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Effects of avian malaria on male behaviour and female visitation in lekking blue-crowned manakins

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Avian malaria, the infection by blood parasites of the genus *Plasmodium,* can reduce host fitness not only through mortality, but also by impairing the expression of sexual selection traits. Although different studies highlight the association of parasitism with a decrease in host reproductive success, few studies have addressed the role of parasites in honest signalling by lekking species. Hence, it is still uncertain which fitness components are affected by parasites in these species. We investigated whether avian malaria is associated with a decrease in mating behaviour of male blue-crowned manakins *Lepidothrix coronata* and whether it affects female visitation in leks of a population in the central Amazon. Through behavioural observations, we estimated the rates of total male activity and social interaction, as well as the frequency of female visits at individual perches. We then examined if individuals were infected with *Plasmodium* spp. using molecular techniques. Avian malaria was associated with a decrease in male mating behaviour in each lek, and mating behaviour correlated with female visitation. Although rates of social interaction were not correlated with avian malaria among males, we observed that interacting with several individuals within a lek may be advantageous for males, as they also vocalized and displayed more, thus increasing their chances of being visited by females. Although female visitation was not associated with avian malaria in individuals or leks, it is still possible that female visitation is indirectly affected by avian malaria through the latter's effects on male activity. We suggest a role for male activity as an honest sexual signal for females. Thus, male display rate could be used by females as cue for the probability of a male being infected.

Avian malaria is a disease caused by protozoans of the genus *Plasmodium* that can modify phenotypic characters of infected individuals, and may lead to reduction in fitness, since it affects the expression of host courtship displays (Buchanan et al. 1999, Gilman et al. 2007, Knowles et al. 2010). Such displays may act as honest indicators of genetic resistance of males to diseases and parasites, representing an important signal for sexual selection (Hamilton and Zuk 1982). Because they indicate indirect benefits for the female and its offspring, these displays may predict female choice (Hamilton and Zuk 1982, Beltran-Bech and Richard 2014). Thus, secondary sexual traits that honestly advertise high genetic quality will increase offspring viability (Hamilton and Zuk 1982).

In species with polygynous lek mating system, males aggregate in arenas to attract females, where comparison among males is facilitated (Bradbury and Gibson 1983). Several hypotheses have been proposed to explain the mechanisms of female choice in leks (Fisher 1915, Lande 1981, Kirkpatrick 1982, Endler and McLellan 1988, Endler and Théry 1996, Gomez and Théry 2004, Anciães and Prum 2008), including the parasite hypothesis (Hamilton and Zuk 1982, review by Kirkpatrick and Ryan 1991). According to this hypothesis, there would be differential signalling among males within leks, reflecting infection occurrence, which would affect female choice. Only a few studies have tested this hypothesis in species with lek mating system, but even considering other mating systems, there is no consensus among their results (Boyce 1990, Gibson 1990, Johnson and Boyce 1991, Höglund et al. 1992, Lebigre et al. 2013). Therefore, it is unclear what are the effects of parasites on secondary sexual traits of males and on female choice in lekking species in particular.

Male blue-crowned manakins *Lepidothrix coronata* (Aves: Pipridae), engage in either solitary or exploded leks, with up to seven individual (Snow 1963, Sick 1967, Skutch 1969, Prum 1994, Anciães et al. 2009, Durães 2009). Aggregations within these leks are stable, with males spending 60% of the time in activities within the leks (Bosholn unpubl.). These aggregations are also lasting, because adult males can participate in the same lek for different reproductive seasons (Durães 2009). Therefore, the typical participation of adult males in leks would be enough for acquiring avian malaria. On the other hand, females visit leks only occasionally

(Durães 2009). During the breeding season, besides performing acrobatic displays, adult males vocalize from display perches as part of their courtship behaviour. These vocalizations were indeed good predictors of male reproductive success within leks in a previous study in Ecuador (Durães et al. 2008, Durães 2009). Furthermore, in some manakin species, females preferred males that interacted with other individuals in vocal duetting and group displays, suggesting that this mating behaviour can promote direct and indirect benefits for females (review by Díaz-Muñoz et al. 2014).

Considering that avian malaria has a negative effect on fitness in different host species studied to date (Hamilton and Zuk 1982, Schall 1983, Marzal et al. 2005, Asghar et al. 2011), it may compromise the behaviour of male and, at least indirectly, of female blue-crowned manakins. Because avian malaria could reduce male body condition (Williams 2005, review by Atkinson and Van Ripper III 1991, Valkiūnas 2005), we tested the predictions that 1) total male activity rates should be reduced at leks with increased prevalence of avian malaria and 2) infected males should exhibit decreased activity rates. Furthermore, because we expected that male activity would be correlated with the rate of interaction among males, we tested the prediction that 3) infection patterns should be correlated with the rate of interaction among males. Considering that in some Manakin species females prefer males that engage in higher activity and social interaction rates (Durães et al. 2008, Durães 2009, review by Díaz-Muñoz et al. 2014), we tested the predictions that 4) females should prefer to visit leks where individuals present higher activity and 5) interaction rates. If indeed these behaviours are preferred, and are associated with avian malaria infection, then female visitation would be also affected by the infection. Consequently, we tested the prediction that 6) females should visit more often leks with low prevalence of malaria, and 7) uninfected males. It is worth mentioning that the presented hypotheses differ from those tested in previous studies, which considered only either individual display rates (Durães et al. 2008, Durães 2009), or the degree of interaction among individual males inside leks (Díaz-Muñoz et al. 2014), but did not consider the variation in such characteristics among leks in predicting female behaviour.

Material and methods

Study area

We carried out fieldwork in the Manaquiri research site, created by the Brazilian Program for Biodiversity Research (PPBio). The site is located aproximatedly 100 km south of Manaus along the 319 Highway, within the Purus-Madeira interfluvium, south of the Amazon River in Amazonas State, Brazil (Fig. 1). The study area lies within a well preserved terra-firme forest composed of dense vegetation typical of ombrophilous forests with emerging canopy. The climate in the region is characterized by high pluviosity (2000 mm), with annual average temperature of 27°C, and the geomorphology consists of large tabular interfluves with plain topography and altitudes ranging from 30 to 50 m. The presence

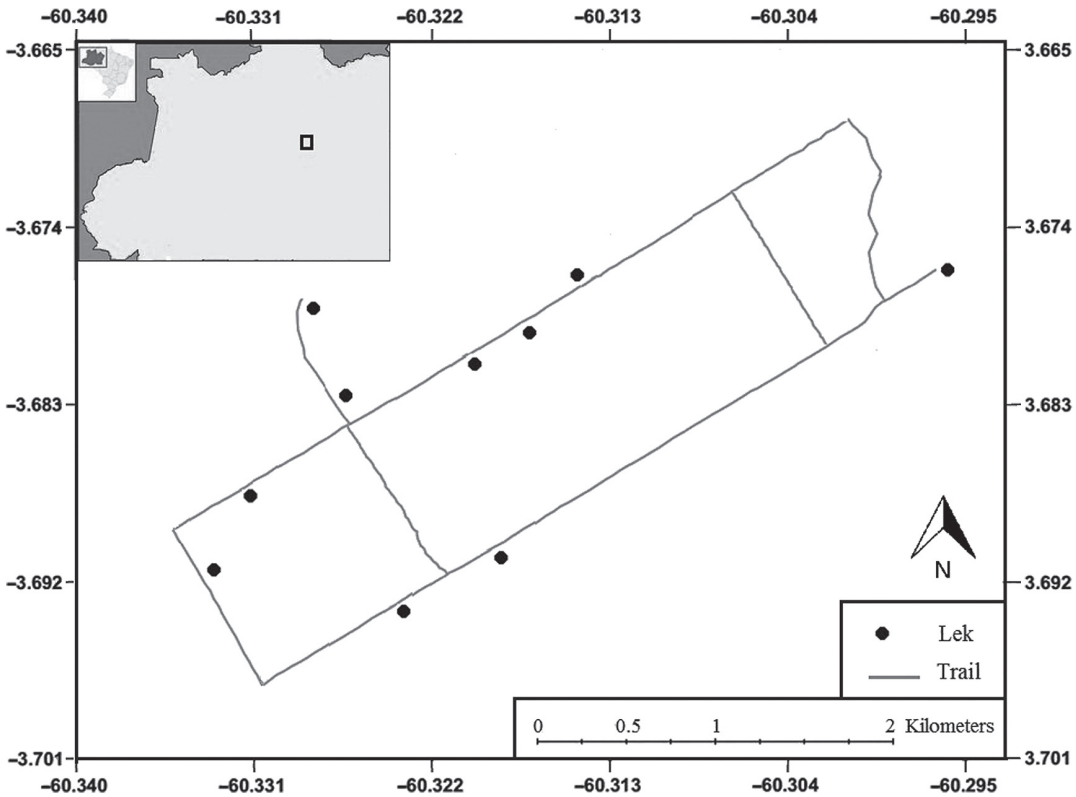

Figure 1. Trail system and sampled leks in the research module 'Manaquiri', Careiro-Castanho municipality, Amazonas state, Brazil. Adapted from <http://ppbio.inpa.gov.br/sitios/br319/infra/km100>.

of micro-terrain with altitudes ranging from one to three meters is frequent and favors the formation of temporary pools on the site (<http://ppbio.inpa.gov.br/sitios/br319/infra/km100>).

Studied population and individual sampling

We sampled individuals from a population of *Lepidothrix coronata arimensis*, in which male plumages present considerable amounts of greenish plumage in the body, wings and tail feathers (Hellmayr 1929, Anciães et al. 2009, review by Kirwan and Green 2012), representing intermediate forms between the black-bodied males typical of the species and the green-bodied forms from southwestern Amazon. In spite of their green plumage feathers, definitive plumage males can easily be differentiated from those in pre-definitive plumages by the blackish plumage in the upper body and their typical blue crown. The reproductive season is June–November in the central Amazon as indicated by mating activity and gonad data (review by Kirwan and Green 2012, Anciães unpubl.).

We collected data for individuals present in ten leks, during the reproductive seasons of 2013 (October and November) and 2014 (June and July). Display perches of adult males were located through continuous acoustic census and visual search along trails. Lekking behaviour was diagnosed by persistence of individuals in perches across consecutive days and by observation of behavioural elements typical of their courtship displays (Durães 2009). After locating individual perches, we used approximately 15 mist nets with 12 m in length by 3 m in height, close to the display perches, for two consecutive days in each lek. We captured 67 blue-crowned manakins, and verified the presence of brood patch for preliminary sexing of green-plumaged birds. We marked individuals with two colored plastic bands on the right tarsus and a metallic band (provided by CEMAVE/IBAMA permit number: 3767) on the left tarsus. We collected one blood sample of approximately 50 µl from each individual through puncture of the brachial vein, using a disposable hypodermic needle and microcapillaries. The blood was stored in microtubes with 1 ml of ethanol 95% until DNA extraction. Because of the conspicuous sexual dimorphism typical of the species (Hellmayr 1929, Sick 1967, Snow 2004, Ryder and Durães 2005, review by Kirwan and Green 2012), we sexed individuals in the field according to the color of their plumage. Males with olive-green and black body plumage with a bright blue crown were classified as adult males in definitive plumage, whereas individuals with predominantly green plumage were classified as individuals of unknown sex. The gender of these individuals was determined through molecular sexing using protocols described elsewhere (Ito et al. 2003). The frequency of males in leks, total male activity, and rate of interaction among males were unaffected after capturing, marking and blood sampling individuals (Bosholn unpubl.).

Behavioural observations

We conducted behavioral observations on display perches in the mornings (07:00–12:00), and afternoons (13:00–16:00) through continuous focal sampling with five-minute intervals (Altmann 1974). The observation protocol included, for each observation interval, sampling the total number of vocalizations emitted by each focal male, occurrence of courtship displays, frequency of interactions among males, and frequency of observed female visits. Courtship displays included all events in which males were observed dancing, solitarily or in group, in display perches in a same observation interval. All behaviours in which a individual was seen dancing or vocalizing with other males, in the same display perches, in a same observation interval, were considered 'interactions among males'. We considered as visiting events 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 and adult males (Durães 2009). That being the case, 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, that individual was also considered as a female. Vocalization, displays, and interaction behaviour among young males were not considered because these individuals present a floater behaviour, visiting several leks during a single reproductive season (Durães 2009) and therefore presenting occasional display patters compared to adult males in a lek, which could lead to bias our comparisons.

DNA extraction, parasite detection and sequencing

DNA from blood samples was extracted with Wizard Genomic DNA Purification Kit (PROMEGA®, Madison, WI, USA) according to the manufacturer's protocol. The DNA pellet was suspended in 50 µl of hydration solution and kept at –20°C until use. DNA quality was checked by electrophoresis by running out 1 µl of the diluted DNA solution on a 1% agarose gel. DNA samples were screened for the presence of haemosporidian parasites by using parasite-specific primers targeting a fragment of the cytochrome *b* gene. We adopted a nested polymerase chain reaction (PCR) method developed by Hellgren et al. (2004) to detect the presence of *Plasmodium* and *Haemoproteus*. Information for detailed laboratory methods, primers' names and PCR's 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 included in runs to check against possible contamination, although none was found in any PCR run. Products from PCR amplifications were run on 1.5% agarose gels, stained with ethidium bromide, and visualized under UV light. PCR products were purified from 1.5% agarose gel using the QIAquick gel extraction kit (Qiagen). Bi-directional sequencing with dye-terminator fluorescent labeling was performed in an ABI Prism 3100 automated sequencer (Applied Biosystems).

Phylogenetic analysis

Phylogenetic relationships among the lineages of *Plasmodium* sp. identified in this study and related morphospecies of *Plasmodium* spp. were inferred by using sequence information from the MalAvi database (Bensch et al. 2009). We used the information about the *cyt b* gene sequences from MalAvi to select

Table 1. Indexes for mating behaviours of adult male blue-crowned manakins.

Indexes	Formula
Total vocalization rate per minute in the lek	[(∑ *Nt of vocalization in lek*/∑ *Nintle*)/5]
Total display rate per minute in the lek	[(∑ *Nt with displays in lek*/∑ *Nintle*)/5]
Total interaction rate per observation interval in the lek	(∑ *Nt vocalizing or displaying in lek*/∑ *Nintle*)
Individual vocalization rate per minute	[(∑ *Nt of vocalization at individual perch*/∑ *Nintind*)/5]
Individual display rate per minute	[(∑ *Nt with displays at individual perch*/∑ *Nintind*)/5]
Individual interaction rate per observation interval	(∑ *Nind vocalizing or displaying*/∑ *Nintind*)
Occurrence of malaria in individual	*Presence* (1) *or absence* (0) *of malaria in individual*
Malaria prevalence in lek	(∑*Nind captured infected in lek*/∑ *Nind captured in lek*)
Frequency of females per observation interval	(*Nt of female visits observed at indivídual perch*/∑ *Nintind*)
Frequency of females per observation interval in lek	(*Nt of female visits observed in lek* ∑ *Nintle*)
Frequency of males in lek	*Nt of males captured in lek*

Abbreviation: Nt = total number; Nintle = total number of observation intervals in the lek; Nind = total number of individuals; Nintind = total number of observation intervals to focal perches.

those sequences for which species were identified based on targeting studies using both morphological and molecular evidences. The sequences were deposited in Genbank (LECOR01 (KU236431), LECOR02 (KU236432), LECOR03 (KU236433), LECOR04 (KU236434)).

The phylogenetic tree was constructed using the Bayesian inference method implemented in MrBayes ver. 3.0b4 (Huelsenbeck and Ronquist 2001). Bayesian inference was performed with four Markov chain Monte Carlo chains that ran simultaneously for 3 000 000 generations, with trees sampled every 100 generations for a total of 30 000 trees. The analyses were conducted until the average standard deviation of the split frequencies reached 0.01. Posterior probabilities were calculated based on the trees retained after the log-likelihood values had stabilized.

Data analysis

We calculated several indexes to summarize the mating behaviours of males and females observed at individual perches and leks (Table 1). Collinearity among behavioural indexes were tested through Pearson correlations (Table 2). Prevalence of infection by *Plasmodium* spp. may vary seasonally (Bensch et al. 2007, Hellgren et al. 2013) and, for this reason, we tested for similarity in the prevalence of infected individuals between years ($\chi^2_1 = 1.11$, $p = 0.34$). Because there was no difference, we pooled the data for both years in subsequent analyses.

We used simple linear regressions to test if total vocalization rates and display behaviours at leks were related to the prevalence of avian malaria. The association between prevalence of avian malaria and total interaction rate at leks was tested through Pearson correlation. We compared the behaviour of infected and uninfected males through *t*-tests for vocalization, display and interaction rates, separately. We also tested the prediction that the frequency of female visits to uninfected males should be higher than to infected males through a *t*-test. Finally, we used simple linear regressions to test the predictions that the frequency of female visits was lower in leks with increased prevalence of avian malaria, and was positively related to male vocalization, display and interaction rates. Non-normal and/or heteroscedastic data were logarithmized. All analyses were done with R ver. 3.1.2 (R Development Core Team).

Results

We captured and observed males in ten leks during the reproductive seasons of 2013 and 2014. The number of females and males (juvenile and adults) varied among leks. We captured a total of 67 individuals of blue-crowned manakins: 29 from which were adult males, 11 were juvenile males and 27 were females. From the total of 29 adult males captured, 28 participated in leks with two to six individuals, and only one belonged to a solitary lek ($\bar{x}$ + SE = 2.9 ± 1.44, n = 29). There was also variation among leks in the number of females captured ($\bar{x}$ + SE = 2.7 ± 1.41, n = 27). The frequency of capture of juvenile males was low on average, when compared to the total number of females and adult males captured ($\bar{x}$ + SE = 1.1 ± 0.99, n = 11). Although there were few female recaptured between leks, we did not recapture juvenile or adult males across leks. The majority of observed females were also banded. Each banded adult male was observed for two consecutive days ($\bar{x}$ + SE = 10 h ± 2.14). The observed adult males were present (vocalizing, displaying or silent) in the leks for 65% of the total observation time.

Table 2. Collinearity among derived indexes for mating behaviours of lekking blue-crowned manakins.

Correlation indexes	Results
Total vocalization rate per minute in lek and total display rate per minute in the lek	$r_9 = 0.56$, $p = 0.08$
Total vocalization rate per minute in lek and total interaction rate in the lek	$r_9 = 0.26$, $p = 0.29$
Total display rate per minute in the lek and total interaction rate in the lek	$r_9 = 0.30$, $p = 0.38$
Individual vocalization rate per minute and individual display rate per minute	$r_{26} = 0.71$, $p = 0.02$
Individual vocalization rate per minute and individual interaction rate	$r_{26} = 0.62$, $p < 0.01$
Individual display rate per minute and interaction rate of individual	$r_{26} = 0.65$, $p = 0.02$
Total interaction rate in the lek and frequency of males in lek	$r^2_9 = 0.52$, $p = 0.01$

The prevalence of avian malaria for the captured birds was 35% within adult males, 90% within young males, and 38% within females. Sequencing of the cyt b DNA fragment in 23 individuals revealed the presence of four different *Plasmodium* lineages: LECOR01 (KU236431), LECOR02 (KU236432), LECOR03 KU236433) and LECOR04 (KU236434). These sequences were 100% similar to sequences previously recovered in different host birds (Passeriforme) from South America and Central America in accordance with GenBank. LECOR 01 was similar to *Plasmodium* sp. 5 MM-2015 (KT373870.1) and *Plasmodium* sp. TACHURIS 01 (KF482356.1) lineages; LECOR 02 was similar to *Plasmodium* sp. G22 (DQ241529.1) lineage; LECOR 03 was similar to *Plasmodium* sp. 9MM-2015 (KT373874.1) and *Plasmodium* sp. HMA-2012 BCRM1 and BCRM2 isolates (JN819330.1 and JN819332.1, respectively); LECOR 04 was similar to *Plasmodium* sp. P-T138 isolate NK162353-T138 (JQ988610.1). The *Plasmodium* sp. LECOR 01 lineage was the most prevalent ($n = 18$) while the other three lineages were recovered in fewer individuals: LECOR 02 ($n = 1$), LECOR 03 ($n = 3$), and LECOR 04 ($n = 1$) (Fig. 2). We did not detect *Haemoproteus* in the sampled individuals.

The phylogenetic analysis of sequences recovered in blue-crowned in this study with morphospecies representatives of five *Plasmodium* subgenus (*Novyella*, *Bennettinia*, *Haemamoeba*, *Giovannolaia*, *Huffia*), recovered from MalAvi and GenBank, showed that LECOR 02 and LECOR 04 form related clades and well supported with morphospecies of *Haemamoeba* subgenus (*P. tejerai* and *P. cathemerium*, respectively) (Fig. 2). Furthermore, LECOR 03 lineage was related to *Novyella* subgenus showing a well-supported clade. The LECOR 01 line did not constitute related clade with other morphospecies suggesting the need for further studies to identify (morphological, molecular and phylogenetic) of this lineage, since it was recovered in 18 of 24 samples analyzed.

The correlations among behavioural indexes estimated for individuals were all significant, but not among the indexes estimated for leks (Table 2). Interaction rates at leks were correlated to the number of individuals at leks. Because individual display rate (correlated with individual vocalization rate) and frequency of female visits to individual males were both correlated with individual interaction rate, we controlled for effects of interaction rates when testing for effects of avian malaria on male display behavior and female visitation. For that, we used the residuals of each of those regressions to compare, separately, individual display rates and frequency of female visits between infected and uninfected males. Similarly, we used the residuals of the regression between total lek interaction rate and frequency of female visits to leks to test the prediction that the frequency of female visits was lower in leks with increased prevalence of avian malaria, controlling for effects of individual interaction rate.

We observed less episodes of vocalization and lower displays rate in leks with high prevalence of infected males ($r^2_9 = 0.34$, $p = 0.04$ and $r^2_9 = 0.43$, $p = 0.02$ respectively; Fig. 3A, B), but the total interaction rate in these leks remained unaltered by the prevalence of infection at leks ($r_9 = 0.09$, $p = 0.78$). The individual interaction rate did not differ between infected and uninfected males ($t_{26} = –0.37$, $p = 0.35$), and uninfected males displayed more than infected males ($t_{26} = –2.37$, $p = 0.01$; Fig. 4), when accounting for the effects of total interaction rate on male display rates.

The prevalence of avian malaria in the leks did not affect the frequency of female visits to the leks ($r^2_9 = –0.11$, $p = 0.82$), even after removing the effects of interaction rate on female visitation, which were positively correlated among leks ($r^2_9 = 0.39$, $p = 0.02$) (Fig. 5). The frequency of female visits to leks was also not correlated to the total display rate ($r^2_9 = 0.04$, $p = 0.26$) observed in leks. Likewise, the frequency of female visits to infected males did not differ from those to uninfected males ($t_{26} = 0.82$, $p = 0.41$), when accounting for effects of individual interaction rate on the frequency of visits, as individuals that displayed more or interacted with more males received more female visits ($r^2 = 0.26$, $p < 0.01$; and $r^2_{26} = 0.23$, $p = 0.01$, Fig. 6, respectively).

Discussion

Our results show that total male activity rates (vocalization and display) were reduced at leks with increased prevalence of avian malaria, and activity rates of infected males were also decreased, corroborating our hypotheses. The pathogenic intensity of avian malaria is associated with the *Plasmodium* species that cause the disease, and with the susceptibility of each host species (Valkiūnas 2005). The effects on host behaviour might be explained by the high pathogenicity of the *Plasmodium* spp. lineages that were found in the studied population, which are in majority phylogenetically related to the clade of morphospecies of the subgenus *Haemamoeba*. This subgenus is composed by different *Plasmodium* species that are considered highly pathogenic, such as *P. tejerai* (Silveira et al. 2013), *P. relictum*, and *P. cathemerium* (Valkiūnas 2005).

Social interaction among males in each lek was not correlated to avian malaria prevalence, against our prediction. This result may be explained by the fact that *Plasmodium* spp. are parasites with indirect life cycles and, therefore, require both an intermediate and final host to complete their cycles. Since the vectors of *Plasmodium* spp. are highly mobile mosquitoes, transmission may become less dependent on the aggregation of individuals and, for that reason, infection patterns may be random (Côté and Poulin 1995, Godfrey et al. 2006). We also did not find any relation between the rate of social interaction among individuals and the occurrence of avian malaria. This may also be explained by the fact that avian malaria transmission depends on the mobility of the vector, without transmission among individuals (Côté and Poulin 1995). Therefore, a male infected with *Plasmodium* spp. will not increase the probability that other males interacting with it will become infected.

Despite the effect of avian malaria on male activity rates of the blue-crowned manakin, courtship behaviour of males was not regulated only by *Plasmodium* spp. infection. The rate of social interaction also affected these behaviours, and masked the effect of the infection on male activity before controlling for the effect o social interaction. This possibly occurred because an infected male could be as active as an

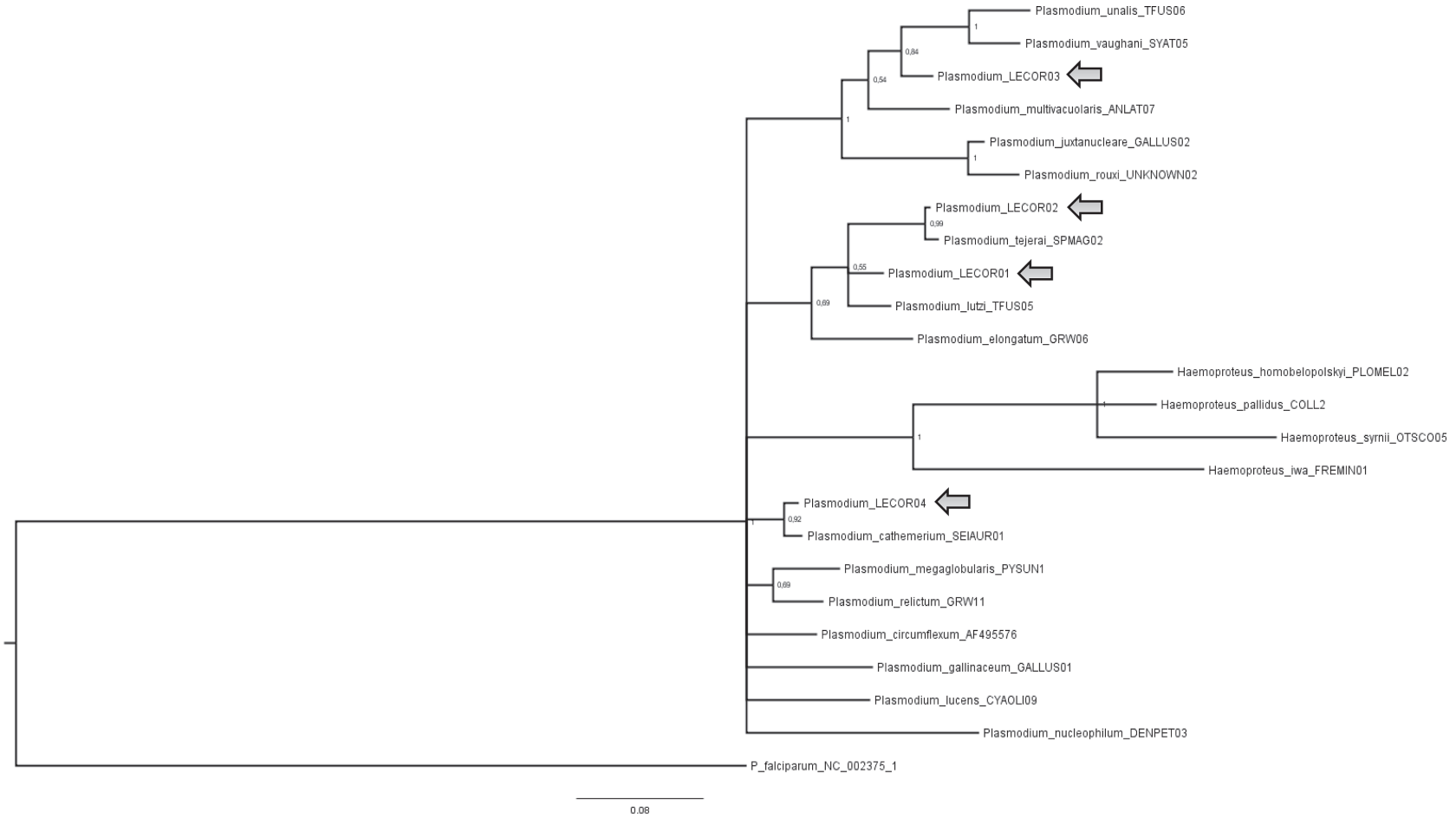

Figure 2. Phylogenetic relationships between morphospecies representatives of five *Plasmodium* subgenus and the *Plasmodium cyt b* lineages in blue-crowned manakins. The four lineages found in blue-crowned manakins LECOR01 ((KU236431), LECOR02 (KU236432), LECOR03 (KU236433) and LECOR04 (KU236434)) are indicated with an arrow. *Plasmodium falciparum* was used as outgroup.

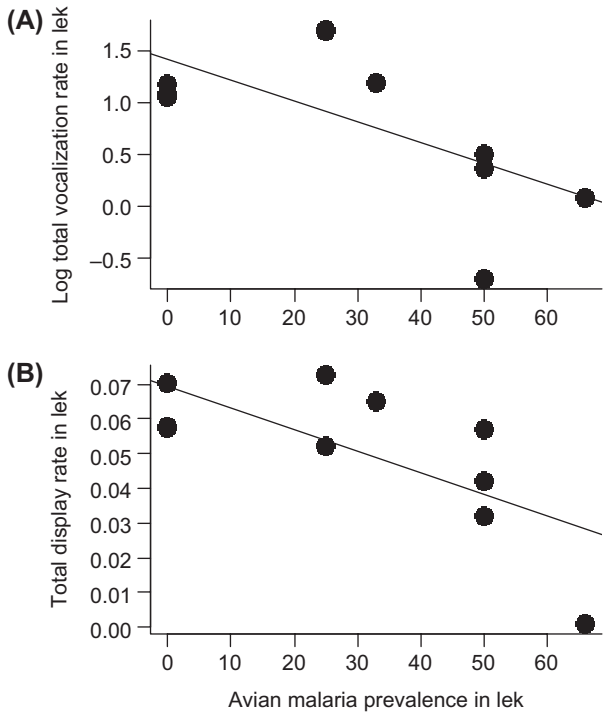

Figure 3. Effect of prevalence of *Plasmodium* spp. in leks on (A) total vocalization rate and (B) total display rate in leks.

uninfected male in leks. After all, individual interaction rate did not differ between infected and uninfected males. Furthermore, the mating behaviour of males could be also impaired by other diseases, and avian malaria could have just a partial effect. Other parasites (e.g. mites, nematodes, trypanosomes, coccidians) could also have a negative effect on mating behaviour of blue-crowned manakins, as observed in other birds species (Moller 1991, Saumier et al. 1991, Dyrcz et al. 2005, Aguilar et al. 2008).

During behavioural sampling we observed that as soon as a male started to vocalize, or even head to the display perches silently, other individuals started to interact with

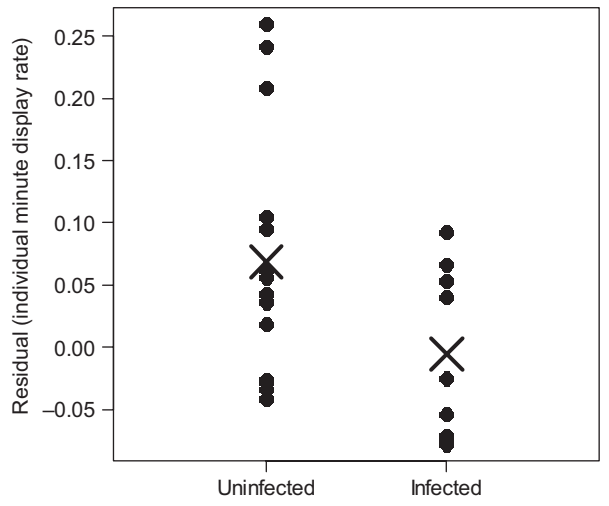

Figure 4. Comparisons of residuals (mean ± 1 SD) obtained from the regression of individual interaction rate and display rate, between infected and uninfected males.

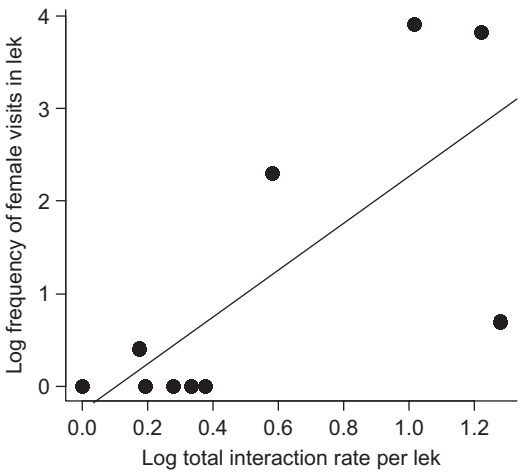

Figure 5. Correlation between the frequency of female visits to leks and the total interaction rate among males at leks.

that male, leading to higher activity rates performed by individuals interacting with other males more frequently, but not necessarily with more individuals. The total rate of social interaction at leks, on the other hand, was dictated by lek size (number of males in the lek) and not associated to individual male activity. These result differs from that of Durães et al. (2009) for the same species studied in Ecuador, where higher vocalization rates were observed at larger leks. Here, we observed males with comparable activity and interaction rates in leks of various sizes and rates of social interaction. Therefore, we suggest that, in the studied population, male activity is regulated by the amount of interactions in which each male engages individually, and not by lek size, indicating geographic variation in the functioning of the lek system within the species.

During our study, females visited more frequently leks where total rate of social interaction was higher, again corroborating our hypothesis. This result is in accordance to the female choice hypothesis (Beehler and Foster 1988, review by Díaz-Muñoz et al. 2014), which predicts that females prefer sites where many males interact, making possible to compare their secondary sexual characteristics before selecting a partner. The displays performed during these

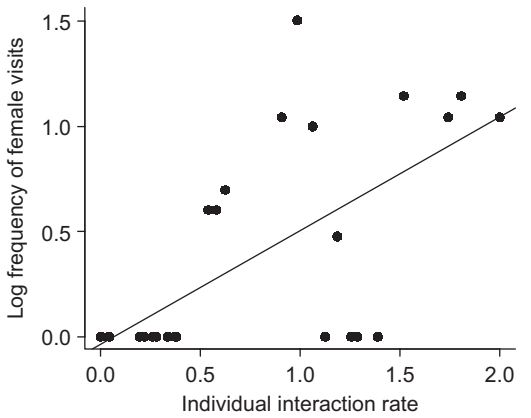

Figure 6. Effects of mating behaviours of individual males on frequency of female visits of individual display rate.

coalitions may allow females to evaluate which male is the best, considering that counter-singing and group displays can serve as an indicator of male genetic quality, showing how coordinated the individuals are. The total vocalization and total display rates at leks, in turn, were not used as cues by the females observed in our study. At the largest sampled lek, for instance, we captured six males and, although the total rate of social interaction was high in this lek, its total vocalization rate was similar to that of smaller leks. This possibly occurred because not all individuals inside the lek vocalized or displayed actively, with considerable variation among males within a single lek. In the smallest sampled lek, only one male was captured and, despite the zero social interaction in this lek, its vocalization rate was considerably high.

Our data suggest that female visitation to males was based on behavioural rates of individuals. Inside leks, the frequency of female visits was higher to males that vocalized and displayed more and that interacted with a higher number of males. However, the frequency of female visits to infected males did not differ from those to uninfected males. Considering that uninfected males were more active, and that females visited them more often, it is possible that vocalization and display behaviours are honest male signals, reliable to indicate male health to females, thus helping in their choice when visiting leks (Zahavi 1975). Furthermore, our results suggest that, although the rate of social interaction of individual males was correlated to their vocalization and display rates, it would not be used by females to distinguish between infected and uninfected males, because of the lack of association between infection and social interaction rates. Therefore, when choosing to visit males that vocalized and displayed more in each lek, female blue-crowned manakins would have a higher probability of choosing a healthy male, even if the frequency of male–male interaction performed by this individual did not reflect its health.

We conclude that avian malaria has an effect on mating behaviour of males from the studied population of the host species *L. coronata*. The mating behaviour of males also had an influence on female preference. However, female preference is not affected by *Plasmodium* spp. prevalence in the lek, nor the occurrence of avian malaria in the individual. Even partially corroborating the parasite hypothesis (Hamilton and Zuk 1982), we only considered the effects of *Plasmodium* infection in the short term, without considering other mechanisms that could be influencing female preferences. Future studies about the effects of infection by *Plasmodium* spp. on blue-crowned manakins or other host species should explore variables directly related to host fitness. Testing the inheritance of female preferences and the activities of infected and uninfected males will be crucial to understand the role of male behaviours described here in the evolution of mechanisms of female choice based on signals that honestly indicate benefits to females and their offspring.

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