bright birds: a role for parasites? *Science (1979)* **218**, 384–387.
13. Hill GE. 1999 Is there an immunological cost to carotenoid-based ornamental coloration? *Am. Nat.* **154**, 589–595. (doi:10.1086/30326)
14. White TE. 2020 Structural colours reflect individual quality: a meta-analysis. *Biol. Lett.* **16**, 20200001. (doi:10.1098/rsbl.2020.0001)
15. Hill GE, Farmer KL, Beck ML. 2004 The effect of mycoplasmosis on carotenoid plumage coloration in male house finches. *J. Exp. Biol.* **207**, 2095–2099. (doi:10.1242/jeb.00998)
16. al Rubaiee Z, al Murayati H, Nielsen JT, Møller AP. 2017 Fungi, feather damage, and risk of predation. *Ecol. Evol.* **7**, 10 797–10 803. (doi:10.1002/ece3.3582)
17. Figuerola J, Muñoz E, Gutiérrez R, Ferrer D. 1999 Blood parasites, leucocytes and plumage brightness in the Cirl Bunting, *Emberiza cirlus*. *Funct. Biol.* **13**, 594–601.
18. Scheuerlein A, Ricklefs RE. 2004 Prevalence of blood parasites in European passeriform birds. *Proc. R. Soc. B* **271**, 1363–1370. (doi:10.1098/rspb.2004.2726)
19. Penha VAS, Rodrigues R, Quaglia AI, Hoepers PG, Del-Claro K, Soares L. 2020 Plumage coloration

predicts haemosporidian infection occurrence in birds plumage coloration predicts haemosporidian infection occurrence in birds. *Ardea* **108**, 1–10. (doi:10.5253/arde.v108i1.a11)

20. Scott DK, Clutton-Brock TH. 1990 Mating systems, parasites and plumage dimorphism in mating systems, parasites and plumage dimorphism in waterfowl. *Behav. Ecol. Sociobiol.* **26**, 261–273.
21. Svensson-Coelho M, Blake JGJG, Loiselle BA, Penrose AS, Parker PG, Ricklefs RE. 2013 Diversity, prevalence, and host specificity of avian plasmodium and haemoproteus in a Western Amazon assemblage. *Ornithol. Monogr.* **76**, 1–47. (doi:10.1525/om.2013.76.1.1)
22. Gupta P, Vishnudas CK, Robin VV, Dharmarajan G. 2020 Host phylogeny matters: examining sources of variation in infection risk by blood parasites across a tropical montane bird community in India. *Parasit. Vectors* **13**, 1–13. (doi:10.1186/s13071-020-04404-8)
23. Ricklefs RE, Swanson BL, Fallon SM, Martínez-Abraín A, Scheuerlein A, Gray J, Latta SC. 2005 Community relationships of avian malaria parasites. *Ecol. Monogr.* **75**, 543–559. (doi:10.1890/04-1820)
24. Garamszegi LZ, Møller AP. 2012 The interspecific relationship between prevalence of blood parasites and sexual traits in birds when considering recent methodological advancements. *Behav. Ecol. Sociobiol.* **66**, 107–119. (doi:10.1007/s00265-011-1259-2)
25. Matthews AE, Ellis VA, Hanson AA, Roberts JR, Ricklefs RE, Collins MD. 2016 Avian haemosporidian prevalence and its relationship to host life histories in eastern Tennessee. *J. Ornithol.* **157**, 533–548. (doi:10.1007/s10336-015-1298-y)
26. Read AF. 1991 Passerine polygyny: a role for parasites? *Am. Nat.* **138**, 434–459.
27. Jaiswal SK, Gupta A, Shafer ABA, PK VP, Vijay N, Sharma VK. 2021 Genomic insights into the molecular basis of sexual selection in birds. *Front. Ecol. Evol.* **9**, 1–17. (doi:10.3389/fevo.2021.538498)
28. Valkiunas G. 2005 *Avian malaria parasites and other haemosporidia*. Boca Raton, FL: CRC Press.
29. Mason NA, Shultz AJ, Burns KJ. 2014 Elaborate visual and acoustic signals evolve independently in a large, phenotypically diverse radiation of songbirds. *Proc. R. Soc. B* **281**, 1–9. (doi:10.1098/rspb.2014.0967)
30. Shultz AJ, Burns KJ. 2017 The role of sexual and natural selection in shaping patterns of sexual dichromatism in the largest family of songbirds (Aves: Thraupidae). *Evolution* **71**, 1061–1074. (doi:10.1111/evo.13196)
31. Drury J, Burroughs N. 2016 Nest shape explains variation in sexual dichromatism in New World blackbirds. *J. Avian Biol.* **47**, 312–320.
32. Fecchio A *et al.* 2019 Avian host composition, local speciation and dispersal drive the regional assembly of avian malaria parasites in South American birds. *Mol. Ecol.* **28**, 2681–2693. (doi:10.1111/mec.15094)
33. Fecchio A, Collins MD, Bell JA, García-Trejo EA, Sánchez-González LA, Dispoto JH, Rice NH, Weckstein JD. 2019*b* Bird tissues from museum collections are reliable for assessing avian haemosporidian diversity. *J. Parasitol.* **105**, 446–453. (doi:10.1645/18-130)
34. Fecchio A *et al.* 2021 Higher infection probability of haemosporidian parasites in Blue-black Grassquits (*Volatinia jacarina*) inhabiting native vegetation across Brazil. *Parasitol. Int.* **80**, 102204. (doi:10.1016/j.parint.2020.102204)
35. Rodrigues RA, Massara RL, Bailey LL, Pichorim M, Moreira PA, Braga ÉM. 2020 Using a multistate occupancy approach to determine molecular diagnostic accuracy and factors affecting avian haemosporidian infections. *Sci. Rep.* **10**, 1–10. (doi:10.1038/s41598-020-65523-x)
36. Burns K, Shultz A. 2012 Widespread cryptic dichromatism and ultraviolet reflectance in the largest radiation of Neotropical songbirds: implications of accounting for avian vision in the study of plumage evolution. *Auk* **129**, 211–221. (doi:10.1525/auk.2012.11182)
37. Soares L, Escudero G, Penha VAS, Ricklefs RE. 2016 Low prevalence of haemosporidian parasites in shorebirds. *Ardea* **104**, 129–141. (doi:10.5253/arde.v104i2.a8)
38. Lacorte GA, Félix GMF, Pinheiro RRB, Chaves AV, Almeida-Neto G, Neves FS, Leite LO, Santos FR, Braga ÃM. 2013 Exploring the diversity and distribution of neotropical avian malaria parasites—a molecular survey from Southeast Brazil. *PLoS ONE* **8**, 1–9. (doi:10.1371/journal.pone.0057770)
39. Ferreira FC, Rodrigues RA, Ellis VA, Leite LO, Borges MA, Braga EM. 2017 Habitat modification and seasonality influence avian haemosporidian parasite distributions in southeastern Brazil. *PLoS ONE* **12**, 1–18. (doi:10.1371/journal.pone.0178791)
40. Fecchio A, Wells K, Bell JA, Tkach VV, Lutz HL, Weckstein JD, Clegg SM, Clark NJ. 2019 Climate variation influences host specificity in avian malaria parasites. *Ecol. Lett.* **22**, 547–557. (doi:10.1111/ele.13215)
41. Lopes VL, Costa FV, Rodrigues RA, Braga ÉM, Pichorim M, Moreira PA. 2020 High fidelity defines the temporal consistency of host-parasite interactions in a tropical coastal ecosystem. *Sci. Rep.* **10**, 1–10. (doi:10.1038/s41598-019-56847-4)
42. Latta SC, Ricklefs RE. 2010 Prevalence patterns of avian haemosporida on hispaniola. *J. Avian Biol.* **41**, 25–33. (doi:10.1111/j.1600-048X.2009.04685.x)
43. Soares L, Latta SC, Ricklefs RE. 2020 Neotropical migratory and resident birds occurring in sympatry during winter have distinct haemosporidian parasite assemblages. *J. Biogeogr.* **47**, 748–759. (doi:10.1111/jbi.13760)
44. Svensson-Coelho M, Ellis VA, Loiselle BA, Blake JG, Ricklefs RE. 2014 Reciprocal specialization in multihost malaria parasite communities of birds: a temperate-tropical comparison. *Am. Nat.* **184**, 624–635. (doi:10.1086/678126)
45. Sambrook J, Russel DW. 2013 Molecular cloning: a laboratory manual. *Meteorolol Z* **22**, 711–728. (doi:10.1127/0941-2948/2013/0507)
46. Bell JA, Weckstein JD, Fecchio A, Tkach VV. 2015 A new real-time PCR protocol for detection of avian haemosporidians. *Parasit. Vectors* **8**, 0–9. (doi:10.1186/s13071-015-0993-0)
47. Hall TA. 1999 BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **41**, 95–98.
48. Bensch S, Hellgren O, PÉrez-Tris J. 2009 MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome b lineages. *Mol. Ecol. Resour.* **9**, 1353–1358. (doi:10.1111/j.1755-0998.2009.02692.x)
49. Braga, Silveira P, Belo NO, Valkiūnas G. 2011 Recent advances in the study of avian malaria: an overview with an emphasis on the distribution of *Plasmodium* spp in Brazil. *Memorias do Instituto Oswaldo Cruz* **106**, 3–11.
50. Ágh N, Csörgő T, Szöllősi E. 2022 Delay in arrival: lineage-specific influence of haemosporidians on autumn migration of European robins. *Parasitol. Res.* **121**, 2831–2840. (doi:10.1007/s00436-022-07621-5)
51. Morand S, Poulin R. 1998 Nematode parasite species richness and the evolution of spleen size in birds. *Canadian J. Zool.* **78**, 1356–1360. (doi:10.1139/z00-076)
52. Shaw AK, Sherman J, Barker FK, Zuk M. 2018 Metrics matter: The effect of parasite richness, intensity and prevalence on the evolution of host migration. *Proc. R. Soc. B* **285**, 1–10. (doi:10.1098/rspb.2018.2147)
53. Martinsen ES, Perkins SL, Schall JJ. 2008 A three-genome phylogeny of malaria parasites (*Plasmodium* and closely related genera): evolution of life-history traits and host switches. *Mol. Phylogenet. Evol.* **47**, 261–273. (doi:10.1016/j.ympev.2007.11.012)
54. Borner J *et al.* 2016 Phylogeny of haemosporidian blood parasites revealed by a multi-gene approach. *Mol. Phylogenet. Evol.* **94**, 221–231. (doi:10.1016/j.ympev.2015.09.003)
55. Galen SC, Borner J, Martinsen ES, Schaer J, Austin CC, West CJ, Perkins SL. 2018 The polyphyly of *Plasmodium*: comprehensive phylogenetic analyses of the malaria parasites (order Haemosporida) reveal widespread taxonomic conflict. *R. Soc.* **5**, 1–16.
56. Burns KJ, Shultz AJ, Title PO, Mason NA, Barker FK, Klicka J, Lanyon SM, Lovette IJ. 2014 Phylogenetics and diversification of tanagers (Passeriformes: Thraupidae), the largest radiation of Neotropical songbirds. *Mol. Phylogenet. Evol.* **75**, 41–77. (doi:10.1016/j.ympev.2014.02.006)
57. Paradis E, Claude J, Strimmer K. 2004 APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**, 289–290. (doi:10.1093/bioinformatics/btg412)
58. Winkler DW, Billerman SM, Lovette IJ. 2020 Tanagers and allies (Thraupidae), version 1.0. In *Birds of the world* (eds SM Billerman, BK Keeney, PG Rodewald, TS Shulenberg). Ithaca, NY: Cornell Lab of Ornithology. (doi:10.2173/bow.thraup2.01)
59. Stoddard MC, Prum RO. 2008 Evolution of avian plumage color in a tetrahedral color space: a phylogenetic analysis of new world buntings. *Am. Nat.* **171**, 755–776. (doi:10.1086/587526)
60. Bischl B, Lang M, Bossek J, Horn D, Richter J, Sumann D. 2022 BBmisc: Miscellaneous Helper Functions for B. Bischl. R package version 1.12. See https://CRAN.R-project.org/package=BBmisc.

61. James G, Witten D, Hastie T, Tibshirani R. 2014 *An introduction to statistical learning: with applications in R. 426*. Berlin, Germany: Springer.
62. Burnham KP, Anderson DR. 2002 *Model selection and inference: a practical information-theoretic approach*, 2nd edn. New York, NY: Springer.
63. Bartoń K. 2019 *MuMIn: multi-model inference. R package version 1.46.0*. See https://CRAN.R-project.org/package=MuMIn.
64. Burnham KP, Anderson DR, Huyvaert KP. 2011 AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behav. Ecol. Sociobiol.* **65**, 23–35. (doi:10.1007/s00265-010-1029-6)
65. R Core Team. 2019 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
66. Morand S, Bordes F. 2015 Parasite diversity of disease-bearing rodents of Southeast Asia: habitat determinants and effects on sexual size dimorphism and life-traits. *Front. Ecol. Evol.* **3**, 1–11. (doi:10.3389/fevo.2015.00110)
67. De Lisle SP, Rowe L. 2015 Parasitism and the expression of sexual dimorphism. *Ecol. Evol.* **5**, 961–967. (doi:10.1002/ece3.1416)
68. Krüger O, Davies NB, Sorenson MD. 2007 The evolution of sexual dimorphism in parasitic cuckoos: sexual selection or coevolution? *Proc. R. Soc. B* **274**, 1553–1560. (doi:10.1098/rspb.2007.0281)
69. Drury JP, Burroughs N. 2016 Nest shape explains variation in sexual dichromatism in New World blackbirds. *J. Avian Biol.* **47**, 312–320. (doi:10.1111/jav.00757)
70. Matysioková B, Remeš V, Cockburn A. 2017 Broad-scale variation in sexual dichromatism in songbirds is not explained by sex differences in exposure to predators during incubation. *J. Avian Biol.* **48**, 1322–1330. (doi:10.1111/jav.01144)
71. Diniz *et al*. 2015 Attractive males are less than adequate dads in a multimodal signalling passerine. *Animal Behav.* **112**, 109–117. (doi:10.1016/j.anbehav.2015.01.006)
72. Edwards DB. 2012 Immune investment is explained by sexual selection and pace-of-life, but not longevity in parrots (Psittaciformes). *PLoS ONE* **7**, e53066. (doi:10.1371/journal.pone.0053066)
73. Liu J, Yan H, Li G, Li S. 2021 Nest concealment is associated with reproductive traits across sympatric bird species. *Ecol. Evol.* **11**, 14 079–14 087. (doi:10.1002/ece3.8117)
74. Sandercock BK. 1997 Incubation capacity and clutch size determination in two calidrine sandpipers: a test of the four-egg threshold. *Oecologia* **110**, 50–59. (doi:10.1007/s004420050132)
75. Sick H. 1997 *Ornitologia Brasileira*. Rio de Janeiro, Brazil: Nova Fronteira.
76. Tobias *et al.* 2012 The evolution of female ornaments and weaponry: social selection, sexual selection and ecological competition. *Phil. Trans. R. Soc. B* **367**, 2274–2293. (doi:10.1098/rstb.2011.0280)
77. Plaza M, Cantarero A, Cuervo JJ, Moreno J. 2018 Female incubation attendance and nest vigilance reflect social signaling capacity: a field experiment. *Behav. Ecol. Sociobiol.* **72**, 24. (doi:10.1007/s00265-017-2423-0)
78. Aguiar de Souza Penha V *et al.* 2022 Data from: Haemosporidian parasites and incubation period influence plumage coloration in tanagers (Passeriformes: Thraupidae). Dryad Digital Repository. (doi:10.5061/dryad.1g1jwsv0d)
79. Aguiar de Souza Penha V *et al.* 2022 Supplementary material from: Haemosporidian parasites and incubation period influence plumage coloration in tanagers (Passeriformes: Thraupidae). Figshare. (doi:10.6084/m9.figshare.c.6292019)