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Avian phenotypic traits related to feeding preferences in two *Culex* mosquitoes

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Abstract Host choice by mosquitoes affects the transmission dynamics of vector-borne infectious diseases. Although asymmetries in mosquito attraction to vertebrate species have been reported, the relative importance of host characteristics in mosquito blood-feeding behavior is still poorly studied. Here, we investigate the relationship between avian phenotypic traits—in particular, morphometry, plumage coloration, and nesting and roosting behavior—and the blood-feeding patterns in two common *Culex* mosquito species on a North American avian community. Forage ratios of the mosquito species were unrelated to the phylogenetic relationships among bird species. *Culex pipiens* fed preferably on birds with lighter-colored plumage and longer tarsi; furthermore, solitary roosting avian species were both bitten by *Cx. pipiens* and *Cx. restuans* more often than expected. These associations may be explained by greater mosquito attraction towards larger birds with a greater color contrast against the background. Although communally roosting birds may release more cues and attract more mosquitoes, individuals may in fact receive fewer bites due to the encounter-dilution effect. Mosquito feeding behavior is a highly complex phenomenon, and our results may improve understanding of the non-random interaction between birds and mosquitoes in natural communities.

Keywords *Culex* mosquitoes · Forage ratio · Host-seeking behavior · Insect vectors · Phylogenetic generalized least squares (PGLS) models · Trait-based approach

Introduction

Mosquitoes (Diptera: Culicidae) are the primary vectors for the transmission of many arthropod-borne pathogens that cause infectious diseases such as malaria and West Nile fever (Marquardt 2004). These pathogens are transmitted between an infected vertebrate host and a new host by mosquito bites but are unable to successfully replicate in all mosquito and vertebrate species. West Nile virus (WNV), for instance, is maintained in an enzootic cycle between ornithophilic mosquitoes, mainly *Culex* species, and birds, which act as amplification hosts. Some WNV-competent bird species may harbor high viral loads, which leads to greater transmission of this pathogen between mosquitoes and birds. However, when amplification reaches a peak, incidental transmission to humans and livestock may occur, possibly facilitated by opportunistic mosquito species capable of shifting their feeding patterns, such as some *Culex* species (e.g., Muñoz et al. 2012, Tempelis et al. 1965; Thiemann et al. 2011). Therefore, the host community ecology and feeding behavior of mosquitoes and the epidemiology of vector-borne pathogens are closely linked, which makes the improved understanding of mosquito feeding patterns in natural communities a crucial issue that needs to be addressed.

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Many mosquitoes show clear preferences for feeding on mammals or birds, while others exhibit a more opportunistic behavior (Farajollahi et al. 2011; Takken and Verhulst 2013). For instance, in Japan and Spain the common house mosquito *Culex pipiens* has been found to feed on both avian and mammalian hosts, frequently on humans, and the Asian tiger mosquito *Aedes albopictus* almost exclusively on mammals (Sawabe et al. 2010; Muñoz et al. 2011). In addition, mosquitoes may show clear preferences for certain host species (Kilpatrick et al. 2006a). For example, in a recent study in Italy, *Cx. pipiens* fed on Common Blackbirds (*Turdus merula*) five times more often than expected, while European Starlings (*Sturnus vulgaris*) were bitten ten times less than expected, based on the relative abundance of these two species (Rizzoli et al. 2015).

These differences in feeding patterns across potential host species clearly affect host-vector contact rates and may have dramatic consequences for the amplification of pathogens and the risk of transmission to other vertebrate species, including humans (Kilpatrick et al. 2006a; Muñoz et al. 2012). Therefore, identifying host species more bitten by mosquitoes could help focus and optimize surveillance programs aimed at detecting zoonotic pathogens, such as WNV and other viruses (Hassan et al. 2003; Figuerola et al. 2008). Despite the need for a better understanding of host utilization by vectors, evidence of the proximate causes of mosquito feeding patterns on different host species is still very limited.

Like other blood-sucking insects, mosquitoes use a combination of visual, thermal, and chemical cues emitted by vertebrate hosts to locate blood meals (Lehane 2005; Hawkes and Gibson 2016). However, the relative importance of each of these stimuli varies among mosquito species, habitats (e.g., mosquito flights path in relation to habitats, see Bidlingmayer 1971), and the spatial scale (i.e., the distance between the vertebrate host and the insect vector) (van Breugel et al. 2015; Cardé 2015). *Culex* mosquitoes, for example, are predominately nocturnal/crepuscular species, and their host-seeking activities usually peak at night or dusk/dawn, but they can also be active during daytime (Becker et al. 2010); they are primarily ornithophilic species, but can also shift their main blood source to mammals (Burkett-Cadena et al. 2011) or humans (Kilpatrick et al. 2006b) depending on seasons and host availability. Darker colors such as black, red, and blue are considered to be more attractive to host-seeking mosquitoes than light colors such as yellow and white (Allan et al. 1987). Likewise, larger hosts may attract more mosquitoes due to their increased release of heat, CO_2, and other olfactory cues (Kleiber 1947; Martínez-de la Puente et al. 2010). Nonetheless, host size can also affect mosquito feeding success due to the amount of bare skin exposed and/or the intensity of host anti-mosquito behavior, given that smaller individuals may be more actively able to defend themselves than larger ones (Edman and Scott 1987; Mooring et al. 2000).

To date, evidences for interspecific trait differences in exposure to vector-borne diseases have been mainly derived from analyses of the prevalence of pathogens or antibodies in their vertebrate hosts (Hamilton and Zuk 1982; Figuerola et al. 2008). However, these analyses of exposure were confounded by interspecific differences in susceptibility to infections and in mortality caused by pathogens. For example, the low prevalence of *Plasmodium* in some avian species may be explained by both low exposures to the pathogen vectors (as suggested by Piersma 1997) and/or the immune capacity of the hosts to fight off infections (Martínez-Abraín et al. 2004). Analyzing bloodmeal origins in female mosquitoes in relation to host abundance provides a less biased estimator of vector feeding patterns on different host species (e.g., Hamer et al. 2009, Kilpatrick et al. 2006a). It also provides an opportunity to incorporate a host trait-based approach into the study of vector preferences affecting pathogen amplification.

The aim of our study was to determine whether host morphological and behavioral traits are able to explain heterogeneities in mosquito feeding patterns. Forage ratios (FR) represent the relative frequency of bloodmeals taken from a bird species in relation to its relative abundance in the study area. We compared the FR of two mosquito species, *Cx. pipiens* and *Cx. restuans*, for different North American bird species as calculated by Hamer et al. (2009) using bloodmeal origins in mosquitoes and bird counts in suburban Chicago, Illinois, USA.

Methods

Data collection

We searched all databases on the ISI Web of Science (http://www.webofknowledge.com) from 1916 to April 2016 for field studies of blood-feeding patterns of mosquitoes in avian communities for the keywords "mosquito blood", "host feeding" and "host foraging". Only those studies with extensive field surveys of avian abundance and host-independent methods (i.e., non-animal baits) for mosquito collection were retained. In total, 13 references were selected as candidate studies, but only three provided data for a FR/feeding index. To analyze the effects of host phenotypic traits on mosquito feeding preference, we used data from Hamer et al. (2009). The other two studies were discarded due to their small sample sizes and methodological differences with the study by Hamer et al. (2009). Specifically, Estep et al. (2011) analyzed 528 bloodmeals from nine different mosquito species, but only 25 bloodmeals derived from eight avian species were from *Cx. restuans*. This mosquito was the only shared species studied by both Hamer et al. (2009) and Estep et al. (2011), and so the data in these two studies could not be analyzed jointly. Mendenhall et al. (2012) analyzed the origin of bloodmeals in 222 *Culex* *erraticus* in Colombia but introduced variations into the FR calculations, which complicates direct comparison of estimates with Hamer et al. (2009).

Hamer et al. (2009) analyzed the origin of bloodmeals from 611 *Cx. pipiens* and 213 *Cx. restuans*. They collected mosquitoes from mid-May to mid-October in 2005–2007 using three types of traps (i.e., light and gravid traps, and aspirators) in suburban southwest Chicago, Illinois (USA). The sampling areas included residential sites, semi-natural sites (cemeteries and a wildlife refuge), and natural sites (landscape mosaic of deforested areas, prairies, savannas, and oak-maple woodland). Bird surveys were performed twice a year from June to mid-July in each mosquito sampling site in both the natural and residential areas.

For each bird and mosquito species, these authors reported a FR calculated as the ratio between the fraction of mosquitoes that had fed on a given bird species and the fraction of the censused individuals belonging to that bird species in the study area. A FR above 1 indicates that the species is bitten more often than expected given its abundance, while FR values below 1 indicate that the species is bitten less often than expected by chance. The mean body mass, tarsus length, bill length, plumage coloration, roosting behavior, and nest type of each bird species were obtained from the literature (Lislevand et al. 2007; Jaramillo and Burke 1999; Clements 2007; del Hoyo et al. 2011; Vuilleumier 2011; Rodewald 2015) (see also Table S1). Attractiveness of bird coloration (plumage and bare parts) was scored according to Yezerinac and Weatherhead (1995). We quantified the total percentage of highly attractive (black, blue, and red; hereafter phac), moderately attractive (light red, light blue, brown, green, orange, pink, and gray; hereafter pmac), and slightly attractive colors (light brown, light green, yellow and white; hereafter psac) by summing the percentages estimated from seven plumage areas defined by Yezerinac and Weatherhead (1995): bill (3%), crown and nape (11%), chin and eye (9%), breast (23%), back, tail, and wings (41%), belly (10%), and legs (3%). The mean values of adult male and female color and morphometry were calculated to minimize the potential effect of sexual trait dimorphism. In addition, we recorded the type of nest used by each bird species and classified each in one of the following categories: cavity or hole-nest, closed nest, and open cup/nest. We also recorded the roosting behavior of these bird species during the non-breeding period in their non-wintering habitats and classified it as either solitary or communal. Although Hamer et al. (2009) conducted bird surveys at the peak of the breeding season (June–July), mosquito sampling was extended well after the breeding season had ended (until mid-October) when the individuals of some avian species aggregate in communal roosts at night as the season progresses (Diuk-Wasser et al. 2010).

Statistical analyses

We used phylogenetic generalized least square (PGLS) models to analyze the relationships between mosquito FR as the dependent variables and host phenotypic traits. Given that phylogenetically closely related bird species share certain phenotypic traits that were not considered in this study (e.g., chemical compounds associated with odor and certain behavioral traits), mosquitoes could potentially prefer to bite certain host groups. PGLS models use the phylogenetic distance between species as a covariance matrix in a linear model to statistically control for the phylogenetic relationships. We obtained a consensus tree (see Fig. S1) from 1000 trees generated on BirdTree (http://birdtree.org) by adopting a 50% majority-rule consensus tree (SumTrees 3.3.1 in DendroPy 3.12.2) using the code described by Rubolini et al. (2015). We estimated the lambda value (λ) using the maximum likelihood method, where λ is related to the strength of the phylogenetic signal (Pagel 1999) and varies between 0 (no phylogenetic signal) and 1 (strong phylogenetic signal) (Kamilar and Cooper 2013). Analyses were conducted using R software v3.2.5 (R Core Development Team 2016) with the packages *ape* v3.5 (Paradis et al. 2004), *car* v2.1 (Fox et al. 2010), and *caper* v0.5.2 (Orme 2013).

The normality of dependent and explanatory variables was examined, and all variables skewed in normal quantile plots were log-transformed. Multi-collinearity among explanatory variables was assessed before fitting the PGLS models by calculating the generalized variance inflation factors (gVIFs). The variables host body mass and pmac were highly correlated with other variables in the dataset as indicated by VIF values > 4 (O'brien 2007) and thus were not included in the PGLS models. Model selection was carried out using Akaike's information criteria (AICc) corrected for small sample sizes to identify the most parsimonious model (lowest AICc) and rank the remaining models (Burnham and Anderson 2003). Delta AICc (ΔAICc) was calculated as the difference in AICc between each model and the best model in the set. Following Burnham and Anderson (2004), we also computed the Akaike weights (ω AICc) to assess the weight of evidence in favor of each candidate model, which can range from 0 (no support) to 1 (full support). We used model averaging to summarize results (Grueber et al. 2011) derived from a global model containing all the predictors. We standardized input variables before model analysis. We then derived a set of submodels (including the null model) from the global model by using the dredge function implemented in the *MuMIn* package v1 (Bartoń 2013). We selected those models with a difference of ΔAICc < 2 to delineate a *top model set*. Finally, the variance explained by each of the selected top models was calculated as the adjusted R^2.

Results

Variation in FR between bird species was not explained by birds' phylogeny ($\lambda = 0$) in either the *Cx. pipiens* or the *Cx.*

restuans models. Since there was no phylogenetic signal in PGLS models, we re-ran the above-described analyses with generalized linear models (GLM) to perform model averaging and summarize results, a procedure that is not compatible with PGLS (hereafter only the results of the GLMs are shown).

For *Cx. pipiens*, three models were selected (Table 1) whose adjusted R^2 values were 23.51, 24.01, and 15.77%. The averaged estimates indicated that FR was positively associated with solitary roosting behavior, tarsus length, and psac, but negatively related to bill length (Table 2). The relative importance of these predictors was 1 for solitary roosting behavior, 0.81 for tarsus length, 0.81 for psac, and 0.29 for bill length. None of the 95% confidence intervals (CI) for the parameter estimates included zero, except for bill length (Table 2), indicating that the three other variables significantly influenced the FR of *Cx. pipiens* (see Fig. 1).

For *Cx. restuans*, five top models were selected (Table 3) and the variance explained by each model was 17.1, 10.16, 12.87, 18.26, and 14.5%. The averaged estimates indicated that FR was positively associated with solitary roosting behavior, psac, phac, and tarsus length (Table 4), and the relative importance of each variable was 1.00, 0.80, 0.46, and 0.32, respectively; however, the 95% CI included zero for psac, phac, and tarsus length (Table 4), thereby indicating that only solitary roosting behavior significantly influenced the FR of *Cx. restuans* (see Fig. 1).

Discussion

We identified correlates of avian phenotypic traits and the blood-feeding patterns of two *Culex* species. Although both *Cx. pipiens* and *Cx. restuans* are considered generalist and opportunistic feeders, they feed more often than expected on some avian species, while others were avoided, regardless of their relative abundance (e.g., Hamer et al. 2009; Kilpatrick et al. 2006a; Rizzoli et al. 2015). Aside from the specific competence of each avian species as a pathogen reservoir, the non-random feeding preferences of mosquitoes may influence the transmission dynamics of vector-borne diseases. This is of great importance given that *Cx. pipiens* is a widely distributed species that acts as the main vector of a number of pathogens affecting humans and wildlife (e.g., WNV, St. Louis encephalitis virus, filarial worms, and avian malaria) (Farajollahi et al. 2011; Ferraguti et al. 2013). The evidence provided here may help clarify the proximate causes of mosquito feeding patterns.

We found that solitary roosting birds were bitten more often than communally roosting species by both *Cx. pipiens* and *Cx. restuans*. Group size may influence the abundance of blood-sucking insects attracted to hosts (Martínez-de la Puente et al. 2010). Although large groups may increase host detection by mosquitoes, they also reduce the individual risk

Table 1 GLMs analyzing the variation in the forage ratio (FR) of *Cx. pipiens* in birds

Explanatory variables						Criterion		
Roosting	Nest	Bill	Phac	Tarsus	Psac	AICc	Δ*i* (AICc)	ω AICc
+				+	+	**187.0**	**0.00**	**0.216**
+		+		+	+	**188.2**	**1.18**	**0.120**
+						**189.0**	**1.99**	**0.080**
+				+		189.2	2.19	0.072
+					+	189.4	2.35	0.067
+			+	+	+	189.6	2.59	0.059
+		+		+		190.2	3.18	0.044
+			+	+		190.7	3.72	0.034
+		+	+	+	+	190.8	3.79	0.032
+			+			190.8	3.82	0.032
+		+				191.4	4.36	0.024
+		+			+	191.7	4.67	0.021
+			+		+	191.9	4.84	0.019
+	+			+	+	191.9	4.87	0.019
+		+	+	+		192.2	5.16	0.016
		+		+	+	192.3	5.30	0.015
+	+	+		+	+	192.8	5.75	0.012
+	+					193.3	6.23	0.010
+		+	+			193.3	6.27	0.009
+	+				+	193.4	6.36	0.009
+	+			+		194.0	6.99	0.007
+		+	+		+	194.3	7.28	0.006
+	+		+			194.5	7.48	0.005
		+		+		194.7	7.64	0.005
				+	+	194.7	7.68	0.005
+	+	+		+		194.7	7.70	0.005
+	+		+	+	+	194.8	7.73	0.005
		+	+	+	+	194.9	7.88	0.004
	+	+		+	+	194.9	7.92	0.004
+	+		+	+		195.2	8.14	0.004
					+	195.7	8.64	0.003
+	+	+	+	+	+	195.8	8.75	0.003
+	+	+				195.8	8.81	0.003
+	+		+		+	195.9	8.91	0.003
+	+	+			+	196.0	9.02	0.002
		+	+	+		196.2	9.16	0.002
						196.2	9.16	0.002
+	+	+	+	+		196.5	9.48	0.002
			+			197.0	9.94	0.002
			+	+	+	197.1	10.06	0.001
+	+	+	+			197.2	10.21	0.001
		+				197.3	10.30	0.001
	+	+		+		197.4	10.37	0.001
				+		197.4	10.38	0.001
	+				+	197.6	10.54	0.001
	+		+			197.6	10.58	0.001
		+			+	197.6	10.63	0.001

Table 1 (continued)

Explanatory variables						Criterion		
	+			+	+	197.7	10.65	0.001
	+	+	+	+	+	197.7	10.70	0.001
			+	+		197.8	10.75	0.001
			+		+	197.9	10.85	0.001
	+	+	+	+		198.0	10.99	0.001
	+					198.6	11.58	0.001
+	+	+	+		+	198.7	11.70	0.001
		+	+			198.8	11.73	0.001
	+		+		+	199.1	12.05	0.001
	+		+	+		199.2	12.20	0.000
	+		+	+	+	199.6	12.58	0.000
	+	+	+			199.6	12.60	0.000
	+	+			+	199.6	12.62	0.000
	+	+				199.6	12.62	0.000
		+	+		+	200.0	13.01	0.000
	+			+		200.5	13.47	0.000
	+	+	+		+	201.5	14.43	0.000

The top models are highlighted in bold. Abbreviations of explanatory variables: Roosting (roosting behavior), Nest (nest type), Bill (log. bill length), Phac (log. percentage of highly attractive colors), Tarsus (log. tarsus length), and Psac (percentage of slightly attractive colors. Δi (AICc) = [AICci − min AICc], ωi (AICc) = the rounded second-order Akaike weights. The variables included in each model are represented by crosses

of being bitten (Cresswell 1994; Janousek et al. 2014), as well as the per capita host exposure to infected vectors due to the encounter-dilution effect (Krebs et al. 2014).

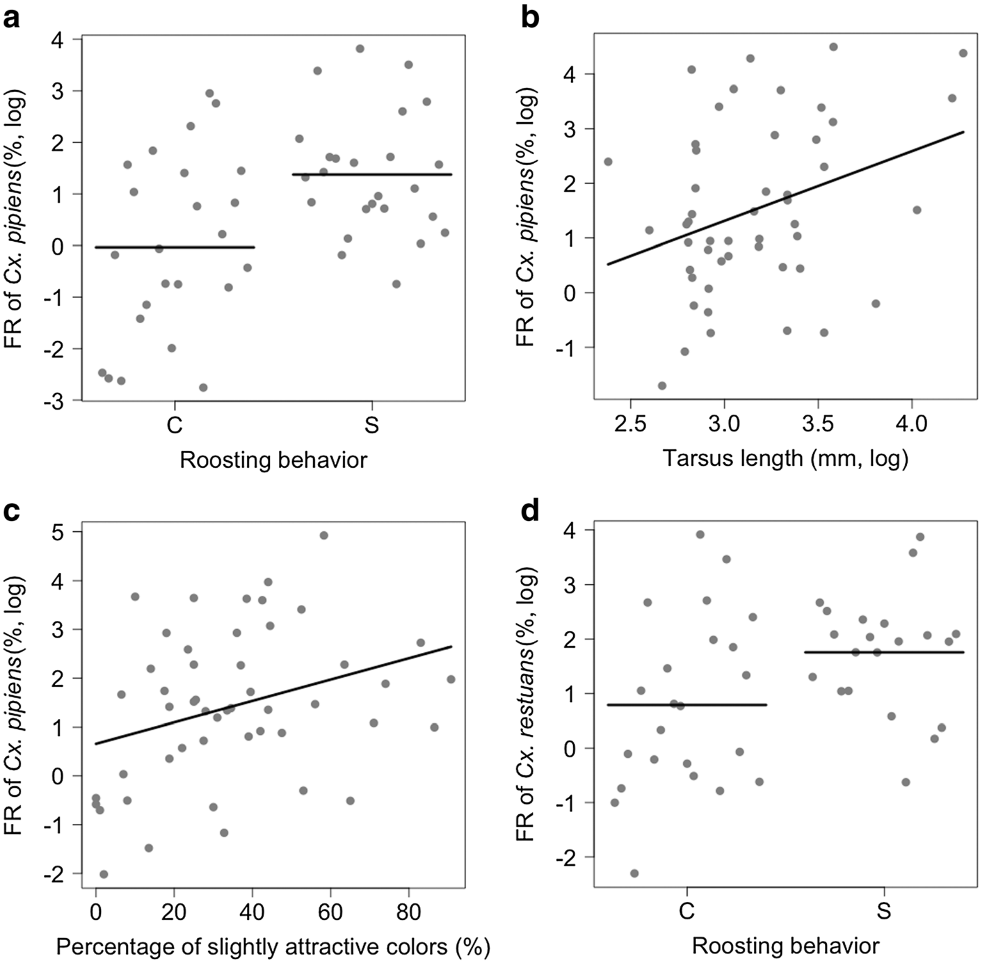
We found that *Cx. pipiens* fed more often on birds with longer tarsi, which may suggest that larger areas of exposed skin are important for determining patterns of host use. This result agrees with the findings of Walker and Edman (1985), who found that mosquito bites on rodents were restricted to areas of bare skin. Most of birds' body surfaces are covered by dense plumage, which is an efficient barrier to mosquito bites. Indeed, unfeathered parts such as tarsi and eye-rings are often targeted by blood-sucking mosquitoes (Blackmore and Dow 1958; authors pers. obs.). Additionally, avian tarsus length is usually positively related to body mass (Green 2001), as found in this study (Pearson correlation = 0.82, t = 9.88, df = 47, $p < 0.01$), and has often been used as a proxy for body size (Senar and Pascual 1997). Therefore, the positive relationship found between tarsus length and body mass suggests a positive association between body mass and mosquito FR. Similar trends have been reported in previous studies with different insect groups including blackflies (Malmqvist et al. 2004), biting midges (Martínez-de la Puente et al. 2009), and mosquitoes (Estep et al. 2012), and are also reflected by higher antibody prevalence reflecting higher previous exposure against mosquito-borne pathogens in larger birds, even after controlling for individuals age (i.e., WNV, Figuerola et al. 2008). In the case of *Cx. restuans*, we also found a positive relationship between FR and tarsus length, although it was not statistically significant. Birds with larger body size may release more cues such as CO_2 and heat, which are all known to be used by host-seeking mosquitoes (Takken and Verhulst 2013). However, visual cues (e.g., a larger silhouette) may be as important as other cues (e.g., CO_2 and heat) for host-seeking mosquitoes, at least at intermediate distances (Cardé 2015).

Contrary to our prediction, we found that birds with a greater percentage of slightly attractive colors, that is, light brown, light green, yellow, and white, were preferred by *Cx. pipiens*. As far as we know, this is the first report of a positive relationship between light-colored plumage in birds and blood-feeding by mosquitoes. Previous studies of blood-sucking mosquitoes found that darker colors were more attractive than lighter ones (Brett 1938; Brown 1954; Gilbert and Gouck 1957; Allan et al. 1987). However, Long et al. (2011) found that pure white and light gray cards attracted more insects, including small dipterans, than other tested colored cards. These contrasting results could be due to methodological differences since these studies were conducted using cloths, traps, or other colored targets (Brett 1938; Brown 1954; Gilbert and Gouck 1957; Allan et al. 1987) and not differently colored live birds moving freely in their natural environments. The initial visual detection of a host by mosquitoes relies on differences in relative brightness and color contrast (Lehane 2005). Browne and Bennett (1981) found that reflected white light attracted 12% more mosquitoes at night than in daytime, while reflected black light attracted 23% fewer mosquitoes at night than in daytime. Under conditions of poor visibility, light colors seem to attract more mosquitoes than in daytime due to the greater brightness and sharper color contrast against dark backgrounds, whereas the attractiveness of dark colors

Table 2 Summary statistics of the averaged model derived from the set of top GLM models (Δi(AICc) < 2) explaining variation in the feeding patterns of *Cx. pipiens*

Parameter	Estimate	SE	z value	95% CI		p
Intercept	0.867	0.218	3.862	0.427	1.307	< 0.001
Roosting behavior	1.355	0.462	2.860	0.426	2.284	0.004
Log. tarsus length	1.164	0.553	2.056	0.054	2.274	0.040
Percentage of slightly attractive colors	0.997	0.473	2.052	0.045	1.949	0.040
Log. bill length	− 0.695	0.610	1.108	− 1.924	0.534	0.268

Fig. 1 Relationship between forage ratios and avian host traits. **a** FR of *Cx. pipiens* and bird roosting behavior (C: communally/S: solitary). **b** FR of *Cx. pipiens* and bird tarsus length (mm). **c** FR of *Cx. pipiens* and percentage of slightly attractive colors (psac) in bird body (%). **d** FR of *Cx. restuans* and bird roosting behavior (C: communally/S: solitary). Estimates were derived from the highest-ranking models according to AICc (Tables 1 and 3). Each conditional relationship was plotted by holding all other variables in multiple regressions at their median values (continuous variables) or at their most common category (categorical variables) using the visreg packages (version 2.2.2) in R. Mean values are shown in **a** and **d**, and regression lines were plotted for **b** and **c**



decreases under these conditions (Browne and Bennett 1981). *Cx. pipiens* and *Cx. restuans* are crepuscular and/or nocturnal, and their host-seeking activity usually peaks at sunrise, sunset, or at night (Becker et al. 2010). However, they may still use visual cues for host-seeking under dark conditions when odor cues indicate a host is nearby (Hawkes and Gibson 2016). The mosquito *Aedes aegypti*, for example, has dichromatic vision, which provides it with good contrast sensitivity (Allan 1994) that allows it to detect hosts and then use other cues to finalize the search process.

Vectors link hosts and pathogens and thus have a great impact on the transmission dynamics of infectious diseases by determining the host-pathogen contact rate (Simpson et al. 2012). Growing evidence suggests that the feeding behavior of mosquitoes, although opportunistic, is not completely random, but rather a complex phenomenon that greatly depends on host abundance and behavior as well as environmental conditions (e.g., Suom et al. 2010; Thiemann et al. 2011). It will also depend on mosquitoes' preference for certain avian species, which leads to the observed heterogeneity in bloodmeals affecting the dynamics of pathogen transmission (Kilpatrick et al. 2006a). Our study highlights how host morphological and behavioral traits contribute to interspecific differences in patterns of host use by mosquitoes, and suggest that larger species roosting alone and/or of lighter colors are good candidate species for vector-borne pathogen surveillance.

Assumption/limitations

Blood acquisition patterns by mosquitoes is a complex phenomenon, which depends on mosquito flight patterns, environmental configuration, host availability in time and space, and blood-feeding success. FR is a widely used index, which takes into account the relative abundance of different hosts, but has some inherent limitations and potential biases. For example, FR will be measured with higher errors for less abundant species where a single bloodmeal may result in a high FR given the low relative abundance of the avian species. Although the density of raptors in avian communities is usually very low, their larger body mass relative to other birds may account for their over-representation in bloodmeal samples (see also Estep et al. 2012), which may give rise to an extremely high FR value according to the calculation method

Table 3 GLMs analyzing the variation in the forage ratio (FR) of *Cx. restuans* in birds. The top models are highlighted in bold

Explanatory variables						Criterion		
Roosting	Nest	Bill	Phac	Tarsus	Psac	AICc	Δ*i* (AICc)	*ω* AICc
+			+		+	**161.8**	**0.00**	**0.137**
+						**162.5**	**0.71**	**0.096**
+					+	**162.5**	**0.72**	**0.095**
+			+	+	+	**162.8**	**0.96**	**0.085**
+				+	+	**163.2**	**1.36**	**0.069**
+		+	+		+	163.9	2.12	0.047
+		+			+	164.2	2.41	0.041
+				+		164.3	2.47	0.040
			+		+	164.5	2.67	0.036
+			+			164.6	2.80	0.034
+		+				164.6	2.82	0.033
					+	164.7	2.87	0.033
+		+	+	+	+	165.6	3.78	0.021
+		+		+	+	165.9	4.05	0.018
						166.0	4.15	0.017
				+	+	166.5	4.63	0.013
			+	+	+	166.5	4.71	0.013
+			+	+		166.7	4.85	0.012
+		+		+		166.8	5.02	0.011
+		+	+			167.0	5.15	0.010
		+	+		+	167.0	5.20	0.010
		+			+	167.1	5.29	0.010
+	+					167.2	5.38	0.009
+	+		+		+	167.3	5.45	0.009
+	+				+	167.3	5.52	0.009
+	+		+	+	+	167.8	5.97	0.007
		+				168.1	6.24	0.006
+	+			+	+	168.2	6.34	0.006
			+			168.2	6.42	0.006
	+				+	168.3	6.44	0.005
				+		168.3	6.44	0.005
		+	+	+	+	168.4	6.61	0.005
		+		+	+	168.5	6.69	0.005
+	+			+		169.0	7.20	0.004
	+		+		+	169.3	7.50	0.003
+		+	+	+		169.4	7.54	0.003
+	+	+			+	169.5	7.71	0.003
	+					169.6	7.76	0.003
+	+		+			169.6	7.83	0.003
+	+	+				169.7	7.85	0.003
+	+	+	+		+	169.7	7.85	0.003
		+		+		170.1	8.28	0.002
		+	+			170.4	8.55	0.002
	+			+	+	170.4	8.57	0.002
			+	+		170.7	8.84	0.002
+	+	+	+	+	+	170.7	8.91	0.002
	+	+			+	170.9	9.12	0.001
+	+	+		+	+	171.1	9.27	0.001
	+		+	+	+	171.6	9.77	0.001
+	+		+	+		171.7	9.87	0.001
+	+	+		+		171.8	9.95	0.001
	+	+				171.8	9.98	0.001
	+		+			172.1	10.30	0.001
	+	+	+		+	172.1	10.30	0.001
	+			+		172.1	10.31	0.001
+	+	+	+			172.3	10.51	0.001
	+	+		+	+	172.4	10.57	0.001
		+	+	+		172.6	10.76	0.001
	+	+	+	+	+	173.2	11.43	0.000
	+	+		+		173.8	12.01	0.000
	+	+	+			174.5	12.67	0.000
+	+	+	+	+		174.6	12.76	0.000
	+		+	+		174.8	12.98	0.000
	+	+	+	+		176.7	14.85	0.000

Abbreviations of explanatory variables: Roosting (roosting behavior), Nest (nest type), Bill (log. bill length), Phac (log. percentage of highly attractive colors), Tarsus (log. tarsus length), and Psac (percentage of slightly attractive colors. Δ*i* (AICc) = [AICc*i* − min AICc], *ωi* (AICc) = the rounded second-order Akaike weights. The variables included in each model are represented by crosses

of FR. Other large birds such as mallards (*Anas platyrhynchos*) were bitten less, probably as a consequence of their resting position (legs and bills hidden) that limits the amount of exposed skin available to mosquitoes (Llopis et al. 2016). To account for the potential effect of extreme FR values in the dataset used in this study, we repeated our analyses but excluded two raptor species (American kestrel *Falco sparverius* and Cooper's hawk *Accipiter cooperii*) and, although results showed similar trends, only roosting behavior significantly affected the FR of *Cx. pipiens* (see Table S2). Aside from the potential impact of the resulting smaller sample size, which could explain why only roosting behavior remained significant after removing these two bird species, this may indicate that larger species were, to some extent, responsible for the detected patterns.

The interruption of mosquitoes' blood-feeding may have important consequences for the transmission of vector-borne pathogens since multiple host-feedings could increase the probability of acquiring and transmitting parasites (Davies 1990; Conway and McBride 1991; Scott et al. 1993). The methods used to trap mosquitoes can also introduce some biases, as CO_2-baited traps may collect more partially engorged mosquitoes corresponding to host species showing intense anti-mosquito behavior, while gravid traps may collect more fully engorged mosquitoes looking for breeding sites

Table 4 Summary statistics of the averaged model derived from the set of top GLM models (Δi(AICc) < 2) explaining variation in the feeding patterns of *Cx. restuans*

Parameter	Estimate	SE	*z* value	95% CI		*p*
Intercept	1.269	0.214	5.753	0.837	1.701	< 0.001
Roosting behavior	1.016	0.443	2.227	0.122	1.910	0.026
Log. percentage of highly attractive colors	0.899	0.520	1.677	− 0.152	1.949	0.094
Percentage of slightly attractive colors	1.039	0.560	1.810	− 0.086	2.164	0.070
Log. tarsus length	0.602	0.467	1.248	− 0.343	1.547	0.212

(Thiemann and Reisen 2012). To address this issue, we also ran models using FR derived from gravid traps instead of FR derived from total traps as both were used by Hamer et al. (2009), and found qualitatively identical results (see Table S3).

Additionally, phenology may have important effects on the capacity to detect significant associations with nest type or roosting behavior, as birds only occupied nest during the first months of mosquito sampling and only roosted communally on the last months of the mosquito sampling.

Given that the explanatory power of our models was relatively low, other avian traits such as the odor profiles or anti-mosquito behavior should also be considered in future studies attempting to assess interspecific differences in host selection by mosquitoes.

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Author contributions

J.Y. conducted the literature review, carried out the analyses, and drafted a first version of the manuscript; L.G. coordinated the analyses and helped collect data and draft the manuscript; J.M.P. helped collect data and draft the manuscript; R.S. coordinated and designed the study; J.F. coordinated and designed the study, and helped in manuscript drafting. All authors have given their approval for publication.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

References

Allan SA (1994) Physics of mosquito vision—an overview. J Am Mosq Control Assoc 10:266–271

Allan SA, Day JF, Edman JD (1987) Visual ecology of biting flies. Annu Rev Entomol 32:297–314. https://doi.org/10.1146/annurev.en.32.010187.001501

Bartoń K (2013) MuMIn: multi-model inference R package version 1

Becker N, Petrić D, Boase C, Lane J, Zgomba M, Dahl C, Kaiser A (2010) Mosquitoes and their control vol 2, 2nd edn. Springer. https://doi.org/10.1007/978-3-540-92874-4

Bidlingmayer W (1971) Mosquito flight paths in relation to the environment. 1. Illumination levels, orientation, and resting areas. Ann Entomol Soc Am 64:1121–1131

Blackmore JS, Dow RP (1958) Differential feeding of *Culex tarsalis* on nestling and adult birds. Mosq News 18:15–17

Brett G (1938) On the relative attractiveness to *Aedes aegypti* of certain coloured cloths. Trans R Soc Trop Med Hyg 32:113–124. https://doi.org/10.1016/S0035-9203(38)90101-4

van Breugel F, Riffell J, Fairhall A, Dickinson MH (2015) Mosquitoes use vision to associate odor plumes with thermal targets. Curr Biol 25:2123–2129. https://doi.org/10.1016/j.cub.2015.06.046

Brown A (1954) Studies on the responses of the female aëdes mosquito. Part VI—the attractiveness of coloured cloths to Canadian species. Bull Entomol Res 45:67–78. https://doi.org/10.1017/S0007485300026808

Browne SM, Bennett GF (1981) Response of mosquitoes (Diptera: Culicidae) to visual stimuli. J Med Entomol 18:505–521. https://doi.org/10.1093/jmedent/18.6.505

Burkett-Cadena ND, McClure CJ, Ligon RA, Graham SP, Guyer C, Hill GE, Ditchkoff SS, Eubanks MD, Hassan HK, Unnasch TR (2011) Host reproductive phenology drives seasonal patterns of host use in mosquitoes. PLoS One 6:e17681

Burnham KP, Anderson DR (2003) Model selection and multimodel inference: a practical information-theoretic approach, 2nd edn. Springer Science & Business Media, New York. https://doi.org/10.1007/b97636

Burnham KP, Anderson DR (2004) Multimodel inference understanding AIC and BIC in model selection. Socio Meth Res 33:261–304. https://doi.org/10.1177/0049124104268644

Cardé RT (2015) Multi-cue integration: how female mosquitoes locate a human host. Curr Biol 25:R793–R795

Clements K (2007) The encyclopedia of birds: 6 volume set. Facts On File, New York

Conway DJ, McBride JS (1991) Genetic evidence for the importance of interrupted feeding by mosquitoes in the transmission of malaria. Trans R Soc Trop Med Hyg 85:454–456

Core Development Team R (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Cresswell W (1994) Flocking is an effective anti-predation strategy in redshanks, *Tringa totanus*. Anim Behav 47: 433–442

Davies C (1990) Interrupted feeding of blood-sucking insects: causes and effects. Parasitol Today 6:19–22

del Hoyo J, Elliott A, Christie DA (2011) The handbook of the birds of the world-volume 16 tanagers to new world blackbirds. Lynx Edicions, Barcelona

Diuk-Wasser MA, Molaei G, Simpson JE, Folsom-O'Keefe CM, Armstrong PM, Andreadis TG (2010) Avian communal roosts as amplification foci for West Nile virus in urban areas in northeastern United States. Am J Trop Med Hyg 82:337–343

Edman JD, Scott TW (1987) Host defensive behaviour and the feeding success of mosquitoes. Int J Trop Insect Sci 8:617–622. https://doi.org/10.1017/S1742758400022694

Estep LK, McClure CJ, Burkett-Cadena ND, Hassan HK, Hicks TL, Unnasch TR, Hill GE (2011) A multi-year study of mosquito feeding patterns on avian hosts in a southeastern focus of eastern equine encephalitis virus. Am J Trop Med Hyg 84:718–726

Estep LK, McClure CJ, Burkett-Cadena ND, Hassan HK, Unnasch TR, Hill GE (2012) Developing models for the forage ratios of *Culiseta melanura* and *Culex erraticus* using species characteristics for avian hosts. J Med Entomol 49:378–387

Farajollahi A, Fonseca DM, Kramer LD, Kilpatrick AM (2011) "Bird biting" mosquitoes and human disease: a review of the role of *Culex pipiens* complex mosquitoes in epidemiology. Infect Genet Evol 11:1577–1585. https://doi.org/10.1016/j.meegid.2011.08.013

Ferraguti M, Martínez-de la Puente J, Muñoz J, Roiz D, Ruiz S, Soriguer R, Figuerola J (2013) Avian *Plasmodium* in *Culex* and *Ochlerotatus* mosquitoes from southern Spain: effects of season and host-feeding source on parasite dynamics. PLoS One 8:e66237. https://doi.org/10.1371/journal.pone.0066237

Figuerola J, Jiménez-Clavero MA, López G, Rubio C, Soriguer R, Gómez-Tejedor C, Tenorio A (2008) Size matters: West Nile Virus neutralizing antibodies in resident and migratory birds in Spain. Vet Microbiol 132:39–46. https://doi.org/10.1016/j.vetmic.2008.04.023

Fox J, Weisberg S, Bates D (2010) Car: companion to applied regression. R package version 2.1–2

Gilbert I, Gouck H (1957) Influence of surface color on mosquito landing rates. J Econ Entomol 50:678–680. https://doi.org/10.1093/jee/50.5.678

Green AJ (2001) Mass/length residuals: measures of body condition or generators of spurious results? Ecology 82:1473–1483

Grueber C, Nakagawa S, Laws R, Jamieson I (2011) Multimodel inference in ecology and evolution: challenges and solutions. J Evol Biol 24:699–711

Hamer GL, Kitron UD, Goldberg TL, Brawn JD, Loss SR, Ruiz MO, Hayes DB, Walker ED (2009) Host selection by *Culex pipiens* mosquitoes and West Nile virus amplification. Am J Trop Med Hyg 80: 268–278

Hamilton WD, Zuk M (1982) Heritable true fitness and bright birds: a role for parasites? Science 218:384–387. https://doi.org/10.1126/science.7123238

Hassan HK, Cupp EW, Hill GE, Katholi CR, Klingler K, Unnasch TR (2003) Avian host preference by vectors of eastern equine encephalomyelitis virus. Am J Trop Med Hyg 69:641–647

Hawkes F, Gibson G (2016) Seeing is believing: the nocturnal malarial mosquito *Anopheles coluzzii* responds to visual host-cues when odour indicates a host is nearby. Parasit Vectors 9:1

Janousek WM, Marra PP, Kilpatrick AM (2014) Avian roosting behavior influences vector–host interactions for West Nile virus hosts. Parasit Vectors 7:1–11

Jaramillo A, Burke P (1999) New world blackbirds: the Icterids. Princeton University Press, Princeton

Kamilar JM, Cooper N (2013) Phylogenetic signal in primate behaviour, ecology and life history. Philos Trans R Soc B 368:20120341

Kilpatrick AM, Daszak P, Jones MJ, Marra PP, Kramer LD (2006a) Host heterogeneity dominates West Nile virus transmission. Proc R Soc B 273:2327–2333. https://doi.org/10.1098/rspb.2006.3575

Kilpatrick AM, Kramer LD, Jones MJ, Marra PP, Daszak P (2006b) West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. PLoS Biol 4:e82

Kleiber M (1947) Body size and metabolic rate. Physiol Rev 27:511–541

Krebs BL, Anderson TK, Goldberg TL, Hamer GL, Kitron UD, Newman CM, Ruiz MO, Walker ED, Brawn JD (2014) Host group formation decreases exposure to vector-borne disease: a field experiment in a 'hotspot'of West Nile virus transmission. Proc R Soc B 281: 20141586

Lehane MJ (2005) The biology of blood-sucking in insects, 2nd edn. Cambridge University Press, New York

Lislevand T, Figuerola J, Szekely T (2007) Avian body sizes in relation to fecundity, mating system, display behavior, and resource sharing. Ecology 88:1605–1605. https://doi.org/10.1890/06-2054

Llopis IV, Tomassone L, Grego E, Serrano E, Mosca A, Vaschetti G, Andrade D, Rossi L (2016) Evaluating the feeding preferences of West Nile virus mosquito vectors using bird-baited traps. Parasit Vectors 9:479

Long C, Flint J, Lepper P (2011) Insect attraction to wind turbines: does colour play a role? Eur J Wildl Res 57:323–331

Malmqvist B, Strasevicius D, Hellgren O, Adler PH, Bensch S (2004) Vertebrate host specificity of wild–caught blackflies revealed by mitochondrial DNA in blood. Proc R Soc Lond B (Suppl) 271: S152–S155. https://doi.org/10.1098/rsbl.2003.0120

Marquardt WH (2004) Biology of disease vectors. Academic Press

Martínez-Abraín A, Esparza B, Oro D (2004) Lack of blood parasites in bird species: does absence of blood parasite vectors explain it all. Ardeola 51:225–232

Martínez-de la Puente J, Merino S, Tomás G, Moreno J, Morales J, Lobato E, Taravera S, Sarto I, Monteys V (2009) Factors affecting *Culicoides* species composition and abundance in avian nests. Parasitology 136:1033–1041. https://doi.org/10.1017/S0031182009006374

Martínez-de la Puente J, Merino S, Lobato E, Rivero-de Aguilar J, del Cerro S, Ruiz-de-Castañeda R, Moreno J (2010) Nest-climatic factors affect the abundance of biting flies and their effects on nestling condition. Acta Oecol 36:543–547. https://doi.org/10.1016/j.actao.2010.07.008

Mendenhall I, Tello S, Neira L, Castillo L, Ocampo C, Wesson D (2012) Host preference of the arbovirus vector *Culex erraticus* (Diptera: Culicidae) at Sonso Lake, Cauca Valley department, Colombia. J Med Entomol 49:1092–1102

Mooring MS, Benjamin JE, Harte CR, Herzog NB (2000) Testing the interspecific body size principle in ungulates: the smaller they come, the harder they groom. Anim Behav 60:35–45. https://doi.org/10.1006/anbe.2000.1461

Muñoz J, Eritja R, Alcaide M, Montalvo T, Soriguer RC, Figuerola J (2011) Host-feeding patterns of native *Culex pipiens* and invasive *Aedes albopictus* mosquitoes (Diptera: Culicidae) in urban zones from Barcelona, Spain. J Med Entomol 48:956–960. https://doi.org/10.1603/ME11016

Muñoz J, Ruiz S, Soriguer R, Alcaide M, Viana DS, Roiz D, Vázquez A, Figuerola J (2012) Feeding patterns of potential West Nile virus vectors in south-west Spain. PLoS One 7:1–9. https://doi.org/10.1371/journal.pone.0039549

O'brien RM (2007) A caution regarding rules of thumb for variance inflation factors. Qual Quant 41:673–690

Orme D (2013) The caper package: comparative analysis of phylogenetics and evolution in R version 0.5.2

Pagel M (1999) Inferring the historical patterns of biological evolution. Nature 401:877–884. https://doi.org/10.1038/44766

Paradis E, Claude J, Strimmer K (2004) APE: analyses of phylogenetics and evolution in R language version 3.5. Bioinformatics 20:289–290

Piersma T (1997) Do global patterns of habitat use and migration strategies co-evolve with relative investments in immunocompetence due to spatial variation in parasite pressure? Oikos:623–631

Rizzoli A, Bolzoni L, Chadwick EA, Capelli G, Montarsi F, Grisenti M, Martínez-de la Puente J, Muñoz J, Figuerola J, Soriguer R (2015) Understanding West Nile virus ecology in Europe: *Culex pipiens* host feeding preference in a hotspot of virus emergence. Parasit Vectors 8:213. https://doi.org/10.1186/s13071-015-0831-4

Rodewald P (2015) The birds of North America. Cornell Laboratory of Ornithology, Ithaca. Online: http://bna.birds.cornell.edu/BNA/ . Accessed August 2015

Rubolini D, Liker A, Garamszegi LZ, Møller AP, Saino N (2015) Using the BirdTree.org website to obtain robust phylogenies for avian comparative studies: a primer. Curr Zool 61:959–965. https://doi.org/10.1093/czoolo/61.6.959

Sawabe K, Isawa H, Hoshino K, Sasaki T, Roychoudhury S, Higa Y, Kasai S, Tsuda Y, Nishiumi I, Hisai N (2010) Host-feeding habits of *Culex pipiens* and *Aedes albopictus* (Diptera: Culicidae) collected at the urban and suburban residential areas of Japan. J Med Entomol 47:442–450. https://doi.org/10.1603/ME09256

Scott TW, Chow E, Strickman D, Kittayapong P, Wirtz RA, Lorenz LH, Edman JD (1993) Blood-feeding patterns of *Aedes aegypti* (Diptera: Culicidae) collected in a rural Thai village. J Med Entomol 30:922–927

Senar J, Pascual J (1997) Keel and tarsus length may provide a good predictor of avian body size. Ardea Wagening 85:269–274

Simpson JE, Hurtado PJ, Medlock J, Molaei G, Andreadis TG, Galvani AP, Diuk-Wasser MA (2012) Vector host-feeding preferences drive transmission of multi-host pathogens: West Nile virus as a model system. Proc R Soc B 279:925–933. https://doi.org/10.1098/rspb.2011.1282

Suom C, Ginsberg HS, Bernick A, Klein C, Buckley P, Salvatore C, LeBrun RA (2010) Host-seeking activity and avian host preferences of mosquitoes associated with West Nile virus transmission in the northeastern USA. J Vector Ecol 35:69–74

Takken W, Verhulst NO (2013) Host preferences of blood-feeding mosquitoes. Annu Rev Entomol 58:433–453. https://doi.org/10.1146/annurev-ento-120811-153618

Tempelis C, Reeves W, Bellamy R, Lofy M (1965) A three-year study of the feeding habits of *Culex tarsalis* in Kern County, California. Am J Trop Med Hyg 14:170–177

Thiemann TC, Reisen WK (2012) Evaluating sampling method bias in *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae) bloodmeal identification studies. J Med Entomol 49:143–149

Thiemann TC, Wheeler SS, Barker CM, Reisen WK (2011) Mosquito host selection varies seasonally with host availability and mosquito density. PLoS Negl Trop Dis 5:e1452

Vuilleumier F (2011) Birds of North America: western region. Dorling Kindersley Publishing, New York

Walker ED, Edman JD (1985) Feeding-site selection and blood-feeding behavior of *Aedes triseriatus* (Diptera: Culicidae) on rodent (Sciuridae) hosts. J Med Entomol 22:287–294. https://doi.org/10.1093/jmedent/22.3.287

Yezerinac SM, Weatherhead PJ (1995) Plumage coloration, differential attraction of vectors and hematozoa infections in birds. J Anim Ecol 64:528–537. https://doi.org/10.2307/5655