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Host group formation decreases exposure to vector-borne disease: a field experiment in a 'hotspot' of West Nile virus transmission

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Animals can decrease their individual risk of predation by forming groups. The encounter-dilution hypothesis extends the potential benefits of gregariousness to biting insects and vector-borne disease by predicting that the *per capita* number of insect bites should decrease within larger host groups. Although vector-borne diseases are common and can exert strong selective pressures on hosts, there have been few tests of the encounter-dilution effect in natural systems. We conducted an experimental test of the encounter-dilution hypothesis using the American robin (*Turdus migratorius*), a common host species for the West Nile virus (WNV), a mosquito-borne pathogen. By using sentinel hosts (house sparrows, *Passer domesticus*) caged in naturally occurring communal roosts in the suburbs of Chicago, we assessed sentinel host risk of WNV exposure inside and outside of roosts. We also estimated *per capita* host exposure to infected vectors inside roosts and outside of roosts. Sentinel birds caged inside roosts seroconverted to WNV more slowly than those outside of roosts, suggesting that social groups decrease *per capita* exposure to infected mosquitoes. These results therefore support the encounter-dilution hypothesis in a vector-borne disease system. Our results suggest that disease-related selective pressures on sociality may depend on the mode of disease transmission.

1. Introduction

A 'selfish herd' may attract more predators owing to greater visibility of the group, but individuals 'dilute' their risk of predation across all other group members [1]. This hypothesis has been extended to biting insects, and risk of exposure to vector-borne disease in a concept known as the 'encounter-dilution effect' [2,3]. Barring an increase in the number of insects attracted to a social group, social groups decrease the *per capita* vector biting rate by distributing the risk of insect bites across many individuals [4,5]. Given that a host's risk of disease exposure increases with the number of insect bites received [6,7], social groups may also decrease an individual's risk of exposure to vector-borne pathogens.

Empirical evidence for the encounter-dilution effect for vector-borne pathogen transmission is mixed and largely observational, although models predict the encounter-dilution effect in vector-borne disease systems [8,9]. Comparative studies of social species and non-social congeners have found higher prevalence of malaria and arboviruses in social species of bird and primates [10–13],

contrary to the predictions of this hypothesis. Conversely, studies of livestock herds and avian flocks indicate that participation in groups does decrease *per capita* vector biting rates [5,14,15]. Nonetheless, definitive evidence of decreased host exposure to vector-borne pathogens as a result of group formation is scant [but see 7]. Vector-borne diseases can exert significant fitness effects on hosts [16,17], resulting in selective pressure favouring gregariousness. Nonetheless, this hypothesis currently lacks empirical support.

West Nile virus (WNV) is a vector-borne pathogen transmitted by *Culex* spp. mosquitoes [18]. WNV is maintained in a seasonal transmission cycle between vectors and wild birds [19,20]. The American robin (*Turdus migratorius*, hereafter 'robin') is an important host species in the WNV transmission cycle owing to high competency, i.e. the ability of robins to transmit and contract the disease during interactions with vectors [21,22]. Robins form communal roosts throughout their breeding season [23,24], which coincides with the peak transmission season of WNV. Previous work suggests communal robin roosts may enable localized transmission of WNV between birds and vectors [25].

We examined communal robin roosts in the west Chicago suburbs, a WNV 'hotspot,' [22,26] and conducted a field experiment to test the encounter-dilution effect . We assessed the major assumption of the encounter-dilution effect, that vector abundances and infection rates were similar inside and outside of social groups, by trapping mosquitoes at roost and non-roost sites during the 2010–2012 WNV transmission seasons. By housing sentinel birds in cages inside and outside of communal roost sites in 2012, we tested the following predictions: if the encounter-dilution effect acts on vector-borne pathogen transmission, then sentinel birds in communal roosts should have lower risk of exposure to WNV than those away from communal roosts. We predicted a lower *per capita* vector index (i.e. the estimated number of interactions a host has with infected mosquitoes per night) for birds in communal roosts.

2. Material and methods

We located five large (200–20 000 birds) communal robin roosts in Cook County, Illinois between May and October of 2010–2012. We trapped mosquitoes inside communal roosts and in residential areas, urban parks and natural areas away from roosts. The total extent of mosquito trapping effort was 21.7 km^2 in 2010, and 7.86 km^2 in 2011 and 2012. Average trap density during all 3 years was at least six traps per km^2 at roost and non-roost locations. We placed 66 traps inside roosts and 270 traps in non-roost areas: this equated to 140 traps total in 2010, 133 in 2011 and 63 in 2012. Vector infection rates from mosquitoes captured in 2012 were also used to estimate the *per capita* vector index [27], i.e. the number of infected mosquitoes encountered per host per night at roost and non-roost sites associated with the sentinel bird experiment.

(a) Mosquito sampling

We estimated vector abundances and infection rates at roost and non-roost sites from 2010–2012 to verify hosts did not encounter more vectors or elevated infection rates in *Culex* spp. vectors (hereafter *Culex*) inside of communal roosts compared with outside of roosts. We deployed Centers for Disease Control and Prevention (CDC) CO_2-baited light traps and infusion-baited gravid traps for one night per week from the beginning of June through to mid-October in 2010–2012. CDC carbon dioxide (CO_2)-baited light traps were used to estimate abundances of host-seeking female mosquitoes of various species, as the CO_2 given off by the traps simulates respiration by hosts. Infusion-baited gravid traps were used to enhance capture of *Culex* vectors already infected with WNV [28]. We identified all captured mosquitoes to species; an exception to this was *Culex pipiens* and *Culex restuans*, which were identified only to genus, as identification to species based on morphology is unreliable [29]. We then pooled mosquitoes based on species, unique trap identification and blood-fed status. We tested *Culex* pools for WNV infection following the protocols previously described [26].

(b) Assessing host risk of West Nile virus exposure

To estimate host risk of exposure to WNV at each site type, we conducted an experiment using sentinel birds. Sentinel birds (typically chickens or other galliforms) are commonly used by public health agencies to survey for transmission of vector-borne diseases such as WNV [30–32]. For this study, we used house sparrows (*Passer domesticus*, hereafter sparrows) as the sentinel species. We used sparrows rather than American robins, because sparrows are competent hosts for WNV [20], contribute significantly to the WNV transmission cycle in Chicago as determined from vector blood meal analysis [19], can be held in captivity with reasonable effort and unlike robins, are not protected by statute in North America.

In 2012, we selected three roost sites and three non-roost sites. The roost sites selected for this study occurred in dense stands of buckthorn or invasive *Phragmites* surrounded by industrial or residential areas. We selected non-roost sites based on similarity to habitats used by non-communally roosting robins observed from previous radiotelemetry work. We selected two residential sites with suitable roosting trees in residential backyards and one natural site with habitat similar to communal roosts used in the study but lacking a communal robin roost. Non-roost sites were at least 0.5 km away from any communal roost or other non-roost sites.

We captured, individually marked with coloured leg bands, and drew blood samples from free-living sparrows to screen for previous WNV exposure. After initial blood draws, we housed sparrows in flight cages at a field laboratory until screening for previous exposure was completed. We screened blood samples for WNV RNA using a quantitative RT-PCR and WNV antibodies using an inhibition ELISA as previously described [26], with one amendment being that viral RNA was extracted using the MagMAX viral RNA isolation kit (Applied Biosystems, Foster City, CA).

Birds that tested negative for WNV antibodies were transferred in groups of five to each of six field cages, three in roost sites and three in non-roost sites. Field cages were built from commercially available bird cages elevated onto four 3 m galvanized steel pipes. We coated the anchor poles with machine grease to prevent disturbance from the public or mammalian predators. Roost cages were placed in central locations within roosts, as determined by nocturnal surveys of the boundaries of the roost. Non-roost cages were placed within 1.5 m of the trunk of a suitable roost tree, within the foliage of the tree and with the top of the cage 3 m off the ground.

We deployed sentinel cages by the third week of July 2012, after which we drew blood samples by jugular venipuncture from each bird weekly for the next eight weeks. The timing of this experiment coincided with the historical period of peak WNV transmission in our study area [26]. Samples drawn from birds in field cages were tested as described above on a weekly basis. We maintained a group of WNV unexposed sparrows protected from exposure to vectors as 'reserve' birds. As birds housed in field cages became exposed to WNV, they were removed and replaced with unexposed birds from the reserve

group. All birds were provided food and water *ad libitum*. Animal care, use and housing were approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign, protocol no. 12047.

In 2012, we estimated the *per capita* vector index, the number of infected vectors encountered per host per night inside and outside of the three communal roosts used for the sentinel bird experiment. To estimate *per capita* biting rates of *Culex* on hosts, we used bird-baited traps. Bird-baited traps are modified CDC light traps that use a single bird as bait in place of CO_2 and attract mosquitoes such as *Culex* that prefer feeding on avian hosts [33]. We constructed bird-baited mosquito traps following Emord & Morris [33]. A single sparrow was placed in the cage attached to the trap, and the trap was placed at least 20 m away from one of the sentinel bird cages for one night per week during the course of the sentinel bird study, which equated to six bird-baited trap nights per week over eight weeks. To protect bait birds from mosquitoes, all cages were lined with insect screening. Each bait bird was used in a trap only one night per week, and bait birds were kept separate from 'reserve' birds used in the sentinel bird experiment.

(c) Estimating vector abundance, infection rates and vector index

To describe overall *Culex* abundance and infection rates from 2010 to 2012, we pooled light and gravid traps into 19 spatially clustered groups; 14 outside of roosts and one inside each of five communal roosts. Spatial groups were assigned based on site type (i.e. roost or non-roost), separation by physical barriers, (e.g. major roadways) and overall habitat type (e.g. natural, residential or industrial areas). All spatial groups covered less than 2.5 km^2 and had an average trap density of six gravid and light traps combined per km^2. Each roost site had between two and four CDC CO_2-baited light traps and two to four gravid traps each, and all traps within the same roost were assigned to the same spatial group. Vector abundance from light traps was estimated as the number of *Culex* captured per light trap night within each spatial group. We derived maximum-likelihood estimates of the minimum infection rate (MIR; number of infected mosquitoes per 1000 mosquitoes trapped [34]) of *Culex* mosquito pools from light and gravid traps with 95% confidence intervals by spatial group using the Pooled Infection Rate v. 3.0 add-in.

To assess the assumption of similar vector abundances within communal roosts and to determine whether communal roosts in our study system increased local infection rates in vectors, we compared infection rates inside and outside communal roosts from 2010 to 2012 from CDC CO_2-baited light traps and gravid traps. We used Poisson regression to analyse *Culex* abundance and MIR in program R using the lme4 package; the response variable was either *Culex* per trap night or MIR, with fixed effects for site type (roost or non-roost), random effects for week nested in year and spatial group, and an offset term equal to the log of the number of traps set in a spatial group during a given week. The use of the offset term accounts for the variation in trap number set in a given week among spatial groups, allowing analysis of untransformed data [35]. For mosquito abundance estimates from bird-baited traps and the per host vector index, we used Poisson regression; we included site type as a fixed effect and a random effect for week.

To compare host encounter rates with infected vectors at roost and non-roost sites, we estimate the vector index at both site types in 2012. We calculated the weekly vector index by multiplying the per light trap night estimates of *Culex* abundance by the maximum-likelihood estimates of vector infection rates from *Culex* captured in the same light traps. To estimate the *per capita* vector index at roost and non-roost sites, we multiplied the MIR estimates from *Culex* pools from the bird-baited traps and the two closest light traps within 200 m of the bird-baited trap by the average number of *Culex* captured per trap night in bird-baited traps. We used MIR estimates from light traps only to estimate per host vector index, because vectors captured in light traps are actively host seeking and therefore capable of transmitting the virus upon their next blood meal. To this end, we assumed host-seeking behaviour of *Culex* to be similar when using bird-baited traps and light traps.

(d) Analysing host risk of West Nile virus exposure

Relative risk of sentinel bird exposure to WNV inside and outside communal roosts in 2012 was analysed using a Cox-proportional hazards mixed model in the coxme package in R. The proportional hazards model assumes the risk of an event occurring, in this case WNV infection of a given bird, is the same across all time intervals. We tested this assumption using the survival package in R, and as this assumption was not violated ($p > 0.1$), we used the proportional hazards model. The length of time an individual remained unexposed to WNV was analysed as dependent on a site type (roost or non-roost) with a random effect for individual birds nested within cage [36].

3. Results

(a) Vector abundance and infection rates at roost and non-roost sites

Our mosquito sampling effort totalled over 4000 trap nights of light and gravid traps across 3 years. We captured 18 158 *Culex* mosquitoes in light traps from 2010 to 2012, which we used to estimate abundances of host-seeking vectors inside and outside of communal roosts. With added sampling using gravid traps, we estimated MIR estimates from 2010 to 2012 using 40 761 *Culex* mosquitoes. Estimated *Culex* abundances were similar at roost and non-roost sites across all 3 years ($z = 0.16$, $p > 0.05$; figure 1*a–c*). Furthermore, the estimated MIR in *Culex* did not differ between roost and non-roost sites ($z = -0.22$, $p > 0.05$; figure 1*d–f*).

(b) Risk of host exposure to West Nile virus and vector indices

Between July and September 2012, we placed 23 sentinel birds in cages near communal roosts and 25 in non-roost cages. Only three sentinel birds near roosts seroconverted to WNV, whereas 11 birds in non-roost cages seroconverted. At every sentinel cage, at least one sentinel bird seroconverted to WNV during the experiment; therefore, *Culex* did feed on sentinel sparrows even at roost cages when numerous robins were present. The risk of WNV exposure for sentinel birds caged within roosts was significantly lower than for birds caged in non-roost locations ($z = -2.17$, $p = 0.03$; figure 2).

Estimated *per capita* vector encounter rates with hosts in 2012 were consistent with results of the sentinel experiment. Vector encounter rates were significantly greater away from roosts ($z = -9.568$, $p < 0.001$). Moreover, the vector index was approximately one-third of that within communal roosts than outside of communal roosts ($z = -17.4$, $p < 0.001$; figure 3*a*). The average estimated *per capita* vector index was 1.1 infected vectors per host per night outside of roosts, which was over 40 times higher than the vector index inside roosts (0.025 infected vectors per host per night; figure 3*b*).

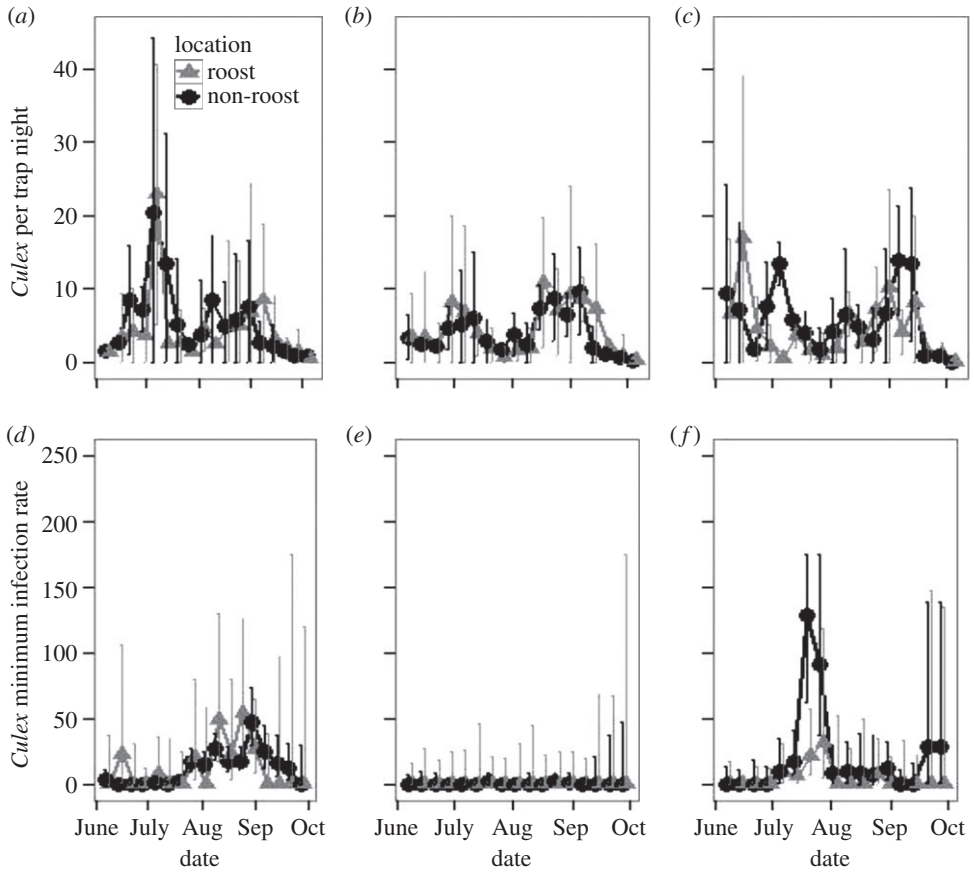

Figure 1. Variation among years in average *Culex* per light trap night (*a*) 2010 (*b*) 2011, and (*c*) 2012 by trap location, ±2 median absolute deviations. (*d–f*) *Culex* minimum infection rate maximum-likelihood estimates (*d*) 2010 (*e*) 2011, and (*f*) 2012 by trap location, ±95% CIs. All figures based on data collected in Chicago, Illinois.

4. Discussion

Our results indicate that the individual risk of exposure to WNV is lower within communal roosts than outside of them. This finding supports the encounter-dilution hypothesis for transmission of vector-borne pathogens. Our data also suggest that decreased individual risk was mediated by decreased *per capita* interactions with infected vectors within social aggregations. Furthermore, *Culex* abundance and MIR did not differ between roost and non-roost sites across 3 years of observation, indicating roosts do not attract more WNV vectors than other areas, nor does host aggregation enable WNV transmission, making the action of the encounter-dilution effect possible in this vector-borne disease system.

Increased exposure to directly transmitted diseases has been considered a selective force acting against sociality [37], but our results support the idea that the mode of disease transmission determines the relationship between diseases and host grouping behaviour. Disease transmission models often consider how transmission responds to host density, which typically falls into one of two categories: density-dependent or frequency-dependent transmission. Diseases transmitted directly from host-to-host or through the environment (e.g. soil or water contamination) are typically density-dependent, meaning locally greater densities of hosts leads to increased disease transmission [38,39]. Transmission of vector-borne pathogens by contrast is frequency-dependent, such that the proportion of infected individuals (vectors or hosts) has more impact on disease transmission than the absolute numbers of hosts or vectors [40,41]. Further, in vector-borne disease systems where vectors are not differentially attracted to groups, vector–host interactions become directly analogous to the predator–prey interactions: larger groups result in a decreased *per capita* risk of interactions with a predator or infectious vector, and vectors or predators may exhibit a functional response at higher host or prey density [1–3,7,42]. In our system, increasing the number of hosts in a roost without changing the number of infected vectors decreases the ratio of infected vectors to available hosts, resulting in decreased risk of host exposure to WNV. Transmission of vector-borne pathogens thus behaves more similarly to predator–prey interactions than does the transmission of directly transmitted pathogens with respect to host grouping behaviour. Our results indicate group formation by hosts in some vector-borne disease systems can provide a selective advantage to social versus non-social individuals.

Many factors can favour gregariousness in animals, including reduced predation risk or increased foraging efficiency in groups [1,3]. Although decreased individual risk of exposure to WNV is a selective advantage to group formation in our system, it is probably not the only advantage to communal roosting. Nuisance feeding by biting insects can alter host behaviour [43], reduce fitness by increasing energy expended during behavioural defences [44,45] and reduce breeding success [46,47]. Consequently, decreased exposure to biting insects within communal roosts may benefit social individuals even in the absence of infection. Under this scenario, the selective advantages of sociality would increase in the presence of vector-borne infections owing to compounding effects of vector feeding and vector-borne infections

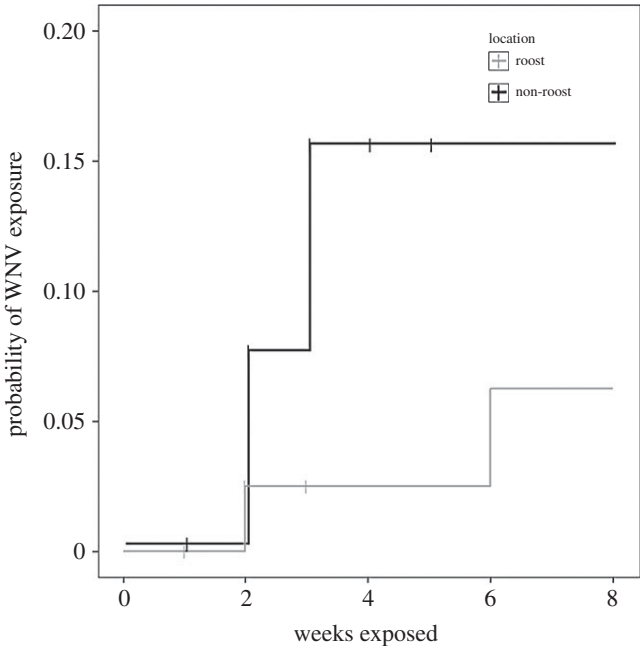

Figure 2. Kaplan–Meier plot of risk to an individual sentinel bird by cage location. Tick marks indicate individuals censored at that interval (e.g. owing to non-West Nile virus related mortality or escape after blood sampling). Plots are offset to ease interpretation.

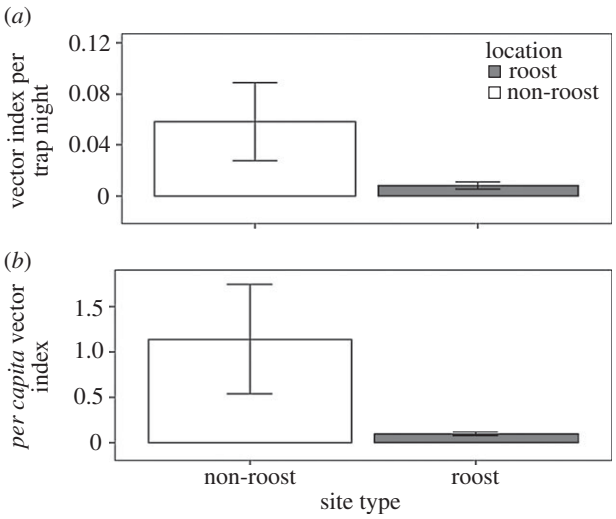

Figure 3. All data were collected in Chicago, Illinois. (*a*) Average vector index, or number of infected *Culex* mosquitoes captured per light trap night, at roost and non-roost locations in 2012 ± 1 standard error. (*b*) Average *per capita* vector index at roost and non-roost locations, between July and September 2012, ± 1 standard error.

[16,17]. At the same time, WNV is a recently introduced selective pressure in our system [19,20], whereas communal robin roosts have been documented throughout North America for over a century [23]. Therefore, it is unlikely WNV has significantly changed communal roosting behaviours in robins since its introduction to North America. Furthermore, WNV prevalence in vectors and hosts varies widely within and among years (figure 3 and [26]), and disease-related benefits of participating in a communal roost may be less pronounced during years of low WNV prevalence. Nonetheless, our results provide evidence of an emerging selective advantage to aggregation in a highly competent host species, which has important implications for future studies of emerging infectious diseases.

A potential confounder in our experimental design is vector preference for robins over house sparrows [18]. Infectious vectors could have fed on robins in communal roosts and avoided the nearby sentinel sparrows. However, sentinel bird exposure to WNV occurred at all sentinel cages throughout the duration of the experiment; therefore, *Culex* were attracted to and fed on sparrows even in the presence of large numbers of robins. In addition, the estimated *per capita* vector index, or number of infected mosquitoes encountered per host per night inside communal roosts was 0.025, compared with 1.1 away from communal roosts. Therefore, any host species using a communal roost would experience decreased risk of contact with infectious vectors. Finally, among a ranking of 25 bird species found to have been fed upon by *Culex* vectors in our study systems, the rate of transmission of WNV was estimated to be highest from American robins and then house sparrows [19], indicating that *Culex* vectors feed readily on house sparrows.

Under the original selfish herd and encounter-dilution hypotheses, more central locations within a group are more desirable owing to decreased exposure to predators [1] or biting insects [2–4]. Therefore, more favourable central positions should be held by dominant individuals or competed for among group members. Similarly, within communal roosts, more dominant individuals should seek to occupy central and more elevated positions, while relegating younger and less dominant birds to less favourable positions [48–50]. Demographic stratification within roosts may have important implications for vector-borne disease exposure, as different vector species feed at different heights within a habitat [51–55]. For example, one study found WNV infection rates in vectors were greater in the canopy than at ground level [55], and another found evidence suggesting differences in roosting positions can influence vector–host interactions [56]. While lower positions within a roost may be less favourable in terms of other potential benefits, less dominant individuals relegated to these positions may still benefit from decreased exposure to vector-borne diseases, particularly when vector feeding patterns or infection rates are vertically stratified. We placed sentinel bird cages within communal roosts at central locations based on nocturnal surveys of roost boundaries, suggesting our results are indicative of the benefits received by individual occupying preferred central locations within roosts. We did not assess the impact of host location within the roost on risk of host exposure to vector-borne diseases. Future work should examine the heterogeneity of host risk of exposure to vector-borne illnesses within groups to examine whether all members of a group benefit equally.

Ethics statement. Animal care, use and housing were approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign, protocol no. 12047.

Data accessibility. Data are available at datadryad.org (doi:10.5061/dryad.fp7s8).

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