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No Evidence that Songbirds Use Odour Cues to Avoid Malaria-infected Conspecifics

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Short title: No avoidance of malaria-infected conspecifics

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Summary

Many animals have evolved mechanisms to detect and avoid parasitized conspecifics, primarily through odour cues, but whether birds are capable of odour-mediated parasite avoidance is unknown. Recently, we showed that exposing song sparrows (*Melospiza melodia*) to avian malaria parasites (*Plasmodium* sp.) alters the chemical composition of their preen oil, which is the major source of body odour in birds. Here, we presented song sparrows with preen oil from uninfected (sham-inoculated) and malaria-infected conspecifics, predicting that birds would spend more time with odour cues from uninfected than infected birds. Birds without detectable malarial infections spent about 50% more time with preen oil from uninfected than infected conspecifics, and females spent nearly twice as much time with preen oil from uninfected than infected conspecifics. However, neither difference was statistically significant. Song sparrows may be unable to detect odour cues of infection, but further experiments are needed to confirm or refute this.

Keywords

Avian malaria; chemical cues; malaria; odour cues; *Plasmodium*; preen oil; songbird; song sparrow

Introduction

Parasitic infections can alter host physiology, behaviour, morphology, odour, or other traits (Dobson, 1988; Penn & Potts, 1998; Moore, 2013). Such alterations can have important effects on disease transmission. For parasites that complete their entire lifecycle within a single host species, transmission between hosts can occur directly (by contact between infected and uninfected conspecifics, as in the case of ectoparasites; Kavaliers et al. 2003), or indirectly (moving from an infected host to the external environment to a new host, as in the case of fecal-oral transmission; Kavaliers et al. 1998; Poirotte et al. 2017).

In these single-host systems, individuals that avoid parasitized conspecifics should benefit from a reduced risk of contagion. Indeed, many animals have evolved mechanisms to detect and avoid parasitized conspecifics (Kavaliers et al., 1995, 2004, 2005a; Poirotte et al., 2017), largely through attending to odour cues of infection (Kavaliers et al., 2004; Olsson et al., 2014; Poirotte et al., 2017; Gordon et al., 2018).

Many parasites have more complex lifecycles involving multiple host species. Malaria parasites (*Plasmodium* spp.), for example, are vector-borne protozoa that require an invertebrate host (primarily Culicid mosquitoes; Atkinson & Van Riper, 1991) and a vertebrate host (notably mammals, birds, or reptiles;

Atkinson, 2008; Perkins & Schaer, 2016; Otero et al., 2019) to complete their lifecycle. The parasite's sexual reproduction occurs in the definitive host (mosquito), asexual reproduction occurs in both host types, and the parasites move between the two hosts during blood feeding (Cox, 2010).

Vector-borne parasites like *Plasmodium* are particularly interesting from the standpoint of alterations to host phenotype because there are multiple potential audiences. The implications for parasite transmission depend on whether the altered phenotype of an infected host is perceived by heterospecific or conspecific receivers. Parasites may manipulate host phenotype to enhance transmission to other host species (Prugnolle et al., 2009). *Plasmodium* parasites produce volatiles that attract mosquitoes when emitted by infected mammals (mice: De Moraes et al., 2014; humans: Kelly et al., 2015; Correa et al., 2017). In birds, *Plasmodium* infection may either increase (Cornet et al., 2013) or reduce (Lalubin et al., 2012) attractiveness to biting insects.

Parasite-mediated alterations in phenotype may also be perceived by conspecifics and used to avoid infected individuals. Although direct transmission is not a risk in multiple-host systems, proximity to infected conspecifics can still increase the likelihood of encountering infected insects (Aron & May, 1982), making it useful to avoid infected conspecifics. For example, rodents avoid

81 the odour of *Plasmodium*-infected conspecifics (Kavaliers, et al.,
82 2005b).
83 Most examples of parasitic infection altering host
84 phenotype and of conspecific or heterospecific responses to the
85 infected individual involve odour cues. In birds, the primary source
86 of body odour is preen oil, a waxy secretion of the uropygial gland
87 (Hagelin & Jones, 2007). In addition to its role in feather
88 maintenance and waterproofing, preen oil is an infochemical that
89 varies among species (Soini et al., 2013), between the sexes
90 (Grieves et al., 2019a), and across populations (Whittaker et al.,
91 2011; Grieves et al., 2019a). This variation is detectable to birds
92 and used in social contexts including species (Zhang et al., 2013;
93 Van Huynh & Rice, 2019) and kin recognition (Coffin et al.,
94 2011).
95 Recently, we found significant changes in the preen oil
96 chemical composition of song sparrows (*Melospiza melodia*)
97 exposed to *Plasmodium* parasites compared to unexposed birds
98 (Grieves et al., 2018). Among birds that were experimentally
99 inoculated with *Plasmodium*, the composition of preen oil changed
100 significantly from pre-infection to two weeks post-infection (the
101 timeframe of maximum parasitemia), regardless of whether
102 infections succeeded or were cleared by the birds. In contrast, no

significant changes to preen oil were seen over this timeframe in sham-inoculated birds (Grieves et al., 2018).

Because song sparrows appear to detect and respond to information available in preen oil (Grieves et al., 2019b,c), and because exposure to *Plasmodium* sp. alters preen oil composition (Grieves et al., 2018), we hypothesized that song sparrows would avoid odour cues from conspecifics infected with *Plasmodium*, consistent with findings in mammals (Kavaliers, et al., 2005b).

To test this hypothesis, we presented song sparrows with preen oil from conspecifics that had been either experimentally infected with *Plasmodium* or sham-inoculated with uninfected blood. These preen oil stimuli were collected from birds at peak-parasitemia (i.e., acutely infected birds), the time frame in which we detected significant shifts in the preen oil composition of *Plasmodium*-exposed birds (Grieves et al. 2018). Using a two-choice design, we monitored the time spent by song sparrows with each sample type (infected or sham-inoculated). Because some test subjects were naturally infected with haematozoan parasites at the time of capture and testing, we also compared responses of haematozoan-infected versus uninfected focal birds to odour cues of *Plasmodium*-infected versus sham-inoculated conspecifics.

Methods

Collection and preparation of preen oil samples

Preen oil samples were collected as part of a prior study investigating the effects of *Plasmodium* infection on preen oil chemical composition (see Grieves et al., 2018 for full details of experimental infections, preen oil collection, and sample processing). In brief, Grieves et al. (2018) collected preen oil from adult song sparrows captured in London, Ontario, Canada (42.98°N, 81.25°W) during July and August 2016 and kept on an ambient photoperiod during September and October. Sparrows were assigned to the experimental or sham-inoculation group in a block-randomized fashion, balancing groups by previous infection status and sex as best as possible. Birds in the experimental group were inoculated with whole blood from song sparrows infected with *Plasmodium* (99% sequence identity to lineage P-SOSP2, GenBank accession no. KT193628); birds in the sham-inoculation group were inoculated with whole blood from uninfected song sparrows.

The present study used preen oil from 8 successfully infected birds (5 males, 3 females) and 9 sham-inoculated birds (7 males, 2 females) in the study described above. Samples were collected thirteen days after inoculation, near the timing of maximum expected parasitemia (Sarquis-Adamson &

MacDougall-Shackleton, 2016). This occurred in September 2016, when birds were no longer in breeding condition. As detailed elsewhere (Grieves et al., 2018), preen oil was expressed from the uropygial gland and stored at -20°C for 2 months. Samples were then thawed, dissolved in 1 – 3 mL of stable organic solvent (pure chloroform, $CHCl_3$), then analyzed using GC-FID. Grieves et al. (2018) tested for differences in the *Plasmodium*-infected and sham-inoculated groups using permutational multivariate analysis of variance.

After analysis, samples dissolved in $CHCl_3$ were stored at 4°C for 15 months. To prepare these preen oil samples for the present study, we carefully allowed them to just dry by loosening the caps under a fume hood at room temperature, checking frequently to re-cap the samples once dry. We then re-dissolved each sample in 250 µL of $CHCl_3$. This method ensured that preen oil samples would be presented to the focal birds (i.e., study subjects) at a comparable concentration to that used in other two-choice odour studies using a similar experimental design (Whittaker et al. 2011; Grieves et al., 2019b,c).

Preen oil samples did not significantly differ between males and females ($F = 1.35$, $p = 0.23$; reported in Grieves et al., 2018); likely because the samples were collected from nonbreeding birds (Grieves et al., 2019a). However, *Plasmodium*-infected individuals

varied in their parasite load (parasites per 10 000 cells examined: range = 4 – 1471). We therefore pooled samples within each treatment group to create two cocktails: one from the 9 *Plasmodium* sp. infected birds and one from the 11 sham-inoculated birds. Average (± SE) parasite loads of birds contributing to the infected and sham-inoculated cocktails were 170.7 ± 162.6, and 0.6 ± 0.3, respectively.

Chemical analysis of preen oil

To ensure pooled samples were not quantitatively different from the original (i.e., individual) samples, we analyzed the pooled samples using GC-FID following Grieves et al. (2018). We conducted chemical analysis on an Agilent 7890A gas chromatograph with flame ionization detector (GC-FID) fitted with a 5% phenyl methyl siloxane column (Agilent Technologies DB-5, 30 m × 0.32 µm ID × 0.25 µm film thickness) using the following program: we injected 1 µL of each sample (N = 2) with a 30 psi pressure pulse (1 min) and, after an initial 1 min hold at 70ºC, eluted with the following temperature profile: increase to 130ºC at 20ºC/min, then to 320ºC at 4ºC/min. The injector and FID temperatures were 200ºC and 310ºC respectively. We used hydrogen as a carrier gas at 2.5 mL/min. The GC-FID run included a blank sample containing solvent only ($CHCl_3$) and a sample of

known composition (i.e., previously analyzed by both GC-FID and gas chromatography-mass spectrometry, GC-MS; Slade et al., 2016).

We quantified the relative size of each chromatogram peak identified by GC-FID (i.e., 8 individual samples from the *Plasmodium*-infected birds and 9 individual samples from the sham-inoculated birds along with the pooled *Plasmodium*-infected cocktail and the pooled sham-inoculated cocktail), retaining for analysis only peaks that comprised ≥ 0.1% of the total chromatogram area. To prevent large peaks from disproportionately influencing distance measures (Leclaire et al., 2014), we normalized the data in R (R Development Core Team, 2017) using the 'range' method in the 'decostand' function in the 'vegan' package (Dixon & Palmer, 2003). We log x + 1 transformed the normalized dataset then constructed pairwise matrices of Bray-Curtis dissimilarity, which we interpret as chemical distances between samples.

Study subjects and housing

Study subjects were 36 adult song sparrows (27 male, 9 female) captured by mist net in August and September 2017 in London, Ontario, Canada (42.98°N, 81.25°W). We determined sex by PCR amplification following Griffiths et al. (1998). We housed subjects

in individual cages in a single room, maintained at 20 ± 1°C, at the Advanced Facility for Avian Research. Birds had ad libitum access to water and food.

Until February 2018, the light schedule mimicked the natural photoperiod. On 22 February 2018, when the natural photoperiod at this latitude is approx. 11 h:13 h light:dark (11L:13D), we altered the photoperiod to 14L:10D to photostimulate the subjects and bring them into breeding condition (Wingfield, 1993). Birds were tested while in breeding condition because odour sensitivity appears greater in breeding than nonbreeding birds (Clark & Smeraski, 1990; de Groof et al., 2010). Birds were maintained on 14L:10D throughout the experiment. Males began singing on 13 February 2018 and continued to sing throughout the duration of trials (26 through 29 March 2018; thus, we considered it likely that all birds were in breeding condition at the time of this experiment.

Parasite screening of test subjects

We used PCR to screen study subjects for haemosporidian malarial parasites. We collected approx. 20 µL of blood via brachial venipuncture from each bird at the time of capture. We extracted DNA using a salt extraction protocol, then used a two-stage nested PCR approach to amplify parasite cytochrome *b* (Hellgren et al.

2004). We used the first-stage primers HAEMNFI and HAEMNR3 (Hellgren et al., 2004) to amplify an initial 617 bp fragment of cytochrome *b* from the haemosporidian genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*. Using 1 µL of first-stage product as template, we then performed two separate second-stage reactions: one used the internally nested primers HAEMF and HAEMR2 to amplify a 478 bp fragment of *Plasmodium* and *Haemoproteus* cytochrome *b*, and the other used primers HAEMFL and HAEMRL to amplify a 480 bp fragment of *Leucocytozoon* cytochrome *b*.

We conducted PCR reactions in a total volume of 10 µL and included 50 ng total genomic DNA as template (or 1 µL of first-stage PCR product for the second-stage PCR), 0.2 mM dNTPs, 2.0 mM $MgCl_2$, 1X Buffer, 0.6 mM of each primer and 0.5 units *Taq* DNA polymerase. Thermocycling conditions included an initial step of 94°C for 3 min; 20 cycles (first-stage) or 35 cycles (second-stage) of 94°C for 30 sec, 50°C for 30 sec and 72°C for 45 sec; and a final extension step of 72°C for 10 min.

We ran 5 µL of second-stage products on a 2% agarose gel including a water-only negative control and a positive control for each of the two second-stage primer sets. We inferred infection status from the presence (infected) versus absence (uninfected) of a band in the second stage reactions for each primer set. Eight of 36

263 birds (6 males, 2 females) were infected with *Plasmodium* and/or
264 *Haemoproteus* at the time of capture and no *Leucocytozoon*
265 infections were detected. To minimize stress to the birds, we
266 screened only once at the initial time of capture. While a lack of
267 replicate screening may have caused us to miss very low level
268 infections, this PCR method reliably detects low-level malarial
269 infections (Perkins et al., 1998; Richard et al., 2002).
270
271 *Behavioural trials of study subjects*
272 We conducted trials in a Plexiglas Y-maze using a design similar
273 to Whittaker et al. (2011); maze arms: 20 (H) × 40 (L) × 20 (W)
274 cm; central area: 20 (H) × 35 (L) × 20 (W) cm. We placed a perch
275 near the end of each arm and placed each odour stimulus (see
276 below) on a cotton ball taped into a dish at the end of each arm (8
277 cm from the perch). The maze contained a start chamber (20 (H)×
278 14 (L) × 20 (W) cm) separated by an opaque Plexiglas barrier that
279 was slid open and closed to release the bird into the maze. We
280 made the side walls opaque by taping brown Kraft paper to the
281 outside of the maze and placed a wire screen on top of the maze so
282 that birds could detect the ceiling. We used a vacuum pump
283 (Neptune DynaPump, Thermoscientific) to circulate air from the
284 odour stimulus (dissolved preen oil applied to clean cotton balls)
285 down the arms of the maze while preventing mixing in the central

area. This was achieved by connecting equal lengths of air tubing near the base of each arm (5.5 (H) × 9 cm from the central area) to the vacuum pump. Because the vacuum pump produced noise, we habituated subjects to the sound by running the pump in their holding room for 1 hr/d from 22 February 2018 to 1 March 2018. All birds used in this study had also participated in four additional odour preference trials during the previous three weeks (Grieves et al. 2019b,c), and were thus familiar with the testing apparatus.

The maze was placed in an observation room such that each side of the maze was equidistant from the wall and the maze was positioned evenly between two overhead lights. All trials were video recorded with an Activeon CX high-definition camera.

At the start of each testing day, we removed preen oil stimuli from 4°C storage and warmed them to room temperature for approx. 5 min. We conducted trials from 08:00–11:30 am daily. From 2 – 5 min before each trial began, we used a Hamilton syringe to apply 50 µL of odour stimulus onto a fresh cotton ball affixed to each arm of the maze. The syringe was rinsed three times with $CHCl_3$ after each stimulus application. We used a random number generator to determine the order in which birds would be tested. We flipped a coin to assign stimulus type to maze arm for the first trial, then alternated stimulus locations for each

subsequent trial. The Y-maze was cleaned with 70% ethanol and air dried between each trial.

Trials lasted 20 min in total and began with the focal bird being placed into a start chamber separated from the rest of the maze by a slidable opaque barrier for a 5 min ‘acclimation period’. Then, the barrier was opened and closed immediately after the bird entered the maze. Most birds entered as soon as the barrier was opened, and all birds entered within a few seconds. The next 5 min constituted the ‘exploration period’. For trials to be considered successful, the focal bird was required to enter both maze arms or to enter one arm and also orient towards the other arm (defined as standing within one body width of the arm with bill oriented toward that arm for at least 10 sec) during the exploration period. The final 10 min were considered the ‘choice period’. In the case of unsuccessful trials (9 birds were re-trialed) we tested the focal bird 24 – 48 h later up to a maximum of two trial attempts. Most birds investigated the maze during the exploration period prior to the start of the trial, such that 75% (27/36) of trials were ultimately successful. For successful trials, we scored the time within the 10 min ‘choice period’ that the focal bird spent in or orienting towards each arm of the maze. Trials were scored blind with respect to bird and stimulus identity.

Statistical analysis

All analyses were performed in R version 3.2.3 (R Development Core Team, 2017). To confirm that pooled preen oil samples were not quantitatively different from the original (i.e., individual) samples, we conducted permutational multivariate analysis of variance (PERMANOVA) with 10 000 permutations using the 'adonis' command in the 'vegan' package (Dixon & Palmer, 2003) on the pairwise Bray-Curtis dissimilarity matrices. This permutation-based approach, analogous to a nonparametric MANOVA, does not make assumptions about the data's distribution and may be less sensitive to group differences in the dispersion of points than other methods (Anderson, 2001; Anderson & Walsh, 2013).

To test for differences in time spent by study subjects with each stimulus (odour) type, we fit a restricted maximum likelihood (REML) linear mixed model using 'lme4' (Bates et al., 2015). Fixed effects included sample type (preen oil from sham-inoculated versus *Plasmodium*-infected birds), sex of the focal bird, malarial infection status of the focal bird at time of capture, and the two-way interactions sample type × sex, and sample type × infection status at time of capture. Focal bird ID was included as a random factor and the dependent variable was time spent in or approaching a maze arm. Visual assessments of qq-plots and residuals

confirmed that data and residuals were distributed approximately normally and the residuals showed no evidence of homoscedasticity. *P*-values were obtained using Wald tests (using the 'Anova' function in the 'car' package).

Because birds had participated in four prior experiments using the same design but different stimuli, we used binomial tests to assess whether individuals had developed any apparent preferences for either maze arm.

Ethical approval

All applicable guidelines for the care and use of animals were followed in accordance with the ethical standards of the Canadian Council on Animal Care guidelines and The University of Western Ontario Animal Use Subcommittee (protocol number 2016-017). All birds were captured under permission from the Canadian Wildlife Service and Environment and Climate Change Canada (Scientific Collection Permit CA 0244; banding subpermit 10691F).

Results

We first confirmed that the pooled preen oil samples (i.e., test stimuli) were not quantitatively different from the original (i.e., individual) samples. We found no significant differences in preen oil chemical composition between our individual sham-inoculated

samples and the pooled sham cocktail (PERMANOVA: $F_{1, 11} = 0.64$, $R = 0.06$, $p = 0.667$) nor between the individual *Plasmodium*-infected samples and the pooled infected cocktail (PERMANOVA: $F_{1, 9} = 2.22$, $R = 0.22$, $p = 0.199$).

There was no significant difference in the amount of time song sparrows spent with preen oil from *Plasmodium*-infected versus uninfected birds (Figure 1a). However, females spent nearly twice as much time with preen oil from uninfected than infected conspecifics (Figure 1b) and birds without detectable malarial infections spent about 50% more time with preen oil from uninfected than infected conspecifics (Figure 1c). We found no significant main effect of sample type (*Plasmodium*-infected versus uninfected), focal bird sex, or focal bird infection status at time of capture on time spent with particular samples, nor were there any significant interactions (Table 1).

(Figure 1 here)

(Table 1 here)

Across all trials, 17.1% (6/35) of song sparrows spent more time in one maze arm than the other significantly more often than expected by chance (binomial test: $p < 0.05$; mean = 4.3 trials per

bird; Supplemental Materials Table S1), possibly indicating a side bias in these individuals.

Discussion

We tested whether song sparrows would avoid the preen oil odour of *Plasmodium*-infected conspecifics. Overall, song sparrows did not appear to discriminate preen oil from *Plasmodium*-infected versus uninfected (sham-inoculated) birds.

While not statistically significant, female song sparrows spent nearly twice as much time with preen oil from uninfected than infected conspecifics (Figure 1b), and we do not dismiss the possibility that a larger sample size might have revealed a preference for uninfected odour. In blue tits (*Cyanistes caeruleus*), the probability of extra-pair paternity is higher when uninfected females are mated to haematozoan-infected males (Podmokła et al., 2015), suggesting that females discriminate between infected and uninfected males.

Malarial parasite infection status of the focal bird was not significantly related to the amount of time birds spent with preen oil from infected versus uninfected conspecifics, although birds that were uninfected at time of capture spent about 50% more time with preen oil from uninfected than infected conspecifics (Figure 1c), suggesting that the lack of significant difference may reflect

low statistical power. Interestingly, studies on fish and mammals have shown that avoidance of parasitized conspecifics can be diminished or abolished when test subjects are themselves infected (Poulin 1994; Poulin & Vickery, 1996; Kavaliers et al. 1998).

Rates of disease transmission are expected to decrease if conspecifics avoid selecting infected individuals as mates or social partners (Kavaliers et al., 2003, 2005a). Therefore, we expected both sexes to avoid the odour of parasitized conspecifics. Recently, we found that male and female song sparrows spend more time with preen oil odour of opposite sex conspecifics (Grieves et al. 2019b) and with preen oil odour of MHC-dissimilar and MHC-diverse potential mates (Grieves et al., 2019c), indicating that both sexes can use preen oil odour cues. While we can only speculate as to why we did not detect evidence of odour-based discrimination of preen oil from *Plasmodium*-infected versus uninfected birds, we propose several potential explanations.

Although focal birds were in breeding condition, odour stimuli were collected from nonbreeding birds. Such stimuli may be nonstimulating to breeding condition birds, especially given that preen oil composition differs between breeding and nonbreeding song sparrows (Grieves et al., 2019a) and other species (Bhattacharyya & Chowdhury, 1995; Fischer et al., 2017). We used samples collected from nonbreeding birds to reduce the

likelihood that preen oil cues of sex or genotype (known to be salient to song sparrows; Grieves et al., 2019b,c) might influence focal birds' responses to the pooled preen oil stimuli. Thus we interpret responses as primarily reflecting social rather than mating preferences.

Preferences for uninfected individuals may be more pronounced in the context of mate choice, a hypothesis that could be tested by presenting breeding condition subjects with odour from breeding condition individuals. Similarly, in contrast to other odour preference experiments in this species (Grieves et al. 2019b,c) we presented focal birds with pooled preen oil from multiple individuals, rather than from a single individual. We pooled stimuli to eliminate variation in parasite loads of stimulus birds, and although we could detect no difference in the chemistry of pooled relative to individual samples, focal birds may have reacted differently to the pooled stimuli.

Our samples were collected during acute-stage infection (Sarquis-Adamson & MacDougall-Shackleton, 2016; Grieves et al., 2018). Mosquitoes (*Culex pipiens*) are more attracted to chronically-infected than acutely-infected (i.e., at peak parasitemia as in this study) or uninfected birds (Cornet et al., 2013), and gametocytes (capable of infecting mosquitoes) are produced and enter red blood cells of the vertebrate host during the chronic, not

the acute, phase of infection (Valkiunas, 2005; Rivero & Gandon, 2018). Although a prior study conducted on the same samples used here as test stimuli revealed significant changes in the preen oil chemical profiles of acutely-infected song sparrows (Grieves et al., 2018), it is possible that chronic-stage infection is more biologically relevant to both hosts and vectors, as this is the time during which the disease can be spread. Thus, it may be that preen oil from acutely-infected birds is nonstimulating to conspecifics.

Birds may discriminate among infected and uninfected conspecifics using odour cues that are not derived from preen oil, such as feather odour, which may chemically differ from preen oil (Sandilands et al., 2004; Zhang et al, 2013). It is also possible that whole body odour is the main odour source used to discriminate between infected and uninfected conspecifics, as has been found for mosquito vectors seeking avian hosts (Lalubin et al., 2012; Cornet et al., 2013; Díez-Fernández et al., 2020).

Finally, we cannot exclude the possibility that birds can detect cues of *Plasmodium* infection, but do not behaviourally discriminate between infected and uninfected conspecifics. Because *Plasmodium* parasites are not transmitted directly from bird to bird or by environmental contamination, the risks of proximity to infected conspecifics may be low. More work is needed to determine the extent to which vectors use chemical cues

of infection status in birds, identify the specific chemical cues that are present, determine whether they are universal across host and vector species, and confirm whether or not avian and other hosts can detect and use these cues.

Alternatively, birds may be unable to detect cues of infection status. Vectors such as mosquitoes may be the sole audience of infection-related shifts in preen oil chemical composition (Robinson et al., 2018). For example, chronically *Plasmodium*-infected domestic canaries (*Serinus canaria*) attract significantly more mosquito (*C. pipiens*) vectors than uninfected and acutely infected birds (Cornet et al., 2013). However, Lalubin et al. (2012) found the opposite: *Plasmodium*-infected great tits (*Parus major*) attracted significantly fewer *C. pipiens* than did uninfected birds; however, chronically and acutely infected birds were not differentiated in this study. *C. pipiens* are generally attracted to preen oil secretions (Russell & Hunter, 2005), but the reasons for this are as yet unknown. Future investigations into exactly how the chemical profile of preen oil differs among uninfected, acutely infected, and chronically infected birds—and the identification of specific compounds that attract mosquitoes—should be a productive area for future research.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figure caption

Figure 1. Time spent by 27 song sparrows with preen oil from uninfected (filled circle) and *Plasmodium*-infected (open circle) conspecifics in two-choice experiments. Values reported are mean ± SE. Filled and open circles connected by black lines are mean ± SE, values in gray show paired data for each individual. A: All focal individuals, B: male and female focal individuals, C: uninfected and haematozoan-infected individuals (based on PCR screening at time of capture).