Ecology of Vector-Borne Diseases:

Effects of Host Traits on Mosquito Feeding Preferences and Its Implications for Disease Transmission

PhD Thesis
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Ecology of vector-borne diseases: effects of host traits on mosquito feeding preferences and its implications for disease transmission

A dissertation submitted
to the University of Seville
in partial fulfillment of the requirements
for the degree of
Doctor of Philosophy

Jiayue Yan
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I certify that I have read this dissertation and that, in my opinion, it is fully adequate in scope and quality as a dissertation for the degree of Doctor of Philosophy.

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Mosquito and bird sampling were conducted with all the necessary permits from the regional Department of the Environments (Consejería de Medio Ambiente, Junta de Andalucía). Entomological surveys and bird sampling on private land were conducted with all the necessary permits and consent, and in the presence of owners.

Contents

Summary

Last decades have seen an accelerated emergence or re-emergence of vector-borne diseases (VBDs) in many geographic areas, representing a major threat to public health and biodiversity conservation involving human, wildlife and domestic animals. Despite the medical and economic impact of VBDs, the ecology of VBDs, especially the interaction between hosts and vectors is still poorly understood. Previous studies on epidemiology assumed a random host-vector interaction and ignored the potential effects of heterogeneity in host traits, such as individual´s health status and evolutionary history, on the transmission rates of pathogens. Failure to recognize and incorporate the potential effects of host traits into epidemiological models could result in biased estimates of disease transmission dynamics. Therefore, the four chapters included in this thesis focus on exploring the effects of host trait heterogeneity on host-vector interactions at both inter- and intra-specific levels.

Although some vector-borne pathogens (VBPs) cause human diseases, most of them are zoonotic with wildlife serving as their primary vertebrate hosts. Mosquitoes are primary vectors of many VBPs, such as avian *Plasmodium* and West Nile virus (WNV). Given the important role of mosquitoes and wildlife hosts in the transmission cycle of VBPs, this thesis mainly used a mosquito-borne pathogen, i.e. avian *Plasmodium*, as the study system, including some of their natural vectors *Culex pipiens* and *Culex restuans* and vertebrate hosts, i.e. the House Sparrow (*Passer domesticus*). Host traits may have profound influence on avian host-mosquito vector interaction, as host-seeking mosquitoes may rely on some host traits to locate potential hosts, resulting in mosquito feeding preferences that produce heterogeneous host-vector contact rates.

Host-seeking activity of mosquitoes is an integrative process that involves multiple senses to detect potential hosts. In this thesis, I first reviewed the role of host morphological, behavioral and physiological traits in mosquito attraction. Mosquito host selection is the outcome of the interaction between innate host preference and extrinsic factors including host attractiveness and availability. Mosquito feeding preference can be readily affected by different host traits producing variations in olfactory, visual, thermal and other cues used in the host-seeking process. I identified knowledge gaps on the role of different host traits in mosquito feeding preference, and highlighted the need of both theoretical and empirical studies on such topic.

To identify the effect of host morphological and behavioral traits on mosquito feeding preference, I conducted a comparative study by combining data of mosquito forage ratios in an avian community from North America with data of avian morphology and behavior. Birds with lighter colors, bigger body size and solitary

roosting behavior were bitten more often than expected from their relative abundance in the community. This study highlights the role of host morphological and behavioral traits in interspecific differences in host use patterns of mosquitoes. Future surveillance program for VBPs could consider the larger species roosting alone and/or of lighter colors as good candidates as focal species.

To figure out the role of host physiological traits in mosquito blood-feeding preference, I performed dual-choice experiments by exposing House Sparrows with different metabolic rates to blood-seeking *Cx. pipiens* and analyzed the relationship between mosquito feeding preference and host metabolism. Individual birds with lower resting metabolic rates were bitten more often than their conspecific counterparts by mosquitoes. This represents the first experimental evidence of a link between host metabolism and vector feeding preference. In addition, birds with greater body mass were bitten more frequently by mosquitoes. As *Cx. pipiens* is a key vector for multiple VBPs, identifying traits affecting the feeding preference of this mosquito species may throw some light on the epidemiology of these pathogens.

To assess the impact of host infection on blood feeding patterns of mosquitoes, I carried out two dual-choice experiments by exposing House Sparrows with different infection status (i.e. *Plasmodium*-infected vs. uninfected) and with different infection intensity (manipulated through a medication treatment to reduce the parasite load, i.e. higher *Plasmodium* load vs. lower – treated- *Plasmodium* load) to blood-seeking mosquitoes respectively. Individual birds infected with *Plasmodium* were bitten more often than those infected but treated counterparts. However, the infection status of birds did not significantly affect mosquito feeding pattern, with *Plasmodium*-infected and uninfected birds bitten similarly by mosquitoes. Our findings partially support the parasite manipulation hypothesis, which probably operates via a reduction in defensive behavior in more intensively infected birds, and highlights the importance of considering parasite load in studies on host-vector-pathogen interactions.

By using interdisciplinary approaches that combine comparative methods, molecular analyses as well as empirical bioassays, this thesis identifies several key links between host traits and mosquito feeding preferences at both inter- and intra-specific levels, which may help to better understand the dynamics of host-vector contact rates and hence, the transmission dynamics of VBPs. Incorporating heterogeneity from host traits in future studies may improve our understanding on the ecology of VBDs as well as the surveillance and control efforts in the complex transmission network of VBDs.

Resumen

Las últimas décadas han presenciado una acelerada emergencia o el resurgimiento de enfermedades transmitidas por vectores (ETVs) en muchas áreas geográficas, afectando a humanos, animales salvajes y domésticos, lo que representa una gran amenaza para la salud pública y la conservación de la biodiversidad. A pesar del impacto médico y económico de las ETVs, la ecología de estas, especialmente las interacciones entre los hospedadores y los vectores, aún son insuficientemente conocidas. Los estudios epidemiológicos previos han asumido tradicionalmente una interacción aleatoria entre hospedadores y vectores, ignorando los posibles efectos de la heterogeneidad en las características del hospedador, tales como el estado de salud del individuo y la historia evolutiva, en las tasas de transmisión de patógenos. La falta de reconocimiento e incorporación de los posibles efectos de las características del huésped en los modelos epidemiológicos podría dar lugar a estimaciones sesgadas de la dinámica de transmisión de ETVs. Por lo tanto, los cuatro capítulos incluidos en esta tesis se centran en el estudio de los efectos de la heterogeneidad de ciertos rasgos del hospedador en las interacciones hospedador-vector a nivel tanto inter como intra-específico.

Aunque algunos patógenos transmitidos por vectores (PTVs) causan enfermedades en humanos, la mayoría de ellos son zoonóticos, siendo la fauna silvestre el hospedador vertebrado primario. Los mosquitos son vectores primarios de muchos PTVs, como el *Plasmodium* aviar y el virus de West Nile (WNV). Dado el importante papel de los mosquitos y los hospedadores silvestres en el ciclo de transmisión de los PTVs, en esta tesis se utilizó principalmente un patógeno transmitido por los mosquitos, el *Plasmodium* aviar, sus vectores naturales *Culex pipiens* y *Culex restuans* y el hospedador vertebrado Gorrión Común (*Passer domesticus*) como sistema de estudio. Las características del huésped pueden tener una profunda influencia en la interacción entre este y los mosquitos, ya que los mosquitos basan su búsqueda de alimento en ciertas características del hospedador, lo que resulta en una preferencia de alimentación que da lugar a tasas de contacto huésped-vector heterogéneas.

La actividad de búsqueda de hospedador por parte de los mosquitos es un proceso integrador que combina múltiples señales. En esta tesis, en primer lugar revisé el papel de las características morfológicas, comportamentales y fisiológicas del huésped en la atracción de los mosquitos. La preferencia de alimentación de los mosquitos es el resultado de las interacciones entre la preferencia por determinados huéspedes y los factores extrínsecos, incluido el atractivo y la disponibilidad de los mismos. La preferencia puede verse afectada por la variación en las características del

hospedador, a través de señales olfativas, visuales, y térmicas, entre otras, utilizadas en el proceso de búsqueda de los huéspedes. Las lagunas aún existentes en el conocimiento sobre los efectos de la heterogeneidad de las características del huésped en la preferencia de alimentación de mosquitos ponen de manifiesto la necesidad de realizar estudios específicos tanto teóricos como empíricos sobre este tema.

Para determinar el efecto de las características morfológicas y comportamentales del huésped en la preferencia de alimentación de los mosquitos, llevé a cabo un estudio comparativo mediante la combinación de datos sobre tasas de alimentación de mosquitos en una comunidad de aves de América del Norte con datos de morfología y comportamiento de dichas aves. Las aves con plumaje de coloración más clara, tamaño corporal más grande y comportamiento de descanso solitario fueron picadas con mayor frecuencia de lo esperado en función de su abundancia relativa en la comunidad. Este estudio destaca el papel de las características morfológicas y comportamentales del huésped en las diferencias inter-específicas en los patrones de alimentación de los mosquitos. Estos resultados resultan de utilidad en la aplicación de programas de vigilancia de PTVs, pudiendo centrarse en especies de aves más grandes, con hábitos de descanso solitarios y / o de colores más claros como especies focales.

Para determinar el papel de las características fisiológicas del huésped en la preferencia de la alimentación de los mosquitos, realicé experimentos de selección doble, consistente en exponer dos gorriones con diferentes tasas metabólicas a *Cx. pipiens* y analicé la relación entre las preferencias de alimentación del mosquito y el metabolismo del hospedador. Las aves con tasas metabólicas basales más bajas fueron picadas por mosquitos con mayor frecuencia que sus conspecíficos. Esto representa la primera evidencia experimental del vínculo entre el metabolismo del huésped y la preferencia de alimentación del vector. Además, las aves con mayor masa corporal fueron picadas con mayor frecuencia por los mosquitos. Como *Cx. pipiens* es un vector clave para múltiples PTVs, la identificación de las características que afectan a la preferencia de alimentación de esta especie de mosquito puede arrojar luz sobre la epidemiología de estos patógenos.

Para evaluar el impacto de infección del huésped por el patógeno *Plasmodium* en los patrones de alimentación de los mosquitos, llevé a cabo dos experimentos de selección doble. Así, expuse dos gorriones con diferente estado de infección (es decir, infectados por *Plasmodium* y no infectados) y con diferente intensidad de infección (a través de un tratamiento que reduce la carga parasitaria, es decir, una carga de *Plasmodium* más alta frente a una carga más baja) a los mosquitos en búsqueda de alimento. Las aves infectadas con *Plasmodium* fueron picadas con mayor frecuencia que aquellas infectadas aunque medicadas. Sin embargo, el estado de infección de las aves en sí mismo no afectó significativamente el patrón de alimentación de los mosquitos, siendo las aves infectadas por *Plasmodium* y no infectadas picadas de manera similar por los mosquitos. Estos resultados apoyan, al menos parcialmente, la hipótesis de la manipulación parasitaria, la cual probablemente opera a través de una reducción en el comportamiento defensivo del hospedador, y destacan la importancia de considerar la carga parasitaria en estudios sobre las interacciones huésped-vector-patógeno.

Mediante el uso de un enfoque interdisciplinar que combina estudios comparativos, análisis moleculares y aproximaciones experimentales, esta tesis logra identificar varias relaciones clave entre las características del huésped y la preferencia de alimentación de los mosquitos, a nivel tanto inter como intra-específico, lo que puede ayudar a comprender mejor la dinámica de tasas de contacto entre hospedadores y vectores y, por tanto, la dinámica de transmisión de PTVs. La incorporación de la heterogeneidad de las características del huésped en futuros estudios sobre la ecología de las ETVs puede mejorar nuestro esfuerzo de vigilancia y control de la compleja red de transmisión de ETVs.

General introduction

Vector-borne diseases and host traits

Vector-borne diseases (VBDs) are caused by a broad array of pathogens that are mostly transmitted by arthropod vectors including mosquitoes, ticks, flies, fleas, midges and mites (Little, 2014). Last decades have seen an accelerated emergence or re-emergence of VBDs in many geographic areas, representing a major threat to public health and biodiversity conservation involving humans, wildlife and domestic animals (Daszak et al., 2000; Taylor et al., 2001; Harrus and Baneth, 2005; Tabachnick, 2010; Kilpatrick and Randolph, 2012). For example, 300 million malaria, 50-100 million dengue and 120 million filariasis cases were annually reported worldwide according to the World Health Organization (WHO, 2009a; WHO, 2009b; WHO, 2000). Overall, these diseases account for 17% of the estimated global infectious-disease burden (Tabachnick, 2010). VBDs have become increasingly important due to the geographic expansion of vector-borne pathogens and their vectors (Little, 2014).

Vector-borne pathogens (VBPs) usually fall into four main categories of microorganisms, that is, viruses, bacteria (including rickettsia), protozoa and nematodes. Biological transmission is the most important way for the circulation of these pathogens. Some VBPs circulate between humans, but others mainly infect wildlife, with humans and domestic animals acting as incidental hosts (Jones et al., 2008; Gubler, 2009). Mosquitoes are primary vectors of many VBPs, such as *Plasmodium*, including avian malaria parasites, and West Nile virus (WNV) (Farajollahi et al., 2011). Some examples of mosquito-borne diseases are listed in Table 1.

Table 1. Some examples of mosquito-borne diseases. Adapted from Hunter 2003 and Gubler 2009.

Pathogen	Disease	Primary vectors	Main hosts	Geographical distribution
Protozoa				
Plasmodium	Malaria	*Anopheles* and *Culex* spp.	Humans, mammals, birds, reptiles	Worldwide
Nematodes				
Dirofilaria	Dirofilariasis	Various mosquito spp.	Canids, felids	Worldwide
Viruses				
Alphavirus				
Eastern Equine encephalitis virus	Eastern equine encephalitis	Various mosquito spp.	Birds	Americas
Flavivirus				

Table 1 (Continued)

Pathogen	Disease	Primary vectors	Main hosts	Geographical distribution
Japanese encephalitis virus	Japanese encephalitis	*Culex* spp.	Birds	Japan, Far East
Mayaro virus	Mayaro	Various mosquito spp.	Birds	South America
Murray Valley encephalitis virus	Murray Valley encephalitis	*Culex annulirostris*	Birds	Australia, New Guinea
Rocio virus	Rocio viral encephalitis	Various mosquito spp.	Birds	South America
Ross River virus	Ross River fever	Various mosquito spp.	Marsupials, humans	Australia, South Pacific
Saint Louis encephalitis virus	St. Louis encephalitis	*Culex* spp.	Birds	Americas
Sindbis virus	Sindbis fever	Various mosquito spp.	Birds	Asia, Africa, Australia, Europe, Americas
West Nile virus	West Nile fever	*Culex* spp.	Birds	Africa, Europe, North America and India
Western equine encephalitis virus	Western equine encephalitis	*Culex and Culiseta* spp.	Birds, rabbits	Africa, Asia, Europe, Americas
Yellow fever virus	Yellow fever	*Aedes aegypti*	Primates, humans	Africa, South and Central America
Phlebovirus				
Rift Valley fever virus	Rift Valley fever	*Culex* and *Aedes* spp.	Mammals, bats and humans	Africa

The transmission dynamics of VBPs may be affected by many extrinsic factors that influence the life history and distribution of hosts, vectors and pathogens, such as climate change (Dobson and Carper, 1992; Githeko et al., 2000; Reiter, 2001; Gage et al., 2008), biodiversity loss (Lips et al., 2006; Keesing et al., 2013) and land-use changes (Norris, 2004; Patz et al., 2004; McFarlane et al., 2013; Ferraguti et al., 2016). Nonetheless, the ecological interactions within the 'disease triangle' formed by hosts, vectors and pathogens (see Figure 1) may largely drive the epidemiology of VBDs, as these interactions directly determine the host-vector contact rates and hence, the pathogen transmission rates (Nuttall et al., 2000; Tabachnick, 2010). Intensive efforts have been devoted to study the prevalence of VBPs in host communities and their pathology in both vertebrate hosts and insect vectors. Nonetheless, factors influencing the interactions between hosts and vectors have been poorly studied. In addition, most studies have assumed that host-vector interactions occur in a random manner. However, host traits affecting mosquito feeding preferences can produce important heterogeneities in the frequency of interactions between hosts and vectors. For example, host body size has been shown to be positively related with the attraction of many vectors, such as mosquitoes (Port et al., 1980; Estep et al., 2012), biting midges

Culicoides (Martínez-de la Puente et al., 2009) and blackflies (Malmqvist et al., 2004; Martínez-de la Puente et al., 2010a). Similarly, other physiological factors including hosts' infection with VBPs may determine the contact rate between vertebrate hosts and insect vectors as shown by Cornet et al. (2013a, b), who reported that *Plasmodium*-infected birds attracted significantly more mosquito bites than their uninfected counterparts. In extreme cases, a few individuals could account for a majority of pathogen transmission contacts, and this has given rise to the concept of superspreaders (Paull et al., 2012; VanderWaal and Ezenwa, 2016). Failure to recognize and, subsequently, incorporate host trait heterogeneity into epidemiological models could result in biased estimates of disease transmission dynamics.

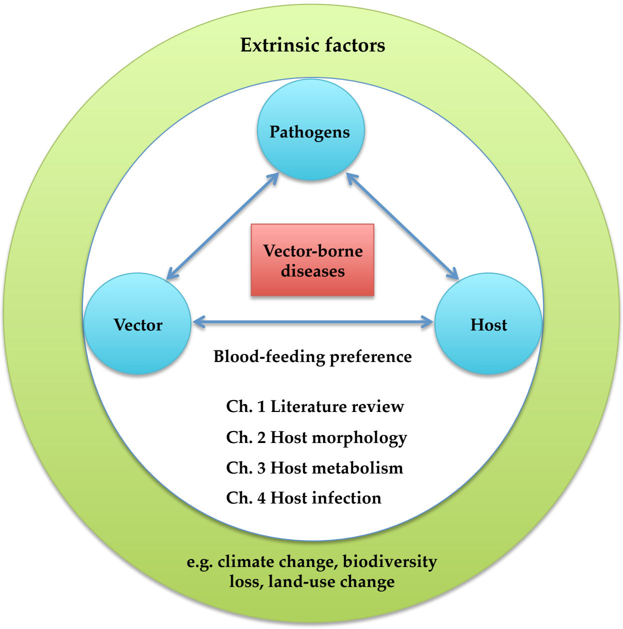

Figure 1. Scheme of the ecological interactions within the disease triangle that drives the transmission of vector-borne diseases (VBDs). Extrinsic variables may influence the abundance and distribution of hosts, vectors and pathogens, but intrinsic factors such as host-vector interactions will directly drive the transmission of VBDs. This thesis mainly focuses on exploring the effects of host-vector interactions. Chapters included in this thesis are reported as 'Ch.' followed by corresponding numbers. In particular, Chapter 1 identifies the role of different host traits in influencing mosquito feeding preferences; Chapter 2, 3 and 4 focus on the effects of host phenotypic and behavioral traits, metabolic traits and the infection on the mosquito feeding preference, respectively.

Host traits may strongly influence vector attraction by affecting their host-seeking behavior. Mosquitoes use olfactory, visual and thermal cues to locate and approach to their vertebrate hosts (Takken and Verhulst, 2013). Under limited light conditions when the activity of many mosquito species peaks (Chiba et al., 1982;

Anderson et al., 2007), olfactory cues emitted by hosts are thought to be the primary stimuli for host-seeking mosquitoes (Bowen, 1991; Smallegange and Takken, 2010). Carbon dioxide (CO_2) is the most important olfactory stimulus for host-seeking mosquitoes, which could be detected by mosquitoes from a long range (>10 m) (Gillies 1980; van Breugel et al., 2015). In addition to CO_2, many host volatile compounds, such as octenol (Kline et al., 2007), nonanal (Syed and Leal, 2009) and the waxy secretion of the uropygial gland (Russell and Hunter, 2005) have been reported to attract mosquitoes in an intermediate to close range. Visual cues, such as color, body size and motion, may also play an important role in mosquito attraction, as mosquitoes may detect these cues in a range between 1- 10 m (van Breugel et al., 2015; Cardé, 2015). The compound eyes of diurnal mosquitoes are well developed to detect visual cues; even nocturnal species are sensitive to twilight in the night (Lehane, 2005; Land et al., 1997; Land et al., 1999). Besides, heat and moisture produced by host metabolism may serve as close range cues (< 1m) for host seeking mosquitoes, which guides the final location of suitable biting sites for mosquitoes (van Breugel et al., 2015; Cardé, 2015). While many studies assessed the role of single cues from hosts in isolation or in association with CO_2, few have explored the effects of the natural combination of all available host cues on mosquito attraction. The release of these cues could be greatly affected by host metabolism, infection, as well as host morphology. In addition, both intra- and inter-specific variation in host traits may affect mosquito feeding patterns. For example, *Culex pipiens* mosquitoes preferably fed on birds with greater body mass across different species (Yan et al., 2017a), and with higher parasite loads at intra-specific level (Yan et al., 2017b). Despite its importance for understanding the ecology of VBDs, few studies have explored the role of host traits in mosquito feeding preference at both intra- and inter-specific levels.

Mosquito feeding preference is a highly complex phenomenon, which could be affected by both innate host preference and host availability (Takken and Verhulst, 2013). The innate host preference by mosquitoes may be determined by both genetics and the detectability of host cues, including visual, olfactory and thermal cues (Takken and Verhulst, 2013; Cardé et al., 2015; van Breugel et al., 2015). In the field, however, the innate host preference may be readily overruled by host availability, given that many mosquitoes are opportunistic and use variable blood sources across different seasons and locations (Kilpatrick et al., 2006a; Takken and Verhulst, 2013). Thus, field data on mosquito feeding patterns without considering host availability may provide a biased estimate of mosquito feeding preference, as host availability under natural conditions can greatly vary across different host species, habitats and seasons.

Bioassays conducted under controlled laboratory conditions with equally abundant hosts could be a more accurate way to identify mosquito feeding preferences in order to determine the importance of particular host traits on host selection by mosquitoes. To do that, different approaches have been used including studies based on "Y" two stimuli choice olfactometers. However, this method had some technical difficulties, such as the lack of mosquito reaction to the stimuli (e.g., Lalubin et al., 2012). In addition, the number of mosquitoes attracted in an olfactometer may not represent the natural feeding preference owing to the lack of interactions between hosts and mosquitoes, especially host defensive behavior (Darbro and Harrington, 2007). Thus, direct night exposure of equally abundant and freely moving birds to unfed mosquitoes combined with blood-meal analyses could be a less biased way to identify mosquito feeding preferences. This approach allows the biological interactions between birds and mosquitoes as well as simulates a common decision-making situation of mosquitoes according to different characteristics of hosts.

The biology of the studied host-vector-pathogen system

The vertebrate hosts

The main study model used as vertebrate host in this thesis is the wild House Sparrow (*Passer domesticus*), as this species is a natural reservoir for multiple VBPs (Komar et al., 2001; Arrigo et al., 2010; LaPointe et al., 2012) and has been reported to be one of the preferred hosts of several mosquito species, including *Culex pipiens* (Hamer et al., 2008; Muñoz et al., 2011). The House Sparrow is a small and widespread songbird species with a geographic distribution including almost all continents of the planet (Anderson, 2006; Clements, 2007; Summers-Smith et al., 2017). Their body length is about 16 cm and the body mass ranges from 24 to 39.5 g. House Sparrows are commonly seen in human settlements but also can be found in a variety of habitats. They are well-known amplification reservoirs for many avian pathogens, such as avian *Plasmodium* and WNV (Hamer et al., 2009; Nemeth et al., 2009; Tompkins and Gleeson, 2006; Loiseau et al., 2011). Over 40 lineages belonging to avian *Plasmodium* and closely-related haemosporidians have been reported in House sparrows to date and the prevalence of *Plasmodium* infection in this species is usually very high (Marzal et al., 2011, Ferraguti, 2017). On the other hand, House Sparrows have also been confirmed as competent hosts for the transmission of WNV (Pérez-Ramírez et al., 2014). Although the House sparrow is the main study model in this thesis, Chapter 1 uses morphological and behavioral data from 49 avian species from North America.

The vectors

Mosquitoes (Diptera: Culicidae), with 111 genera containing 3557 species worldwide (according to Mosquito Taxonomic Inventory: http://mosquito-taxonomic-inventory. Info; accessed 8 September 2017), are at the center of entomological studies because of their main role as vectors of a large number of viral and parasitic pathogens affecting humans, livestock and wildlife (Reinert et al., 2009; Becker et al., 2010). Many *Culex* species, for example, feed mainly on birds and thus, can transmit many avian pathogens, such as avian *Plasmodium*, WNV and St. Louis encephalitis virus (Farajollahi et al., 2011; Ferraguti et al., 2013). The studied insect vectors , i.e. *Culex restuans* and mainly *Cx. pipiens* (Figure 2), are predominately crepuscular/nocturnal species showing a host-seeking behavior usually peaking at twilight conditions (e.g. sunset, sunrise and starlit nights). However, these species may remain active during daytime (Allan et al., 1987; Becker et al., 2010). Both species are generally opportunistic, feeding mainly on different bird species (Martínez-de la Puente et al. 2015; 2016), but also on mammals (Burkett-Cadena et al., 2011) including humans (Kilpatrick et al., 2006a) under the pressure of limited host availability (e.g. Ferraguti et al., 2013).

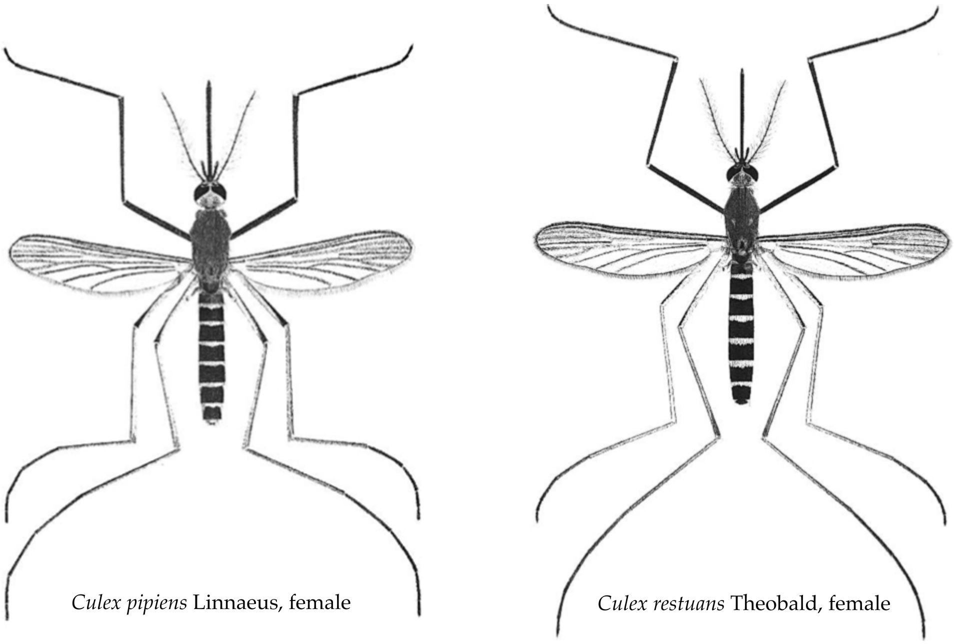

Figure 2. Morphology of two vector species of avian *Plasmodium* and West Nile virus (Modified from Carpenter 1965).

The pathogens

Avian *Plasmodium* are a group of vector-borne pathogens that cause malaria in many avian families (Valkiūnas, 2005). There is a great diversity of avian *Plasmodium* lineages all over the world with the highest diversity found in low latitude tropical areas (Clark et al., 2014). The life cycle of avian *Plasmodium* is very complex, including sexual stages of reproduction in mosquito vectors and asexual stages in bird hosts (Valkiūnas, 2005). Briefly, the sexual stages begin with the blood feeding mosquitoes that ingest the blood meal as well as gametocytes from an infected bird. These gametocytes develop to gametes and fuse as zygotes to form ookinetes that can penetrate into the midgut wall of mosquitoes. In the inner wall of midgut, ookinetes develop into oocytes. Sporozoites developed within oocysts may release from the midgut cells and invade into the salivary gland of mosquitoes. A new bite of the mosquitoes that survive long enough until the emergence of sporozoites can pass the parasites onto birds (Figure 3). The asexual stages in birds start from the sporozoites, which can go through three types of merogony to develop into merozoites, that is: i) primary exoerythrocytic merogony that takes place in reticuloendothelial cells; ii) erythrocytic merogony that takes place in erythrocytes; and iii) secondary exoerythrocytic merogony that takes place in endothelial cells (Figure 3). Merozoites can further develop into macrogametocyte and microgametocyte in the blood stream of birds.

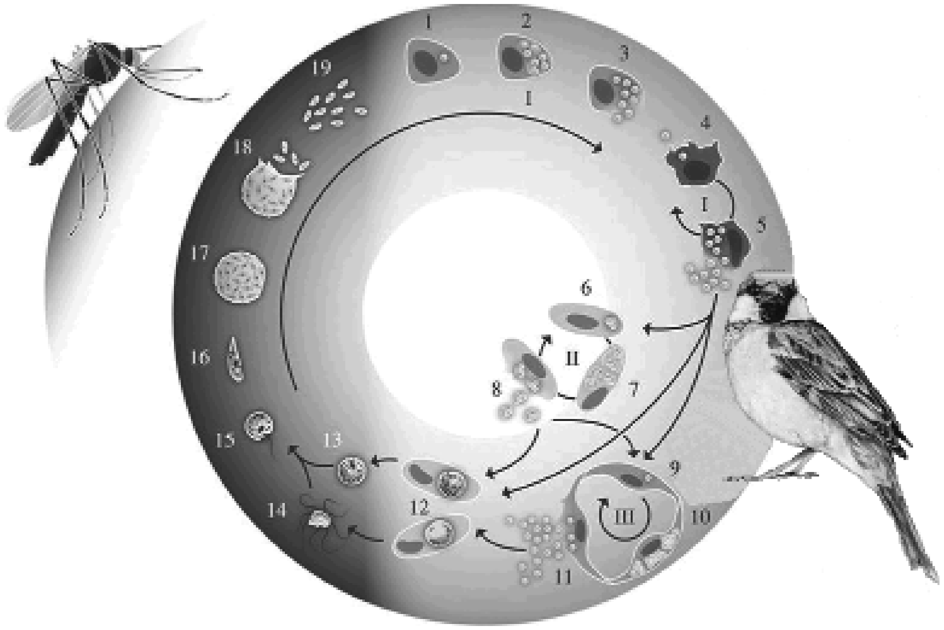

Figure 3. Life cycle of the avian malaria parasite *Plasmodium relictum* (modified from Grilo et al. 2016). Stages 1-12 in birds and stages 13-19 in mosquitoes. I (1-5): Sporozoites from mosquitoes invading reticuloendothelial cells of birds via mosquito bites and developing to merozoites; II (6-8): merozoites invading erythrocytes and cycling to reproduce more merozoites; III (9-11): merozoites invading endothelial cells and cycling to reproduce more merozoites; 12: macrogametocytes and microgametocytes; 13 and 14: gametocytes invading mosquitoes via blood feeding and developing to macrogametes and exoflagellation of microgametes; 15: zygotes; 16: ookinetes; 17 and 18: oocysts in mosquito midgut; 19: sporozoites in the salivary glands of mosquitoes.

Avian haemosporidians have been studied intensely by parasitologists in major zoogeographic regions. In the case of *Plasmodium* spp., they have often been used as model systems to the study of human malaria, as they are closely related to those producing this disease (Atkinson and van Riper III, 1991; Valkiūnas, 2005; García-Longoria et al., 2016). Avian haemotozoa, including *Plasmodium,* are diverse, widespread and abundant, and are easily sampled without disrupting the host population. Thus, they are valuable model systems for the study of VBPs (Valkiūnas, 2005). The effects of avian haemotozoa on wildlife hosts have been intensely studied, particularly to determine the effects of parasites on natural populations and hence, their epidemiological implications (Merino et al., 2000; Knowles et al., 2010; Wood et al., 2007; Lachish et al., 2011; Marzal et al., 2005; Asghar et al., 2015). A number of studies have characterized the diversity of malaria infections in avian hosts (Bensch, et al., 2000; Fallon et al., 2003; Fallon et al., 2005; Latta and Ricklefs, 2010; Ricklefs et al., 2005; Beadell et al., 2004; Beadell et al., 2009). However, factors affecting parasite transmission through host-vector interactions are poorly studied. Phenotypic changes in hosts induced by pathogen infections have been reported to enhance vector attraction in plants (Eigenbrode et al., 2002; Shapiro et al., 2012), invertebrates (Stafford et al., 2011) and vertebrates (O'Shea et al., 2002; De Moraes et al., 2014), including humans (Lacroix et al., 2005; Batista et al., 2014). This supports the parasite manipulation hypothesis that states that parasites/pathogens may adaptively alter host traits in a way to increase host-vector interactions and ultimately, the transmission rates of the parasites/pathogens. To date, few studies have investigated the effects of avian *Plasmodium* infection on mosquito attraction (but see Cornet et al., 2013 a, b; Lalubin et al., 2012). Avian *Plasmodium* spp. and related lineages are highly abundant worldwide, as 38 valid species were recognized (Valkiūnas, 2005) and 488 recognized *cytochrome b* lineages were described (Clark et al., 2014). Among others, *Plasmodium relictum* SGS1 is thought to be one of the most common avian *Plasmodium* lineages in birds and mosquitoes in Europe (Schoener et al., 2017), and this is the case in this thesis. *Plasmodium relictum* SGS1 has been reported to occur in a number of hosts from all continents except Antarctica (Bennett et al., 1993; Beadell et al., 2006). In addition, many ornithophilic mosquito species such as *Cx. pipiens* are the natural vectors for this parasite (Santiago-Alarcon et al., 2012). Thus, it represents an ideal study system for investigating the effects of host infection on mosquito feeding behavior.

Objectives

A wealth of previous models for the transmission dynamics of infectious diseases assumed the existence of homogeneous host communities and considered that different individual hosts have the same contact rates with their vectors, and hence, host-vector interactions are often thought to be random (Aron and May, 1982; Anderson et al., 1992; Grenfell and Dobson, 1995). These models tended to ignore the effects of host-trait heterogeneity on vector feeding preferences. However, growing evidence have shown that host-vector contact rates are not completely random, and vectors may prefer to feed on certain hosts displaying some traits that make them more attractive to vectors (Kingsolver, 1987; Burkot, 1988; Lacroix et al., 2005; Cornet et al., 2013a; Yan et al., 2017a, b). Therefore, the impact of vector feeding preferences based on host characteristics on disease transmission is poorly understood. Incorporating host traits in this thesis and identifying what traits affect vector feeding preference will be of great importance. The general goal of this thesis is to understand how host traits affect the feeding preferences of mosquito vectors. To fulfill this goal, this thesis investigates the effects of host morphology, behavior, metabolism and infection with VBPs on mosquitoes' blood feeding behavior. I predicted that these host traits might influence mosquito feeding patterns, as they may affect host seeking and feeding behavior of mosquitoes.

In **Chapter 1,** I conducted a systematic review of literature on host cues involved in mosquito host detection and blood feeding behavior. Host seeking activity of mosquitoes is an integrative process that combines multiple senses to detect suitable hosts. Thus, I reviewed the effects of visual, olfactory and thermal cues from the vertebrate hosts on the mosquitoes' behavior to identify knowledge gaps for future studies on mosquito feeding preferences, including the role of potentially neglected cues. I also proposed new approaches to identify mosquito feeding preferences. In order to assess the role of inter-specific variation in different host traits in mosquito feeding preferences **Chapter 2** investigates how avian morphological and behavioral traits affect mosquito feeding preferences under natural conditions, while considering the potential importance of phylogenetic relationships of bird species. Bird plumage coloration and body size are consistent and heritable characteristics that relate to many aspects of their life history. Mosquitoes may discriminate different visual cues to select the most suitable host to maximize blood feeding. I hypothesize that inter-specific variation in avian traits will significantly affect mosquito feeding preference; particularly, I predict that birds with bigger body size will receive more mosquito bites, as the surface available to mosquito bites is larger and bigger birds will release more heat and CO_2, both being well-known cues used by mosquitoes to detect vertebrates. Birds with darker plumage coloration will attract more mosquitoes, as many studies assumed that darker colors are more attractive than lighter ones. Finally, avian roosting behavior will significantly affect mosquito bites; in particular, birds roosting communally will attract more mosquitoes, as they will release a greater amount of cues used by host-seeking mosquitoes.

In order to figure out the role of intra-specific variation in host traits in mosquito feeding preferences, **Chapter 3** assessed the effects of individual metabolism on mosquito feeding patterns. Avian metabolism may greatly vary among different individuals, even within the same species. A higher metabolism is often positively related to the release of multiple host cues, such as CO_2 and heat. I hypothesize that individual birds with higher metabolic rates will receive more mosquito bites, as a higher metabolic rate is positively related to body mass as well as greater emission of cues for host-seeking mosquitoes. In addition, **Chapter 4** assesses the effects of intra-specific variation in host infection status and intensity with VBPs on mosquito feeding preference. Avian *Plasmodium* infection and medication treatment provide useful experimental models to test the parasite manipulation hypothesis, which states that parasites may alter host phenotypic traits in a way that increase host-vector contact rates and hence, the transmission of parasites. The aim of this study is precisely to test this hypothesis, by exploring whether there is a mosquito feeding preference for infected and/or more severely infected birds. I hypothesize that mosquitoes will preferably bite *Plasmodium*-infected birds and/or birds with higher parasite loads, as birds under these conditions may be more attractive/susceptible to mosquitoes.

Study approaches

Literature review and comparative analysis

In order to identify knowledge gaps for the experimental exploration in the thesis as well as for future research directions, I performed a literature review in Chapter 1 to systematically summarize the currently available knowledge on potential attractants/repellants for host-seeking mosquitoes. In total, I reviewed 138 highly relevant articles or books that have been published on scientific journals and presses. To test the role of avian phenotypic traits in mosquito feeding preferences, I conducted comparative analyses in Chapter 2 by combining data on bird morphology and behavior with avian phylogenetic relationship using Phylogenetic Generalized Least Squares (PGLS) models. As some phylogenetically-close birds may potentially share certain morphological and behavioral traits as considered here, it was necessary to incorporate avian phylogeny in the analyses to minimize these biases. I also used model selection and averaging approaches to obtain the best models explaining the variation in mosquito feeding preferences and to summarize the final set of significant predictors following Burnham and Anderson(2002) and Grueber et al. (2011).

Mosquito sampling and rearing

In Chapter 3 and 4, I used different experimental approaches to test whether host physiological (i.e., metabolism and infection) traits affect mosquito feeding preferences. To perform experimental bioassays, the first step is to establish a stable production of experimental mosquitoes. To do that, I first collected mosquito larvae at Cañada de los Pájaros Nature Reserve (Seville, Spain) using standard dippers/nets. Subsequently, they were maintained in a climatic chamber under standard conditions (see details in Chapter 3 and 4). The use of the climatic chamber can keep larvae at their optimum growth conditions and maximize the yield of adult mosquitoes. Emerged adult mosquitoes were anaesthetized with diethyl ether (Lipnick, 1991), sexed and identified to species level following Schaffner et al. (2001) and Becker et al. (2010). Female *Cx. pipiens* were maintained in insect rearing cages until 24h before the experiments. In total, I used 2851 and 6308 female *Cx. pipiens* for Chapter 3 and 4 and obtained 779 and 1589 engorged mosquitoes in each of the chapters respectively.

Bird sampling and acclimation

In Chapter 3 and 4, I used juvenile House Sparrows as the experimental vertebrate hosts to assess the role of host metabolism and infection in attracting mosquitoes. To do that, 80 juvenile birds were captured using mist-nets in the Huelva province (southern Spain) and were individually ringed. In the field, their weight and wing length were measured and their age was estimated based on plumage characteristics (Svensson, 2006). From each bird I extracted a volume of blood equivalent to less than 1% of its body mass using sterile syringes. Blood samples were maintained in Eppendorf tubes and kept in iceboxes during the fieldwork. Samples were transported to the laboratory and maintained at 4 °C for 24 h until the separation of serum and cellular fractions by centrifugation at 4000 rpm for 10 min. The cellular fractions were maintained at -20 °C for further molecular analyses. Birds were transported to the facilities of the Unit of Animal Experimentation at the Doñana Biological Station (EBD-CSIC) and were maintained in a vector-free room with *ad libitum* access to a standard mixed diet (see details in Chapter 3 and 4) for 7 days to acclimate to the new environment. Bird sex and infection with blood parasites were molecularly determined. Subsequently, birds were exposed to mosquito bites for 12 hours during the night of the experiment. All the birds were blood-sampled again and then released into the field. In the second experiment of Chapter 4, blood smears were used to estimate the intensity of infection with blood parasites. All experimental procedures were approved by the CSIC Ethics Committee and Animal Health authorities according to Spanish legislation (CEBA-EBD-12-40).

Bird metabolism measurement and bioassays

In Chapter 3, the resting metabolic rate (RMR) of House Sparrows, estimated as the minimum oxygen consumption under post-absorptive digestive conditions during its resting cycle (Hill, 1972; McNab, 1997; Rodríguez et al., 2014), was measured during a 12-hour period prior to the bioassays of mosquito exposure. RMR was measured using an open-circuit respirometer (Sable Systems International). In addition, birds were weighed with a digital balance shortly before performing RMR measurements. The night following the RMR measurements, half of the birds were randomly injected with 2, 4-Dinitrophenol (DNP), an artificial decoupler of oxidative phosphorylation (Williams, 1966), which may induce an increase in the metabolic rate (Nicholls and Ferguson, 2013). Immediately after injection, pairs of birds consisting of a DNP and a control bird were exposed to mosquitoes in insect-rearing tents for 12 hours in complete darkness (see Figure 4).

In both Chapter 3 and 4, birds were molecularly sexed and the infection with blood parasites was analyzed to establish individual infection status before exposing experimental birds to mosquitoes. The physiological conditions of the experimental birds used in the two chapters are different. In Chapter 3, healthy House Sparrows without detectable infection with *Plasmodium*, *Haemoproteus* and *Leucocytozoon* were used in bioassays; while in Chapter 4, two types of bioassays were performed: i) exposure of *Plasmodium*-infected and uninfected (as previous "healthy") birds to mosquitoes; and ii) exposure of *Plasmodium*-infected and infected but anti-malaria treated birds to mosquitoes. The first experiment compared birds with different infection status, and the second with different infection intensity. Seven days before the mosquito exposure bioassays, birds assigned to anti-malaria treatment group were subcutaneously injected with primaquine, a commonly used anti-malaria compound that could reduce the blood parasite load in birds including House Sparrows (Merino et al., 2000; 2004; Marzal et al., 2005; Tomás et al., 2007; Martínez-de la Puente et al., 2010b). The biological half-life of primaquine is short (Baird and Hoffman, 2004) and hence, this drug was not likely to have any direct effect on bird behavior one week post medication (Cauchard et al., 2016). Experimental birds were exposed to an average of 172 and 151 unfed female *Cx. pipiens* in the two experiments, respectively (more details see in Chapter 3 and 4).

Figure 4. Bioassay with House Sparrows (*P. domesticus*) and *Cx. pipiens* mosquitoes. Bird in pairs are exposed to unfed female mosquitoes during 12h. Exposed birds differed in their conditions, for example, with different metabolic rate or infection status. Mosquitoes could freely interact with birds without any restriction.

Molecular analyses: bird sexing, genotyping and pathogen detection

In Chapter 3 and 4, birds were first blood-sampled for genomic DNA following Gutiérrez-López et al. (2015) and molecularly sexed according to Ellegren (1996) and Griffiths et al. (1998). PCR amplifications were conducted (see reaction conditions in Griffiths et al., 1998) and the positive amplifications were resolved in 3% agarose gels (more details see Chapter 3 and 4). Eight different primer pairs were used to genotype birds following Garnier et al. (2009). To identify homozygous (one band) and heterozygous (two bands) individuals for each microsatellite, positive amplifications were resolved in 3% agarose gels and the amplification pattern was compared between birds from the same trial.

I use avian *Plasmodium* as the studied pathogen systems. To determine whether a bird is infected by *Plasmodium* or not, bird blood samples were screened with a nested-PCR according to Hellgren et al. (2004). Infection with avian *Plasmodium* was determined again after completion of the experiments. The presence of amplicons was verified in 1.8% agarose gels and positive amplifications were DNA sequenced. Sequences were edited and assigned to parasite lineages/morphospecies after comparison with sequences in GenBank (more details see Chapter 3 and 4). Any birds infected by *Plasmodium*, *Haemoproteus* or *Leucocytozoon* were not included in Chapter 3; all birds infected with *Haemoproteus* or *Leucocytozoon* were excluded from Chapter 4, except one bird co-infected with *Plasmodium* and *Haemoproteus*. To estimate the parasite load of the infected birds, blood smears were made using a drop of avian blood stained according to Bennett (1970) and parasitemia were counted as the number of *Plasmodium*-infected cells per 10,000 RBCs in a blood smear (Carl Zeiss-Imager A1).

Chapter 1

Understanding blood-feeding preferences in mosquitoes: determinants and challenges

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Manuscript

Abstract

Mosquito blood-feeding preference represents a key factor in the transmission of vector-borne pathogens because it greatly influences host-vector contact rates. Blood-feeding patterns of mosquitoes are not completely random, but rather mosquitoes show clear preference for biting particular species and/or individuals. Factors including host-seeking cues and host availability may be determinants of the observed pattern of mosquito feeding preferences. Mosquitoes use different cues emitted by vertebrate hosts to identify their blood meal source, including visual, olfactory and thermal stimuli. Thus, differences in the quality/intensity of the released cues may drive the host selection by mosquitoes at both inter- and intraspecific levels. In this study, we review current evidence on the potential factors affecting the attractiveness of hosts to mosquitoes. In addition, we propose the use of a novel functional trait-based approach to study mosquito feeding preferences in future studies conducted under natural conditions.

Keywords: Vector-borne diseases; Host selection; Blood feeding; Host-seeking cues; Host availability; Functional traits; Trait-based approaches

Introduction

Mosquitoes (Diptera: Culicidae) are the primary vectors of a number of pathogens causing severe infectious diseases, including malaria and West Nile fever (Becker et al. 2010). The spread of these pathogens from an infected host to a new one is bridged by the blood-feeding behavior of mosquitoes, which harbor the disease agents in their salivary glands and inject them into targeted hosts' blood (Marquardt 2004). However, some pathogens show particular requirements, being not able to thrive in all vertebrate hosts and mosquitoes. For example, West Nile virus (WNV) primarily circulates between *Culex* mosquitoes and some competent birds that are able to develop sufficient viral level for the transmission to mosquitoes (Kramer et al. 2008; Pérez-Ramírez et al. 2014). Therefore, the contacts between competent vertebrate hosts and insect vectors are vital for vector-borne pathogens to successfully complete their life cycle and maximize their transmission (Takken and Verhulst 2013). Some bird species, such as the American Robin, are competent to harbor high WNV loads, which favor a greater transmission of the virus (Kilpatrick et al. 2006a). For mosquito females, blood feeding is essential to obtain metabolic resources and proteins for egg laying and hence, reproductive fitness (Clements 1992; Takken et al. 1998; Chaves et al. 2010). Some mosquito species feed opportunistically on a wide range of hosts and express nonspecific host preferences; however, others are specialists showing preference to feed predominantly on certain host species (Gibson and Torr 1999). In addition, many mosquito-borne pathogens are host specialist and, therefore, are able to only develop in particular taxa. Given these necessities of host selection by mosquitoes and disease agents, blood-feeding preferences of insect vectors could be more common than expected in nature, representing an essential step in vector-borne pathogen amplification and transmission.

What is blood-feeding preference?

Takken and Verhulst (2013) defined host preference as "the trait to preferentially select certain host species over others". Thus, blood-feeding preference is the result of mosquitoes biting certain hosts more frequently than others, which may greatly affect host-vector-pathogen interactions in the wild. A recent study reported that the common house mosquito *Culex pipiens* from Italy fed on European Starlings (*Sturnus vulgaris*) ten times less than expected, while fed on Common Blackbirds (*Turdus merula*) five times more often than expected given their relative abundances (Rizzoli et al. 2015). However, increasing evidence showed that blood-feeding preferences of mosquitoes not only occur among different host species but also among different classes of animals. For example, studies in different countries including Spain, Italy and Japan demonstrated that the Asian tiger mosquito *Aedes albopictus* fed almost solely on mammals, while *Cx. pipiens* fed on both avian and mammalian hosts, including humans (Sawabe et al. 2010; Muñoz et al. 2011; Martínez-de la Puente et al. 2015a). Besides interspecific preferences, mosquitoes may preferentially feed on some individuals over other conspecific counterparts (Kelly 2001; Gervasi et al. 2016). In humans, some individuals were bitten more often than others due to even a slight difference in their olfactory profiles (Kelly 2001).

In the field, the actual preference of mosquitoes to feed on a particular vertebrate may be determined by the innate host preference of insects, host-seeking cues and host availability (Fig 1; Takken and Verhulst 2013). Although in some cases, innate host preference may even be readily overruled by host-seeking cues and host availability in the field (Takken and Verhulst 2013; McBride 2016). For example, many *Culex* species are ornithophilic but they can switch to feed predominantly on other hosts, including humans, when the availability of their preferred hosts decreases (Edman 1971; Kilpatrick et al. 2006b; Simpson et al. 2012). Such changes in feeding behavior could partially explain the phenology of diseases in human caused by zoonotic pathogens. This is the case of WNV in North America, where human cases appear shortly after mosquitoes switch feeding from their preferred migratory avian hosts to humans (Kilpatrick et al. 2006b). These changes in mosquito blood-feeding patterns may affect pathogen transmission. In extreme cases, 20% of competent hosts that preferably fed by mosquitoes may contribute to at least 80% of effective transmission cases (Woolhouse et al. 1997). To better understand blood-feeding preferences of mosquitoes, it is of great importance to figure out what factors may be related to the release of host-seeking cues and host availability and how these factors may vary among different vertebrate species and individuals.

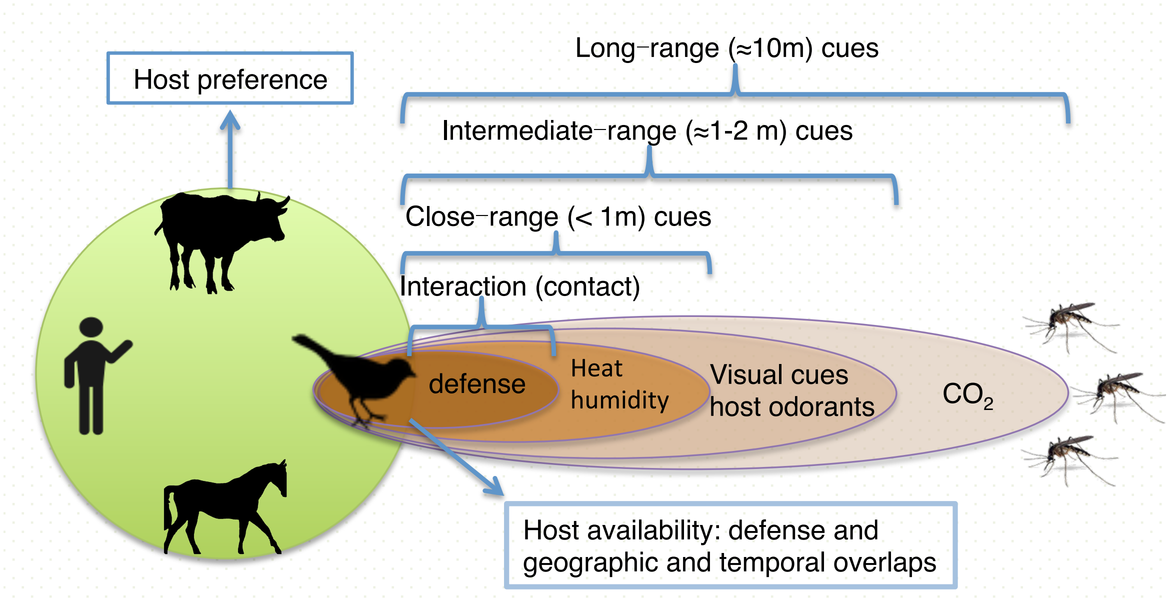

Fig 1. Mosquito blood-feeding preference is the outcome of the interactions among innate host preference, host-seeking cues and host availability. Host preference may limit what sort of hosts mosquitoes feed on; blood-feeding preference of mosquitoes could largely depend on the integrative effects of a combination of host-seeking cues and host availability.

Factors influencing the release of host-seeking cues

Mosquito blood-feeding preference is a highly complex phenomenon that could be affected by both intrinsic and extrinsic factors (Takken and Verhulst 2013). Some intrinsic factors, especially genetic differences among mosquito species may greatly affect host choice. For example, *Aedes aegypti*, *Anopheles gambiae* and *Anopheles funestus* express a strong innate host preference for humans. This preference for humans is genetically fixed and may not switch to other available hosts even if there are more abundant hosts other than humans (Takken and Knols 1999). For some blood-sucking insects, such as *Culicoides* biting midges, the role of phylogeny in vector blood-feeding behavior has been assessed (Martínez-de la Puente et al. 2015b); but we still know very little about the extent to which phylogenetic history is associated with mosquito feeding preferences. Under natural conditions, however, many mosquitoes show some degree of opportunistic feeding behavior, and rely on detectable cues that indicate the presence of a suitable blood source (Takken and Verhulst 2013). Mosquitoes use multiple sensilla to detect olfactory, visual, thermal and other cues in order to find hosts. Therefore, those factors potentially affecting the release of these cues may play a central role in host selection by mosquitoes. Intra- and/or interspecific differences in host traits that govern the release of these cues may determine the host attractiveness/ susceptibility to mosquitoes.

Table 1. **Avian attractants/ repellants affecting mosquito host-seeking behavior**. Abbreviations or symbols used in the table: $\pm CO_2$ functioning either with or without carbon dioxide; $-CO_2$ functioning without carbon dioxide; $+CO_2$ functioning with carbon dioxide; *Cx.* the genus *Culex*; *Ae.* the genus *Aedes; An.* the genus *Anopheles; Oc.* the genus *Ochlerotatus; Cs.* the genus *Culiseta; Co.* the genus *Coquilletidia.*

Materials	Effect	Host	Vector	Reference
Nonanal	Attractive$\pm CO_2$	Pigeon (*Columba livia*) Chicken (*Gallus gallus*)	*Cx. quinquefasciatus*	Syed and Leal 2009
Heptanal	Attractive$-CO_2$	Pigeon (*Columba livia*) Chicken (*Gallus gallus*)	*Cx. quinquefasciatus*	Syed and Leal 2009
Octanal	Attractive$-CO_2$	Pigeon (*Columba livia*) Chicken (*Gallus gallus*)	*Cx. quinquefasciatus*	Syed and Leal 2009
Decanal	Attractive$-CO_2$	Pigeon (*Columba livia*) Chicken (*Gallus gallus*)	*Cx. quinquefasciatus*	Syed and Leal 2009; Cooperband et al. 2008
Undecanal	Attractive$-CO_2$	Chicken (*Gallus gallus*)	*Cx. quinquefasciatus*	Cooperband et al. 2008
Dodecanal	Attractive$-CO_2$	Chicken (*Gallus gallus*)	*Cx. quinquefasciatus*	Cooperband et al. 2008
Tetradecanal	Attractive$-CO_2$	Chicken (*Gallus gallus*)	*Cx. quinquefasciatus*	Cooperband et al. 2008
Pentadecanal	Attractive$-CO_2$	Chicken (*Gallus gallus*)	*Cx. quinquefasciatus*	Cooperband et al. 2008
Hexadecanal	Attractive$-CO_2$	Chicken (*Gallus gallus*)	*Cx. quinquefasciatus*	Cooperband et al. 2008
Heptadecanal	Attractive$-CO_2$	Chicken (*Gallus gallus*)	*Cx. quinquefasciatus*	Cooperband et al. 2008
Octadecanal	Attractive$-CO_2$	Chicken (*Gallus gallus*)	*Cx. quinquefasciatus*	Cooperband et al. 2008
Feather	Attractive$\pm CO_2$	Chicken (*Gallus gallus*)	*Cx. quinquefasciatus,* *Ae. aegypti,* *Cx. nigripalpus,* *Cx. tarsalis*	Allan et al. 2006
Bacterial-volatiles	Attractive$+CO_2$	Chicken (*Gallus gallus*)	*An. gambiae,* *An. arabiensis*	Busula et al. 2017
Uropygial-secretions	Attractive$+CO_2$	American Crow (*Corvus brachyrhynchos*)	*Cx. pipiens,* *Cx. restuans*	Russell and Hunter 2005
Blood	Attractive$-CO_2$	Chicken (*Gallus gallus*)	*Cx. quinquefasciatus,* *Cx. nigripalpus,* *Ae. aegypti*	Allan et al. 2006b

Table 1 (continued)

Materials	Effect	Host	Vector	Reference
Intact host	Attractive+CO_2	Turkey (*Meleagris* spp.) Chicken (*Gallus gallus*) House Sparrow (Passer domesticus) Pigeon (*Columba livia*)	*Cx. pipiens,* *Ae. aegypti,* *Cx. quinquefasciatus,* *Cx. nigripalpus,* *Cx. tarsalis,* *Cx. restuans,* *Oc. trivittatus* *Cs. morsitans* *Co. perturbans* *Oc. c. canadensis*	Braverman et al. 1991, Allan et al. 2006, Darbro and Harrington 2006, Faraji and Gaugler 2015
Alcohols	Attractive-CO_2	Chicken (*Gallus gallus*)	*Culex*. spp.	Bernier et al. 2008
Ketones	Attractive-CO_2	Chicken (*Gallus gallus*)	*Culex*. spp.	Bernier et al. 2008
Diones	Attractive-CO_2	Chicken (*Gallus gallus*)	*Culex*. spp.	Bernier et al. 2008
Intact host	Repelling+CO_2	Chicken (*Gallus gallus*)	*An. arabiensis*	Jaleta et al. 2016
Isobutyl-butyrate	Repelling±CO_2	Chicken (*Gallus gallus*)	*An. arabiensis*	Jaleta et al. 2016
Naphthalene	Repelling±CO_2	Chicken (*Gallus gallus*)	*An. arabiensis*	Jaleta et al. 2016
Hexadecane	Repelling±CO_2	Chicken (*Gallus gallus*)	*An. arabiensis*	Jaleta et al. 2016
Trans-limonene-oxide	Repelling±CO_2	Chicken (*Gallus gallus*)	*An. arabiensis*	Jaleta et al. 2016
Limonene	Repelling+CO_2	Hosts including chicken	*An. arabiensis*	Jaleta et al. 2016
Cis-limonene-oxide	Repelling+CO_2	Hosts including chicken	*An. arabiensis*	Jaleta et al. 2016
β-myrcene	Repelling+CO_2	Hosts including chicken	*An. arabiensis*	Jaleta et al. 2016
Fecal sacs	Repelling±CO_2	Common Blackbird (*Turdus merula*)	No trapped mosquitoes	Ibáñez-Álamo et al. 2016
Bird odorants	Repelling-CO_2	Crested Auklet (*Aethia cristatella*)	*Ae. aegypti*	Douglas III et al. 2005

Olfactory cues

Olfactory cues are thought to play the most important role in host detection by mosquitoes (Bowen 1991; Smallegange and Takken 2010; Olanga et al. 2010). Mosquitoes are able to detect specific host odorants using three types of olfactory receptors (i.e. odorant, gustatory and ionotropic receptors) located on maxillary palpi, antennae and labellum (Kwon et al. 2006, Pitts and Zwiebel 2006, Leal 2013, Potter 2014). There are many odorants that have been reported to attract mosquitoes (see Table 1). According to the distance that they start functioning as host location cues for mosquitoes, they can be roughly divided into two categories: long-range cues, i.e. carbon dioxide (CO_2), and intermediate/close-range cues, i.e. host volatile compounds (Fig 1; van Breugel et al. 2015; Cardé 2015).

1) Carbon dioxide

Carbon dioxide (CO_2) is considered the most important olfactory stimulus for host-seeking mosquitoes (Gillies 1980). CO_2 exhalation from vertebrates is usually higher than the atmospheric level of CO_2 (normally 0.03-0.04%), which can be diluted and dispersed more than 15 m before falling to background levels (Gillies and Wilkes 1972; Gillies 1980; Zöllner et al. 2004). Hence, mosquitoes could use CO_2 as a long-range cue to locate their hosts in the wild. Odorant receptor neurons in mosquitoes can detect changes in CO_2 concentrations as low as 0.01% in relation to background concentration, and generate electrophysiological signals for activation and orientation towards hosts (Gillies 1980). Under laboratory conditions, mosquitoes did not display orientation flight unless CO_2 was intermittently present (Payne et al. 1986; Bowen 1991). In the field, CO_2 is released from hosts that act like point sources that generate filamentous plumes of increased CO_2 concentration, rather than broad and homogeneous CO_2 increases (Bowen 1991). In experiments conducted in dual-choice olfactometers, mosquitoes are less reactive to CO_2 stimulus than when performed in wind tunnels, and this could be due to the continuous flow and unnaturally high concentration of CO_2 reached in dual-choice olfactometers. As the primary cue of activation and orientation, CO_2 may function jointly with other odorants during the process of attraction and, in many cases, mosquitoes will not react to the presence of other odorant stimuli unless a flow of CO_2 is present. This is the case of the Lactic acid, a substance that attracts mosquitoes in combination with a CO_2 flow, but produces no reaction when CO_2 is at atmospheric concentration (Acree et al. 1968; Smith et al. 1970). However, as a general cue exhaled by all vertebrates, CO_2 is thought to be a cue indicating the existence of a host but not a cue that can help discriminate a suitable host from others, e.g. a bird from a mammal (Mboera and Takken 1997).

2) Other olfactory stimuli

Lactic acid (L-lactic acid) is one of the best-known cues used by blood seeking mosquito females to locate their hosts, as supported by both field and laboratory studies (see review in Takken and Knols 1999). In the laboratory, L-lactic acid alone was not attractive to mosquitoes but became attractive to these insects in presence of CO_2 and other odorants, including ammonia, supporting a strong synergistic effect of these compounds (Takken 1991; Geier et al. 1999a; Dekker et al. 2002). This phenomenon had been reported in *Ae. aegypti* (Acree et al. 1968), *An. gambiae* (Dekker et al. 2002) and *Anopheles quadrimanulatus* (Price et al. 1979). In the field, however, few studies have examined the role of L-lactic acid as a bait/attractant in trapping mosquitoes (Takken and Knols 1999). However, a few results obtained in the field were very similar to those found in the laboratory, supporting the attractiveness of L-lactic acid combined with CO_2 and 1-octen-3-ol (Kline et al. 1990; Poulin et al. 2017) but not when the component was tested alone (Stryker and Young 1970). As one of the main components of human sweat, ammonia had also been reported as an important attractant to mosquitoes, including *An. gambiae* (Braks et al. 2001), and *Ae. aegypti* (Geier et al. 1999b). Given the limited distance reached by these two volatile compounds, they may serve as intermediate to close-range cues for host seeking mosquitoes, although to the best of our knowledge, all the experiments carried out so far with lactic acid and ammonia have involved mammophilic mosquito species and information is lacking for ornithophilic species. There are other substances that may provide attractive or repelling cues for mosquitoes, such as volatile compounds from the preen wax secreted by the uropygial gland of birds, feathers, feces and skin bacteria (see Table 1). Bacterial volatiles could greatly affect host attractiveness to mosquitoes (Verhulst et al. 2010), but little evidence comes from studies on birds (see Busula et al. 2017). Chicken is the most common avian species used in this sort of studies, and few wild bird species, have been assayed for the attractiveness of their odors to mosquitoes (Table 1). It is noteworthy that there are both attractive and repelling substances from the same host species for mosquitoes, and so simply testing mosquito attraction in relation to interspecific differences in host cues may not fully represent the causes of mosquito feeding preference. More studies performed at intra-specific host level are clearly needed in order to clarify the true relevance of differences in olfactory cues in mosquito attraction.

Visual cues

Visual cues are considered as important stimuli in the activation, orientation and landing of blood-sucking insects including mosquitoes. They are though to be intermediate-range cues that usually function in synergy with other stimuli (Lehane 2005; Cardé 2015; van Breugel et al. 2015). The main visual organs of mosquitoes are compound eyes, which can detect color, light intensity, pattern and motion of hosts as well as interpret sensory information as indicators for host discrimination (Lehane 2005). The relative importance of the visual cues depends on the habits of each mosquito species. For example, nocturnal mosquitoes are unlikely to depend on visual stimuli to the same extent than diurnal and crepuscular species (Wen et al. 1997).

However, nocturnal mosquitoes are more sensitive to light than diurnal species and their eyes can function in a wide range of light intensities, including extremely low light conditions such as starlit nights (Land et al. 1997; Land et al. 1999). Consequently, visual cues could be important for both diurnal and nocturnal mosquitoes, as they may affect their flight paths (Bidlingmayer 1975; Bidlingmayer and Hem 1981) and lead them to finish the final stages of orientation (Cardé 2015). Despite the prominent role of olfactory cues, the initial detection of a host by mosquitoes often relies on visual stimuli and depends on the differences in color/ intensity contrast or relative motion between the host and the background (Lehane 2005).

1) Color/ intensity contrast

Many mosquitoes are crepuscular or nocturnal and their host-seeking behavior usually peaks at sunrise, sunset, or at night with twilight (Allan et al. 1987; Becker et al. 2010). However, they can still detect visual cues under dark conditions (Hawkes and Gibson 2016). *Aedes aegypti*, for example, has dichromatic vision with high sensitivity in intensity contrast (Allan 1994), which may allow host detection under dark condition and then use other cues to approach to their hosts. In fact, the flight activity of nocturnal mosquito species appeared to be greatly enhanced during full moon nights (Bidlingmayer 1964; Allan et al. 1987; Rubio-Palis 1992; Kampango et al. 2011). Previous studies on 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) demonstrated that pure white and light grey cards attracted more insects, including small dipterans, than other tested colored cards. These contrasting results could be due to methodological differences since these studies used different objects without controlling object shape, size and color/intensity contrast of objects against the background. 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 decreases under these conditions (Browne and Bennett 1981). Thus, rather than color or light intensity themselves, color/ intensity contrast against the background may play a more important role in mosquito attraction. Perhaps this could explain why many nocturnal insects, including mosquitoes, respond to artificial lights and color/ light contrast, a fact that has been successfully exploited in the design of traps for mosquitoes (Barr et al. 1963; Haufe 1964; Reinert 1989; Silver 2007; Muirhead-Thompson 2012; Costa-Neta et al. 2017).

2) Other visual stimuli

Mosquito eyes are very sensitive to motion and thus, hosts' movements may affect their location by mosquitoes (Allan et al. 1987; Lehane 2005). For example, Gillett (1972) reported that walking workers in the field attracted host-seeking mosquitoes due to their movement. Additionally, Tomás et al. (2008) reported a positive correlation between the abundance of flying blood sucking insects in bird nests and adult provisioning rate to their nestlings. However, it is difficult to identify the relative role of this stimulus, as moving animals may increase the release of other cues (e.g. CO_2), which complicate the experimental design for this sort of studies. After the initial detection, the color contrast of the target against the background may favor detection by host seeking mosquitoes. In other blood-sucking insects, patterned targets were thought to be less attractive to vectors than plain targets, as patterned ones are less visible from a long distance (Lehane 2005). In mosquitoes, however, this relationship still needs to be tested and the role of avian plumage pattern in mosquito attraction remains poorly understood.

Other cues: heat and humidity

Heat of mammals and birds can function as close-range cues for host seeking mosquitoes and the distance that body heat becomes attractive is roughly smaller than 1m (van Breugel et al. 2015; Cardé 2015). Mosquitoes can be very sensitive to heat as supported by Davis and Sokolove (1975), who recorded a maximal spike frequency changes in sensilla of *Ae. aegypti* in response to a temperature change of + 0.2 ℃. Heat produced by metabolic activity may act in three ways to indicate the presence of hosts: radiant heat emitted by hosts, heat gradient between hosts or mosquitoes and the body heat of the contacted hosts. All of them may attract mosquitoes and help mosquitoes to find suitable landing sites (Lehane 2005; van Breugel et al. 2015). In the field, the addition of heat to the traps has been demonstrated to significantly increase the number of captured mosquitoes (Kline and Lemire 1995). Experimental studies also showed that *Ae. aegypti* may not orient to a mimic human except heat was also added at a close proximity (van Breugel et al. 2015). However, it is still not known whether the difference in body heat among different targets may affect mosquito host choice.

Mosquitoes are also sensitive to water vapor and can detect the small change in relative humidity at a close range (Clements 1992; Laarman 1958). Humidity has been suggested as a synergistic agent with other stimuli, rather than an independent cue to attract mosquitoes alone (Lehane 2005; Takken and Verhulst 2013). However, to the best of our knowledge, there is no evidence showing that mosquitoes are attracted by water vapor generated by host metabolism under field condition. In a laboratory experiment, Olanga et al. (2010) found that combining odor baits with moisture and heat did not increase the attractiveness of the bait to mosquitoes. Nonetheless, a study on *Culicoides* found that both humidity and temperature in bird nests could affect the abundance of blood-sucking insects (Martínez-de la Puente et al. 2010).

Factors influencing host availability

Host abundance

Mosquito blood-feeding preference is not only a behavioral decision making process by itself, but is also strongly affected by relative host availability (Lehane 2005). Host density influences host choice of mosquitoes, especially of those opportunistic species (Kilpatrick et al. 2006b; Simpson et al. 2012). But even for specialist species, their feeding preference may also change when the abundance of the preferred hosts decreases (Lefèvre et al. 2009; Wekesa et al. 1997). For mammals and birds, migration could strongly affect the degree of geographic and temporal overlap between hosts and mosquitoes (see Fig 1) and rapidly change the relative abundance of the preferred hosts of mosquitoes (Lehane 2005). For example, in North America, *Culex nigripalpus* and *Culex tarsalis* can switch their blood-feeding source from birds in the winter and spring to mammals in the summer (Tempelis and Washino 1967; Edman and Taylor 1968; Edman 1974). Kilpatrick et al. (2006b) documented a clear shift of *Cx. pipiens* feeding habits from birds to humans, which was coincident with the migration of mosquito's preferred bird species (American robin *Turdus migratorius*). In addition, vertical distribution of hosts at small scale may also affect the relative availability of hosts and so mosquito feeding preference. For example, *Aedes triseriatus* and *Aedes hendersoni* are sibling species living sympatrically, but the former species feed mainly on ground dwelling animals such as deer while the latter feed mainly on canopy dwelling animals such as squirrels (Nasci 1982).

Defensive behavior

Host defensive behavior may play an important role in determining blood-feeding preferences of mosquitoes by reducing the feeding success on potential hosts to mosquitoes. When a mosquito approaches to a potential host, anti-mosquito behavior could strongly affect mosquito feeding success (Day and Edman 1984; Edman and Scott 1987; Darbro and Harrington 2007). A study on defensive behavior of ciconiiform birds concluded that green heron and the crowned night heron displayed less anti-mosquito movement than other host species and thus, received far more bites from mosquitoes (Webber and Edman 1972). Among others, foot-pecking and foot-slapping were the most effective behaviors to prevent mosquitoes from feeding on their legs (Webber and Edman 1972). It is a great risk for mosquitoes to feed on a host with high level of defense; they may thus avoid these individuals when having the alternative of feeding on less defensive hosts (Day and Edman 1984) or may feed on less defensive individuals due to blood-sucking interruption (Hodgson et al. 2001). The degree of defensive behavior may be related to host body size; smaller animals may protect themselves more effectively from mosquito attacks by a higher level of grooms/preens (Mooring et al. 2000). On the other hand, the intensity of host defensive behaviors increase with the number of mosquitoes attacking the host, which may in turn limit the blood meal ingested by mosquitoes (Edman et al. 1972; Waage and Nondo 1982; Darbro and Harrington 2007).

Challenges in studies of mosquito feeding preference

Although the effort of searching for potential stimuli affecting mosquito blood-feeding preference has lasted, at least, four decades, there are still many challenges hampering the progress. Among others, limitations in the research methodology used could account for a considerable part of the slow progress. Particularly, there is a lack of effective approaches for recording mosquito host seeking behaviors without significantly impacting the immediate environment of mosquitoes (Gibson and Torr 1999). A new technology based on video tracking system has been recently applied to study the movement of mosquitoes (i.e. Angarita-Jaimes et al. 2016), which can be applied in the future for better understanding mosquito host seeking behavior. In addition, the interaction between hosts and mosquitoes become more complex when considering the circadian rhythms and geographic distribution of both hosts and mosquitoes. For example, it could be hard to measure the attractiveness to mosquitoes in the field of some cryptic/nocturnal species, because of the problems associated to the observation/counting of such vertebrate species.

A popular way to examine mosquito blood-feeding preference is the determination of the blood meal origin in engorged mosquitoes captured in the field. Previous studies used CDC light traps, BG traps, gravid traps and aspirators to sample wild engorged mosquitoes (e.g. Hamer et al. 2009; Muñoz et al. 2012), but in many of such studies, no information is available on the relative abundance of the different vertebrate species at the moment and place of mosquito capture (e.g. Apperson et al.

2004; Gringrich et al. 2005; Molaei et al. 2006). Spatial distribution of mosquito collection may also affect estimates of mosquito feeding patterns. For example, indoor mosquito collections tend to detect more blood meals originated from humans, while mosquitoes collected from farms may get more blood meals from livestock (Takken et al. 2002). However, the vertebrate species accounting for the largest proportion of blood meal composition may not necessarily be ranked as the mosquitoes' most preferred host without considering their relative abundance in relation to all available hosts. Therefore, robust studies combining field mosquito collection and host surveys at the same localities are in high demand.

A number of studies used Forage Ratios (FR) to measure mosquito blood-feeding preferences. FR is calculated as the proportion of blood meals from a host divided by the relative abundance of the host in the study area. A host with a FR greater than 1 means that this species is bitten more often than expected from its abundance (i.e. preferred host), while a host with a FR smaller than 1 means that this species is bitten less, regardless of its abundance (i.e. avoided host). Compared to previous studies, FR is a less biased way to examine mosquito blood-feeding preferences, since it takes host availability into account (Kilpatrick et al. 2006a; Hamer et al. 2009). However, extreme values of FR may occur when dealing with rare host species (see Hess et al. 1968; Hamer et al. 2009). In addition, FR calculation presents other drawbacks including the fact that estimates for rare species have a high error interval, and difficulties in the estimation of the abundances of the different vertebrate species (e.g. nocturnal species) may also bias FR estimates. In addition, FR is not an absolute value related to species across localities but is highly dependent on the presence/ availability of other less or more preferred host species in the study area.

Host-choice assays in the laboratory or the field may provide a more objective approach to understand host preference of mosquitoes, since host availability is well controlled under experimental conditions. These tests are often conducted with live hosts or host's odor in dual-choice olfactometers. The dual-choice settings may simulate some of the situations that a host-seeking mosquito may encounter in the wild and so, may give an indication of host preference. These bioassays, however, face two major technical difficulties as well: one is that most of the mosquitoes within the olfactometer may not respond to hosts or hosts' odor inside the olfactometer (Lalubin et al. 2012; Cornet et al. 2013); the other is that host preference is usually measured as the number of activated or oriented mosquito instead of engorged mosquitoes. In the wind tunnels, mosquitoes may show upwind flight even in clean air, complicating the observation of their responses to odors (Gibson and Torr 1999). These limitations may hamper the olfactometer approach to truly reflect the nature of mosquito blood-feeding preference, especially by omitting that hosts may perform anti-mosquito behavior, which may greatly influence mosquitoes' host choice (Edman and Scott, 1987; Darbro and Harrington, 2007). In addition, using these techniques in the high number of vertebrate species usually present in natural communities is technically challenging. Combining host-choice assays of live animals directly exposed to mosquitoes with blood meal genotyping of engorged mosquitoes that have fed on tested animals could shed new light on understanding mosquito blood-feeding preference (e.g. Burkett-Cadena et al. 2010; Cornet et al. 2013; Yan et al. 2017a). This approach could simulate the natural situation with mosquitoes facing host choices while effectively controlling the variation in host abundance. In addition, it will allow biological contact between hosts and mosquitoes without changing their innate traits, such as host' intact olfactory profile and anti-mosquito behavior, which may truly reflect the natural interactions between hosts and vectors.

Avian functional traits as integrative cues affecting mosquito feeding preferences

Laboratory studies on mosquito feeding preferences tended to test the attractiveness of each volatile compound (see examples above) in isolation or in combination with CO_2. However, mosquitoes use multiple cues jointly to seek for blood sources (Cardé 2015) and so, the synergic effects of these compounds may be more important than the independent effect of any single compound. For example, an intact host is usually much more attractive than the sum of single cues to mosquitoes (Allan et al. 2006). In addition, most mosquitoes usually feed on a great range of hosts and the multihost-multivector assemblages complicate the ecological interactions in disease transmission (Johnson et al. 2015). These facts hamper the identification of the drivers of heterogeneities in mosquito blood-feeding preferences, which is vital for understanding the transmission dynamics of vector-borne diseases. Thus, it could be useful to group different host species based on their functional traits (see Box 1) that may influence mosquitoes' preferences. This could be a more practical way to monitor the epidemiology of vector-borne pathogens in the field. For instance, Dallas and Presley (2014) found that host functional traits, such as longevity and abundance, rather than factors related to dispersal such as home range size have driven the parasite community structure. A set of functional traits in a certain species may represent a type of niche, and so we can use niche-based or functional trait-based approaches to predict the host-vector interactions across different communities. In this context, host traits are no longer species-specific characteristics limited to different individuals but higher-level functional traits that allow us to identify their effects beyond simple taxonomic record within species. For example, Han et al. (2015) found that "trait profiles" of known reservoir species instead of taxonomic labels had more power in classifying susceptible hosts for future zoonotic infections.

Box 1. Incorporating functional trait-based approaches into studies of mosquito feeding preferences

What are functional traits?

Functional traits are an array of phenotypic characteristics of individual organisms, such as morphological, physiological and behavioral attributes, which influence performance and fitness of such organisms as well as the interactions between organisms and their surrounding environments (Violle et al. 2007; Díaz et al. 2013; Nock et al. 2016). Grouping different species by functional traits and using trait-based approaches to understand the complexity in ecological interactions has rapidly developed into application (Nock et al. 2016). Avian plumage coloration, body size and health conditions are measurable and inferable functional traits that can be applied into understanding host-vector interactions, which may govern the transmission of vector-borne infectious diseases.

Application of avian functional traits in understanding mosquito feeding patterns

1) Plumage coloration

Few studies have examined the role of avian plumage coloration in mosquito attraction (but see Yezerinac and Weatherhead 1995). This is due, at least in part, to the underestimation of the importance of visual stimuli in mosquito host seeking behavior. However, there is increasing evidence showing that both diurnal and nocturnal mosquito species can respond to visual cues even in dark conditions (van Breugel et al. 2015; Hawkes and Gibson 2016). Several studies have suggested that darker colors may attract more mosquitoes than lighter ones, based on the attraction of mosquitoes towards colored objects (e.g. cloth patches) (Sippell and Brown 1953; Brown 1954; Gilbert and Gouck 1957; Browne and Bennett 1981), but none of these studies have used live or stuffed birds.

In a comparative study, Yezerinac and Weatherhead (1995) did not find any relationship between the relative attractiveness of plumage colors to mosquitoes and the prevalence of haematozoa. By contrast, Scheuerlein and Ricklefs (2004) concluded that the prevalence of blood parasites was higher in avian species with male birds of lighter plumage coloration. Yan et al. (2017b) found that avian species with lighter plumage coloration were more preferred by mosquitoes than those with darker plumage as estimated by mosquito FR. These studies suggest a direct link between plumage coloration and vector attraction that may affects the prevalence of vector-borne parasites.

Box 1 (continued)

2) Body size

Host body size was positively associated with the attraction of a number of blood-sucking insects, including blackflies (Malmqvist et al. 2004), biting midges (Martínez-de la Puente et al. 2009), and mosquitoes (Estep et al. 2012). Larger avian species also presented a higher prevalence of antibodies against West Nile virus in Spain, ever after controlling for individual age (Figuerola et al. 2008), suggesting a higher exposure to mosquito-borne pathogens. Hosts of larger body size could be more easily detected from a longer distance by mosquitoes (Lehane 2005) and may release more cues such as CO_2 and heat, which could be used as tracking cues by host-seeking mosquitoes (Takken and Verhulst 2013). In birds, body size is often positively correlated with tarsus length, and species with larger body size may provide larger unfeathered areas for mosquito bites (Yan et al. 2017b).

3) Malaria infection

Pathogen infections in plants (Eigenbrode et al. 2002; Shapiro et al. 2012), invertebrates (Stafford et al. 2011), vertebrates (O'Shea et al. 2002; De Moraes et al. 2014) and humans (Lacroix et al. 2005; Batista et al. 2014) have been reported to enhance vector attraction, which supports the parasitic manipulation hypothesis. This hypothesis states that pathogens could adaptively manipulate host phenotypes to facilitate their transmission (Poulin 1995; Hurd 2003). Avian malaria parasites of the genus *Plasmodium* are vector-borne pathogens that may cause changes in birds' behavior and physiology, such as reduced activity (Cauchard et al. 2016), anaemia and enlargement of liver and spleen (Valkiūnas 2005). These changes may affect the release of the olfactory cues used by mosquitoes and reduce bird anti-mosquito behavior, which may potentially alter mosquito feeding preference for uninfected or infected birds. However, little is known about the mechanisms affecting the relationship between avian malaria infection and bird attractiveness to mosquitoes. Different studies have provided contradictory results as both enhanced and reduced mosquito attraction towards *Plasmodium* infected birds had been reported (Cornet et al. 2013; Lalubin et al. 2012). For example, Cornet et al. (2013) documented that chronically infected birds by *Plasmodium relictum* attracted more bites of the vector *Cx. pipiens* when exposed simultaneously with acutely infected or uninfected birds. Nevertheless, Lalubin et al. (2012) found that uninfected birds were more attractive to *Cx. pipiens* than *Plasmodium*-infected birds. Yan et al. (2017a) tested the effects of both avian malaria infection prevalence and the intensity of infection on mosquito bites and found that only the last variable was positively associated with mosquito biting rates. Thus, how avian malaria infection may affect mosquitoes feeding preference is still an open research question. Future studies should pay more attention to the role of parasite load in influencing mosquito feeding preferences as an alternative beyond parasite prevalence.

Concluding remarks

The application of knowledge on blood feeding preferences of mosquitoes in infectious disease programs highlights the use of measureable host traits in predicting mosquito feeding patterns. Although there are an increasing number of studies on blood- feeding preferences of mosquitoes, the comprehensive understanding of this behavior, especially the host-related factors influencing blood-feeding preferences, is still poorly known. There are still some challenges to study blood-feeding preferences of mosquitoes resulting from methodological difficulties, which highlights the importance of the use of novel tools to combine host functional traits and mosquito feeding bioassays. Trait-based approaches could provide a practical tool to deal with these difficulties and facilitate the monitor of mosquito-preferred hosts across different species and communities. Vector feeding preference may be the key link between host functional traits and the transmission of infectious diseases. Perkins et al. (2003) used "large-bodied" and "sexually active" traits in mice to successfully explain a large variation (93%) of observed transmission potential for vector-borne encephalitis virus, although these individuals only represented ~20% of the host population. Thus, identifying what measurable traits of hosts may affect mosquito feeding preferences could dramatically benefit our understanding of infectious diseases ecology as well as the development of highly efficient measures for infectious diseases control by targeting the susceptible hosts with mosquito-preferred traits.

Summary points

1. Mosquito blood-feeding preferences are usually affected by both innate host preferences of mosquitoes and extrinsic factors including host attractiveness and availability in the field. Some mosquito species express inherent preference for feeding on mammals, birds or certain individuals within the same species. However, many mosquitoes are opportunistic feeders and hence, their innate host preference could be readily overruled by the variation in host-seeking cues and availability.
2. Olfactory cues are the most important host-seeking cues for mosquitoes. CO_2 and many volatile compounds isolated from hosts have been tested for mosquito attraction. However, more studies on attractiveness of intact hosts, especially wild birds are needed, as these cues function jointly in host seeking process of mosquitoes. Many studies on mosquito attraction are based on interspecific comparisons; further research effort at intra-specific level is also needed.

3. Visual cues can also affect mosquito feeding preferences at an intermediate range and the role of visual cues in mosquito attraction may have been underestimated, as increasing evidence show that even crepuscular/nocturnal mosquitoes can respond to visual stimuli. In fact, color/ intensity contrast against background may play an important role in mosquito attraction. The effect of avian plumage coloration on mosquito attraction calls for further research.
4. Heat and humidity released by host metabolism may also affect mosquito feeding preferences, but their role in mosquito attraction in wild animals has not been properly studied.
5. Mosquito feeding preference may best be assessed combining host-choice assays on live animals with blood meal tracking techniques to calculate mosquito feeding patterns both in the field and laboratory.
6. Functional trait-based approaches could be the solution to deal with the joint effect of multiple cues on host-seeking process of mosquitoes and the ecological complexity in multihost-multivector interactions across different communities in the wild, which may shed new light in understanding mosquito feeding preference and the transmission dynamics of vector-borne infectious diseases.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JY, JMP, LG, JF conceived of the study and JY wrote the first draft with valuable contribution from other coauthors. All authors contributed to the writing of the work and approved the final version of the manuscript.

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References

ACREE, F., TURNER, R., GOUCK, H., BEROZA, M. & SMITH, N. (1968). L-Lactic acid: a mosquito attractant isolated from humans. *Science* **161,** 1346-1347.

ALLAN S. A. (1994). Physics of mosquito vision--an overview. *Journal of the American Mosquito Control Association* **10,** 266-271.

ALLAN S. A., DAY, J. F. & EDMAN, J. D. (1987). Visual ecology of biting flies. *Annual Review of Entomology* **32,** 297-314.

ALLAN S., BERNIER, U. R. & KLINE, D. (2006). Attraction of mosquitoes to volatiles associated with blood. *Journal of Vector Ecology* **31,** 71-78.

ANGARITA-JAIMES, N., PARKER, J., ABE, M., MASHAURI, F., MARTINE, J., TOWERS, C. E., MCCALL, P. & TOWERS, D. (2016). A novel video-tracking system to quantify the behaviour of nocturnal mosquitoes attacking human hosts in the field. *Journal of The Royal Society Interface* **13,** 20150974.

APPERSON C. S., HASSAN, H. K., HARRISON, B. A., SAVAGE, H. M., ASPEN, S. E. & FARAJOLLAHI, A. (2004). Host Feeding Patterns of Established and Potential Mosquito Vectors of West Nile Virus in the Eastern United States. *Vector Borne and Zoonotic Diseases* **4.**

BARR, R. A., SMITH, T. A., BOREHAM, M. M. & WHITE, K. E. (1963). Evaluation of some factors affecting the efficiency of light traps in collecting mosquitoes. *Journal of Economic Entomology* **56,** 123-127.

BATISTA, E. P., COSTA, E. F. & SILVA, A. A. (2014). *Anopheles darlingi* (Diptera: Culicidae) displays increased attractiveness to infected individuals with *Plasmodium vivax* gametocytes. *Parasites & Vectors* **7,** 251.

BECKER N., PETRIĆ, D., BOASE, C., LANE, J., ZGOMBA, M., DAHL, C. & KAISER, A. (2010). *Mosquitoes and their control*, 2nd edition. Springer.

BERNIER, U. R., ALLAN, S. A., QUINN, B. P., KLINE, D. L., BARNARD, D. R. & CLARK, G. G. (2008). Volatile compounds from the integument of White Leghorn Chickens (*Gallus gallus domesticus* L.): candidate attractants of ornithophilic mosquito species. *Journal of Separation Science* **31,** 1092-9.

BIDLINGMAYER, W. (1964). The effect of moonlight on the flight activity of mosquitoes. *Ecology* **45,** 87-94.

BIDLINGMAYER, W. (1975). Mosquito flight paths in relation to the environment. Effect of vertical and horizontal visual barriers. *Annals of the Entomological Society of America* **68,** 51-57.

BIDLINGMAYER, W. & HEM, D. G. (1981). Mosquito flight paths in relation to the environment. Effect of the forest edge upon trap catches in the field. *Mosquito News* **41,** 55-59.

BOWEN, M. F. (1991). The sensory physiology of host-seeking behavior in mosquitoes. *Annual Review of Entomology* **36,** 139-158.

BRAKS M. A. H., MEIJERINK, J. & TAKKEN, W. (2001). The response of the malaria mosquito, *Anopheles gambiae*, to two components of human sweat, ammonia and L-lactic acid, in an olfactometer. *Physiological Entomology* **26,** 142-148.

BRAVERMAN, Y., KITRON, U. & KILLICK-KENDRICK, R. (1991). Attractiveness of vertebrate hosts to *Culex pipiens* (Diptera: Culicidae) and other mosquitoes in Israel. *Journal of Medical Entomology* **28,** 133-138.

BRETT G. (1938). On the relative attractiveness to *Aedes aegypti* of certain coloured cloths. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **32,** 113-124.

BROWN A. (1954). Studies on the responses of the female aëdes mosquito. Part VI—The attractiveness of coloured cloths to Canadian species. *Bulletin of Entomological Research* **45,** 67-78.

BROWNE, S. M. & BENNETT, G. F. (1981). Response of mosquitoes (Diptera: Culicidae) to visual stimuli. *Journal of Medical Entomology* **18,** 505-521.

BURKETT-CADENA, N. D., LIGON, R. A., LIU, M., HASSAN, H. K., HILL, G. E., EUBANKS, M. D. & UNNASCH, T. R. (2010). Vector–host interactions in avian nests: do mosquitoes prefer nestlings over adults? *The American Journal of Tropical Medicine and Hygiene* **83,** 395-399.

BUSULA, A., TAKKEN, W., BOER, J., MUKABANA, W. & VERHULST, N. (2017). Variation in host preferences of malaria mosquitoes is mediated by skin bacterial volatiles. *Medical and Veterinary Entomology* **31,** 320-326.

CARDÉ R. T. (2015). Multi-cue integration: how female mosquitoes locate a human host. *Current Biology* **25,** R793-R795.

CAUCHARD, L., ANGERS, B., BOOGERT, N. J. & DOLIGEZ, B. (2016). Effect of an anti-malaria drug on behavioural performance on a problem-solving task: An experiment in wild great tits. *Behavioural Processes* **133,** 24-30.

CHAVES, L. F., HARRINGTON, L. C., KEOGH, C. L., NGUYEN, A. M. & KITRON, U. D. (2010). Blood feeding patterns of mosquitoes: random or structured? *Frontiers in Zoology* **7,** 1-11.

CLEMENTS A. N. (1992). *The biology of mosquitoes. Volume 2: Sensory reception and behaviour*. CABI Publishing, Wallingford, UK.

COOPERBAND, M. F., MCELFRESH, J. S., MILLAR, J. G. & CARDÉ, R. T. (2008). Attraction of female *Culex quinquefasciatus* Say (Diptera: Culicidae) to odors from chicken feces. *Journal of Insect Physiology* **54,** 1184-1192.

CORNET, S., NICOT, A., RIVERO, A. & GANDON, S. (2013). Malaria infection increases bird attractiveness to uninfected mosquitoes. *Ecology Letters* **16,** 323-329.

COSTA-NETA, B., DA SILVA, A., BRITO, J., MORAES, J., REBÊLO, J. & SILVA, F. (2017). Light-Emitting Diode (LED) traps improve the light-trapping of Anopheline mosquitoes. *Journal of Medical Entomology***,** tjx148.

DALLAS T. & PRESLEY, S. J. (2014). Relative importance of host environment, transmission potential and host phylogeny to the structure of parasite metacommunities. *Oikos* **123,** 866-874.

DARBRO J. M. & HARRINGTON, L. C. (2007). Avian defensive behavior and blood-feeding success of the West Nile vector mosquito, *Culex pipiens*. *Behavioral Ecology* **18,** 750-757.

DARBRO, J. M. & HARRINGTON, L. C. (2006). Bird-baited traps for surveillance of West Nile mosquito vectors: effect of bird species, trap height, and mosquito escape rates. *Journal of Medical Entomology* **43,** 83-92.

DAVIS E. E. & SOKOLOVE, P. G. (1975). Temperature responses of antennal receptors of the mosquito, *Aedes aegypti*. *Journal of Comparative Physiology* **96,** 223-236.

DAY J. F. & EDMAN, J. D. (1984). The importance of disease induced changes in mammalian body temperature to mosquito blood feeding. *Comparative Biochemistry and Physiology Part A: Physiology* **77,** 447-452.

DE MORAES, C. M., STANCZYK, N. M., BETZ, H. S., PULIDO, H., SIM, D. G., READ, A. F. & MESCHER, M. C. (2014). Malaria-induced changes in host odors enhance mosquito attraction. *Proceedings of the National Academy of Sciences of the United State of America* **111,** 11079-11084.

DEKKER T., STEIB, B., CARDÉ, R. & GEIER, M. (2002). L-lactic acid: a human-signifying host cue for the anthropophilic mosquito *Anopheles gambiae*. *Medical and Veterinary Entomology* **16,** 91-98.

DÍAZ S., PURVIS, A., CORNELISSEN, J. H., MACE, G. M., DONOGHUE, M. J., EWERS, R. M., JORDANO, P. & PEARSE, W. D. (2013). Functional traits, the phylogeny of function, and ecosystem service vulnerability. *Ecology and Evolution* **3,** 2958-2975.

DOUGLAS III, H., CO, J., JONES, T., CONNER, W. & DAY, J. (2005). Chemical odorant of colonial seabird repels mosquitoes. *Journal of Medical Entomology* **42,** 647-651.

EDMAN J. D. (1971). Host-feeding patterns of Florida mosquitoes I. *Aedes, Anopheles, Coquillettidia, Mansonia* and *Psorophora*. *Journal of Medical Entomology* **8,** 687-695.

EDMAN J. D. (1974). Host-Feeding Patterns of Florida Mosquitoes: III. *Culex* (*Culex*) and *Culex* (*Neoculex*). *Journal of Medical Entomology* **11,** 95-104.

EDMAN J. D. & SCOTT, T. W. (1987). Host defensive behaviour and the feeding success of mosquitoes. *International Journal of Tropical Insect Science* **8,** 617-622.

EDMAN J. D. & TAYLOR, D. J. (1968). *Culex nigripalpus*: seasonal shift in the bird-mammal feeding ratio in a mosquito vector of human encephalitis. *Science* **161,** 67-68.

EDMAN J., WEBBER, L. & KALE, H. (1972). Effect of mosquito density on the interrelationship of host behavior and mosquito feeding success. *The American Journal of Tropical Medicine and Hygiene* **21,** 487-491.

EIGENBRODE, S. D., DING, H., SHIEL, P. & BERGER, P. H. (2002). Volatiles from potato plants infected with potato leafroll virus attract and arrest the virus vector, *Myzus persicae* (Homoptera: Aphididae). *Proceedings of the Royal Society of London B: Biological Sciences* **269,** 455-460.

ESTEP L. K., MCCLURE, C. J., BURKETT-CADENA, N. D., HASSAN, H. K., UNNASCH, T. R. & HILL, G. E. (2012). Developing models for the forage ratios of *Culiseta melanura* and *Culex erraticus* using species characteristics for avian hosts. *Journal of Medical Entomology* **49,** 378-387.

FARAJI, A., EGIZI, A., FONSECA, D. M., UNLU, I., CREPEAU, T. & HEALY, S. P. (2014). Comparative host feeding patterns of the Asian tiger mosquito, *Aedes albopictus*, in urban and suburban northeastern USA and implications for disease transmission. *PLoS Neglected Tropical Diseases* **8,** e3037.

FIGUEROLA J., JIMÉNEZ-CLAVERO, M. A., 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. *Veterinary Microbiology* **132,** 39-46.

GEIER M., BOSCH, O. J. & BOECKH, J. (1999a). Influence of odour plume structure on upwind flight of mosquitoes towards hosts. *Journal of Experimental Biology* **202,** 1639-1648.

GEIER M., BOSCH, O. J. & BOECKH, J. (1999b). Ammonia as an attractive component of host odour for the yellow fever mosquito, *Aedes aegypti*. *Chemical Senses* **24,** 647-653.

GERVASI, S. S., BURKETT-CADENA, N., BURGAN, S. C., SCHREY, A. W., HASSAN, H. K., UNNASCH, T. R. & MARTIN, L. B. (2016). Host stress hormones alter vector feeding preferences, success, and productivity. *Proceedings of the Royal Society of London B: Biological Sciences* **283,** 20161278.

GIBSON, G. & TORR, S. (1999). Visual and olfactory responses of haematophagous Diptera to host stimuli. *Medical and Veterinary Entomology* **13,** 2-23.

GILBERT I. & GOUCK, H. (1957). Influence of surface color on mosquito landing rates. *Journal of Economic Entomology* **50,** 678-680.

GILLETT, J. (1972). The mosquito: its life, activities, and impact on human affairs. Doubleday & Company. 358pp.

GILLIES M. (1980). The role of carbon dioxide in host-finding by mosquitoes (Diptera: Culicidae): a review. *Bulletin of Entomological Research* **70,** 525-532.

GILLIES M. & WILKES, T. (1972). The range of attraction of animal baits and carbon dioxide for mosquitoes. Studies in a freshwater area of West Africa. *Bulletin of Entomological Research* **61,** 389-404.

GINGRICH J. B. & WILLIAMS, G. M. (2005). Host-feeding patterns of suspected West Nile virus mosquito vectors in Delaware, 2001-2002. *Journal of the American Mosquito Control Association* **21,** 194-200.

HAMER G. L., KITRON, U. D., GOLDBERG, T. L., BRAWN, J. D., LOSS, S. R., RUIZ, M. O., HAYES, D. B. & WALKER, E. D. (2009). Host Selection by *Culex pipiens* Mosquitoes and West Nile Virus Amplification. *American Journal of Tropical Medicine and Hygiene* **80,** 268-278.

HAN B. A., SCHMIDT, J. P., BOWDEN, S. E. & DRAKE, J. M. (2015). Rodent reservoirs of future zoonotic diseases. *Proceedings of the National Academy of Sciences of the United States of America* **112,** 7039-7044.

HAUFE, W. (1964). Visual attraction as a principle in design of mosquito traps. *The Canadian Entomologist* **96,** 118-118.

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. *Parasites & Vectors* **9,** 1.

HESS, A., HAYES, R. O. & TEMPELIS, C. (1968). The use of the forage ratio technique in mosquito host preference studies. *Mosquito News* **28,** 386-389.

HODGSON, J. C., SPIELMAN, A., KOMAR, N., KRAHFORST, C. F., WALLACE, G. T. & POLLACK, R. J. (2001). Interrupted blood-feeding by *Culiseta melanura* (Diptera: Culicidae) on European starlings. *Journal of Medical Entomology* **38,** 59-66.

HURD, H. (2003). Manipulation of medically important insect vectors by their parasites. *Annual Review of Entomology* **48,** 141-161.

IBÁÑEZ-ÁLAMO, J. D., RUIZ-RAYA, F., RODRÍGUEZ, L. & SOLER, M. (2016). Fecal sacs attract insects to the nest and provoke an activation of the immune system of nestlings. *Frontiers in Zoology* **13,** 3.

JALETA, K. T., HILL, S. R., BIRGERSSON, G., TEKIE, H. & IGNELL, R. (2016). Chicken volatiles repel host-seeking malaria mosquitoes. *Malaria Journal* **15,** 354.

KAMPANGO, A., CUAMBA, N. & CHARLWOOD, J. (2011). Does moonlight influence the biting behaviour of *Anopheles* funestus? *Medical and Veterinary Entomology* **25,** 240-246.

KELLY D. W. (2001). Why are some people bitten more than others? *Trends in Parasitology* **17,** 578-81.

KILPATRICK A. M., DASZAK, P., JONES, M. J., MARRA, P. P. & KRAMER, L. D. (2006a). Host heterogeneity dominates West Nile virus transmission. *Proceedings of the Royal Society of London B: Biological Sciences* **273,** 2327-33.

KILPATRICK A. M., KRAMER, L. D., JONES, M. J., MARRA, P. P. & DASZAK, P. (2006b). West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. *PLoS Biology* **4,** e82.

KLINE D. L. & LEMIRE, G. F. (1995). Field evaluation of heat as an added attractant to traps baited with carbon dioxide and octenol for *Aedes taeniorhynchus*. *Journal of the American Mosquito Control Association* **11,** 454-456.

KLINE D., TAKKEN, W., WOOD, J. & CARLSON, D. (1990). Field studies on the potential of butanone, carbon dioxide, honey extract, l-octen-3-ol, L-lactic acid and phenols as attractants for mosquitoes. *Medical and Veterinary Entomology* **4,** 383-391.

KRAMER L. D., STYER, L. M. & EBEL, G. D. (2008). A global perspective on the epidemiology of West Nile virus. *Annual Review of Entomology* **53,** 61-81.

KWON H.-W., LU, T., RÜTZLER, M. & ZWIEBEL, L. J. (2006). Olfactory responses in a gustatory organ of the malaria vector mosquito *Anopheles gambiae*. *Proceedings of the National Academy of Sciences of the United State of America* **103,** 13526-13531.

LAARMAN J. (1958). The host-seeking behaviour of anopheline mosquitoes. *Tropical and Geographical Medicine* **10,** 293-305.

LACROIX, R., MUKABANA, W. R., GOUAGNA, L. C. & KOELLA, J. C. (2005). Malaria infection increases attractiveness of humans to mosquitoes. *Plos Biology* **3,** 1590-1593.

LALUBIN F., BIZE, P., VAN ROOYEN, J., CHRISTE, P. & GLAIZOT, O. (2012). Potential evidence of parasite avoidance in an avian malarial vector. *Animal Behaviour* **84,** 539-545.

LAND M., GIBSON, G. & H , J. (1997). Mosquito eye design: conical rhabdoms are matched to wide aperture lenses. *Proceedings of the Royal Society of London B: Biological Sciences* **264,** 1183-1187.

LAND, M., GIBSON, G., HORWOOD, J. & ZEIL, J. (1999). Fundamental differences in the optical structure of the eyes of nocturnal and diurnal mosquitoes. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* **185,** 91-103.

LEAL W. S. (2013). Odorant reception in insects: roles of receptors, binding proteins, and degrading enzymes. *Annual Review of Entomology* **58,** 373-391.

LEFÈVRE T., GOUAGNA, L.-C., DABIRÉ, K. R., ELGUERO, E., FONTENILLE, D., RENAUD, F., COSTANTINI, C. & THOMAS, F. (2009). Beyond nature and nurture: phenotypic plasticity in blood-feeding behavior of *Anopheles gambiae* s.s. when humans are not readily accessible. *The American Journal of Tropical Medicine and Hygiene* **81,** 1023-1029.

LEHANE M. J. (2005). *The biology of blood-sucking in insects,* Second edition. Cambridge University Press, New York.

LONG C., FLINT, J. & LEPPER, P. (2011). Insect attraction to wind turbines: does colour play a role? *European Journal of Wildlife Research* **57,** 323-331.

MALMQVIST, B., STRASEVICIUS, D., HELLGREN, O., ADLER, P. H. & BENSCH, S. (2004). Vertebrate host specificity of wild–caught blackflies revealed by mitochondrial DNA in blood. *Proceedings of the Royal Society of London B: Biological Sciences (Suppl.)* **271,** S152-S155.

MARQUARDT, W. H. (2004). *Biology of disease vectors*. Academic Press.

MARTÍNEZ-DE LA PUENTE, J., MERINO, S., TOMÁS, G., MORENO, J., MORALES, J., LOBATO, E., TALAVERA, S. & SARTO I MONTEYS, V. (2009). Factors affecting *Culicoides* species composition and abundance in avian nests. *Parasitology* **136,** 1033-1041.

MARTÍNEZ-DE LA PUENTE, J., FIGUEROLA, J. & SORIGUER, R. (2015b). Fur or feather? Feeding preferences of species of *Culicoides* biting midges in Europe. *Trends in Parasitology* **31,** 16-22.

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 Oecologica* **36,** 543-547.

MARTÍNEZ-DE LA PUENTE, J., MUÑOZ, J., CAPELLI, G., MONTARSI, F., SORIGUER, R., ARNOLDI, D., RIZZOLI, A. & FIGUEROLA, J. (2015a). Avian malaria parasites in the last supper: identifying encounters between parasites and the invasive Asian mosquito tiger and native mosquito species in Italy. *Malaria Journal* **14,** 32.

MBOERA, L. & TAKKEN, W. (1997). Carbon dioxide chemotropism in mosquitoes (Diptera: Culicidae) and its potential in vector surveillance and management programmes. *Medical and Veterinary Entomology* **7,** 355-368.

MCBRIDE, C. S. (2016). Genes and odors underlying the recent evolution of mosquito preference for humans. *Current Biology* **26,** R41-R46.

MOLAEI G., ANDREADIS, T. G., ARMSTRONG, P. M., ANDERSON, J. F. & VOSSBRINCK, C. R. (2006). Host feeding patterns of *Culex* mosquitoes and West Nile virus transmission, northeastern United States. *Emerging Infectious Diseases* **12,** 468-474.

Mooring M. S., Benjamin, J. E., Harte, C. R. & Herzog, N. B. (2000). Testing the interspecific body size principle in ungulates: the smaller they come, the harder they groom. *Animal Behaviour* **60,** 35-45.

Muirhead-Thompson, R. (2012). *Trap responses of flying insects: the influence of trap design on capture efficiency*. Academic Press.

Muñoz J., Eritja, R., Alcaide, M., Montalvo, T., Soriguer, R. C. & Figuerola, J. (2011). Host-feeding patterns of native *Culex pipiens* and invasive *Aedes albopictus* mosquitoes (Diptera: Culicidae) in urban zones from Barcelona, Spain. *Journal of Medical Entomology* **48,** 956-960.

MuñÑoz, J., Ruiz, S., Soriguer, R., Alcaide, M., Viana, D. S., Roiz, D., Vázquez, A. & Figuerola, J. (2012). Feeding patterns of potential West Nile virus vectors in south-west Spain. *PLoS One* **7,** 1-9.

Nasci R. S. (1982). Differences in host choice between the sibling species of treehole mosquitoes *Aedes triseriatus* and *Aedes hendersoni*. *The American Journal of Tropical Medicine and Hygiene* **31,** 411-415.

Nock C. A., Vogt, R. J. & Beisner, B. E. (2016). Functional traits. In *eLS*. John Wiley & Sons, Ltd, Chichester, UK.

O'Shea, B., Rebollar-Tellez, E., Ward, R., Hamilton, J., El Naiem, D. & Polwart, A. (2002). Enhanced sandfly attraction to Leishmania-infected hosts. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **96,** 117-118.

Olanga, E. A., Okal, M. N., Mbadi, P. A., Kokwaro, E. D. & Mukabana, W. R. (2010). Attraction of *Anopheles gambiae* to odour baits augmented with heat and moisture. *Malaria Journal* **9,** 6.

Payne, T. L., Birch, M. C. & Kennedy, C. (1986). *Mechanisms in insect olfaction*. Clarendon Press Oxford.

Perkins S. E., Cattadori, I. M., Tagliapietra, V., Rizzoli, A. P. & Hudson, P. J. (2003). Empirical evidence for key hosts in persistence of a tick-borne disease. *International Journal for Parasitology* **33,** 909-917.

Pitts R. J. & Zwiebel, L. J. (2006). Antennal sensilla of two female anopheline sibling species with differing host ranges. *Malaria Journal* **5,** 26.

Potter C. J. (2014). Stop the biting: targeting a mosquito's sense of smell. *Cell* **156,** 878-881.

Poulin, B., Lefebvre, G., Muranyi-Kovacs, C. & Hilaire, S. (2017). Mosquito traps: an innovative, environmentally friendly technique to control mosquitoes.

International Journal of Environmental Research and Public Health **14,** 313.

Poulin, R. (1995). "Adaptive" changes in the behaviour of parasitized animals: a critical review. *International Journal for Parasitology* **25,** 1371-1383.

Price G. D., Smith, N. & Carlson, D. A. (1979). The attraction of female mosquitoes (*Anopheles quadrimaculatus* Say) to stored human emanations in conjunction with adjusted levels of relative humidity, temperature, and carbon dioxide. *Journal of Chemical Ecology* **5,** 383-395.

Reinert, W. C. (1989). The New Jersey light trap: an old standard for most mosquito control programs. *Journal of the American Mosquito Control Association* **76,** 17-25.

Rizzoli, A., Bolzoni, L., Chadwick, E. A., 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. *Parasites & Vectors* **8,** 213.

Rubio-Palis, Y. (1992). Influence of moonlight on light trap catches of the malaria vector *Anopheles nuneztovari* in Venezuela. *Journal of the American Mosquito Control Association* **8,** 178-180.

Russell, C. B. & Hunter, F. F. (2005). Attraction of *Culex pipiens/restuans* (Diptera : Culicidae) mosquitoes to bird uropygial gland odors at two elevations in the Niagara Region of Ontario. *Journal of Medical Entomology* **42,** 301-305.

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. *Journal of Medical Entomology* **47,** 442-450.

Scheuerlein, A. & Ricklefs, R. E. (2004). Prevalence of blood parasites in European passeriform birds. *Proceedings of the Royal Society of London B: Biological Sciences* **271,** 1363-1370.

Shapiro, L., Moraes, C. M., Stephenson, A. G. & Mescher, M. C. (2012). Pathogen effects on vegetative and floral odours mediate vector attraction and host exposure in a complex pathosystem. *Ecology Letters* **15,** 1430-1438.

Silver, J. B. (2007). *Mosquito ecology: field sampling methods*. Springer Science & Business Media.

Simpson J. E., Hurtado, P. J., Medlock, J., Molaei, G., Andreadis, T. G., Galvani, A. P. & Diuk-Wasser, M. A. (2012). Vector host-feeding preferences drive transmission of multi-host pathogens: West Nile virus as a model system. *Proceedings of the Royal Society of London B: Biological Sciences* **279,** 925-33.

Sippell, W. & Brown, A. (1953). Studies of the responses of the female *Aedes* mosquito.

Part V. The role of visual factors. *Bulletin of Entomological Research* **43,** 567-574.

SMALLEGANGE, R. C. & TAKKEN, W. (2010). Host-seeking behaviour of mosquitoes: responses to olfactory stimuli in the laboratory. In *Olfaction in vector-host interactions* (ed. W. Takken and B. G. J. Knols). Wageningen Academic Publishers, Wageningen, The Netherlands.

SMITH C. N., SMITH, N., GOUCK, H. K., WEIDHAAS, D., GILBERT, I., MAYER, M., SMITTLE, B. & HOFBAUER, A. (1970). L-lactic acid as a factor in the attraction of *Aedes aegypti* (Diptera: Culicidae) to human hosts. *Annals of the Entomological Society of America* **63,** 760-770.

STAFFORD, C. A., WALKER, G. P. & ULLMAN, D. E. (2011). Infection with a plant virus modifies vector feeding behavior. *Proceedings of the National Academy of Sciences of the United State of America* **108,** 9350-9355.

STRYKER R. G. & YOUNG, W. W. (1970). Effectiveness of carbon dioxide and L-lactic acid in mosquito light traps with and without light. *Mosquito News* **30,** 388-393.

SYED, Z. & LEAL, W. S. (2009). Acute olfactory response of *Culex* mosquitoes to a human- and bird-derived attractant. *Proceedings of the National Academy of Sciences of the United State of America* **106,** 18803-18808.

TAKKEN W. (1991). The role of olfaction in host-seeking of mosquitoes: a review. *International Journal of Tropical Insect Science* **12,** 287-295.

TAKKEN W. & KNOLS, B. G. (1999). Odor-mediated behavior of Afrotropical malaria mosquitoes. *Annual Review of Entomology* **44,** 131-157.

TAKKEN W. & VERHULST, N. O. (2013). Host preferences of blood-feeding mosquitoes. *Annual Review of Entomology* **58,** 433-53.

TAKKEN, W., GEENE, R., ADAM, W., JETTEN, T. H. & VAN DER VELDEN, J. A. (2002). Distribution and dynamics of larval populations of *Anopheles messeae* and *A. atroparvus* in the delta of the rivers Rhine and Meuse, The Netherlands. *AMBIO: A Journal of the Human Environment* **31,** 212-218.

TAKKEN, W., KLOWDEN, M. & CHAMBERS, G. (1998). Effect of body size on host seeking and blood meal utilization in *Anopheles gambiae* sensu stricto (Diptera: Culicidae): the disadvantage of being small. *Journal of Medical Entomology* **35,** 639-645.

TEMPELIS G. & WASHINO, R. (1967). Host-feeding patterns of *Culex tarsalis* in the Sacramento Valley, California, with notes on other species. *Journal of Medical Entomology.*

Tomás, G., Merino, S., Martínez-de la Puente, J., Moreno, J., Morales, J. & Lobato, E. (2008). Determinants of abundance and effects of blood-sucking flying insects in the nest of a hole-nesting bird. *Oecologia* **156,** 305-312.

Valkiūnas G. (2005). *Avian malaria parasites and other haemosporidia*. CRC press.

van Breugel, F., Riffell, J., Fairhall, A. & Dickinson, M. H. (2015). Mosquitoes use vision to associate odor plumes with thermal targets. *Current Biology* **25,** 2123-9.

Verhulst, N. O., Andriessen, R., Groenhagen, U., Kiss, G. B., Schulz, S., Takken, W., van Loon, J. J. A., Schraa, G. & Smallegange, R. C. (2010). Differential attraction of malaria mosquitoes to volatile blends produced by human skin bacteria. *PLoS One* **5,** e15829.

Violle C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., HummeL, I. & Garnier, E. (2007). Let the concept of trait be functional! *Oikos* **116,** 882-892.

Waage J. & Nondo, J. (1982). Host behaviour and mosquito feeding success: an experimental study. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **76,** 119-122.

Webber L. & Edman, J. (1972). Anti-mosquito behaviour of ciconiiform birds. *Animal Behaviour* **20,** 228-232.

Wekesa J., Yuval, B., Washino, R. & de Vasquez, A. (1997). Blood feeding patterns of *Anopheles freeborni* and *Culex tarsalis* (Diptera: Culicidae): effects of habitat and host abundance. *Bulletin of Entomological Research* **87,** 633-641.

Wen Y., Muir, L. E. & Kay, B. H. (1997). Response of *Culex quinquefasciatus* to visual stimuli. *Journal of the American Mosquito Control Association* **13,** 150-152.

Woolhouse M. E., Dye, C., Etard, J.-F., Smith, T., Charlwood, J., Garnett, G., Hagan, P., Hii, J., Ndhlovu, P. & Quinnell, R. (1997). Heterogeneities in the transmission of infectious agents: implications for the design of control programs. *Proceedings of the National Academy of Sciences of the United State of America* **94,** 338-342.

Yan, J., Gangoso, L., Martínez-de la Puente, J., Soriguer, R. & Figuerola, J. (2017b). Avian phenotypic traits related to feeding preferences in two *Culex* mosquitoes. *The Science of Nature* **104,** 76.

Yan, J., Martínez-de la Puente, J., Gangoso, L., Gutiérrez-López, R., Soriguer, R. & Figuerola, J. (2017a). Avian malaria infection intensity influences mosquito feeding patterns. *International Journal for Parasitology***,** in press.

Yezerinac, S. M. & Weatherhead, P. J. (1995). Plumage coloration, differential attraction of vectors and hematozoa infections in birds. *Journal of Animal Ecology* **64,** 528-

537.

ZOLLNER G. E., TORR, S. J., AMMANN, C. & MEIXNER, F. X. (2004). Dispersion of carbon dioxide plumes in African woodland: implications for host-finding by tsetse flies. *Physiological Entomology* **29,** 381-394.

Chapter 2

Avian phenotypic traits in relation 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 pat- terns 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.

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).

Table 1. GLMs analyzing the variation in the forage ratio (FR) of *Cx. pipiens* in birds. 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. Δ*i* (AICc) = [AICc*i* –min AICc], ω*i* (AICc) = the rounded second-order Akaike weights. The variables included in each model are represented by crosses.

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

Table 1 (Continued)

Explanatory variables						Criterion		
+	+		+		+	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
	+			+	+	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

Table 2. Summary statistics of the averaged model derived from the set of top GLM models (Δ*i*(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

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).

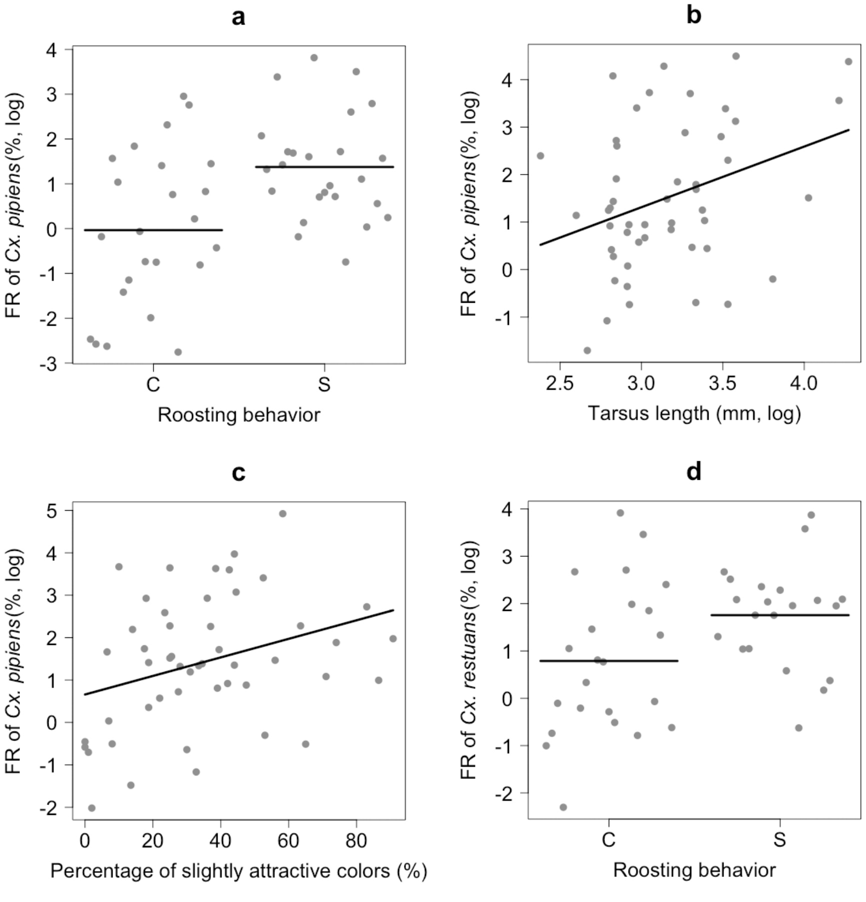

Fig 1. Relationship between forage ratios and avian host traits: **a**. FR of *Cx. pipiens* and bird roosting behavior (C: communally/S: solitary); **b**. FR of *Cx. pipiens* and bird tarsus length (mm); **c**. FR of *Cx. pipiens* and percentage of slightly attractive colors (psac) in bird body (%); **d**. FR of *Cx. restuans* and bird roosting behavior (C: communally/S: solitary). Estimates were derived from the highest-ranking models according to AICc (Table 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**.

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 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).

Table 3. GLMs analyzing the variation in the forage ratio (FR) of *Cx. restuans* in birds. 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. Δ*i* (AICc) = [AICc*i* –min AICc], ω*i* (AICc) = the rounded second-order Akaike weights. The variables included in each model are represented by crosses

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

Table 3 (Continued)

Explanatory variables						Criterion		
	+				+	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

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

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 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 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 (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.

Supplementary information

Table S1. Phenotypic attributes for the 49 avian species included in this study. The forage ratio is given for mosquitoes of the species *C. pipiens* and *Cx. restuans*. Abbreviations used: Mass: body mass (g); Tarsus: tarsus length (mm); Bill: bill length (mm); Phac: the percentage of highly attractive colors; Pmac: the percentage of moderately attractive colors; Psac: the percentage of slightly attractive colors; R.: roosting behavior (C-communal, S-solitary); Nest: nest type (C-close cup, H-hole or cavity-nester, O-open cup/nest); *Cp.*: forage ratio for *Cx. pipiens*; *Cr.*: forage ratio for *Cx. restuans*.

Additional references cited in the table:

1. Richards GL (1971) The common crow, *Corvus brachyrhynchos*, in the Great Basin. Condor 73:116-118. doi:10.2307/1366136
2. Layne JN, Smith DR (1992) Size Comparison of Resident and Wintering American Kestrels in South-Central Florida (Comparaciones en el Tamaño de Individuos de Poblaciones Residentes e Invernales de Falco sparverius en la Parte Sur-central de Florida). J Field Ornithol 63:256-263
3. Cramp S, Perrins CM, Brooks DJ (1994) The Birds of the Western Palearctic-Volume VIII: Crows to Finches. Oxford: University Press
4. Murphy MT (1989) Life history variability in North American breeding tyrant flycatchers: phylogeny, size or ecology? Oikos 54:3-14. doi:10.2307/3565891
5. Aldrich JW, James FC (1991) Ecogeographic variation in the American Robin (Turdus migratorius). The Auk 108:230-249
6. Lapiedra O, Sol D, Carranza S, Beaulieu JM (2013) Behavioural changes and the adaptive diversification of pigeons and doves. Proc R Soc B 280: 20122893
7. Janousek WM, Marra PP, Kilpatrick AM (2014) Avian roosting behavior influences vector–host interactions for West Nile virus hosts. Parasit Vectors 7:1-11
8. Poulin R (1991) Group-living and infestation by ectoparasites in passerines. Condor:418-423
9. Harrap S, Quinn D (1996) Tits, nuthatches & treecreepers. A&C Black.
10. Audubon: Guide to North American Birds Online (2016) National Audubon Society. http://www.audubon.org/bird-guide. Accessed 28 October 2016

Table S1

Common name	Species name	Mass	Tarsus	Bill	Phac	Pmac	Psac	R.	Nest	*Cp.*	*Cr.*
Cooper's Hawk	*Accipiter cooperii*	452	67.6	17.6	13.5	69	17.5	S	O^{10}	25.17	
Red-winged Blackbird	*Agelaius phoeniceus*	48.45	28.05	21.45	66.5	20	13.5	C	O^{10}	0.08	0.41
Mallard	*Anas platyrhynchos*	1161.5	45	40.2	23.75	43.5	32.75	C	O^{10}	0.2	0.51
Cedar Waxwing	*Bombycilla cedrorum*	32.35	16.55	6.76	16	53	31	C	O^{10}	0.59	0.75
Northern Cardinal	*Cardinalis cardinalis*	41.35	28.1	12.65	55.5	11	33.5	S^{7}	O^{10}	5.5	6.2
American Goldfinch	*Carduelis tristis*	12.15	14.4	9.28	20	15	65	C	O^{10}	0.09	0.22
House Finch	*Carpodacus mexicanus*	21.75	17.2	8.7	11.25	36.25	52.5	C	O^{10}	5.69	3.42
Veery	*Catharus fuscescens*	30.9	29.2	13.5	8.5	64	27.5	S	O^{10}	3.12	7.97
Swainson's Thrush	*Catharus ustulatus*	29.8	27.1	8.67	2.5	55	42.5	S^{8}	O^{10}	50.34	
Killdeer	*Charadrius vociferus*	96.55	35.8	20.25	21	54	25	C	O^{10}	4.68	11.94
Northern Flicker	*Colaptes auratus*	135.7	28.075	26.225	32.5	39.5	28	S	H^{10}	5.4	13.78
Rock Pigeon	*Columba livia*	280.5	30.05	18.8	37	62	1	C	O^{10}	0.19	0.49
Eastern Wood-Pewee	*Contopus virens*	14.1	13.45	11.55	21.5	53	25.5	S	O^{10}	2.67	6.8
American Crow	*Corvus brachyrhynchos*	399.7	56.05^{1}	32.4^{1}	100	0	0	C	O^{10}	0.54	1.37
Blue Jay	*Cyanocitta cristata*	71.65	34.155	24.98	36	39	25	S	O^{10}	8.44	3.08
Yellow Warbler	*Dendroica petechia*	9.8	18.4	7.955	6	7.5	86.5	S	C^{10}	2.27	5.8
Gray Catbird	*Dumetella carolinensis*	32.15	27.4	11	13	87	0	S^{7}	O^{10}	0.78	1.99
Willow Flycatcher	*Empidonax traillii*	12.6	16.57	8.985	13.5	23	63.5	S	O^{10}	7.17	18.3
American Kestrel	*Falco sparverius*	119.9^{2}	35.9^{2}	12.75^{2}	16.5	58.5	25	S	H^{10}	75.51	
Common Yellowthroat	*Geothlypis trichas*	10.1	20.5^{3}	13.6^{3}	6.25	3	90.75	S^{8}	O^{10}	6.95	17.73
Barn Swallow	*Hirundo rustica*	19.1	10.8	12.25	56.5	25.5	18	C	C^{10}	1.94	4.96
Baltimore Oriole	*Icterus galbula*	35.4	23.5	17.9	30.5	35	34.5	C^{8}	C^{10}	1.12	2.85
Song Sparrow	*Melospiza melodia*	24.2	24.175	15.68	8	70	22	S^{8}	O^{10}	2.11	2.69
Brown-Headed Cowbird	*Molothrus ater*	41.65	24.1	16.35	36	25	39	C	O^{10}	0.65	3.34
Great-Crested Flycatcher	*Myiarchus crinitus*	34.4^{4}	18.4^{4}	15.4^{4}	6	38	56	S	H^{10}	3.64	9.28
Monk Parakeet	*Myiopsitta monachus*	120	18.65	19.5	3.5	66.5	30	C	C^{10}	0.11	0.28
Black-Capped Chickadee	*Parus atricapillus*	12.95	16.7	9.05	23	35	42	S	H^{10}	1.86	2.37
House Sparrow	*Passer domesticus*	30.2	19.75	15.3	13	68.25	18.75	C	C^{10}	0.32	0.33
Indigo Bunting	*Passerina cyanea*	14.7	16.885	10.875	51.25	30	18.75	S	O^{10}	3.09	7.89
Ring-Necked Pheasant	*Phasianus colchicus*	1090	71.7	40.7	19	36.5	44.5	C	O^{10}	25.17	64.25

Table S1 (continued)

Common name	Species name	Mass	Tarsus	Bill	Phac	Pmac	Psac	R.	Nest	*Cp.*	*Cr.*
Downy Woodpecker	*Picoides pubescens*	28.2	16.25	15.86	47	0	53	S	H[10]	0.53	1.35
Hairy Woodpecker	*Picoides villosus*	65.5	21.085	32.39	56	0	44	C	H[10]	12.96	33.08
Eastern Towhee	*Pipilo erythrophthalmus*	40.5	26.25	9.85	37.5	48.5	14	S	O[10]	11.85	30.24
Scarlet Tanager	*Piranga olivacea*	28.3	19.5[3]	10.15	50	11.5	38.5	S	O[10]	34.09	87
Blue-Gray Gnatcatcher	*Polioptila caerulea*	6	17.2	21.34	7	56	37	C	C[10]	1.81	4.62
Common Grackle	*Quiscalus quiscula*	105.9	34.15	28.15	27	71	2	C	O[10]	0.06	0.24
Common Canary	*Serinus canaria*	15.25[3]	16.85[3]	11.85[3]	9.75	32	58.25	C	O[10]	25.17	
Eastern Bluebird	*Sialia sialis*	29.5	23.05	9.8	47.5	42.5	10	C	H[10]	10.75	27.43
White-Breasted Nuthatch	*Sitta carolinensis*	21.1	18.6	19.1	13.5	42.5	44	S[9]	H[10]	3.29	8.39
Chipping Sparrow	*Spizella passerina*	12.75	17.05	9.4	18	74	8	S	O[10]	0.46	0.59
Eurasian Collared-Dove	*Streptopelia decaocto*	193	25.05	16.75	6	11	83	C	O[10]	4.65	11.86
European Starling	*Sturnus vulgaris*	86	29.6	21.34	70	23	7	C	H[10]	0.39	0.91
Brown Thrasher	*Toxostoma rufum*	68.5	33.65	24.65	8.5	68	23.5	S	O[10]	24.17	
House Wren	*Troglodytes aedon*	11.45	16.9	11.9	11.5	41	47.5	S[8]	H[10]	1.82	1.55
American Robin	*Turdus migratorius*	79.95	32.75[5]	22.4[5]	20	73.5	6.5	C[7]	O[10]	2.26	1.92
Eastern Kingbird	*Tyrannus tyrannus*	40.6[4]	16.4[4]	13.9[4]	31.5	29	39.5	S	O[10]	4.04	10.32
Warbling vireo	*Vireo gilvus*	12.55	17.27	16.9	12	52	36	C	C[10]	3.54	9.02
Red-eyed Vireo	*Vireo olivaceus*	20.3	18.45	9.6	2	27	71	S	C[10]	2.49	6.35
Mourning Dove	*Zenaida macroura*	135[6]	20.5[6]	13.5[6]	6	20	74	C	O[10]	1.55	0.8

Table S2. 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* after omitting two raptor species.**

Parameter	Estimate	SE	z value	2.50%	97.50%	*P*
Intercept	0.743	0.221	3.272	0.298	1.188	0.001
Roosting behavior	1.186	0.451	2.556	0.276	2.095	0.011
Log. tarsus length	0.239	0.432	0.548	-0.271	1.649	0.584
Percentage of slightly attractive colors	0.678	0.570	1.173	-0.045	1.865	0.241

Table S3. 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* derived from gravid trap.**

Parameter	Estimate	SE	z value	2.50%	97.50%	*P*
Intercept	1.169	0.216	5.267	0.734	1.604	<0.001
Roosting behavior	1.317	0.455	2.819	0.401	2.232	0.005
Log. tarsus length	1.136	0.533	2.078	0.064	2.207	0.038
Percentage of slightly attractive colors	1.000	0.472	2.060	0.049	1.951	0.039
Log. bill length	-0.568	0.611	0.903	-1.799	0.664	0.366

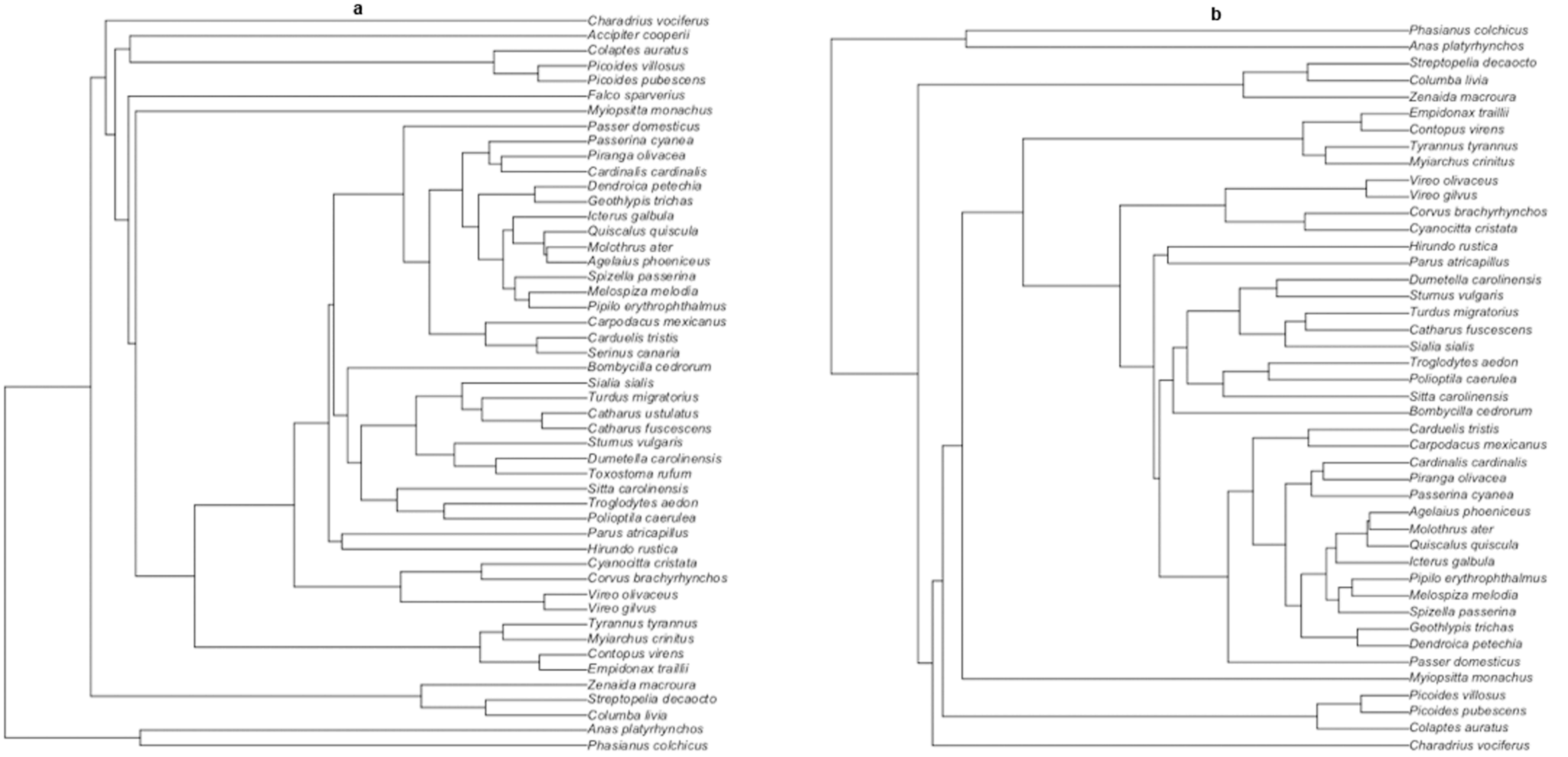

Fig S1. Consensus trees obtained from 1000 trees generated on BirdTree (http://birdtree.org). The trees generated by adopting a 50% majority-rule consensus tree (SumTrees 3.3.1 in DendroPy 3.12.2) using the code described by Rubolini *et al*. (2015 *Curr Zool* **61**). Working phylogenies for PGLS analyses included **a)** 49 avian species with the estimated forage ratio for *Cx. pipiens*; **b)** 44 avian species with the estimated forage ratio for *Cx. restuans*.

References

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

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

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. 2 edn. Springer doi: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. doi:10.1016/S0035-9203(38)90101-4

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. doi: http://dx.doi.org/10.1017/S0007485300026808

Browne SM, Bennett GF (1981) Response of mosquitoes (Diptera: Culicidae) to visual stimuli. J Med Entomol 18:505-521. doi: http://dx.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. 2 edn. Springer Science & Business Media, New York. doi: 10.1007/b97636

Burnham KP, Anderson DR (2004) Multimodel inference understanding AIC and BIC in model selection. Socio Meth Res 33:261-304. doi: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, NJ

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

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. doi:http://dx.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. doi: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. doi: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. doi: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. doi: http://dx.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. doi: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, NJ

Kamilar JM, Cooper N (2013) Phylogenetic signal in primate behaviour, ecology and life history. Phil 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. doi: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. 2 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. doi:http://dx.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. doi: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, 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. doi:10.1016/j.actao.2010.07.008

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. doi:10.1017/S0031182009006374

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. doi: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. doi: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. doi: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. doi: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

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

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. doi:10.1186/s13071-015-0831-4

Rodewald P (2015) The Birds of North America Online: http://bna.birds.cornell.edu/BNA/. Cornell Laboratory of Ornithology, Ithaca, NY. 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. doi: 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. doi:http://dx.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-Wageningen 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. doi: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. doi: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, Wheeler SS, Barker CM, Reisen WK (2011) Mosquito host selection varies seasonally with host availability and mosquito density. PLoS Negl Trop Dis 5:e1452

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

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. doi:10.1016/j.cub.2015.06.046

Vuilleumier F (2011) Birds of North America: Western Region. Dorling Kindersley Publishing, New York, NY

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. doi: http://dx.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. doi:10.2307/5655

Chapter 3

Does bird metabolic rate affect mosquito feeding preference?

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Parasites & Vectors, under review

Abstract

Host selection by mosquitoes plays a central role in the transmission of vector-borne infectious diseases. Although inter-specific variation in mosquito attraction has often been reported, the mechanisms underlying intra-specific differences in host's attractiveness to mosquitoes are still poorly known. Metabolic rate is related to several physiological parameters used as location cues by mosquitoes, and so potentially affect host-vector contact rates. Therefore, individual hosts with higher metabolic rates should be more attractive to host-seeking mosquitoes. Here, we investigated the role of bird metabolic rate in the feeding preferences of *Culex pipiens* (Linnaeus), a widespread mosquito vector of many pathogens affecting human and wildlife health. *Passer domesticus* (Linnaeus) pairs containing one bird treated with 2,4-Dinitrophenol (DNP) and the other injected with phosphate-buffered saline solution (PBS) (i.e. control) were simultaneously exposed overnight to mosquitoes. Contrary to our expectation, the treatment did not affect the proportion of mosquitoes biting on each individual. This may be due to the absence of a significant effect of DNP treatment on bird resting metabolic rate. However, birds with lower resting metabolic rates prior to the DNP injection, regardless of the treatment, were bitten more often than their counterparts. In addition, larger individuals were bitten more frequently than smaller ones. This intra-specific difference in mosquito feeding preferences could be explained by enhanced anti-mosquito behaviour associated with higher metabolic rate. In a host community, individuals with high metabolism may actively avoid being bitten by mosquitoes, despite releasing more attractant cues. Since metabolic rates can be related to individual differences in personality and life history traits, differences in mosquitoes' feeding preferences may determine intra-specific differences in exposure to vector-borne pathogens.

Keywords: Host choice; Resting metabolic rate; Vector attraction; Body mass; Defensive behaviour; Bloodmeal origin; Intra-specific difference; *Passer domesticus*; 2,4-Dinitrophenol, Blood feeding.

Background

Mosquitoes (Diptera: Culicidae) are responsible for the transmission of multiple vector-borne pathogens that cause diseases such as malaria, West Nile fever and yellow fever (Becker et al., 2010). Host selection by mosquitoes is recognized as a key factor affecting pathogen amplification and transmission risk since it drives host-vector contact rates (Kilpatrick et al., 2006; Muñoz et al., 2012). Differential mosquito biting preferences have been reported at host inter-specific level (Gingrich et al., 2005; Molaei et al., 2007; Farajollahi et al., 2011; Muñoz et al., 2012; Martiínez-de la Puente et al., 2015; Rizzoli et al., 2015), but also among individuals within species (Kelly, 2001; Gervasi et al., 2016). Understanding the individual characteristics underlying these asymmetries is of great importance as variability in contact rates could result in heterogeneous individual risk for pathogen transmission (Paull et al., 2012; VanderWaal et al., 2016). However, the mechanisms underlying host intra-specific differences in mosquito attraction are still poorly understood (Paull et al., 2012).

Mosquitoes use visual, thermal and chemical cues to detect their hosts (Takken and Verhulst, 2013). Metabolic rates of host animals are related to the activity and physiology of an individual (Nilsson, 2002). This in turn is directly linked to the emission of CO_2, heat and humidity (Blaxter, 1989), which may enhance mosquito attraction (van Breugel et al., 2015). On the other hand, metabolic rate may also be associated with defensive behaviour, with individuals of higher metabolism being more restless and hence more difficult for mosquitoes to bite. In addition, body mass (BM) is usually a positive correlate of metabolic rate in many organisms (Gillooly et al., 2001). In birds, metabolic rate is positively associated with BM as supported by a study on 231 species (McKechnie et al., 2006), but a negative association was also reported within small-size hummingbirds (Opazo et al., 2005). Owing to the potential link with metabolic rate, BM may also affect the emission of multiple host-seeking cues as well as the defensive behaviour, which may affect mosquito attraction. A positive link between BM and blood-sucking insect attraction has been reported by a high number of authors working with groups of arthropod vectors such as mosquitoes (Port et al., 1980; Estep et al., 2012), biting midges *Culicoides* (Martiínez-de la Puente et al., 2009) and blackflies (Malmqvist et al., 2004; Martiínez-de la Puente et al., 2010). However, the specific role of avian BM in mosquito attraction at intra-specific level remains poorly studied. Finally, a potential bias in studies of mosquito preference is that much research to date has focused on mosquito attractants and whether or not one particular individual will be chosen as a host before another one. However, under natural conditions individuals exposed to mosquito bites are in many cases surrounded by other potential hosts. Therefore, the likelihood of being bitten may not only depend on these attractant factors but also on the overall host community, that is, whether or not they are surrounded by more attractive and/or susceptible counterparts.

Despite the potential importance of host metabolic rate in mosquito feeding preferences, to the best of our knowledge no study has yet experimentally tested the link between host metabolic rate and mosquito host selection. Here we tested the hypothesis that mosquitoes preferentially bite individuals with higher metabolic rates, as these may release more attractant clues. Alternatively, individuals with higher metabolic rates may more effectively avoid being bitten than their counterparts with lower metabolic rates. To do that we assigned house sparrows *Passer domesticus* (Linnaeus) to two experimental treatments: birds injected with 2,4-Dinitrophenol (DNP) or treated as controls, and exposed them to the bites of the mosquito *Culex pipiens* (Linnaeus) in pairs. The mosquito species *Culex pipiens* is widespread and acts as the main bridge vector for a number of pathogens affecting humans and wildlife including house sparrows (e.g. West Nile virus, Saint Louis encephalitis virus, filarial worms and avian malaria) (Farajollahi et al., 2011). Our experimental approach simulates a situation of host selection in the wild where birds with different physiological conditions grouped together during the breeding season or at roosts, and mosquitoes are active in seeking hosts.

Results

The percentage of fed mosquitoes in relation to total mosquitoes introduced in each trial varied from 5.46% to 40.6% (mean = 27.2% ± 2.51% SE; see also Additional file 1). The mean percentages of fed mosquitoes in heterogeneous (containing one male and one female bird) and homogenous (containing two males) trials did not differ significantly (one-way ANOVA, $F_{1, 14} = 1.44$, $p = 0.25$). The blood meal origin of 429 mosquitoes was identified to the individual level. The mean (± SE) number of mosquitoes with a blood meal derived from a single individual was 12.63 (±1.74; range: 0-30; Additional file 1) with non-significant differences between heterogeneous and homogenous trials (one-way ANOVA, $F_{1, 29} = 0.16$, $p = 0.69$). An average of 3.33 (range = 0 - 7) mosquitoes contained mixed blood meals per trial. In subsequent analyses we only present the results excluding these mixed blood meals as results including this data were qualitatively the same (data not shown).

Prior to the treatment, the resting metabolic rate (RMR) did not differ significantly between birds assigned to the DNP and control groups (one-way ANOVA, $F_{1, 29} = 0.012$, $p = 0.92$). Two top models (Table 1) were selected according to the AICc criterion. The explained variance (conditional R^2) was 62.76% (model with BM and RMR (logistic-transformed) as explanatory variables) and 54.84% (model with only BM). Neither of these models included the experimental treatment and the treatment had no significant effect when added to the model with the lowest AICc (estimates ± SE = -0.086 ± 0.766, z = -0.112, p = 0.91). The averaged estimates indicated that feeding preference was positively associated with BM but negatively correlated to RMR (Table 2). The relative importance of BM and RMR was 1.00 and 0.63, respectively. The 95% confidence intervals for the parameter estimates did not include zero, indicating that these two predictors significantly influenced feeding preference (Table 2, Fig. 1). These results were not an artefact caused by collinearity between both variables since the correlation coefficient between RMR and BM was very low and not significant (cor = 0.223, p = 0.235), and the results did not change qualitatively when using the residuals of RMR against BM as a predictor instead of RMR (results not shown).

Table 1. **Model selection from the set of GLMMs analyzing the variation in mosquito feeding preferences**. All models include bird pair and bird identity as random terms. Explanatory variables: BM-body mass, Sex-bird sex, RMR-resting metabolic rate (logistic transformation), T-treatment, sex*T-interaction between sex and treatment, RMR*T-interaction between RMR and treatment, ΔAICc = [AICc*i* –min AICc]. The variables included in each model are represented by crosses.

BM	Sex	RMR	T	Sex*T	RMR*T	AICc	ΔAICc	ω AICc
+		**+**				**194.7**	**0.00**	**0.364**
+						**195.8**	**1.10**	**0.210**
+	+	+				197.9	3.12	0.077
+		+	+			197.9	3.14	0.076
+	+					198.5	3.75	0.056
+			+			198.7	3.97	0.050
+		+	+		+	199.2	4.42	0.040
						199.7	4.95	0.031
+	+	+	+			201.3	6.55	0.014
		+				201.3	6.58	0.014
+	+		+			201.6	6.86	0.012
	+					201.6	6.91	0.012
			+			201.9	7.14	0.010
+	+	+	+		+	202.7	7.92	0.007
+	+	+	+	+	+	202.9	8.15	0.006
+	+	+	+	+		203.2	8.42	0.005
		+	+			203.7	8.93	0.004
	+	+				203.9	9.12	0.004
	+		+			203.9	9.19	0.004
+	+		+	+		204.3	9.60	0.003

Table 1 (Continued)

BM	Sex	RMR	T	Sex*T	RMR*T	AICc	ΔAICc	ω AICc
		+	+		+	206.0	11.25	0.001
	+	+	+			206.4	11.64	0.001
	+		+	+		206.5	11.74	0.001
	+	+	+	+		208.8	14.10	0.000
	+	+	+		+	209.2	14.43	0.000
	+	+	+	+	+	211.3	16.61	0.000

In order to identify causes explaining the non-significant effect of the experimental treatment, we measured the RMR of eight birds immediately after the injection with DNP (n=4) or treated as controls (n=4). The RMR of birds did not statistically differ between the two experimental groups (one-way ANOVA, $F_{1,\,7} = 0.075$, $p = 0.79$).

Table 2. Summary statistics of the averaged model explaining the variation in feeding patterns of *Cx. pipiens*. Explanatory variables: BM-bird body mass and RMR-resting metabolic rate (logistic transformation).

Explanatory variable	Estimate	SE	z value	95% CI		*P*
Intercept	-0.033	0.384	0.081	-0.240	1.014	0.936
BM	2.453	0.835	2.804	0.608	3.311	0.005
RMR	-1.612	0.777	1.974	-2.533	-0.003	0.048

Discussion

In this study we tested the relationship between host metabolic rate and the feeding preference of *Cx. pipiens* mosquitoes. We found that these mosquitoes preferred to feed on birds with higher BM but lower RMR. To date this is the first evidence that host metabolic rate does affect mosquito feeding preference. As our focal species *Cx. pipiens* is an important vector for multiple infectious diseases, identifying factors affecting the biting preferences of this mosquito species may throw some light on the epidemiology of these pathogens.

The positive association between feeding preference and BM suggests that larger individuals may release more cues that facilitate their detection and location by mosquitoes along multiple pathways, such as vision, motion and odour. In our case, birds and mosquitoes were kept in close proximity but under complete darkness. In this context, motion (including anti-mosquito behaviour) rather than vision or odour could be the most important factor determining mosquito feeding patterns. Smaller individuals tend to move more frequently than larger ones (Mooring et al., 2000), and avian defensive behaviour can greatly affect mosquito feeding success (Klowden and Lea, 1979; Day and Edman, 1984; Edman and Scott, 1987; Darbro and Harrington, 2007). This could explain why birds with higher BM were bitten more frequently by mosquitoes. Another non-mutually exclusive explanation could be that larger individuals are an easier prey for mosquitoes since, when compared to smaller or more active (higher RMR) individuals, they may offer larger biting surfaces and be less proficient at avoiding bites. Our study provides new evidence for the importance of host size on mosquito blood-feeding at the intra-specific level.

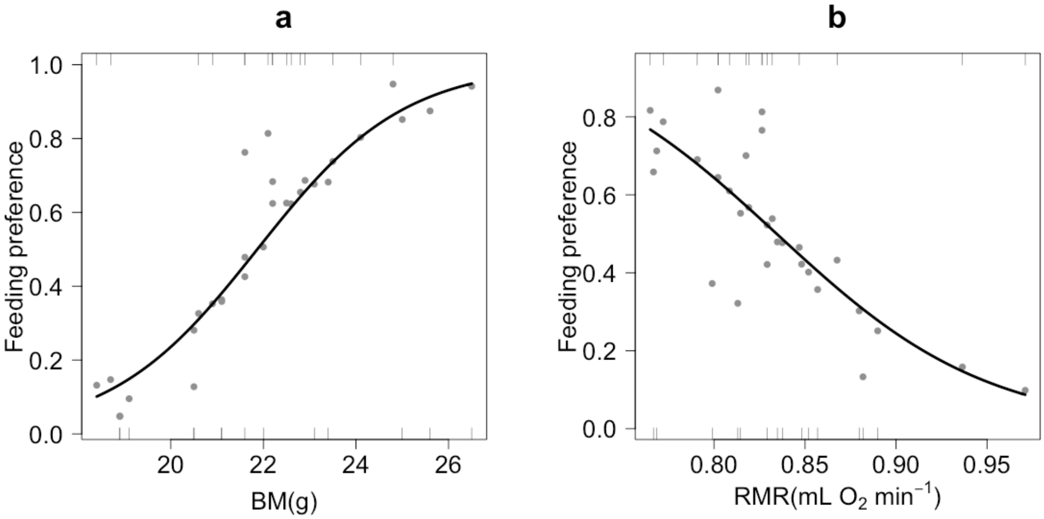

Fig. 1 Relationship between mosquito feeding preferences and a) body mass (BM); b) resting metabolic rate (**RMR**, logistic transformation). Bloodmeal origin was determined from 429 engorged mosquitoes. Total sample size of house sparrows was 30, with 15 replicates for control and DNP groups, respectively. Estimates were derived from the highest-ranked models according to the AICc. Each conditional relationship was plotted by holding the median value of the other variable using the visreg package (version 2.2.2) in R.

Contrary to our prediction, birds with lower RMR suffered more mosquito bites than individuals with higher RMR. Higher metabolic rate is expected to be associated with the increased emission of the cues used by host-seeking mosquitoes (Takken and Verhulst, 2013). Mosquito blood-feeding is a complex behaviour that includes different phases, from appetitive behaviour to a consummatory reaction and the cessation of feeding (Browne and Bennett, 1981). In our study, birds were placed close (within 60 cm) to mosquitoes, and initially the heat and humidity released by hosts may have been used as clues for the detection by mosquitoes (Takken and Verhulat, 2013; Cardé, 2015). However, after approaching their hosts, the success of blood feeding is largely determined by bird behaviour since mosquitoes avoid those individuals/species that are more active at the time of biting (Day and Edman, 1984).

In our study, birds were able to move freely during the exposure to mosquitoes and hence may have performed anti-mosquito behaviour to protect themselves from bites. The defensive behaviour displayed by birds against mosquitoes (i.e. foot stomping, head and wing movement, tail shaking; Darbro and Harrington, 2007) may reduce the ability of mosquitoes to complete a blood meal (Klowden and Lea, 1979; Edman and Scott, 1987; Darbro and Harrington, 2007) but are also energetically costly (Biro and Stamps, 2010). Animals with high RMR may be more active, aggressive, explorative and bold, while their low RMR counterparts may be calmer and shyer and have the tendency to avoid novel situations (Careau et al., 2008). Therefore, intensive movements powered by higher RMR could explain why mosquitoes bite preferably birds with lower RMR.

Despite the initial differences in RMR, we did not find any significant effect of the experimental treatment on the mosquito feeding preference. The DNP administration did not significantly affect the RMR of birds, as no differences in RMR were found between DNP-treated and control birds during the following 12 hours after injection. Previous studies have recorded an increase in metabolic rates as a result of mild mitochondrial uncoupling by DNP administration in species including invertebrates (Padalko, 2005), amphibians (Salin et al., 2012), birds (Stier et al., 2014) and mammals (Caldeira de Silva et al., 2008). However, the efficacy of DNP in most of the cases was very short in time (Harper et al., 2001). DNP can be quickly eliminated from the organism, and, for example, within 24 h up to 98% of DNP have been eliminated in ducks and rabbits (Gehring and Buerge, 1969), and the metabolic rate returned to normal values a day after injection (Dominguez et al., 1993). If the efficacy of treatment did not significantly affect the RMR of birds or/and the efficacy lasted only during the period before mosquitoes were able to bite birds, this could explain –at least in part– why no significant effects were found in this study. Indeed, the efficacy of DNP on RMR of animals may depend on the dose (Harper et al., 2001) and experimental conditions, such as temperature (Cassuto, 1968). Further studies are necessary in order to identify the effective dose of DNP to modify the RMR of wild house sparrows without increasing mortality nor producing long-term damages in bird health.

Our study simulates the natural social environment of birds in which an individual is surrounded by others that emit different physiological and behavioural clues for host-seeking mosquitoes. Although a bird with higher metabolism may give off more cues that attract mosquitoes, the final outcome of mosquito feeding patterns may also depend on the social group size, which could reduce the individual risk of being attacked (Cresswell, 1994; Janousek et al., 2014), i.e., the *per capita* bird exposure to infected mosquitoes is less given the encounter-dilution effect (Krebs et al., 2014). The broader host community in which an individual is exposed to blood-sucking mosquitoes may be a relevant factor – as is the production of attractant cues – when determining whether an individual will be bitten or not.

Conclusions

Mosquito feeding preference at intra-specific host level may be affected by hosts' metabolic rates and body mass, as well as by the host community. As metabolism is closely related to individual differences in personality (Careau et al., 2008), behaviour (Biro and Stamps, 2010) and life history traits (Stier et al., 2014), individuals may vary greatly in their exposure to mosquito bites and consequently in the amount of exposure to vector-borne diseases.

Methods

Bird and mosquito sampling and maintenance

Mosquito larvae were collected from Cañada de los Pájaros (Seville, Spain) in the summer 2014 and were reared in plastic trays containing water in climatic chambers. Larvae were supplied with shrimp food (Mikrozell 20ml/22g; Dohse Aquaristik GmbH & Co. KG, D-53501, Gelsdorf, Germany). Mosquitoes were kept at 27(±1)°C and 65–70% relative humidity (RH) under a photoperiod of 12:12 (L: D) h. One-to-five days after emergence, adult mosquitoes were anaesthetized with diethyl ether (Lipnick, 1991) and then sexed and identified to species level (Schaffner et al., 2001) on chilly Petri dishes using a stereomicroscope (Nikon SMZ-645). Female *Cx. pipiens* were retained and placed in insect-rearing cages (BugDorm-43030F, 32.5×32.5×32.5 cm) in the same chamber conditions as above with *ad libitum* access to 1% sugar solution until 10–19 days old. Mosquitoes were deprived of the sugar solution 24 hours prior to the experiment and maintained with water until 12 hours before the experiment.

Thirty juvenile house sparrows were trapped in Huelva province (southern Spain) in July 2014 using mist nets. We chose wild house sparrows as vertebrate hosts because this species is a natural reservoir for multiple vector-borne pathogens (Komar et al., 2001; Arrigo et al., 2010; LaPointe et al., 2012) and has been reported to be one of the preferred hosts of several mosquito species including *Cx. pipiens* (Hamer et al., 2008). Yearling birds were individually marked with metal rings and weighed with a digital scale (Pesola-MS500). A small blood sample from each bird was taken using jugular venipuncture for future molecular analyses (see below). Subsequently, birds were transported to the Animal Experimentation Unit at the Estación Biológica de Doñana (EBD-CSIC) and kept in pairs in cages (58.5×25×36 cm) in a vector-free room at 22(±1)°C and a 12L: 12D light cycle. Water and food (mixed grain) were provided *ad libitum*. Two-to-five days after finishing the experiment, birds were released at the site of capture.

Experimental procedure

The 30 house sparrows used in this study consisted of 21 males (10 DNP and 11 control) and nine females (five DNP and four control). Of the 15 pairs, nine contained a male and a female bird and six pairs included two male birds.

The RMR of each bird was measured as the minimum oxygen consumption under post-absorptive digestive conditions during its resting cycle (McNab, 1997; Rodríguez et al., 2014). RMR was measured during a 12-hour period from 20:00 to 08:00 using an open-circuit respirometer (Sable Systems International). Oxygen consumption (mL O_2 min^{-1}) was estimated as the lowest value of the averages of 10 min runs (Hill, 1972). Birds' BM was recorded before RMR measurements were taken.

The night following the RMR measurements, half of the birds (n=15) were randomly injected subcutaneously with 0.2 mg of DNP diluted in 0.04 ml of phosphate-buffered saline solution (PBS)(DNP group), while the remaining birds (n=15) were injected with the same volume of PBS (control group). DNP is an artificial decoupler of oxidative phosphorylation (Williams, 1966) and, acting as a protonophore, facilitates the leak of the protons that build up the force to drive ATP synthesis and results in poor connection between oxidation and phosphorylation. This induces an increase in the metabolic rate (i.e. oxygen consumption) to compensate for mitochondrial inefficiency and to meet energy demands (Nicholls et al., 2013). Immediately after injection, a pair of birds consisting of a DNP and a control bird was exposed to 10-19-days-old unfed female mosquitoes in the dark for 12 hours from 20:00 to 08:00 (activity peak of *Cx. pipiens, see* Chiba et al., 1982; Anderson et al., 2007). In all, 15 trials over three nights were conducted. In each trial, a birdcage (38.5×26 ×5.5 cm) containing a pair of birds was exposed to an average of 190 (range: 181–198) unfed *Cx. pipiens* females in insect-rearing tents (BugDorm-3120, white, 60x60x60 cm). Mosquitoes and birds were allowed to move and come into contact without any restrictions, as mosquitoes were able to freely enter the birdcages. At the end of each trial, blood-engorged mosquitoes were aspirated from inside tents, counted and stored at -20 °C.

The RMR could not be measured immediately following DNP and PBS injection because the mosquito exposure trials were taking place. Thus, in order to assess the effect of DNP injection on bird RMR, we captured eight additional house sparrows; four of them were injected with DNP and the other four with PBS as in the previous experiment. Immediately after the injection, the RMR of these individuals was recorded during 12 hours using the same approach reported above.

Molecular analyses

We isolated genomic DNA from blood samples taken from birds using the DNA Kit Maxwell® 16LEV (Gutiérrez-López et al., 2015). Birds were molecularly sexed and their *Plasmodium*, *Haemoproteus* and *Leucocytozoon* infection status were determined (Hellgren et al., 2004). To reduce any potential effect of host infection status on mosquito host selection (*see* Cornet et al., 2013), only birds without detectable infection by these parasites were included in the experimental procedure.

Thirty engorged mosquitoes were randomly selected after each trial to determine the origin of their blood meals — the only exception was one trial that produced only nine engorged mosquitoes, which were all analysed. Mosquito abdomens were separated from the head-thorax using sterile pipette tips and Petri dishes on an ice surface. Genomic DNA of the blood meal was isolated using the HotSHOT procedure (*see* Alcaide et al., 2009; Martínez-de la Puente et al., 2013). DNA samples were stored at -20°C until PCR amplification analyses.

The sex of birds bitten by each mosquito was determined from blood meal (Ellegren, 1996; Griffiths et al., 1998). We used the primer pair P2 (5'-TCTGCATCGCTAAATCCTTT-3') and P8 (5'-CTCCCAAGGATGAGRAAYTG-3') that targets the sex-related chromo-helicase-DNA-binding gene (*CHD*). PCR amplification was carried out in a total volume of 25 μL in thermal cyclers (BIO-RAD T100 and Agilent Sure Cycler 8800). The reaction conditions and cycle temperatures are described in (Griffiths et al., 1998). Positive amplifications were visualized in 3% agarose gels. This procedure was used to partially identify the origin of the mosquito blood meals. In particular, for the nine pairs containing a male and a female bird, the blood meals with one-band amplification were identified as male-derived blood meals. Blood meals providing two bands of amplification (i.e. bloodmeals from a female or a mixed bloodmeal from both a male and a female) and those from six bird pairs including two males were processed using eight different primer pairs to target different microsatellite fragments of the genotyped birds (*see* Additional file 2; Garnier et al., 2009). Microsatellite amplifications were conducted with a total volume of 20 μL for each sample containing 2 μL of extracted DNA sample, 2 μL of PCR buffer (10×), 0.6 μL of $MgCl_2$ (50mM), 0.16 μL of dNTPs (25mM), 0.1 μL of Taq, 13.54 μL of H_2O and 0.8 μL of primer for two DNA strands respectively. Positive amplifications were visualized in 3% agarose gels to identify homozygous (one band) and heterozygous (two bands) individuals for each microsatellite and compared between birds from the same trial pair.

For pairs composed of two males, we selected pairs of microsatellite primers having mutually exclusive amplification patterns for each bird of the pair. This procedure allows birds to be identified and reduce the cost of sequencing. Samples with one-band amplification for either of the pair of primers were identified as blood meals from either one of the pair of birds, while two-banded amplifications for the two microsatellites were identified as mixed-blood meals. For those cases where birds showed a similar amplification pattern, we sequenced four different microsatellites (Pdo A08, B01, D09 and F09; see also Table S1) from bird blood samples and mosquito blood meals using the 3130*xl* ABI Genetic Analyzer (Applied Biosystems). Alleles were scored using GENEMAPPER v3.7 (Applied Biosystems). The origin of the remaining samples was resolved by comparing the size of alleles amplified by multiple primer pairs. Consequently, for each trial we obtained the number of mosquitoes that had bitten each individual and the number of mosquitoes that had bitten both birds. To assess the reliability of the assignment of blood origin, both the sex determination and microsatellite genotyping were run in duplicate for 52 and 12 samples, respectively. No inconsistent results were found.

Statistical analyses

A one-way ANOVA was used to compare the feeding percentage/number between heterogeneous (containing one male and one female bird) and homogenous (containing two males) trials. The RMR in the DNP and control groups prior to the experimental injection was compared with one-way ANOVA. The same procedure was also used to test for differences in RMR between control and DNP birds immediately after injection. We used Generalized Linear Mixed Models (GLMMs) with binomial error and logit link function to test the relationship between bird RMR and mosquito feeding preference. This dependent variable was calculated as the number of mosquitoes that fed exclusively on one of the two birds (without mixed blood meals) within a pair in relation to the total analysed number of engorged mosquitoes that fed on the same pair. Before fitting models, bird RMR was logistic-transformed to attain normality (Maxwell, 1974). In the models, BM and RMR were introduced as covariates;

bird sex, treatment, the interaction between sex and treatment and between treatment and RMR were incorporated as explanatory factors; bird identity was included as a random factor in order to cope with the over-dispersion found in models with a count response (Harrison, 2014); in addition, bird pair was also included as a random factor as some pairs were composed of birds of different gender and so direct comparisons between pair members could not be conducted without controlling for confounding variables such as bird sex and BM. The multi-collinearity of explanatory variables was first assessed by calculating the generalized variance inflation factors (gVIFs) and, as these gVIF values were < 4 for the two continuous variables, both were incorporated into further analyses (O'brien, 2007). Model selection was based on the second order Akaike's Information Criteria (AICc). Delta AICc (ΔAICc) was calculated as the difference in AICc between the model with the lowest AICc and other models.

First, we fitted a global model containing all the predictors using the *lme4* package v1.1 (Bates et al., 2014). We standardized input variables before model analysis using the *arm* package v1.8 (Gelman et al., 2009). We then derived a set of sub-models (including the null model, which contained only the intercept) from the global model by using the dredge function implemented in the *MuMIn* package v1.15 (Bartoń, 2013). A 'top model set' was created by selecting those models with a difference of $\Delta \mathrm{AICc} < 2$. If more than one model was selected in the top model set, we performed a model-averaging approach to summarize the results using the *MuMIn* package v1.15 (Bartoń, 2013). Finally, as a measure of goodness-of-fit for mixed models, we calculated the explained variance (conditional R^2) for each of the selected top models (Nakagawa et al., 2013). All analyses were carried out in R software v3.2.5 (R Core Development team, 2016).

Abbreviations

AICc: Akaike information criterion with a correction for finite sample sizes; ANOVA: analysis of variance; ATP: Adenosine triphosphate; BM: body mass; *CHD*: chromo helicase DNA binding gene; DNA: deoxyribonucleic acid; DNP: 2,4-Dinitrophenol; dNTP: deoxynucleotide; GLMMs: generalized linear mixed models; gVIF: generalized variance-inflation factors; PBS: phosphate buffered saline Solution; PCR: polymerase chain reaction; RMR: resting metabolic rate

Declarations

Ethics approval and consent to participate

No human subjects were involved in the study. All experimental procedures were approved by the CSIC Ethics committee and Animal Health authorities and complied with Spanish legislation (CEBA-EBD-12-40).

Consent for publication

Not applicable

Availability of data and material

All data are reported in the article and contained in additional files submitted along with the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

JY, JB, JMP, LG, RS and JF designed the study. JY, JB, JMP, LG and RGL conducted fieldwork. JY, JB and JMP performed the bioassays. JY, JMP and RGL carried out molecular analyses. JY, LG and JMP carried out statistical analyses. All authors contributed to writing the manuscript and approved the final version.

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Supplementary information

Additional file 1. Bird characteristics, experimental settings and mosquito blood-feeding data. Abbreviations used in the table listed as following. ID: bird identity; M/F: male/female; C/DNP: control/2,4-Dinitrophenol; BM: body mass (g); RMR: resting metabolic rate (mL O_2 min^{-1}); TIM: total introduced mosquitoes (count); TFM: total fed mosquitoes (count); PTFM: percentage of total fed mosquitoes (%); TAM: total analysed mosquitoes (exclusively non-mixed bloodmeals, count); FM: fed mosquitoes (exclusively non-mixed bloodmeals, count); FP: feeding preference (ratio).

Bird pair	ID	Gender	Treatment	BM	RMR	TIM	TFM	PTFM	TAM	FM	FP
1	1	M	C	20.6	1.71	183	10	5.46	9	3	0.33
	2	F	DNP	18.4	1.18					6	0.67
2	3	M	C	25.6	1.58	181	41	22.65	25	6	0.24
	4	F	DNP	25	1.2					19	0.76
3	5	M	DNP	21.1	1.62	185	41	22.16	27	6	0.22
	6	F	C	22.6	1.4					21	0.78
4	7	M	C	22.2	1.5	186	59	31.72	28	27	0.96
	8	F	DNP	19.1	1.19					1	0.04
5	9	M	DNP	20.5	1.72	186	67	36.02	28	4	0.14
	10	F	C	22.2	1.88					24	0.86
6	11	M	C	23.1	1.64	191	41	21.47	23	13	0.57
	12	F	DNP	20.9	1.44					10	0.43
7	13	M	DNP	21.6	2.69	196	70	35.71	25	5	0.20
	14	F	C	24.1	1.58					20	0.80
8	15	M	C	22.9	3.51	194	64	32.99	25	9	0.36
	16	F	DNP	26.5	2.09					16	0.64
9	17	M	DNP	23.4	1.79	198	30	15.16	27	6	0.22
	18	F	C	23.5	1.51					21	0.78
10	19	M	C	18.9	1.38	197	80	40.61	26	0	0.00
	20	M	DNP	22.1	1.56					26	1.00
11	21	M	C	21.6	1.48	189	34	17.99	28	7	0.25
	22	M	DNP	18.7	1.22					21	0.75
12	23	M	C	22.5	1.6	190	58	30.53	28	22	0.79
	24	M	DNP	22	1.99					6	0.21
13	25	M	C	22.8	1.33	191	74	38.74	24	20	0.83
	26	M	DNP	21.1	1.75					4	0.17
14	27	M	C	24.8	1.56	192	58	32.21	26	26	1.00
	28	M	DNP	20.5	2.01					0	0.00
15	29	M	C	18.9	1.47	192	52	27.08	30	0	0.00
	30	M	DNP	21.6	1.4					30	1.00

Additional file 2. Primers used in this study for genotyping of house sparrows. *Primers used in DNA sequencing; ⁺Primer labelled with FAM, VIC, NED or PET; Adapted from Garnier et al. (2009)

Locus name	Primer sequence (5´-3´)	Genbank Accession no.
PdoA08*	AGCTTTTCAGGTCTCCTTCT+VIC CTACACCAGCAAGATCCATT	FJ422589
PdoB01*	GCCTGCTTAAACTATCTTGG+PET GATATAGGGAGCAGAGTTCTTG	FJ422590
PdoB04	ATTTGGGTGGTTAGTTCAAA+FAM CAAATACAGTGCATCTACAACC	FJ422591
PdoC11	GCAGCATGTCATAATAGCAG+FAM TTTTCCTTTGCATACACCA	FJ422592
PdoD09*	CTCTCCTGCTATGCTTCCT+PET CTTGGGATATGATGGAAATG	FJ422593
PdoE09	TGACTAAAATAGATCAAGGCTTTT+FAM TGCAAAGATACCAGAACTCAT	FJ422594
PdoF05	GCATATTTCTGGCATTCTTC+VIC TCAAATAAAGTGCTCCACAA	FJ422595
PdoF09*	CACGGGTGGTATTTTATATG+NED ATGTTGCAGATTGAAAAGTG	FJ422596

References

Alcaide M, Rico C, Ruiz S, Soriguer R, Muñoz J, Figuerola J. Disentangling vector-borne transmission networks: a universal DNA barcoding method to identify vertebrate hosts from arthropod bloodmeals. PLoS One. 2009; 4(9):e7092.

Anderson JF, Main AJ, Ferrandino FJ, Andreadis TG. Nocturnal activity of mosquitoes (Diptera: Culicidae) in a West Nile virus focus in Connecticut. J Med Entomol. 2007; 44(6):1102-1108.

Arrigo NC, Adams AP, Watts DM, Newman PC, Weaver SC. Cotton rats and house sparrows as hosts for North and South American strains of eastern equine encephalitis virus. Emerg Infect Dis. 2010; 16(9):1373-1380.

Bartoń K. MuMIn: multi-model inference. R package version. 2013; 1(5).

Bates D, Maechler M, Bolker B, Walker S. lme4: Linear mixed-effects models using Eigen and S4. R package version. 2014; 1(7).

Becker N, Petrić D, Boase C, Lane J, Zgomba M, Dahl C, Kaiser A. Mosquitoes and their control. 2nd edn. Springer; 2010.

Biro PA, Stamps JA. Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? Trends Ecol Evol. 2010; 25(11):653-659.

Blaxter K. Energy metabolism in animals and man. CUP Archive; 1989.

Browne SM, Bennett GF. Response of mosquitoes (Diptera: Culicidae) to visual stimuli. J Med Entomol. 1981; 18(6):505-521.

Caldeira da Silva CC, Cerqueira FM, Barbosa LF, Medeiros MH, Kowaltowski AJ. Mild mitochondrial uncoupling in mice affects energy metabolism, redox balance and longevity. Aging Cell. 2008; 7(4):552-560.

Cardé RT. Multi-cue integration: how female mosquitoes locate a human host. Curr Biol. 2015; 25(18):R793-R795.

Careau V, Thomas D, Humphries M, Réale D. Energy metabolism and animal personality. Oikos. 2008; 117(5):641-653.

Cassuto Y. Metabolic adaptations to chronic heat exposure in the golden hamster. American Journal of Physiology--Legacy Content. 1968; 214(5):1147-1151.

Chiba Y, Kubota M, Nakamura Y. Differential effects of temperature upon evening and morning peaks in the circadian activity of mosquitoes, *Culex pipiens pallens* and *C. pipiens molestus*. Biol Rhythm Res. 1982; 13(1):55-60.

Cornet S, Nicot A, Rivero A, Gandon S. Malaria infection increases bird attractiveness to uninfected mosquitoes. Ecol Lett. 2013; 16(3):323-329. doi: 10.1111/ele.12041.

Cresswell W. Flocking is an effective anti-predation strategy in redshanks, *Tringa* *totanus*. Anim Behav. 1994; 47(2):433-442.

Darbro JM, Harrington LC. Avian defensive behavior and blood-feeding success of the West Nile vector mosquito, *Culex pipiens*. Behav Ecol. 2007; 18(4):750-757. doi: 10.1093/beheco/arm043.

Day JF, Edman JD. Mosquito engorgement on normally defensive hosts depends on host activity patterns. J Med Entomol. 1984; 21(6):732-740.

Dominguez S, Menkel J, Fairbrother A, Williams B, Tanner R. The effect of 2, 4-dinitrophenol on the metabolic rate of bobwhite quail. Toxicol Appl Pharmacol. 1993; 123(2):226-233.

Edman JD, Scott TW. Host defensive behaviour and the feeding success of mosquitoes. Int J Trop Insect Sci. 1987; 8(5-6):617-622. doi: http://dx.doi.org/10.1017/S1742758400022694.

Ellegren H. First gene on the avian W chromosome (CHD) provides a tag for universal sexing of non-ratite birds. Proc R Soc Lond B. 1996; 263(1377):1635-1641.

Estep LK, McClure CJ, Burkett-Cadena ND, Hassan HK, Unnasch TR, Hill GE. Developing models for the forage ratios of *Culiseta melanura* and *Culex erraticus* using species characteristics for avian hosts. J Med Entomol. 2012; 49(2):378-387.

Farajollahi A, Fonseca DM, Kramer LD, Kilpatrick AM. "Bird biting" mosquitoes and human disease: a review of the role of *Culex pipiens* complex mosquitoes in epidemiology. Infect Genet Evol. 2011; 11(7):1577-1585. doi: 10.1016/j.meegid.2011.08.013.

Garnier S, Durand P, Arnathau C, Risterucci AM, Esparza-Salas R, Cellier-Holzem E, Sorci G. New polymorphic microsatellite loci in the house sparrow, *Passer domesticus*. Mol Ecol Resour. 2009; 9(3):1063-1065. doi: 10.1111/j.1755-0998.2009.02552.x.

Gehring P, Buerge J. The cataractogenic activity of 2, 4-dinitrophenol in ducks and rabbits. Toxicol Appl Pharmacol. 1969; 14(3):475-486.

Gelman A, Su Y-S, Yajima M, Hill J, Pittau MG, Kerman J, Zheng T, Dorie V: arm: Data analysis using regression and multilevel/hierarchical models (R package, version 9.01). 2009.

Gervasi SS, Burkett-Cadena N, Burgan SC, Schrey AW, Hassan HK, Unnasch TR, Martin LB: Host stress hormones alter vector feeding preferences, success, and productivity. Proc R Soc B. 2016; 283(1836): 20161278.

Gillooly JF, Brown JH, West GB, Savage VM, Charnov EL. Effects of size and temperature on metabolic rate. Science. 2001; 293(5538):2248-2251.

Gingrich JB, Williams GM. Host-feeding patterns of suspected West Nile virus mosquito vectors in Delaware, 2001-2002. J Am Mosq Control Assoc. 2005; 21(2):194-200.

Griffiths R, Double MC, Orr K, Dawson RJG. A DNA test to sex most birds. Mol Ecol. 1998; 7(8):1071-1075. doi: 10.1046/j.1365-294x.1998.00389.x.

Gutiérrez-López R, Martínez-de la Puente J, Gangoso L, Soriguer RC, Figuerola J. Comparison of manual and semi-automatic DNA extraction protocols for the barcoding characterization of hematophagous louse flies (Diptera: Hippoboscidae). J Vector Ecol. 2015; 40(1): 11-15.

Hamer GL, Kitron UD, Brawn JD, Loss SR, Ruiz MO, Goldberg TL, Walker ED. *Culex pipiens* (Diptera: Culicidae): a bridge vector of West Nile virus to humans. J Med Entomol. 2008; 45(1):125-128.

Harper J, Dickinson K, Brand M. Mitochondrial uncoupling as a target for drug development for the treatment of obesity. Obes Rev. 2001; 2(4):255-265.

Harrison XA. Using observation-level random effects to model overdispersion in count data in ecology and evolution. PeerJ. 2014; 2:e616

Hellgren O, Waldenström J, Bensch S. A new PCR assay for simultaneous studies of *Leucocytozoon*, *Plasmodium*, and *Haemoproteus* from avian blood. J Parasitol. 2004; 90(4):797-802.

Hill RW. Determination of oxygen consumption by use of the paramagnetic oxygen analyzer. J Appl Physiol. 1972; 33(2):261-263.

Janousek WM, Marra PP, Kilpatrick AM. Avian roosting behavior influences vector-host interactions for West Nile virus hosts. Parasit Vectors. 2014; 7: 399. doi: 10.1186/1756-3305-7-399.

Kelly DW. Why are some people bitten more than others? Trends Parasitol. 2001; 17(12):578-581.

Kilpatrick AM, Daszak P, Jones MJ, Marra PP, Kramer LD. Host heterogeneity dominates West Nile virus transmission. Proc R Soc B. 2006; 273(1599):2327-2333. doi: 10.1098/rspb.2006.3575.

Klowden MJ, Lea AO. Effect of defensive host behavior on the blood meal size and feeding success of natural populations of mosquitoes (Diptera: Culicidae). J Med Entomol. 1979; 15(5-6):514-517. doi:http://dx.doi.org/10.1093/jmedent/15.5-6.514.

Komar N, Panella NA, Burns JE, Dusza SW, Mascarenhas TM, Talbot TO. Serologic evidence for West Nile virus infection in birds in the New York City vicinity during an outbreak in 1999. Emerg Infect Dis. 2001; 7(4):621.

Krebs BL, Anderson TK, Goldberg TL, Hamer GL, Kitron UD, Newman CM, Ruiz MO, Walker ED, Brawn JD. Host group formation decreases exposure to vector-borne disease: a field experiment in a 'hotspot'of West Nile virus transmission. Proc R Soc B. 2014; 281(1796):20141586.

LaPointe DA, Atkinson CT, Samuel MD. Ecology and conservation biology of avian malaria. Ann N Y Acad Sci. 2012; 1249(1):211-226.

Lipnick RL. Narcosis induced by ether and chloroform. In: Lipnick RL, editor. Studies of Narcosis. 1 ed. Dordrecht: Springer; 1991. p. 93-107.

Malmqvist B, Strasevicius D, Hellgren O, Adler PH, Bensch S. Vertebrate host specificity of wild–caught blackflies revealed by mitochondrial DNA in blood. Proc R Soc lond B. 2004; 271 Suppl 4: S152-S155. doi: 10.1098/rsbl.2003.0120.

Martínez-de la Puente J, Merino S, Lobato E, Rivero-de Aguilar J, del Cerro S, Ruiz-de-Castañeda R, Moreno J. Nest-climatic factors affect the abundance of biting flies and their effects on nestling condition. Acta Oecol. 2010; 36(6):543-547. doi: 10.1016/j.actao.2010.07.008.

Martínez-de la Puente J, Merino S, Tomás G, Moreno J, Morales J, Lobato E, Talavera S, Sarto i Monteys V. Factors affecting Culicoides species composition and abundance in avian nests. Parasitology. 2009; 136(9):1033-1041. doi: 10.1017/S0031182009006374.

Martínez-de la Puente J, Muñoz J, Capelli G, Montarsi F, Soriguer R, Arnoldi D, Rizzoli A, Figuerola J. Avian malaria parasites in the last supper: identifying encounters between parasites and the invasive Asian mosquito tiger and native mosquito species in Italy. Malar J. 2015; 14(1):32.

Martínez-de la Puente J, Ruiz S, Soriguer R, Figuerola J. Effect of blood meal digestion and DNA extraction protocol on the success of blood meal source determination in the malaria vector *Anopheles atroparvus*. Malar J. 2013; 12(1):109.110.

Maxwell A. The logistic transformation in the analysis of paired-comparison data. Br J Math Stat Psychol. 1974; 27(1):62-71.

McKechnie AE, Freckleton RP, Jetz W. Phenotypic plasticity in the scaling of avian basal metabolic rate. Proc R Soc B. 2006; 273(1589):931-937.

McNab BK. On the utility of uniformity in the definition of basal rate of metabolism. Physiol Zool. 1997; 70(6):718-720.

Molaei G, Andreadis TG, Armstrong PM, Bueno R, Dennett JA, Real SV, Sargent C, Bala A, Randle Y, Guzman H. Host feeding pattern of *Culex quinquefasciatus* (Diptera: Culicidae) and its role in transmission of West Nile virus in Harris

County, Texas. Am J Trop Med Hyg. 2007; 77(1):73-81.

Mooring MS, Benjamin JE, Harte CR, Herzog NB. Testing the interspecific body size principle in ungulates: the smaller they come, the harder they groom. Anim Behav. 2000; 60(1):35-45. doi: 10.1006/anbe.2000.1461.

Muñoz J, Ruiz S, Soriguer R, Alcaide M, Viana DS, Roiz D, Vázquez A, Figuerola J. Feeding patterns of potential West Nile virus vectors in south-west Spain. PLoS One. 2012; 7(6):1-9. doi: 10.1371/journal.pone.0039549.

Nakagawa S, Schielzeth H. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. Methods Ecol Evol. 2013; 4(2):133-142.

Nicholls DG, Ferguson S. Bioenergetics. 4th edn: Academic Press; 2013.

Nilsson JÅ. Metabolic consequences of hard work. Proc R Soc Lond B. 2002; 269(1501):1735-1739.

O'brien RM. A caution regarding rules of thumb for variance inflation factors. Qual Quan. 2007; 41(5):673-690.

Opazo JC, Soto-Gamboa M, Fernández MJ. Cell size and basal metabolic rate in hummingbirds. Rev Chil Hist Nat. 2005; 78(2): 261-265.

Padalko V. Uncoupler of oxidative phosphorylation prolongs the lifespan of *Drosophila*. Biochemistry (moscow). 2005; 70(9):986-989.

Paull SH, Song S, McClure KM, Sackett LC, Kilpatrick AM, Johnson PT. From superspreaders to disease hotspots: linking transmission across hosts and space. Front Ecol Environ. 2012; 10(2): 75-82.

Port G, Boreham P, Bryan JH. The relationship of host size to feeding by mosquitoes of the *Anopheles gambiae* Giles complex (Diptera: Culicidae). Bull Entomol Res. 1980; 70(1):133-144.

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

Rizzoli A, Bolzoni L, Chadwick E, Capelli G, Montarsi F, Grisenti M, Martínez-de la Puente J, Muñoz J, Figuerola J, Soriguer R. Understanding West Nile virus ecology in Europe: *Culex pipiens* host feeding preference in a hotspot of virus emergence. Parasit Vectors. 2015; 8(1): 213. doi: 10.1186/s13071-015-0831-4.

Rodríguez A, Broggi J, Alcaide M, Negro JJ, Figuerola J. Determinants and short-term physiological consequences of PHA immune response in lesser kestrel nestlings. J Exp Zool Part A. 2014; 321(7):376-386.

Salin K, Luquet E, Rey B, Roussel D, Voituron Y. Alteration of mitochondrial efficiency affects oxidative balance, development and growth in frog (*Rana temporaria*) tadpoles. J Exp Biol. 2012; 215(5):863-869.

Schaffner F, Angel G, Geoffroy B, Hervy J, Rhaiem A, Brunhes J. The mosquitoes of Europe, an identification and training programme. Montpellier, France: IRD. 2001.

Stier A, Bize P, Roussel D, Schull Q, Massemin S, Criscuolo F. Mitochondrial uncoupling as a regulator of life-history trajectories in birds: an experimental study in the zebra finch. J Exp Biol. 2014; 217(19):3579-3589.

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

van Breugel F, Riffell J, Fairhall A, Dickinson MH. Mosquitoes Use Vision to Associate Odor Plumes with Thermal Targets. Curr Biol. 2015; 25(16):2123-2129. doi: 10.1016/j.cub.2015.06.046.

VanderWaal KL, Ezenwa VO. Heterogeneity in pathogen transmission: mechanisms and methodology. Funct Ecol. 2016; 30(10): 1606-22. doi: 10.1111/1365-2435.12645.

Williams GC. Natural selection, the costs of reproduction, and a refinement of Lack's principle. Am Nat. 1966; 100(916):687-690.

Chapter 4

Avian malaria infection intensity influences mosquito feeding patterns

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Abstract

Pathogen-induced host phenotypic changes are widespread phenomena that can dramatically influence host-vector interactions. Enhanced vector attraction to infected hosts has been reported in a variety of host-pathogen systems, and has given rise to the parasite manipulation hypothesis whereby pathogens may adaptively modify host phenotypes to increase transmission from host to host. However, host phenotypic changes do not always favour the transmission of pathogens, as random host choice, reduced host attractiveness and even host avoidance after infection have also been reported. Thus, the effects of hosts' parasitic infections on vector feeding behaviour and on the likelihood of parasite transmission remain unclear. Here, we experimentally tested how host infection status and infection intensity with avian *Plasmodium* affect mosquito feeding patterns in house sparrows (*Passer domesticus*). In separate experiments, mosquitoes were allowed to bite pairs containing i) one infected and one uninfected bird and ii) two infected birds, one of which treated with the antimalarial drug, primaquine. We found that mosquitoes fed randomly when exposed to both infected and uninfected birds. However, when mosquitoes were exposed only to infected individuals, they preferred to bite the non-treated birds. These results suggest that the malarial parasite load rather than the infection itself plays a key role in mosquito attraction. Our findings partially support the parasite manipulation hypothesis, which probably operates via a reduction in defensive behaviour, and highlights the importance of considering parasite load in studies on host-vector-pathogen interactions.

Keywords: Adaptive avoidance; *Culex* mosquitoes; Host choice; Infection status; *Plasmodium*; Parasite manipulation

Introduction

Pathogen-induced phenotypic changes in host morphology, behaviour and physiology may greatly affect interactions between hosts and insect vectors, and may in turn have an impact on the transmission dynamics of vector-borne pathogens (Hurd, 2003; Libersat et al., 2009; Poulin, 2010; Lafferty and Kuris, 2012). Despite not having a full understanding of its underlying mechanisms, the parasite manipulation hypothesis (Poulin, 1995; Hurd, 2003) has received increasing attention during the last decade (e.g. Lefèvre et al., 2008, 2009). This hypothesis proposes that pathogens manipulate a host's phenotype to increase host-vector contact rates, thereby enhancing both the probability of pathogen acquisition and the transmission to a new host (Lefèvre et al., 2006; Lefèvre and Thomas, 2008; Mauck et al., 2010, 2012). Indeed, the enhanced attractiveness of infected hosts to vectors has been reported in plants (Eigenbrode et al., 2002; Shapiro et al., 2012), invertebrates (Stafford et al., 2011) and vertebrates (O'Shea et al., 2002; Cornet et al., 2013 a, b; De Moraes et al., 2014) including humans (Lacroix et al., 2005; Batista et al., 2014).

Malaria parasites of the genus *Plasmodium* are vector-borne pathogens that require the bite of a competent mosquito to spread from an infected to a new host (Valkiūnas, 2005). A number of studies have reported vector preference for mammalian hosts already infected by malaria parasites. For example, in humans, children harbouring *Plasmodium falciparum* parasites in transmissible stages (i.e. gametocytes) were more attractive to mosquitoes (measured as a reaction to odours) than those harbouring parasites in non-transmissible stages (i.e. trophozoites) or uninfected children (Lacroix et al., 2005). Similarly, Day and Edman (1983) found that mosquitoes fed almost exclusively on malaria-infected mice when both infected and uninfected individuals were made available. However, whether host infection affects vector feeding behaviour remains an open question since contrasting results were also reported. For instance, mosquitoes preferred to feed on bats infected with the mildest stages of the malaria-like parasite *Polychromophilus murinus* (Witsenburg et al., 2014) or even preferred to feed on uninfected hosts to the detriment of their infected counterparts (Daugherty et al., 2011).

Avian malaria parasites have recently been used to test the parasite manipulation hypothesis since they may alter host behaviour (e.g. reduced activity, Cauchard et al., 2016) and physiology (e.g. anaemia and enlargement of the liver and spleen, Valkiūnas, 2005), which could potentially affect mosquito attraction. Nonetheless, contradictory trends have been also reported and, for instance, Cornet et al. (2013a) found that birds chronically infected by *Plasmodium relictum* were bitten more frequently by the avian malaria vector *Culex pipiens* than their uninfected or acutely infected counterparts. This finding would support the parasite manipulation hypothesis, as this behaviour would increase the fitness of parasites (transmission success) (Poulin, 1995). On the other hand, Lalubin et al. (2012) reported that *Cx. pipiens* were more attracted to uninfected birds than to *Plasmodium*-infected birds, a finding that cannot be explained by parasitic manipulation but rather by the hypothesis of vector adaptive avoidance (Hart, 1990; Martínez-de la Puente et al., 2009; Lalubin et al., 2012). This latter hypothesis is based on the costs induced by parasites in their vectors, such as decreased fecundity (Vézilier et al., 2012) and survival (Ferguson and Read, 2002; Lalubin et al., 2014). However, Cornet et al. (2013a) used birds infected in the laboratory that were deprived of movement, which may not reflect the situation that occurs in the field. The study by Lalubin et al. (2012) suffers from the technical problem when using olfactometers, i.e. the lack of physical interaction between birds and mosquitoes (e.g. feeding attempts and hosts' defence). Therefore, the actual effects of parasitic infection on vectors' feeding patterns remain to be clarified.

Here, we conducted two separate experiments to determine the effects of avian *Plasmodium* infection on the feeding behaviour of the avian malaria vector *Cx. pipiens*. Firstly, we exposed naturally infected and uninfected house sparrows (*Passer domesticus*) to mosquitoes to assess the effect of birds' infection status on mosquito biting rates. Secondly, we assessed the effect of host parasite load on the probability of mosquito bites by treating half of the *Plasmodium*-infected birds (hereafter, the treated in this experiment) with an antimalarial drug and then exposing both infected (hereafter, the control in this experiment) and treated birds to mosquito bites. In both cases, the pairs of birds representing dual conditions of malaria infection (i.e. infected versus uninfected or control versus treated) were exposed simultaneously to mosquitoes to simulate a common situation of making choices as faced by mosquitoes in the field. In addition, birds were allowed to move freely in their cages to avoid hampering anti-mosquito behaviour that could greatly affect the feeding success of mosquitoes (Darbro and Harrington, 2007). According to the parasite manipulation hypothesis, we predicted that *Plasmodium*-infected control (non-treated) birds would be bitten more often than uninfected and treated individuals, respectively, as parasite-induced changes (e.g. hosts' odours, anti-mosquito behaviours) would facilitate mosquito bites (Day and Edman, 1983; De Moraes et al., 2014). Alternatively, and according to the adaptive avoidance hypothesis, we predicted that mosquitoes would bite infected control birds less often than uninfected and treated birds, since mosquitoes may adaptively select uninfected birds or those with less intense infections to avoid the costs of infection.

Materials and methods

Mosquito and bird collection and rearing

Mosquito larvae were collected in the Cañada de los Pájaros nature reserve (37 °14′03″ N, 6°07′50″ W, Seville, Spain) during the summer of 2014 and then transported to the laboratory, where they were supplied with shrimp food (Mikrozell 20 ml/22 g; Dohse Aquaristik GmbH & Co. KG, D-53501, Gelsdorf, Germany) and maintained under controlled conditions (65–70% relative humidity (RH), 27 ± 1 °C and a light (L): dark (D) cycle of 12:12 h). Emerged mosquitoes were anaesthetized with diethyl ether (Lipnick, 1991), sexed and identified following Schaffner et al. (2001). Female *Cx. pipiens* were maintained in insect rearing cages (BugDorm-43030F, 32.5×32.5×32.5 cm) with ad libitum access to 1% sucrose solution. Mosquitoes were deprived of sucrose solution 24 h before the experiment took place and henceforth only had access to water.

In July 2014, 78 juvenile house sparrows were captured using mist nets in Huelva province (southern Spain). Birds were ringed upon capture and their body mass and wing length were measured using a digital scale (Pesola-MS500) and a 30 cm end-stop ruler, respectively. A blood sample was obtained for further molecular analyses (see Section 2.3). Birds were transferred to the Unit of Animal Experimentation at Estación Biológica de Doñana-Consejo Superior de Investigaciones Científicas (EBD-CSIC), Spain where they were maintained in pairs in cages (58.5×25×36 cm) within a vector-free room programmed with a photoperiod cycle of 12:12 h L: D at 22 ± 1 °C. Birds were housed for 1 week before the start of the experiments and had ad libitum access to fresh water and a standard mixed diet for seed and insect-eater birds (KIKI; GZM S.L., Alicante, Spain). Birds were released at their capture site 2–5 days after the completion of the experiments. All experimental procedures were approved by the CSIC Ethics Committee and Animal Health authorities as per Spanish legislation (CEBA-EBD-12-40).

Experimental procedure

Before performing the experiments, birds were molecularly sexed (see Section 2.3) and their infection status with blood parasites (i.e. *Plasmodium*, *Haemoproteus* and *Leucocytozoon*) was determined using primer pairs HaemNF1/HaemNR3 and HaemF/HaemR2 following Hellgren et al. (2004). Their infection status with blood parasites was determined again after completion of the experiments. The presence of amplicons was verified in 1.8% agarose gels. Positive amplifications were sequenced using the BigDye technology (Applied Biosystems, USA) or by the Macrogen sequencing service (Macrogen Inc., The Netherlands). Sequences were edited using the software Sequencher™ v 4.9 (Gene Codes Corp. © 1991–2009, Ann Arbor, MI, USA 48108) and assigned to parasite lineages/morphospecies after comparisons with sequences in GenBank (National Center for Biotechnology Information, (NCBI), USA). Birds infected with *Haemoproteus* or *Leucocytozoon* were not included in this study. In the first experiment, 20 pairs of birds consisting of a *Plasmodium*-infected (10 males and 10 females) and an uninfected bird (10 males and 10 females) were exposed to unfed female *Cx. pipiens* (mean number = 172, range = 156–183). In this experiment, a *Plasmodium*-infected bird was also co-infected with *Haemoproteus* as determined by sequencing of blood after the completion of the experiment. Besides this co-infected bird, 15 individuals were infected with the lineages Rinshi-1 (*P. relictum*), three infected with Rinshi-7 (*P. relictum*), and one bird co-infected with both Rinshi-1 and Donana07 (*Plasmodium* spp.). Each pair contained one male and one female bird, with one infected and one uninfected individual. Eight to 18 day old female mosquitoes were used in all experiments to reduce the potential effect of mosquito age on host location capacity (Bohbot et al., 2013). In the second experiment carried out 15 days after completing the first experiment, only *Plasmodium*-infected birds were used; 16 infected birds (including the above-mentioned co-infected bird) from the first experiment were re-used to minimize the number of animals employed, nine of which were assigned to the non-treated control group and seven to the treated group (including the above-mentioned co-infected bird). Seven days before exposure to mosquitoes, birds were randomly assigned to either the treated or control group. Besides the *Plasmodium* and *Haemoproteus* co-infected bird, the lineages infecting medicated birds in experiment 2 were Rinshi-1 (14 individuals), Rinshi-7 (1), Rinshi-8 (*P. relictum,* 2) and co-infection by Rinshi-1 and Donana07 (1). In the case of control birds, nine individuals were infected with Rinshi-1, four with Rinshi-7, one with Rinshi-8, one with Donana07, two individuals were co-infected with Rinshi-1 and Donana07, one with Rinshi-7 and PADOM1 (*Plasmodium* spp.) and one with Rinshi-7 and Donana07. Treated birds were s.c. injected with 0.1 mg of primaquine (Sigma, St. Louis, MO, USA) diluted in 0.1 ml of saline solution, while control birds were injected with the same volume of saline solution (see Merino et al., 2000; Tomás et al., 2007 for a similar procedure). Primaquine is a chemical compound that can bind and modify malaria parasites' DNA (López-Antuñano, 1999), as well as disrupt malaria parasites' mitochondrial membranes (Baird and Rieckmann, 2003), thereby effectively reducing the blood parasite load in birds such as house sparrows (Merino et al., 2004; Marzal et al., 2005; Tomás et al., 2007; Martínez-de la Puente et al., 2010). In humans, the effect of a single and low dose of primaquine can clear most of the malaria gametocytes 7 days after treatment (Burgess and Bray, 1961). The biological half-life of primaquine in plasma is approximately 4-9 h (Baird and Hoffman, 2004), hence this drug was not likely to have any direct effect on behaviour 1 week post treatment (Cauchard et al., 2016). Seven days later, 19 treated birds (12 males and seven females) and 19 control birds (10 males and nine females) were exposed to unfed female *Cx. pipiens* (mean number = 151, range = 136–171). Sixteen pairs consisted of one male and one female bird, and three pairs of two males. In both experiments, pairs were maintained in a cage (38.5x26x25.5 cm) within an insect-rearing tent (BugDorm-3120, white, 60x60x60 cm). Birds were able to move freely and mosquitoes within each tent were similarly able to freely enter the birds' cage. Trials were carried out under dark conditions from 20:00 h until 08:00 h during the peak mosquito activity period. The following morning, blood-fed mosquitoes were collected, counted and stored at -20 °C; all the birds were immediately blood-sampled again and then released into the field 1–2 days after exposure during the second experiment. In the second experiment, blood smears were used to estimate the intensity of infection with blood parasites. The number of infected cells per 10,000 red blood cells (RBCs) was estimated in visual fields under 10,000X magnification (Carl Zeiss-Imager A1).

Molecular analyses

Genomic DNA from bird blood samples was isolated using a Maxwell® 16LEV Blood DNA Kit (Gutiérrez-López et al., 2015). Birds were molecularly sexed using primers P2 (5'-TCTGCATCGCTAAATCCTTT-3') and P8 (5'-CTCCCAAGGATGAGRA AYTG-3') following Ellegren (1996) and Griffiths et al. (1998). PCR amplifications were conducted with a total volume of 25 μL in thermal cyclers (Agilent sure cycler 8800, USA and BIO-RAD T100, USA). The cycle temperatures and other reaction conditions were as given in Griffiths et al. (1998); the positive amplifications were resolved in 3% agarose gels (TBE (Tris/Borate/EDTA buffer solution) 1X, 110V, 45 min). Eight different primer pairs targeting different microsatellite fragments were used to genotype birds (Table 1; Garnier et al., 2009). The amplification of each sample was carried out in a total volume of 20 μL containing 13.54 μL of H_2O, 2 μL of extracted DNA, 2 μL of PCR buffer (10×), 0.6 μL of $MgCl_2$ (50 mM), 0.16 μL of total dNTPs (25 mM), 0.1 μL of Taq and 0.8 μL of primer for each of the two DNA strands. To identify homozygous (one band) and heterozygous (two bands) individuals for each microsatellite, positive amplifications were resolved in 3% agarose gels (TBE 1X, 110V, 60 min) and the amplification pattern was compared between birds from the same trial.

Table 1. Primers used in this study for genotyping house sparrows. Adapted from Garnier et al. (2009)

Locus name	Primer sequence (5´-3´)	Genbank Accession no.
PdoA08[a]	AGCTTTTCAGGTCTCCTTCT [b] VIC CTACACCAGCAAGATCCATT	FJ422589
PdoB01 [a]	GCCTGCTTAAACTATCTTGG [b] PET GATATAGGGAGCAGAGTTCTTG	FJ422590
PdoB04	ATTTGGGTGGTTAGTTCAAA [b] FAM CAAATACAGTGCATCTACAACC	FJ422591
PdoC11	GCAGCATGTCATAATAGCAG [b] FAM TTTTCCTTTGCATACACCA	FJ422592
PdoD09 [a]	CTCTCCTGCTATGCTTCCT [b] PET CTTGGGATATGATGGAAATG	FJ422593
PdoE09	TGACTAAAATAGATCAAGGCTTTT [b] FAM TGCAAAGATACCAGAACTCAT	FJ422594
PdoF05	GCATATTTCTGGCATTCTTC [b] VIC TCAAATAAAGTGCTCCACAA	FJ422595
PdoF09 [a]	CACGGGTGGTATTTTATATG [b] NED ATGTTGCAGATTGAAAAGTG	FJ422596

[a] Primers used in DNA sequencing.

[b] Primer labelled with FAM, VIC, NED or PET.

To identify the origins of mosquitoes' blood meals, we isolated DNA from engorged mosquitoes using the HotSHOT procedure (see Alcaide et al., 2009; Martínez-de la Puente et al., 2013 for further details). In those cases where less than 30 engorged mosquitoes were obtained, we isolated the DNA from all engorged mosquitoes. When there were more engorged mosquitoes, we isolated DNA from 30 randomly selected individuals. Overall, an average of 20.2 ± 1.68 (mean ± S.E.) (range = 4 – 30) and 27.2 ± 1.29 (range = 9 – 30) engorged mosquitoes per pair were selected from the first and second experiments, respectively. The abdomen of each mosquito was separated from the head and thorax using sterile tips on chilled Petri dishes. One negative control of DNA extraction (i.e. without any tissue) was included for each plate. We applied the molecular sexing protocol detailed above for mosquito blood meals to partially identify the hosts of mosquitoes for those trials containing a male and a female bird. One-band amplifications were identified as male-derived blood meals. Given that samples with the amplification of two bands could be derived from a blood meal from a female bird or a mixed blood meal from both a male and female bird, these blood meals were processed by further analyses. Mosquitoes from trials containing two males (see results) were processed as follows: after genotyping birds, we analysed mosquitoes' blood meals using microsatellite primer pairs that had mutually exclusive amplification patterns for each bird within a pair, that is, bird A in a pair had a one-band amplification for one microsatellite but two bands for the other microsatellite, whereas bird B in that pair displayed the opposite pattern. Thus, samples with one-band amplification for either of the pair of primers were identified as blood meals from either one of the pair of birds, while those with two-banded amplifications for the two microsatellites were identified as mixed-blood meals. When birds showed a similar amplification pattern, four different microsatellites (Pdo A08, B01, D09 and F09; see also Table 1) from bird blood samples and mosquito blood meals were sequenced using the 3130*xl* ABI Genetic Analyzer (Applied Biosystems, USA) and the alleles were scored using GENEMAPPER v3.7 (Applied Biosystems). We managed to identify the origins of these remaining samples by comparing the sizes of alleles in birds and in mosquitoes' blood meals.

Statistical analyses

Generalized Linear Mixed Models (GLMMs) with binomial error and logit link function were used to assess the effect of bird infection status (i.e. infected versus uninfected birds) and intensity (i.e. control versus treated birds) on mosquito biting rates. The dependent variable 'biting rate' was incorporated into models as the number of mosquitoes that fed on one bird with respect to the number of mosquitoes that fed on the other bird within a pair (including mixed blood meals) using the *cbind* function. In any given trial, the number of engorged mosquitoes on a particular bird was analysed as a binomial variable with the total number of engorged mosquitoes as the binomial denominator. Bird body mass and log-transformed parasite load (only available for the second experiment) were included as covariates. We ran alternative models using body condition, estimated as the standardized residuals of linear regressions of body mass on wing length fitted separately for males and females (to control sexual dimorphism in wing length), instead of body mass. As the results did not change qualitatively, we only present the results for the models fitted with body mass. Bird sex and infection status (infected versus uninfected) or intensity (control versus treated) were included as fixed factors, while bird pair and bird identity were included as random terms. No evidence of collinearity between the two continuous independent variables included in the models was found, as the generalized variance inflation factors (gVIFs) were < 4 (O'Brien, 2007). Model selection was based on the second order Akaike's Information Criteria (AICc). Delta AICc(ΔAICc) was calculated as the difference in AICc between the model with the lowest AICc and the other models. Model averaging of all models with ΔAICc < 2 was performed following the zero method in Grueber et al. (2011). The variance explained for each model was calculated by conditional R^2 (Nakagawa and Schielzeth, 2013). Paired t-tests were used to compare the mean infection intensity between control and treated birds (Experiment 2). To account for any potential effect of the mixed blood meals, we repeated the same analyses by excluding these data from the calculation of biting rates. As results were qualitatively the same (data not shown), hereafter we only show the results including data of mixed blood meals. To account for any potential effects of outliers, we excluded a trial that contained a bird with a very high parasite load in the second experiment and repeated the above-mentioned analyses. As results remained qualitatively the same, we only present the results without the outlier. In addition, to account for any potential effect of co-infection by *Plasmodium* and *Haemoproteus*, we repeated the analyses without the trial that contained the co-infected bird in experiment 1, and results showed no qualitative change (data not shown); in experiment 2 the co-infected bird was assigned to the treated group and treated with an anti-malarial drug before exposure to mosquitoes. Hence, the results we present below include the co-infected bird. All analyses were performed in R 3.2.5 (R Core Development Team, 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.) using the packages *arm* (Gelman, A., Su, Y.-S., Yajima, M., Hill, J., Pittau, M.G., Kerman, J., Zheng, T., Dorie, V., 2009. arm: Data analysis using regression and multilevel/hierarchical models. R package, version 9.01.), *lme4* (Bates, D., Maechler, M., Bolker, B., Walker, S., 2014. lme4: Linear mixed-effects models using Eigen and S4. R package, version 1.) and *MuMIn* (Bartoń, K., 2013. MuMIn: multi-model inference. R package, version 1.)

Results

The percentage of engorged mosquitoes from the total mosquitoes introduced in each trial varied from 3.45% to 37.6% (mean = 14.2% ± 1.92% S.E.) for the first experiment and from 13.8% to 71.0% (mean = 38.2% ± 4.06% S.E.) for the second experiment. In total, the bloodmeal origin for each of 403 and 508 mosquitoes for the first and second experiment, respectively, was identified to the individual bird level. The mean number of engorged mosquitoes with known bloodmeal origin was 11.0 ± 1.02 S.E. per individual for the first experiment and 15.3 ± 1.46 S.E. per individual for the second experiment. The average number of mixed bloodmeals was 1.85 per trial in the first experiment and 2.44 in the second experiment.

In the first experiment, the mean biting rate was 0.52 ± 0.06 S.E. (range = 0.05 – 1.00) and 0.60 ± 0.06 S.E. (range = 0.05 – 0.95) for *Plasmodium*-infected and uninfected groups, respectively. Three GLMMs analysing the variation in biting rates between *Plasmodium*-infected and uninfected birds were selected based on AICc criteria (Table 2). However, none of the explanatory variables significantly affected the biting rate, as indicated by 95% confidence intervals (CIs), which included zero in all cases (Table 3). We did not find any significant relationship between *Plasmodium* infection status and biting rate (Table 3; Fig 1A).

Table 2. Results of Generalized Linear Mixed Models (GLMMs) analysing the variation in mosquito biting rate in relation to bird sex, infection status (uninfected or infected) and body mass, and the interaction between sex and infection status. Individual and pair identities were included as random terms. Δ*i* (AICc) = [AICci –min AICc]; ω_i (AICc) = the rounded second-order Akaike weights. Models were ranked by AICc values. Crosses indicate variables included in each model. Bold indicates top models (Δ*i* (AICc) ≤ 2).

Explanatory variables				Criterion		
Sex	Status	Body mass	Sex×Status	AICc	Δ*i* (AICc)	ω*i* AICc
				238.4	**0.00**	**0.324**
	+			**239.4**	**1.00**	**0.196**
		+		**240.1**	**1.72**	**0.137**
+				240.6	2.18	0.109
	+	+		241.0	2.56	0.090
+	+			241.8	3.40	0.059
+		+		242.7	4.25	0.039
+	+	+		243.7	5.31	0.023
+	+		+	244.5	6.03	0.016
+	+	+	+	246.2	7.78	0.007

Table 3. Summary of the averaged model derived from the top model set explaining the variation in mosquito biting rates in relation to bird infection status (infected versus uninfected birds). Model-averaged coefficients (conditional average) ± S.E., 95% Confidence intervals (CI), z value and *P* values of the averaged model are shown.

Explanatory variables	Estimate	S.E.	95% CI		z value	*P*
Intercept	0.240	0.200	-0.165	0.645	1.163	0.245
Infection status	0.484	0.393	-0.312	1.280	1.191	0.234
Body mass	0.349	0.400	-0.462	1.160	0.842	0.400

In the second experiment, the mean biting rate was 0.65 ± 0.07 S.E. (range = 0.10-1.00) and 0.44 ± 0.07 S.E. (range = 0.00-0.93) for control and treated groups respectively. The infected birds treated with primaquine had significantly lower parasitaemia levels than the controls (t = 2.14, d.f. = 17, *P* = 0.046; Fig 2). Based on AICc criteria, three different models were selected to explain the variation in mosquito biting rate (Table 4). The variance explained was 30.0 % for the first model, 22.7 % for the second model, and 6.56 % for the third model. The averaged model indicated that the biting rate was lower in treated than in control birds (Fig. 1B), lower in males than in females, and positively correlated to log-parasitaemia (Table 5). The relative importance of the independent variables was 1.0 for medication treatment, 0.25 for log-parasitaemia and 0.21 for sex. The 95% CI included zero for the variables log-parasitaemia and sex, thus indicating that treatment was the only explanatory variable that significantly affected the mosquito biting rate (Table 5).

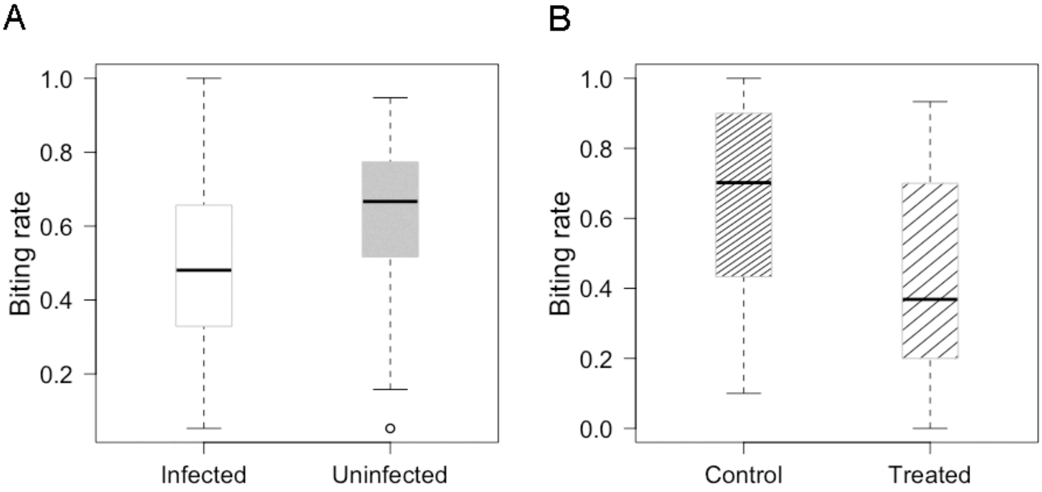

Fig 1. Comparison of biting rate between (A) ***Plasmodium*****-infected and uninfected birds and (B) control (non-treated) and treated birds**. The line within each box indicates the median and the edges of each box the first (Q1) and third (Q3) quartiles; the whiskers extend over 1.5 times the interquartile range.

Discussion

We combined two experiments to assess the role of *Plasmodium* infection prevalence and intensity in mosquito feeding patterns. Contrary to our predictions derived from the parasite manipulation and the adaptive avoidance hypotheses, in the first experiment we found that mosquitoes bit uninfected and infected birds with a similar probability. However, in the second experiment, mosquitoes were found to feed predominantly on control (infected) individuals when birds with high and experimentally reduced infection intensities were exposed simultaneously. These results highlight the need to perform experimental manipulations of the parasite load when attempting to assess the impact of parasite infection levels on host selection by mosquitoes.

Although previous studies have assessed the role of the malarial infection status on mosquito feeding patterns, methodological differences complicate comparisons of their conclusions. For instance, Day and Edman (1983) used a rodent malaria model and found that infected individuals suffered more bites from *Anopheles stephensi* mosquitoes than uninfected individuals. A similar pattern is to be expected in birds, as shown by Cornet et al. (2013a), who found that mosquitoes fed more frequently on birds suffering experimental infections with *Plasmodium* than on uninfected individuals. The stage of infection was important as mosquitoes feed preferentially on birds in the chronic phase of infection, in comparison with control uninfected birds and those in the acute phase of infection with *Plasmodium* (Cornet et al., 2013a). The acute phase of infection occurs immediately after infection with a high-proliferation of *Plasmodium* in host blood, which lasts for 10-13 days (Cornet et al., 2013a). Given that the birds had been maintained in a mosquito-free room for 13-19 days in experiment 1 and 32-35 days in experiment 2 before mosquito exposure we can assume that all the individuals were in the chronic phase of infection. Cornet et al. (2013a, b), however, immobilized birds to avoid anti-mosquito behaviour, which is known to seriously affect mosquito feeding success (Day, 1984; Edman and Scott, 1987;

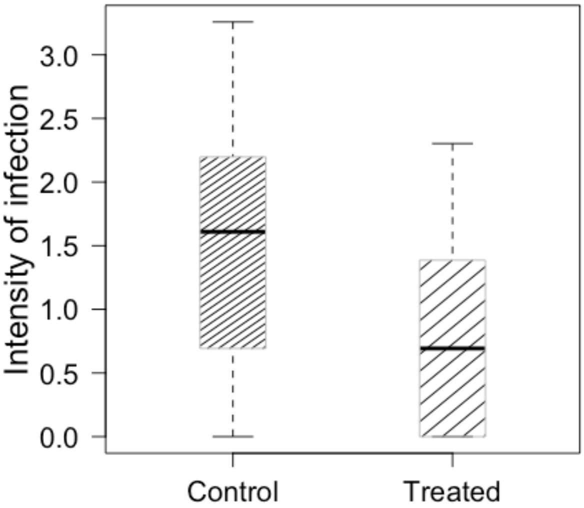

Fig 2. Intensity of infection (log-transformed) **with *Plasmodium* in control (non-treated) and treated birds**. The intensity of infection was measured as the number of infected cells per 10,000 red blood cells. The line within each box indicates the median and the edges of each box the first (Q1) and third (Q3) quartiles; the whiskers extend over 1.5 times the interquartile range.

Darbro and Harrington, 2007). In addition, the experiments by Cornet et al. (2013a) were performed within a short time span (just 2 h). In our study, however, mosquitoes were allowed to feed on birds for 12 h to reproduce more accurately the natural interactions between mosquito feeding attempts and host defence. Moreover, fitness costs of experimentally infected birds could be milder than those experienced by naturally infected birds due to lower selection pressure (e.g. predation, see Møller and Nielsen, 2007; and food supply, see Appleby et al., 1999). This probably allowed these birds to tolerate more severe infections and led to profound changes in their phenotypes (Medzhitov et al., 2012), while most naturally infected birds, with the exception of some individuals that have strong immune defences, may not be able to survive a severe infection (Appleby et al., 1999; Woodworth et al., 2005; Valkiūnas et al., 2006; Bensch et al., 2007; Møller and Nielsen, 2007). In the study by Cornet et al. (2013a), the naïve birds infected in the laboratory may be sicker than the wild naturally-infected

Table 4. Results of Generalized Linear Mixed Models (GLMMs) analysing variation in the biting rate in relation to bird parasitaemia, body mass, sex, treatment (control or treated) and the interaction between sex and treatment (Sex×Treatment). Individual and pair identities were included as random terms. Δi (AICc) = [AICci –min AICc]; ω_i (AICc) = the rounded second-order Akaike weights. Models were ranked by AICc values. Crosses indicate variables included in each model. Bold indicates top models (Δi (AICc) ≤ 2).

Explanatory variables					Criterion		
log. Parasitaemia	Body mass	Sex	Treatment	Sex×Treatment	AICc	Δ*i* (AICc)	ω*i* AICc
			+		**246.1**	**0.00**	**0.257**
+			**+**		**247.6**	**1.49**	**0.122**
		+	**+**		**247.9**	**1.83**	**0.103**
+					248.2	2.09	0.091
					248.7	2.61	0.070
	+		+		248.8	2.69	0.067
+		+	+		249.7	3.58	0.043
+		+			249.9	3.75	0.039
		+			250.0	3.86	0.037
+	+		+		250.5	4.37	0.029
	+	+	+		250.7	4.61	0.026
		+	+	+	250.8	4.71	0.024
+	+				250.9	4.79	0.023
	+				251.3	5.14	0.020
	+	+			252.4	6.32	0.011
+	+	+			252.5	6.44	0.010
+		+	+	+	252.6	6.46	0.010
+	+	+	+		252.7	6.57	0.010
	+	+	+	+	253.8	7.69	0.005
+	+	+	+	+	255.8	9.67	0.002

birds we used here, which were in the asymptomatic stage of chronic infection (Zehtindjiev et al., 2008). This difference in infection-induced phenotypic changes could dramatically alter the mosquito feeding patterns. Consequently, we cannot determine whether the differences between our results and those reported by Cornet et al. (2013a) are due to the immobilization of birds, to differences in the development status of *Plasmodium* in the naturally infected individuals used in our study or to the experimental manipulation of parasite load. The outcome of mosquito feeding patterns could vary greatly owing to different phases of infection or the relative use of host cues by mosquitoes. These factors may even produce contradictory findings since mosquitoes may be more attracted to uninfected than to infected birds (Lalubin et al., 2012). In addition, it is important to highlight the fact that birds included in our study were naturally infected and that their infection status was assessed based on molecular amplification of parasite DNA. The nested-PCR method used here provided positive amplifications in birds with infections corresponding to only one parasite per 100,000 host blood cells (Hellgren et al., 2004). Although we only measured parasite load rather than gametocyte load, recent studies have shown that parasitaemia is positively correlated to gametocytemia in different lineages of avian *Plasmodium* (Pigeault et al., 2015). In addition, parasitaemia is thought to be a better predictor of mosquito infection rather than gametocytemia, due to its correlation with host immunity and metabolic profiles (Pigeault et al., 2015). Parasitaemia has successfully been used as a proxy for the intensity of avian *Plasmodium* infection to assess its effects on mosquito attraction (Cornet et al., 2013a, b). Therefore, our result that mosquitoes more often bit birds with a higher parasite load might suggest a greater chance of pathogen transmission to mosquitoes, which could in turn increase pathogen transmission among hosts. Results from this experiment may, at least partially, support the parasite manipulation hypothesis.

Table 5. Summary statistics of the averaged model derived from the top model set, which explains the variation in the mosquito biting rate in relation to bird infection intensity (control non-treated versus treated birds). Model-averaged coefficients (conditional average) ± S.E., 95% Confidence intervals (CI), z value and *P* values of the averaged model are shown.

Explanatory variables	Estimate	S.E.	95% CI		z value	*P*
Intercept	0.270	0.276	0.942	-0.292	0.832	0.346
Treatment	-1.219	0.568	2.066	-2.376	-0.062	0.039
log.Parasitaemia	0.668	0.598	1.074	-0.552	1.888	0.283
Sex	-0.526	0.552	0.916	-1.651	0.599	0.360

Our study underlines the role of the intensity of infection by *Plasmodium* rather than the infection itself in mosquito feeding preferences. Mosquito feeding behaviour is a complex phenomenon that includes flight activation, attraction to hosts, landing on selected hosts, and biting of specific parts of the hosts' bodies. Host-seeking mosquitoes use visual, thermal and olfactory cues to discriminate different hosts, which also depend on the specific environment in which host-vector interactions occur (Day, 2005; Takken and Verhulst, 2013; Cardé, 2015; van Breugel et al., 2015). In our study, birds and mosquitoes interacted closely with each other (within 60 cm) and it is likely that cues such as moisture and heat acted as signals for host localization and selection by mosquitoes (Cardé, 2015; van Breugel et al., 2015). However, anti-mosquito behaviour may in fact have ultimately determined the number of bites received by each bird (Day, 1984; Edman and Scott, 1987; Darbro and Harrington, 2007). Avian malaria can cause high mortality in early stages of infection, which implies that only birds with strong immune systems can survive with chronic infections in the wild (Nordling et al., 1998; Knowles et al., 2009). Wild birds with chronic infections are usually asymptomatic and often only display mild changes in behavioural traits, although olfactory profiles, for example, may be affected (Palinauskas et al., 2008; Cauchard et al., 2016). This could explain why there were no significant differences in our study on mosquito biting rates between naturally infected birds and uninfected birds. However, bird activity such as anti-mosquito behaviour may vary over time and could be enhanced by the reduction of parasite load induced by the anti-malaria treatment (Cauchard et al., 2016). This could explain why infected birds treated with primaquine in our study were bitten less than infected non-treated birds. The alternative that side effects of primaquine, instead of its effect on parasite load, alter mosquito behaviour is poorly supported by previous studies. Bird activity levels did not differ between uninfected-treated and uninfected-control Great tits (*Parus major*) (Cauchard et al., 2016), suggesting that bird susceptibility to mosquito bites could not be affected by the treatment itself. In addition, the biological half-life of primaquine in plasma is approximately 4-9 h (Baird and Hoffman, 2004). Given that we treated the infected birds with primaquine 7 days prior to mosquito exposure, the potential side effects of anti-malarial treatment on mosquito attraction are poorly supported. Our finding that birds with higher parasite loads were bitten more often was in agreement with Day and Edman (1983), who found that mice infected with *Plasmodium* displayed less anti-mosquito behaviour than uninfected individuals. Similarly, Yorinks and Atkinson (2000) reported that infected birds devoted less time to both locomotory and stationary activities that may contribute to avoiding mosquito bites.

Infection with different *Plasmodium* lineages and mixed infections with multiple lineages are commonly recorded in wild birds (Valkiūnas et al., 2003, 2006; Clark et al., 2016). This is the case in our study, and the birds used in our experiments were infected with different lineages or with double lineages. Unfortunately, the high diversity of lineages presented here does not allow incorporation of this factor into our analyses. Consequently, we cannot exclude the possibility that the diversity of lineages may have added some noise to our results. Thus it is advisable that future studies consider lineage identity as a factor in the experimental design. However, to date and to the best of our knowledge, there is no evidence for lineage-specific effects on mosquito attraction.

To date, many studies have focused on host-parasite interactions, but host-vector interactions may also be important, as enhanced feeding on infected hosts will increase the likelihood of parasite transmission. In conclusion, our results partially support the parasite manipulation hypothesis by way of a quantitative association between biting rate and parasite load rather than qualitative comparison of infection status, that is, the *Plasmodium* load in birds influences blood-feeding patterns of mosquito vectors.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

J.Y., J.M.P., L.G., J.F. and R.S. designed the study. J.Y., J.M.P., L.G. and R.G.L. conducted the fieldwork. J.Y. and J.M.P. performed the bioassays. J.Y. and J.M.P. carried out the molecular analyses. J.Y., L.G. and J.M.P. carried out the statistical analyses. All authors contributed to writing the manuscript and approved the final version.

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References

Alcaide, M., Rico, C., Ruiz, S., Soriguer, R., Munoz, J., Figuerola, J., 2009. Disentangling vector-borne transmission networks: a universal DNA barcoding method to identify vertebrate hosts from arthropod bloodmeals. PLoS One 4, e7092.

Appleby, B., Anwar, M., Petty, S., 1999. Short-term and long-term effects of food supply on parasite burdens in Tawny Owls, *Strix aluco*. Funct. Ecol. 13, 315-321.

Baird, J.K., Hoffman, S.L., 2004. Primaquine therapy for malaria. Clin. Infect. Dis. 39, 1336-1345.

Baird, J.K., Rieckmann, K.H., 2003. Can primaquine therapy for *vivax* malaria be improved? Trends Parasitol. 19, 115-120.

Batista, E.P., Costa, E.F., Silva, A.A., 2014. *Anopheles darlingi* (Diptera: Culicidae) displays increased attractiveness to infected individuals with *Plasmodium vivax* gametocytes. Parasit. Vectors. 7, 251.

Bensch, S., Waldenström, J., Jonzén, N., Westerdahl, H., Hansson, B., Sejberg, D., Hasselquist, D., 2007. Temporal dynamics and diversity of avian malaria parasites in a single host species. J. Anim. Ecol. 76, 112-122.

Bohbot, J.D., Durand, N.F., Vinyard, B.T., Dickens, J.C., 2013. Functional Development of the Octenol Response in *Aedes aegypti*. Front. Physiol. 4, 39.

Burgess, R.W., Bray, R., 1961. The effect of a single dose of primaquine on the gametocytes, gametogony and sporogony of *Laverania falciparum*. Bull. W.H.O. 24, 451.

Cardé, R.T., 2015. Multi-cue integration: how female mosquitoes locate a human host. Curr. Biol. 25, R793-R795.

Cauchard, L., Angers, B., Boogert, N.J., Doligez, B., 2016. Effect of an anti-malaria drug on behavioural performance on a problem-solving task: An experiment in wild great tits. Behav. Processes. 133, 24-30.

Clark, N.J., Wells, K., Dimitrov, D., Clegg, S.M., 2016. Co□infections and environmental conditions drive the distributions of blood parasites in wild birds. J. Anim. Ecol. 85, 1461-1470.

Cornet, S., Nicot, A., Rivero, A., Gandon, S., 2013a. Malaria infection increases bird attractiveness to uninfected mosquitoes. Ecol. Lett. 16, 323-329.

Cornet, S., Nicot, A., Rivero, A., Gandon, S., 2013b. Both infected and uninfected mosquitoes are attracted toward malaria infected birds. Malar. J. 12, 179.

Darbro, J.M., Harrington, L.C., 2007. Avian defensive behavior and blood-feeding success of the West Nile vector mosquito, *Culex pipiens*. Behav. Ecol. 18, 750-757.

Daugherty, M.P., Rashed, A., Almeida, R.P., Perring, T.M., 2011. Vector preference for hosts differing in infection status: sharpshooter movement and *Xylella fastidiosa* transmission. Ecol. Entomol. 36, 654-662.

Day, J.F., 2005. Host-seeking strategies of mosquito disease vectors. J. Am. Mosq. Control Assoc. 21, 17-22.

Day, J.F., Edman, J.D., 1983. Malaria renders mice susceptible to mosquito feeding when gametocytes are most infective. J. Parasitol. 69, 163-170.

Day, J.F., Edman, J.D., 1984. Mosquito engorgement on normally defensive hosts depends on host activity patterns. J. Med. Entomol. 21, 732-740.

De Moraes, C.M., Stanczyk, N.M., Betz, H.S., Pulido, H., Sim, D.G., Read, A.F., Mescher, M.C., 2014. Malaria-induced changes in host odors enhance mosquito attraction. Proc. Natl. Acad. Sci. USA 111, 11079-11084.

Eigenbrode, S.D., Ding, H., Shiel, P., Berger, P.H., 2002. Volatiles from potato plants infected with potato leafroll virus attract and arrest the virus vector, *Myzus persicae* (Homoptera: Aphididae). Proc. R. Soc. Lond. B 269, 455-460.

Ellegren, H., 1996. First gene on the avian W chromosome (CHD) provides a tag for universal sexing of non-ratite birds. Proc. R. Soc. Lond. B 263, 1635-1641.

Ferguson, H.M., Read, A.F., 2002. Why is the effect of malaria parasites on mosquito survival still unresolved? Trends Parasitol. 18, 256-261.

Garnier, S., Durand, P., Arnathau, C., Risterucci, A.M., Esparza-Salas, R., Cellier-Holzem, E., Sorci, G., 2009. New polymorphic microsatellite loci in the house sparrow, *Passer domesticus*. Mol. Ecol. Res. 9, 1063-1065.

Griffiths, R., Double, M.C., Orr, K., Dawson, R.J.G., 1998. A DNA test to sex most birds. Mol. Ecol. 7, 1071-1075.

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

Gutiérrez□López, R., Martínez□de la Puente, J., Gangoso, L., Soriguer, R.C., Figuerola, J., 2015. Comparison of manual and semi-automatic DNA extraction protocols for the barcoding characterization of hematophagous louse flies (Diptera: Hippoboscidae). J. Vector Ecol. 40, 11-15.

Hart, B.L., 1990. Behavioral adaptations to pathogens and parasites: five strategies.

Neurosci. Biobehav. Rev. 14, 273-294.

Hellgren, O., Waldenström, J., Bensch, S., 2004. A new PCR assay for simultaneous studies of *Leucocytozoon*, *Plasmodium*, and *Haemoproteus* from avian blood. J. Parasitol. 90, 797-802.

Hurd, H., 2003. Manipulation of medically important insect vectors by their parasites. Annu. Rev. Entomol. 48, 141-161.

Knowles, S.C., Nakagawa, S., Sheldon, B.C., 2009. Elevated reproductive effort increases blood parasitaemia and decreases immune function in birds: a meta-regression approach. Funct. Ecol. 23, 405-415.

Lacroix, R., Mukabana, W.R., Gouagna, L.C., Koella, J.C., 2005. Malaria infection increases attractiveness of humans to mosquitoes. PLoS Biol. 3, 1590-1593.

Lafferty, K.D., Kuris, A.M., 2012. Ecological consequences of manipulative parasites. In: Hughes, D.P., Brodeur, J., Thomas, F. (Ed.), Host manipulation by parasites. Oxford University Press, Oxford, UK, pp. 158-168.

Lalubin, F., Bize, P., van Rooyen, J., Christe, P., Glaizot, O., 2012. Potential evidence of parasite avoidance in an avian malarial vector. Anim. Behav. 84, 539-545.

Lefèvre, T., Koella, J.C., Renaud, F., Hurd, H., Biron, D.G., Thomas, F., 2006. New prospects for research on manipulation of insect vectors by pathogens. PLoS Pathog. 2, e72.

Lefèvre, T., Lebarbenchon, C., Gauthier-Clerc, M., Misse, D., Poulin, R., Thomas, F., 2009. The ecological significance of manipulative parasites. Trends Ecol. Evol. 24, 41-48.

Lefèvre, T., Thomas, F., 2008. Behind the scene, something else is pulling the strings: emphasizing parasitic manipulation in vector-borne diseases. Infect., Genet. Evol. 8, 504-519.

Libersat, F., Delago, A., Gal, R., 2009. Manipulation of host behavior by parasitic insects and insect parasites. Annu. Rev. Entomol. 54, 189-207.

Lipnick, R.L., 1991. Narcosis induced by ether and chloroform. In: Lipnick, R.L. (Ed.), Studies of Narcosis. Springer, Dordrecht, The Netherlands, pp. 93-107.

López-Antuñano, F.J., 1999. Is primaquine useful and safe as true exo-erythrocytic merontocidal, hypnozoitocidal and gametocidal antimalarial drug? Salud Publica Mex. 41, 410-419.

Martínez-de la Puente, J., Merino, S., Tomás, G., Moreno, J., Morales, J., Lobato, E., García-Fraile, S., Belda, E.J., 2010. The blood parasite *Haemoproteus* reduces survival in a wild bird: a medication experiment. Biol. Lett, rsbl20100046.

Martínez-de la Puente, J., Merino, S., Tomás, G., Moreno, J., Morales, J., Lobato, E., Talavera, S., Sarto i Monteys, V., 2009. Factors affecting *Culicoides* species composition and abundance in avian nests. Parasitology 136, 1033-1041.

Martínez-de la Puente, J., Ruiz, S., Soriguer, R., Figuerola, J., 2013. Effect of blood meal digestion and DNA extraction protocol on the success of blood meal source determination in the malaria vector *Anopheles atroparvus*. Malar. J. 12, 109.110.

Marzal, A., De Lope, F., Navarro, C., Møller, A.P., 2005. Malarial parasites decrease reproductive success: an experimental study in a passerine bird. Oecologia 142, 541-545.

Mauck, K., Bosque□Pérez, N.A., Eigenbrode, S.D., Moraes, C.M., Mescher, M.C., 2012. Transmission mechanisms shape pathogen effects on host–vector interactions: evidence from plant viruses. Funct. Ecol. 26, 1162-1175.

Mauck, K.E., De Moraes, C.M., Mescher, M.C., 2010. Deceptive chemical signals induced by a plant virus attract insect vectors to inferior hosts. Proc. Natl. Acad. Sci. USA 107, 3600-3605.

Merino, S., Moreno, J., Sanz, J.J., Arriero, E., 2000. Are avian blood parasites pathogenic in the wild? A medication experiment in blue tits (*Parus caeruleus*). Proc. R. Soc. Lond. B 267, 2507-2510.

Merino, S., Tomas, G., Moreno, J., Sanz, J.J., Arriero, E., Folgueira, C., 2004. Changes in *Haemoproteus* sex ratios: fertility insurance or differential sex lifespan? Proc. R. Soc. Lond. B 271, 1605-1609.

Møller, A.P., Nielsen, J.T., 2007. Malaria and risk of predation: a comparative study of birds. Ecology 88, 871-881.

Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining R^2 from generalized linear mixed□effects models. Methods Ecol. Evol. 4, 133-142.

Nordling, D., Andersson, M., Zohari, S., Lars, G., 1998. Reproductive effort reduces specific immune response and parasite resistance. Proc. R. Soc. Lond. B 265, 1291-1298.

O'Shea, B., Rebollar-Tellez, E., Ward, R., Hamilton, J., El Naiem, D., Polwart, A., 2002. Enhanced sandfly attraction to *Leishmania*-infected hosts. Trans. R. Soc. Trop. Med. Hyg. 96, 117-118.

O'Brien, R.M., 2007. A caution regarding rules of thumb for variance inflation factors. Qual. Quan. 41, 673-690.

Palinauskas, V., Valkiūnas, G., Bolshakov, C.V., Bensch, S., 2008. *Plasmodium relictum* (lineage P-SGS1): effects on experimentally infected passerine birds. Exp. Parasitol. 120, 372-380.

Pigeault, R., Vézilier, J., Cornet, S., Zélé, F., Nicot, A., Perret, P., Gandon, S., Rivero, A.,

2015. Avian malaria: a new lease of life for an old experimental model to study the evolutionary ecology of *Plasmodium*. Phil. Trans. R. Soc. B 370, 20140300.

Poulin, R., 1995. "Adaptive" changes in the behaviour of parasitized animals: a critical review. Int. J. Parasitol. 25, 1371-1383.

Poulin, R., 2010. Parasite manipulation of host behavior: an update and frequently asked questions. Adv. Study Behav. 41, 151-186.

Schaffner, F., Angel, G., Geoffroy, B., Hervy, J., Rhaiem, A., Brunhes, J., 2001. The mosquitoes of Europe, an identification and training programme. IRD, Montpellier, France.

Shapiro, L., Moraes, C.M., Stephenson, A.G., Mescher, M.C., 2012. Pathogen effects on vegetative and floral odours mediate vector attraction and host exposure in a complex pathosystem. Ecol. Lett. 15, 1430-1438.

Stafford, C.A., Walker, G.P., Ullman, D.E., 2011. Infection with a plant virus modifies vector feeding behavior. Proc. Natl. Acad. Sci. USA 108, 9350-9355.

Takken, W., Verhulst, N.O., 2013. Host preferences of blood-feeding mosquitoes. Annu. Rev. Entomol. 58, 433-453.

Tomás, G., Merino, S., Moreno, J., Morales, J., Martinez□De La Puente, J., 2007. Impact of blood parasites on immunoglobulin level and parental effort: a medication field experiment on a wild passerine. Funct. Ecol. 21, 125-133.

Valkiūnas, G., 2005. Avian malaria parasites and other haemosporidia. CRC press, USA.

Valkiūnas, G., Bensch, S., Iezhova, T., Križanauskienė, A., Hellgren, O., Bolshakov, C., 2006. Nested cytochrome b PCR diagnostics underestimate mixed infections of avian blood Haemosporidian parasites: microscopy is still essential. J. Parasitol. 92, 418-422.

Valkiūnas, G., Iezhova, T., Shapoval, A.P., 2003. High prevalence of blood parasites in hawfinch *Coccothraustes coccothraustes*. J. Nat. Hist. 37, 2647-2652.

Valkiūnas, G., Križanauskienė, A., Iezhova, T.A., Hellgren, O., Bensch, S., 2007. Molecular phylogenetic analysis of circumnuclear hemoproteids (Haemosporida: Haemoproteidae) of sylviid birds, with a description of *Haemoproteus parabelopolskyi* sp. nov. J. Parasitol. 93, 680-687.

van Breugel, F., Riffell, J., Fairhall, A., Dickinson, M.H., 2015. Mosquitoes Use Vision to Associate Odor Plumes with Thermal Targets. Curr. Biol. 25, 2123-2129.

Vézilier, J., Nicot, A., Gandon, S., Rivero, A., 2012. *Plasmodium* infection decreases fecundity and increases survival of mosquitoes. Proc. R. Soc. B. 279, 4033-4041.

Westerdahl, H., Asghar, M., Hasselquist, D., Bensch, S., 2011. Quantitative disease resistance: to better understand parasite-mediated selection on major histocompatibility complex. Proc. R. Soc. Lond. B, rspb20110917.

Witsenburg, F., Schneider, F., Christe, P., 2014. Signs of a vector's adaptive choice: on the evasion of infectious hosts and parasite-induced mortality. Oikos 124, 668-676.

Woodworth, B.L., Atkinson, C.T., LaPointe, D.A., Hart, P.J., Spiegel, C.S., Tweed, E.J., Henneman, C., LeBrun, J., Denette, T., DeMots, R., 2005. Host population persistence in the face of introduced vector-borne diseases: Hawaii amakihi and avian malaria. Proc. Natl. Acad. Sci. USA 102, 1531-1536.

Yorinks, N., Atkinson, C.T., 2000. Effects of malaria on activity budgets of experimentally infected juvenile Apapane (*Himatione sanguinea*). Auk 117, 731-738.

Zehtindjiev, P., Ilieva, M., Westerdahl, H., Hansson, B., Valkiūnas, G., Bensch, S., 2008. Dynamics of parasitemia of malaria parasites in a naturally and experimentally infected migratory songbird, the great reed warbler *Acrocephalus arundinaceus*. Exp. Parasitol. 119, 99-110.

General discussion

The transmission of VBPs could be affected by many intrinsic and extrinsic factors. This process seems to be specially intricate in recent times, given that environmental perturbation associated with climate change and human activities, such as land use changes, is altering the distribution and abundance of interacting hosts, vectors and pathogens worldwide (Dobson and Carper, 1992; Gage et al., 2008; Keesing et al., 2013; Ferraguti et al., 2016). Among others, the ecology of VBDs, especially the interactions between hosts and vectors could largely drive the emergence, transmission and dynamics of VBPs (Nuttall et al., 2000; Kilpatrick et al., 2006a,b; Kim and Tsuda, 2010; Kilpatrick and Randolph, 2012; Simpson et al., 2012). During last decades, a great number of studies have focused on host-pathogen or vector-pathogen assemblages (e.g. Komar et al., 2001; Reisen et al., 2005; Marzal et al., 2008). However, the host-vector interactions receive relatively less attention, probably due to the complexity of the behaviors of both hosts and vectors. This thesis delves into the relationships between hosts and vectors, by assessing the role of intra-specific and inter-specific variation in host traits in the heterogeneity of mosquito feeding preferences.

To simplify the complex interactions affecting the transmission of VBPs, previous studies on host-vector interactions usually focus on a single host or vector and epidemiological models often assume random host-vector interactions (Aron and May, 1982; Anderson et al., 1992; Grenfell and Dobson, 1995). Hence, the epidemiological models based on random host-vector interactions could produce biased estimates of VBPs transmission dynamics (see Kilpatrick et al., 2006b; Simpson et al., 2012). In nature, however, host-vector interactions are more complicated, as vectors interact with a variety of hosts depending on their availability, and many vectors express an opportunistic behavior (Takken and Verhulst, 2013). In addition, as shown in this thesis, host-vector interactions are far from random, and many vectors have clear feeding preferences for certain hosts, species or even individuals. By using an integrative approach that combines fieldwork, comparative analyses and laboratory experiments; this thesis explores the proximate causes of non-random host-vector interactions at both intra-specific and inter-specific levels.

Mosquito feeding behavior is a complex process that comprises flight activation, attraction to hosts, landing on selected hosts, and biting of specific parts of the hosts' bodies (Lehane, 2005). In any of these phases, blood-seeking mosquitoes take advantage of any detectable host trait such as visual, olfactory and thermal cues to locate and discriminate hosts (Day, 2005; Takken and Verhulst, 2013; van Breugel et al., 2015; Cardé, 2015). In addition, any host trait that are related to the emission of these cues could influence mosquito feeding behavior and hence, host-vector interactions.

Combining both empirical and theoretical approaches, this thesis is able to identify the links between some key host traits and mosquito feeding preferences.

To understand the links between host traits and mosquito feeding preferences, the theoretical first step is to identify what particular host characteristics may affect mosquito seeking and feeding behaviors. To this end, Chapter 1 systematically reviewed the current progress in the studies on mosquito attraction and blood feeding behavior. Based on this review, I highlighted that mosquito feeding preferences are usually affected by both innate host preference of mosquitoes and extrinsic factors, including host attractiveness and availability in the field. Some mosquito species express inherent preference for feeding on, for instance, mammals or, birds, but also for certain individuals within the same species. Nonetheless, many mosquitoes are opportunistic feeders, and hence, their innate host preference could be readily overruled by the variation in host availability or attractiveness (Takken and Verhulst, 2013). Olfactory cues are the most important host-seeking clues used by mosquitoes. CO_2 and many volatile compounds isolated from hosts have been tested for mosquito attraction and differences in the release of these cues may drive the host-selection patterns by mosquitoes. However, many studies on mosquito attraction tested the effect of each of these substances in isolation or in combination only with CO_2 in laboratory (e.g. Cooperband et al., 2008; Syed an Leal, 2009), despite that these substances often function jointly during the host seeking process of mosquitoes (Cardé, 2015). Thus, future studies should pay more attention to the integrative effects of theses substances on mosquito attraction/feeding preference. As natural blends of all potential cues, intact hosts such as wild animals are ideal candidates for exploring mosquito attraction/feeding preference. Among others, wild birds are relatively rarely tested for mosquito attraction, although they are the primary vertebrate hosts of several highly important VBPs, such as WNV and avian malaria parasites (McLean et al., 2001; Valkiūnas, 2005). Visual cues can also affect mosquito feeding choice in an intermediate range and the role of visual cues in mosquito attraction may have been underestimated, as increasing evidence shows that even crepuscular/nocturnal mosquitoes can respond to visual stimuli (e.g. Wen et al., 1997; Hawkes and Gibson, 2016). In fact, color/intensity contrast against background may play an important role in mosquito attraction. The effect of avian plumage coloration on mosquito attraction remains rarely explored (but see Chapter 2). Given the complexity of the mechanisms affecting mosquito feeding preference, this may best be assessed using approaches combining host-choice assays of live animals with blood meal tracking techniques to calculate mosquito feeding patterns both in the field and laboratory. I also compared several methods for studying mosquito feeding preference and proposed functional trait-based approaches to deal with the joint effect of multiple cues on the host-seeking process of mosquitoes and the ecological complexity in multihost-multivector interactions across different communities in the wild. From a practical point of view, this may have important implications, especially with regard to the transmission dynamics of vector-borne infectious diseases.

Once we knew the knowledge gaps in the links between host traits and mosquito feeding preferences, the next step is to explore these gaps using both theoretical and empirical approaches. Increasing evidence suggests that the mosquito feeding behavior is not completely random, but rather a complex combination that depends on mosquitoes' preference for certain host species, host availability as well as environmental conditions (e.g. Suom et al., 2010; Thiemann et al., 2011). However, important gaps still exist in our understanding about the factors affecting mosquito feeding behavior. For example, despite the great importance of host-vector contact rates in the transmission dynamics of pathogens, little is known about the role of host morphological and behavioral traits and mosquito feeding preference. I identified several associations between these traits and mosquito feeding patterns using comparative analysis. In this regard, plumage coloration emerged as an important trait for mosquito attraction, providing the first evidence that lighter colors may be more attractive to mosquitoes than darker ones. I also confirmed the already-found positive correlation between host body size and vector bites (see Malmqvist et al., 2004; Martínez-de la Puente., 2009; Estep et al., 2012). In addition, I predicted birds roosting together could attract more mosquito bites as they may release more cues used by host seeking mosquitoes than solitary roosting species. In contrast to this prediction, I found a higher preference of mosquitos for solitary roosting species. This supports the idea that communal roosting may reduce exposure to mosquito bites. I conclude that host traits indeed can affect mosquito feeding preference and this may improve our understanding of the non-random interactions between hosts and mosquitoes in natural communities. Although this study is limited to a particular avian community, it may still have implications for broader host communities, as similar functional traits could be found in many animals and the trait-based approaches could link host traits to vector feeding patterns across different geographic scales (Johnson et al., 2015). Altogether, the interactions of these factors lead to the heterogeneity in mosquito feeding patterns affecting the dynamics of pathogen transmission (Kilpatrick et al., 2006b). This study represents an example of the use of trait-based approaches in the study of vector-borne disease ecology. From a practical perspective, these results could be easily applied in, for instance, surveillance programs for VBPs, which could consider that larger species roosting alone and/or of lighter plumage coloration as good candidates as focal species for VBP monitoring.

Host metabolism is another important trait that may influence mosquito searching and feeding behaviors as it could affect individual host profiles including the release of some key location cues for mosquitoes, such as CO_2, odor, and temperature. Metabolic rate, however, is difficult to measure, particularly under field conditions. Therefore, to explore this potential association, I conducted an experimental approach by manipulating the resting metabolic rate of assayed birds. I identified a significant and negative association between resting metabolic rate and mosquito feeding preference, which is, to date, the first experimental evidence that host metabolic rate can affect mosquitoes' blood feeding behavior. Nonetheless, the underlying mechanisms of this detected link could be highly complex, as host metabolism may co-vary with multiple cues that may in turn alter mosquito behavior. Among others, the most likely mechanism in our case is the host defensive behavior against mosquitoes, which is energetically costly and can greatly affect mosquito feeding success (Darbro and Harrington, 2007). Hence, individual birds with higher metabolic rates may show more intense defensive anti-mosquito behavior and this may lead to the observed feeding patterns. Other mechanisms, such as host odors and temperature, may also have the potential to influence mosquito feeding patterns, but they could be of less importance given that my experimental approach should have minimized these possibilities. Again, a positive and significant association was also found between avian body mass and mosquito feeding preference, which seems to be related with the fact that larger individuals are also larger targets (with larger available skin surface) and produce more cues used by mosquitoes. This could account for the recurrent detected link between bird body mass and mosquito feeding patterns. This finding adds new evidence for the importance of host size on mosquito blood-feeding at the intra-specific level. The experimental approach used here simulates the natural environment where individuals with different physiological and behavioral traits roost together. In such a context, a bird with higher metabolic rate may release more location cues for host-seeking mosquitoes, but the actual mosquito feeding patterns may often depend on the surrounding group size, which could reduce the individual risk of being attacked (Cresswell, 1994; Janousek et al., 2014), making the per capita bird exposure to mosquitoes lower given the encounter-dilution effect (Krebs et al., 2014). The broader host community in which an individual is exposed to blood-sucking mosquitoes may be a relevant factor – as is the production of attractant cues – when determining whether an individual will be bitten or not. Another aspect of host physiological traits that may potentially affect mosquito feeding behavior is host infection. Host infection by different pathogens is a widespread trait of wild animals. For example, many wild birds are infected by blood parasites, including avian *Plasmodium* (Valkiūnas, 2005). Pathogens may alter the odor profile of infected hosts, as this has been reported in rodents (De Moraes et al., 2014) and humans (Emami et al., 2017), which may change host attractiveness to vectors and hence, host-vector contact rates (Emami et al., 2017). On the other hand, pathogens may alter the behavior of infected hosts, such as defensive and explorative behaviors, which may render the infected hosts less defensive or more susceptible to mosquito bites (Day and Edman 1983; Yorinks and Atkinson, 2000). As a result, host infection may dramatically change the host-vector contact rates. These phenotypic changes induced by pathogens in infected hosts gave rise to the parasitic manipulation hypothesis. This hypothesis states that pathogens could adaptively alter host phenotype in a way that increases host-vector contact rates and thus the fitness (transmission) of pathogens (Poulin, 1995; Hurd, 2003). To test this hypothesis, I combined two experiments to assess the role of *Plasmodium* infection prevalence and intensity in mosquito feeding patterns. I found that mosquitoes fed randomly when facing infected and uninfected birds, which could be explained by the mild changes induced by natural and chronic *Plasmodium* infection. This result alone did not support the parasitic manipulation hypothesis, as the lack of preference for infected birds could not increase the fitness of the parasites. However, when mosquitoes were exposed only to infected individuals, they preferentially bit those with higher parasite loads. These results suggest that the malarial parasite load rather than the infection itself plays a key role in mosquito attraction. Therefore, these findings as a whole partially support the parasitic manipulation hypothesis, which probably operates via a reduction in defensive behavior, and highlights the importance of considering parasite load in studies on host-vector-pathogen interactions. More studies are needed to explore the underlying mechanism of the disproportionate host use by mosquitoes toward heavier infected birds. Nonetheless, in nature there is a great diversity of parasite lineages (Clark et al., 2014) and given their different pathology, host infections with different lineages may not have the same effects on mosquito attraction. In addition, many wild birds exhibit a simultaneous infection with different pathogens (Valkiūnas et al., 2006; Clark et al., 2016), but the effects of mixed-infections on avian phenotype may be far more complicated than single infection. For example, the effects of co-infection by two different *Plasmodium* lineages may not equal the sum of two single infections (Marzal et al., 2008). As a result, the extent to which mixed infections may influence mosquito feeding behavior is still poorly understood. Future studies could take different pathogen lineages and/or mix-infections into account and further explore their potential role in mosquito attraction.

Altogether, this thesis identified several key links in host-vector interactions acting in the disease triangle, which may potentially influence the transmission dynamics of VBPs. In particular, some important links of host morphological and behavioral traits (Chapter 2) and physiological traits (Chapter 3-4) through the emission of different cues (Chapter 1) to mosquito feeding preferences were identified. This thesis highlights the role of host traits in generating heterogeneities in the exposure of host to mosquito bites and hence, in host-vector contact rates, which may ultimately influence the ecology and dynamics of VBDs. Trait-based approaches may provide a practical tool in future epidemiological studies, and enhance the efficiency of surveillance programs by focusing on a group of individuals and/or species that present traits that make them more likely to enter in contact with the mosquitoes and consequently, with the pathogens they can transmit.

Conclusions

1. Mosquito feeding preference is a compromise between innate host preference and extrinsic factors including host attractiveness and availability. Innate host preference can be readily affected by variable host traits such as morphology, behavior and physiology, which produce olfactory, visual, thermal and other cues for host-seeking mosquitoes. The joint effects of multiple host traits on mosquito attraction and the complexity of multihost-multivector assemblages call for novel methodological approaches. This study highlighted the benefits of using functional trait-based approaches in monitoring host-vector contact rates.

2. Inter-specific differences in host morphological and behavioral traits drive mosquito feeding preferences, while host phylogeny do not affect mosquito behavior. Particularly, birds with lighter plumage coloration, bigger body size and solitary roosting behavior were bitten more often than expected from their relative abundance in the community.

3. Avian resting metabolic rate and body mass affect the host selection by mosquitoes at the intra-specific level. Birds with lower resting metabolic rate and higher body mass were bitten more often by *Cx. pipiens* mosquitoes. These bird traits affect the feeding preference of this mosquito species, a major vector of multiple VBPs, which may throw light on the epidemiology of pathogens with ecological and public health relevance.

4. Host infection with the common avian pathogen *Plasmodium* affect mosquito feeding preference, although different effects could be found when considering the two facets of infection, including prevalence and intensity of infection. While no differences were found in mosquito bites between infected and un-infected birds, the experimental reduction of the parasite load reduced the susceptibility of birds to mosquito bites. These findings partially support the parasite manipulation hypothesis, which probably operates via a reduction in defensive behavior of heavily infected hosts, and highlights the importance of considering parasite load in studies on host-vector-pathogen interactions.

References

Allan, S. A., Day, J. F., & Edman, J. D. (1987). Visual ecology of biting flies. *Annual Review of Entomology, 32*(1), 297-314.

Anderson, J. F., Main, A. J., Ferrandino, F. J., & Andreadis, T. G. (2007). Nocturnal activity of mosquitoes (Diptera: Culicidae) in a West Nile virus focus in Connecticut. *Journal of Medical Entomology, 44*(6), 1102-1108.

Anderson, R. M., May, R. M., & Anderson, B. (1992). *Infectious diseases of humans: dynamics and control* (Vol. 28): Wiley Online Library.

Anderson, T. R. (2006). *Biology of the ubiquitous house sparrow: from genes to populations*: Oxford University Press.

Aron, J. L., & May, R. M. (1982). The population dynamics of malaria. In R. B. Anderson (Eds.), *The population dynamics of infectious diseases: theory and applications* (pp. 139-179). Boston, USA: Springer.

Arrigo, N. C., Adams, A. P., Watts, D. M., Newman, P. C., & Weaver, S. C. (2010). Cotton rats and house sparrows as hosts for North and South American strains of eastern equine encephalitis virus. *Emerging Infectious Diseases, 16*(9), 1373-1380.

Asghar, M., Hasselquist, D., Hansson, B., Zehtindjiev, P., Westerdahl, H., & Bensch, S. (2015). Hidden costs of infection: chronic malaria accelerates telomere degradation and senescence in wild birds. *Science, 347*(6220), 436-438.

Atkinson, C. T., & van Riper III, C. (1991). Pathogenicity and epizootiology of avian haematozoa: *Plasmodium*, *Leucocytozoon*, and *Haemoproteus*. In J. E. Loye & M. Zuk (Eds.), *Bird–parasite interactions: ecology, evolution, and behaviour* (pp. 19-48). Oxford, UK: Oxford University Press.

Baird, J. K., & Hoffman, S. L. (2004). Primaquine therapy for malaria. *Clinical Infectious Diseases, 39*(9), 1336-1345.

Batista, E. P., Costa, E. F., & Silva, A. A. (2014). *Anopheles darlingi* (Diptera: Culicidae) displays increased attractiveness to infected individuals with *Plasmodium vivax* gametocytes. *Parasit Vectors, 7*, 251. doi:10.1186/1756-3305-7-251

Beadell, J. S., Covas, R., Gebhard, C., Ishtiaq, F., Melo, M., Schmidt, B. K., Perkins, S. L., Graves, G. R., & Fleischer, R. C. (2009). Host associations and evolutionary relationships of avian blood parasites from West Africa. *International Journal for Parasitology, 39*(2), 257-266.

Beadell, J. S., Gering, E., Austin, J., Dumbacher, J. P., Peirce, M. A., Pratt, T. K., Atkinson, C. T., & Fleischer, R. C. (2004). Prevalence and differential host-specificity of two avian blood parasite genera in the Australo-Papuan region. *Molecular Ecology, 13*(12), 3829-3844.

Beadell, J. S., Ishtiaq, F., Covas, R., Melo, M., Warren, B. H., Atkinson, C. T., Bensch, S., Graves, G. R., Jhala, Y. V., & Peirce, M. A. (2006). Global phylogeographic limits of Hawaii's avian malaria. *Proceedings of the Royal Society of London B: Biological Sciences, 273*(1604), 2935-2944.

Becker, N., Petrić, D., Boase, C., Lane, J., Zgomba, M., Dahl, C., & Kaiser, A. (2010). *Mosquitoes and their control* (2nd ed. Vol. 2): Springer.

Bennett, G. F. (1970). Simple techniques for making avian blood smears. *Canadian Journal of Zoology, 48*(3), 585-586.

Bennett, G. F., Bishop, M. A., & Peirce, M. A. (1993). Checklist of the avian species of *Plasmodium* Marchiafava & Celli, 1885 (Apicomplexa) and their distribution by avian family and Wallacean life zones. *Systematic Parasitology, 26*(3), 171-179.

Bensch, S., Stjernman, M., Hasselquist, D., Örjan, Ö., Hannson, B., Westerdahl, H., & Pinheiro, R. T. (2000). Host specificity in avian blood parasites: a study of *Plasmodium* and *Haemoproteus* mitochondrial DNA amplified from birds. *Proceedings of the Royal Society of London B: Biological Sciences, 267*(1452), 1583-1589.

Bowen, M. F. (1991). The sensory physiology of host-seeking behavior in mosquitoes. *Annual Review of Entomology, 36*, 139-158.

Burkett-Cadena, N. D., McClure, C. J. W., Ligon, R. A., Graham, S. P., Guyer, C., Hill, G. E., Ditchkoff, S. S., Eubanks, M. D., Hassan, H. K., & Unnasch, T. R. (2011). Host reproductive phenology drives seasonal patterns of host use in mosquitoes. *PLoS One, 6*(3), e17681.

Burkot, T. (1988). Non-random host selection by anopheline mosquitoes. *Parasitology Today, 4*(6), 156-162.

Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: a practical information-theoretic approach* (second ed.): Springer Science & Business Media.

Cardé, R. T. (2015). Multi-cue integration: how female mosquitoes locate a human host. *Current Biology, 25*(18), R793-R795.

Cauchard, L., Angers, B., Boogert, N. J., & Doligez, B. (2016). Effect of an anti-malaria drug on behavioural performance on a problem-solving task: An experiment in wild great tits. *Behavioural Processes, 133*, 24-30.

Chiba, Y., Kubota, M., & Nakamura, Y. (1982). Differential effects of temperature upon evening and morning peaks in the circadian activity of mosquitoes, *Culex pipiens pallens* and *C. pipiens molestus*. *Biological Rhythm Research, 13*(1), 55-60.

Clark, N. J., Clegg, S. M., & Lima, M. R. (2014). A review of global diversity in avian haemosporidians (*Plasmodium* and *Haemoproteus*: Haemosporida): new insights from molecular data. *International Journal for Parasitology, 44*(5), 329-338.

Clark, N. J., Wells, K., Dimitrov, D., & Clegg, S. M. (2016). Co-infections and environmental conditions drive the distributions of blood parasites in wild birds. *Journal of Animal Ecology, 85*(6), 1461-1470.

Clements, K. (2007). *The Encyclopedia of Birds: 6 volume set*. New York, NJ: Facts On File.

Cooperband, M. F., McElfresh, J. S., Millar, J. G., & Cardé, R. T. (2008). Attraction of female *Culex quinquefasciatus* Say (Diptera: Culicidae) to odors from chicken feces. *Journal of Insect Physiology, 54*(7), 1184-1192.

Cornet, S., Nicot, A., Rivero, A., & Gandon, S. (2013a). Malaria infection increases bird attractiveness to uninfected mosquitoes. *Ecology Letters, 16*(3), 323-329. doi:10.1111/ele.12041

Cornet, S., Nicot, A., Rivero, A., & Gandon, S. (2013b). Both infected and uninfected mosquitoes are attracted toward malaria infected birds. *Malaria Journal, 12*, 179. doi:10.1186/1475-2875-12-179

Cresswell, W. (1994). Flocking is an effective anti-predation strategy in redshanks, *Tringa totanus*. *Animal Behaviour, 47*(2), 433-442.

Darbro, J. M., & Harrington, L. C. (2007). Avian defensive behavior and blood-feeding success of the West Nile vector mosquito, *Culex pipiens*. *Behavioral Ecology, 18*(4), 750-757. doi:10.1093/beheco/arm043

Daszak, P., Cunningham, A. A., & Hyatt, A. D. (2000). Emerging infectious diseases of wildlife--threats to biodiversity and human health. *Science, 287*(5452), 443.

Day, J. F. (2005). Host-seeking strategies of mosquito disease vectors. *Journal of the American Mosquito Control Association, 21*(sp1), 17-22.

Day, J. F., & Edman, J. D. (1983). Malaria renders mice susceptible to mosquito feeding when gametocytes are most infective. *The Journal of parasitology*, 163-170.

De Moraes, C. M., Stanczyk, N. M., Betz, H. S., Pulido, H., Sim, D. G., Read, A. F., & Mescher, M. C. (2014). Malaria-induced changes in host odors enhance mosquito attraction. *Proc Natl Acad Sci U S A, 111*(30), 11079-11084. doi:10.1073/pnas.1405617111

Dobson, A., & Carper, R. (1992). Global warming and potential changes in host-parasite and disease-vector relationships. In R. L. Peters & T. E. Lovejoy (Eds.), *Global warming and biodiversity* (pp. 201-220). New Haven, CT: Yale University Press.

Eigenbrode, S. D., Ding, H., Shiel, P., & Berger, P. H. (2002). Volatiles from potato plants infected with potato leafroll virus attract and arrest the virus vector, *Myzus persicae* (Homoptera: Aphididae). *Proceedings of the Royal Society of London B: Biological Sciences, 269*(1490), 455-460.

Ellegren, H. (1996). First gene on the avian W chromosome (CHD) provides a tag for universal sexing of non-ratite birds. *Proceedings of the Royal Society of London B: Biological Sciences, 263*(1377), 1635-1641.

Emami, S. N., Lindberg, B. G., Hua, S., Hill, S. R., Mozuraitis, R., Lehmann, P., Birgersson, G., Borg-Karlson, A. K., Ignell, R., & Faye, I. (2017). A key malaria metabolite modulates vector blood seeking, feeding, and susceptibility to infection. *Science, 355*(6329), 1076-1080.

Estep, L. K., McClure, C. J., Burkett-Cadena, N. D., Hassan, H. K., Unnasch, T. R., & Hill, G. E. (2012). Developing models for the forage ratios of *Culiseta melanura* and *Culex erraticus* using species characteristics for avian hosts. *Journal of Medical Entomology, 49*(2), 378-387.

Fallon, S. M., Bermingham, E., & Ricklefs, R. E. (2005). Host specialization and geographic localization of avian malaria parasites: a regional analysis in the Lesser Antilles. *The American Naturalist, 165*(4), 466-480.

Fallon, S. M., Bermingham, E., Ricklefs, R. E., & Poulin, R. (2003). Island and taxon effects in parasitism revisited: avian malaria in the Lesser Antilles. *Evolution, 57*(3), 606-615.

Farajollahi, A., Fonseca, D. M., Kramer, L. D., & Kilpatrick, A. M. (2011). "Bird biting" mosquitoes and human disease: a review of the role of *Culex pipiens* complex mosquitoes in epidemiology. *Infections, Genetics and Evolution, 11*(7), 1577-1585. doi:10.1016/j.meegid.2011.08.013

Ferraguti, M. (2017). *Biodiversity and vector-borne diseases: effects of landscape, mosquito and vetebrate communities on the transmission of West Nile virus and avian malaria parasites.* (PhD Thesis), Universidad de Pablo de Olavide, Seville, Spain.

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*(6), e66237. doi: 10.1371/journal.pone.0066237

Ferraguti, M., Martínez-de La Puente, J., Roiz, D., Ruiz, S., Soriguer, R., & Figuerola, J. (2016). Effects of landscape anthropization on mosquito community composition and abundance. *Scientific Reports, 6*, 29002.

Gage, K. L., Burkot, T. R., Eisen, R. J., & Hayes, E. B. (2008). Climate and vectorborne diseases. *American Journal of Preventive Medicine, 35*(5), 436-450.

García-Longoria, L., Magallanes, S., González-Blázquez, M., Refollo, Y., de Lope, F., & Marzal, A. (2016). New approaches for an old disease: studies on avian malaria parasites for the twenty-first century challenges. In *Current Topics in Malaria*: InTech.

Garnier, S., Durand, P., Arnathau, C., Risterucci, A. M., Esparza-Salas, R., Cellier-Holzem, E., & Sorci, G. (2009). New polymorphic microsatellite loci in the house sparrow, *Passer domesticus*. *Molecular Ecology Resources, 9*(3), 1063-1065. doi:10.1111/j.1755-0998.2009.02552.x

Gillies, M. (1980). The role of carbon dioxide in host-finding by mosquitoes (Diptera: Culicidae): a review. *Bulletin of Entomological Research, 70*(04), 525-532.

Githeko, A. K., Lindsay, S. W., Confalonieri, U. E., & Patz, J. A. (2000). Climate change and vector-borne diseases: a regional analysis. *Bulletin of The World Health Organization, 78*(9), 1136-1147.

Grenfell, B. T., & Dobson, A. P. (1995). *Ecology of infectious diseases in natural populations* (Vol. 7): Cambridge University Press.

Griffiths, R., Double, M. C., Orr, K., & Dawson, R. J. G. (1998). A DNA test to sex most birds. *Molecular Ecology, 7*(8), 1071-1075. doi:10.1046/j.1365-294x.1998.00389.x

Grilo, M., Vanstreels, R., Wallace, R., García-Párraga, D., Braga, É., Chitty, J., Catão-Dias, J. L., & Madeira de Carvalho, L. M. (2016). Malaria in penguins–current perceptions. *Avian Pathology, 45*(4), 393-407.

Grueber, C., Nakagawa, S., Laws, R., & Jamieson, I. (2011). Multimodel inference in ecology and evolution: challenges and solutions. *Journal of Evolutionary Biology, 24*(4), 699-711.

Gubler, D. (2009). Vector-borne diseases. *Revue scientifique et technique, 28*(2), 583.

Gutiérrez-López, R., Martínez-de la Puente, J., Gangoso, L., Soriguer, R. C., & Figuerola, J. (2015). Comparison of manual and semi-automatic DNA extraction protocols for the barcoding characterization of hematophagous louse flies (Diptera: Hippoboscidae). *Journal of Vector Ecology, 40*(1), 11-15.

Hamer, G. L., Kitron, U. D., Brawn, J. D., Loss, S. R., Ruiz, M. O., Goldberg, T. L., & Walker, E. D. (2008). *Culex pipiens* (Diptera: Culicidae): a bridge vector of West Nile virus to humans. *Journal of Medical Entomology, 45*(1), 125-128.

Hamer, G. L., Kitron, U. D., Goldberg, T. L., Brawn, J. D., Loss, S. R., Ruiz, M. O., Hayes, D. B., & Walker, E. D. (2009). Host Selection by *Culex pipiens* Mosquitoes and

West Nile Virus Amplification. *American Journal of Tropical Medicine and Hygiene, 80*(2), 268-278.

Harrus, S., & Baneth, G. (2005). Drivers for the emergence and re-emergence of vector-borne protozoal and bacterial diseases. *International Journal for Parasitology, 35*(11), 1309-1318.

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. *Parasites & Vectors, 9*(1), 1.

Hayes, E. B., Komar, N., Nasci, R. S., Montgomery, S. P., O'Leary, D. R., & Campbell, G. L. (2005). Epidemiology and transmission dynamics of West Nile virus disease. *Emerging Infectious Diseases, 11*(8).

Hellgren, O., Waldenström, J., & Bensch, S. (2004). A new PCR assay for simultaneous studies of *Leucocytozoon*, *Plasmodium*, and *Haemoproteus* from avian blood. *Journal of Parasitology, 90*(4), 797-802.

Hill, R. W. (1972). Determination of oxygen consumption by use of the paramagnetic oxygen analyzer. *Journal of Applied Physiology, 33*(2), 261-263.

Hunter, P. (2003). Climate change and waterborne and vector-borne disease. *Journal of Applied Microbiology, 94*(s1), 37-46.

Hurd, H. (2003). Manipulation of medically important insect vectors by their parasites. *Annual Review of Entomology, 48*(1), 141-161.

Janousek, W. M., Marra, P. P., & Kilpatrick, A. M. (2014). Avian roosting behavior influences vector-host interactions for West Nile virus hosts. *Parasites & Vectors, 7*. doi:10.1186/1756-3305-7-399

Johnson, P. T., De Roode, J. C., & Fenton, A. (2015). Why infectious disease research needs community ecology. *Science, 349*(6252), 1259504. doi:10.1126/science.1259504

Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., Gittleman, J. L., & Daszak, P. (2008). Global trends in emerging infectious diseases. *Nature, 451*(7181), 990-993.

Keesing, F., Belden, L. K., Daszak, P., Dobson, A., Harvell, C. D., Holt, R. D., Hudson, P., Jolles, A., Jones, K. E., & Mitchell, C. E. (2010). Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature, 468*(7324), 647-652.

Kilpatrick, A. M., Kramer, L. D., Jones, M. J., Marra, P. P., & Daszak, P. (2006a). West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. *PLoS Biology, 4*(4), e82.

Kilpatrick, A. M., & Randolph, S. E. (2012). Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. *The Lancet, 380*(9857), 1946-1955.

Kilpatrick, A. M., Daszak, P., Jones, M. J., Marra, P. P., & Kramer, L. D. (2006b). Host heterogeneity dominates West Nile virus transmission. *Proceedings of the Royal Society of London B: Biological Sciences,* 273(1599), 2327-2333. doi:10.1098/rspb.2006.3575.

Kim, K., & Tsuda, Y. (2010). Seasonal changes in the feeding pattern of *Culex pipiens pallens* govern the transmission dynamics of multiple lineages of avian malaria parasites in Japanese wild bird community. *Molecular Ecology, 19*(24), 5545-5554.

Kingsolver, J. G. (1987). Mosquito host choice and the epidemiology of malaria. *The American Naturalist, 130*(6), 811-827.

Kline, D., Allan, S., Bernier, U., & Welch, C. (2007). Evaluation of the enantiomers of 1-octen-3-ol and 1-octyn-3-ol as attractants for mosquitoes associated with a freshwater swamp in Florida, USA. *Medical and Veterinary Entomology, 21*(4), 323-331.

Knowles, S., Palinauskas, V., & Sheldon, B. (2010). Chronic malaria infections increase family inequalities and reduce parental fitness: experimental evidence from a wild bird population. *Journal of Evolutionary Biology, 23*(3), 557-569.

Komar, N., Panella, N. A., Burns, J. E., Dusza, S. W., Mascarenhas, T. M., & Talbot, T. O. (2001). Serologic evidence for West Nile virus infection in birds in the New York City vicinity during an outbreak in 1999. *Emerging Infectious Diseases, 7*(4), 621.

Krebs, B. L., Anderson, T. K., Goldberg, T. L., Hamer, G. L., Kitron, U. D., Newman, C. M., Ruiz, M. O., Walker, E. D., & Brawn, J. D. (2014). Host group formation decreases exposure to vector-borne disease: a field experiment in a 'hotspot'of West Nile virus transmission. *Proceedings of the Royal Society of London B: Biological Sciences, 281*(1796), 20141586.

Lachish, S., Knowles, S. C., Alves, R., Wood, M. J., & Sheldon, B. C. (2011). Infection dynamics of endemic malaria in a wild bird population: parasite species-dependent drivers of spatial and temporal variation in transmission rates. *Journal of Animal Ecology, 80*(6), 1207-1216.

Lacroix, R., Mukabana, W. R., Gouagna, L. C., & Koella, J. C. (2005). Malaria infection increases attractiveness of humans to mosquitoes. *PLoS Biology, 3*(9), 1590-1593. doi:10.1371/journal.pbio.0030298

Lalubin, F., Bize, P., van Rooyen, J., Christe, P., & Glaizot, O. (2012). Potential evidence of parasite avoidance in an avian malarial vector. *Animal Behaviour, 84*(3), 539-545. doi:10.1016/j.anbehav.2012.06.004

Land, M., Gibson, G., & Horwood, J. (1997). Mosquito eye design: conical rhabdoms are matched to wide aperture lenses. *Proceedings of the Royal Society of London B: Biological Sciences, 264*(1385), 1183-1187.

Land, M., Gibson, G., Horwood, J., & Zeil, J. (1999). Fundamental differences in the optical structure of the eyes of nocturnal and diurnal mosquitoes. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology, 185*(1), 91-103.

LaPointe, D. A., Atkinson, C. T., & Samuel, M. D. (2012). Ecology and conservation biology of avian malaria. *Annals of the New York Academy of Sciences, 1249*(1), 211-226.

Latta, S. C., & Ricklefs, R. E. (2010). Prevalence patterns of avian haemosporida on Hispaniola. *Journal of Avian Biology, 41*(1), 25-33.

Lehane, M. J. (2005). *The biology of blood-sucking in insects* (Second ed.). New York: Cambridge University Press.

Lipnick, R. L. (1991). Narcosis induced by ether and chloroform. In R. L. Lipnick (Eds.), *Studies of Narcosis* (1 ed., pp. 93-107). Dordrecht, The Netherland: Springer.

Lips, K. R., Brem, F., Brenes, R., Reeve, J. D., Alford, R. A., Voyles, J., Carey, C., Livo, L., Pessier, A. P., & Collins, J. P. (2006). Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proceedings of the National Academy of Sciences of the United States of America, 103*(9), 3165-3170.

Little, S. E. (2014). Vector-Borne Diseases In D. D. Bowman (Eds.), *Georgis' Parasitology for Veterinarians-E-Book* (pp. 240-253): Elsevier Health Sciences.

Loiseau, C., Zoorob, R., Robert, A., Chastel, O., Julliard, R., & Sorci, G. (2011). *Plasmodium relictum* infection and MHC diversity in the house sparrow (*Passer domesticus*). *Proceedings of the Royal Society of London B: Biological Sciences, 278*(1709), 1264-1272.

Malmqvist, B., Strasevicius, D., Hellgren, O., Adler, P. H., & Bensch, S. (2004). Vertebrate host specificity of wild–caught blackflies revealed by mitochondrial DNA in blood. *Proceedings of the Royal Society London B: Bilogical Sciences, 271*(Suppl 4), S152-S155. doi:10.1098/rsbl.2003.0120

Martínez-de la Puente, J., Ferraguti, M., Ruiz, S., Roiz, D., Soriguer, R. C., & Figuerola, J. (2016). *Culex pipiens* forms and urbanization: effects on blood feeding sources and transmission of avian *Plasmodium*. *Malaria Journal, 15*(1), 589.

Martínez-de la Puente, J., Merino, S., Lobato, E., Rivero-de Aguilar, J., del Cerro, S., Ruiz-de-Castañeda, R., & Moreno, J. (2010a). Nest-climatic factors affect the abundance of biting flies and their effects on nestling condition. *Acta Oecologica, 36*(6), 543-547. doi:10.1016/j.actao.2010.07.008

Martínez-de la Puente, J., Merino, S., Tomás, G., Moreno, J., Morales, J., Lobato, E., García-Fraile, S., & Belda, E. J. (2010b). The blood parasite *Haemoproteus* reduces survival in a wild bird: a medication experiment. *Biology Letters*, rsbl20100046.

Martínez-de la Puente, J., Merino, S., Tomás, G., Moreno, J., Morales, J., Lobato, E., Talavera, S., & Sarto I Monteys, V. (2009). Factors affecting *Culicoides* species composition and abundance in avian nests. *Parasitology, 136*(09), 1033-1041. doi:10.1017/S0031182009006374

Martínez-de la Puente, J., Muñoz, J., Capelli, G., Montarsi, F., Soriguer, R., Arnoldi, D., Rizzoli, A., & Figuerola, J. (2015). Avian malaria parasites in the last supper: identifying encounters between parasites and the invasive Asian mosquito tiger and native mosquito species in Italy. *Malaria Journal, 14*(1), 32.

Marzal, A., Bensch, S., Reviriego, M., Balbontin, J., & De Lope, F. (2008). Effects of malaria double infection in birds: one plus one is not two. *Journal of Evolutionary Biology, 21*(4), 979-987.

Marzal, A., De Lope, F., Navarro, C., & Møller, A. P. (2005). Malarial parasites decrease reproductive success: an experimental study in a passerine bird. *Oecologia, 142*(4), 541-545.

Marzal, A., Ricklefs, R. E., Valkiūnas, G., Albayrak, T., Arriero, E., Bonneaud, C., Czirják, G. A., Ewen, J., Hellgren, O., Hořáková, D., Iezhova, T. A., Jensen, H., Križanauskienė, A., Lima, M. R., de Lope, F., Magnussen, E., Martin, L. B., Møller, A. P., Palinauskas, V., Pap, P. L., Pérez-Tris, J., Sehgal, R. N. M., Soler, M., Szöllősi, E., Westerdahl, H., Zetindjiev, P., & Bensch, S. (2011). Diversity, Loss, and Gain of Malaria Parasites in a Globally Invasive Bird. *PLoS One, 6*(7), e21905. doi:10.1371/journal.pone.0021905

McFarlane, R. A., Sleigh, A. C., & McMichael, A. J. (2013). Land-use change and emerging infectious disease on an island continent. *International Journal of Environmental Research and Public Health, 10*(7), 2699-2719.

McLean, R. G., Ubico, S. R., Docherty, D. E., Hansen, W. R., Sileo, L., & McNamara, T. S. (2001). West Nile virus transmission and ecology in birds. *Annals of the New York Academy of Sciences, 951*(1), 54-57.

McNab, B. K. (1997). On the utility of uniformity in the definition of basal rate of metabolism. *Physiological Zoology, 70*(6), 718-720.

Merino, S., Moreno, J., Sanz, J. J., & Arriero, E. (2000). Are avian blood parasites pathogenic in the wild? A medication experiment in blue tits (*Parus caeruleus*). *Proceedings of the Royal Society of London B: Biological Sciences, 267*(1461), 2507-2510.

Merino, S., Tomás, G., Moreno, J., Sanz, J. J., Arriero, E., & Folgueira, C. (2004). Changes in *Haemoproteus* sex ratios: fertility insurance or differential sex lifespan? *Proceedings of the Royal Society of London B: Biological Sciences, 271*(1548), 1605-1609.

Muñoz, J., Eritja, R., Alcaide, M., Montalvo, T., Soriguer, R. C., & Figuerola, J. (2011). Host-feeding patterns of native *Culex pipiens* and invasive *Aedes albopictus* mosquitoes (Diptera: Culicidae) in urban zones from Barcelona, Spain. *Journal of Medical Entomology, 48*(4), 956-960. doi:10.1603/ME11016

Nemeth, N., Young, G., Ndaluka, C., Bielefeldt-Ohmann, H., Komar, N., & Bowen, R. (2009). Persistent West Nile virus infection in the house sparrow (*Passer domesticus*). *Archives of Virology, 154*(5), 783-789.

Nicholls, D. G., & Ferguson, S. (2013). *Bioenergetics* (4 ed.): Academic Press.

Norris, D. E. (2004). Mosquito-borne diseases as a consequence of land use change. *EcoHealth, 1*(1), 19-24.

Nuttall, P., Paesen, G., Lawrie, C., & Wang, H. (2000). Vector-host interactions in disease transmission. *Journal of Molecular Microbiology and Biotechnology, 2*(4), 381-386.

O'Shea, B., Rebollar-Tellez, E., Ward, R., Hamilton, J., El Naiem, D., & Polwart, A. (2002). Enhanced sandfly attraction to *Leishmania*-infected hosts. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 96*(2), 117-118.

Patz, J. A., Daszak, P., Tabor, G. M., Aguirre, A. A., Pearl, M., Epstein, J., Wolfe, N. D., Kilpatrick, A. M., Foufopoulos, J., & Molyneux, D. (2004). Unhealthy landscapes: policy recommendations on land use change and infectious disease emergence. *Environmental Health Perspectives, 112*(10), 1092.

Paull, S. H., Song, S., McClure, K. M., Sackett, L. C., Kilpatrick, A. M., & Johnson, P. T. (2012). From superspreaders to disease hotspots: linking transmission across hosts and space. *Frontiers in Ecology and the Environment, 10*(2), 75-82.

Pérez-Ramírez, E., Llorente, F., & Jiménez-Clavero, M. Á. (2014). Experimental infections of wild birds with West Nile virus. *Viruses, 6*(2), 752-781.

Port, G., Boreham, P., & Bryan, J. H. (1980). The relationship of host size to feeding by mosquitoes of the *Anopheles gambiae* Giles complex (Diptera: Culicidae). *Bulletin of Entomological Research, 70*(01), 133-144.

Poulin, R. (1995). "Adaptive" changes in the behaviour of parasitized animals: a critical review. *International Journal for Parasitology, 25*(12), 1371-1383.

Reinert, J. F. (2009). List of abbreviations for currently valid generic-level taxa in family Culicidae (Diptera). *European Mosquito Bulletin, 27*, 68-76.

Reisen, W., Fang, Y., & Martinez, V. (2005). Avian host and mosquito (Diptera: Culicidae) vector competence determine the efficiency of West Nile and St. Louis encephalitis virus transmission. *Journal of Medical Entomology, 42*(3), 367-375.

Reiter, P. (2001). Climate change and mosquito-borne disease. *Environmental Health Perspectives, 109*(Suppl 1), 141.

Ricklefs, R. E., Swanson, B. L., Fallon, S. M., Martínez-Abraín, A., Scheuerlein, A., Gray, J., & Latta, S. C. (2005). Community relationships of avian malaria parasites in southern Missouri. *Ecological Monographs, 75*(4), 543-559.

Rizzoli, A., Bolzoni, L., Chadwick, E. a., Capelli, G., Montarsi, F., & Grisenti, M. (2015). Understanding West Nile virus ecology in Europe: *Culex pipiens* host feeding preference in a hotspot of virus emergence. *Parasites & Vectors, 8*. doi:10.1186/s13071-015-0831-4.

Rodríguez, A., Broggi, J., Alcaide, M., Negro, J. J., & Figuerola, J. (2014). Determinants and short-term physiological consequences of PHA immune response in lesser kestrel nestlings. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology, 321*(7), 376-386.

Russell, C. B., & Hunter, F. F. (2005). Attraction of *Culex pipiens/restuans* (Diptera : Culicidae) mosquitoes to bird uropygial gland odors at two elevations in the Niagara Region of Ontario. *Journal of Medical Entomology, 42*(3), 301-305. doi:10.1603/0022-2585(2005)042[0301:aocrdc]2.0.co;2

Santiago-Alarcon, D., Palinauskas, V., & Schaefer, H. M. (2012). Diptera vectors of avian Haemosporidian parasites: untangling parasite life cycles and their taxonomy. *Biological Reviews, 87*(4), 928-964.

Schaffner, F., Angel, G., Geoffroy, B., Hervy, J., Rhaiem, A., & Brunhes, J. (2001). The mosquitoes of Europe, an identification and training programme. *Montpellier, France: IRD*.

Schoener, E., Uebleis, S. S., Butter, J., Nawratil, M., Cuk, C., Flechl, E., Kothmayer, M., Obwaller, A. G., Zechmeister, T., & Rubel, F. (2017). Avian *Plasmodium* in Eastern Austrian mosquitoes. *Malaria Journal, 16*(1), 389.

Shapiro, L., Moraes, C. M., Stephenson, A. G., & Mescher, M. C. (2012). Pathogen effects on vegetative and floral odours mediate vector attraction and host exposure in a complex pathosystem. *Ecology Letters, 15*(12), 1430-1438.

Simpson, J. E., Hurtado, P. J., Medlock, J., Molaei, G., Andreadis, T. G., Galvani, A. P., & Diuk-Wasser, M. A. (2012). Vector host-feeding preferences drive transmission of multi-host pathogens: West Nile virus as a model system. *Proceedings of the Royal Society of London B: Biological Sciences, 279*(1730), 925-933. doi:10.1098/rspb.2011.1282

Smallegange, R. C., & Takken, W. (2010). Host-seeking behaviour of mosquitoes: responses to olfactory stimuli in the laboratory. In W. Takken & B. G. J. Knols (Eds.), *Olfaction in vector-host interactions* (pp. 143-180). The Netherland: Wageningen Academic Publishers.

Stafford, C. A., Walker, G. P., & Ullman, D. E. (2011). Infection with a plant virus modifies vector feeding behavior. *Proceedings of the National Academy of Sciences, 108*(23), 9350-9355.

Summers-Smith, D., Christie, D. A., & Garcia, E. F. J. (2017). House Sparrow (*Passer domesticus*). In J. del Hoyo, A. Elliott, J. Sargatal, D. A. Christeie, & E. de Juana (Eds.), *Handbook of the Birds of the World Alive*. Barcelona: Lynx Editions.

Suom, C., Ginsberg, H. S., Bernick, A., Klein, C., Buckley, P., Salvatore, C., & LeBrun, R. A. (2010). Host-seeking activity and avian host preferences of mosquitoes associated with West Nile virus transmission in the northeastern USA. *Journal of Vector Ecology, 35*(1), 69-74.

Svensson, L. (2006). *Identification guide to European passerines*. Thetford, UK: British Trust for Ornithology.

Syed, Z., & Leal, W. S. (2009). Acute olfactory response of *Culex* mosquitoes to a human-and bird-derived attractant. *Proceedings of the National Academy of Sciences, 106*(44), 18803-18808.

Tabachnick, W. (2010). Challenges in predicting climate and environmental effects on vector-borne disease episystems in a changing world. *Journal of Experimental Biology, 213*(6), 946-954.

Takken, W., & Verhulst, N. O. (2013). Host preferences of blood-feeding mosquitoes. *Annual Review of Entomology, 58*, 433-453. doi: 10.1146/annurev-ento-120811-153618

Taylor, L. H., Latham, S. M., & Mark, E. (2001). Risk factors for human disease emergence. *Philosophical Transactions of the Royal Society of London B: Biological Sciences, 356*(1411), 983-989.

Thiemann, T. C., Wheeler, S. S., Barker, C. M., & Reisen, W. K. (2011). Mosquito host selection varies seasonally with host availability and mosquito density. *PLoS Neglected Tropical Diseases, 5*(12), e1452.

Tomás, G., Merino, S., Moreno, J., Morales, J., & Martínez-de la Puente, J. (2007). Impact of blood parasites on immunoglobulin level and parental effort: a medication field experiment on a wild passerine. *Functional Ecology, 21*(1), 125-133.

Tompkins, D., & Gleeson, D. (2006). Relationship between avian malaria distribution and an exotic invasive mosquito in New Zealand. *Journal of the Royal Society of New Zealand, 36*(2), 51-62.

Valkiūnas, G. (2005). *Avian malaria parasites and other haemosporidia*: CRC press.

Valkiūnas, G., Bensch, S., Iezhova, T., Križanauskienė, A., Hellgren, O., & Bolshakov, C. (2006). Nested cytochrome b PCR diagnostics underestimate mixed infections of avian blood Haemosporidian parasites: microscopy is still essential. *Journal of Parasitology, 92*, 418-422.

van Breugel, F., Riffell, J., Fairhall, A., & Dickinson, M. H. (2015). Mosquitoes Use Vision to Associate Odor Plumes with Thermal Targets. *Current Biology, 25*(16), 2123-2129. doi:10.1016/j.cub.2015.06.046

VanderWaal, K. L., & Ezenwa, V. O. (2016). Heterogeneity in pathogen transmission: mechanisms and methodology. *Functional Ecology, 30*(10), 1606-1622.

Wen, Y., Muir, L. E., & Kay, B. H. (1997). Response of *Culex quinquefasciatus* to visual stimuli. *Journal of the American Mosquito Control Association, 13*(2), 150-152.

Williams, G. C. (1966). Natural selection, the costs of reproduction, and a refinement of Lack's principle. *The American Naturalist, 100*(916), 687-690.

Wood, M. J., Cosgrove, C. L., Wilkin, T. A., Knowles, S. C., Day, K. P., & Sheldon, B. C. (2007). Within-population variation in prevalence and lineage distribution of avian malaria in blue tits, *Cyanistes caeruleus*. *Molecular Ecology, 16*(15), 3263-3273.

World Health Organization (2000). Lymphatic filariasis. WHO Fact Sheet No. 102.

World Health Organization (2009a). Dengue and dengue hemorrhagic fever. WHO Fact Sheet No. 117.

World Health Organization (2009b). Malaria. WHO Fact Sheet No. 94.

Yan, J., Gangoso, L., Martínez-de la Puente, J., Soriguer, R., & Figuerola, J. (2017a). Avian phenotypic traits related to feeding preferences in two *Culex* mosquitoes. *The Science of Nature, 104*(9-10), 76.

Yan, J., Martínez-de la Puente, J., Gangoso, L., Gutiérrez-López, R., Soriguer, R., & Figuerola, J. (2017b). Avian malaria infection intensity influences mosquito feeding patterns. *International Journal for Parasitology,* in press.

Yorinks, N., & Atkinson, C. T. (2000). Effects of malaria on activity budgets of experimentally infected juvenile Apapane (*Himatione sanguinea*). *The Auk, 117*(3), 731-738.

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List of publications

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I. **Yan, J**., Martínez-de la Puente, J., Gangoso, L., Ruiz, S., Soriguer, R., & Figuerola, J. Understanding blood-feeding preferences in mosquitoes: determinants and challenges. *Manuscript*.

II. **Yan, J**., Gangoso, L., Martínez-de la Puente, J., Soriguer, R., & Figuerola, J. (2017). Avian phenotypic traits related to feeding preferences in two *Culex* mosquitoes. *The Science of Nature*, 104(9-10), 76.

III. **Yan, J**., Broggi, J., Martínez-de la Puente, J., Gutiérrez-López, R., Gangoso, L., Soriguer, R., & Figuerola, J. Does bird metabolic rate affect mosquito feeding preference? *Parasites & Vectors*, under review.

IV. **Yan, J**., Martínez-de la Puente, J., Gangoso, L., Gutiérrez-López, R., Soriguer, R., & Figuerola, J. (2017). Avian malaria infection intensity influences mosquito feeding patterns. *International Journal for Parasitology*, in press.

Publications resulting from collaborative projects during PhD

V. Gutiérrez-López, R., Martínez-de la Puente, J., Gangoso, L., **Yan, J**., Soriguer, R. C., & Figuerola, J. (2016). Do mosquitoes transmit the avian malaria-like parasite *Haemoproteus*? An experimental test of vector competence using mosquito saliva. *Parasites & Vectors*, 9(1), 609.

VI. Gangoso, L., **Yan, J**., Martínez-de la Puente, J., Jiménez-Clavero, M. A., Soriguer, R., & Figuerola, J. What makes a host competent for WNV transmission? The role of avian phylogeny. *Manuscript*.

VII. Gutiérrez-López, R., Martínez-de la Puente, J., Gangoso, L., **Yan, J**., Soriguer, R. C., & Figuerola, J. Effects of infection intensity with *Plasmodium* on the transmission risk of avian malaria. *Manuscript*.