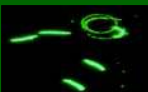


PLASMODIUM IN THE MOSQUITO: CONTRIBUTION TO DRUG RESISTANCE (THE SELFISH GAMETOCYTE)

Dr Peter Billingsley
Sanaria Inc.
9800 Medical Center Drive,
Rockville, MD 20850, USA.



INTRODUCTION

- **Malaria in the mosquito**
- **Dynamics of transmission through the mosquito**
- **Manipulation of transmission by the parasite**
- **Drug treatments, gametocytes and transmission**
- **Mosquitoes and drug resistance**



PLASMODIUM SPOROGONIC CYCLE



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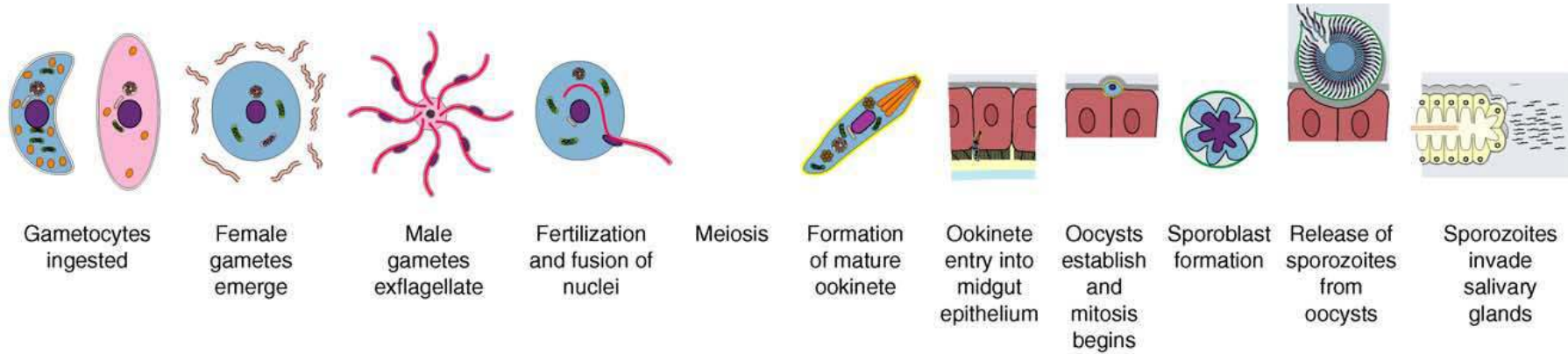
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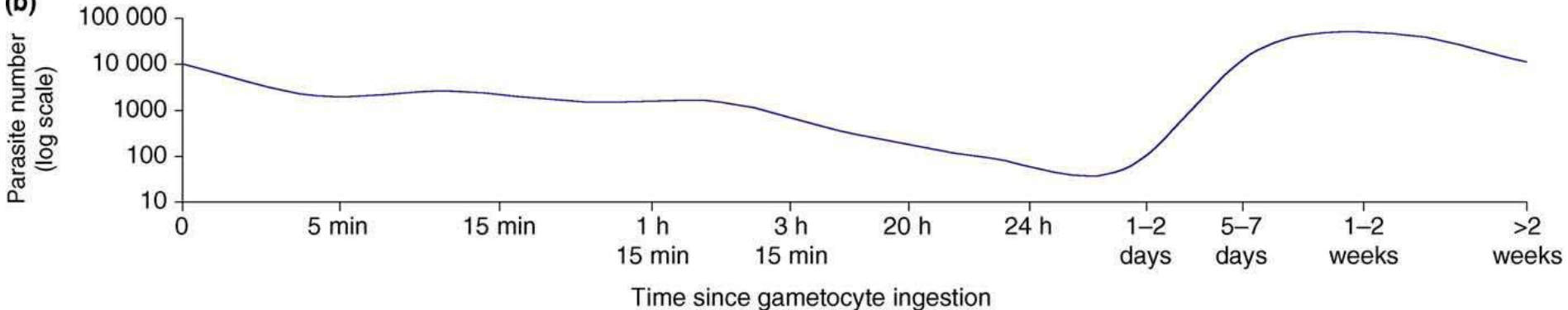


LIFE AND DEATH IN THE MOSQUITO

(a)



(b)



TRENDS in Parasitology

Baton & Ranford-Cartwright 2005 Trends Parasitol 22:574-580

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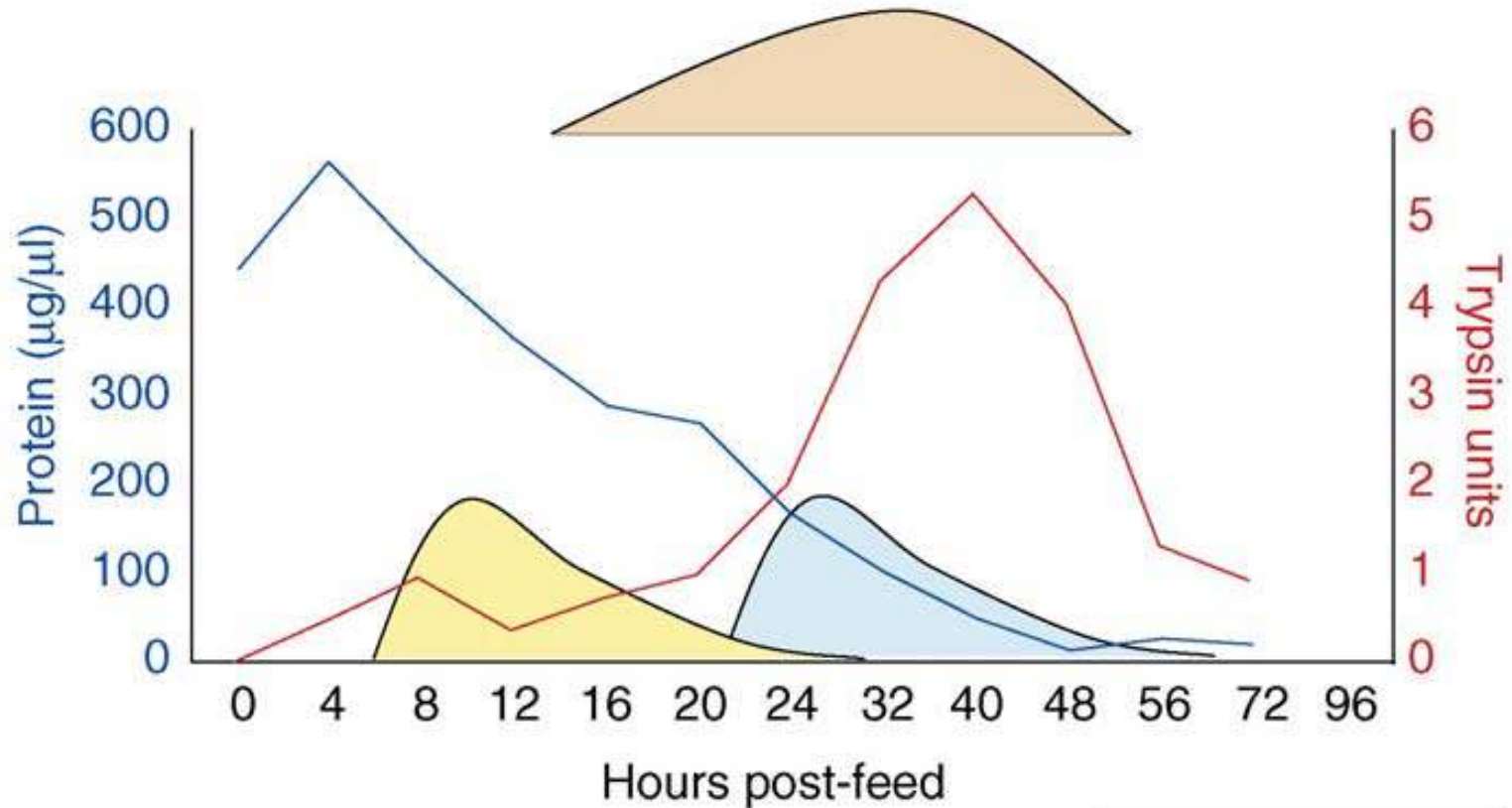
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LIFE AND DEATH IN THE MOSQUITO



TRENDS in Parasitology

Vaughan 2006 Trends Parasitol 23:63-70



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LIFE AND DEATH IN THE MOSQUITO

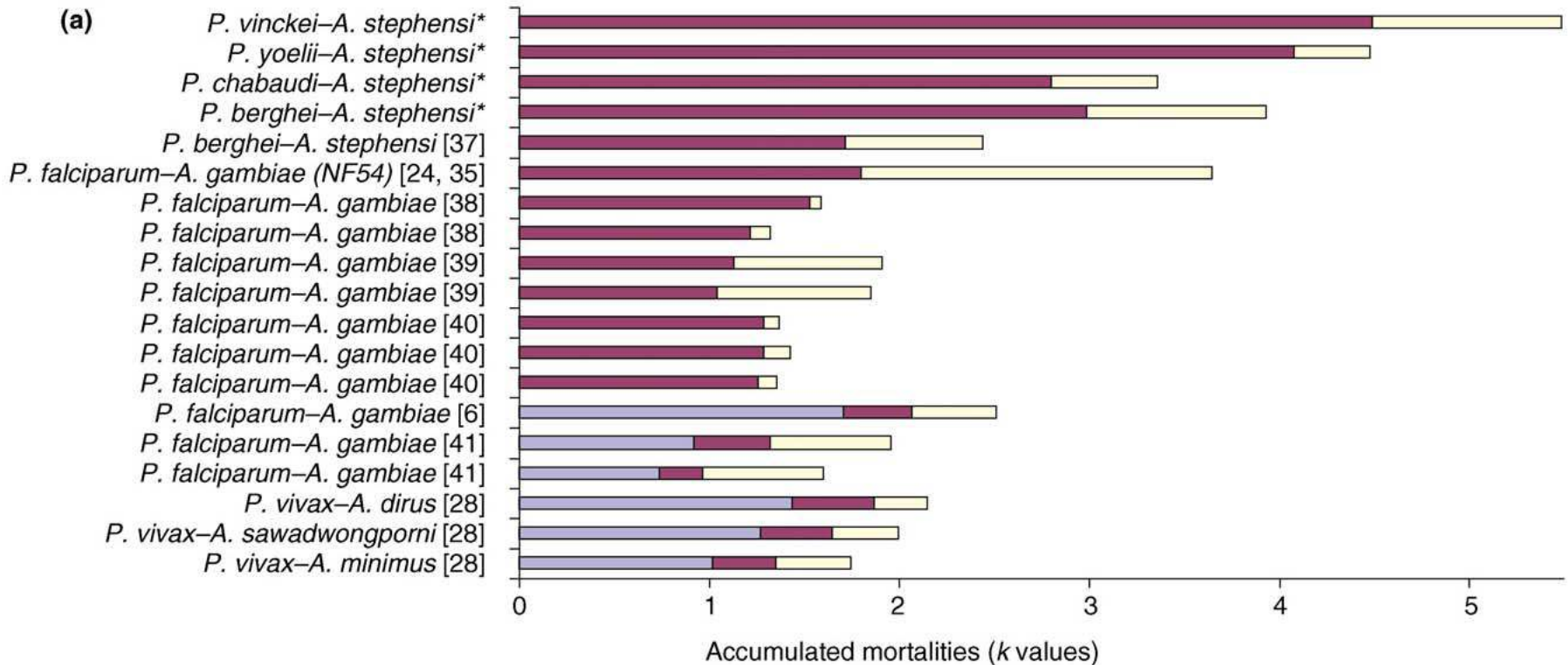
Table I. A life table for early sporogony of *Plasmodium vivax* parasites developing within *Anopheles dirus* mosquitoes

	Macrogametocyte	$k-1$	Zygote	$k-2$	Ookinete	$k-3$	Oocyst	K
Density	359		15		10		2	
Log ₁₀	2.55		1.18		1.00		0.31	
K values		1.37		0.18		0.70		2.25
		23-fold (95.7%)		1.5-fold (33.9%)		5-fold (80.0%)		178-fold (99.4%)

- k = “killing power”
- Logarithmic
- Here $K = 2.25 = 178$ -fold loss



LIFE AND DEATH IN THE MOSQUITO



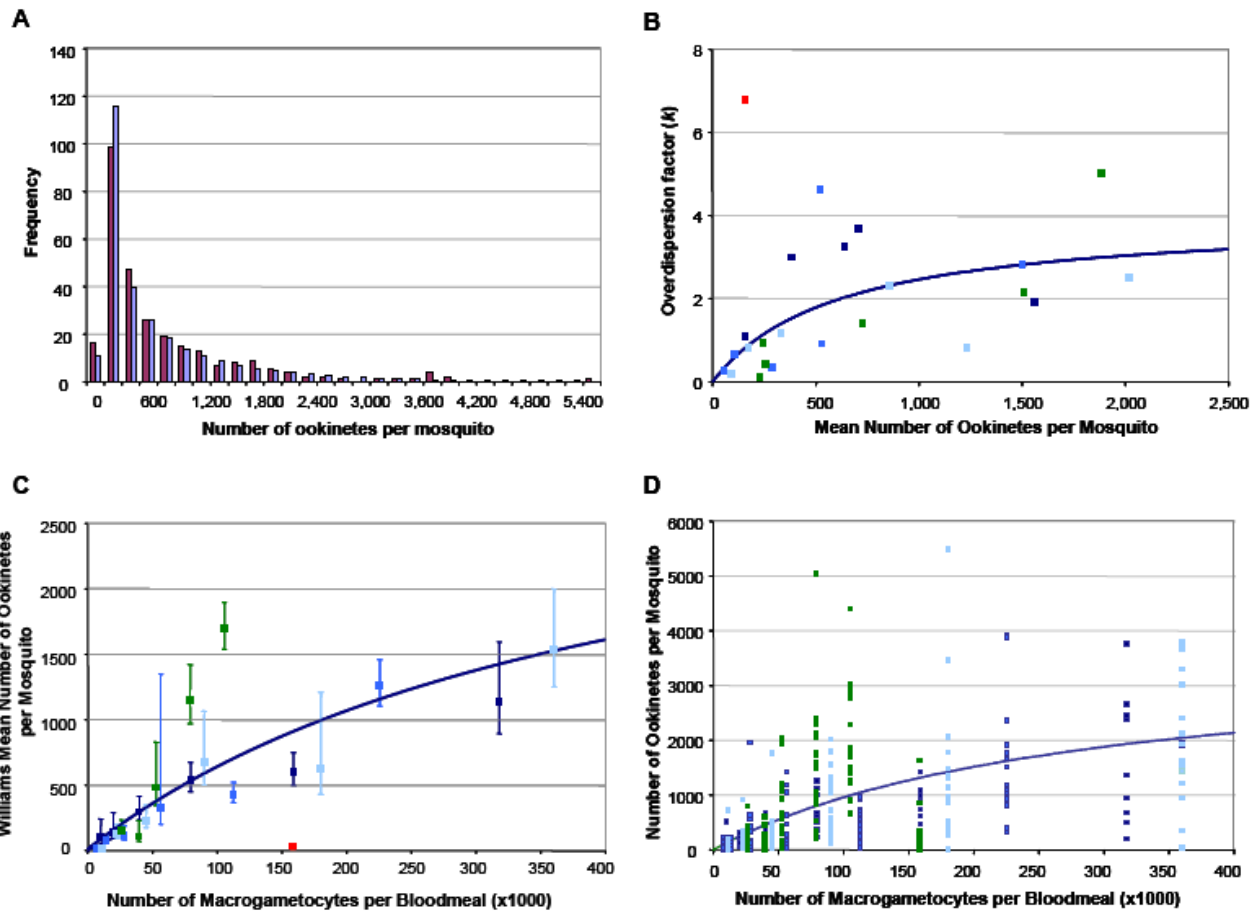
LIFE AND DEATH IN THE MOSQUITO

	GAM	RF	Y_1	OOK	Y_2	OOC2	Y_3	OOC7	Y_T
Prevalence	—	100%		91.9%		48.6%		37.8%	
Average	433.5	12.6		5.5		1.8		2	
SD	456.1	16.2		7.7		3		4.2	
Yield			41.6% (20–85)		61.4% (22–169)		91.2% (54–154)		25.7% (7–83)

GAM, gametocyte; RF, round form; OOK, ookinete; OOC2, young oocyst (day 2); OOC7, mid-size oocyst (day 7). $Y_1 = 1/\text{antilog } k_1$ with $k_1 = \Sigma[\log(\text{RF}) - \log(\text{OOK})]/n$; $Y_2 = 1/\text{antilog } k_2$ with $k_2 = \Sigma[\log(\text{OOK}) - \log(\text{OOC2})]/n$; $Y_3 = 1/\text{antilog } k_3$ with $k_3 = \Sigma[\log(\text{OOC2}) - \log(\text{OOC7})]/n$; $Y_T = 1/\text{antilog } k_T$ with $k_T = \Sigma(k_1 + k_2 + k_3)/n$, represents the total parasite loss from round form to oocyst day 7.



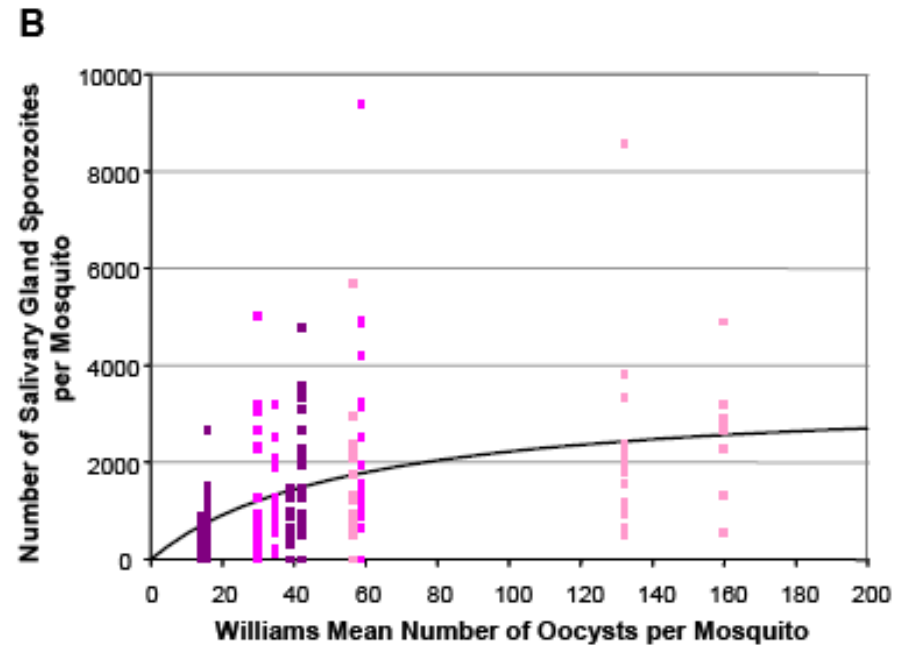
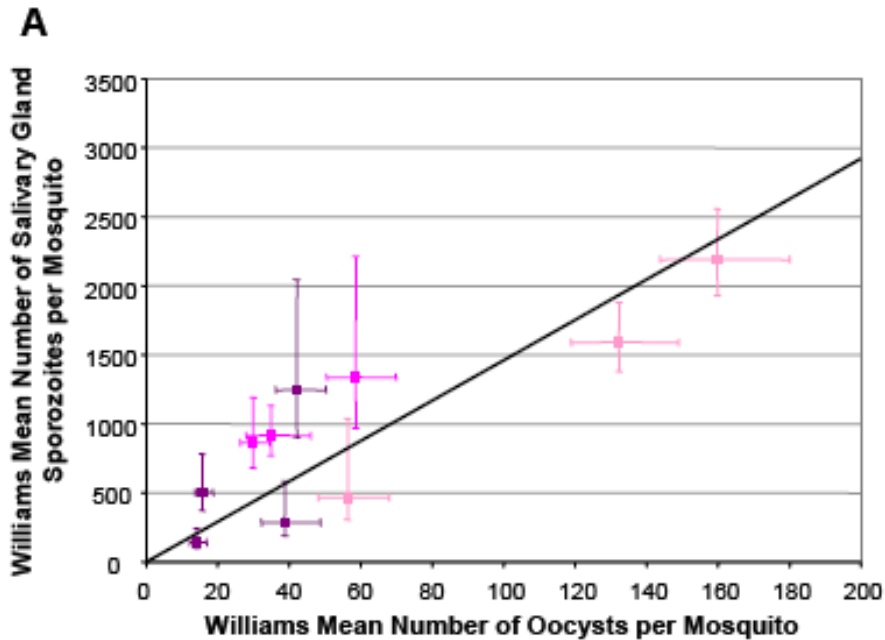
LIFE AND DEATH IN THE MOSQUITO



Sinden *et al.* 2007 PLoS Pathogens 3(12): e195. doi:10.1371/journal.ppat.0030195



LIFE AND DEATH IN THE MOSQUITO



Sinden *et al.* 2007 PLoS Pathogens 3(12): e195. doi:10.1371/journal.ppat.0030195



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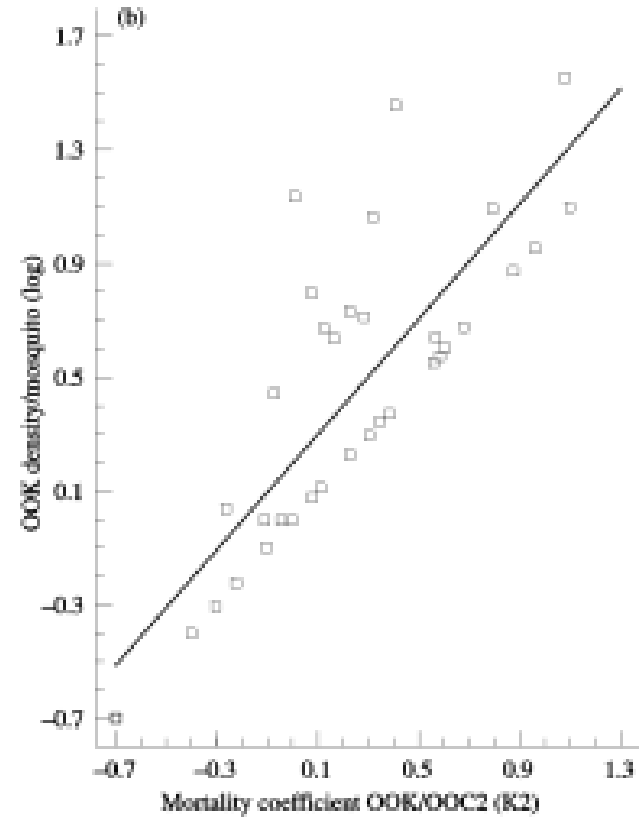
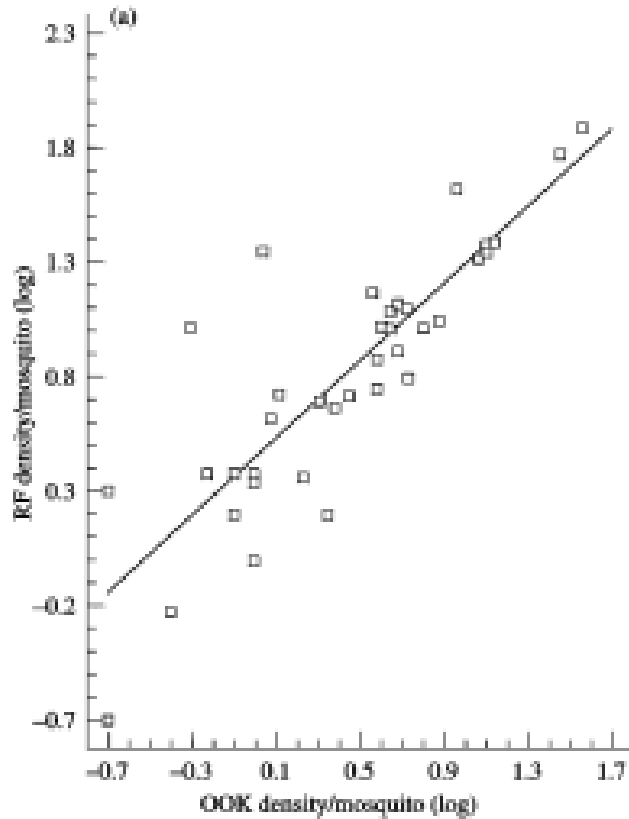
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LIFE AND DEATH IN THE MOSQUITO



Gouagna et al. 1998 Trop Med Int Health 3:21-28



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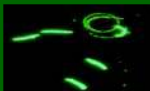
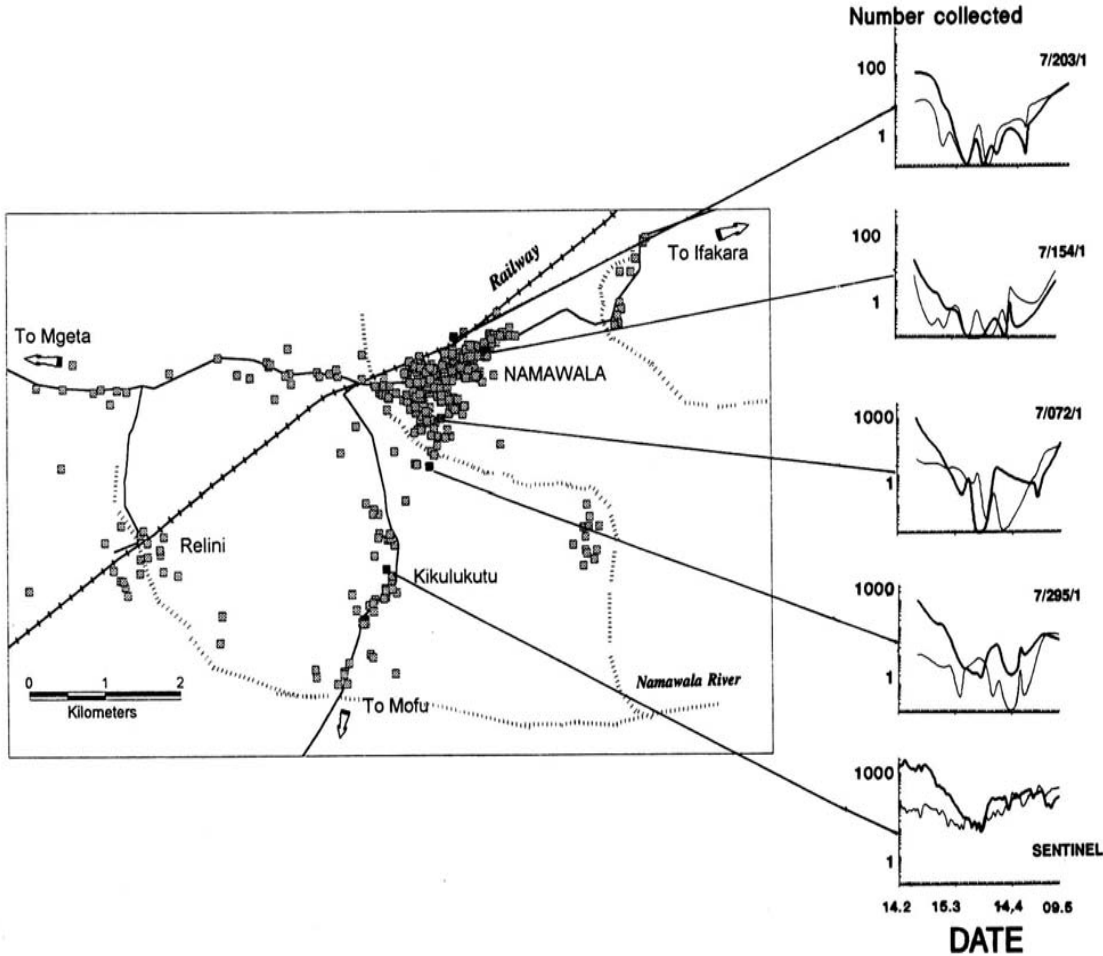
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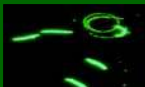
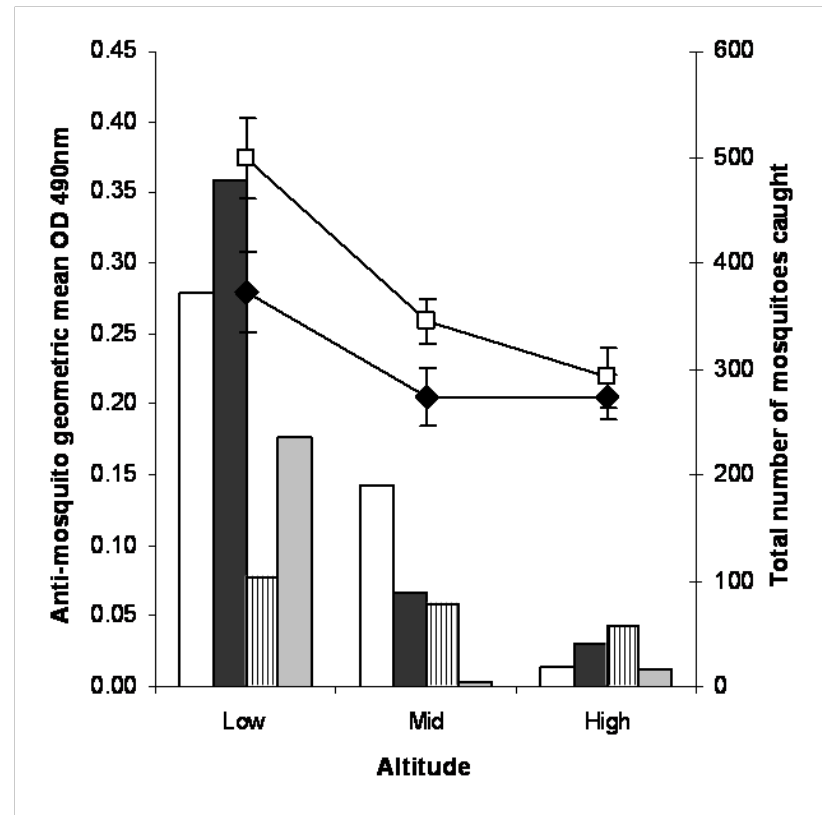
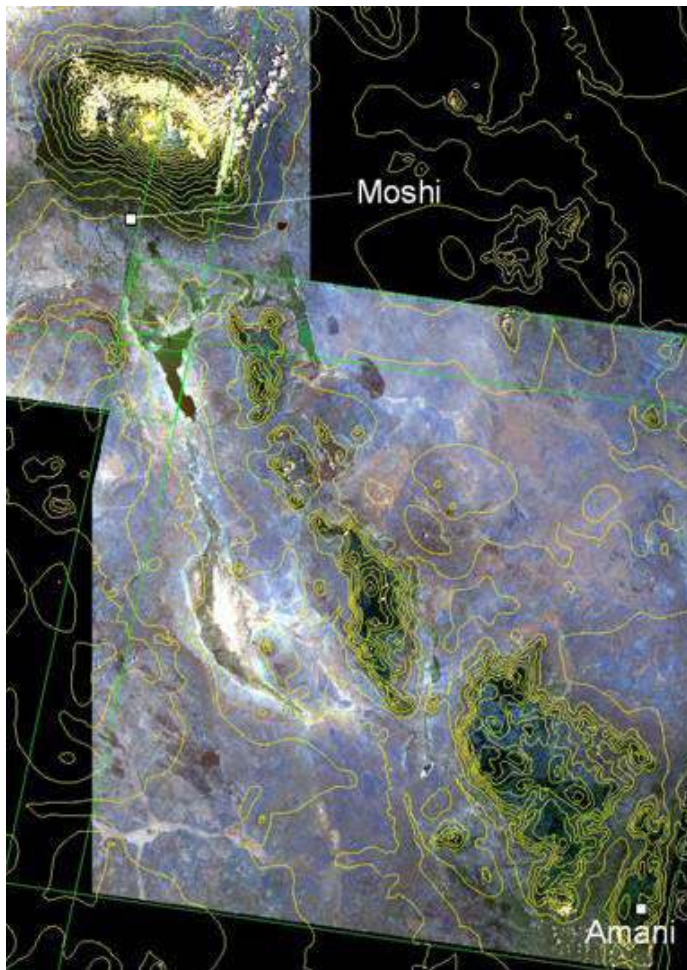




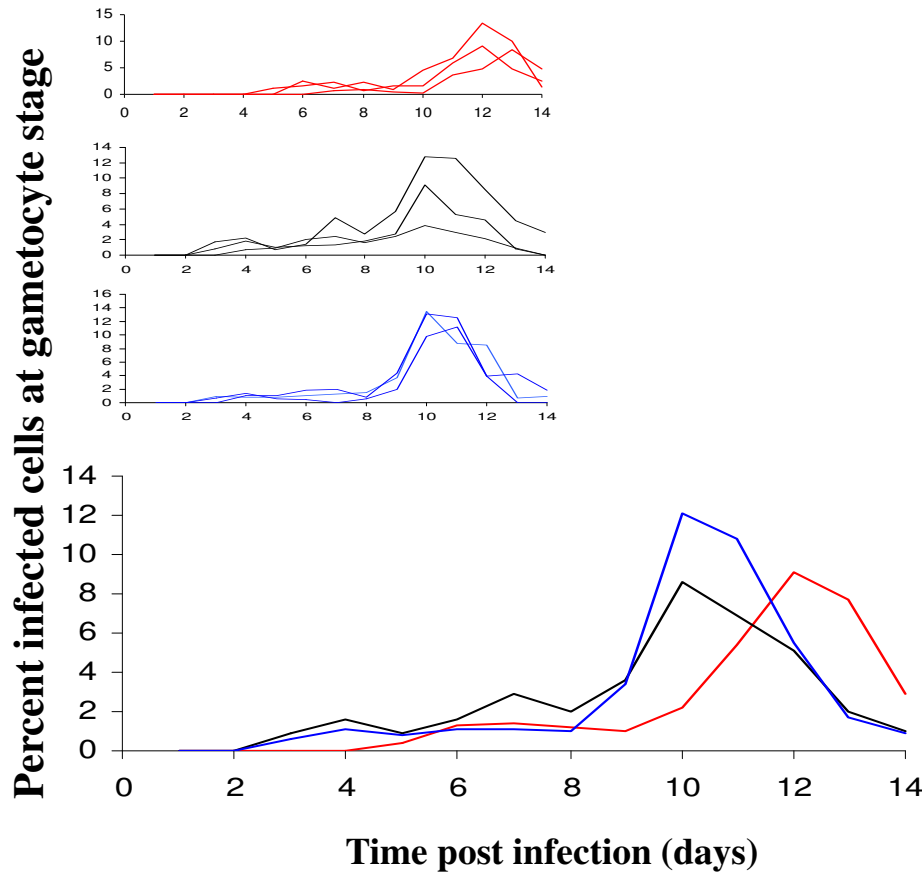
HETEROGENEITY OF EXPOSURE



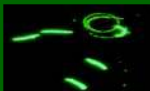
SPATIAL HETEROGENEITY OF ANTIBODY RESPONSES TO MOSQUITO SALIVA



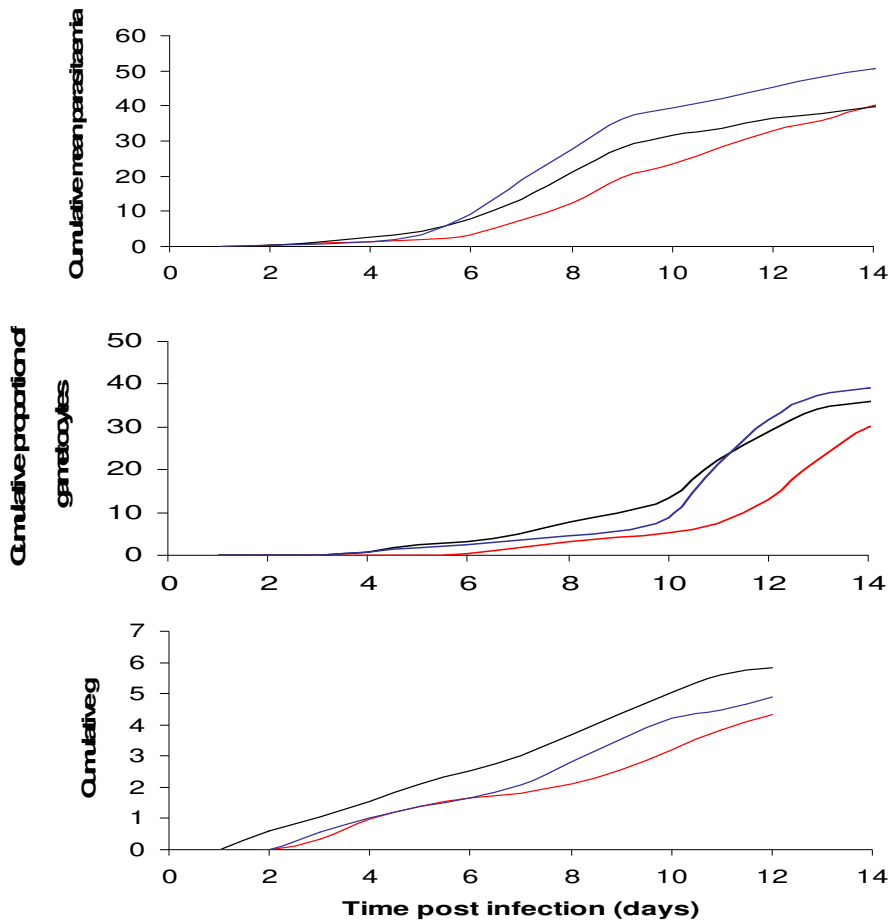
MOSQUITO PROBING AND GAMETOCYTE KINETICS



- Proportion of parasites at the gametocyte stage is higher in mice exposed to 50 probing mosquitoes.
- Peak gametocytes are advanced by 2 days in mice exposed to mosquitoes



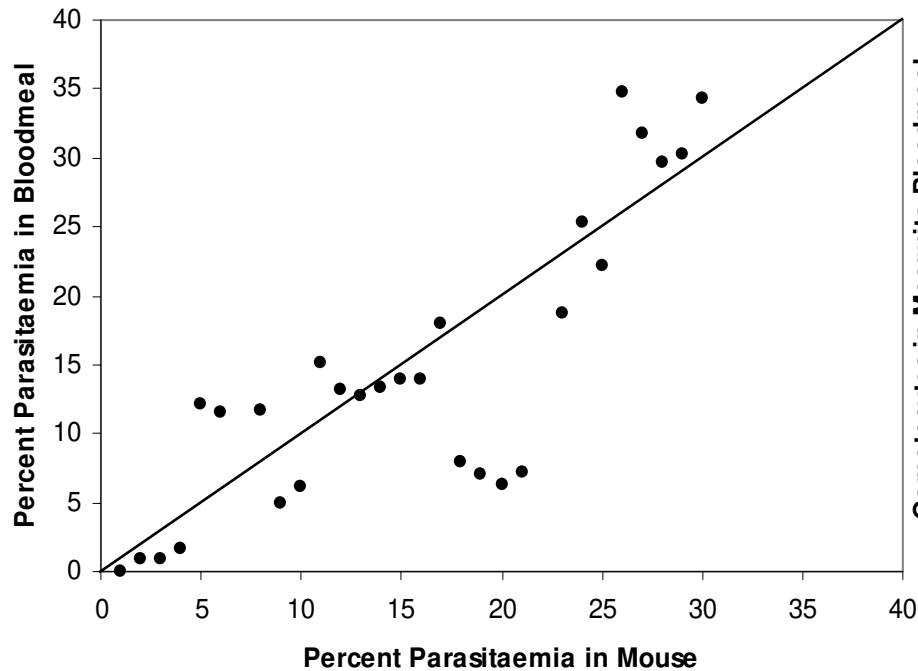
MOSQUITO PROBING AND GAMETOCYTE KINETICS



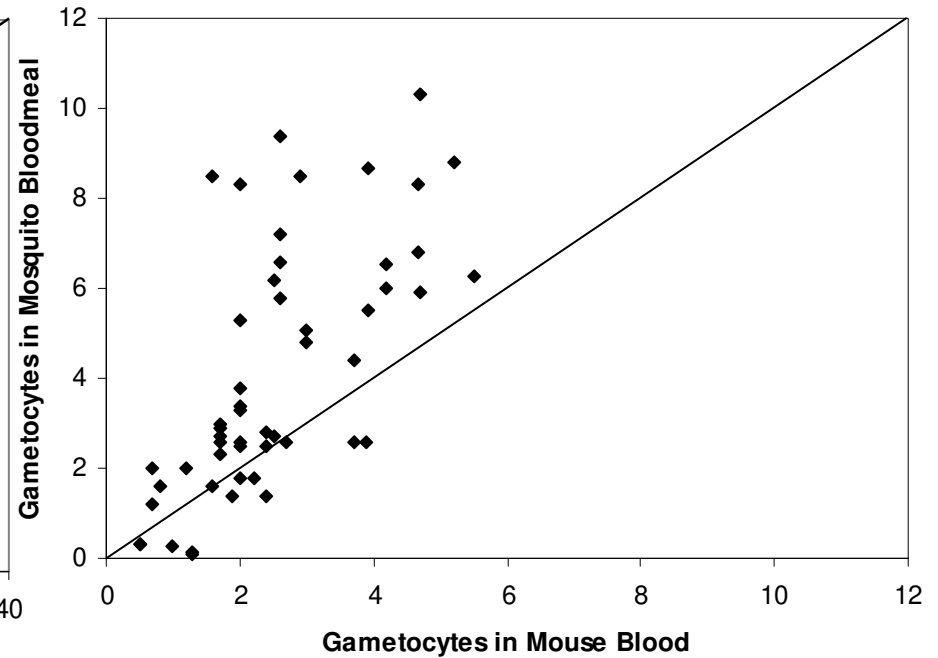
- Total number parasites increased; $T_{50\%}$ advanced by 1.2-1.64 days
- Total number of gametocytes increased; $T_{50\%}$ advanced by 2.0-2.47 days
- Increased rate of gametocyte production



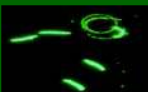
P. BERGHEI INGESTION: GAMETOCYTE BIAS IN THE BLOODMEAL



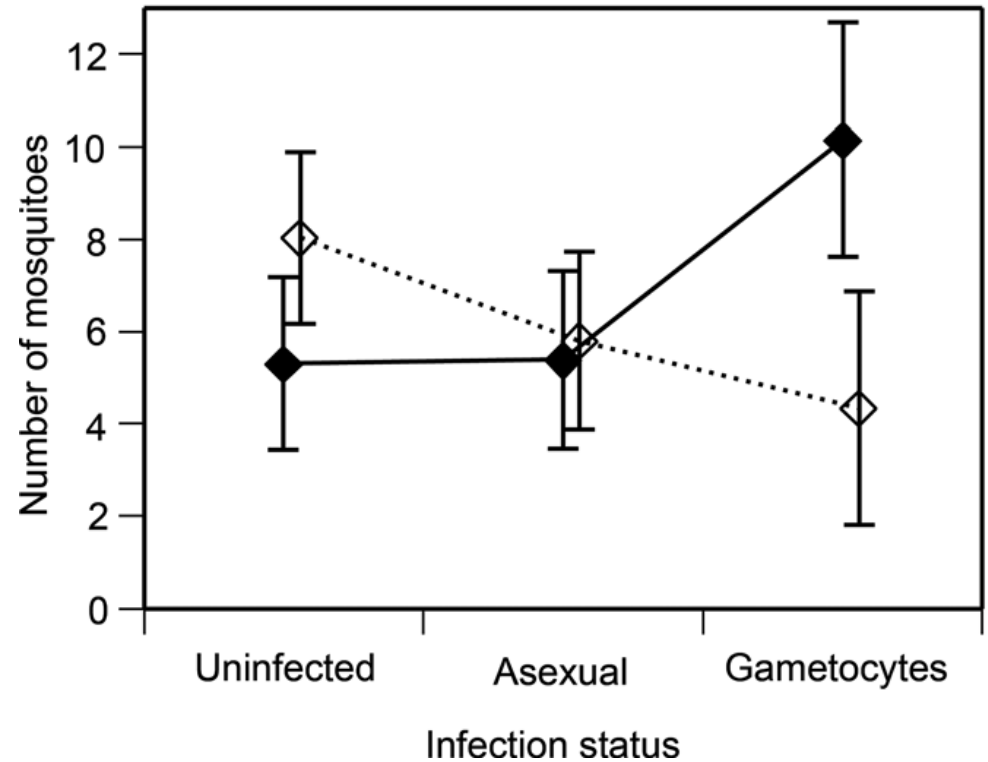
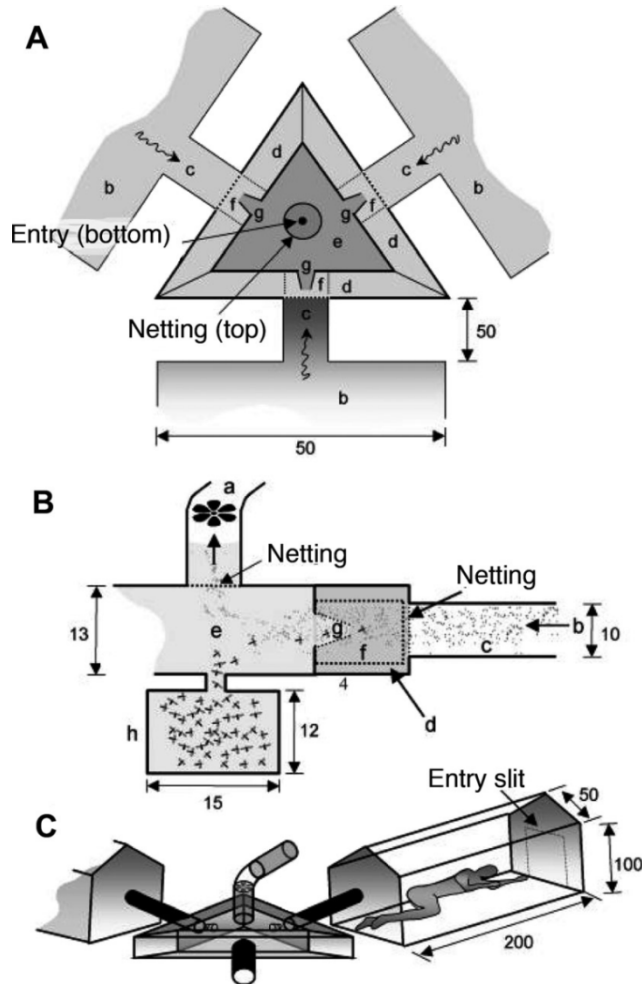
Parasites



Gametocytes



MOSQUITOES PREFER GAMETOCYTE CARRIERS

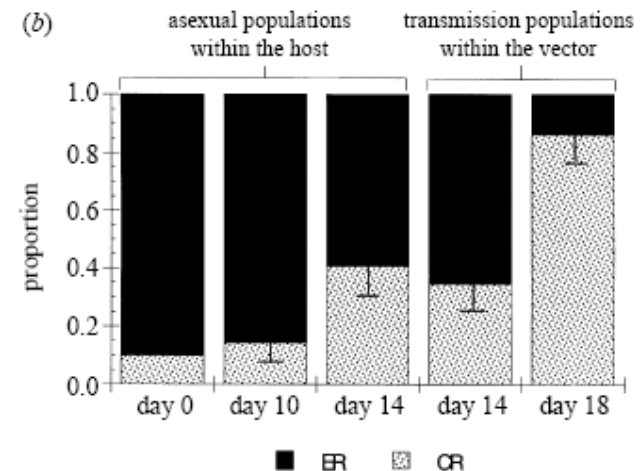
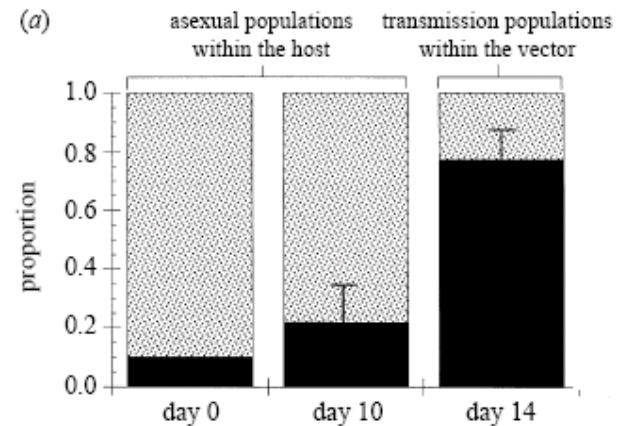


Lacroix et al. (2005) 3: e298.

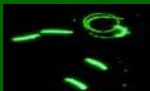


EMERGENCE OF COMPETING CLONES

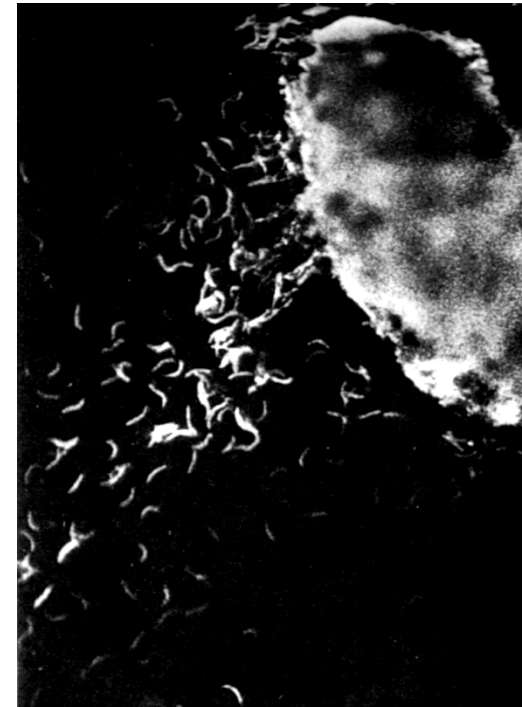
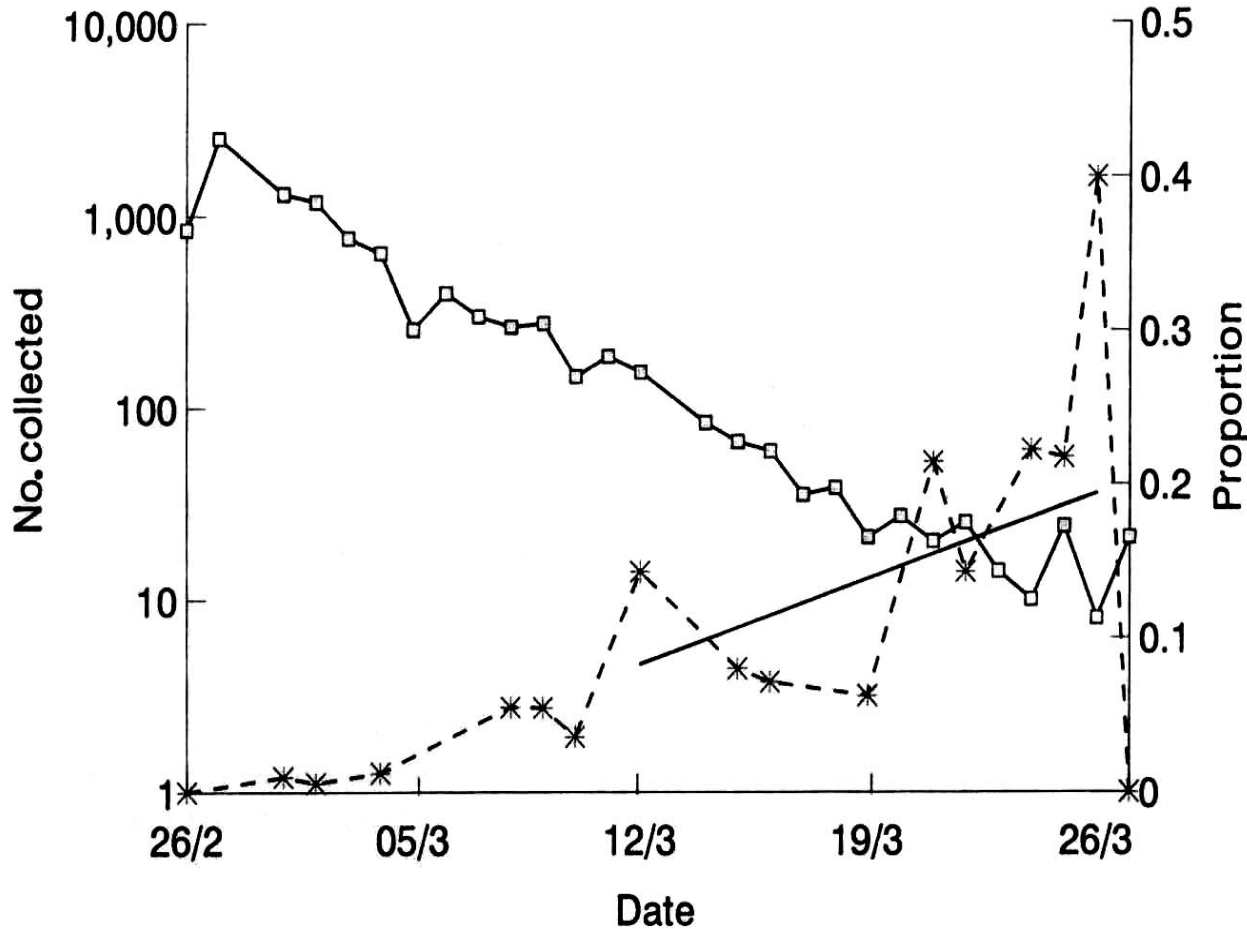
- *P. chabaudi*
- 'Minority' clones in host transmitted to mosquito
- Population in vector is disproportionately higher than in host.
- Mixed infections may transmit at higher rate than clonal infections



Taylor et al., Proc. R. Soc. Lond. B (1997) 264, 927-935



OLD MOSQUITOES ARE DANGEROUS....



EPIDEMIOLOGICAL IMPLICATIONS

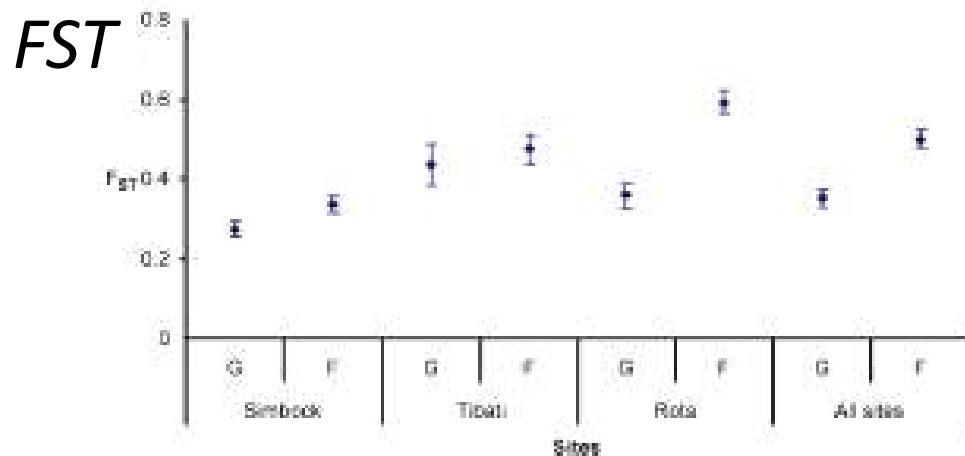
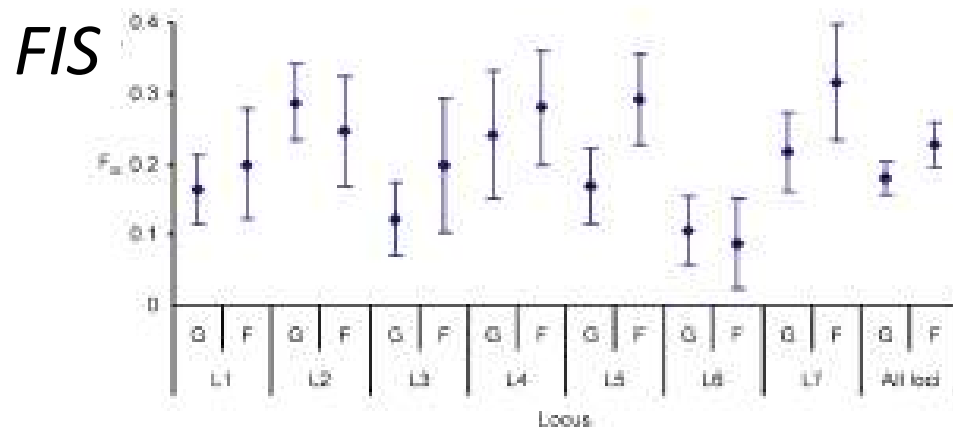
- **Individuals exposed to more mosquitoes make even greater contribution to transmission than thought.**
 - Greater induction of gametocytes
 - Greater infection to the mosquito (proportional and absolute)
 - Greater infection from the mosquito (absolute)
- **Mosquito-infective gametocytes produced even when 'clone' is in minority**
- **Gametocyte-infected individuals attract mosquitoes**





STRUCTURE OF PARASITE POPULATIONS IN MOSQUITOES

Inbreeding coefficient statistics for *P. falciparum* oocysts collected from *A. gambiae* (G) and *A. funestus* (F) in Cameroon and Kenya.



Annan et al., 2007 PNAS 104: 7987–7992



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STRUCTURE OF PARASITE POPULATIONS IN MOSQUITOES

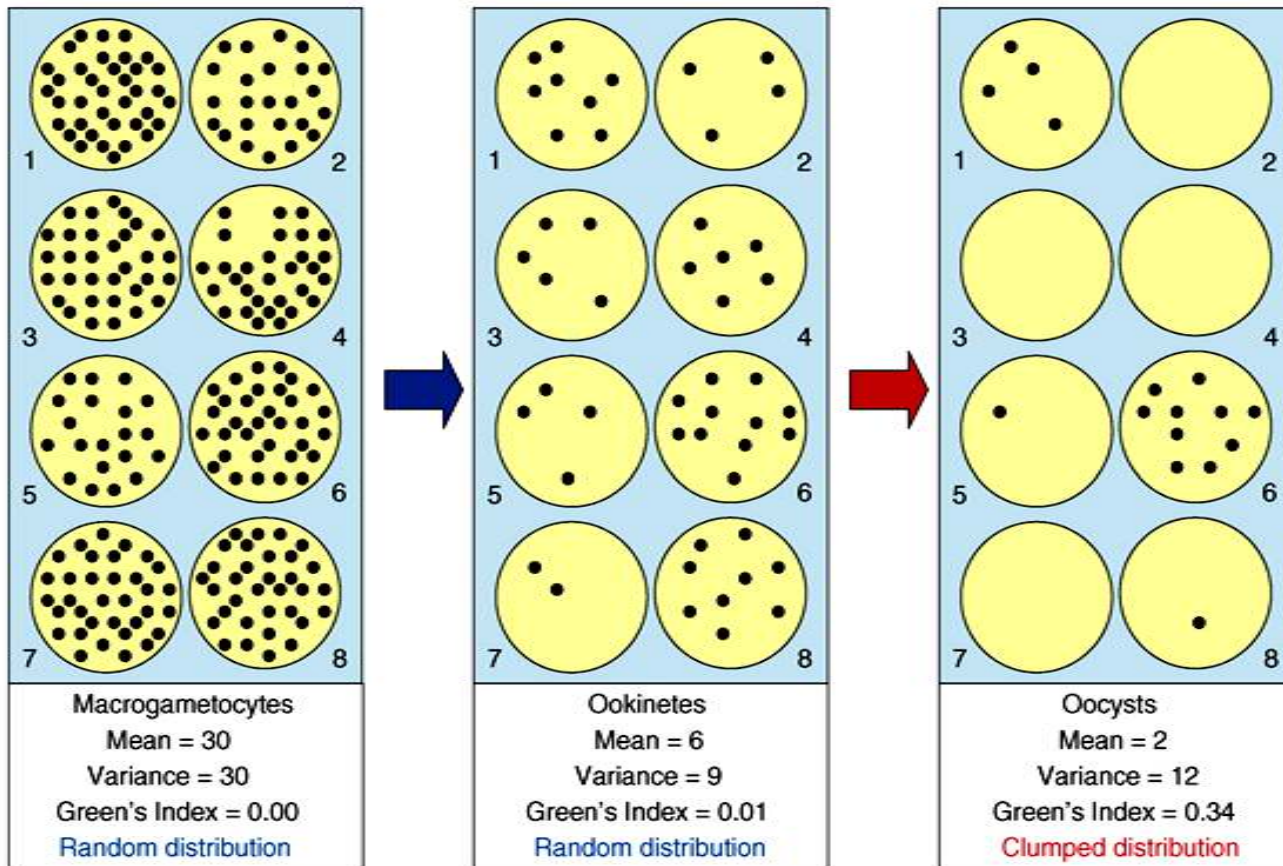
	Selfing rate	Random mating in midgut	Genotypic LD (overall)	Genotypic LD (outcrossed oocysts)	Haplotypic LD (selfed oocysts)	Clonal relatedness (within people)
Tanzania	0.33	n/a*	n/a	n/a	n/a	n/a
PNG	0.5	Yes (a/c* nulls)	n/a	n/a	n/a	n/a
Kenya	0.4	No	Yes	n/a	n/a	n/a
Malawi	0.5	Yes (a/c nulls)	Yes	No	No	inconclusive

- *P. falciparum* is a fully 'sexual' organism
- Oocysts collected in Malawi (and PNG) show evidence of random mating and no linkage disequilibrium.

Mzilahowa T, et al., 2007) PLoS ONE 2(7): e613.doi:10.1371/journal.pone.0000613



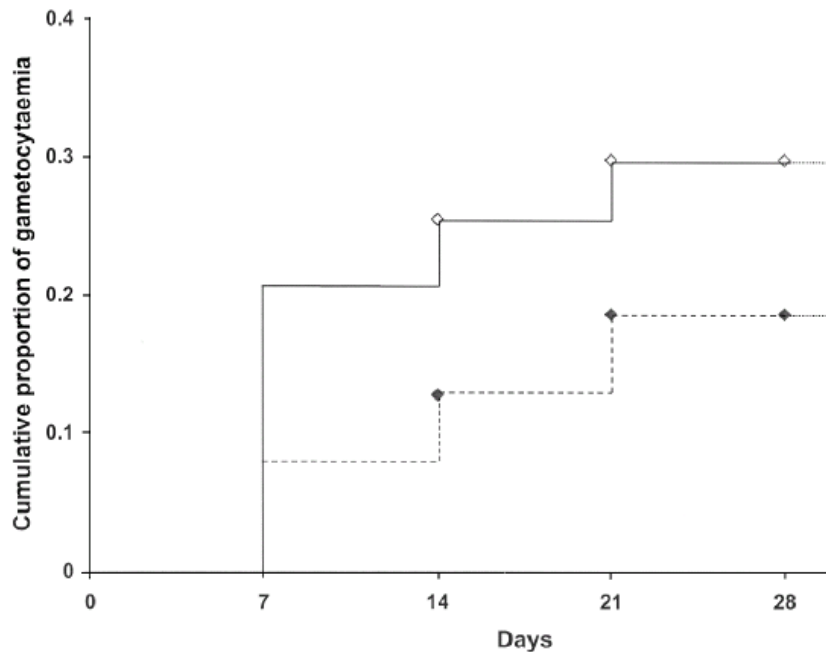
PARASITE AGGREGATION AND TRANSMISSION



TRENDS in Parasitology



GAMETOCYTAEMIA IS INDUCED BY ANTIMALARIALS



- Kaplan-Meier estimator of the time to gametocytaemia for untreated children (dotted line) and SP-treated children (solid line).
- Groups differed in asexual parasite density at enrolment. Gametocyte carriers on day 0 were excluded, as well as children who reported the use of antimalarial drugs prior to enrolment. Log-rank $P = 0.05$

Bouesma et al., Malaria Journal 2004, 3:18

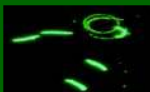


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GAMETOCYTES ARE INFECTIVE AFTER MALARIA THERAPY

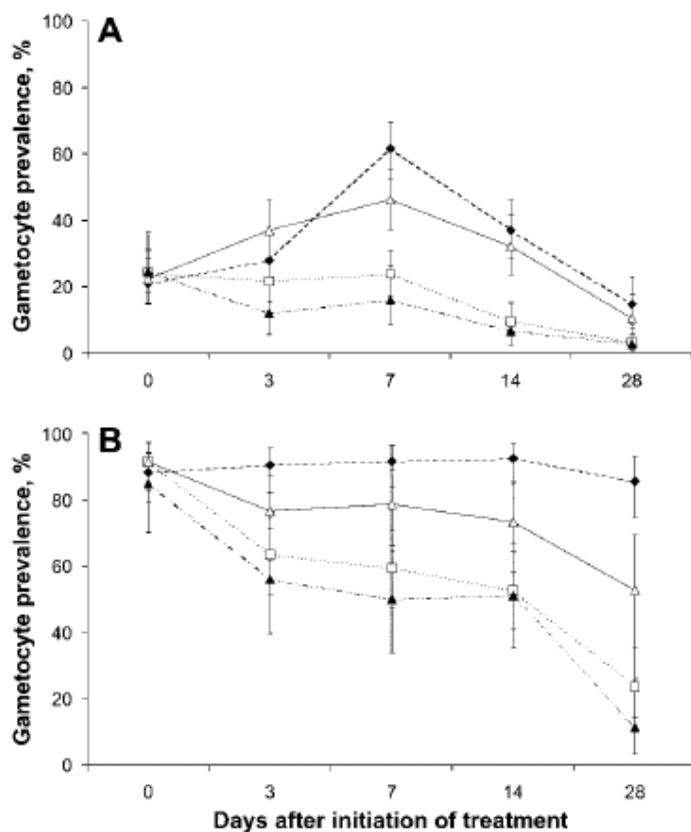


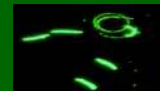
Table 4. Percentage of mosquitoes that became infected in membrane-feeding assays, by treatment arm.

Treatment arm	Infected mosquitoes, % (proportion)	RR (95% CI) ^a
SP monotherapy	6.9 (52/750)	1
SP plus AQ	5.5 (41/750)	0.79 (0.53–1.17)
SP plus AS	2.3 (17/750)	0.33 (0.19–0.56)
AL	3.6 (27/750)	0.52 (0.33–0.82)

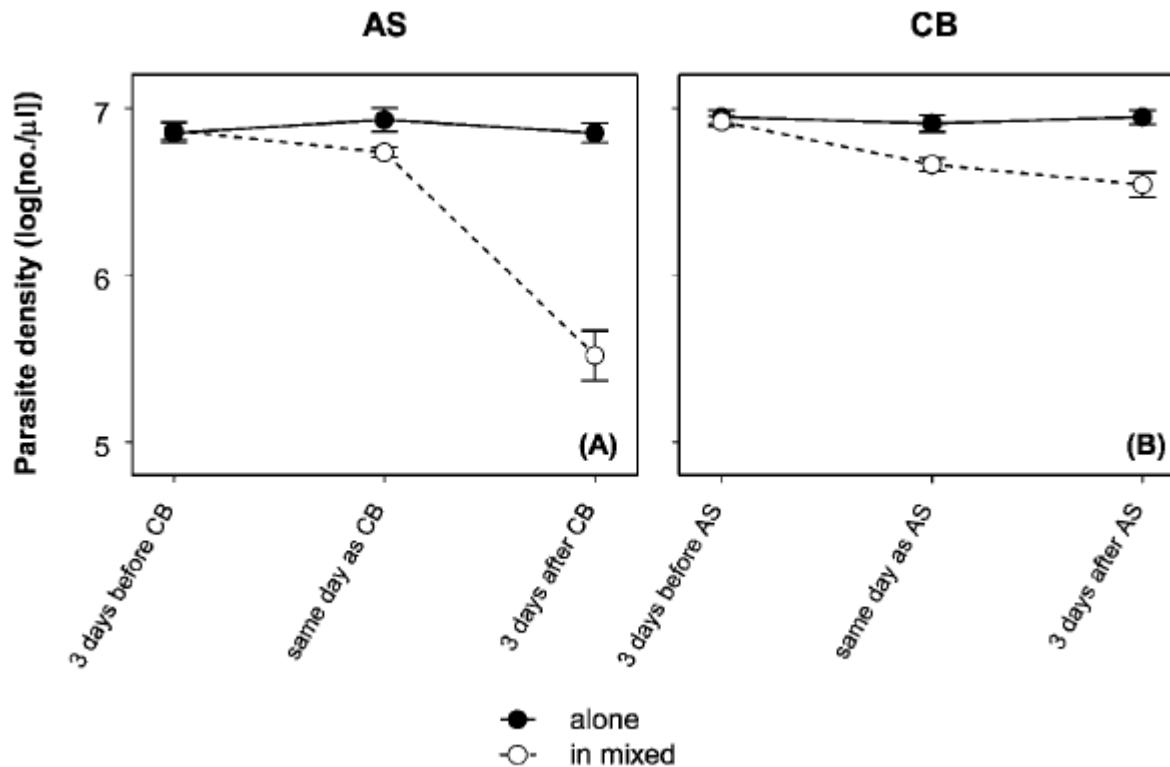
NOTE. Twenty-five randomly selected children from each treatment arm were included in the assays. AL, artemether-lumefantrine; AQ, amodiaquine; AS, artesunate; CI, confidence interval; RR, relative risk; SP, sulfadoxine-pyrimethamine.

^a The RR for the probability of a mosquito becoming infected, with the SP monotherapy arm as the reference group.

Bouesma et al., J Inf Dis 2006; 193:1151–9

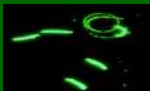


EMERGENCE OF DRUG RESISTANT-PARASITES FROM MIXED INFECTIONS



- Total numbers of AS (A) and CB (B) parasites produced over the whole infection in single and mixed (AS and CB simultaneously or 3 days apart) infections in experiment 1 (mean \pm SEM). See table 1 for the numbers of mice on which data points are based.

De Roode et al., 2005 166: 531-542



EMERGENCE OF GAMETOCYTES POST-COMBINATION THERAPY

Table 2. Sub-patent parasitaemia and gametocyte carriage before and after Treatment.

Group		Day 0	Day 3	Day 7*	Day 14
AS+SP	Parasitaemia detected by PCR	84.6% (44/52)	25.5% (12/47)	6.5% (3/46)	4.4% (2/45)
	Gametocytaemia detected by RT-PCR	11.5% (6/52)	-	6.5% (3/46)	4.4% (2/45)
AS+SP+PQ	Parasitaemia detected by PCR	100% (52/52)	15.7% (8/51)	8.3% (4/48)	6.4% (3/47)
	Gametocytaemia detected by RT-PCR	11.5% (6/52)	-	8.3% (4/48)	6.4% (3/47)

*Risk difference between the two treatment groups on day 7: 1.9% (95%CI -8.5% to +12.3%), on day 14: 2% (95%CI -7.2% to +11.2%).

doi:10.1371/journal.pone.0001311.t002

El-Sayed et al., (2007) PLoS ONE 2:e1311. doi:10.1371/journal.pone.0001311



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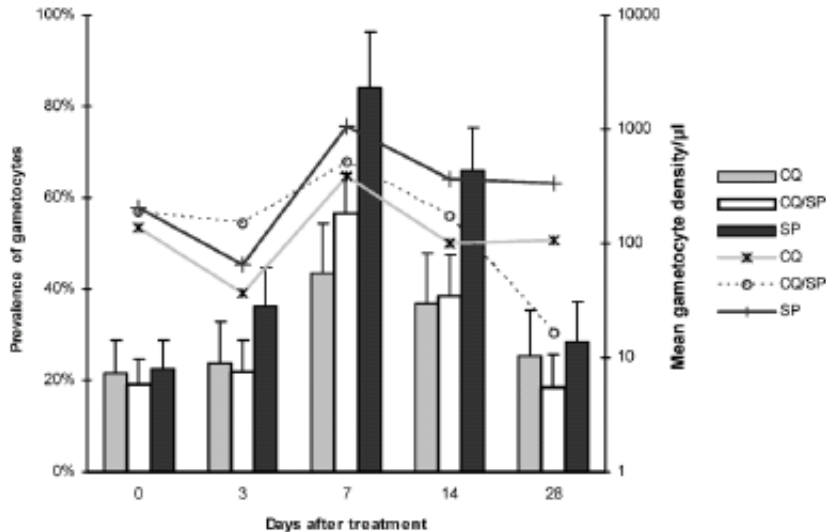
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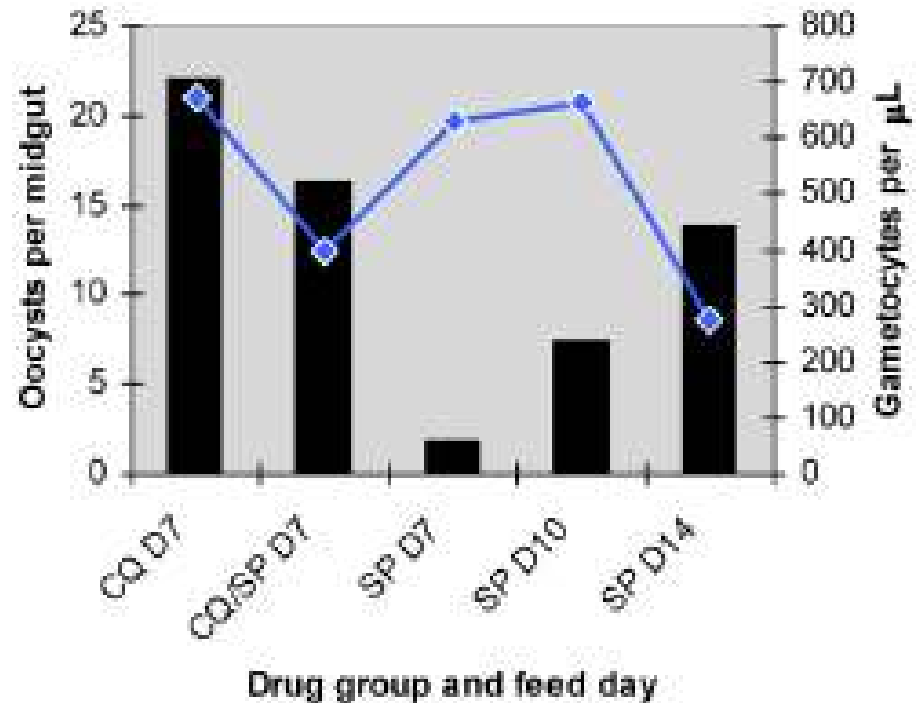
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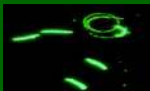
PROLONGED GAMETOCYTE INFECTIVITY FOLLOWING DRUG TREATMENTS



	Number of children evaluated				
Drug group	D0	D3	D7	D14	D28
CQ	126	88	85	76	75
CQ/SP	193	138	124	117	109
SP	181	127	38	106	99

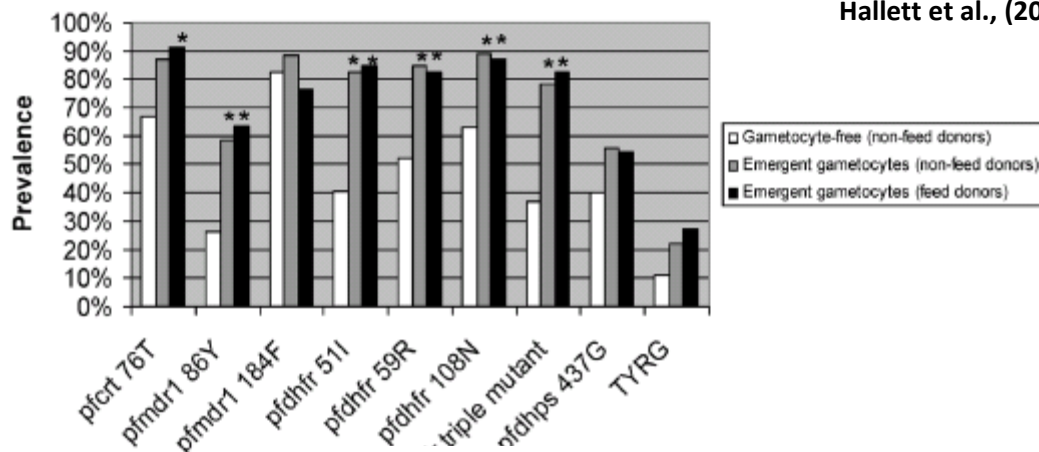


Hallett et al., (2006) PLoS Clin Trials 1(3): e15. DOI: 10.1371/journal.pctr.0010015



TRANSMISSION ADVANTAGE OF RESISTANT GENOTYPES

Hallett et al., (2006) PLoS Clin Trials 1(3): e15. DOI: 10.1371/journal.pctr.0010015



“..the presence of the four-locus multidrug-resistant haplotype TYRG (consisting of mutations pfcr1-76T, pfmdr1-86Y, pfdhfr-59R, and pfdhps-437G) was associated with significantly higher oocyst burdens after treatment with the combination CQ/SP.

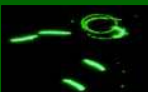
Drug Group	TYRG in Feed Sample Gametocytes			TYRG in Midgut Oocysts		
	Mean Oocyst Burden		Oocyst Ratio, TYRG Genotype versus Others (95% CI) [Number Mosquitoes Dissected]	Mean Oocyst Burden		Oocyst Ratio, TYRG Genotype versus Others (95% CI) [Number Midguts Positive]
	TYRG	Other		TYRG	Other	
CQ	0.095	1.06	0.090 (0.009–0.886) p = 0.039 [368]	2.00	30.80	0.065 (0.008–0.508) p = 0.009 [12]
CQ+SP	4.03	0.081	49.4 (4.38–557) p = 0.002 [370]	36.85	3.75	9.83 (3.260–29.63) p < 0.001 [18]
SP	0.067	0.27	0.249 (0.016–1.01) p = 0.051 [942]	6.58	13.67	0.482 (0.168–1.382) p = 0.174 [18]



DRUG RESISTANT GENOTYPES AND TRANSMISSION

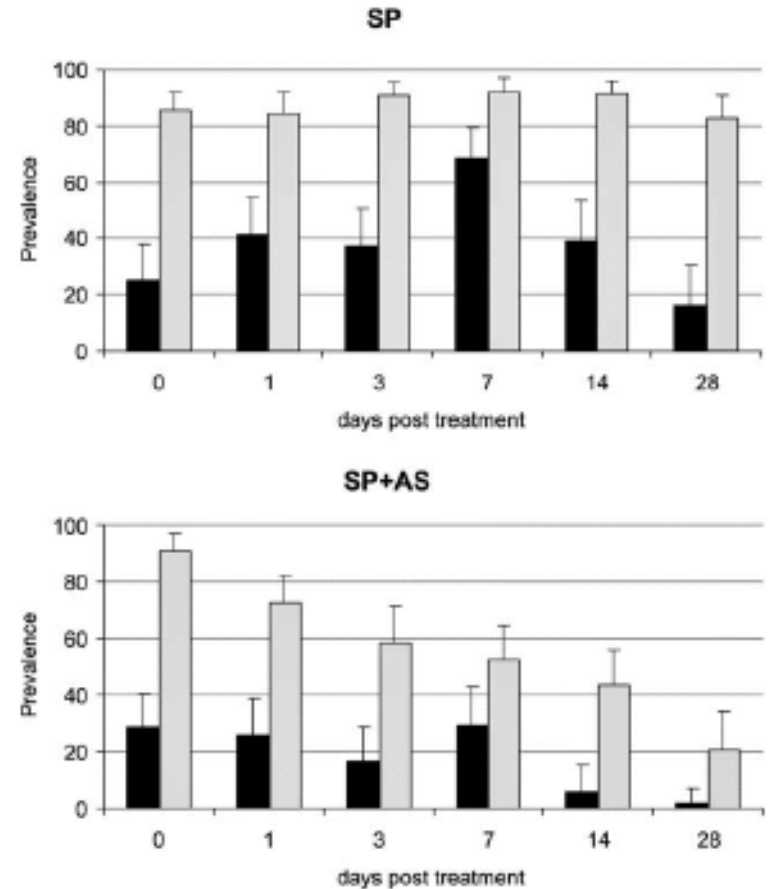
- Treated with CQ and SP (Fansidar)
- Parasites examined pre-treatment and days 7, 14 , 21 (transmission season), and 80 (start of the dry season).
- Gametocyte carriage
 - Microscopy: increased significantly following CQ and SP treatment, days 7 and 14.
 - RT-PCR: no significant difference in gametocyte rate days 0 through 14.
- Day 80: 33% in CQ- and 8% in SP-treated group.
- Alleles associated with drug resistance of *P. falciparum* to chloroquine (pfprt and pfmdr1) and pyrimethamine (dhfr) increased in frequency through time following both drug treatments.
- Infections with drug-resistant parasites tended to have higher gametocyte prevalence than drug-sensitive infections.

Ali et al., 2006 Trans R Soc Trop Med Hyg. 100:176-83



EXTENDING THE INFECTIOUS WINDOW

- Microscopically detectable parasites observed to day 28
- PCR detectable parasites always present
- Still approx. 20 % prevalence after 28 days



Schneider et al., (2006) Int J Parasitol 36: 403–408

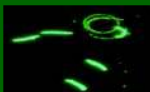


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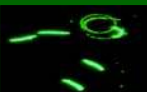
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THE SELFISH GAMETOCYTE AND DRUG RESISTANCE

- **Gametocyte manipulates:**
 - Mosquito host location
 - Mosquito blood feeding
 - Gametocyte uptake
- **Increases probability of sexual recombination and infection through the mosquito.**
- **Gametocytes**
 - increase in number post-drug treatment
 - maintain low densities for prolonged periods in host
 - Are infectious long after treatment
- **Drug resistant gametocytes**
 - Increase in frequency in host and mosquitoes post-treatment
 - Have an extended period in circulation even after combination treatments





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