Mathematical Modeling of Malaria Transmission by Mosquitoes

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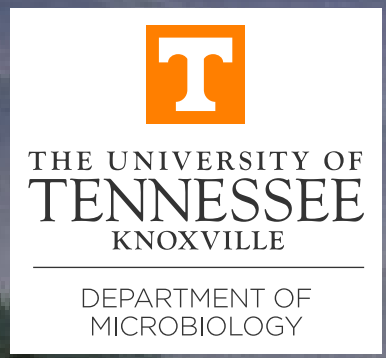
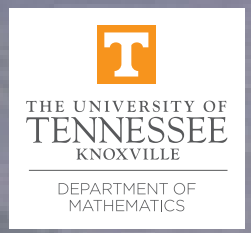
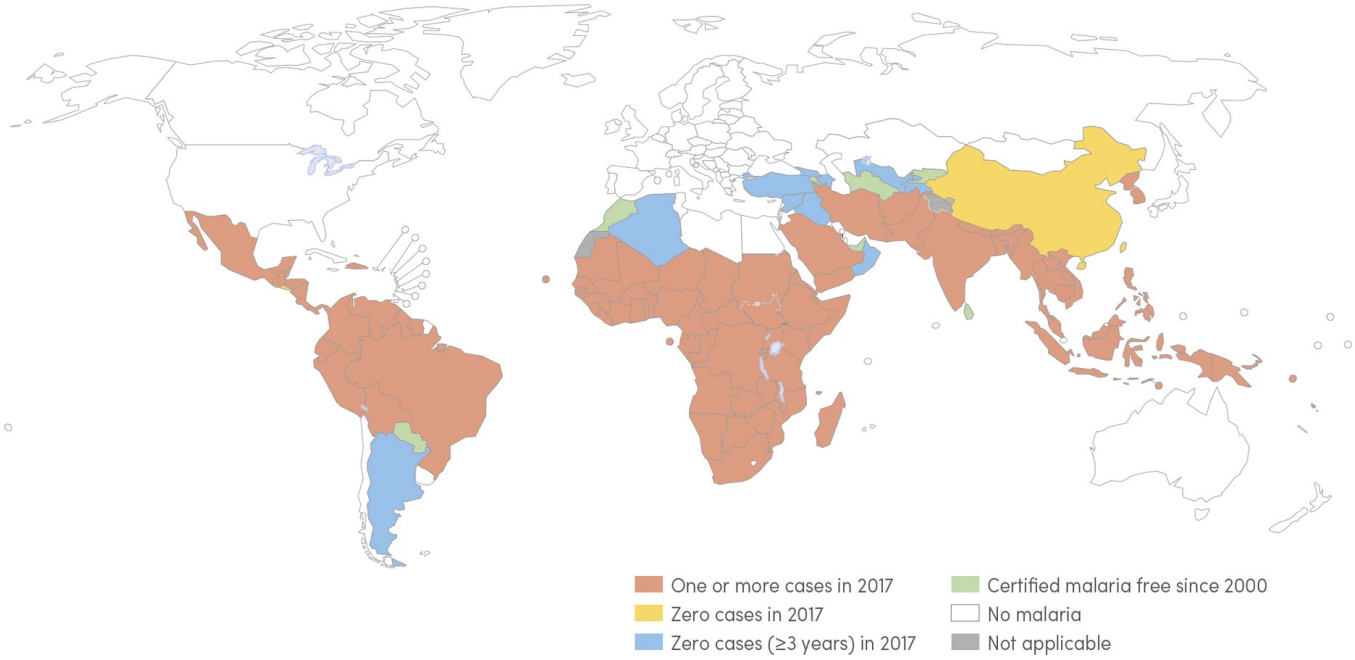




Outline of the talk

Malaria incidence in the world (2017)

Countries with indigenous cases in 2000 and their status by 2017 Countries with zero indigenous cases over at least the past 3 consecutive years are considered to be malaria free. All countries in the WHO European Region reported zero indigenous cases in 2016 and again in 2017. In 2017, both China and El Salvador reported zero indigenous cases. *Source: WHO database.*



WHO: World Health Organization.

WHO 2020

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Murray et al. Lancet 2014; WHO 2020

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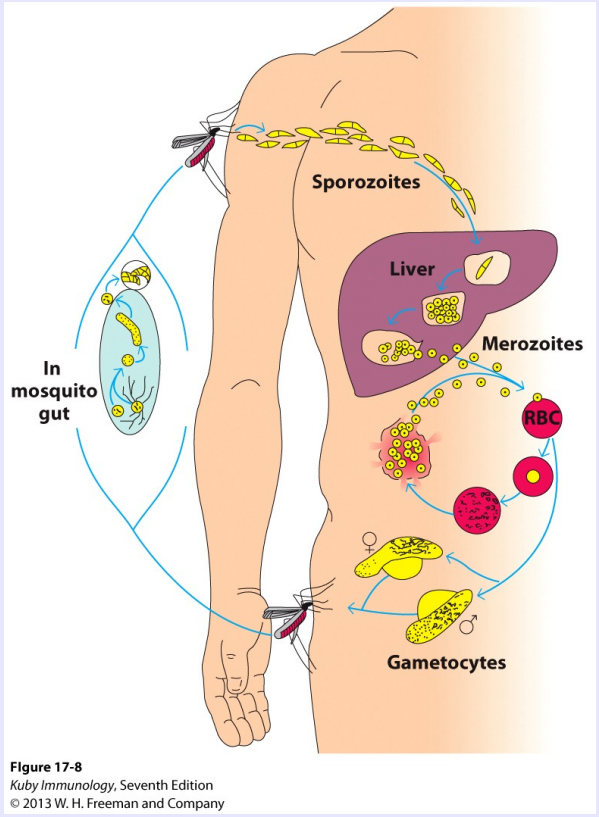
- Control measures include prevention of transmission (bednets, vector control) and treatment of symptomatic infections (antiparasitic drugs such as artemisinin).
- In our experiments we used parasites *Plasmodium yoelii* (Py) that are natural pathogens of rodents (e.g., mice).

Murray et al. Lancet 2014; WHO 2020

Basic steps of malaria life cycle

- Infection starts when an infected mosquito probes for blood and injects sporozoites. Generally, a median of 20 parasites are injected by the mosquito (in mice).



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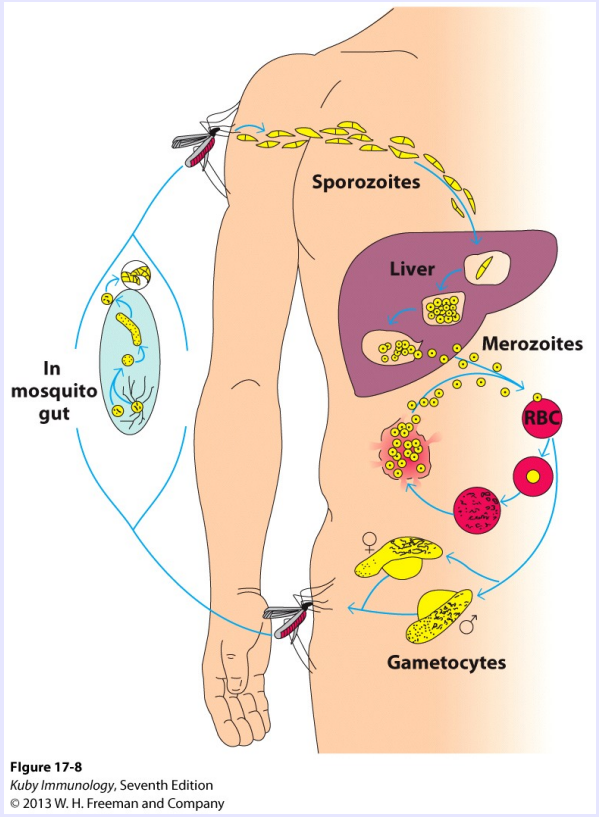

Figure 17-8
Kuby Immunology, Seventh Edition

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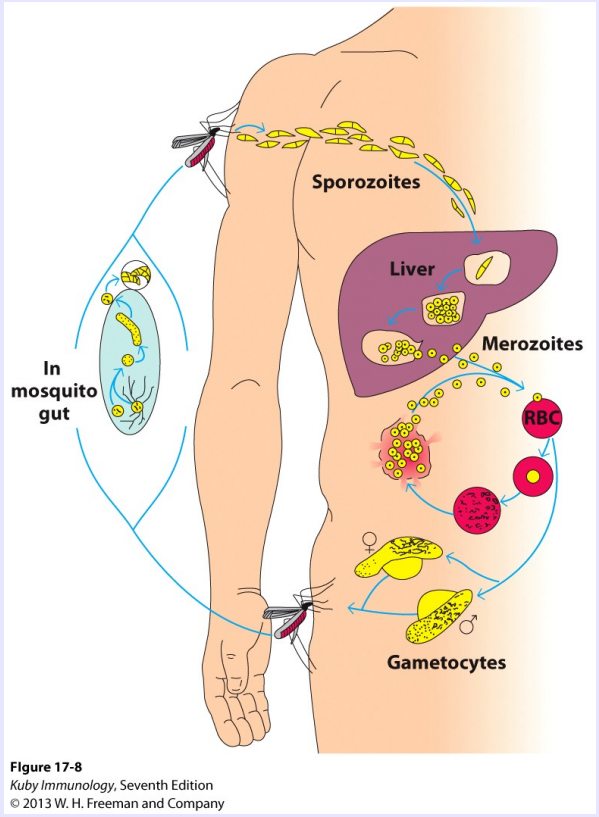

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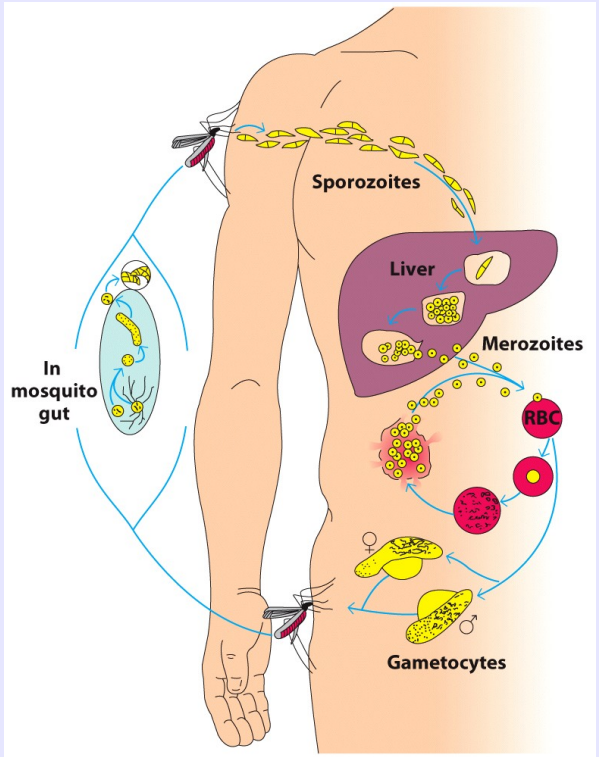

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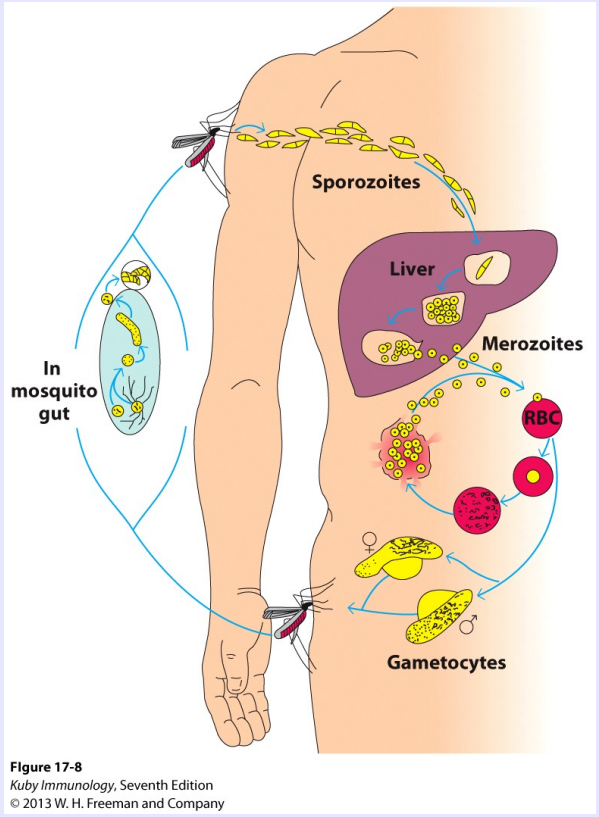

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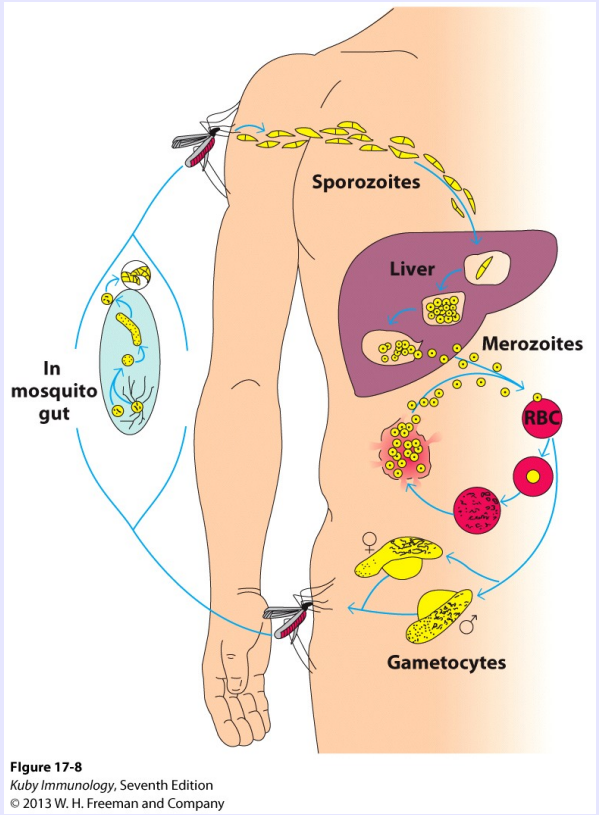
- Over the course of infection, merozoites differentiate into gametocytes which are picked up by mosquitoes and differentiate into sporozoites in the mosquito's gut.
- Sporozoites migrate from the gut to the salivary glands where they wait until the mosquito searches for blood on another (human) host.



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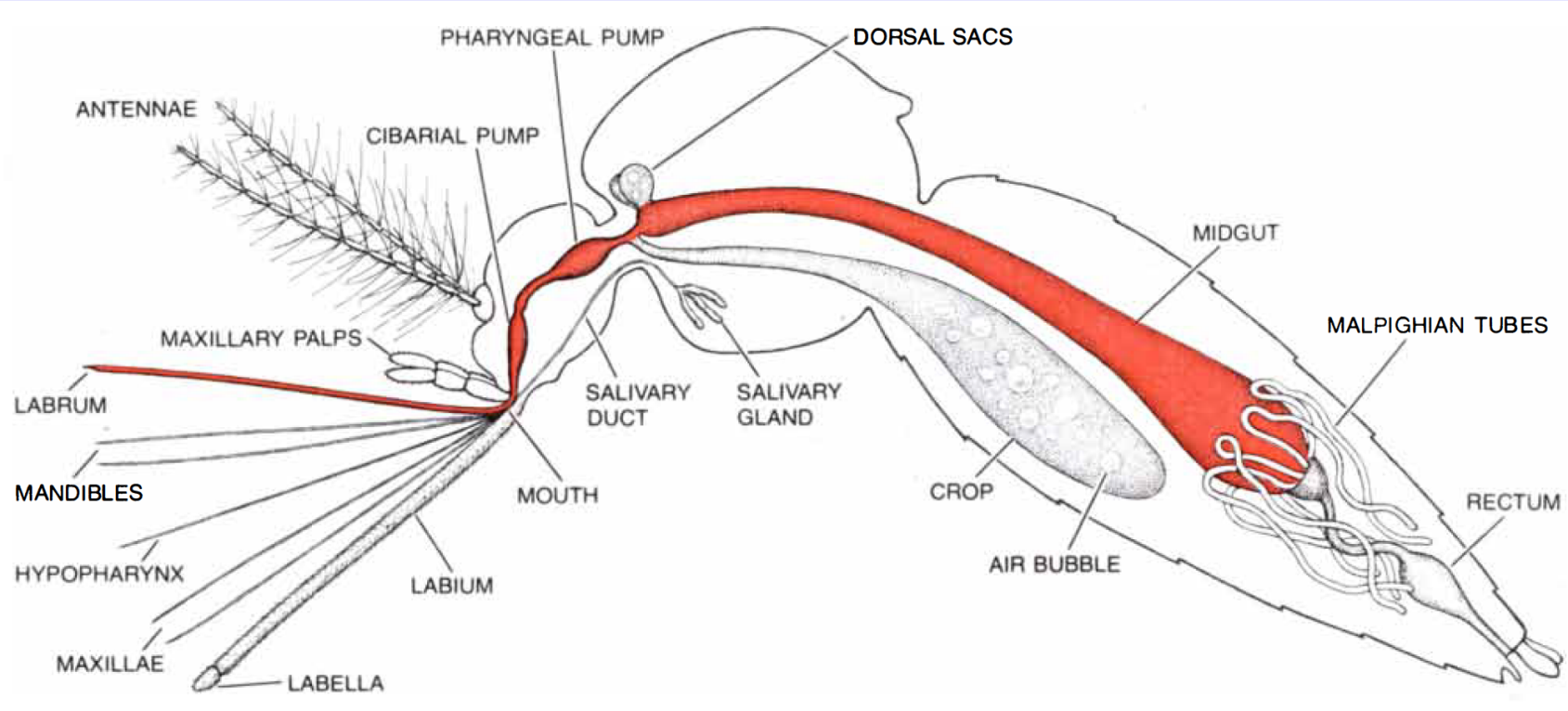
Mosquito: basic anatomy (Anopheles stephensi)

Jones Sci Amer 1978

Mosquito: basic anatomy



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How do mosquitoes initiate the infection?

- Importance of controlling mosquitoes to stop malaria epidemics has been recognized over 100 years ago.

Smith et al. PLoS Biol 2007; Smith PLoS Path 2012

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- Basic mathematical models, as those developed by Ross and Macdonald, show the dependence of the transmission potential of malaria, given by the basic reproductive number R_0, as the function of parameters:

$$R_0 = \frac{ma^2\boldsymbol{b}c}{gr}\mathrm{e}^{-gv}$$

where m is the ratio of mosquitoes to humans, a is the mosquito biting rate, b is the probability that infectious mosquito bite results in human infection, c is the probability that mosquito becomes infected following a bite of an infected human, g is mosquito's death rate, v is the time between mosquito becoming infected and becoming infectious, r is the human recovery rate from the infection.

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- Change in the biting rate a or the mortality of mosquito g have the nonlinear impact on the overall infectiousness of the infection.
- One important parameter $\boldsymbol{b}$ – the probability that a bite by an infectious mosquito results in infection – has not been directly estimated.

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Unresolved issues with malaria transmission

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- It is often assumed that infected mosquitoes inject sporozoites while taking the blood meal. For example, in malaria vaccine trials, volunteers are bid by infectious mosquitoes and bite is deemed “successful” if the mosquito takes the blood meal. Volunteers (vaccinated and controls) are exposed to several (e.g., 5) successful bites.

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- It is generally assumed that infectious mosquito (i.e., carrying sporozoites) is always capable of cause infection in the host.
- However, it has been noted in early studies of malaria epidemiology that counted the average number of infectious bites humans receive and disease incidence and found 10 fold difference (between predicted and observed disease incidence). This was attributed to maternal immunity (because disease was mostly observed in young children).

Biting rate and malaria incidence rate

- Early studies could accurately quantify the number of infectious bites humans receive per day in malaria endemic regions (about 0.06-0.9 per day).

TABLE II.—DATA ON ANOPHELINE PREVALENCE AND INFECTION

	Tanganyika (Mngeza)		Uganda (Lira)	
	A. gambiæ	*A. funestus*	*A. gambiæ*	*A. funestus*
Mosquito density (per person) ..	4·25	50	3·2	3·1
Man-biting frequency (per day) ..	0·5	0·5	0·5	0·33–0·5
Sporozoite rate (proportion) ..	0·047	0·034	0·146	0·062
"Infective" bites per person per day ..	0·1	0·85	0·23	0·06–0·1

NOTE.—Mosquito density in the Mpakane area in Tanganyika was less than in Mngeza, about half. Mngeza data are given for illustration.

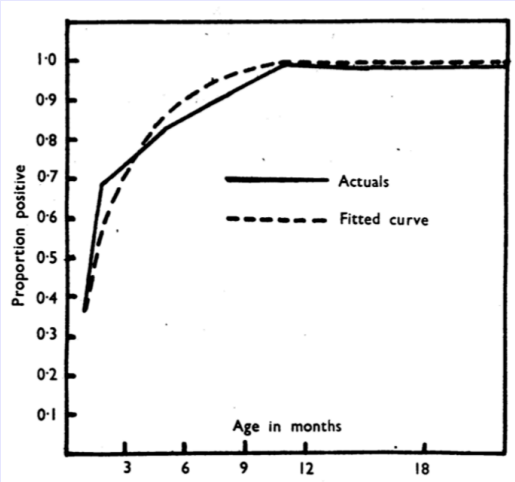

FIG. 1.—Infant parasite rates in Uganda.

Macdonald Proc Roy Soc Med 1956; Macdonald Bull Wld Hlth Org 1956

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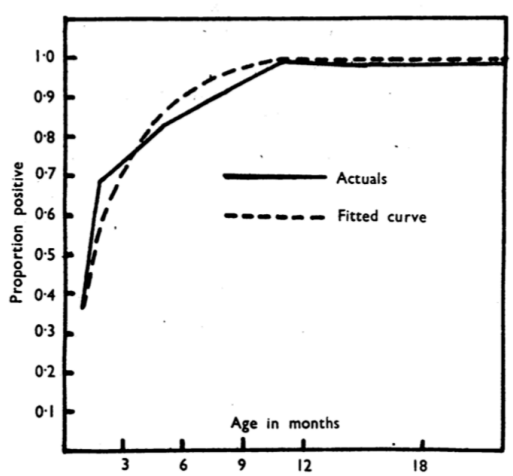

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- "There is also evidence, which appears conclusive to the writer, that in this area only about 1 in every 100 bites inflicted on infants by sporozoite-infected mosquitos resulted in establishment of infection, and in another area only 1 in 20 did so. There may be many causes for this failure, and their relative importance cannot at present be assessed, but among them some considerable weight must be given to the very small numbers of oocysts and sporozoites typically found in mosquitos in such places, numbers which are almost negligible when compared with those commonly seen in experimental infections."

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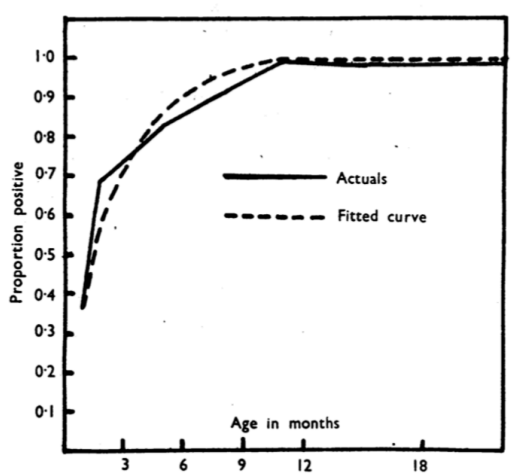

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Experiments to probe mosquito's ability to infect mice

Team:

Maya Aleshnick
(Johns Hopkins University)

Photini Sinnis
(Johns Hopkins University)

and Gayane Yenokyan at JH helped with statistics.

Aleshnick et al. PLoS Path 2020 (in press)

Experimental design: basic details

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- Mice bid by infected mosquitoes were observed for the presence of blood stage infection by Giemsa-stained blood smears on days 5, 10 and 15 post-feed.

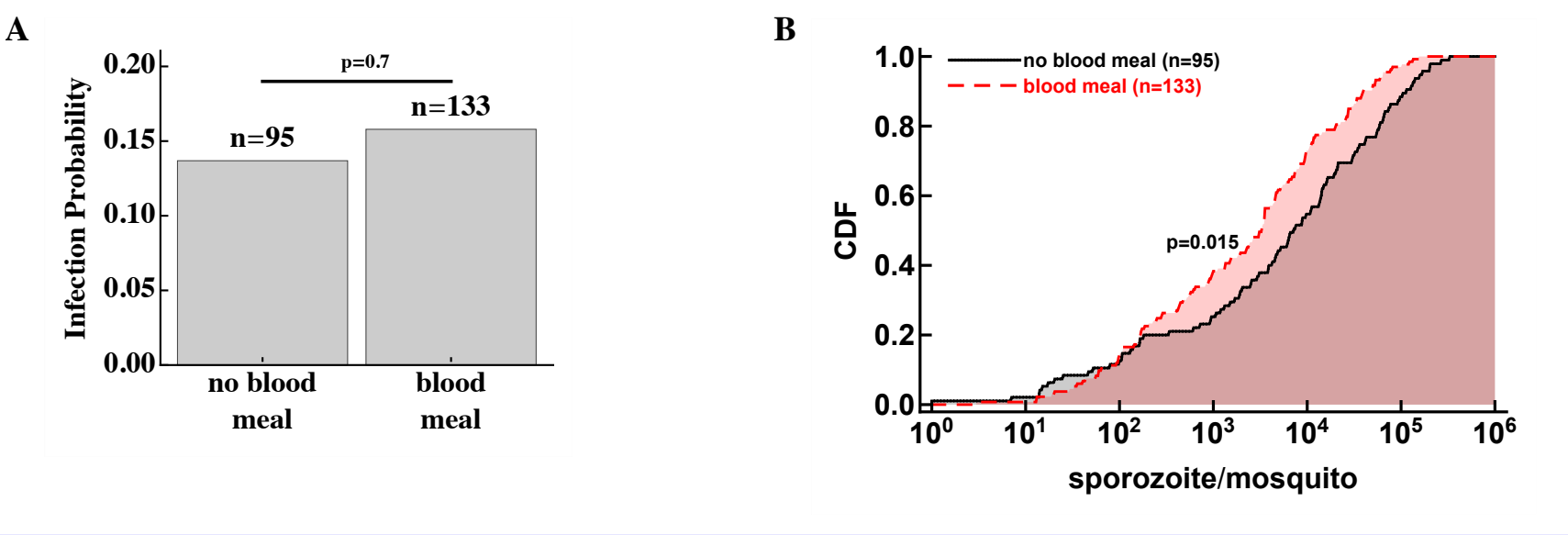
Data example (available as supplement to the paper)

	A	B	C	D	E	F	G	H	I
1	ID	Probe time	PT, sec	Bloodmeal	SG load	Malaria	experiment	feeding	location
2	SMFE-1/CA-4	ND	ND	0	2,951	0	1	observed	Ear
3	SMFE-1/CA-5	ND	ND	0	17	0	1	observed	Ear
4	SMFE-1/CB-1	ND	ND	1	168	1	1	observed	Ear
5	SMFE-1/CB-2	ND	ND	0	19,059	0	1	observed	Ear
6	SMFE-1/CB-3	ND	ND	0	1,176	0	1	observed	Ear
7	SMFE-1/CB-4	ND	ND	1	80,942	1	1	observed	Ear
8	SMFE-1/CB-5	ND	ND	1	76,525	1	1	observed	Ear
9	SMFE-2/CA-1	1:12	72	1	4,562	0	2	observed	Ear
10	SMFE-2/CA-2	0:53	53	0	612	0	2	observed	Ear
11	SMFE-2/CA-3	5:41	341	1	468	0	2	observed	Ear
12	SMFE-2/CA-4	20:48	1248	0	11,099	1	2	observed	Ear
13	SMFE-2/CA-5	10:20	620	0	164	0	2	observed	Ear
14	SMFE-2/CB-1	14:42	882	0	7	0	2	observed	Ear
15	SMFE-2/CB-2	4:31	271	0	2,515	0	2	observed	Ear
16	SMFE-2/CB-3	23:00	1380	0	98	0	2	observed	Ear
17	SMFE-2/CB-4	5:50	350	1	15,734	0	2	observed	Ear
18	SMFE-2/CB-5	12:00	720	0	54,131	1	2	observed	Ear
19	SMFE-2/CC-1	8:05	485	0	30,190	1	2	observed	Ear
20	SMFE-2/CC-2	5:35	335	0	21,365	0	2	observed	Ear
21	SMFE-2/CC-3	8:19	499	1	4,578	1	2	observed	Ear

Aleshnick et al. PLoS Path 2020 (in press)

DOES TAKING THE BLOOD MEAL INFLUENCE INFECTION PROBABILITY?

Taking the blood meal does not influence infection

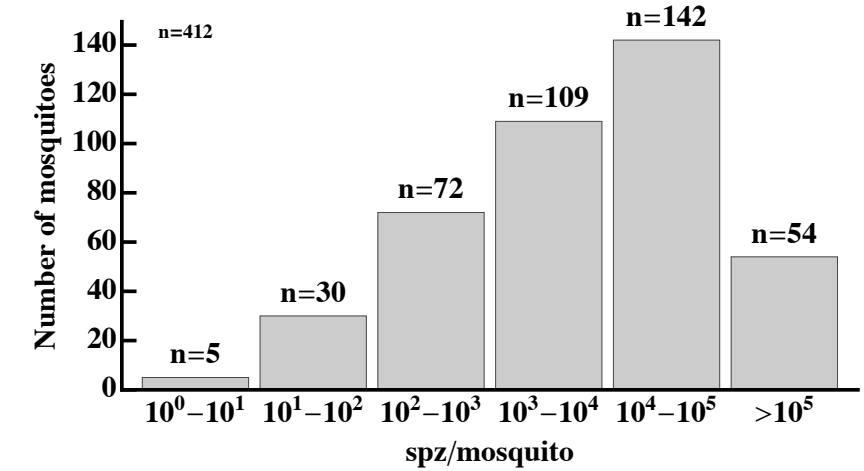


1. Power analysis suggests the difference in infection (14% vs. 16%) may be significant (at $p = 0.05$) if about 600 mice were used in the experiment.
2. Mosquitoes that did not take the blood meal had a higher sporozoite load than those that did take the blood meal (panel B; KS test).
3. Intravital imaging experiments (Vanderberg and Frevert (2004) and Sinnis et al. (unpublished)) showed that sporozoites are readily injected during probing.

Vanderberg and Frevert Int J Paras 2004; Hopp et al. eLife 2015; Sinnis et al. (unpublished)

DOES SALIVARY GLAND SPOROZOITE NUMBER INFLUENCE INFECTION PROBABILITY?

Sporozoite numbers and infection probability



The median load was 8865 sporozoites, with a range of 1-647,714.

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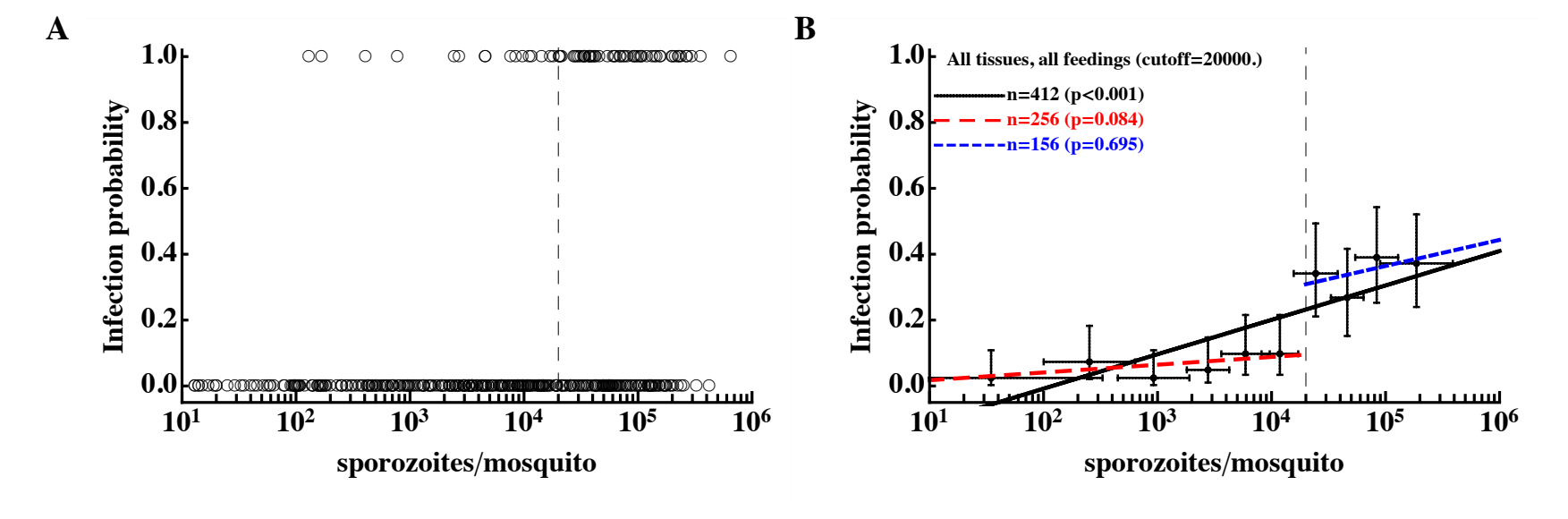

On average, 17.5% became infected after a mosquito bite. Spearman rank correlation test was used.

Mathematical modeling of malaria infection

- To better understand the relationship between sporozoite numbers and infection probability per bite we developed a series of mathematical models. In a “single hit model” infection occurs if one of S infectious agents can initiate the infection. Then

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- Finally, the "threshold model" assumes that infection probability is low at low sporozoite numbers but increases to a limit after crossing a threshold:

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Mathematical modeling of malaria infection

- To better understand the relationship between sporozoite numbers and infection probability per bite we developed a series of mathematical models. In a "single hit model" infection occurs if one of S infectious agents can initiate the infection. Then

$$p(S) = 1 - \mathrm{e}^{-\lambda S}$$

- In the "powerlaw model" sporozoites may compete in the mosquito or the mouse (the competition is described by parameter n):

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- Other tested models also include slope-threshold model, logistic model, double logistic model, models with "soft" threshold, etc.

Fitting models to data and comparing models

- We fitted mathematical models to the data using likelihood approach in which the likelihood of the model given the data is defined as

$$L \sim \prod_{i=1}^{N} p(S_i)^{D_i} (1 - p(S_i))^{1-D_i}$$

where $p(S_i)$ is the infection probability, S_i and $D_i = (0, 1)$ is the sporozoite load in SG and infection probability of a mouse bid by an i^{th} mosquito, respectively, and $N = 412$.

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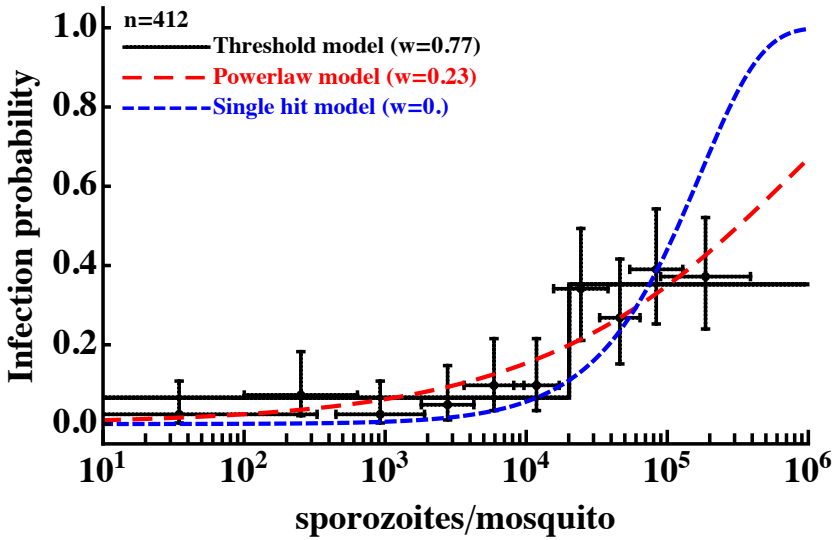
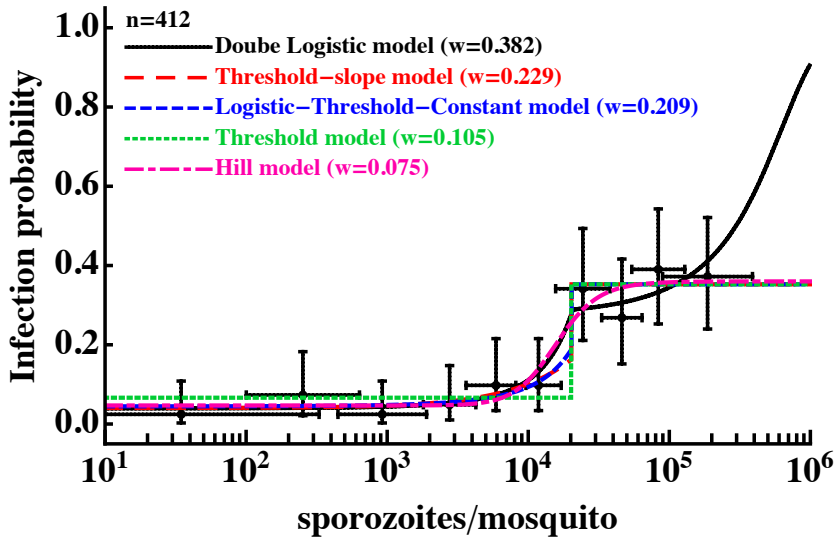
- Quality of model fits to data was evaluated using Hosmer-Lemeshow test (goodness-of-fit test) by binning the data and model predictions into 6-8 bins.

Models including a threshold describe the data best





Parameter estimates: single hit ($\lambda = 5.8 \times 10^{-6}$), powerlaw ($\lambda = 3.8 \times 10^{-3}$, $n = 0.41$), threshold model ($p_{\text{min}} = 0.066$, $p_{\text{max}} = 0.35$, $S^* = 20166$).

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Alternative hypotheses:

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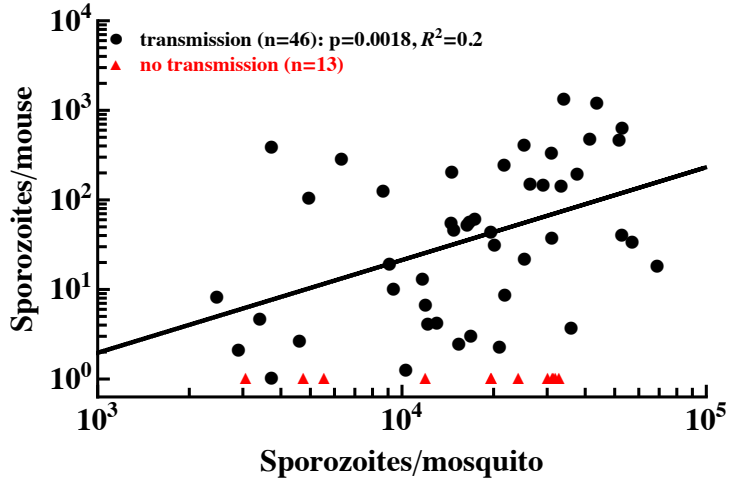

Medica and Sinnis Infec Immun 2005

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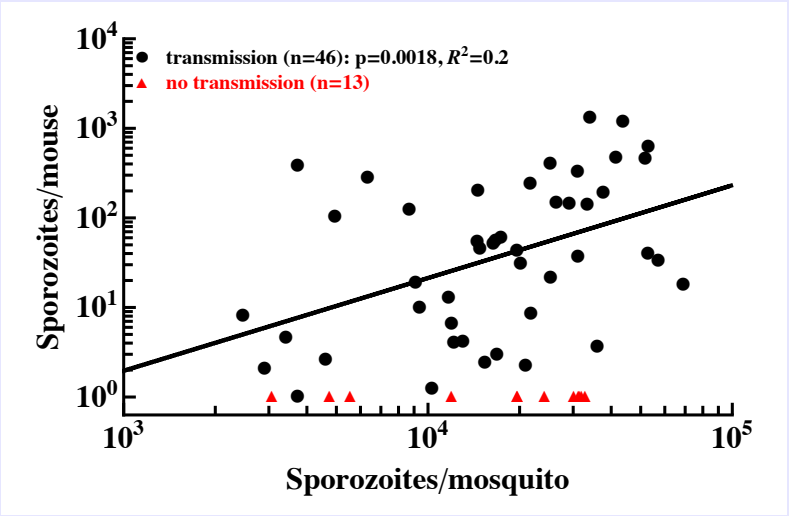

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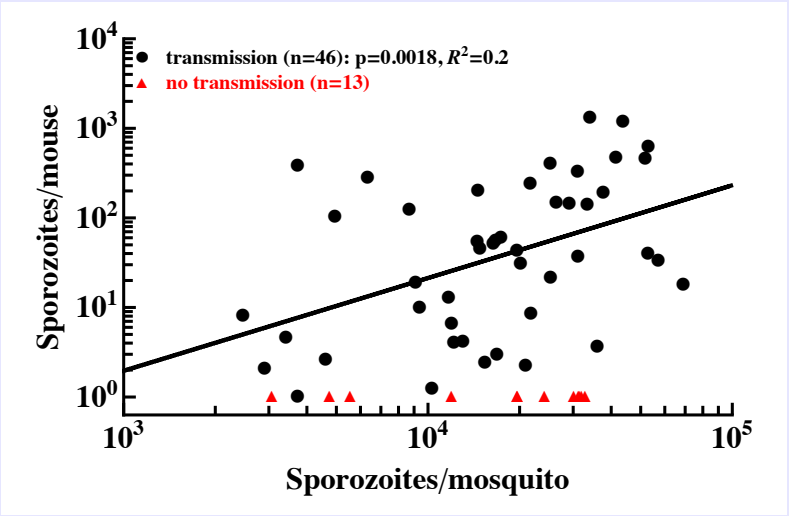

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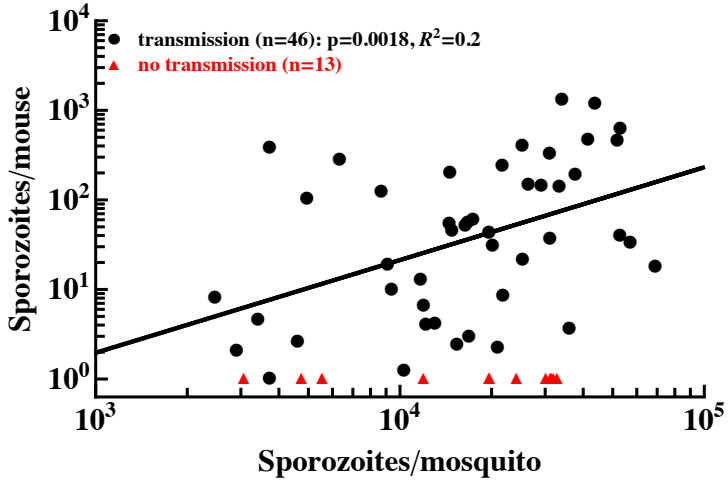

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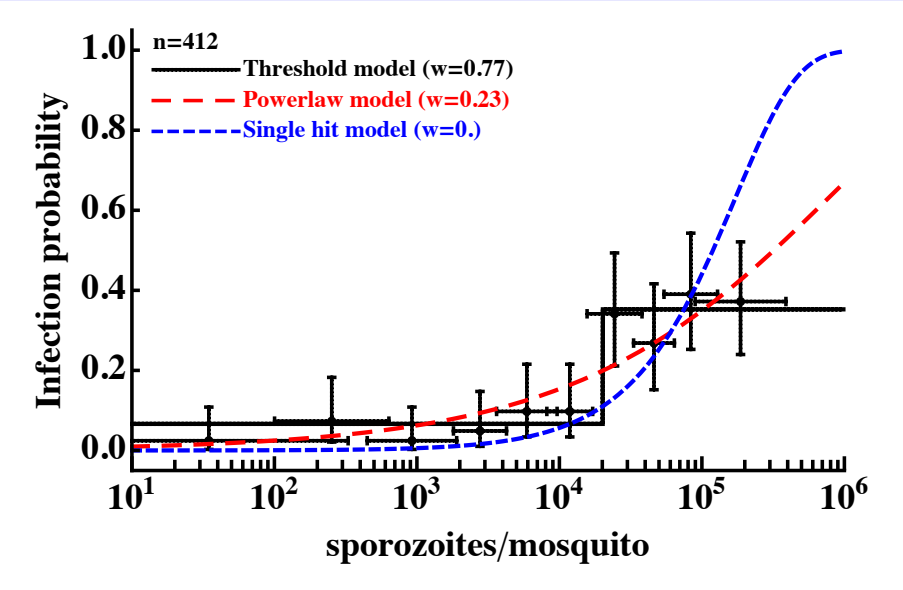
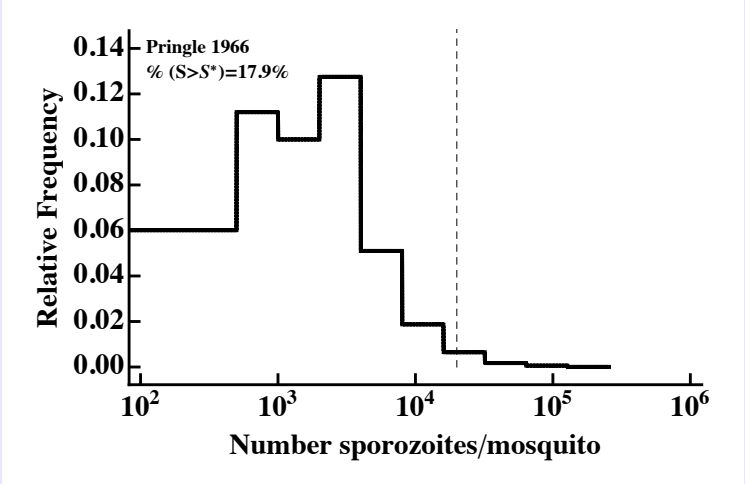
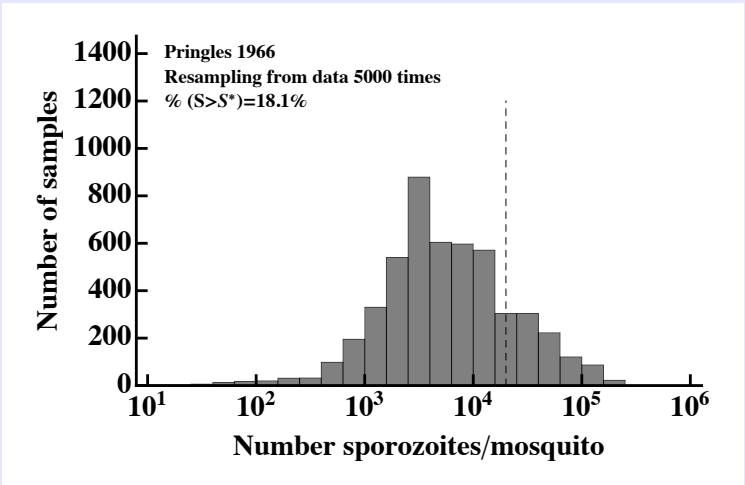
Highly infected mosquitoes are present in nature

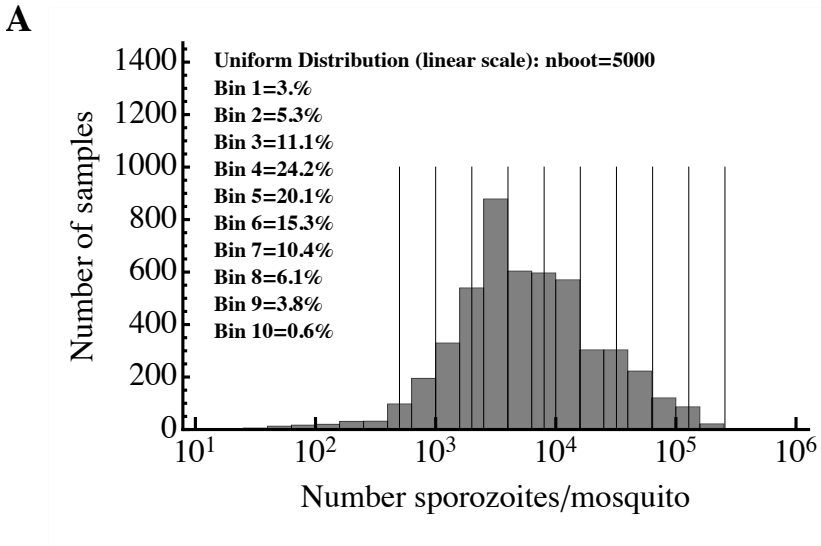


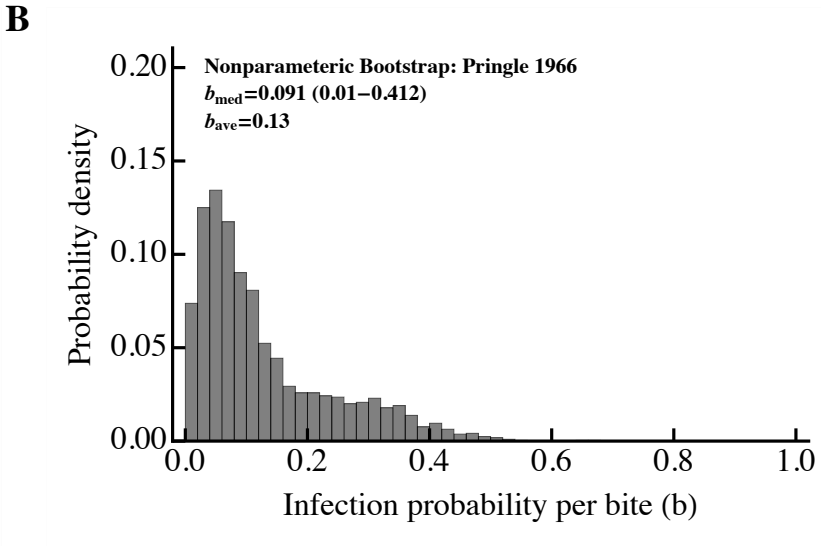




Pringle Trans Roy Soc Hyg Med 1966

Estimating b for actual data (Pringle 1966 dataset)





- We assume that "mouse" data can be directly used to predict infection of humans with Plasmodium parasites.
- Pringle data are for north-east Tanzania (2-3% infected, $n \sim 400$ dissected).
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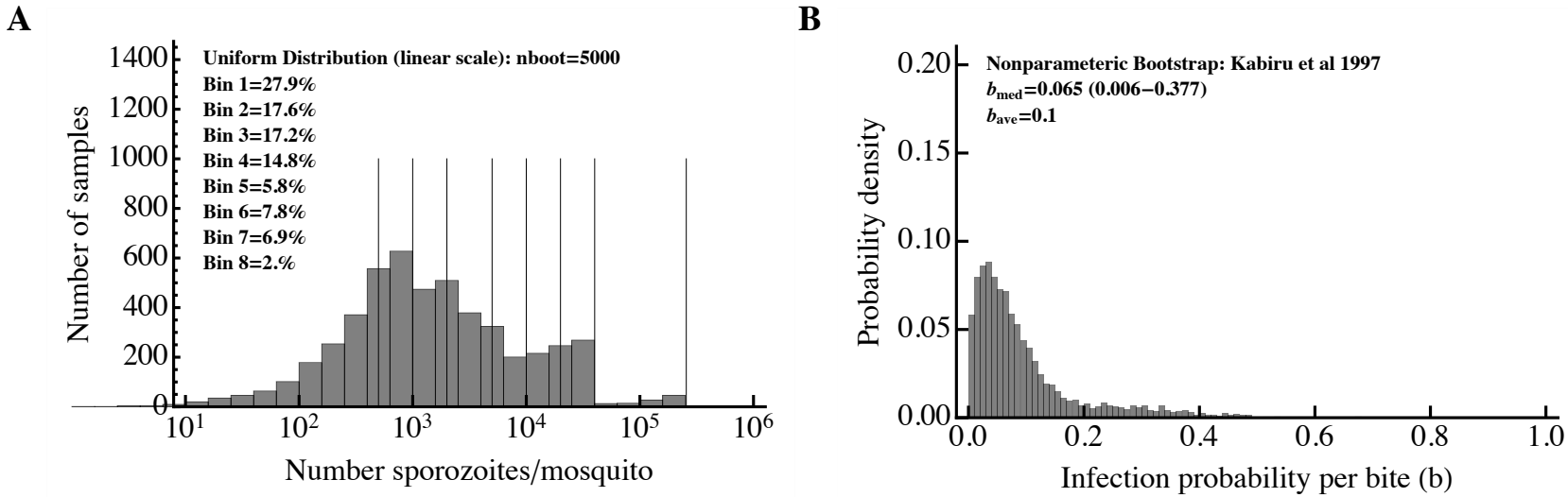
Estimating b for actual data (Kabiru 1997 dataset)



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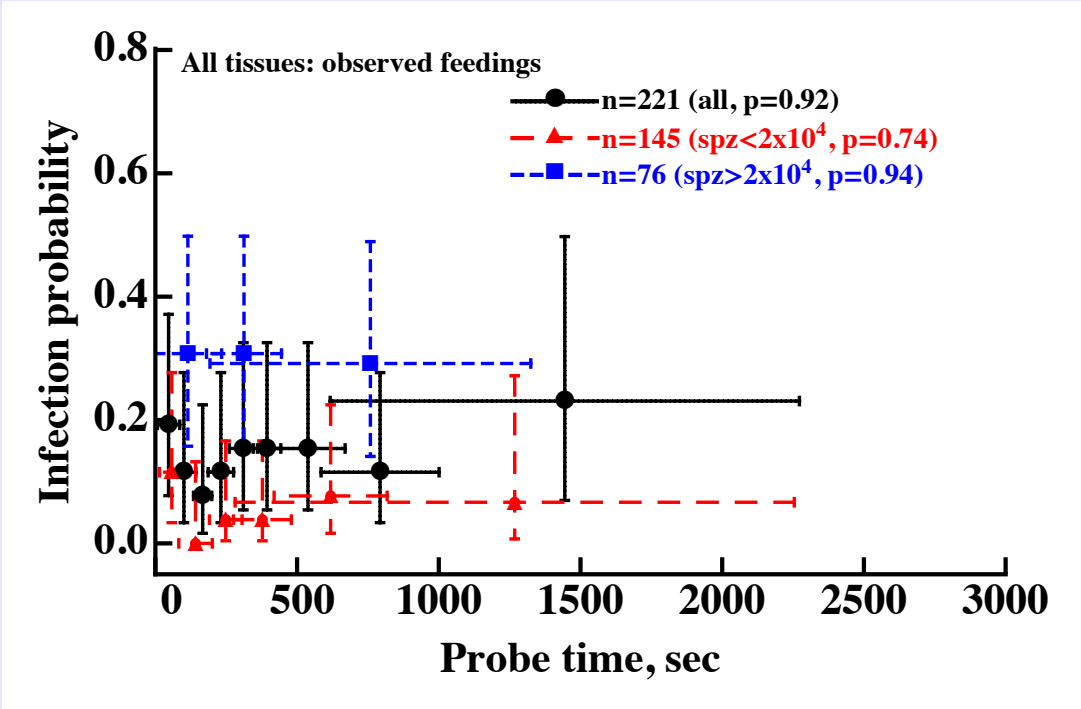
DOES PROBING TIME IMPACT INFECTION PROBABILITY?

No influence of probing time on infection for observed feedings

One set of experiments:

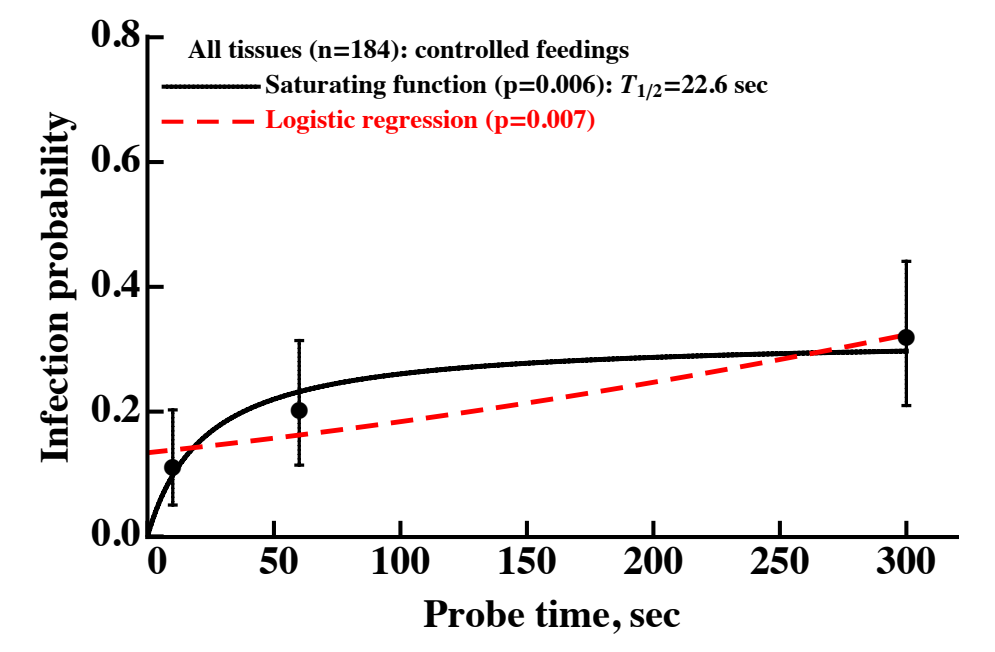
- In “uncontrolled” (observed) feeding experiments, mosquitoes were allowed to probe for any time.



Short probing results in efficient transmission

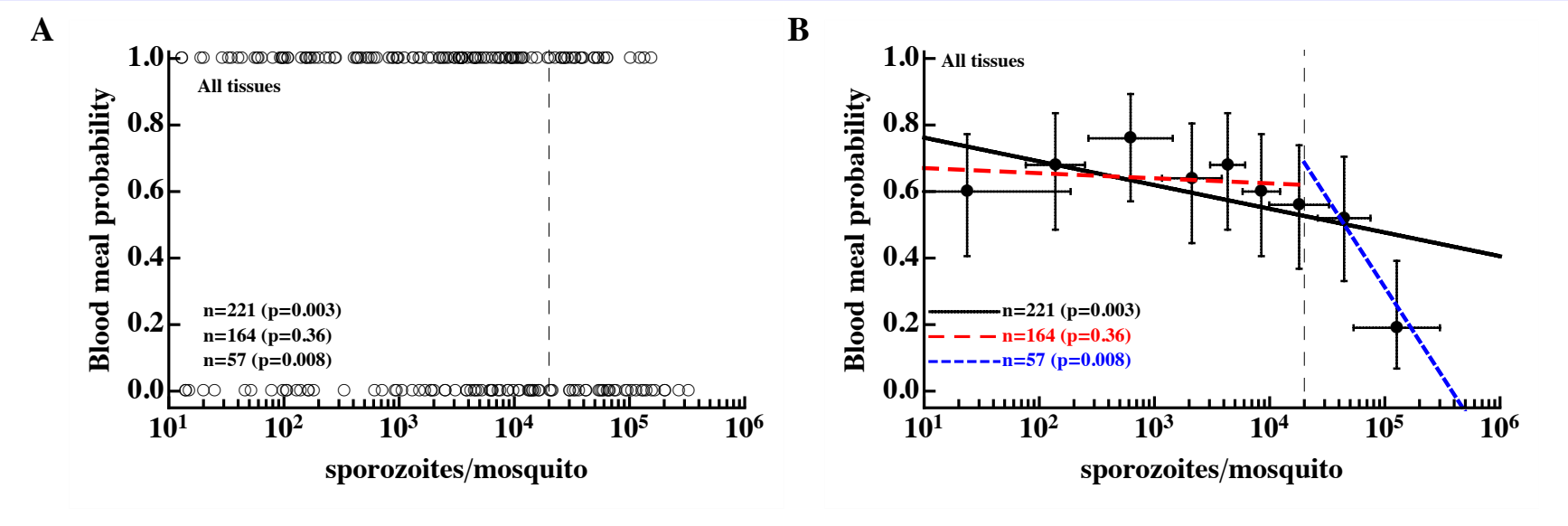
Another set of experiments:

- In “controlled” feeding experiments, mosquitoes were allowed to probe for 10 sec, 1 min, or 5 min on individual mice.
- We fitted a saturating ($p = p_{\max} t/(h+t)$) or logistic ($p = 1/(1+\mathrm{e}^{\beta_0 - \beta_1 t})$) function to the infection data using the likelihood method (treating as infection process as a Bernoulli trial).

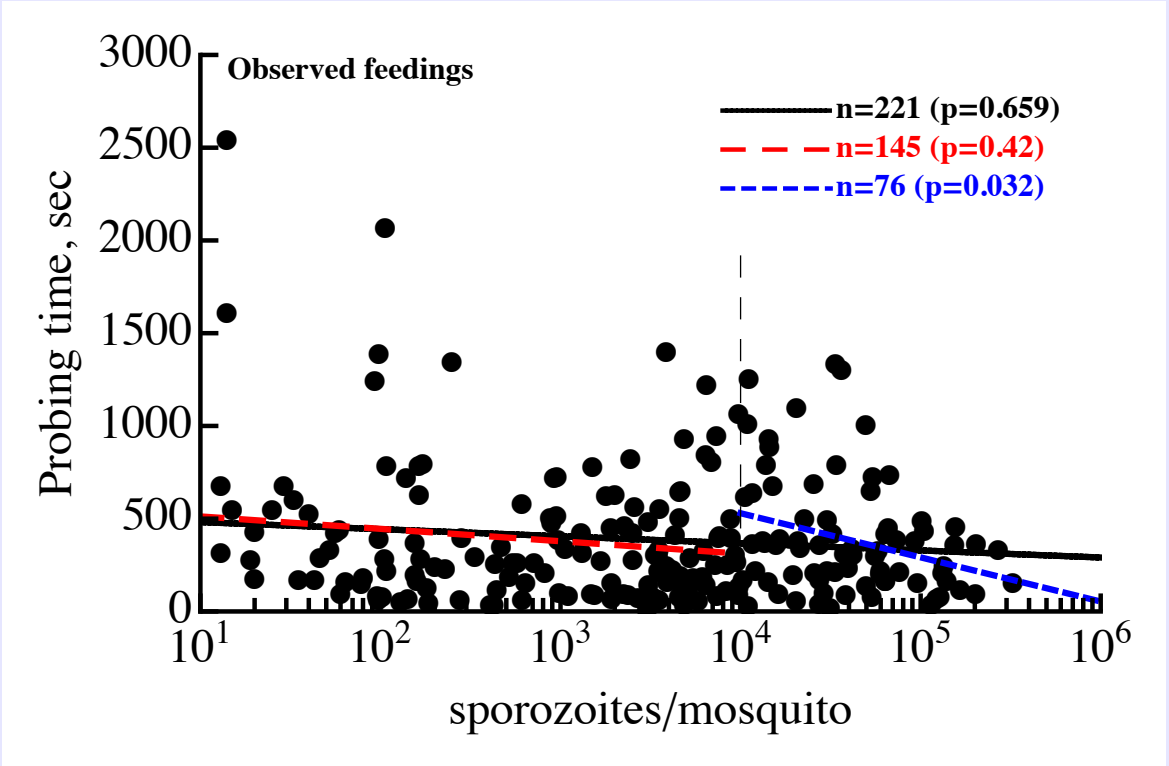


DOES SALIVARY GLAND SPOROZOITE NUMBER INFLUENCE BLOOD MEAL TAKE PROBABILITY?

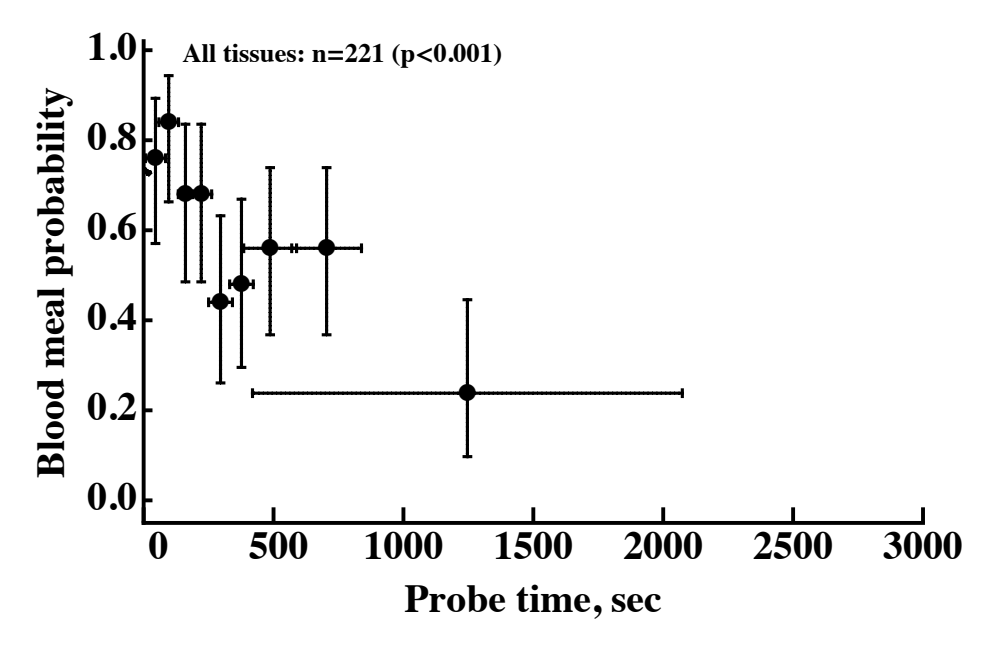
Some mosquitoes fail to take a blood meal



No impact of sporozoite number on probing time



Not taking a blood meal results in longer probing times



- Data were binned for visualization purposes. Spearman rank correlation analysis was done on raw (unbinned) data.
- Short probing times (< 5 min) do not influence blood meal taking probability.

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6. Prevalence of infection in mosquitoes in natural settings allows to calculate the probability of infection per infectious mosquito bite b in the Ross-Macdonald model. For two analyzed datasets, $b_{\text{med}} = 0.065 - 0.1$.

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- Whether our result hold for other Plasmodium species including those infecting humans and other mosquito species (such as *Aëdes aegypti* or *Anopheles gambiae*) remains to be determined.

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- We only analyzed impact of few variables on transmission (blood meal take, probing time, sporozoite number per mosquito). It is possible that other parameters are also important in determining infection probability (e.g., age of mosquitoes, health of mosquitoes, etc.)

Acknowledgements

- Maya Aleshnik and Photini Sinnis (for performing experiments)
- GanusovLab for discussion of this and other malaria-related work.
- You for joining in!
- This work was supported by the NIH.

QUESTIONS?

Other areas we are working on in malaria

- Do activated or memory CD8 T cells utilize unique strategies to search for pathogens in the liver?
- Are CD8 T cells attracted to sites of infection in the liver (e.g., malaria liver stages)?
- How many memory CD8 T cells are needed to protect against malaria infection?
- What is the role of chemokine receptors (CXCR3 and CCR5) in controlling malaria liver stages?

Three important philosophy papers/ideas

- Chamberlin's method of multiple working hypotheses.

The Method of Multiple Working Hypotheses

With this method the dangers of parental affection for a favorite theory can be circumvented.

T. C. Chamberlin

Chamberlin Science 1890; Platt Science 1964; Oreskes et al. Science 1994

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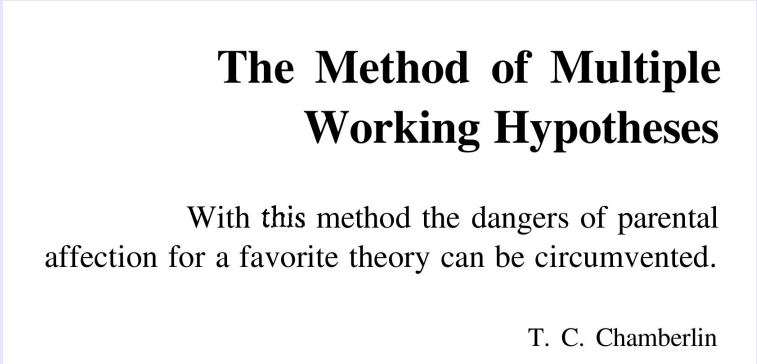

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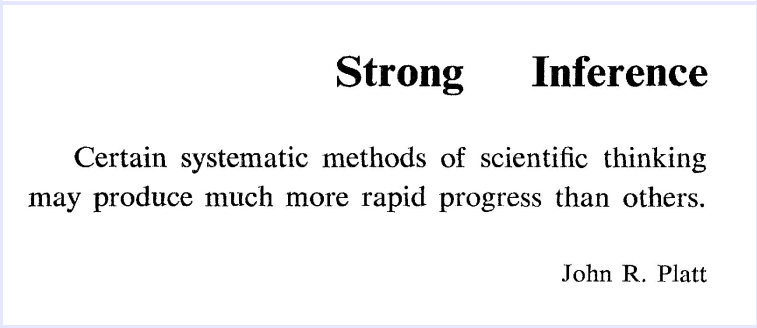

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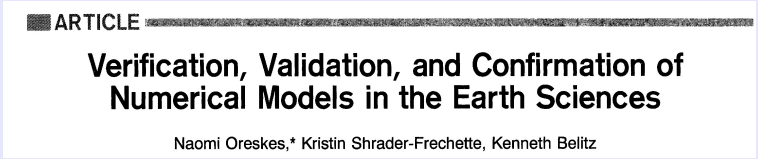

ARTICLE

Verification, Validation, and Confirmation of Numerical Models in the Earth Sciences

Naomi Oreskes,* Kristin Shrader-Frechette, Kenneth Belitz

Chamberlin Science 1890; Platt Science 1964; Oreskes et al. Science 1994

(more) QUESTIONS?