Avian malaria: a new lease of life for an old experimental model to study the evolutionary ecology of *Plasmodium*

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Supplementary materials

Includes Supplementary Tables S1 and S2, and Supplementary Figures S1-S7

Table S1 - Summary of the experiments included in the paper. The table indicates: the year in which they were carried out, the number of birds used and whether they were infected or uninfected, the average number of mosquitoes per bird, whether the birds where at the chronic or acute stage of the infection, and the traits measured in both the bird and mosquito hosts. *par*: parasiaemia, *gam*: gametocytaemia, *conv*: conversion rate, *pcv*: packed cell volume (anaemia), *prev*: oocyst prevalence, *burd*: oocyst burden, *hm*: haematin, *size*: mosquito size, *surv*: survival, *fec*: fecundity

Exp nb.	Year	Nb birds		Mosquitoes/ bird (mean)	Bird infection stage	Mosquito strain	Traits measured		REF
		Uninf	Inf				In the bird	In the mosquito	
1	2009	0	9	24	Chronic	Wild	*par*	*prev, burd*	[1]
2	2009	0	13	15	Chronic	Wild	*par*	*prev, burd*	[1]
3	2009	5	5	55	Acute	SLab	*par, gam, conv*	*prev, burd, hm, size, surv*	[2]
4	2010	6	6	44	Acute	SLab	*par, gam ,conv*	*prev, burd, hm, size, surv*	[2]
5	2010	0	3	150	Acute	SLab	*par*	*prev, burd, hm*	[3]
6	2010	0	5	10	Acute	SLab	*par, gam, conv*	*prev, burd, hm, size, fec*	[4]
7	2011	5	4	54	Acute	SLab	*par, gam, conv, pcv*	*prev, burd, hm, size, fec, surv*	[5]
8	2012	0	14	21	Chronic	SLab	*par, pcv*	*prev, burd*	[6]
9	2012	0	4	15	Acute	SLab	*par*	*prev, burd, hm,*	[4]
10	2013	0	5	47	Acute	SLab	*par, gam, conv, pcv*	*prev, burd, hm, size*	[7]
11	2013	0	6	50	Acute	SLab	*par, gam, conv, pcv*	*prev, burd, hm, size, fec*	[5]
12	2013	10	9	63	Acute	SLab	*par, gam, conv, pcv*	*prev, burd, hm, size, fec*	[8]
13	2014	0	5	23	Acute	SLab	*par, gam, conv, pcv*	*prev, burd, hm, size*	[7]
14	2014	0	10	31	Acute	Wild	*par, gam, conv*	*prev, burd, hm*	[9]

References cited in Table S1:

[1] Vézilier, J., Nicot, A., Gandon, S. & Rivero, A. (*in prep*) The impact of insecticide resistance on *Plasmodium* development in *Culex pipiens:* lessons from chronic infections. Currently a chapter in J Vézilier's thesis: *Resistance aux insecticides et transmission de la malaria chez le moustique Culex pipiens.* Université de Montpellier (2011)

[2] Zélé, F., Nicot, A., Duron, O & Rivero, A (2012) Infection with *Wolbachia* protects mosquitoes against *Plasmodium*-induced mortality in a natural system. *J. Evol. Biol.* 25, 1243-1252. doi: 10.1111/j.1420-9101.2012.02519.x

[3] Vézilier, J., Nicot, A., Gandon, S. & Rivero, A. 2010 Insecticide resistance and malaria transmission: infection rate and oocyst burden in Culex pipiens mosquitoes infected with Plasmodium relictum. *Malar. J.* **9**, 379. (doi:10.1186/1475-2875-9-379)

[4] Vézilier, J., Nicot, A., Gandon, S. & Rivero, A. 2012 Plasmodium infection decreases fecundity and increases survival of mosquitoes. *Proc. R. Soc. B Biol. Sci.* **279**, 4033–4041. (doi:10.1098/rspb.2012.1394)
[5] Zélé, F., Nicot, A., Duron, O & Rivero, A (*in prep*) Fecundity effects of *Wolbachia* on *Plasmodium*-infected mosquitoes. Currently a chapter in F. Zélé's PhD thesis: *Interaction entre la bactérie endosymbiotique Wolbachia et le parasite responsable de la malaria aviaire, Plasmodium relictum chez le moustique Culex pipiens.* Université de Montpellier (2012)
[6] Cornet, S., Nicot, A., Rivero, A., & Gandon, S. (2014). Evolution of plastic transmission strategies in avian malaria. *PLoS pathogens*, *10*(9), e1004308.
[7] Pigeault, R., Nicot, A., Gandon, S., Rivero, A. 2015. (*in prep*) Impact of mosquito age on *Plasmodium* transmission in the avian malaria model
[8] Pigeault, R., Vézilier, J., Nicot, A., Gandon, S., Rivero, A. 2015 Transgenerational effect of infection in Plasmodium-infected mosquitoes, submit Bio. Let
[9] Pigeault, R., Nicot, A., Gandon, S., Rivero, A. unpublished data

Table S2: Description of statistical models used. The response variable was not transformed unless otherwise stated. N gives the number of mosquitoes included in each analysis. "Maximal model" gives the complete set of explanatory variables included in the model. "Minimal model" gives the model containing only the significant variables and their interactions. Square brackets indicate variables fitted as random factors (nested variables are indicated with a backslash). Curly brackets indicate the error structure used (n: normal errors, b: binomial errors). Please see Table S1 for the acronyms of the variables fitted in the models. Other acronyms: *exp* = experiment, *asex* = proportion of asexuals, $para_{t-1}$ = parasitaemia at the previous time point, *pass1* = all passages (starting at 1 finishing at 103), *pass2* = passages within each segment (each segment starts with a mosquito passage followed by a variable number of standard passages), *pass3* = passages within each segment but excluding the mosquito passage, *pass4* = passages within each segment but excluding the mosquito passage and the first standard passage immediately following it, *pass5* = passages within each segment as a categorical variable with 3 levels (mosquito p fitted as a categorical variable with three values: first passage (mosquito), second passage (standard), and the rest (standard), *S* = segment number.

Variable of interest	Resp. variable	Model nb	N (mosquit)	N (birds)	Maximal model	Minimal model	R subroutine {error struct}
Serial passages							
Parasitemia	log (*par*)	*1*	-	414	par_{t-1} + *pass1*	par_{t-1} + *pass1*	glm {n}
	log (*par*)	2	-	414	par_{t-1} + *pass2*S*	par_{t-1} + *pass2* + *S*	glm {n}
	log (*par*)	3	-	414	par_{t-1} + *pass3*S*	par_{t-1} + *pass3* + *S*	glm {n}
	log (*par*)	4	-	414	par_{t-1} + *pass4*S*	par_{t-1}	glm {n}
	log (*par*)	5	-	414	par_{t-1} + *pass5*S*	par_{t-1} + *pass5*	glm {n}
Gametocytemia	log (*gam*)	6	-	338	gam_{t-1} + *pass1*	gam_{t-1}	glm {n}
	log (*gam*)	7	-	338	gam_{t-1} + *pass2*S*	gam_{t-1}	glm {n}
Trait correlations within the bird							
Parasitemia	*par*	8	-	62	*exp*	*exp*	glm {n}
Gametocytaemia	*gam*	9	-	54	*exp*	*exp*	glm {n}
	sqrt (*gam*)	10	-	54	sqrt (*par*) + [1|*exp*]	sqrt (*par*) + [1|*exp*]	lme {n}
	sqrt (*gam*)	11	-	54	sqrt (*asex*) + [1|*exp*]	sqrt (*asex*) + [1|*exp*]	lme {n}
Conversion rate	*conv*	12	-	54	*exp*	exp	glm {n}
Anaemia (packed cell volume)	*pcv*	13	-	42	*inf* + [1|*exp*]	*inf* + [1|*exp*]	lme {n}
	pcv	14	-	27	sqrt (*par*) + [1|*exp*]	1 + [1|*exp*]	lme {n}
	pcv	15	-	54	*conv* + [1|*exp*]	1 + [1|*exp*]	lme {n}

Table S2: Description of statistical models used (cont'd)

Predictors of mosquito infection							
Bird-related factors							
Oocyst prevalence	prev	16	1681	-	stage + [1|bird]	stage + [1|bird]	lmer {b}
	prev	17	-	52	sqrt (par) + sqrt (par^2) + [1|exp]	1 + [1|exp]	lmer {b}
	prev	18	-	44	sqrt (gam)+ [1|exp]	sqrt (gam)+ [1|exp]	lmer {b}
	prev	19	-	27	pcv + [1|exp]	1 + [1|exp]	lmer {b}
Oocyst burden	$burd^{-0.12}$	20	1209	-	stage + [1|exp]	stage + [1|exp]	lme {n}
	log (burd)	21	-	47	sqrt (par) + sqrt (par^2) + [1|exp]	sqrt (par) + sqrt (par^2) + [1|exp]	lme {n}
	log (burd)	22	-	39	sqrt (gam) + [1/exp]	1 + [1|exp]	lme {n}
	log (burd)	23	-	47	prev + [1|exp/bird]	prev + [1|exp/bird]	lme {n}
	log (burd)	24	-	27	pcv + [1|exp]	1 + [1|exp]	lme {n}
Blood meal size (haematin)	sqrt (hm)	25	-	52	sqrt (par) + [1|exp]	sqrt (par) + [1|exp]	lme {n}
Mosquito-related factors							
Oocyst prevalence	prev	26	1110	-	sqrt (hm) + sqrt (hm^2) + [1|exp/bird]	sqrt (hm) + [1|exp/bird]	lmer {b}
Oocyst burden	log (burd)	27	1110	-	sqrt (hm) + sqrt (hm^2) + [1|exp/bird]	sqrt (hm) + [1|exp/bird]	lme {n}
Blood meal size (haematin)	sqrt (hm)	28	-	42	pcv + [1|exp]	pcv + [1|exp]	lme {n}
	sqrt (hm)	29	1596		size*inf + [1|exp/bird]	size*inf + [1|exp/bird]	lme {n}
Predictors of mosquito fitness							
Fecundity	fec	30	1480		inf + [1|exp/bird]	inf + [1|exp/bird]	lme {n}
	fec	31	1480		hm*inf + hm^2 + [1|exp/bird]	hm*inf + hm^2 + [1|exp/bird]	lme {n}
Survival	surv	32	442		hm*inf + [1|exp/bird]	1+ [1|exp/bird]	lme {n}
	surv	33	-	11	sqrt (par) + [1|exp]	sqrt (par) + [1|exp]	lme {n}

Supplementary Figures

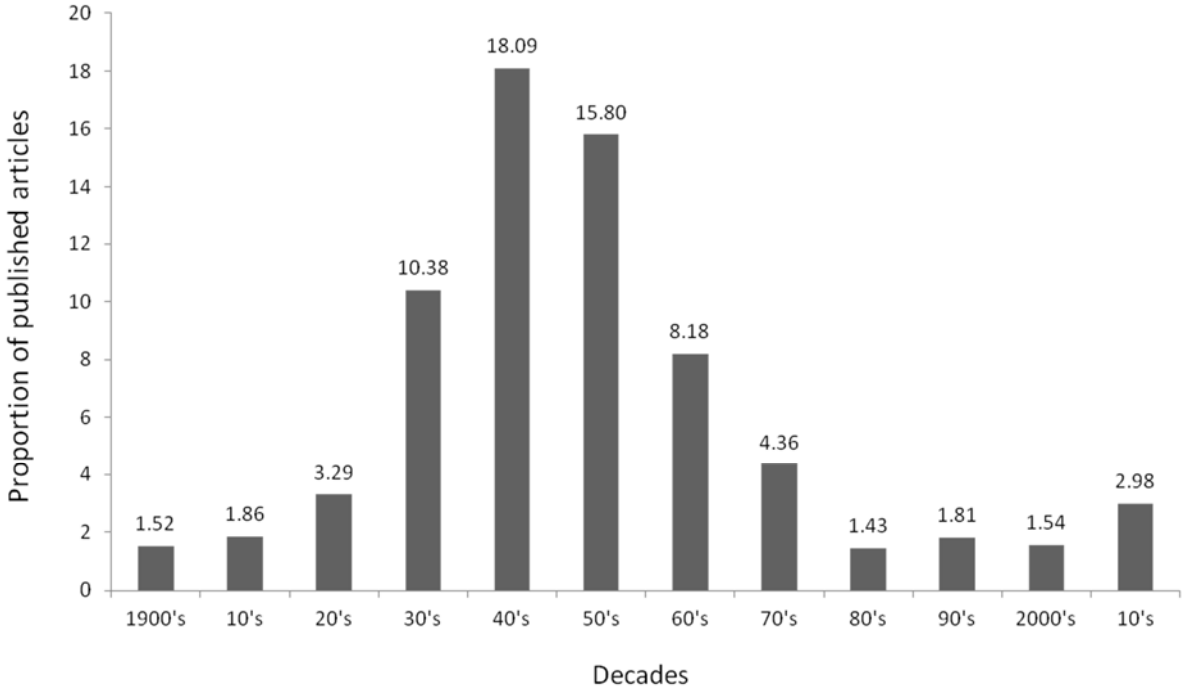

Figure S1. Proportion of total malaria papers published between 1900 and 2014 that deal with avian malaria. Search carried out on the Web of Science using the search terms "*Plasmodium* or malaria" (for total malaria papers) and "((plasmodium or malaria*) and (avian or bird*)) or (plasmodium and (gallinaceum or relictum or elongatum or cathemerium or lophurae or fallax))" (for avian malaria papers).

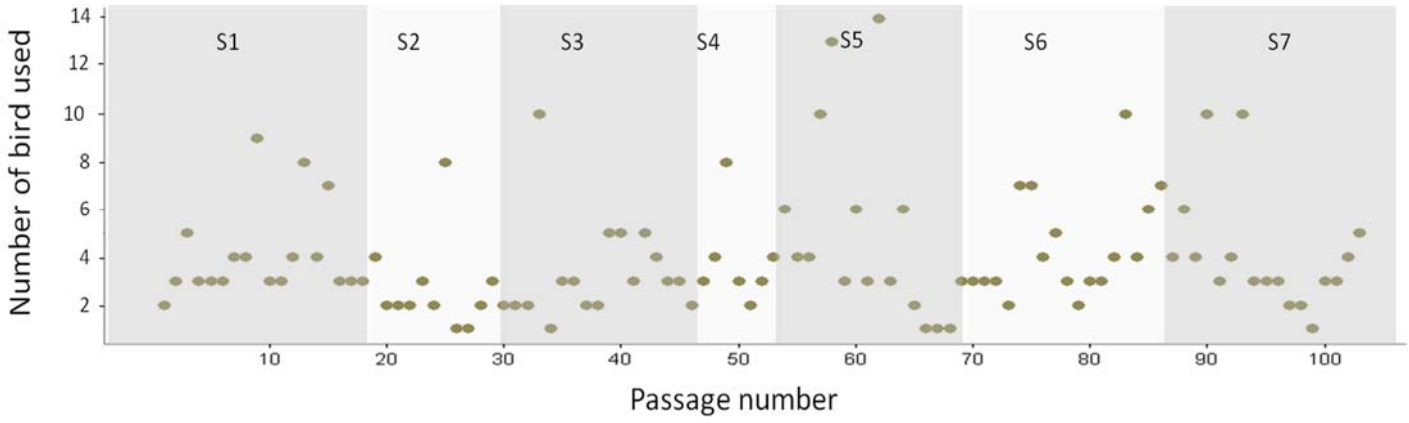

Figure S2. Number of bird used in the serial passage analysis.

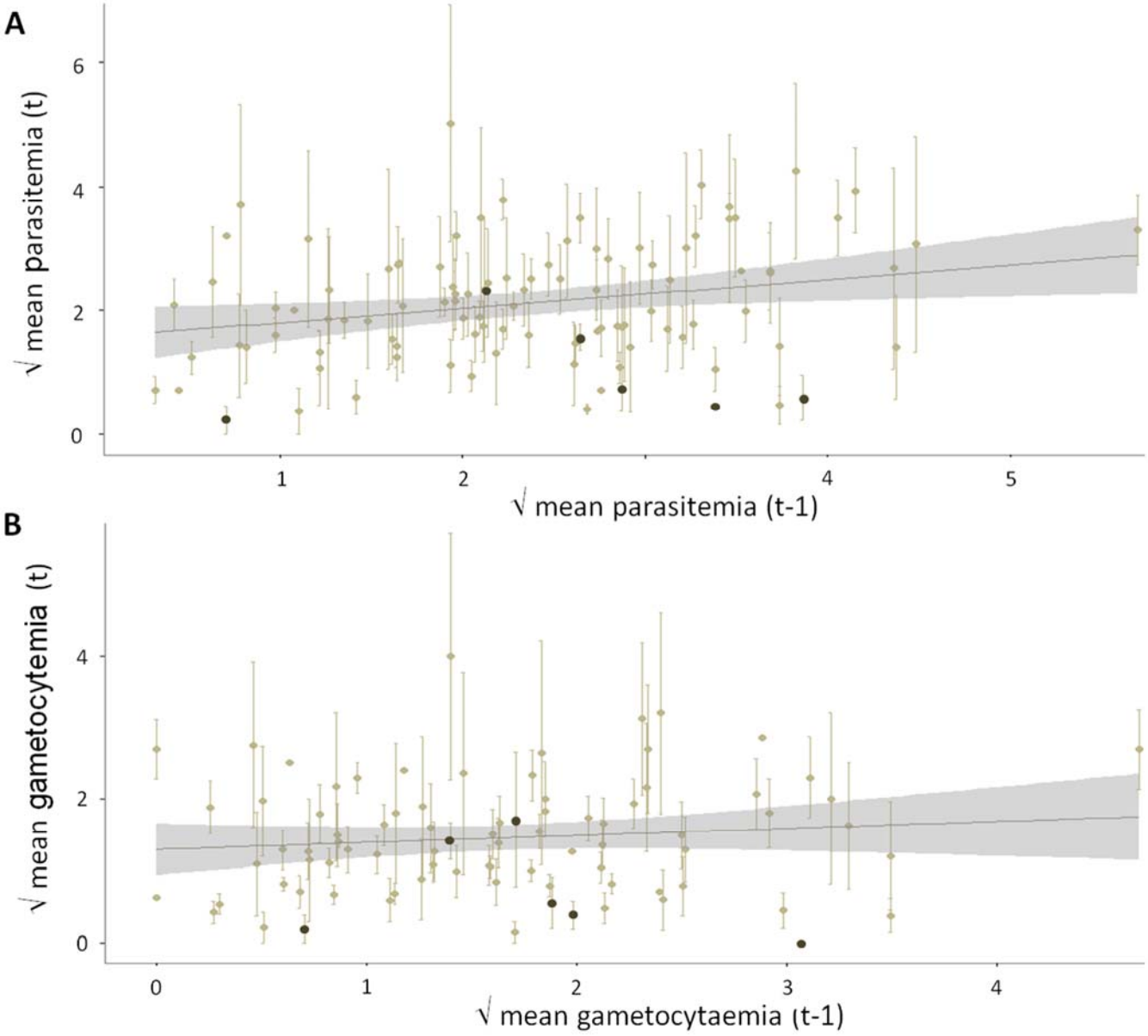

Figure S3. Correlation between mean parasitaemia (A) or gametocytaemia (B) at time (t) and mean parasitaemia or gametocytaemia at time (t-1) over the whole passage sequence since the isolation of the parasite strain from wild sparrows in 2009. Bars represent standard errors around the mean. Both variables have been square root transformed to improve the fit of the model. Dark grey dots represent the parasitaemias and gametocytaemias immediately following a mosquito passages (see **Figure 1**)

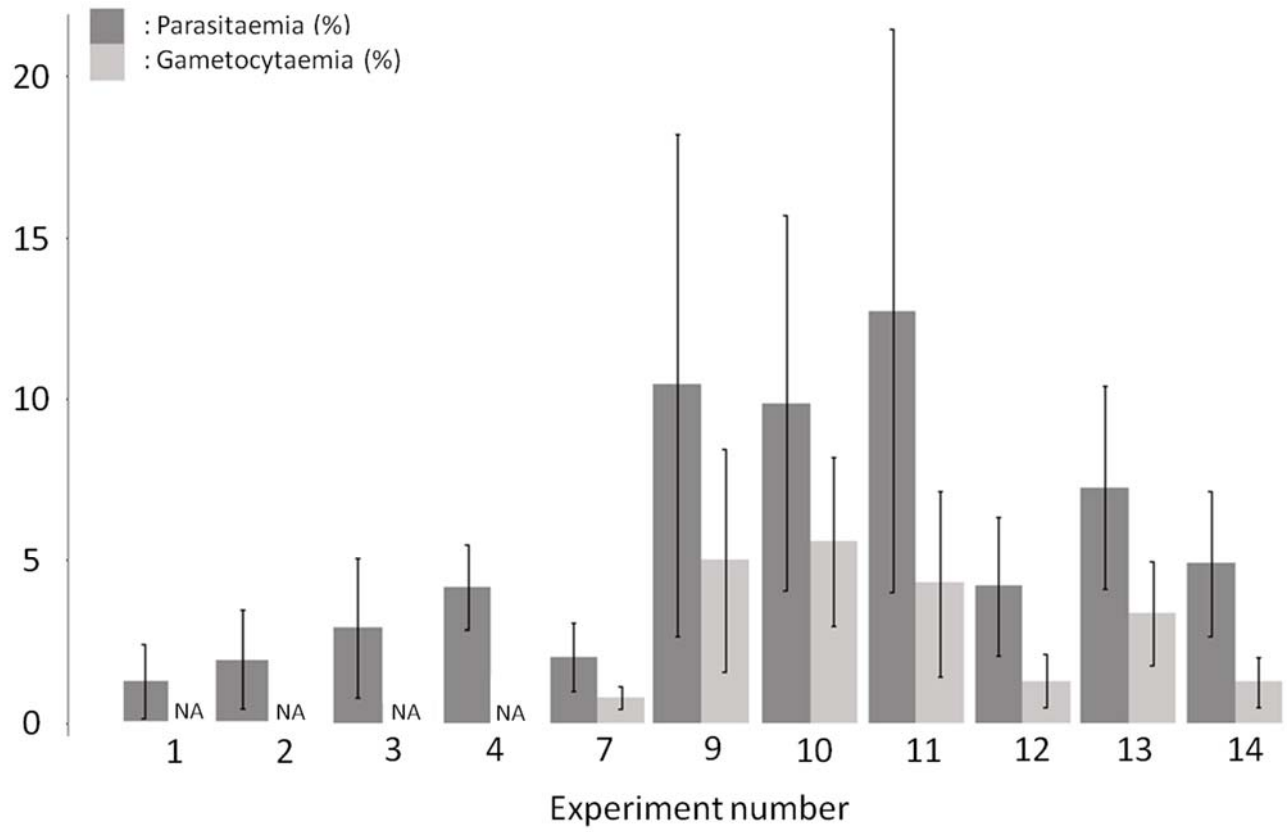

Figure S4. Mean parasitaemia (dark grey bars) and gametocytaemia (pale grey bars) in birds used in each of the 11 experiments reported in this paper. Bars represent standard errors around the means.

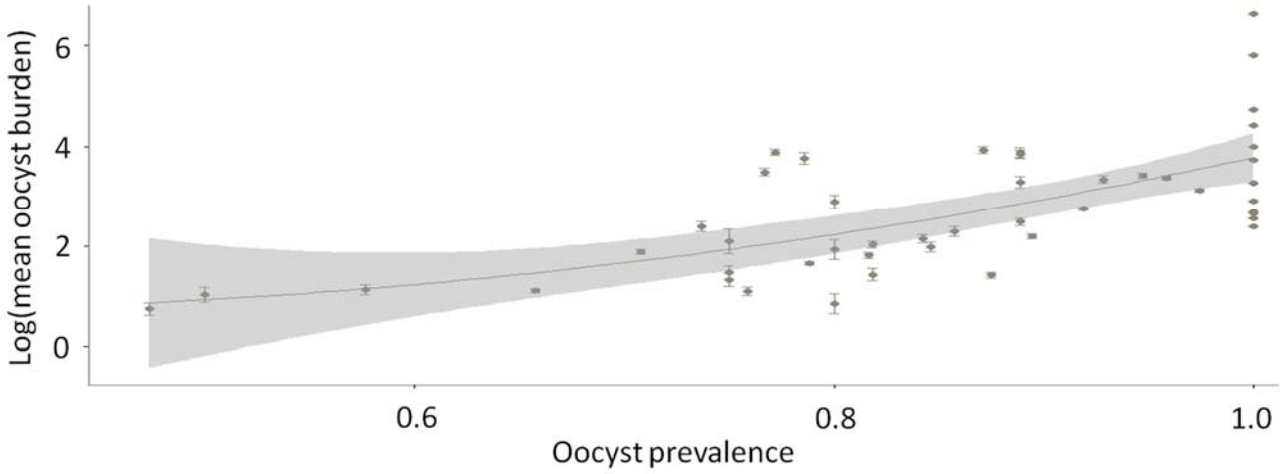

Figure S5. Relationship between oocyst prevalence and mean oocyst burden. Each point represents a different bird. Shaded areas on either side of the regression line represent 95% confidence intervals.

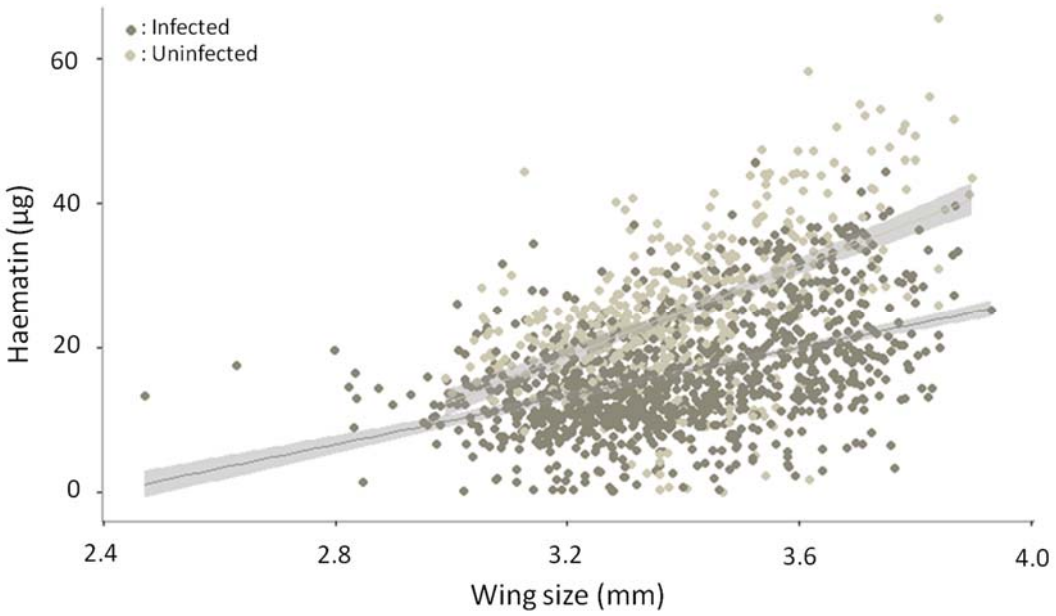

Figure S6. Relationship mosquito size (wing length) and blood meal size quantified as the amount of haematin excreted. Light grey dots: uninfected mosquitoes, dark grey dots: infected mosquitoes. Shaded areas on either side of the regression line represent 95% confidence intervals.

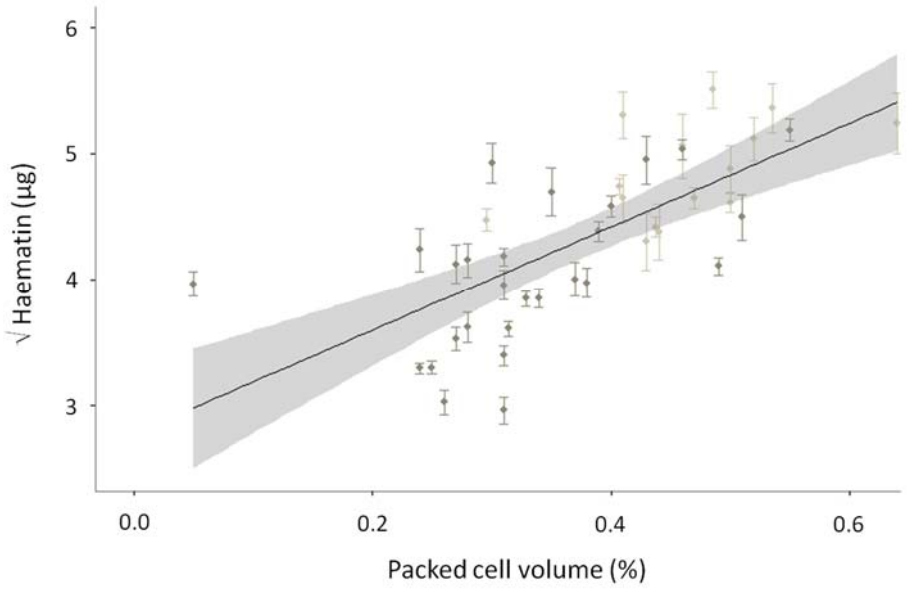

Figure S7. Relationship mosquito anaemia (quantified as packed cell volume) and blood meal size (quantified as the amount of haematin excreted). Light grey dots: uninfected mosquitoes, dark grey dots: infected mosquitoes. Shaded areas on either side of the regression line represent 95% confidence intervals.