Supplementary Appendix

This appendix accompanies the following manuscript:

Plasmodium falciparum infection in humans and mosquitoes influence natural Anopheline biting behavior and transmission

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Note: Source data for supplementary figures are found in the Source Data file.

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Supplemental Methods

P. falciparum csp amplicon deep sequencing

All *P. falciparum*-positive DBS and reared mosquito abdomens were genotyped across a variable segment of the parasite circumsporozoite gene (*csp*) as previously described (1). Dual-indexed libraries were sequenced on an Illumina MiSeq platform, sequencing reads were demultiplexed and quality-filtered as previously described, and haplotype inference was performed using DADA2 as implemented in BRAVA (https://github.com/duke-malaria-collaboratory/BRAVA) (1). Initial haplotypes were quality-filtered as follows: haplotype length of 288 bp, minimum read depth of 125, minimum read proportion in a sample of 0.01, and minimum ratio *k/j* where *k* and *j* are haplotypes in the same sample such that *k* is one nucleotide different from *j* of 0.125. The sequence data are available from NCBI (BioProject PRJNA1064031).

Multilevel model analysis

For the multilevel risk factor analysis, we included as risk factors: gender, categorized age as of February 28, 2021 (<5, 5-15, >15), whether the person slept under a net (taken from the most recent monthly survey before the date of a matched bite; missing data was filled in using the monthly survey after the bite), and whether the person was infected with *P. falciparum* (nearest DBS result between -30 to +7 days of the bite, except if RDT+ -14 to 0 days from bite then negative after receiving treatment). We included as adjustors the number of STR-typed mosquitoes collected in the household that night, transmission season (high transmission season was defined as March to August), number of household members, whether any household member tested positive for *P. falciparum* by RDT in the previous month, and number of people sleeping in the same sleeping space as the person. Sensitivity analyses were performed on the *P. falciparum* infection risk factor by restricting to samples where the nearest DBS result was between -14 to +7 days or -7 to +7 days, or where a person was considered positive only when the nearest DBS before and after the bite were positive. Identical models were used with data subsetted to (1) only individuals infected with *P. falciparum* to identify the proportion of onward transmission to mosquitoes contributed by each group of individuals, and (2) only infectious mosquitoes to estimate relative entomological inoculation rates (EIRs).

Data structure for discrete choice models

Suppose we have a household consisting of 8 members with the following characteristics:

Member	Age category (yrs)	Gender	Slept under net	*P. falciparum* infection status
A	> 15	Male	Yes	Negative
B	> 15	Female	Yes	Negative
C	5-15	Female	Yes	Positive
D	5-15	Male	No	Positive
E	> 15	Female	No	Negative
F	< 5	Male	Yes	Positive
G	< 5	Female	Yes	Negative
H	< 5	Female	Yes	Negative

Now suppose all 8 household members were sleeping in the household on a given night in which 3 mosquito bloodmeals matched to the following people:

Mosquito ID	Mosquito species	Multisource	Mosquito *Pf* status	Member(s) bitten
M1	*An. gambiae s.s.*	No	Positive	D
M2	*An. funestus*	No	Negative	D
M3	*An. funsetus*	Yes	Positive	B & F

Then, the data structure for the weighted discrete choice model multivariable on mosquito characteristics and univariate on human gender would be as follows:

Mosquito ID	Mosquito species	Multisource	Mosquito *Pf* status	Human Gender	Choice	Weight
M1	*An. gambiae s.s.*	No	Positive	Female	0	5/8
M1	*An. gambiae s.s.*	No	Positive	Male	1	3/8
M2	*An. funestus*	No	Negative	Female	0	5/8
M2	*An. funestus*	No	Negative	Male	1	3/8
M3a	*An. funestus*	Yes	Positive	Female	1	5/8
M3a	*An. funestus*	Yes	Positive	Male	0	3/8
M3b	*An. funestus*	Yes	Positive	Female	0	5/8
M3b	*An. funsetus*	Yes	Positive	Male	1	3/8

To estimate the relative risk ratio of choosing a male host over a female host for each mosquito co-variate, we run the following using the mlogit R package:

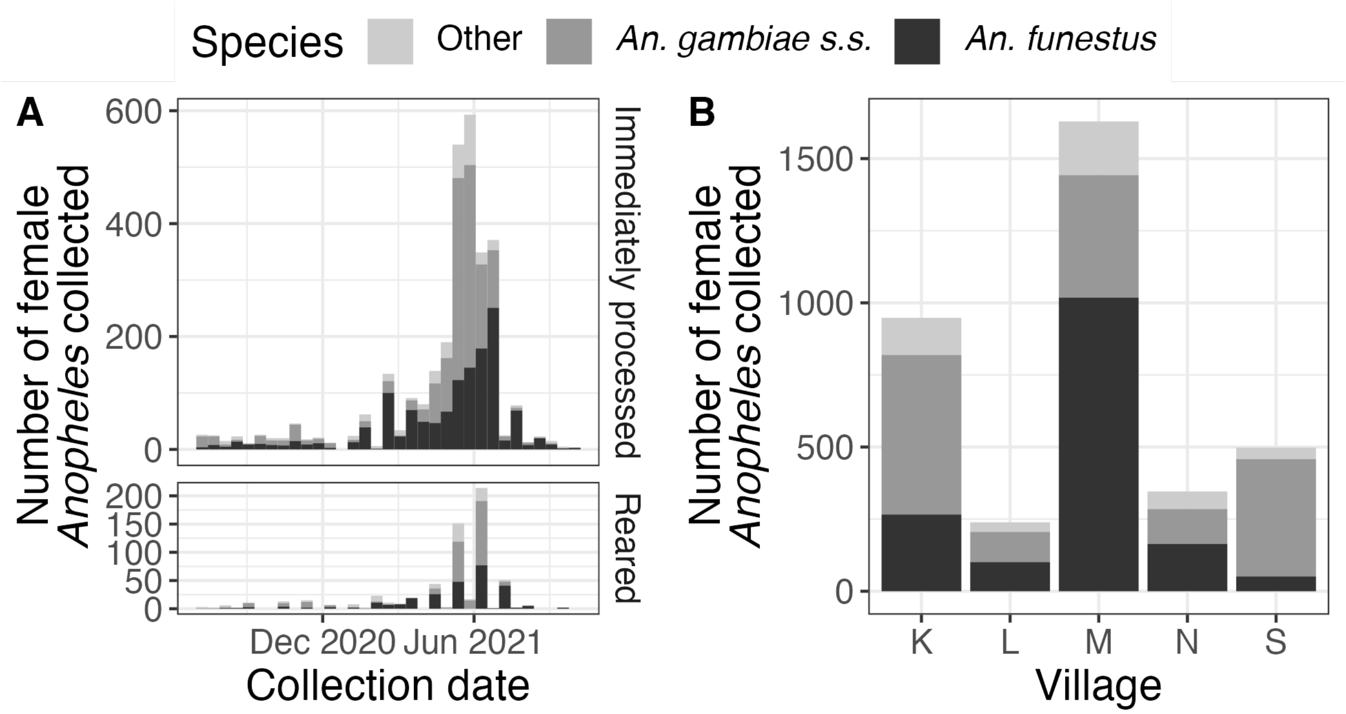
```
mlogit(choice ~ 1 | multisource + moz_head_pf_status + species, chid.var =
"index_mosquito", alt.var = 'gender', weights = weight, data = gender)
```

Multisource meals and sleeping spaces

To assess whether human characteristics associated with mosquitoes taking multisource meals were driven by shared sleeping spaces, we first generated pairs of cohort members living in the same households and assessed the relative risk that DNA from household member pairs who share sleeping spaces appeared in multisource bloodmeals compared to pairs who did not share sleeping spaces. Similarly, we assessed the unadjusted relative risk of individuals sharing a sleeping space with someone else by gender and age group. Unadjusted measures of association, wald confidence intervals, and chi squared tests were determined using the epi.2by2 function (epiR v2.0.66 (2)).

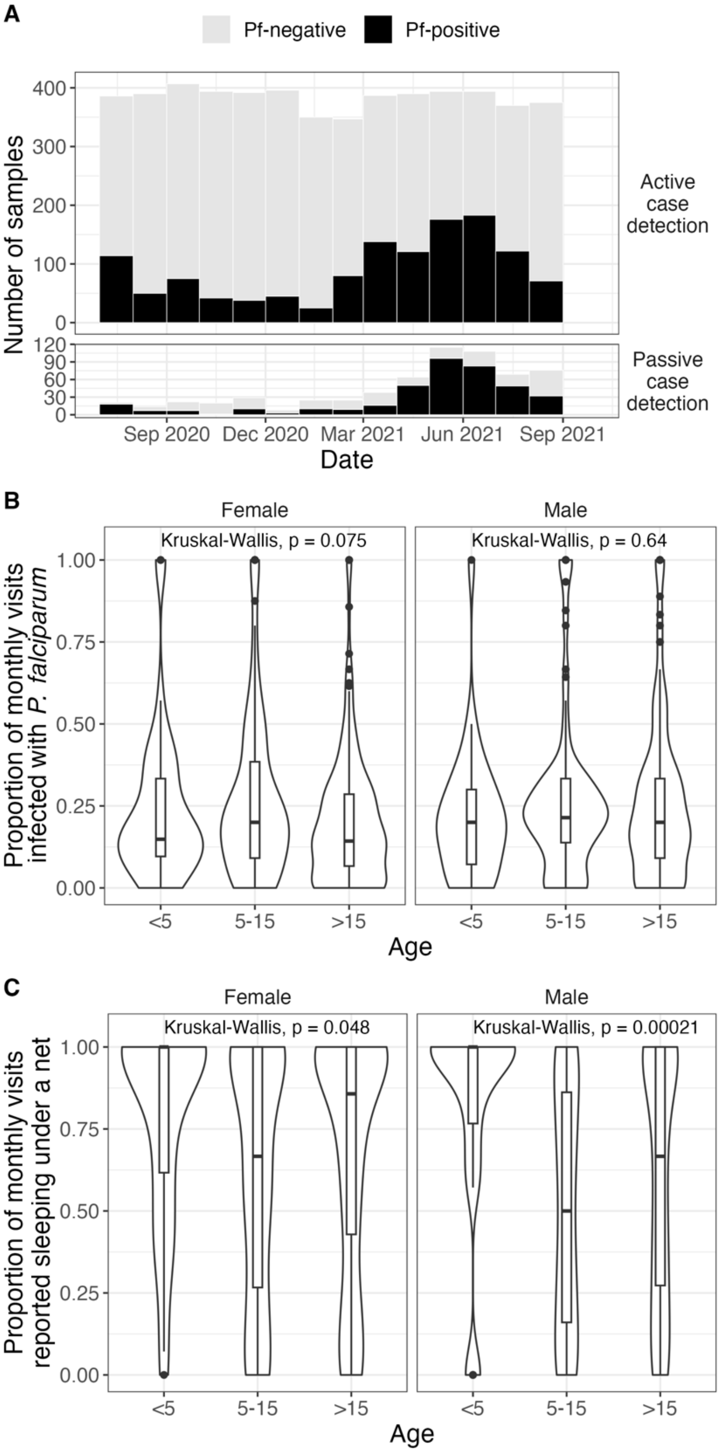
Supplemental Figures

Figure S1: Overview of female *Anopheles* collected



(A) Number of female *Anopheles* collected over time, colored by species and faceted by immediately processed vs. reared. Bin width is 2-week intervals. (B) Number of female *Anopheles* collected by village, colored by species.

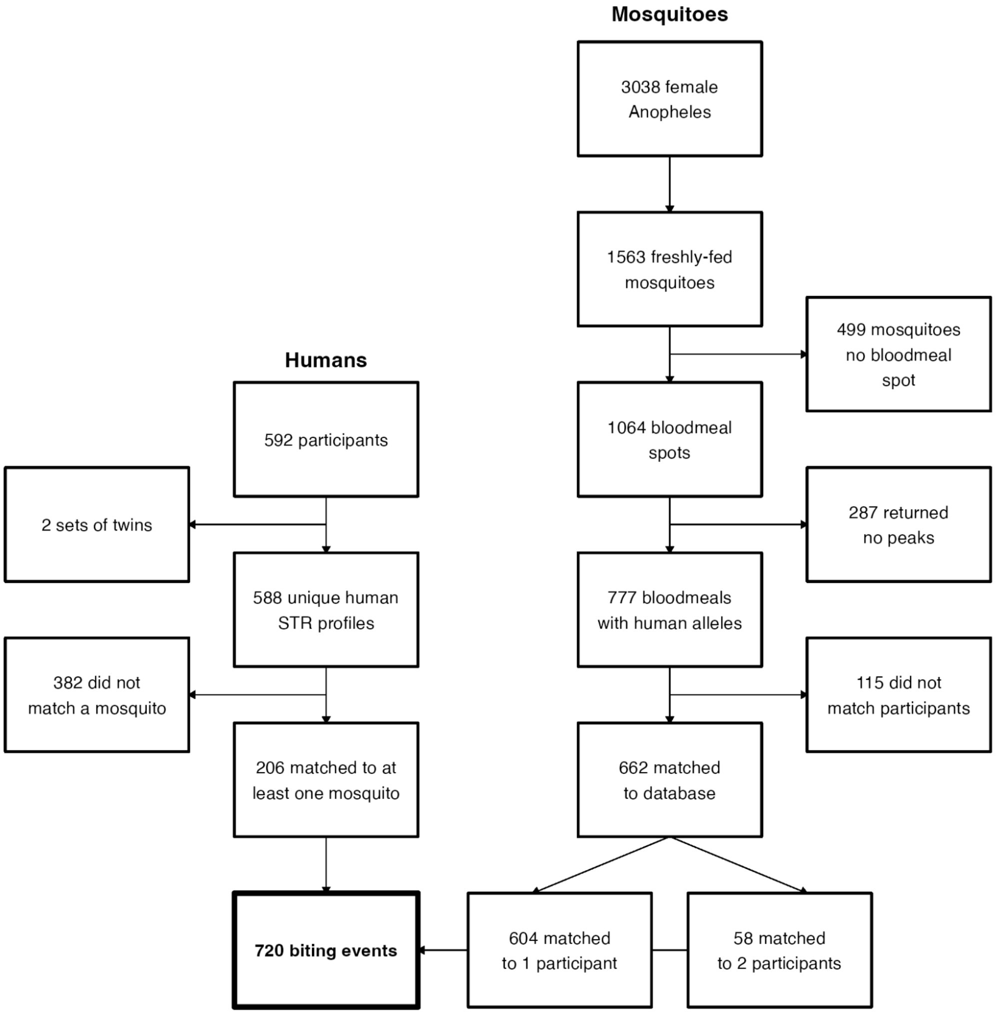
Figure S2: Overview of human infections and demographics during the study period.



(A) Human infections over time for active and passive case detection. (B) Proportion of monthly visits from active case detection that an individual was infected with *P. falciparum*, by age and gender. Kruskal-Wallis p-values between ages within a gender are shown on the plot. Kruskal-Wallis p-values between genders within age groups were 0.93 (<5 years), 0.82 (5-15 years), and 0.03 (>15 years). (C)

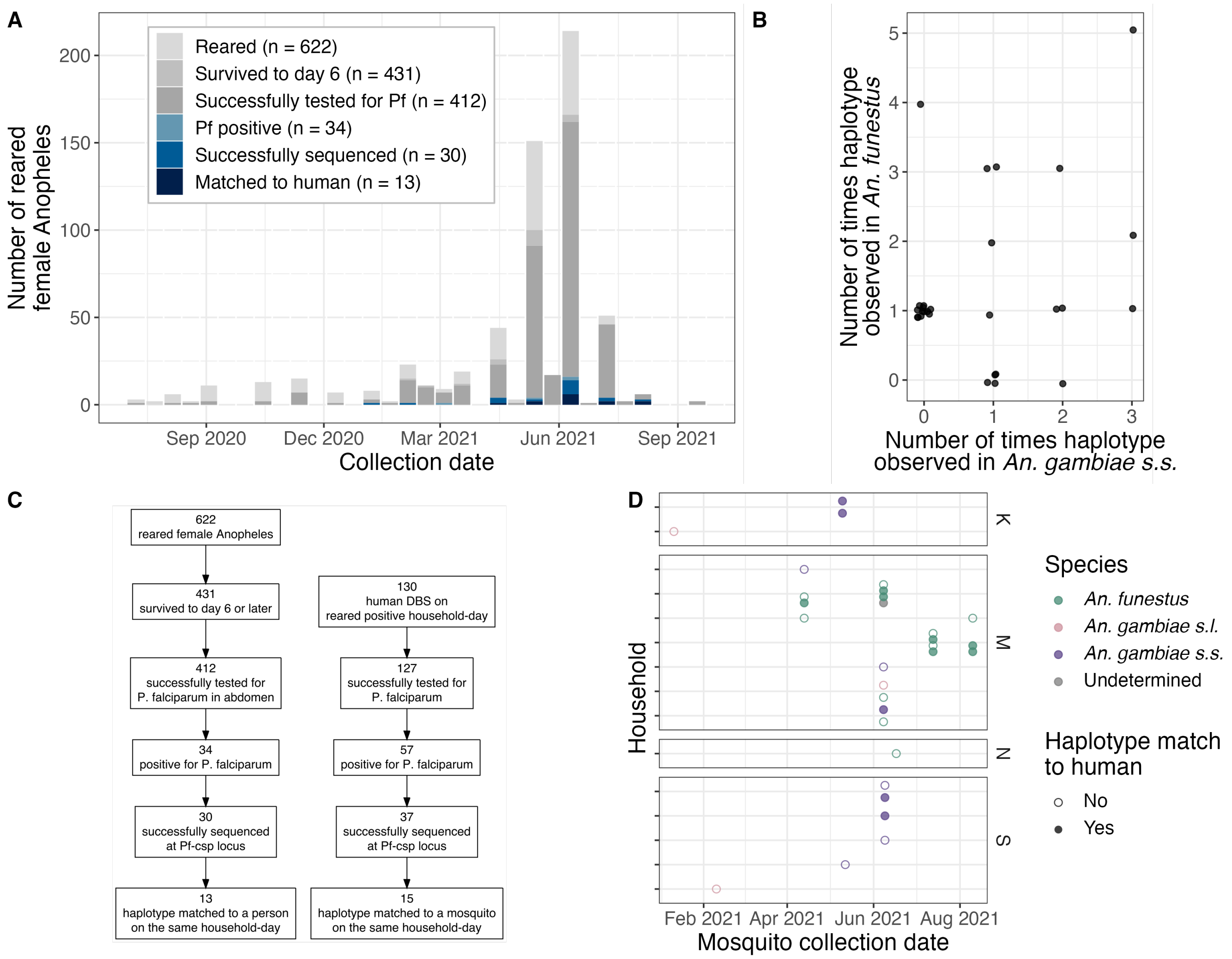
Proportion of monthly visits from active case detection where an individual reported sleeping under a bed net, by age and gender. Kruskal-Wallis p-values between ages within a gender are shown on the plot. Kruskal-Wallis p-values between genders within age groups were 0.7 (<5 years), 0.02 (5-15 years), and 0.08 (>15 years).

Figure S3: Sample flowchart for immediately processed mosquitoes



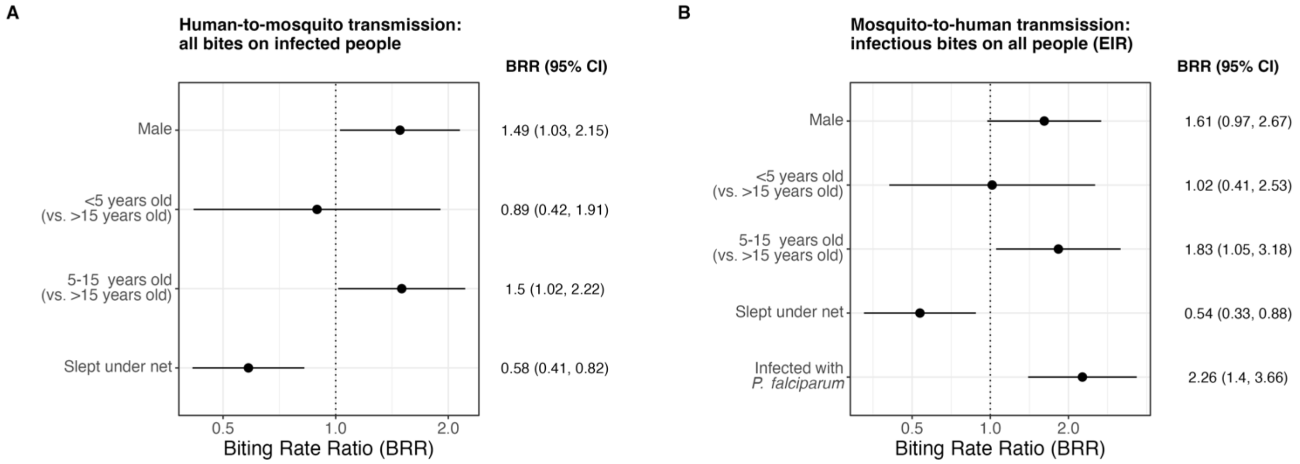
Overview of the number of mosquito bloodmeals and human participants included in analyses.

Figure S4: Overview of reared mosquito spatiotemporal *P. falciparum* haplotype matching to humans



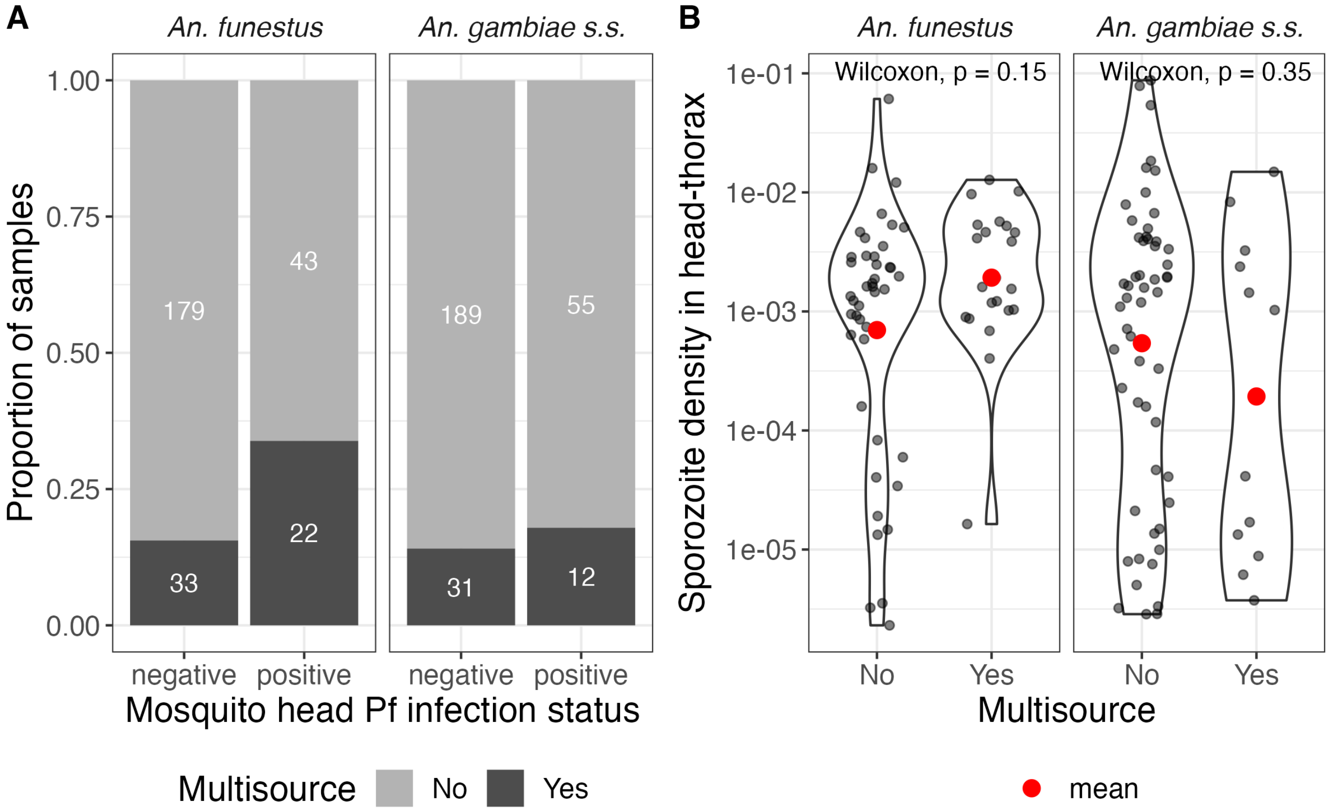
(A) Number of reared processed mosquitoes (y) by collection date (x), colored by whether they were infected with *P. falciparum* and whether their haplotypes matched to an infected person from the same household-day. (B) Number of times a haplotype was observed in *An. gambiae s.s.* vs. *An. funestus*. (C) Sample flowchart for reared mosquitoes and humans from the household-dates where and when the mosquitoes were collected. (D) Mosquitoes that had a haplotype match to a human from the same household-day over time, colored by species.

Figure S5: Biting rate ratios from multilevel sub-models



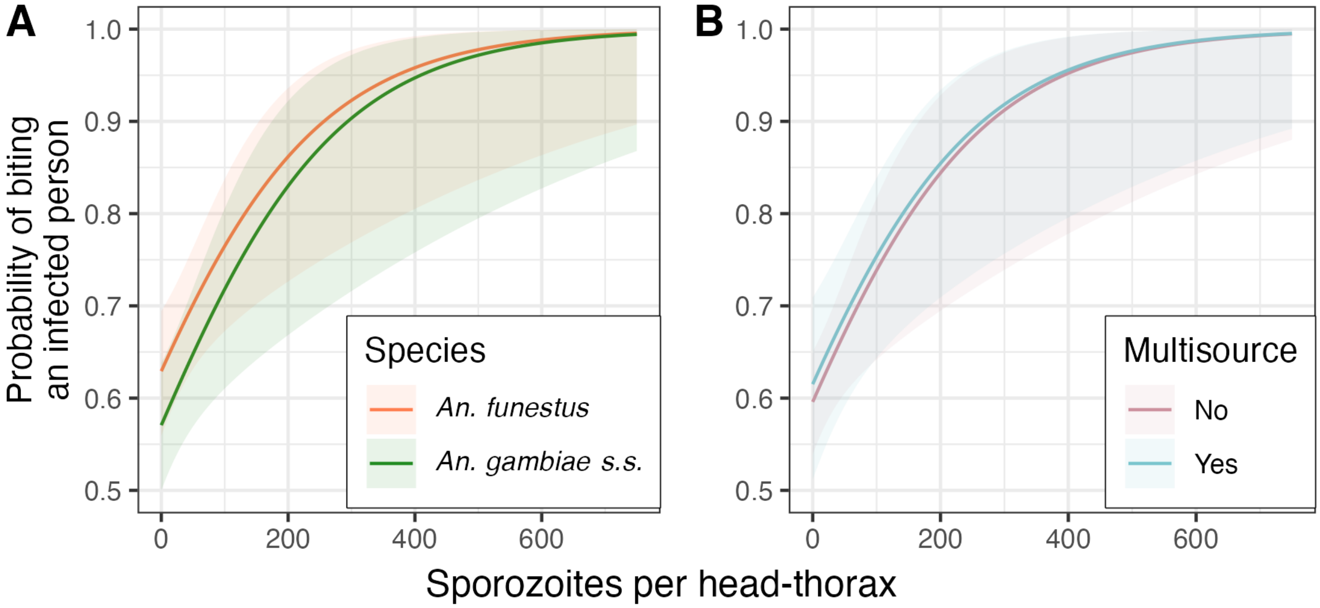
(A) Including only people infected with *P. falciparum.* (B) Including only infectious mosquitoes.

Figure S6: Sporozoite density in head-thorax by multisource and species



(A) Proportion of bloodmeals that were multisouce (dark grey) and single-source (light grey) for mosquitoes with and without P. falciparum parasites detected in the head-thorax, faceted by mosquito species. (B) Estimated sporozoite density in the head-thorax for single-source and multisource bloodmeals faceted by mosquito species (n = 65 *An. funestus*, n = 67 *An. gambiae* s.s.). Red points indicate the mean sporozoite density for each category.

Figure S7: Probability of biting an infected person as a function of sporozoite density



Faceted by species (A) and multisource bloodmeal (B).

Supplemental Tables

Table S1: Risk factor analysis biting rate ratios

Risk factor or adjustor	**Biting Rate Ratio (95% CI)**
Male	1.68 (1.28-2.19)
<5 years old (vs. >15 years old)	0.75 (0.44-1.29)
5-15 years old (vs. >15 years old)	1.49 (1.13-1.98)
Slept under net	0.51 (0.40-0.65)
Infected with *P. falciparum*	1.25 (1.01-1.55)
High transmission season	1.31 (0.98-1.75)
Number of STR-typed mosquitoes in household	1.13 (1.11-1.15)
Number of household members	0.86 (0.81-0.90)
RDT+ household member in prior month	0.86 (0.68-1.08)
Number of people in sleeping space	0.90 (0.79-1.03)

Table S2: Sensitivity analyses decreasing the amount of time allowed between monthly visit and bite

	Biting Rate Ratio (95% CI)			
Model term	**DBS window: -28 to +7 days**	**DBS window: -14 to +7 days**	**DBS window: -7 to +7 days**	**DBS+ before and after bite**
Male	1.68 (1.28-2.20)	1.58 (1.20-2.09)	1.75 (1.24-2.46)	1.76 (1.35-2.29)
<5 (vs. >15) years old	0.75 (0.44-1.29)	0.78 (0.45-1.36)	0.28 (0.10-0.84)	0.70 (0.41-1.19)
5-15 (vs. >15) years old	1.49 (1.12-1.98)	1.43 (1.07-1.92)	1.35 (0.95-1.92)	1.37 (1.04-1.80)
Slept under net	0.51 (0.40-0.65)	0.46 (0.36-0.60)	0.43 (0.31-0.60)	0.51 (0.40-0.65)
Infected with *P. falciparum*	1.25 (1.01-1.56)	1.22 (0.97-1.53)	1.22 (0.89-1.67)	1.69 (1.32-2.17)
High transmission season	1.30 (0.98-1.74)	1.29 (0.93-1.78)	1.33 (0.87-2.02)	1.25 (0.94-1.66)
Number of STR-typed mosquitoes in household	1.13 (1.11-1.15)	1.13 (1.11-1.15)	1.12 (1.09-1.14)	1.13 (1.11-1.15)
Number of household members	0.86 (0.81-0.90)	0.85 (0.80-0.90)	0.86 (0.80-0.93)	0.86 (0.82-0.91)
RDT+ household member in prior month	0.86 (0.68-1.09)	0.95 (0.73-1.22)	0.88 (0.63-1.22)	0.79 (0.62-1.00)
Number of people in sleeping space	0.90 (0.79-1.03)	0.89 (0.78-1.03)	0.93 (0.78-1.11)	0.90 (0.79-1.04)

Table S3: Choice models relative risk ratios

Mosquito feature		Relative Risk Ratio (95% CI)
Human *P. falciparum* infection: positive vs. negative (ref)		
An. funestus vs. *An. gambiae s.s.* (ref)		1.30 (0.87, 1.94)
Multisource vs. single-source bloodmeal (ref)		1.04 (0.64, 1.66)
Sporozoite positive vs. negative (ref)		2.76 (1.65, 4.61)
Slept under a net vs. did not sleep under a net (ref)		
An. funestus vs. *An. gambiae s.s.* (ref)		0.69 (0.41, 1.19)
Multisource vs. single-source bloodmeal (ref)		1.75 (0.97, 3.14)
Sporozoite positive vs. negative (ref)		0.45 (0.23, 0.86)
Human gender: male vs. female (ref)		
An. funestus vs. *An. gambiae s.s.* (ref)		1.19 (0.82, 1.72)
Multisource vs. single-source bloodmeal (ref)		0.50 (0.33, 0.77)
Sporozoite positive vs. negative (ref)		2.02 (1.28, 3.18)
Age vs > 15 years (ref)		
	< 5 years	5 - 15 years
An. funestus vs. *An. gambiae s.s.* (ref)	0.73 (0.32, 1.66)	1.72 (1.17, 2.52)
Multisource vs. single-source bloodmeal (ref)	4.10 (1.80, 9.35)	1.45 (0.89, 2.35)
Sporozoite positive vs. negative (ref)	2.69 (1.19, 6.09)	2.21 (1.37, 3.59)

Table S4: Sensitivity analysis increasing the *P. falciparum* density threshold for defining a mosquito-head thorax as sporozoite-positive.

Mosquito feature	**Sporozoite load threshold (genomes)**	**Relative Risk Ratio (95% CI)**
		Human *P. falciparum* infection: positive vs. negative (ref)
Sporozoite positive vs. negative (ref)	0	2.76 (1.65, 4.61)
Sporozoite positive vs. negative (ref)	10	3.37 (1.82, 6.25)
Sporozoite positive vs. negative (ref)	50	2.71 (1.42, 5.19)
Sporozoite positive vs. negative (ref)	100	3.25 (1.42, 7.43)
Sporozoite positive vs. negative (ref)	200	20.26 (1.57, 65.70)

Table S5: Choice model with continuous sporozoites (per 100 in the head-thorax)

Mosquito feature	**Relative Risk Ratio (95% CI)**
	Human *P. falciparum* infection: positive vs. negative (ref)
An. funestus vs. *An. gambiae s.s.* (ref)	1.28 (0.86, 1.90)
Multisource vs. single-source bloodmeal (ref)	1.08 (0.68, 1.73)
Sporozoite density in head-thorax (per 100)	1.92 (1.23, 2.98)

Supplemental References

1. Z. Lapp, *et al.*, Analytic optimization of *Plasmodium falciparum* marker gene haplotype recovery from amplicon deep sequencing of complex mixtures. 2023.08.17.23294237 (2023).

2. M. Stevenson, *et al.*, epiR: Tools for the Analysis of Epidemiological Data (2023).