

Attraction of *Culex pipiens* to uropygial gland secretions does not explain feeding preference for American robins

Mary C. Garvin¹✉, Amy L. Austin^{1,2}, Norberth H. Stracker¹, Samuel P. Slowinski¹, Jordan E. Rutter¹, Maxwell Butler¹, Megan Michel¹, and Rebecca J. Whelan²

¹Department of Biology, Oberlin College, Oberlin OH 44074, U.S.A., Mary.Garvin@oberlin.edu

²Department of Chemistry and Biochemistry, Oberlin College, Oberlin OH 44074, U.S.A.

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ABSTRACT: *Culex pipiens*, the endemic mosquito vector of West Nile virus in eastern North America, is responsible for maintenance of the virus in avian reservoir hosts, the most important of which appears to be the American robin. One reason for the greater involvement of robins is believed to be the feeding preference of *Cx. pipiens*, however, the basis of this preference is not understood. We tested the hypothesis that the species-specific chemical profile of avian uropygial gland secretions are used by *Cx. pipiens* as cues to locate birds and, therefore, may contribute to the observed feeding preferences. We used gas chromatography-mass spectrometry to identify the semi-volatile components of the uropygial gland secretions of American robins and two other common reservoir host species, the house sparrow and European starling. We found that the chemical composition of the robin secretions was different from those of the sparrows and starlings. Through behavioral choice trials conducted in a dual-port olfactometer, we also found that *Cx. pipiens* did not prefer the secretions of robins over the other two species. Surprisingly, however, we found that *Cx. pipiens* were more often attracted to live starlings over robins and to the secretions of starlings over those of robins. **Journal of Vector Ecology 43 (1): 110-116. 2018.**

Keyword Index: Birds, uropygial gland, West Nile virus, *Culex*, reservoir host.

INTRODUCTION

Transmission of West Nile virus (WNV), first detected in North America in 1999 (Anderson et al. 2001), involves two distinct phases. In the enzootic phase, mosquitoes transmit the virus among avian reservoir hosts, and in the epizootic phase a mosquito bridge vector transmits the virus from birds to humans and other large mammals. The primary enzootic vector in the eastern United States, *Culex pipiens*, maintains the virus in bird populations (Apperson et al. 2004). This species survives high titers of the virus (Turell et al. 2001), is abundant in many regions (Apperson et al. 2002), and feeds primarily on birds (Turell et al. 2001). Although American robins, European starlings, and house sparrows are all competent WNV hosts (Anderson et al. 2001), robins appear to be most important. In their models of WNV transmission, Simpson et al. (2012) found that host availability alone did not explain feeding preferences and suggested that behavioral and ecological factors, including olfactory cues, may contribute to the preference of *Cx. pipiens* for American robins over other species. They concluded that the feeding preference of *Cx. pipiens* is the most important factor in WNV transmission. Studies conducted on WNV reservoir hosts to date report preferences for robins over other passerine bird species, including house sparrows and starlings (Hamer et al. 2009, Apperson et al. 2002, Molaei et al. 2006, Simpson et al. 2009). The basis for these preferences is not known.

Although mosquitoes clearly use respired CO₂ to locate their hosts, it alone cannot explain the observed preferences (Scott et al. 1990, Costantini et al. 1998, Mboera and Takken 1999); other chemical compounds are also likely to be important (Costantini et al. 1998, Zwiebel and Takken 2004). One source of volatile chemical compounds produced by birds that might

serve as olfactory cues for mosquitoes to locate blood meals is the uropygial gland. Located at the base of the tail, this gland is the source of a diverse mixture of organic compounds (Haribal et al. 2005) that birds spread over their feathers during preening. In addition to waterproofing and conditioning the feathers (Giraudeau et al. 2010), the secretions may also function in intraspecific and interspecific communication (see reviews by Rajchard 2007 and Hagelin and Jones 2007). Russell and Hunter (2005) reported a higher capture rate of *Cx. pipiens* in light traps baited with crow uropygial secretions than in secretion-free traps operated at an elevation of 5 m. Allan et al. (2006) evaluated attraction of *Culex spp.* to chicken odors and found that while extracted feather volatiles and uropygial diols were not attractive in isolation, whole feathers were attractive, thus suggesting that other feather volatiles, such as epidermal compounds, might be attractive. Bernier et al. (2008) further explored the attraction of *Culex spp.* to components of chicken uropygial secretions, and while they found that secretions were not attractive, they suggest that the microbial degradation of secretions may result in volatiles that attract mosquitoes.

Here, we use chemical analysis and bioassays to explore the role of uropygial secretions in the attraction of *Cx. pipiens* to American robins over two other common WNV avian reservoir hosts: house sparrow and European starling. We examine the hypothesis that the reported preference of *Cx. pipiens* for robins over house sparrows and starlings is influenced by the semi-volatile chemical compounds in the avian species-specific uropygial gland secretions. Based on this hypothesis, we tested the prediction that *Cx. pipiens* will be more attracted to the American robin uropygial secretions than to those of either the house sparrow or European starling. As the first step towards testing this prediction, we described and compared the semi-volatile chemical

profiles of three WNV avian reservoir host species, American robin (AMRO), house sparrow (HOSP), and European starling (EUST) using gas chromatography and mass spectrometry. We then performed choice trial bioassays in a dual port olfactometer using both whole uropygial secretions and live birds.

MATERIALS AND METHODS

Chemical analysis of uropygial gland secretions

The following chemicals were used, either directly or after methyl esterification, as gas chromatography-mass spectrometry (GC-MS) standards: hexadecanoic acid (Fisher Scientific, Pittsburgh, PA, U.S.A.); tetradecanoic acid (98+%), hexadecanol (99%) (Eastman Chemical, Kingsport, TN, U.S.A.); eicosanoic acid (99+%) (Sigma-Aldrich Chemical, St. Louis, MO, U.S.A.).

Protocols involving live birds were approved by Oberlin College IACUC # S09RBMG-5 and #S15RMG-2. For secretion analysis, birds were captured and sampled from May to August, 2010. Adult robins (N=13) were caught using mist nets at two field sites, Killbuck Marsh Wildlife Area (40°41'N, 81°58'W), in Wayne County, OH and at a residence approximately ten miles north of Oberlin, OH (41°17'N, 82°13'W). Adult house sparrows (N=20) and starlings (N=21) were caught using both mist nets and traps at a dairy farm approximately one mile south of Oberlin and on the Oberlin College campus. Sex was determined via brood patch, plumage, or identification of a sex-specific CHD gene using a polymerase chain reaction (PCR) and gel electrophoresis (Griffiths et al. 1998). For the latter protocol, approximately 0.05 ml of blood was collected via brachial venipuncture into a heparinized microhematocrit tube and one drop was placed in 300 µL of cell lysis solution. After the collection of uropygial gland secretions, birds were measured, weighed, aged according to Pyle (1997), and released at the site of capture.

Uropygial gland secretions were collected in 1-inch capillary tubes (ID=1.5mm, OD=1.8 mm; Kimble, Vineland, NJ, U.S.A.), which were stored in labeled and pre-weighed 1.8 ml chromatography vials that were baked at 110° C for at least 8 h prior to use. To avoid contamination, capillary tubes were held with metal forceps and placed directly over the gland opening. The base of the gland was gently massaged until the secretion had transferred into the capillary tube, which was immediately placed back in its gas chromatography vial. Vials were returned to the laboratory and re-weighed to determine secretion sample weight. Secretion samples from American robins were found to weigh 6 ± 2 mg (average $\pm$ standard deviation; N = 9); European starling secretion samples weighed 14 ± 4 mg (N = 22); secretions from house sparrows weighed 5 ± 2 mg (N = 31). Samples were stored at -80° C until they were analyzed.

Compounds from the uropygial samples were separated and identified using a Trace GC gas chromatograph combined with a Polaris Q quadrupole ion trap mass analyzer (Thermo Finnigan, Waltham, MA, U.S.A.). Prior to analysis, samples were dissolved to a concentration of 20 mg/ml in dichloromethane (DCM) containing 0.005% benzyl alcohol by volume as an internal standard. After being dissolved in DCM, samples were heated at 40° C for 30 min and transferred to a glass HPLC vial insert in a microcentrifuge tube, followed by centrifugation at 14,000×g for 2 min to pellet insoluble material. 1 µl of dissolved secretion was

injected manually into the chromatograph in splitless mode with a 1 ml/min flow of helium carrier gas. The gas chromatograph contained a nonpolar DB-5MS column (30 m length, 0.25 mm ID, 0.25 µm film thickness; Alltech, Deerfield, IL, U.S.A.). Initial column temperature was 50° C, followed immediately by a temperature ramp of 4° C/min to 90° C. A second ramp of 30° C/min then occurred to 280° C, after which a third and final ramp of 1° C/min to 320° C occurred. The final temperature of 320° C was typically held for 5 min, but extended to 20 min for some of the AMRO samples to enable elution of strongly retained material. Detection began after a 5 min solvent delay. Electron impact ionization (EI) detection was performed in full-scan, positive ion mode over a mass-to-charge ratio (m/z) range of 30 to 600. Solvent blanks were run each day to check for sample carry-over.

Chromatographic features were identified by examining their mass spectra, by post-analysis filtering of the total ion chromatogram, and by comparison to authentic standards. A methyl esterification reaction, modified from Sweeney et al. (2004), was performed on secretion samples of each species to further classify the esters. Briefly, a solution of sodium methoxide in methanol was prepared at a concentration of 15 mg/ml. A volume of 500 µl of this solution was added to the uropygial secretion and vortexed to dissolve. The reaction mixture was incubated at 40° C for 25 min, after which the reaction was quenched by adding 100 µl deionized water and vortexing. The resulting methyl esters and alcohols were extracted into GC-grade hexanes, and the hexane fraction was analyzed by GC-MS using the temperature program described above. The mass spectra of the methyl esters and alcohols produced by this reaction were examined for chain length and branching. Chain length was determined by the molecular ion, and branching was determined using reported m/z values from Haribal et al. (2005), Sweeney et al. (2004), and Jacob and Poltz (1974).

Additionally, a methyl esterification reaction adapted from Pugh and Schultz (2000) was performed on three carboxylic acids (tetradecanoic acid, hexadecanoic acid, and eicosanoic acid) to confirm the characteristic fragmentation pattern of straight-chain methyl esters. In this reaction, approximately 1 mg of each acid was dissolved in 4:1 methanol:toluene and treated with acetyl chloride to initiate esterification. Reaction proceeded at 80° C for 75 min, after which the reaction was quenched by the addition of cold aqueous sodium carbonate solution. The resulting methyl esters were extracted into toluene, 1 µl of which was analyzed by GC-MS.

All statistical analyses were performed using PASW Statistics 18 (2009). P-values of < 0.05 were considered significant.

Bioassays

Culex pipiens were acquired from the Ohio State University colony (established in 1993) and were reared in an environmental chamber on the Oberlin College campus under the conditions of 24° C, 70-80% humidity, and a 14:10 (L/D) photoperiod. Adults were provided with fresh water and 10% sugar solution *ad libitum* in 50 ml vials with cotton wicks. Assays were conducted with female mosquitoes between 7 and 14 days of age deprived from access to sugar meals for 24 h. All trials were run during the first 2 h period of the dark cycle. A red light was operated in the room to allow workers to conduct trials.

Choice trials were conducted in a dual port olfactometer (Figure 1) modified from Posey et al. (1998). Airflow into the plenum was maintained between 378 and 471 m³/h as measured by an anemometer (Model 00AW7S, Dwyer Instruments, Michigan City, IN). Thirty female mosquitoes were aspirated into the flight chamber and allowed to acclimate for 15 min prior to the start of the trial. Secretions were collected from birds during June and July, 2012. Birds used live in bioassays were collected during June and July, 2016; birds were captured, assayed, and released at site of capture on the same day. Birds or secretions were placed in the two stimulus ports. Each test comprised multiple trials in which approximately 30 mosquitoes were placed in the flight chamber and allowed to choose either the right or left port, one of which contained a live bird (in live bird trials) or uropygial secretions of a robin (in secretion trials). Replicates were run for each pairing of live birds, and secretions only, as follows: robin:robin, robin:house sparrow, and robin:starling. For live bird assays, birds were captured as described above and released within 12 h. Secretions were collected as described above and placed inside the stimulus ports on filter paper-lined sterile petri dishes. After the capillary tube was removed from the glass vial, a small bulb was used to void the secretion from the tube onto the filter paper, then tube, vial, and lid were also placed on the petri dish. This process was completed within the stimulus port to ensure that the volatiles in the head space of the vial were not lost. For secretion-only trials, a plume of carbon dioxide was released into the incurrent air entering the stimulus ports to activate mosquito feeding. The screen doors separating the flight chamber and two stimulus ports were then opened and the trials were conducted for 45 min. Upon completion of the trial, the screen doors were closed, the stimulus ports were removed, and mosquitoes entering each port were counted. Mosquitoes that did not respond to a stimulus were excluded from the analysis. To avoid residual odors from the previous trial, the entire olfactometer was cleaned between trials with 70% ETOH and allowed to dry for 20 min. In addition, for within-treatment trials, stimuli were alternated between the left and right port so that no two trials were run with the same stimuli in the ports as during the previous trial. We controlled for the age of the bird by using only after hatching-year birds for all live and secretion assays.

Because the choices of mosquitoes within a trial are not independent of one another, we used a generalized linear mixed model (SPSS) with trial as a random factor and individual mosquitoes as subjects within trials. We modeled a binary probability distribution with a logit link function in which the port (right or left) that a mosquito moved into during the trial was the response variable, and the side that the robin was on was the fixed effect predicting mosquito choice. *P*-values of < 0.05 were considered significant.

RESULTS

Comparison of uropygial secretions from robins, house sparrows, and starlings

The uropygial secretions from the three species were qualitatively different in the volume of sample collected and the viscosity of the samples. Starlings produced the most secretion by weight and it was the most fluid (least viscous). House sparrow

secretions on average weighed less than those of starlings and were slightly less viscous. Secretions from robins were observed to be the most thick and viscous of the three species and were comparable, on average, to the mass of secretions produced by house sparrows. Chromatographic analysis of uropygial secretions revealed striking qualitative differences in the chemical profiles of the three species examined in this study (Figure 2). Consistent with their macroscopic viscosity, secretions from robins (bottom trace, red) contained far fewer semi-volatile compounds than the other two species but more non-volatile compounds. The compounds identified in robin secretions were hexadecanol (the major peak, with retention time 15.89 min) and a 23-carbon diester containing a C11 alcohol. Hexadecanol was confirmed by comparison with an authentic standard. The diester was confirmed by examination of its mass spectrum: diester spectra contain an obvious parent ion peak at $M + 2$ and a prominent fragment derived from the acid portion of the molecule. In addition, robin secretions contained large non-volatile esters that were strongly retained on the GC column, even when the temperature program approached the column's operating limit. Because these compounds, owing to their extremely low volatility, were poorly resolved as intact esters, we characterized them after methyl esterification, as described below.

The secretions of sparrows (middle trace, orange) and starlings (top trace, green) contained complex mixtures of semi-volatile esters that were not found in secretions from robins. We classified these esters according to total chain length by examining the mass spectrum for each chromatographic feature, noting that esters consistently produce a parent ion at $M + 1$. Examination of the total ion chromatograms revealed that several chromatographic features shared the same m/z value, indicative of different branching patterns giving rise to varied retention times. Since fragmentation in the mass analyzer varies with molecular branching, there was no single characteristic mass spectrum for esters. The esters in the house sparrow secretions were found to follow a repeating pattern which corresponds to five different structural isomers of the same molecular weight. Because of the regularity of this pattern, we hypothesize that within each set of esters of the same total molecular weight, each peak possesses the same branching pattern as the corresponding ester in the other sets. No such regularly repeating pattern was observed in the starling secretions.

To further characterize the semi-volatile esters in sparrow and starling secretions and the non-volatile esters in robin secretions, we performed methyl esterification reactions on representative secretion samples. GC-MS analysis of the resulting methyl esters enabled the identification of the chain length of the carboxylic acids and alcohols comprising the esters (Table 1). We used selected ion monitoring and published fragmentation values (Jacob and Poltz 1974, Sweeney et al. 2004) to determine the basic branching pattern of the methyl esterified carboxylic acids. The values used to characterize branching were: $m/z = 101$ for 3-methyl branching, $m/z = 88$ for 2-methyl branching, $m/z = 87$ for 4-methyl branching, and $m/z = 143$ for straight chain methyl esters. We were unable to identify branching in the alcohol portions. The methyl esterification reaction decouples the alcohol component of the ester from the acid component; we are therefore able to provide a maximum-to-minimum range for the non-

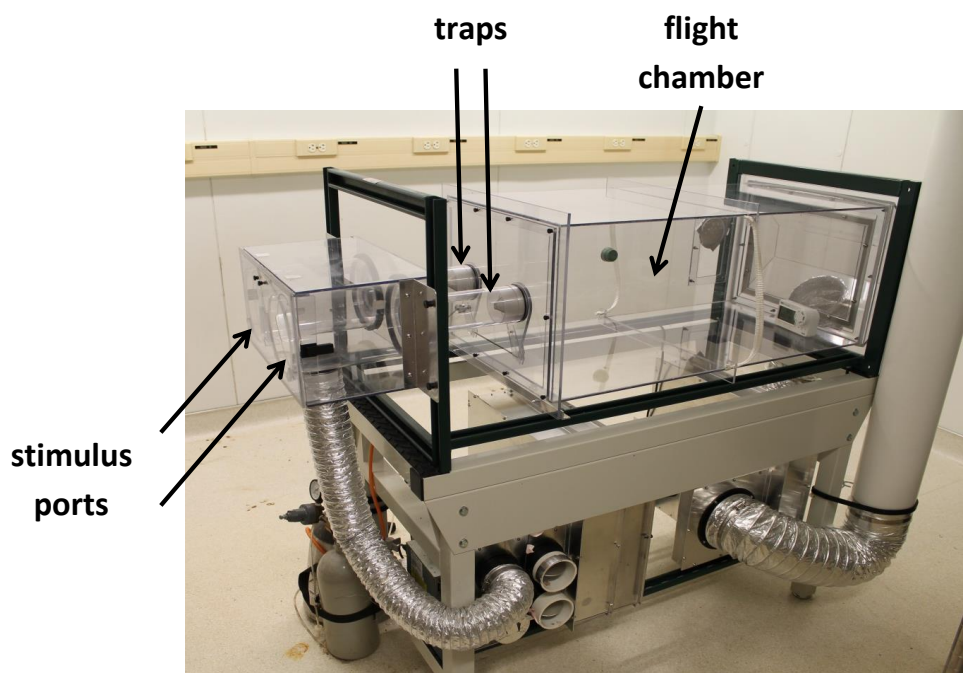


Figure 1. Dual port olfactometer used for mosquito choice trial bioassays. Birds or secretions were placed in the stimulus ports into which filtered air entered. Mosquitoes were placed in the flight chamber on the right, from which air exited. Mosquitoes, choosing one stimulus over the other, were captured in the cylindrical traps connecting each of the two stimulus ports to the flight chamber.

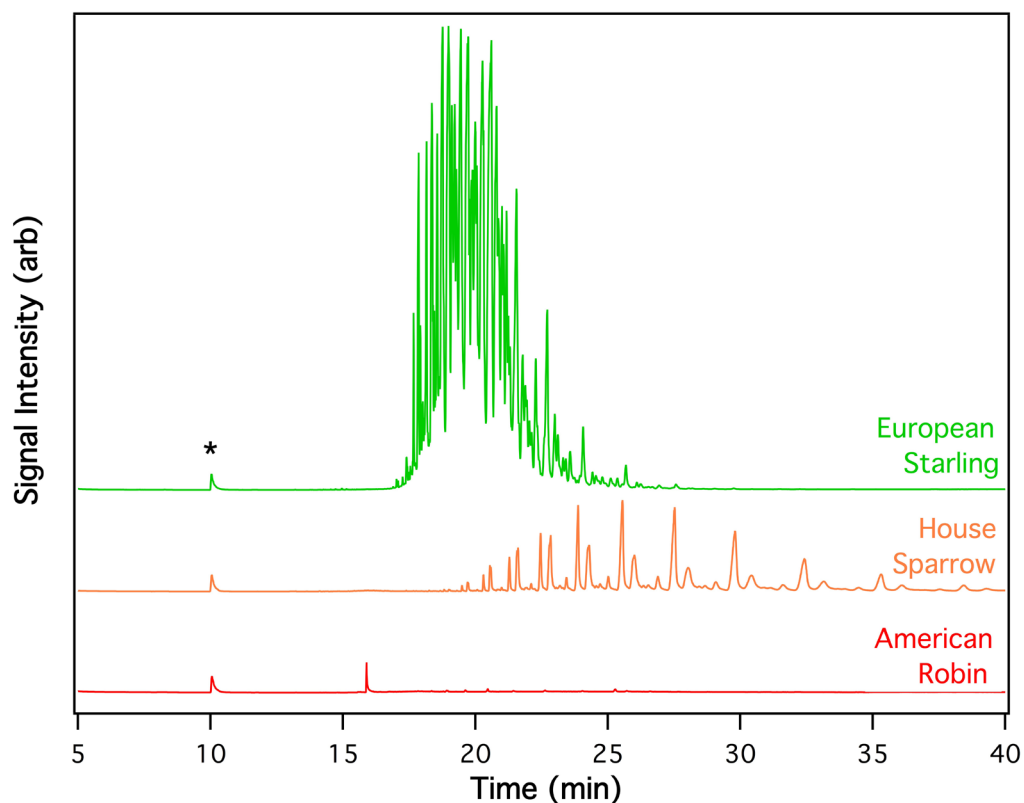


Figure 2. Representative gas chromatograms from the uropygial gland secretions of American robin (bottom, red), house sparrow (middle, orange) and European starling (top, green). The asterisk designates the internal standard, benzyl alcohol. Note the appearance of hexadecanol (16 min) in robins only, and the abundant and diverse esters in sparrow and starling secretions.

Table 1. Carbon content of esters, composition of acids and alcohol moieties comprising the esters, and most frequently observed branching in the three species. The most abundant ester chain length in each secretion is shown in parentheses. Acids and alcohols were determined through transesterification reactions, while intact esters were examined in the original samples. The alcohol portion had branching that we could not characterize.

Species	Ester composition (most abundant ester)	Acids	Alcohols	Observed branching in carboxylic acid portion
American robin	C30-C49 ^{a,b}	C14-C26	C16-C23	unbranched and 2-methyl
House sparrow	C24-C37 (C31) ^c	C13-C20	C13-C19	unbranched and 3-methyl
European starling	C23-C33 (C29) ^d	C10-C18	C11-C16	unbranched and 2- and 4-methyl

^aEster composition for robins is an estimate based on the acids and alcohols. Intact esters were too heavy to examine.

^bRobin 2-methyl branched esters contained further uncharacterized branching.

^cSparrow 3-methyl branched esters contained further uncharacterized branching.

^dStarling 2-methyl and 4-methyl contained further uncharacterized branching.

volatile esters produced by robins. The intact ester composition for robins was estimated by combining the shortest acid with the shortest alcohol and the longest acid with the longest alcohol. This calculation provides an estimated range of esters contained in the robin secretions.

Bioassay

In trials in which mosquitoes were provided a choice between live robins and live house sparrows, the stimulus port containing the robin significantly predicted mosquito choice; most mosquitoes chose the port containing the robin (Table 2). In trials during which mosquitoes were presented with live robins and live starlings, the stimulus port containing the starlings significantly predicted mosquito choice.

In trials during which mosquitoes were presented with secretions only, the stimulus port containing robin secretions did not predict mosquito choice. Likewise, during trials with secretions from robins and starlings, the stimulus port containing robin secretions did not significantly predict mosquito choice. However, choice was predicted by the starling secretion port.

DISCUSSION

We found that the semi-volatile compounds in the uropygial gland secretions of American robins differed from those of European starlings and house sparrows, lending support to our hypothesis that these compounds may contribute to the observed feeding preference of *Cx. pipiens* for robins over the latter two species. The primary difference that we observed was

the relative simplicity of secretions from robins: the secretion profile is dominated by hexadecanol, a long-chain, semi-volatile alcohol, and contains only minor amounts of other semi-volatile compounds. Although volatile short-chain alcohols are reported to be attractive to mosquitoes (Zweibel and Takken 2004), to our knowledge no studies have directly investigated the effect of long-chain alcohols on mosquito feeding preferences.

However, choice trial olfactometer bioassays did not provide support for our hypothesis. Although we found that *Cx. pipiens* showed a strong preference for live robins over live house sparrows, confirming earlier field-choice trials (Simpson et al. 2009) and blood meal studies (Apperson et al. 2004, Kilpatrick et al. 2006, Hamer et al. 2009), we found no support for our hypothesis that uropygial secretions were driving this preference. Mosquitoes were not more often attracted to secretions of either species. These results suggest that factors other than the volatile components of uropygial secretions are contributing to the preference of *Cx. pipiens* for robins. Such factors may include other olfactory cues, including the products resulting from bacterial degradation of uropygial secretions as suggested by Bernier et al. (2008) in their study of mosquito attraction to chicken feathers. Alternatively, this result could be explained by the difference in body mass between the two species, which may have resulted in greater emission of both heat and carbon dioxide by the larger robin.

Contrary to literature on mosquito feeding preference noted above, we found that *Cx. pipiens* demonstrated a strong preference for live starlings over robins. Moreover, in the secretion-only assay, we found that starling secretions were more attractive to mosquitoes than robin secretions. That our findings contradict

Table 2. Result of live bird and secretion-only bioassay.

Bird species pairing	Assay Type	Number of Trials	Number of mosquitoes		F-value (d.f.)	p-value
			Port 1 (robin)	Port 2		
robin-sparrow	live birds	9	197	29	89.295 (1, 225)	< 0.001
	secretions only	5	20	19	0.000 (1, 37)	0.995
robin-starling	live birds	11	108	193	24.51 (1, 300)	< 0.001
	secretions only	5	28	59	10.447 (1, 85)	0.002

preference studies conducted in nature might be explained by a number of factors as summarized by Hamer et al. (2009), who suggest that the feeding preferences of *Cx. pipiens* are likely context-specific and heavily influenced by ecological and behavioral factors, including genetic variation among *Cx. pipiens* populations (Kent et al. 2007, Kilpatrick et al. 2007, Farajollahi et al. 2011). Choice trials comparing the response of wild and laboratory colony *Cx. pipiens* to starlings and their uropygial secretions would be helpful in elucidating this potential source of variation. Moreover, given the reported influence of diet on the non-volatile components of uropygial secretions (Thomas et al. 2010), starling diet could be an important variable contributing to our results. The starlings used in this study were captured at a local dairy, where they forage on the nutritious silage provided to the dairy cows; this diet is likely very different from that of the urban starlings used in other studies.

House sparrow and starling secretions differed from those of robins in the presence of diverse mixtures of semi-volatile esters. Haribal et al. (2005) suggested that the ester component of uropygial secretions may be associated with plumage maintenance and conditioning. If so, the shared ester profiles of these two species may reflect the similar environmental stresses that they face, especially given their preference for human-modified habitats, colonial nature, and propensity to build cavity nests (Lowther and Cink 1992, Cabe 1993). Robins produce much lower levels of semi-volatile esters; most esters produced by robins are heavier than those observed in sparrow and starling secretions and are therefore non-volatile.

Species-specific profiles that we report here have also been reported in a number of other studies (see Haribal et al. 2005) and may be attributed to a number of factors including diet (Thomas et al. 2010). House sparrows and starlings generally have similar food preferences (Lowther and Cink 1992, Cabe 1993). Robins, however, are more likely to consume large numbers of invertebrates and fruit (Sallabanks and James 1999).

We also found differences between house sparrows and starlings in the esters produced. These differences include the pronounced repetitious branching pattern of esters in sparrow secretions corresponding to similar isomers of the same chain length. No analogous pattern was found in starling secretions. Another difference is the complexity of branching in the esters, as revealed by transesterification reactions. The esters in the sparrow secretions contained acid portions with 3-methyl branching, whereas the esters in the starling secretions contained a mixture of 2-methyl and 4-methyl branching.

The most striking aspect of the house sparrow uropygial secretions was the regular and repeating pattern of five peaks, corresponding to five ester isomers of the same carbon chain length with different branching patterns. Although the exact branching patterns that differentiate the individual peaks are unknown, it is possible that branching affects the physical properties of the various esters (Kulkarni and Sawant 2002) and their chemical function (Epand et al. 2005). In each of the sets of five isomers, the most prominent peak is the last, which presumably has the least amount of branching. The involvement of esters in host-seeking behavior is not clear. Gravid female mosquitoes seeking oviposition sites are reported to be both attracted to and repelled by esters (Hwang et al. 1982, Sharma et al. 2009). However, females seeking a blood

meal may respond very differently than gravid females. Moreover, because we were unable to completely characterize branching patterns or relative chain lengths, identification of esters in this study was not possible. Still, the qualitative variation that we observed in esters from robins compared to house sparrows and starlings is notable and worthy of further study.

In summary, we found no support for the hypothesis that the preference of *Cx. pipiens* for robins over house sparrows is a result of the semi-volatile components of uropygial gland secretions. Surprisingly, and in contrast to other studies, we found that *Cx. pipiens* were more often attracted to live starlings over live robins. As noted above, this difference might be explained by context-specific environmental factors or genetic variation in either the host or mosquito populations. The *Cx. pipiens* used in bioassays in this study were colony-reared and may vary genetically from mosquitoes in earlier studies. Moreover, relatively few *Cx. pipiens* host studies have been completed to date, thus this difference might reflect our limited understanding of such sources of variation. This preference is especially interesting in light of the strong mosquito preference for starling secretions over robin secretions. The fact that European starlings are a pest species in North America (Cabe 1993), especially in dairies and other agricultural operations where they roost and breed in large numbers, underscores the importance of further study to more fully understanding their role as sources of blood meals for *Cx. pipiens*.

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REFERENCES CITED

- Allan, S.A., U.R. Bernier, and D.L. Kline. 2006. Laboratory evaluation of avian odors for mosquito (Diptera: Culicidae) attraction. *J. Med. Entomol.* 43: 225-231.
- Anderson, J.F., C. R. Vossbrinck, T.G. Andreadis, A. Itom, W.H. Beckwith, and D.R. Mayo. 2001. Characterization of West Nile virus from five species of mosquitoes, nine species of birds, and one mammal. *Ann. NY Acad. Sci.* 951: 328-331.
- Apperson, C.S., B.A. Harrison, T.R. Unnasch, H.K. Hassan, W.S. Irby, H.M. Savage, S.E. Aspen, D.W. Watson, L.M. Rueda, B.R. Engber, and R.S. NASCI. 2002. Host-feeding habits of *Culex* and other mosquitoes (Diptera: Culicidae) in the borough of Queens in New York City, with characters and techniques for identification of *Culex* mosquitoes. *J. Med. Entomol.* 39: 777-785.
- Apperson, C.S., H.K. Hassan, B.A. Harrison, H.M. Savage, S.E.

- Aspen, A. Farajollahi, W. Crans, T.J. Daniels, R.C. Falco, M. Benedict, M. Anderson, L. McMillen, and T.R. Unnasch. 2004. Host feeding patterns of established and potential mosquito vectors of West Nile virus in the eastern United States. *Vector-Borne Zoonot. Dis.* 4: 71-82.
- Bernier, U.R., S.A. Allan, B.P. Quinn, D.L. Kline, D.R. Barnard, and G.G. Clark. 2008. Volatile compounds from the integument of white leghorn chickens (*Gallus gallus domesticus* L.): Candidate attractants of ornithophilic mosquito species. *J. Sep. Sci.* 31: 1092-1099.
- Cabe, P.R. 1993. European Starling. *The Birds of North America.* 48: 1-15.
- Costantini, C., N. Sagnon, A.D. Torre, M. Diallo, J. Brady, G. Gibson, and M. Coluzzi. 1998. Odor-mediated host preferences of West African mosquitoes, with particular reference to malaria vectors. *Am. J. Trop. Med. Hyg.* 58: 56-63.
- Epanand, R.M., S.D. Rychnovsky, J.D. Belani, and R.F. Epanand. 2005. Role of chirality in peptide-induced formation of cholesterol-rich domains. *Biochem. J.* 390: 541-548.
- Farajollahi, A., D. M. Fonseca, L.D. Kramer, and A.M. Kilpatrick. 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.
- Giraudeau, M., C. Duval, N. Guillon, V. Bretagnolle, C. Gutierrez, and P. Heeb. 2010. Effects of access to preen gland secretions on mallard plumage. *Naturwissenschaften* 97: 277-581.
- Griffiths, R., M.C. Double, K. Orr, and R.J.G. Dawson. 1998. A DNA test to sex most birds. *Mol. Ecol.* 7: 1071-1075.
- Hagelin, J.C. and I.L. Jones. 2007. Bird odors and other chemical substances: a defense mechanism or overlooked mode of intraspecific communication? *Auk* 124: 741-761.
- Hamer, G.L., U.D. Kitron, T.L. Goldberg, J.D. Brawn, S.R. Loss, M.O. Ruiz, D.B. Hayes, and E.D. Walker. 2009. Host selection by *Culex pipiens* mosquitoes and West Nile virus amplification. *Am. J. Trop. Med. Hyg.* 80: 268-278.
- Haribal, M., A.A. Dhondt, D. Rosane, and E. Rodriguez. 2005. Chemistry of preen gland secretions of passerines: different pathways to the same goal? Why? *Chemoecology* 15: 251-260.
- Hwang, Y., G.W. Schultz, H. Axelrod, W.L. Kramer, and M.S. Mulla. 1982. Oviposition repellency of fatty acids and their derivatives against *Culex* and *Aedes* mosquitoes. *Environ. Entomol.* 11: 223-226.
- Jacob, J. and J. Poltz. 1974. Composition of uropygial gland secretions of birds of prey. *Lipids* 10: 1-8.
- Kent, R.J., L.C. Harrington, and D.E. Norris. 2007. Genetic differences between *Culex pipiens* f. molestus and *Culex pipiens* f. pipiens (Diptera: Culicidae) in New York. *J. Med. Entomol.* 44: 50-59.
- Kilpatrick, A.M., L.D. Kramer, M.J. Jones, P.P. Marra, and P. Daszak. 2006. West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. *PLoS Biol.* 4: e82.
- Kilpatrick, A.M., L.D. Kramer, M.J. Jones, P.P. Marra, P. Daszak, and D.M. Fonseca. 2007. Genetic influences on mosquito feeding behavior and the emergence of zoonotic pathogens. *Am. J. Trop. Med. Hyg.* 77: 667-671.
- Kulkarni, M.G. and S.B. Sawant. 2002. Physico-chemical properties of wax esters synthesised from corresponding alcohols using hydrobromic acid and hydrogen peroxide action. *Eur. J. Lipid Sci. Tech.* 104: 387-393.
- Lowther, P.E. and C.L. Cink. 1992. House Sparrow. In: *The Birds of North America.* 12: 1-15.
- Mboera, L.E.G. and W. Takken. 1999. Odour-mediated host preference of *Culex quinquefasciatus* in Tanzania. *Entomol. Exp. Appl.* 92: 83-88.
- Molaei, G., T.G. Andreadis, P.M. Armstrong, P.M., J.F. Anderson, and C.R. Vossbrink. 2006. Host feeding patterns of *Culex* mosquitoes and West Nile virus transmission, northeastern United States. *Emerg. Infect. Dis.* 12: 468-475.
- Posey, K.H., D.R. Barnard, and C.E. Shreck. 1998. Triple cage olfactometer for evaluating mosquito (Diptera: Culicidae) attraction responses. *J. Med. Entomol.* 35: 330-334.
- Pugh, M.E. and E. Schultz. 2000. A laboratory experiment demonstrating the dynamic character of membranes. *Biochem. Mol. Biol. Ed.* 28: 332-326.
- Pyle, P. 1997. *Identification Guide to the North American Birds.* Slate Creek Press, Bolinas, CA.
- Rajchard, J. 2007. Intraspecific and interspecific chemosignals in birds: a review. *Vet. Med.* 52: 385-391.
- Russell, C.B. and F.F. Hunter. 2005. Attraction of *Culex pipiens/restuans* (Diptera: Culicidae) mosquitoes to bird uropygial gland odors at two elevations in the Niagara region of Ontario. *J. Med. Entomol.* 42: 301-305.
- Sallabanks, R. and F.S. James. 1999. American Robin. In: *The Birds of North America.* 462: 1-26.
- Scott, T.W., L.H. Lorenz, and J.D. Edman. 1990. Effects of house sparrow age and arbovirus infection on attraction of mosquitoes. *J. Med. Entomol.* 27: 856-863.
- Sharma, K.R., T. Seenivasagan, A.N. Rao, K. Ganesan, O.P. Agrawal, and S. Prakash. 2009. Mediation of oviposition responses in the malaria mosquito *Anopheles stephensi* Liston by certain fatty acid esters. *Parasitol. Res.* 104: 281-286.
- Simpson, J.E., C.M. Folsome-O'Keefe, J.E. Childs, L.E. Simons, T.G. Andreadis, and M.A. Diuk-Wasser. 2009. Avian host-selection by *Culex pipiens* in experimental trials. *PLoS One.* 4: 1-5.
- Simpson, J.E., P.J. Hurtado, J. Medlock, M. Gourdarz, T.G. Andreadis, A.P. Galvani, and A. Diuk-Wasser. 2012. Vector host-feeding preference drive transmission of multi-host pathogens: West Nile virus as a model system. *Proc. R. Soc. B.* 279: 925-933.
- Sweeney, R.J., I.J. Lovette, and E.L. Harvey. 2004. Evolutionary variation in feather waxes of passerine birds. *Auk* 121: 435-445.
- Thomas, R.H., E.R. Price, G.L. Seewagen, S.A. Mackenzie, M.A. Bernards, and C.G. Guglielmo. 2010. Use of TLC-FID and GC-MS/FID to examine the effects of migratory state, diet, and captivity on preen wax composition in white-throated sparrows *Zonotrichia albicollis*. *Ibis* 152: 782-792.
- Turell, M.J., M.L. O'Guinn, D.J. Dohm, and J.W. Jones. 2001. Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile virus. *J. Med. Entomol.* 38: 130-134.
- Zwiebel, L.J. and W. Takken. 2004. Olfactory regulation of mosquito-host interactions. *Insect Biochem. Molec. Biol.* 34: 645-652.