The effects of host availability and fitness on *Aedes albopictus* blood feeding patterns in New York

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Abstract

Aedes albopictus is a competent vector of numerous pathogens, representing a range of transmission cycles involving unique hosts. Despite the important status of this vector, variation in its feeding patterns is poorly understood. We examined the feeding patterns of *Ae. albopictus* utilizing resting collections in Long Island, New York, and contextualized blood meal sources with host availability measured by household interviews and camera traps. We identified 90 blood meals, including 29 human, 22 cat, 16 horse, 12 opossum, 5 dog, 2 goat, and 1 rabbit, rat, squirrel and raccoon. Our study is the first to quantitatively assess *Ae. albopictus* feeding patterns in the context of host availability of wild animals in addition to humans and domestic animals. Host feeding indices showed that cats and dogs were fed upon disproportionately often compared to humans. Forage ratios suggested a tendency to feed on cats and opossums and to avoid raccoons, squirrels, and birds. This feeding pattern was different from another published study from Baltimore, where *Ae. albopictus* fed more often on rats than humans. To understand if these differences were due to host availability or mosquito population variation, we compared the fitness of Long Island and Baltimore *Ae. albopictus* after feeding on rat and human blood. In addition, we examined fitness within the Long Island population after feeding on human, rat, cat, horse, and opossum blood. Together, our results do not show major mosquito fitness differences by blood hosts, suggesting that fitness benefits do not drive Northeastern *Ae. albopictus* feeding patterns.

Introduction

Aedes albopictus is a globally invasive mosquito of human and veterinary health importance. This species is capable of transmitting over 20 pathogens in laboratory assays[1], and is a confirmed natural vector of dengue, Zika, and chikungunya viruses, and dog heart worm[1, 2]. *Aedes albopictus* is a suspected vector of numerous additional viruses, including Eastern equine encephalitis and West Nile due to virus detection in field-collected mosquitoes, although there is no direct evidence of transmission to humans yet[1]. These pathogens encompass vastly different transmission cycles, including anthroponoses (e.g. Zika: human to mosquito) and zoonoses (e.g. West Nile virus: primarily bird to mosquito to human; dog heartworm: dog or wild canid to mosquito). In light of the broad vector potential of *Ae. albopictus* and variation in feeding

patterns in nature, it is critical to perform host feeding studies in locations relevant to human and animal health risk.

Variation in mosquito host feeding patterns can be influenced by a number of factors including innate host preference, environmental conditions, host availability, and the design of the studies themselves. These factors may explain the variation in host feeding reported for *Ae. albopictus* in the literature.

Published results for *Ae. albopictus* range from generalist or mammalophilic to highly anthropophagic (=human feeding) feeding patterns. For example, a high percentage of mosquitoes with human-derived blood meals were identified in tropical countries such as Thailand (100%) and Cameroon (99.4%)[3, 4]. In Thailand, aspirator collections were conducted around human dwellings, however, in Cameroon, mosquitoes were collected at a leisure and equestrian center, both of which were surrounded by human dwellings. In some parts of the USA, human feeding frequency was much lower, such as at a tire dump in Missouri (6.5%), urban Baltimore, Maryland (13.6%), urban and rural sites in Hawaii (18.1%), and suburban North Carolina (20%)[5, 6, 7, 8]. Additional studies have reported moderate human feeding rates such as in urban and peripheral sites in Brazil, urban and suburban Japan, and suburban New Jersey, USA[9, 10, 11]. Of those populations that did not feed predominantly on humans, most fed on a diverse array of animals, with the exception of Baltimore, where a striking number of *Ae. albopictus* fed on rats (72.3%)[6].

One notable consistency amongst all published studies (with a sample size over 75) is a tendency for *Ae. albopictus* to feed primarily on mammals compared to birds and reptiles[3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18]. About half of studies report feeding on birds at low rates (1.7% to 25.6% of all blood meals)[5, 7, 8, 11, 13, 14, 16, 17, 18]. A tendency to feed even sporadically on birds is

74 particularly important because of their role as amplifying hosts of arboviruses such as West Nile
75 and Eastern equine encephalitis.

76 Host availability is rarely considered in the design of mosquito blood feeding studies
77 despite its importance in driving mosquito blood feeding patterns and thus interpreting study
78 results. In Italy, *Ae. albopictus* from urban and rural sites had replicable differences in feeding
79 patterns, mirroring differences in host availability at these sites[14]. However, the authors only
80 made qualitative note of site type and did not quantify host availability. We are aware of only
81 two published studies (in North Carolina and Brazil) that have quantitatively assessed the link
82 between host availability and blood feeding for *Ae. albopictus*[8, 13]. Their results do not provide a
83 clear picture of whether *Ae. albopictus* feeds disproportionately often on humans compared to
84 other mammals, with results varying depending on measurement type, stratification level, and
85 non-human animal in question.

86 In addition to host availability, host attraction may be a major driver influencing blood
87 feeding patterns[19]. Unfortunately, only two published studies have explored host attraction in *Ae.*
88 *albopictus*[20, 21]. The authors reported higher attraction to humans compared to numerous other
89 species including dogs and chickens. Preferential attraction to hosts is determined genetically,
90 and may evolve as a result of elevated mosquito fitness after ingesting a given species' blood[19,
91 22]. This has been demonstrated for *Ae. aegypti*, which maximizes reproductive fitness on human
92 blood, its preferred host[23]. Only two studies have addressed the impact of blood from different
93 species on *Ae. albopictus* egg production[24, 25], but none have compared both survival and
94 fecundity using the most ecologically relevant hosts.

95 We sought to determine *Ae. albopictus* feeding patterns in suburban and farm landscapes
96 along its front of active northward expansion in New York State[26]. Our aim was to investigate

these feeding patterns in the context of host availability and their consequences for mosquito fitness. Ultimately, we wanted to fill a gap in our understanding of *Ae. albopictus* feeding ecology along its Northeast USA range limit and how it might relate to public health risk. To rigorously address blood feeding patterns, we performed host censes to calculate host feeding indices and forage ratios. We then assessed whether fitness of Long Island, NY *Ae. albopictus* varied by host blood species ingested in the laboratory through a series of life table studies. To explore population differences, we compared fitness of Long Island and Baltimore populations fed human and rat blood meals.

Methods

Field Sites

Eight sites were selected in Suffolk County on Long Island, NY: four farms and four residential areas, each containing between nine and seventeen collection properties. *Ae. albopictus* has been present in Suffolk County since 2004, although its distribution is not uniform or complete across the county (Moses Cucura, pers comm). Residential sites were selected based on *Ae. albopictus* presence reported by the Suffolk County Vector Control and Arthropod-Borne Disease Laboratory and larval distribution data[27]. All residential sites were suburban, with variable human population density: Central Islip (1,853 people/sq km), Bay Shore (1,853 people/sq km), Babylon (1,660 people/sq km), and Hauppauge (734 people/sq km). All four farms were partially bordered by suburban residential and forested natural landscapes.

Collection

Weekly collections were conducted at each site between 20 June and 15 August, 2018 with large custom-designed aspirators (30.5 cm diameter, 114 cm height, 12 V PM DC 2350 RPM, 1/35 Horse power, 3.7 amp motor)[3]. One mosquito was collected with a hand net while host-seeking near collectors and was included in analysis because blood was partially digested upon collection. Mosquitoes were immobilized in acetone-treated jars (3 min) and sorted in the field to remove non-mosquito by-catch. The samples were transported on ice to the laboratory for identification according to a taxonomic key[28]. *Aedes albopictus* were considered engorged if blood was visible in the abdomen upon examination. Mosquitoes were stored at -20°C and transported to Cornell University on dry ice for blood meal identification.

Blood Meal Identification:

Abdomens were removed from mosquitoes using forceps and transferred to sterile microcentrifuge tubes. To avoid cross-contamination, forceps were dipped in ethanol and flame-sterilized between each sample. DNA was extracted from abdomens using Qiagen Puregene Cell kit (Qiagen Sciences, Germantown, MD, USA). To identify blood meals, we amplified templates from the vertebrate-specific cytochrome c oxidase subunit I (*COI)* "barcoding" gene. Primers designed by Reeves et al. (2018) were used to amplify a 395 base pair amplicon[29](Table 1).

Table 1: Primer sequences designed by Reeves et al. (2018)

Primer Name	**Sequence**
VertCOI_7194_F	5'- CGM ATR AAY AAY ATR AGC TTC TGA Y -3'
Mod_RepCOI_R	5'- TTC DGG RTG NCC RAA RAA TCA -3'

Other Reeves *COI* primers were not used due to co-amplification of *Ae. albopictus* DNA. Co-amplification is a recurrent issue with identifying *Ae. albopictus* blood meals due to matching sequences between its own genome and primers designed for use in blood meal studies of other mosquito species[15]. Notably, cytochrome b primers designed by Egizi et al. (2013) were used initially, but due to low success rate in our hands, we switched to the Reeves primers[29]. Three blood meals identified with the Egizi primers were not successfully amplified by the Reeves primers; results with both primer sets were combined for our data analysis.

PCR conditions were slightly modified from Reeves et al. (2018) in order to minimize co-amplification of *Ae. albopictus* DNA and maximize amplification of desired amplicon[29]. Reactions were performed with total volume of 20 µL, consisting of 10 µL of 2.0X Apex Taq RED Master Mix (Genesee Scientific Corp., San Diego, CA), 0.75 µL of VertCOI_7194_F forward primer (10 µM), 0.75 µL of Mod_RepCOI_R reverse primer (10 µM), 6.5 µL sterile nuclease-free H_2O, and 2 µL of extracted DNA. Most reactions were conducted with the following thermocycling conditions: 94°C for 3 min, followed by 40 cycles of 94°C for 40 s, 53.5°C for 30 s, and 72°C for 60 s, and a final extension step at 72°C for 7 min. The annealing temperature was modified from Reeves et al. (2018) in order to minimize amplification of *Ae. albopictus* DNA according to a temperature gradient test conducted on positive (human-fed) and negative (non-fed) mosquito controls. Conditions were further modified for a subset of reactions to optimize amplification: 94°C for 3 min, followed by 5 cycles of 94°C for 40 s, 45°C for 30 s, and 72°C for 60 s, and then 35 cycles of 94°C for 40 s, 48.5°C for 30 s, and 72°C for 60 s, and a final extension step at 72°C for 7 min. All reactions were conducted alongside a positive (human-fed mosquito) and negative (sterile nuclease-free water) control. PCR products (5 µL)

were loaded onto a 1% agarose gel stained with gelRED, electrophoresed, and visualized with UV light (Mighty Bright, Hoefer Scientific Instruments, San Francisco, CA, USA).

Samples with positive bands after gel electrophoresis were purified with FastAP and Exonuclease (ThermoFisher Scientific, Waltham, MA, USA) and submitted for Sanger sequencing at the Cornell University Biotechnology Resources Center. Sequences were compared to the available database in NCBI Basic Local Alignment Search Tool (BLASTn) and were identified to a source if matches were ≥98% with a sequence of known origin (with the exception of an eastern gray squirrel (*Sciurus carolinensis*) sequence, which had a 95.5% match).

Host Availability

Household Interviews:

To estimate host availability, household interviews were conducted weekly at time of collection (see Supplemental Materials S1). Residents were asked about the number of people and pets living in their house and the amount of time spent outside by species that day and the two days prior. Interviews were conducted in English or Spanish depending on homeowner preference.

Camera Traps:

Two motion-triggered camera traps (Moultrie M-880, #MCG-12691, Calera, AL, USA) were set at each site from 16 July to 13 August 2018 on selected properties in residential sites and different locations within farm sites. Cameras were operated according to the setting, height, and angle specifications described by Linske et al.[30], with the exclusion of scent lures. Camera data were used to estimate host abundance by determining the number of animal encounters with the camera per trap day. If a given species was photographed within 30 min of the last image of that animal, it was considered the same individual and was not counted separately. If multiple

individuals were captured in one image within 30 min of last sighting, the count was equal to the maximum number captured together in an image.

Fitness by Host Species

Mosquito Rearing: Mosquitoes were collected from four towns on Long Island, NY and reared in colony for six to ten generations. Eggs from Baltimore, MD (between F_3 and F_6 depending on replicate) were reared synchronously with the Long Island colony in order to assess between population differences. For each replicate, eggs were vacuum hatched, provided with a pinch of pulverized fish food (crushed Cichlid Gold™ fish food pellets; Hikari, Himeji, Japan), and one day later, separated into trays of 200 larvae, with 1L of distilled water, and 4 Cichlid Gold™ fish food pellets. Adult mosquitoes were maintained in an environmental chamber (28°C, 71.9% ± 9.5% relative humidity, 10 hr light, 10 hr dark, 2 hr dusk/dawn). Cups of 200 pupae were placed into cages inside the chamber, and upon eclosion, 10% sucrose was provided for 2-4 d. Males were removed and sucrose was replaced with distilled water for 1 d prior to blood feeding.

Blood: Human (Lampire Biologicals; Pipersville, PA, USA), opossum (The Janet L. Swanson Wildlife Health Center; Ithaca, NY, USA), rat (The Center for Animal Resources and Education, Cornell University), cat (The Center for Animal Resources and Education at Cornell University; Ithaca, NY, USA) and horse (Lampire Biologicals; Pipersville, PA, USA) blood treated with anticoagulant (sodium citrate) was stored at -20°C upon arrival. Blood was thawed in warm water immediately before use. Mosquito blood feeding was conducted with artificial feeders (water reservoir at 37°C and de-salted sausage casings as membrane) as described previously[31].

Within-population differences of Long Island Ae. albopictus

In order to determine whether fitness advantages for different host blood sources influence feeding patterns of Long Island *Ae. albopictus*, we assessed fecundity and survival of females after feeding on human, cat, horse, opossum, and rat blood. These species were chosen based on commonly identified blood sources in our study or in Baltimore, MD[6].

Fecundity and Survival: Fully engorged mosquitoes (approximately 35 per blood species per replicate and 3-4 replicates per group) were gently transferred individually into 0.5L paper cups with a dry oviposition vessel. Mosquitoes were maintained in the environmental chamber as described above. One day after blood feeding, strained larval rearing water was added to oviposition vessels to encourage egg lay. No additional water or sugar was provided. Each mosquito was checked daily for presence of eggs (first day of egg lay) and mortality until all females had died. Total number of eggs laid per female was recorded at the end of experiment. Dead mosquitoes were frozen at -20°C and later dissected to determine number of mature retained eggs, if any. We compared the total eggs produced (retained + laid eggs). In replicate two, mosquitoes with a large number of retained eggs were not counted and were therefore not included in the egg analyses but were included in survival analyses. For individuals where egg retention data was not available, number of eggs laid was used. The following blood types were tested: replicate one included human, rat, cat, and horse; replicates two and three included human, rat, cat, horse, and opossum; replicate four included human, rat and opossum.

Between-population differences of Long Island and Baltimore Ae. albopictus

Because of the striking differences in field-collected host blood meal sources between our study and a prior Baltimore study[6], we assessed whether fitness varied between *Ae. albopictus* from these two locations after feeding on rat (source of 72.3% of blood meals in Baltimore, and 1.1%

of blood meals in our current study of mosquitoes on Long Island) and human blood (source of 13.6% of blood meals in Baltimore and 32.2% in Long Island)[6].

Fecundity and survival: Long Island and Baltimore *Ae. albopictus* were fed rat and human blood and observed synchronously. The rat and human-fed Long Island individual mosquitoes from replicates 1-3 of the within-population fitness assessment described above were used to compare both between-population fitness of Long Island and Baltimore *Ae. albopictus* and within-population fitness of Long Island *Ae. albopictus.* The wing length of a subset of Long Island and Baltimore individuals was measured to control for body size differences between the two colonies[32, 33].

Data Analysis

Host availability

Residential Host Feeding Index: Abundance and time-weighted host feeding indices (HFI) were calculated using blood meal identification data from residential areas and household interview data for humans, cats and dogs. Feeding indices were calculated according to equations described by Kay et al. (1979) and modified by Richards et al. (2006) as follows[8, 34]:

$$\mathrm{HFI} = \frac{\mathrm{B}_x / \mathrm{B}_y}{\mathrm{H}_x / \mathrm{H}_y}$$

where B_x and B_y represent the average number of blood meals from host *x* and host *y* per household and H_x and H_y represent the average number of host *x* and host *y* residing per household. Averages were calculated with data from households positive for at least one

bloodmeal. Data were aggregated across all four residential sites because household and site-specific calculations frequently resulted in non-real values due to zeroes in the denominators.

A time-weighted feeding index [8] was calculated as follows:

$$\mathrm{HFI_T} = \mathrm{HFI}\left(\frac{\mathrm{T}_y}{\mathrm{T}_x}\right)$$

where T_y and T_x represent the time spent outside by hosts *y* and *x*, respectively. When household interview data was missing on the date of bloodmeal collection (26 of 66 surveys), the average of all other interview responses from that household was used as an approximation.

An HFI or HFI_T greater than 1 indicated that host *x* was fed upon more often than expected compared to host *y* given their abundance or time spent outside. An HFI or HFI_T equal to 1 indicated that the hosts were fed upon in proportion to their availability and an HFI or HFI_T less than 1 indicated that host *y* was fed upon more often than expected compared to host *x*. Note that while an HFI or HFI_T greater or less than 1 may reflect *Ae. albopictus* preference, it does not conclusively demonstrate it, as we cannot rule out influences from other factors such as host defenses, timing of host availability, or host location in the yard.

Residential Forage Ratio: Forage ratios are another method for determining host feeding frequency by host availability[3]. In our study, these were calculated using blood meal identification data and camera trap images from residential sites. Forage ratios were calculated for each animal species that was captured by camera traps as follows[35]:

$$\frac{\text{Number of blood meals from host } x \ / \ \text{Total number of all blood meals}}{\text{Number of host } x \text{ in the population} \ / \text{Total number of all hosts in population}}$$

In the case of this study, the proportion of all hosts represented by host *x* was approximated by the proportion of all camera trap images that were taken of host *x*. Because camera traps were only placed in 2 properties per site, forage ratio calculations were limited to animals that tend to cross freely between yards, including all wild animals and cats, but excluding humans and dogs.

A forage ratio greater than one suggests that the host was fed upon more often than expected given its abundance and less than one suggests that the host was fed upon less often than expected. A forage ratio equal to one indicates that the host was fed upon in proportion to its abundance in the population. As with host feeding indices, forage ratios may reflect preference but do not prove it because the same sources of bias may impact these results.

Farm Host Availability: At the farm sites, host feeding indices and forage ratios were not calculated due to small sample sizes and technical difficulties of defining host availability. Interviews of human and domestic animal availability were only conducted once at farms during the last week of collections. Farm owners could not accurately estimate human exposure due to unpredictable influx of people on site for riding lessons and farm work. Animal exposure could not be reliably measured because of inconsistent use of fenced paddocks and semi-enclosed barns. Camera traps were positioned in order to picture wild animals at the outskirts of the fenced paddocks and therefore did not often picture domestic farm animals. Interview and camera trap data is reported for each but are only qualitatively compared to blood meal data; no further calculations were conducted.

Life table studies- fitness by host species

Within-population differences: The effect of host blood source on egg production (fecundity) was assessed with a linear model, including replicate and mosquito survival as covariates. The effect of host blood source on mosquito survival was also determined using a linear model, including replicate as a covariate. Estimated marginal means *post hoc* analyses were conducted using the emmeans package[36]. Survival curves were created with the average proportion surviving across the replicates and compared for each host blood species. The basic reproductive rate (R_0) was calculated for each blood type and replicate according to previously described equations [37]. The effect of blood type on R_0 was compared via a linear model.

Between-population differences: Egg production and survival were compared between human/rat, Long Island/ Baltimore groups using linear models, as described above. However, in this case, number of eggs produced by each individual was divided by average wing length of the cohort, reported as eggs per mm wing length (eggs/mm wl), in order to control for the effect of body size, which differed between Baltimore and Long Island colonies despite identical rearing.

Ethics approval:

Survey protocols were reviewed and considered exempt by Cornell University's Institutional Review Board (IRB).

Results

Blood Meal Identification: 3,241 *Ae. albopictus* were collected over the course of the summer (1,575 female and 1,666 male) and 182 (14% of aspirator-collected females) were blood-fed. Of these, 152 blood meals were less than half digested. Host identity was successfully assigned to

90 samples (49.5%), including 29 human (*Homo sapiens;* 32.2%), 22 cat (*Felis catus;* 24.4%), 16 horse (*Equus caballus;* 17.8%), 12 opossum (*Didelphis virginiana;* 13.3%), 5 dog (*Canis lupus familiaris;* 5.6%), 2 goat (*Capra hircus;* 2.2%), and 1 each of rabbit (*Sylvilagus floridanus*; 1.1%), rat (*Rattus norvegicus*; 1.1%), squirrel (*Sciurus carolinensis*; 1.1%), and racoon (*Procyon lotor*; 1.1%). When divided into residential (n=66) and farm sites (n=24), most of the residential blood meals were from humans (40.9%), followed by cat (31.8%) and opossum (18.2%). The majority of farm blood meals were from horses (66.7%), followed by human (8.3%) and goat (8.3%) (Figure 1).

Host availability

Residential Host Feeding Index: Household interview and blood meal data were used to calculate host feeding indices (HFIs), indicative of relative tendency to feed on certain vertebrate hosts at all residential properties where blood meals were collected (n=28) (Table 2). The most human blood meals were collected per property (0.96 ± 0.21), followed by cat (0.75 ± 0.17), and dog (0.18 ± 0.09). Similarly, there were the most human residents per property (3.18 ± 0.36), followed by cat (0.39 ± 0.19), and dog (0.29 ± 0.10). However, cats spent the most time outside over the 2 days prior to collection (278.74 ± 232.93 min), followed by humans (234.26 ± 49.83 min), and dogs (53.61 ± 22.05 min). The standard error in cat time was large because some individuals were outdoor cats (24 hrs/d) while others were only allowed outside for short periods of time.

Table 2

Mean (± SE) number of blood meals, residents, and time spent outside for humans, cats and dogs per property

	Mean (± SE) per property		
Host	Blood meal	Residents	Time outside (min)
Human	0.96 (0.21)	3.18 (0.36)	234.26 (49.83)
Cat	0.75 (0.17)	0.39 (0.19)	278.74 (232.93)
Dog	0.18 (0.09)	0.29 (0.10)	53.61 (22.05)

Mean numbers of blood meals and residents were used to calculate pairwise comparisons of feeding between humans, cats, and dogs through abundance and time-weighted HFIs (Table 3). Human vs cat HFI and HFI_T both demonstrate a tendency to feed on cats compared to humans (0.16 and 0.20). Likewise, human vs dog HFI and HFI_T both suggest that *Ae. albopictus* feeds disproportionately often on dogs compared to humans (0.49 and 0.14). However, cat vs dog HFI and HFI_T produced opposite results: according to abundance measures, cats were fed upon disproportionately more often compared to dogs (3.05), but when time-weighted, dogs were fed upon disproportionately more often compared to cats (0.73). On average, cats spent much more time outside than dogs, causing the directionality change of the index. Furthermore, neither HFI metric demonstrates a particularly strong deviance from the expected feeding proportions, suggesting that *Ae. albopictus* may not have a strong preference between cats and dogs.

Table 3

346 Abundance and time-weighted host feeding indices

Index	Human vs Cat	Human vs Dog	Cat vs Dog
HFI	0.16	0.49	3.05
HFI_T	0.20	0.14	0.73

347

348 **Residential Forage Ratio**: Forage ratios (FRs) were calculated from camera trap data at the 4
349 residential sites for all animals for which camera trap images were taken or blood meals
350 collected (Table 4). Cats and opossums were fed upon more often than expected given their
351 relative abundance in the host population. Of all residential blood meals taken from free roaming
352 species (i.e., not humans and dogs), 65.7 ± 10.2% were derived from cats, but only 27.4 ± 10.9%
353 of all images were taken of cats, resulting in a 3.56 ± 0.98 FR (above the FR=1 threshold to infer
354 preference). Opossum blood meals accounted for 31.8 ± 10.8% of all blood meals but no
355 opossums were pictured, resulting in an undefined FR, suggesting preference for opossums.
356 Raccoons, the other nocturnal animal, were pictured often (24.8 ± 16.4% of all images) but only
357 represented 2.5 ± 2.5% of all blood meals, resulting in a FR below 1 (0.046 ± 0.046), suggesting
358 avoidance. Squirrels and birds were also pictured often (21.6 ± 10.5% and 26.2 ± 11.2%
359 respectively) but no blood meals were collected at residential sites, resulting in a FR of 0,
360 suggesting avoidance.

361

362 **Table 4**

363 Mean (± SE) percentage of all blood meals, percentage of all animals and forage ratio for all
364 animals for which camera trap images were taken or blood meals collected at residential sites
365 (n=4) in Suffolk Country, NY.

	Mean (± SE)		
	% of all blood meals	% of all images	Forage Ratio
Cat	65.7 (10.2)	27.3 (10.9)	3.6 (1.0)
Possum	31.8 (10.8)	0 (0)	∞*
Raccoon	2.5 (2.5)	24.8 (16.4)	0.05 (0.05)
Squirrel	0 (0)	21.6 (10.5)	0 (0)
Bird	0 (0)	26.2 (11.2)	0 (0)

366 *FR was infinite because division by zero is undefined

367

368 **Farm Host Availability**: Approximate numbers and time spent outside for humans and domestic
369 animals were reported by the farm owners. At Farm A, approximately nine people spent time at
370 the farm for a total of 52 hours per day. The farm also had 40 horses, spending a total of 70 hrs/d
371 outside. At Farm A, 3.6% of camera trap images were of cats, 67.9% of raccoons, 17.9% of
372 foxes, 3.6% of deer, and 7.1% of squirrels. Blood meals collected at Farm A included 6 horse
373 and 1 squirrel.

374 Farm B estimated that 30 people (180 hrs), 100 horses (200 hrs), 2 dogs (26 hrs), and 2
375 goats (26 hrs) were outside on the property per day. Of all camera trap images at Farm B, 37.1%

were of cats, 44.3% of raccoons, 4.1% of opossums, 5.2% of deer, 5.2% of squirrels, and 4.1% of rabbits. The blood meals consisted of 5 horses, 1 human, and 1 rabbit.

Farm C estimated that 7 people (11 hrs), 46 horses (420 hrs), 2 dogs (12 hrs), 18 chickens (171 hrs), 4 ducks (38 hrs), and 1 goose (24 hrs) spent time outside per day. The most images were taken of cats (48.8%), followed by birds (23.3%), raccoons (14.0%), squirrels (9.3%) and rabbits (4.7%). Blood meals included 4 horses and 1 cat.

Farm D estimated that 3 people (14 hrs), 8 horses (48 hrs), 2 dogs (8 hrs), 20 goats (260 hrs), 4 sheep (52 hrs), 1 alpaca (24 hrs), 1 llama (24 hrs), 20 rabbits (260 hrs), 9 ducks (117 hrs), and 30 chickens (720 hrs) spent time outside per day. The camera trap pictured raccoons (33.3%) and birds (66.7%). Blood meals collected included: 2 goat, 1 horse, 1 human, and 1 rat.

Despite the diversity of hosts available at the 4 farm sites, the predominant blood meal identified at three of these sites was horse. The fourth farm was an anomaly, with more blood meals collected from goats than horses, but it was also the only farm where more goats were available than horses. Once again, raccoons were pictured at all sites, but no blood meals were collected, further suggesting avoidance of this animal. Birds were pictured frequently at 2 sites, and no blood meals collected, also suggesting avoidance.

Fitness by Host Species

Within-population differences of Long Island Aedes albopictus

The proportions of *Ae. albopictus* that laid and retained mature eggs and mean (± SE) number of eggs produced are reported in Table 5.

Table 5: Egg production by blood meal source for Long Island *Ae. albopictus*

Blood Source	Proportion which laid eggs (%)	Proportion with retained eggs (%)*	Mean eggs produced (± SE)
Cat	57/89 (64.0)	10/89 (11.2)	40.3 (4.0)
Horse	70/97 (72.2)	11/97 (11.3)	48.5 (3.9)
Human	104/121 (86.0)	23/121 (19.0)	61.0 (2.9)
Rat	100/122 (82.0)	16/122 (13.1)	53.5 (3.7)
Opossum	64/86 (74.4)	10/86 (11.6)	58.7 (4.8)

*Includes mosquitoes with any number of retained eggs

Females that ingested cat blood resulted in lower fecundity compared to those fed human and opossum blood (β= -17.3, SE=5.3, *P*=0.01 and β= -20.9, SE=5.9, *P*=0.004, respectively). There was no significant difference between any other blood group (Figure 2a). There was also no significant effect of survival time on number of eggs produced (although only one blood meal was provided in this study, which may limit impact of extended survival). On average, all blood groups began laying on day 3 post-blood meal, and all blood groups survived for 7-9 days. Notably, there were significant differences between replicates; mosquitoes in replicate 1 produced more eggs than replicate 2 and 3 (β= 30.67, SE=447, *P*<0.0001 and β= 35.69, SE=4.41, *P*<0.0001, respectively) and mosquitoes in replicates 2 and 3 produced fewer eggs than replicate 4 (β= -30.37, SE=5.37, *P*<0.0001 and β= -35.39, SE=5.38, *P*<0.0001, respectively).

There were no significant differences in survival time between any of the host blood groups (Figure 2b). Mosquitoes fed cat blood survived (± SE) 7.6 (±0.45) days, horse-fed survived 8.6 (±0.48) days, human-fed survived 9.6 (±0.3) days, rat-fed survived 8.7 (±0.4) days, and opossum-fed survived 9.5 (±0.6) days. There were significant differences in survival by replicate: replicate 1 had higher survival than replicate 3 (β=1.7, SE=0.5, *P*=0.006) and replicates 1, 2, and 3 had lower survival than replicate 4 (β=-3.5, SE=0.6, *P*<0.0001 ; β= -4.5, SE=0.6, *P*<0.0001 ; β=-5.1, SE=0.6, *P*<0.0001 respectively). Daily survival curves averaged over the three or four replicates are presented in Figure 3.

The mean (±SE) R_0 across replicates was 19.5 (±6.5) for Long Island *Ae. albopictus* fed cat blood, 22.9 (±5.7) for horse blood, 29.7 (±4.1) for human, 27.1 (±8.9) for opossum, and 27.0 (±4.1) for rat. No significant differences in (R_0) were found by host blood group.

Between-population differences of Long Island and Baltimore Ae. albopictus

The proportions of *Ae. albopictus* that laid and retained mature eggs, mean (± SE) eggs, and mean (± SE) eggs/mm wl is reported in Table 6.

Table 6: Egg production for Long Island and Baltimore *Aedes albopictus* females fed human or rat blood

Origin and Blood Source	Proportion which laid eggs (%)	Proportion with retained eggs (%)	Mean eggs produced (± SE)	Mean eggs/mm w produced (± SE)
Baltimore Human	73/89 (82.0)	12/89 (13.5)	41.4 (3.2)	14.7 (1.1)
Baltimore Rat	70/95 (73.7)	11/95 (11.6)	38.2 (3.5)	13.6 (1.3)
Long Island Human	76/89 (85.4)	17/89 (19.1)	58.8 (3.6)	20.7 (1.3)
Long Island Rat	75/95 (78.9)	13/95 (13.7)	46.1 (4.0)	16.2 (1.4)

The only significant differences in eggs produced per mm wing length were between Long Island mosquitoes fed human blood and the three other groups (Figure 4a). Baltimore mosquitoes fed human (β= -6.0, SE=1.8, *P*=0.0008) and rat blood (β= -6.9, SE=1.8, *P*=0.0001) produced fewer eggs/mm wl than Long Island mosquitoes fed human blood. Long Island mosquitoes fed human blood produced more eggs per mm wl than those fed rat blood (β= 3.8, SE=1.8, *P*=0.03). Baltimore mosquitoes fed rat blood produced marginally fewer eggs/mm wl than Long Island mosquitoes fed rat blood (β= -3.1, SE=1.7, *P*=0.07). There was no significant difference in eggs produced/mm wl between Baltimore mosquitoes fed human and rat blood (β= 1.0, SE=1.8, *P*=0.6) or Baltimore mosquitoes fed human blood and Long Island mosquitoes fed rat blood (β= -2.1, SE=1.8, *P*=0.2).

436 The mean (± SE) survival time of Baltimore *Ae. albopictus* was significantly higher for
437 human blood (9.6 days ±0.4) compared to rat blood (7.2 days ±0.4) (β= 2.3, SE=0.5, *P*=0.0001).
438 The same survival trend was observed for Long Island *Ae. albopictus* where mosquitoes fed
439 human blood survived marginally longer than those fed rat blood (9.0 days ±0.3 and 7.7 days
440 ±0.4 respectively: β= 1.3, SE=0.5, *P*=0.08) (Figure 4b). Baltimore mosquitoes fed human blood
441 survived significantly longer compared to Long Island mosquitoes fed rat blood (β=1.9, SE=0.5,
442 *P*=0.002). Survival time was significantly lower for Baltimore mosquitoes fed rat blood
443 compared to Long Island mosquitoes fed human blood (β=-1.7, SE=0.5, *P*=0.008). There was no
444 significant difference in survival time between mosquitoes fed human blood from both sites (β=
445 0.6, SE=0.5, *P*=0.6) or fed rat blood from both sites (β= -0.4, SE=0.5, *P*=0.8). We did detect
446 differences by replicate, where replicate 1 had a higher survival than replicate 2 (β= 1.4, SE=0.5,
447 *P*=0.006) while paired replicates 1-3 and 2-3 had no significant difference in survival (β= 0.9,
448 SE=0.4, *P*=0.1 and β= -0.6, SE=0.5, *P*=0.4 respectively). Daily survival curves averaged over
449 the three replicates are presented in Figure 5.

450 The mean (±SE) basic reproductive rate (R_0) (averaged over 3 replicates) of Baltimore
451 *Ae. albopictus* fed human blood is 20.4 (±1.2), 19.7 (± 4.6) for Baltimore rat, 29.3 (± 5.7) for
452 Long Island, and 24.5 (± 4.5) for Long Island rat. No significant differences occurred between R_0
453 of any of the blood groups.

454

455 **Discussion**

456 Mosquito feeding behavior plays a vital role in disease transmission, however, it can be difficult
457 to understand and predict because there are diverse factors that influence feeding behavior in

458 nature. We investigated the feeding patterns of the globally invasive vector, *Ae. albopictus*, in
459 farm and residential habitats at the northern edge of its range in the United States. In tandem, we
460 addressed two factors that may influence these patterns: host availability and variation in
461 mosquito fitness from different host blood sources.

462 The ten host species we detected in *Ae. albopictus* blood meals from Long Island, NY are
463 hosts previously reported for this species elsewhere in the world. The proportion of human blood
464 meals (32.2%) identified in Long Island was lower than reported in many other locations
465 worldwide[3, 4, 10, 11, 13, 14, 15, 16, 17, 18, 38], but was higher than in other studies from the United States
466 (Hawaii, Missouri, North Carolina, and Maryland)[5, 6, 7, 8]. More *Ae. albopictus* fed on cats in our
467 study on Long Island than in any other location previously reported. The third most common
468 host for this mosquito species on Long Island, the horse, has only been detected in four of
469 sixteen previous *Ae. albopictus* blood meal studies and at lower levels[7, 8, 13, 14]. Similarly, the
470 fourth most common host, opossum, has been reported in four previous studies, also at lower
471 levels[5, 8, 10, 15]. Long Island *Aedes albopictus* fed less frequently on dogs compared to the
472 representative proportion in numerous other studies[7]. Notably absent from the Long Island blood
473 meals were cows, deer, and birds, all of which were present on at least one site in our study and
474 have been detected in at least six previous blood meal studies. It is possible that a larger
475 sampling of blood meals may have revealed these hosts, however, birds have also been absent
476 from other studies in Northeastern USA[6, 10, 15]. Notably, only about half of collected blood meals
477 were successfully identified to species, but the reason for the low success rate is unknown. It is
478 possible that this may have biased the species that were identified, however, tests of primer
479 versatility performed by Reeves et al. (2018) showed amplification for the majority of vertebrate
480 species (90/93)[29].

481 This is only the third study of *Ae. albopictus* blood feeding biology that quantitatively
482 assessed host availability, and the first to do so with wild animals. Abundance and time-weighted
483 host feeding indices (HFIs) calculated using household interview data revealed
484 disproportionately high levels of feeding on cats and dogs compared to humans. Richards et al.
485 (2006) reported a similar trend for HFIs based on host abundance in North Carolina, but when
486 time-weighted, found that humans were fed upon disproportionately often compared to cats and
487 dogs[8]. In Brazil, HFIs based on host abundance showed the opposite trend to ours, suggesting
488 that *Ae. albopictus* fed disproportionately often on humans compared to cats and dogs[13]. These
489 results highlight the need for additional studies that measure host availability and also suggest a
490 need for caution when extrapolating these results to make conclusions about innate mosquito
491 preference. In both Long Island and North Carolina, collections were only conducted at a subset
492 of houses per neighborhood, allowing for the movement of blood fed mosquitoes from properties
493 where interviews were not conducted. Flight range for engorged blood fed *Ae. albopictus* is not
494 known, but reported range of other blood fed species suggest that movement between properties
495 is possible after feeding, as do records of *Ae. albopictus* dispersal between blood feeding and
496 oviposition[39, 40, 41, 42]. Furthermore, household interview data depends on accurate self-reporting
497 of outdoor activity, which may be unreliable[43]. This inaccuracy of outdoor time estimates is
498 compounded if the interview is only administered once for the entire sampling period, such as in
499 Richards et al. (2006)[8].

500 We also assessed host availability through camera traps in order to calculate forage ratios
501 for free-roaming animals, which suggest a tendency to feed on cats and opossums and to avoid
502 raccoons, squirrels, and birds compared to their relative abundance in residential sites. While
503 camera traps do not provide a perfect measure of host abundance, it is considered a robust

504 method for mammal inventories[44]. Camera traps may be less useful in estimating bird
505 abundance[45], however, birds were one of the most frequently photographed groups of animals in
506 our study, but were not fed upon, so improved accuracy in estimating bird abundance would not
507 have altered conclusions drawn from forage ratio calculations.

508 Despite limitations, estimating host availability and abundance in conjunction with blood
509 meal studies is much more informative than studies that lack such data. By understanding more
510 about the context in which a certain feeding pattern arose, more general conclusions can be
511 drawn about feeding behavior. The patterns revealed after accounting for host availability can be
512 caused by numerous factors, such as host defenses. This may explain the high number of
513 opossum blood meals because this nocturnal marsupial would likely be asleep, with decreased
514 self-defense, during *Ae. albopictus* daytime biting activity. However, raccoons are also nocturnal
515 and in contrast, were fed upon less often than expected, suggesting that innate preferences or
516 other factors could potentially also be at play. Only two preference studies have been conducted
517 for *Ae. albopictus*; in La Reunion Island, a no-choice blood feeding experiment on 12 animal
518 species found chicken, human, dog and cow were fed upon more often than duck, shrew, rat, pig,
519 mouse, goat, gecko, and chameleon[20]. Subsequently, a choice experiment showed higher
520 attraction to humans compared to chicken, dog, cow and goat[20]. However, large and small
521 animals were treated differently and were not given equal opportunities for self-defense,
522 potentially affecting results. In Thailand, landing catches demonstrated preference for humans
523 compared to pigs, buffalo, dogs, and chickens; however, the use of a second human to catch
524 mosquitoes from the non-human animals may have impacted results. It therefore remains
525 unclear whether *Ae. albopictus* has innate host preference.

One mechanism by which host preferences may evolve is through natural selection whereby feeding on a certain host enhances reproductive fitness, leading selection to favor genetic variants with preference for that host[46]. This is known to be the case for other species, such as *Ae. aegypti*[23]. We investigated the potential role of fitness in driving *Ae. albopictus* feeding patterns by assessing survival and egg production of mosquitoes fed on several host species in the Northeastern United States. Within the Long Island *Ae. albopictus* population, we found that host species had very limited impact on survival, egg production, or basic reproductive rate. The only significant differences were lower egg production after feeding on cats compared to humans and opossums, and no significant differences in survival. Interestingly, the reduced fecundity on cat blood is opposite to what we might expect based on the feeding index, which suggested a tendency to feed more often on cats compared to humans. A previous report from Baltimore of high feeding rates on rats, led us to compare the fitness of Long Island and Baltimore *Ae. albopictus* after feeding on human and rat blood. Specifically, we investigated whether differences in fitness may be driving the striking differences in feeding patterns between the two locations. However, the only significant difference was higher egg production by Long Island mosquitoes fed human blood than all three other groups. If egg production was driving this difference, we would expect to also see higher egg production for Baltimore mosquitoes fed rat compared to human blood, but this was not the case. Furthermore, survival of mosquitoes fed on human blood was longer than those fed on rat blood for both Baltimore and Long Island *Ae. albopictus.* Together, these results suggest that fitness advantage does not drive different feeding patterns in these two locations.

The impact of host species on *Ae. albopictus* egg production has only been assessed twice before. Gubler (1970) found greater fecundity for mouse-fed females, followed by guinea pig,

549 rat, and chicken; however, the study was not replicated and no statistical analyses were
550 conducted[25]. In another study, chicken-fed *Ae. albopictus* were less fecund than those offered
551 guinea pig or human blood and, consistent with our results, no differences between the two
552 mammal species were found[24]. These results do not demonstrate a selective pressure for *Ae.*
553 *albopictus* to evolve preferences within mammalian hosts. However, preference can evolve
554 through other pathways and should be assessed directly. Other specialist feeders lack apparent
555 fitness advantages for their preferred host. For example, *Anopheles gambiae* has a well-
556 established preference for humans, but in a single study conducted to date, there is no fitness
557 advantage provided by a human-only diet compared to a generalist diet[47].

558 It is also possible that when assessed under different conditions, differences in fitness by
559 host species may be revealed. For instance, we did not provide the mosquitoes with sugar after
560 blood feeding; the presence of sugar has been shown to reduce reproductive fitness in *Ae.*
561 *albopictus* compared to human blood alone and mosquitoes on Long Island feed frequently on
562 sugar[33, 48]. For *Ae. aegypti*, the addition of sugar changed the directionality of host species effects
563 on fitness, shifting the fitness benefits from human to mouse blood[23]. If a similar phenomenon
564 exists for *Ae. albopictus*, the absence of sugar in our experiments would maximize the fitness of
565 human blood compared to other species. We also only provided the mosquitoes with one blood
566 meal. Providing a more natural series of blood meals may have influenced our results.

567 *Aedes albopictus* is often referred to as anthropophilic due to the high percentage of
568 human blood meals in numerous field studies and the preference assessments conducted by
569 Delatte et al. (2010)[20]. However, this classification remains unproven. In fact, our results are
570 more indicative of a generally mammalophilic feeding behavior for *Ae. albopictus*. It is
571 important to understand the underlying blood feeding behavior and physiology of *Ae. albopictus*

572 because it influences and modulates the feeding patterns in the field, which will ultimately
573 influence pathogen transmission[19]. In Long Island, the diverse utilization of hosts in residential
574 and farm settings demonstrates that *Ae. albopictus* could serve as an enzootic bridge vector.
575 However, the absence of bird blood meals suggests that *Ae. albopictus* may be of limited concern
576 as a vector of West Nile and Eastern equine encephalitis viruses in the Northeastern US.
577 Populations of *Ae. albopictus* in this region have sufficient vector competence to transmit
578 numerous anthroponotic viruses[49, 50, 51], but transmission of these pathogens may be limited due
579 to lower rates of human feeding compared to other regions[52].

580 Our results provide insight into disease transmission risk by *Ae. albopictus* in
581 Northeastern United States. Additionally, our observations reveal that host availability has a
582 major impact on feeding patterns, but did not fully explain blood meal distribution. Fitness
583 benefits did not explain the feeding patterns observed in Long Island or Baltimore, highlighting
584 the need for further research on determinants of *Ae. albopictus* feeding behavior.

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597

598 **Figure Legends:**

599 **Figure 1. Distribution of blood meals by site type**

600 At residential sites, most *Ae. albopictus* fed on human, followed by cat and opossum. At farm
601 sites, the majority fed on horse, followed by human and goat.

602

603 **Figure 2 a)** Box plot of number of eggs produced by Long Island mosquitoes fed cat, horse,
604 human, rat, and opossum blood. Blood types that do not share a letter above boxes are
605 significantly different. **b)** Box plot of survival time in days. Blood types that do not share a letter
606 inside boxes are significantly different.

607

608 **Figure 3:** Survival of Long Island *Ae. albopictus* by host blood ingested

609

610 **Figure 4 a)** Box plot of number of eggs produced by Baltimore and Long Island mosquitoes fed
611 rat and human blood. Groups that do not share a common letter are significantly different. **b)**
612 Box plot of survival in days. Groups that do not share a common letter are significantly different.

613

614 **Figure 5:** Survival of Baltimore and Long Island *Ae. albopictus* fed human and rat blood. Curves
615 are averaged over three replicates.

616

617 **References Cited**

618

619 1. Gratz N, 2004. Critical review of the vector status of *Aedes albopictus*. Med vet entomol
620 18: 215-227.
621 2. Kraemer MU, Sinka ME, Duda KA, Mylne AQ, Shearer FM, Barker CM, Moore CG,
622 Carvalho RG, Coelho GE, Van Bortel WJe, 2015. The global distribution of the arbovirus
623 vectors *Aedes aegypti* and *Ae. albopictus*. eLife 4: e08347.

3. Ponlawat A, Harrington LC, 2005. Blood feeding patterns of *Aedes aegypti* and *Aedes albopictus* in Thailand. J Med Entomol 42: 844-849.
4. Kamgang B, Nchoutpouen E, Simard F, Paupy C, 2012. Notes on the blood-feeding behavior of *Aedes albopictus* (Diptera: Culicidae) in Cameroon. Parasite Vector 5: 4.
5. Savage HM, Niebylski ML, Smith GC, Mitchell CJ, Craig GB, 1993. Host-feeding patterns of *Aedes albopictus* (Diptera, Culicidae) at a temperate North American site. Journal of Medical Entomology 30: 27-34.
6. Goodman H, Egizi A, Fonseca DM, Leisnham PT, LaDeau SL, 2018. Primary blood-hosts of mosquitoes are influenced by social and ecological conditions in a complex urban landscape. Parasite Vector 11: 218.
7. Tempelis CH, Hayes RO, Hess AD, Reeves WC, 1970. Blood-feeding habits of 4 species of mosquito found in Hawaii. Am J Trop Med Hyg 19: 335-&.
8. Richards SL, Ponnusamy L, Unnasch TR, Hassan HK, Apperson CS, 2006. Host-feeding patterns of *Aedes albopictus* (Diptera : Culicidae) in relation to availability of human and domestic animals in suburban landscapes of central North Carolina. Journal of Medical Entomology 43: 543-551.
9. Gomes AC, Silva NN, Marques G, Brito M, 2003. Host-feeding patterns of potential human disease vectors in the Paraiba Valley Region, State of Sao Paulo, Brazil. J Vector Ecol 28: 74-78.
10. Faraji A, Egizi A, Fonseca DM, Unlu I, Crepeau T, Healy SP, Gaugler R, 2014. Comparative Host Feeding Patterns of the Asian Tiger Mosquito, *Aedes albopictus*, in Urban and Suburban Northeastern USA and Implications for Disease Transmission. Plos Neglect Trop D 8: 11.
11. Sawabe K, Isawa H, Hoshin K, Sasaki T, Roychoudhury S, Higa Y, Kasai S, Tsuda Y, Nishiumi I, Hisai N, Hamao S, Kobayashi M, 2010. Host-Feeding Habits of *Culex pipiens* and *Aedes albopictus* (Diptera: Culicidae) Collected at the Urban and Suburban Residential Areas of Japan. J Med Entomol 47: 442-450.
12. Niebylski ML, Savage HM, Nasci RS, Craig GB, 1994. Blood hosts of *Aedes albopictus* in the United States. Journal of the American Mosquito Control Association 10: 447-450.
13. Gomes AC, Silva NN, Marques G, Brito M, 2003. Host-feeding patterns of potential human disease vectors in the Paraiba Valley Region, State of Sao Paulo, Brazil. Journal of Vector Ecology 28: 74-78.
14. Valerio L, Marini F, Bongiorno G, Facchinelli L, Pombi M, Caputo B, Maroli M, della Torre A, 2010. Host-Feeding Patterns of *Aedes albopictus* (Diptera: Culicidae) in Urban and Rural Contexts within Rome Province, Italy. Vector-Borne Zoonot 10: 291-294.
15. Egizi A, Healy SP, Fonseca DM, 2013. Rapid blood meal scoring in anthropophilic *Aedes albopictus* and application of PCR blocking to avoid pseudogenes. Infect Genet Evol 16: 122-128.
16. Guo XX, Li CX, Wang G, Zheng Z, Dong YD, Zhang YM, Xing D, Zhao TY, 2014. Host feeding patterns of mosquitoes in a rural malaria-endemic region in Hainan Island, China. J Am Mosquito Contr 30: 309-311.
17. Sivan A, Shriram AN, Sunish IP, Vidhya PT, 2015. Host-feeding pattern of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in heterogeneous landscapes of South Andaman, Andaman and Nicobar Islands, India. Parasitology Research 114: 3539-3546.
18. Kim H, Yu HM, Lim HW, Yang SC, Roh JY, Chang KS, Shin EH, Ju YR, Lee WG, 2017. Host-feeding pattern and dengue virus detection of *Aedes albopictus* (Diptera:

Culicidae) captured in an urban park in Korea. Journal of Asia-Pacific Entomology 20: 809-813.

19. Takken W, Verhulst NO, 2013. Host preferences of blood-feeding mosquitoes. Annu rev entomol 58: 433-453.
20. Delatte H, Desvars A, Bouétard A, Bord S, Gimonneau G, Vourc'h G, Fontenille D, 2010. Blood-feeding behavior of *Aedes albopictus*, a vector of Chikungunya on La Réunion. Vector-Borne Zoonot 10: 249-258.
21. Sullivan MF, Gould DJ, Maneechai S, 1971. Observations on host range and feeding preferences of *Aedes albopictus* (Skuse). J Med Entomol 8: 713-+.
22. McBride CS, Baier F, Omondi AB, Spitzer SA, Lutomiah J, Sang R, Ignell R, Vosshall LBJN, 2014. Evolution of mosquito preference for humans linked to an odorant receptor. 515: 222.
23. Harrington LC, Edman JD, Scott TW, 2001. Why do female *Aedes aegypti* (Diptera: Culicidae) feed preferentially and frequently on human blood? J Med Entomol 38: 411-422.
24. Xue RD, Barnard DR, Ali A, 2009. Influence of multiple blood meals on gonotrophic dissociation and fecundity in *Aedes albopictus*. J Am Mosquito Contr 25: 504-507.
25. Gubler D, 1970. Comparison of reproductive potentials of *Aedes (Stegomyia) albopictus* Skuse and *Aedes (Stegomyia) polynesiensis* Marks. Mosq News 30: 201-9.
26. Harrington LC, Shragai T, 2016. New York State Tiger Mosquito Education Network (Tiger NET).
27. Shragai T, Harrington LC, 2018. *Aedes albopictus* (Diptera: Culicidae) on an Invasive Edge: Abundance, Spatial Distribution, and Habitat Usage of Larvae and Pupae Across Urban and Socioeconomic Environmental Gradients. J Med Entomol.
28. Andreadis TG, Thomas MC, Shepard JJ, 2005. Identification guide to the mosquitoes of Connecticut: Connecticut Agricultural Experiment Station.
29. Reeves LE, Gillett-Kaufman JL, Kawahara AY, Kaufman PE, 2018. Barcoding blood meals: New vertebrate-specific primer sets for assigning taxonomic identities to host DNA from mosquito blood meals. PLoS Neglect Trop D 12: e0006767.
30. Linske MA, Williams SC, Stafford III KC, Ortega IM, 2018. *Ixodes scapularis* (Acari: Ixodidae) Reservoir Host Diversity and Abundance Impacts on Dilution of *Borrelia burgdorferi* (Spirochaetales: Spirochaetaceae) in Residential and Woodland Habitats in Connecticut, United States. J Med Entomol 55: 681-690.
31. Ledesma N, Harrington L, 2015. Fine-scale temperature fluctuation and modulation of *Dirofilaria immitis* larval development in *Aedes aegypti*. Veterinary parasitology 209: 93-100.
32. Nasci RS, 1990. Relationship of wing length to adult dry weight in several mosquito species (Diptera: Culicidae). J Med Entomol 27: 716-719.
33. Fikrig K, Peck S, Deckerman P, Dang SR, St Fleur K, Goldsmith H, Qu S, Rosenthal H, Harrington LC, 2020. Sugar feeding patterns of New York *Aedes albopictus* mosquitoes are affected by saturation deficit, flowers, and host seeking. Plos Neglect Trop D 14: 16.
34. Kay BH, Boreham PFL, Edman JD, 1979. Application of the feeding index concept to studies of mosquito host-feeding patterns. Mosquito News 39: 68-72.
35. Hess A, Hayes RO, Tempelis C, 1968. The use of the forage ratio technique in mosquito host preference studies. Mosq News 28: 386-9.

36. Lenth R, 2019. emmeans: estimated marginal means, aka least-squares means. R package v. 1.3. 4.
37. Southwood T, 1978. Introduction to the study of animal populations. Ecological Methods: Springer, 1-6.
38. Kek R, Hapuarachchi HC, Chung CY, Bin Humaidi M, Razak M, Chiang S, Lee C, Tan CH, Yap G, Chong CS, Lee KS, Ng LC, 2014. Feeding Host Range of *Aedes albopictus* (Diptera: Culicidae) Demonstrates Its Opportunistic Host-Seeking Behavior in Rural Singapore. Journal of Medical Entomology 51: 880-884.
39. Tuten HC, Bridges WC, Paul KS, Adler PH, 2012. Blood-feeding ecology of mosquitoes in zoos. Med Vet Entomol 26: 407-416.
40. Greenberg JA, DiMenna MA, Hanelt B, Hofkin BV, 2012. Analysis of post□blood meal flight distances in mosquitoes utilizing zoo animal blood meals. J Vector Ecol 37: 83-89.
41. Marini F, Caputo B, Pombi M, Tarsitani G, Della Torre A, 2010. Study of *Aedes albopictus* dispersal in Rome, Italy, using sticky traps in mark–release–recapture experiments. Med Vet Entomol 24: 361-368.
42. Honório NA, Silva WdC, Leite PJ, Gonçalves JM, Lounibos LP, Lourenço-de-Oliveira R, 2003. Dispersal of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in an urban endemic dengue area in the State of Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz 98: 191-198.
43. Alshurafa N, Jain J, Stump TK, Spring B, Robinson JK, 2019. Assessing recall of personal sun exposure by integrating UV dosimeter and self-reported data with a network flow framework. Plos one 14: e0225371.
44. Silveira L, Jacomo AT, Diniz-Filho JAF, 2003. Camera trap, line transect census and track surveys: a comparative evaluation. Biol conserv 114: 351-355.
45. O'Brien TG, Kinnaird MF, 2008. A picture is worth a thousand words: the application of camera trapping to the study of birds. Bird Conservation International 18: S144-S162.
46. Lyimo IN, Ferguson HM, 2009. Ecological and evolutionary determinants of host species choice in mosquito vectors. Trends in Parasitology 25: 189-196.
47. Lyimo I, Keegan S, Ranford□Cartwright L, Ferguson H, 2012. The impact of uniform and mixed species blood meals on the fitness of the mosquito vector *Anopheles gambiae* ss: does a specialist pay for diversifying its host species diet? Journal of Evolutionary Biology 25: 452-460.
48. Braks MAH, Juliano SA, Lounibos LP, 2006. Superior reproductive success on human blood without sugar is not limited to highly anthropophilic mosquito species. Med Vet Entomol 20: 53-59.
49. Sanchez-Vargas I, Harrington LC, Black WC, Olson KE, 2019. Analysis of salivary glands and saliva from *Aedes albopictus* and *Aedes aegypti* infected with chikungunya viruses. Insects 10: 39.
50. Dieme C, Ciota AT, Kramer LD, 2020. Transmission potential of Mayaro virus by *Aedes albopictus*, and *Anopheles quadrimaculatus* from United States. Parasite Vector 13: 1-6.
51. Kaczmarek ME, Herzog NL, Noval MG, Zuzworsky J, Shah Z, Bajwa WI, Stapleford KA, 2020. Distinct New York City *Aedes albopictus* Mosquito Populations Display Differences in Salivary Gland Protein D7 Diversity and Chikungunya Virus Replication. Viruses 12: 698.
52. Olson MF, Ndeffo-Mbah ML, Juarez JG, Garcia-Luna S, Martin E, Borucki MK, Frank M, Estrada-Franco JG, Rodríguez-Pérez MA, Fernández-Santos NA, 2020. High rate of

761 non-human feeding by *Aedes aegypti* reduces Zika virus transmission in South Texas.
762 Viruses 12: 453.

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765

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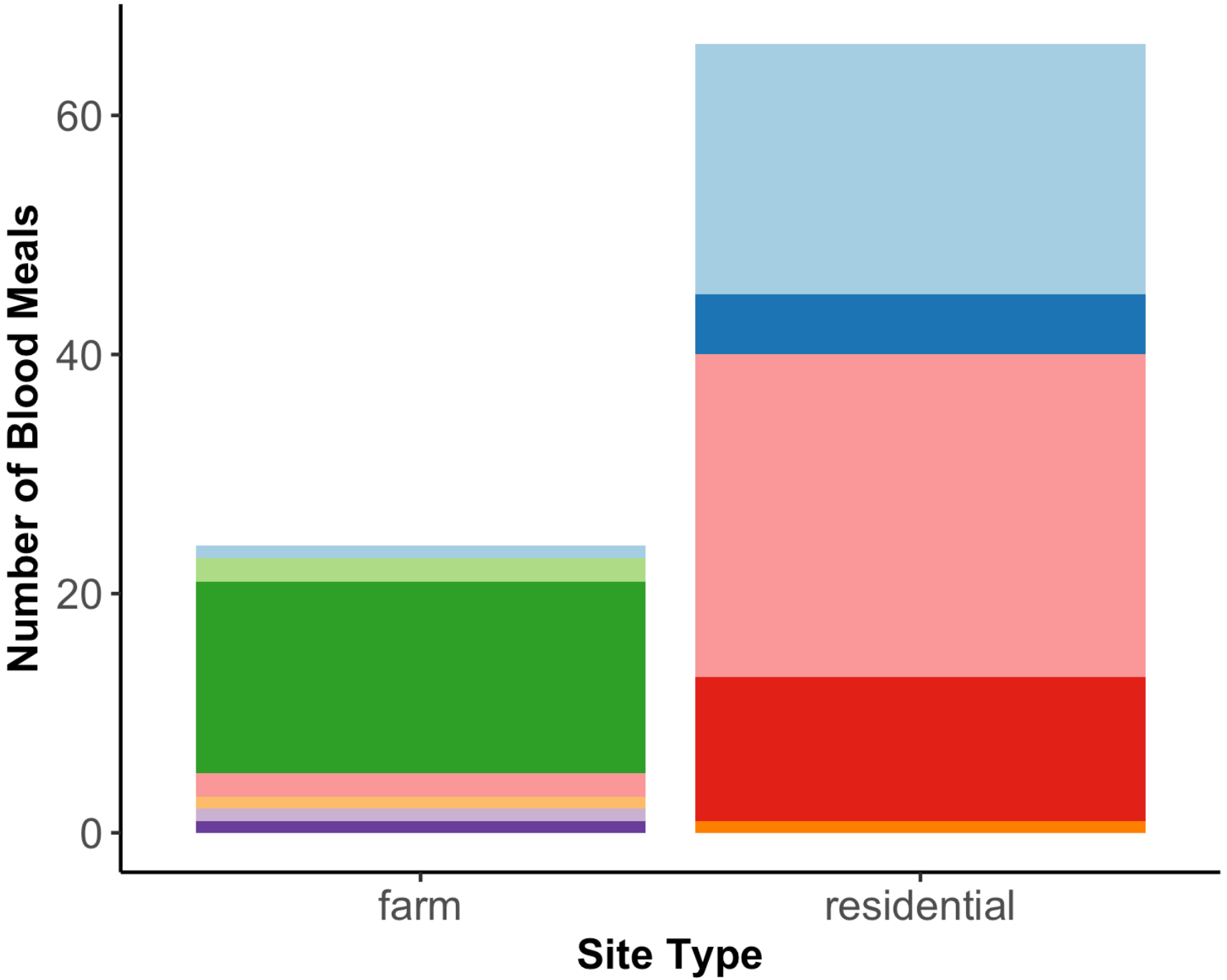
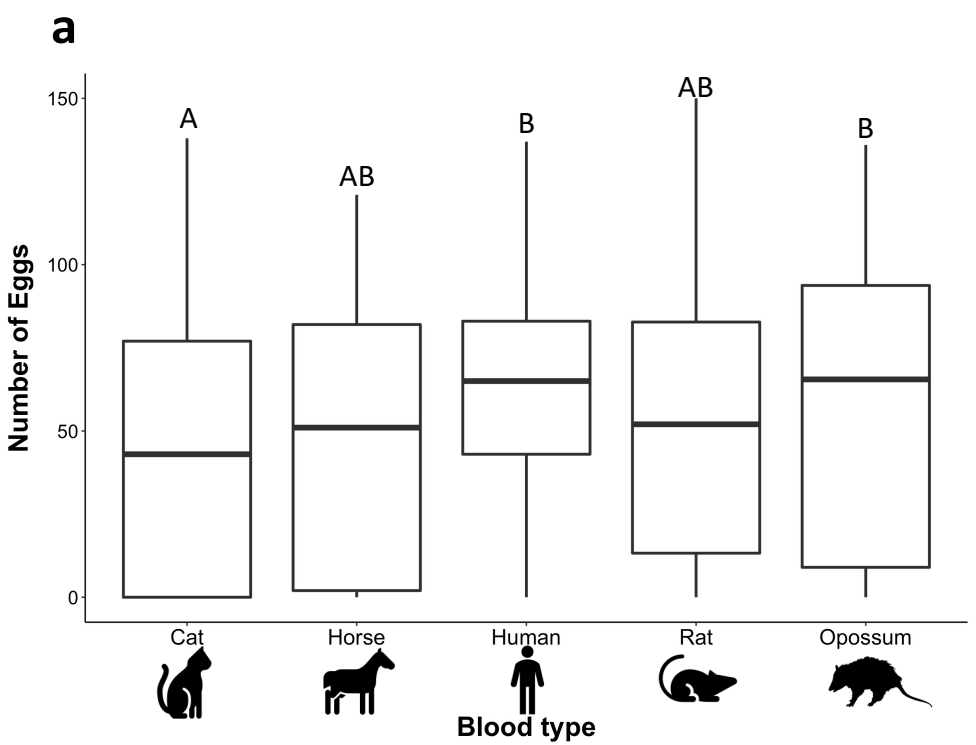
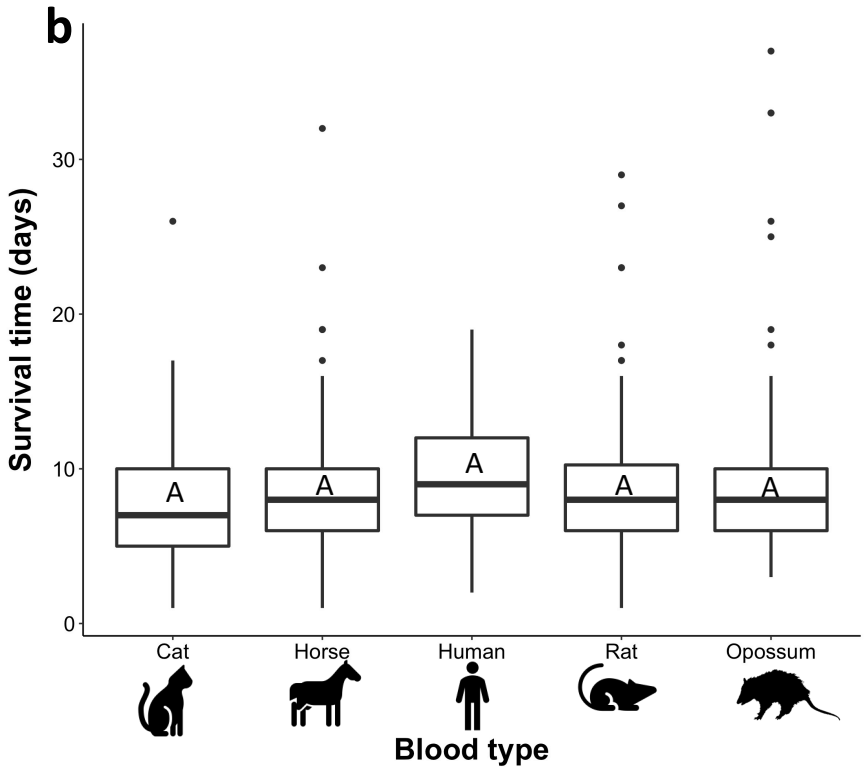
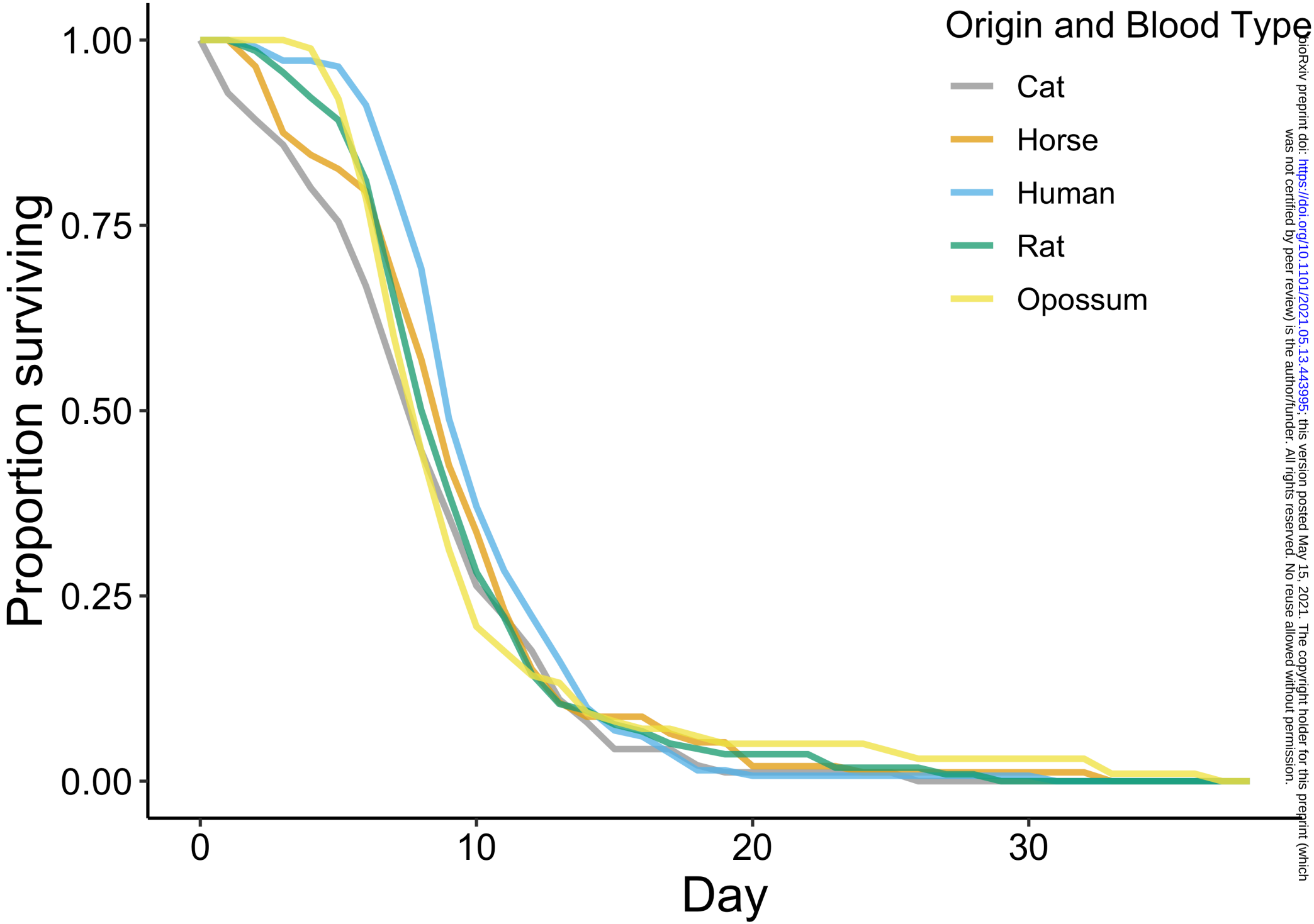
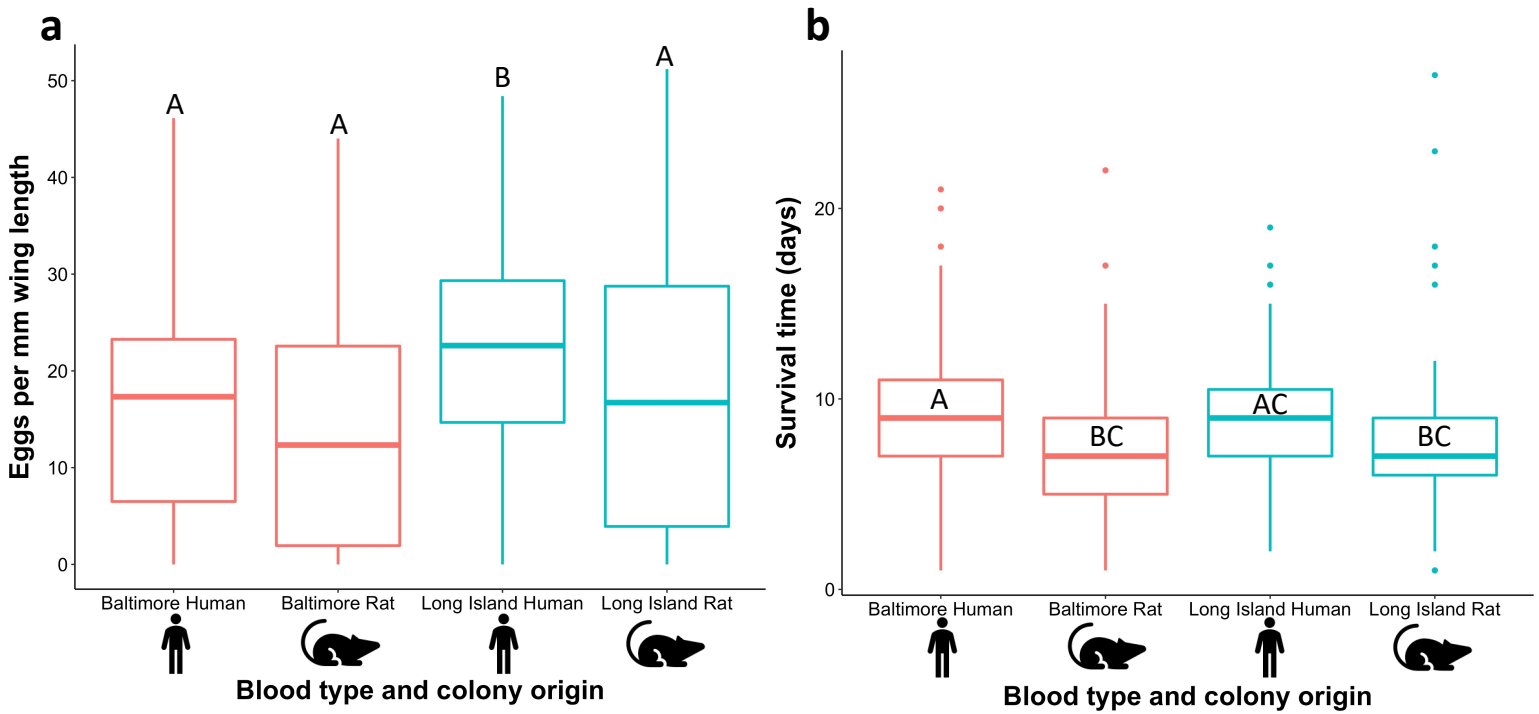



a
Number of Eggs
150
100
50
0
A
AB
B
AB
B
Cat
Horse
Human
Rat
Opossum
Blood type


b
Survival time (days)
30
20
10
0
A
A
A
A
A
Cat
Horse
Human
Rat
Opossum
Blood type




a
Eggs per mm wing length
50
40
30
20
10
0
A
A
B
A
Baltimore Human
Baltimore Rat
Long Island Human
Long Island Rat
Blood type and colony origin
b
Survival time (days)
20
10
0
A
BC
AC
BC
Baltimore Human
Baltimore Rat
Long Island Human
Long Island Rat
Blood type and colony origin