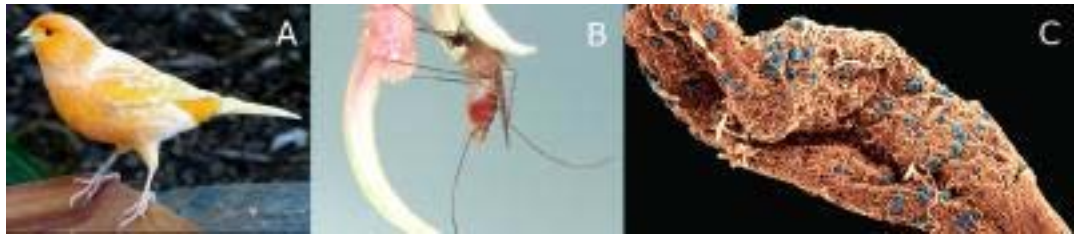


# *Adaptations for transmission in avian malaria: parasitic manipulation and plasticity*

Stéphane Cornet

Sylvain Gandon, Ana Rivero
Antoine Nicot

IRD Institut de recherche pour le développement

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erc

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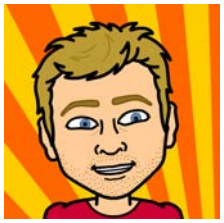
cnrs



# *About me*

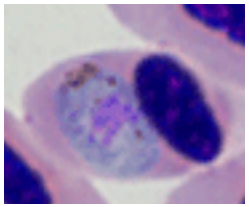
Immune variation and parasite-induced immunodepression
(Université de Bourgogne, 2006-2009)

Evolutionary ecology of avian malaria transmission
(CEFE-MIVEGEC Montpellier, 2010-2012)

Ecoimmunology and bioinvasions
(CBGP Montpellier-Sénégal, 2014- )

- PART 1 -

# What is avian malaria?

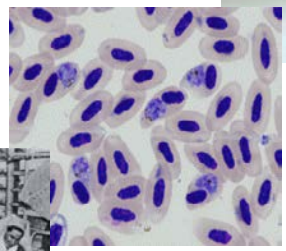


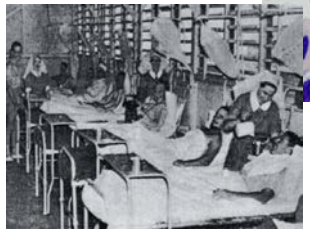
# *Malaria parasites*

Male-aria = bad air

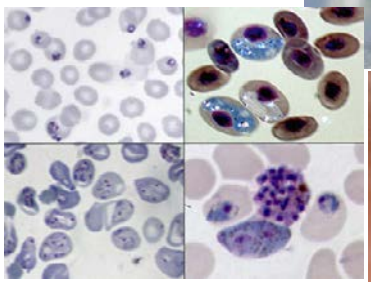
Alphonse Laveran (1880)
**Discovers *Plasmodium***

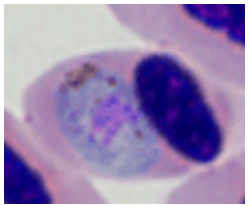
Ronald Ross (1898)
**Transmission by mosquitoes**





1940's

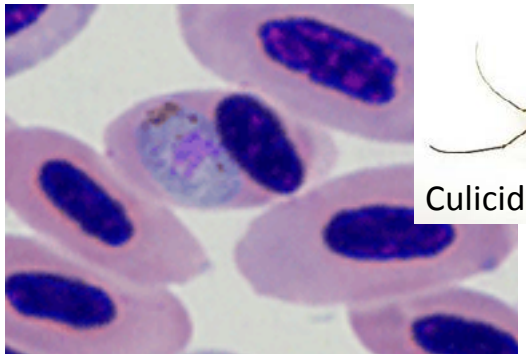




# *Avian malaria parasites (Haemosporidia)*

## Plasmodiidae

*Plasmodium (40 morphosp / 290 cyt-b lineages)*

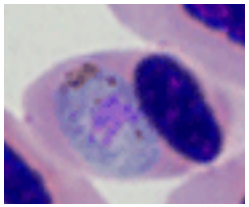



## Haemoproteidae

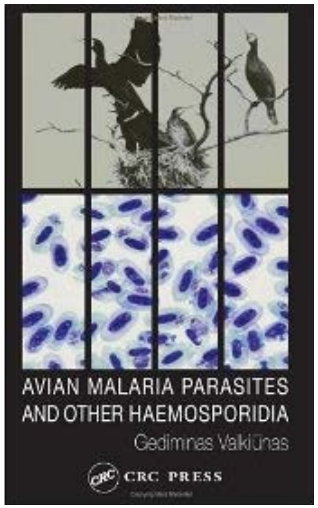
*(Para)Haemoproteus (74/436)*


## Leucocytozoidae

*Leucocytozoon (11/158)*

# *Avian malaria: molecular approach*



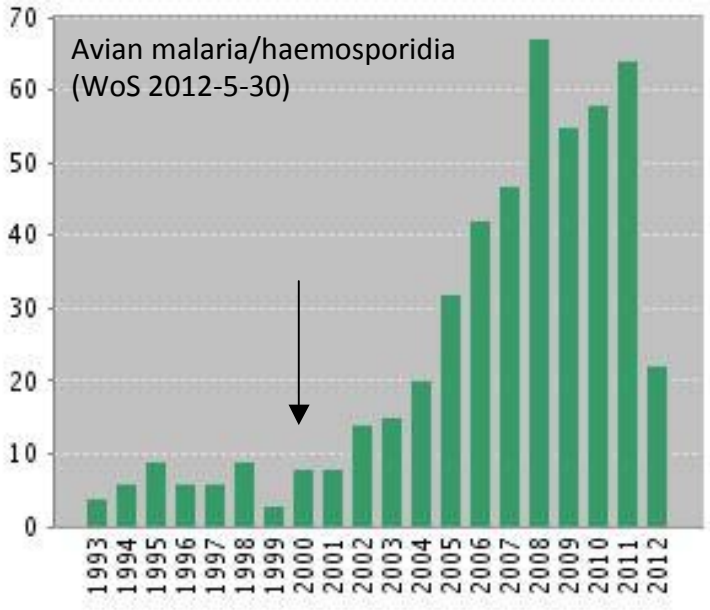

PERMANENT GENETIC RESOURCES ARTICLE

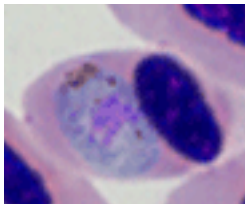
## MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome *b* lineages

STAFFAN BENSCH,* OLOF HELLGREN† and JAVIER PÉREZ-TRIS‡

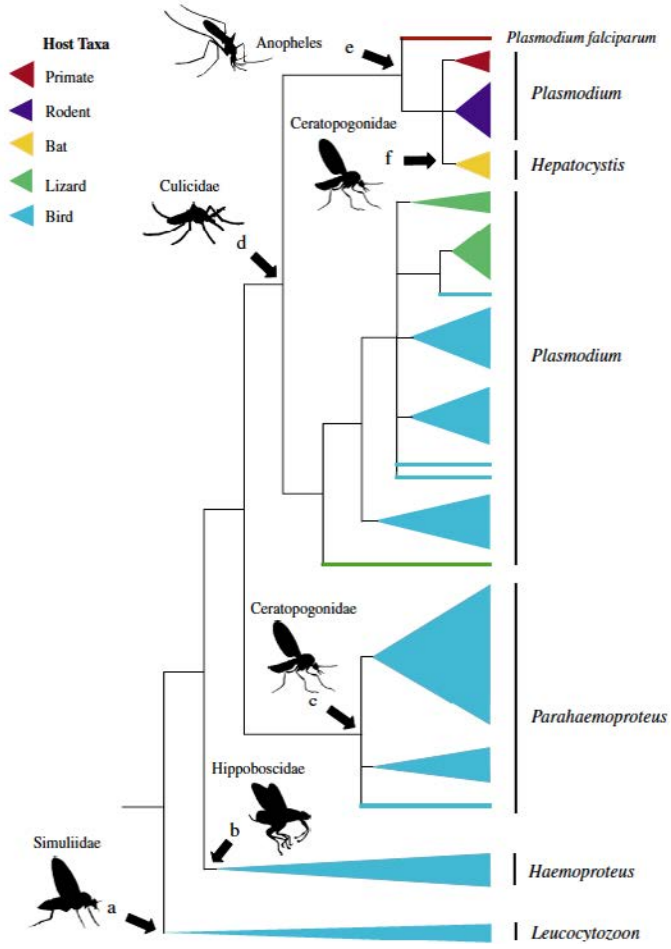
*Mol Ecol Res* 2009

- > 40 *Plasmodium* morphospecies (290 cyt-b lineages)
- *Plasmodium relictum* (9 cyt-b lineages)
- SGS1 most common *P. relictum* lineage in Europe (46 different hosts, 14 families of Passeriformes)

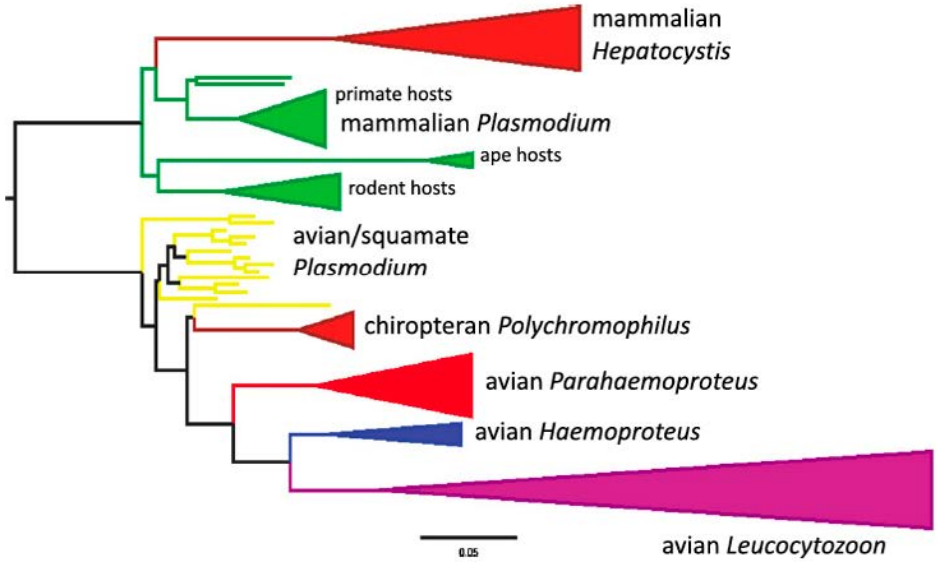




# *Avian malaria - Phylogenetic relationships*



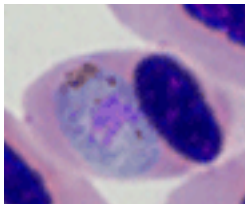

Martisen *et al.* 2008 *Mol Phylogen Evol*
3-genome phylogeny with 4 markers (2 mit, 1 apicompl, 1 nucl) (outgroup = *Leucocytozoon*) / major clades associated with vector shifts + other 'traditional' characteristics



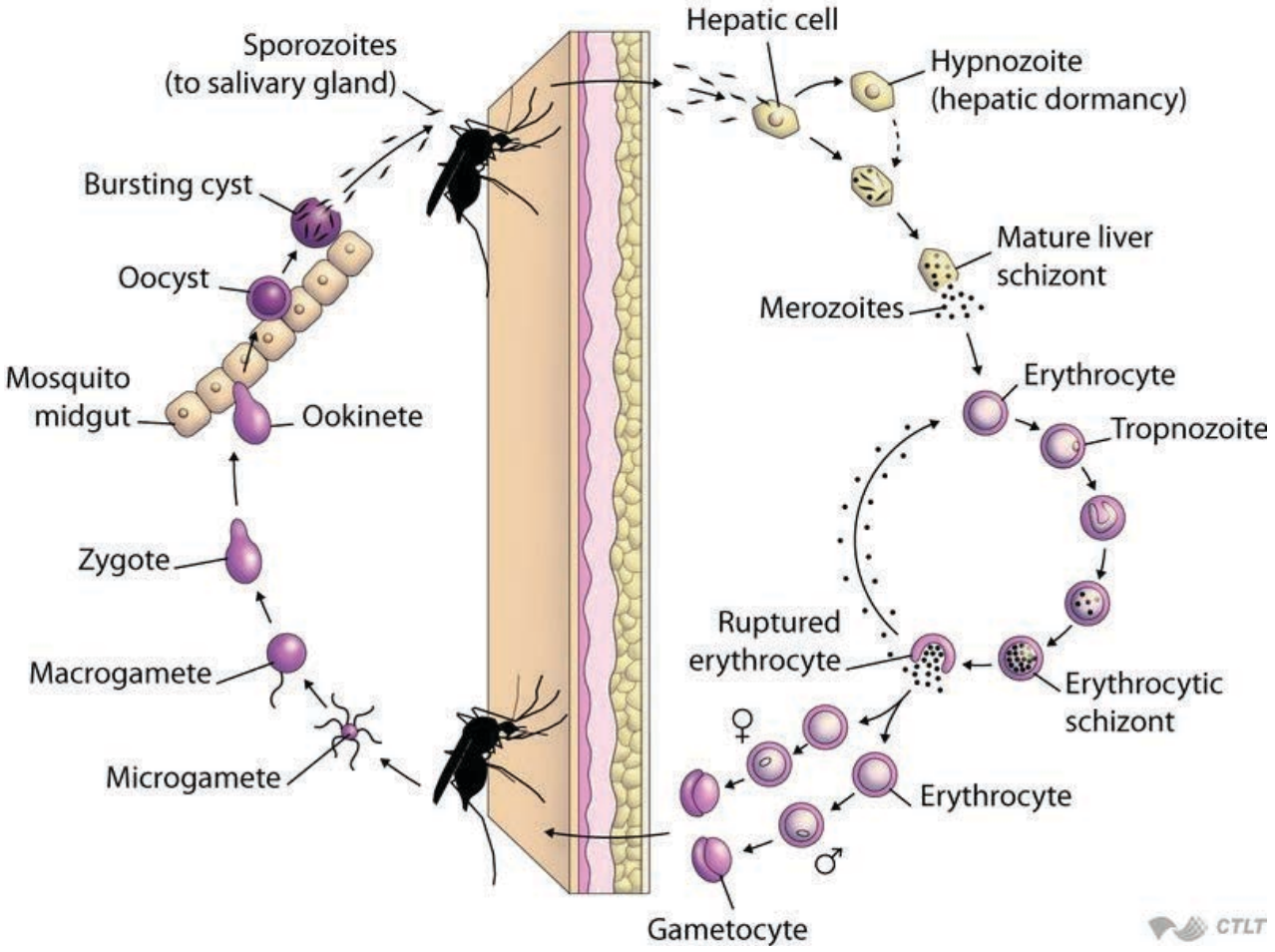

Outlaw & Ricklefs 2011 *PNAS*
Outgroup-free phylogenetic recontruction using relaxed molecular clock with uncorrelated rates

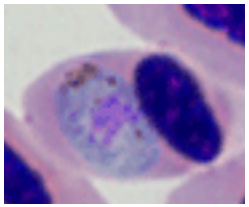
- Huge diversity of avian haemosporidia, influenced by vector, host, ecology…

- Promising model to investigate host-parasite interactions in the wild, coevolutionary processes and parasite-mediated effects on host life histories



# *Malaria: the life cycle*





# *Malaria: the life cycle of* Plasmodium relictum

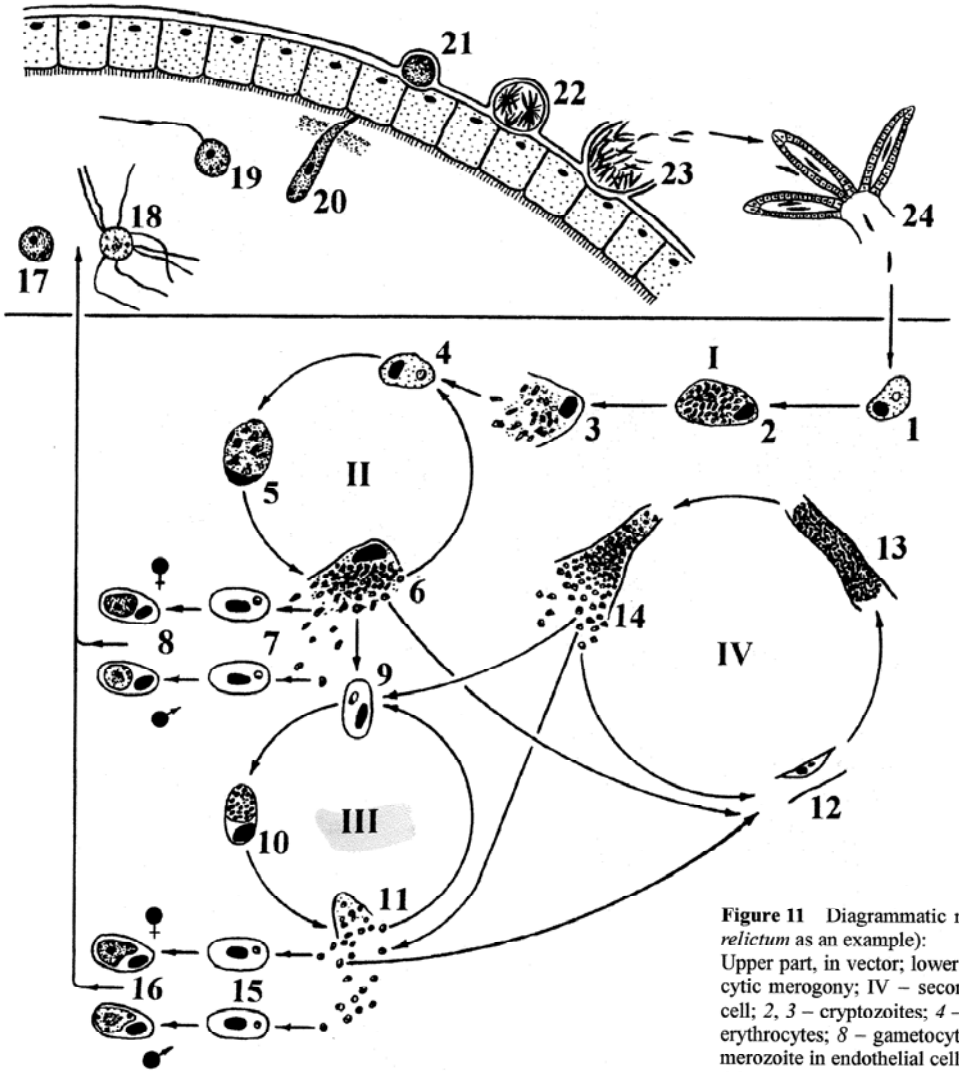
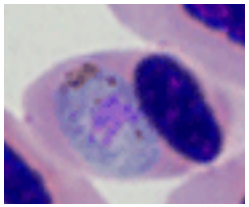



**Figure 11** Diagrammatic representation of the life cycle of bird malaria parasites (*Plasmodium relictum* as an example):
Upper part, in vector; lower part, in bird: I, II – primary exoerythrocytic merogony; III – erythrocytic merogony; IV – secondary exoerythrocytic merogony; *1* – sporozoite in reticuloendothelial cell; *2*, *3* – cryptozoites; *4* – merozoite in macrophage; *5*, *6* – metacryptozoites; *7* – merozoites in erythrocytes; *8* – gametocytes; *9* – merozoite in erythrocyte; *10*, *11* – erythrocytic meronts; *12* – merozoite in endothelial cell of capillaries; *13*, *14* – phanerozoites; *15* – merozoites in erythrocytes; *16* – gametocytes; *17* – macrogamete; *18* – exflagellation of microgametes; *19* – fertilization of macrogamete; *20* – ookinete penetrating the peritrophic membrane; *21* – young oocyst; *22*, *23* – sporogony; *24* – sporozoites in the salivary glands of vector.

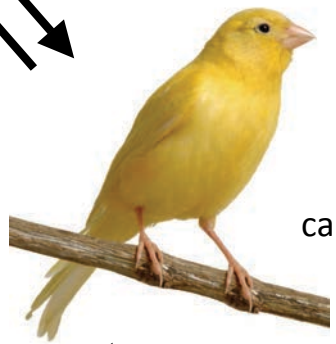
Valkiunas 2005



# *A semi-natural system in the lab*

*Culex pipiens*
(field & lab)

*P. relictum* lineage SGS1
isolated from house sparrows



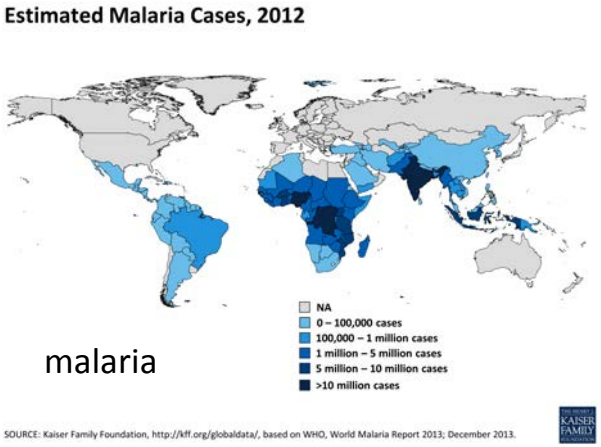
canaries

In the field (*Plasmodium sp.*)

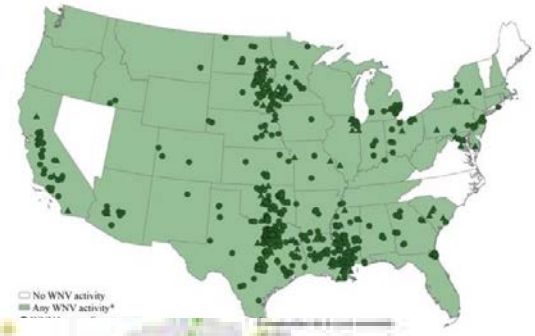
- 30-75 % in Passeriformes
- 5-10 % in *Culex* mosquitoes

natural mosquito-*Plasmodium* association

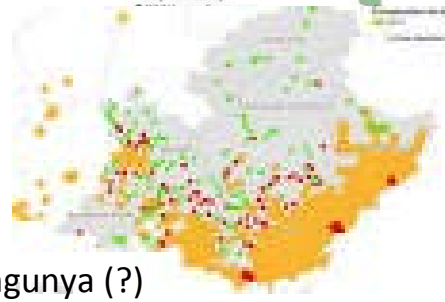
# *Epidemiology of vector-borne pathogens*




malaria




West Nile



chikungunya (?)

Infectious diseases: to set efficient public health/veterinary policies, need a better understanding of the factors affecting their spread

Models and predictions of epidemics
>> Accurate estimation of the parameters

>> Factors influencing malaria transmission

Quantitative effects

Qualitative effects

density of vectors

vectorial capacity

$$R_0 = \frac{m\,VC}{r}$$

duration of infection in the host

mosquito susceptibility

mosquito behaviour (biting rate)

mosquito infectiousness

length sporogonic cycle

$$VC = \frac{a_u\, a_i\, b\, c\, e^{-gn}}{g}$$

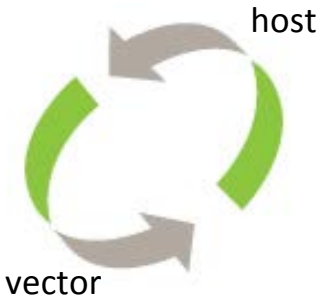
mosquito mortality (1/g longevity)

$R_0$ expected number of secondary infections produced by a single infection in a susceptible population

# *Epidemiology of vector-borne pathogens*

Two ways of manipulating (increasing transmisison, $R_0$)

- host-to-vector transmission
- vector-to-host transmission




**AVIAN MALARIA**

- Alternative system to study the ecology and epidemiology of malaria parasites
- Important role for the understanding of human malaria epidemiology

- Can malaria manipulate vector behaviour?
- Can malaria react plastically to vector stimuli (biting)?

- PART 2 -

# Can malaria manipulate vector behaviour?

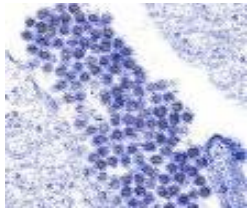
## *Parasitic manipulation and vector-borne diseases*

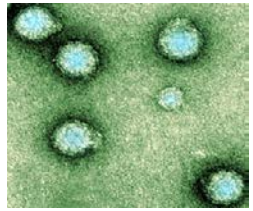
- Contact rate between hosts and vectors is a fundamental determinant of parasite transmission and epidemiology

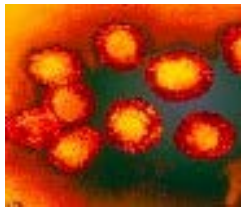
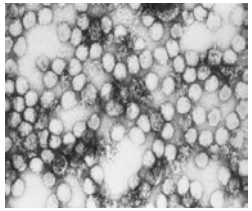
- Parasitic manipulation in vectors: alterations of phenotypic traits increasing the rate of contact and the success of vector-to-host transmission

- Parasites could also manipulate their vertebrate hosts to render them more attractive to vectors (selective advantage for parasites)

# *Parasitic manipulation and vector-borne diseases*



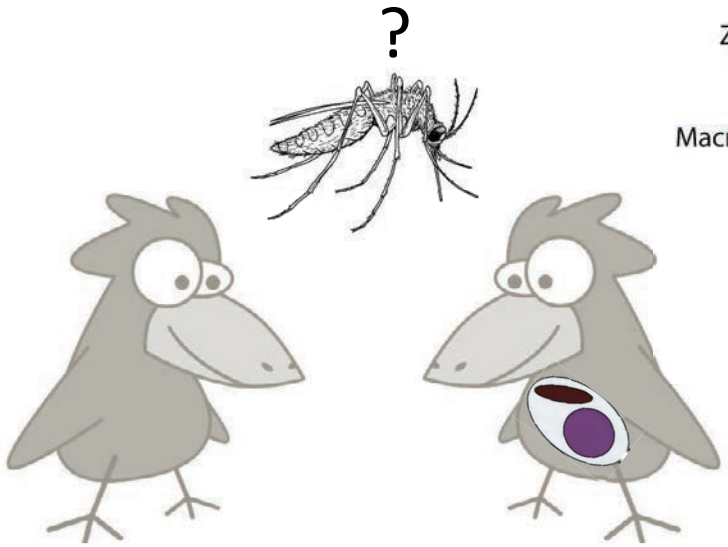



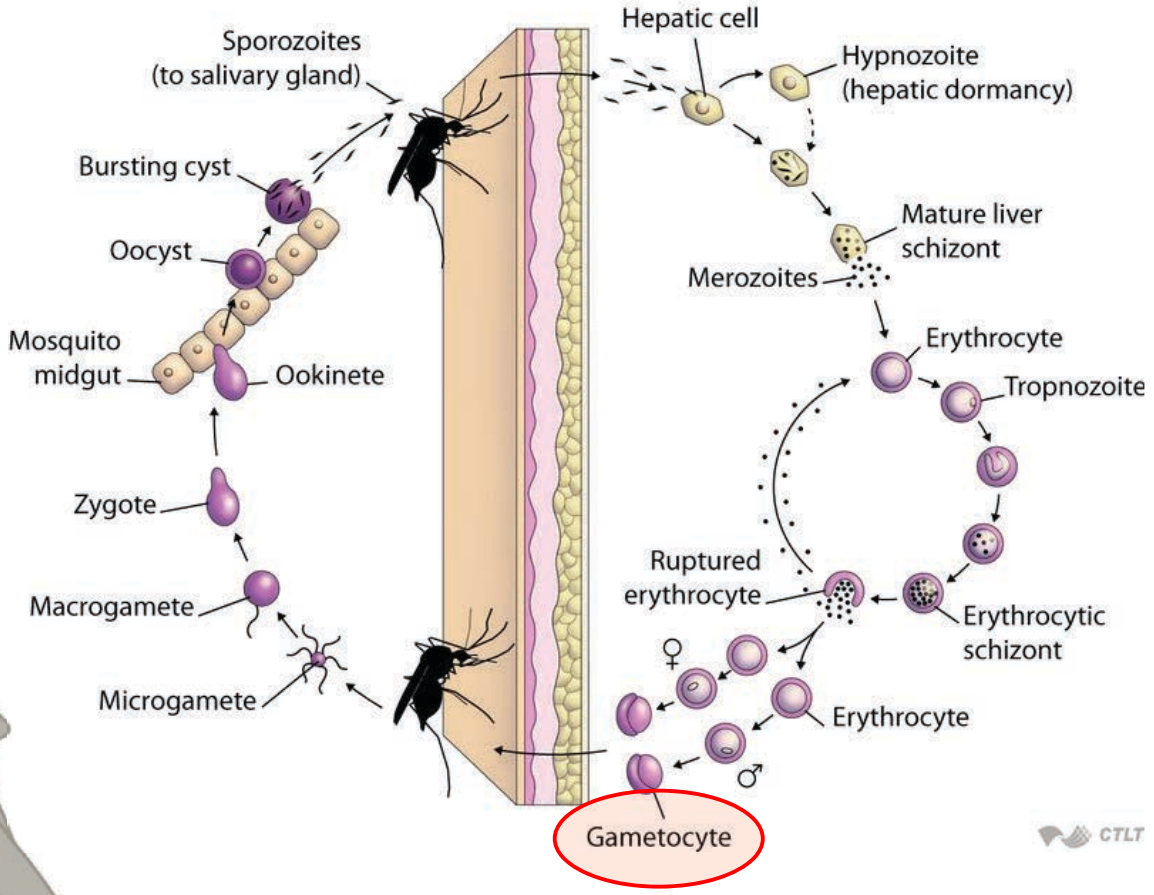


- Non-random biting if mosquitoes feed disproportionately more often on infected hosts

- Qualitatively and quantitatively affects the dynamics of vector-borne parasites

# *Do malaria infection increase bird attractiveness?*



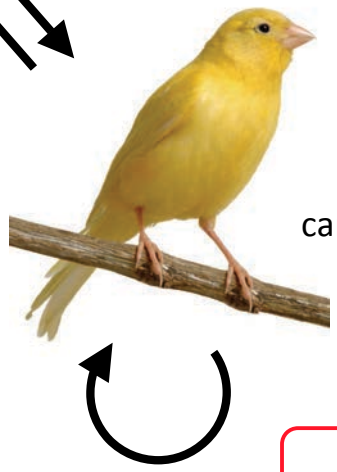




Biting behaviour of uninfected mosquitoes?

# *A semi-natural system in the lab*

*Culex pipiens*
(field & lab)

*P. relictum* lineage SGS1
isolated from house sparrows

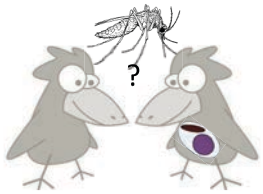


canaries

30-75 % infection
*Plasmodium sp.* in the field

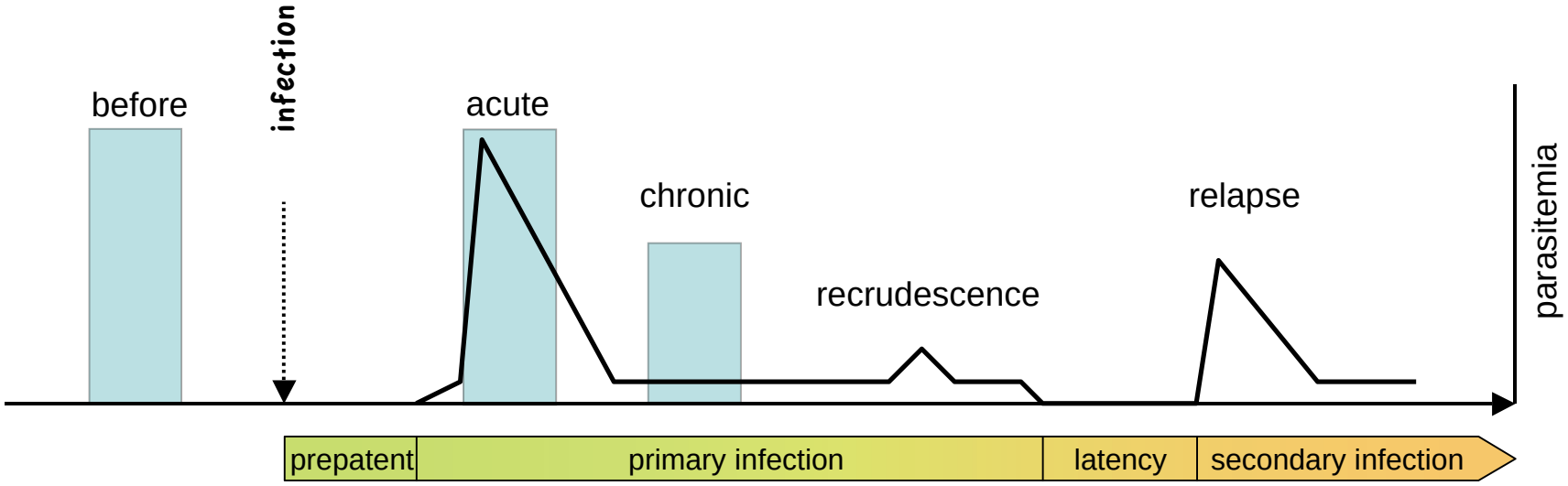
>> opportunity for a choice / the evolution of manipulation

natural mosquito-*Plasmodium* association



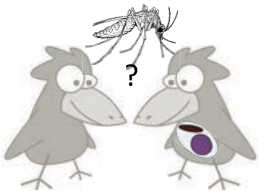

# *Test for infection-induced changes in attractiveness*

Mosquito behaviour: uninfected *vs. Plasmodium*-infected birds … but when??

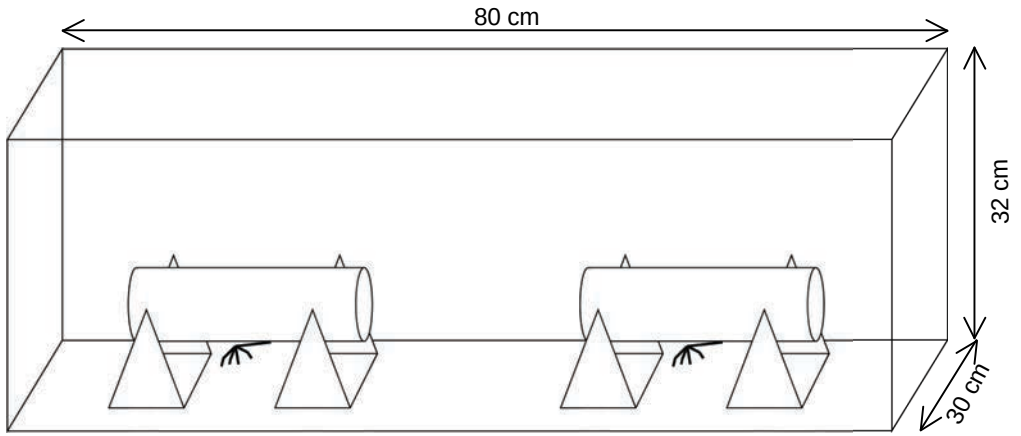



- Pairs tested 3 times - before infection: initial attractiveness
  - acute infection (10 dpi): high parasitemia
  - chronic infection (24 dpi): low parasitemia, "natural" situation

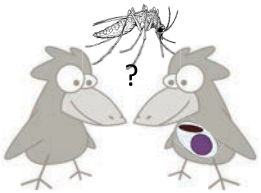
- Presence of gametocytes in both acute and chronic infections




# *Methods - Experimental set-up*

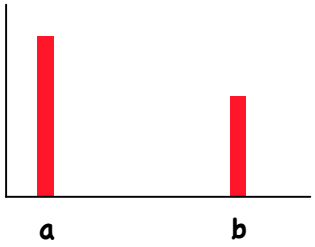


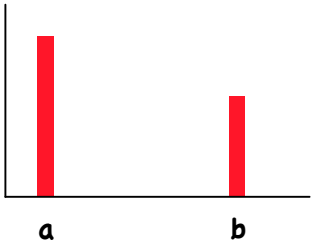

- 70 uninfected mosquitoes released, allowed to feed for 2 hours (≠ olfactometry)
- Choice inferred using a molecular method by genotyping the ingested blood
- Pairs of birds with dissimilar microsat profile, matched for sex and condition



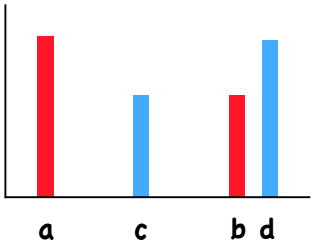
# *Methods - Molecular diagnosis*

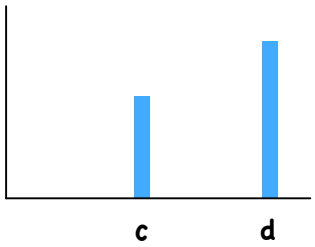
Genotyping the blood meal to assess mosquito's choice (microsat locus)

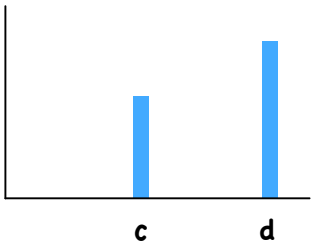


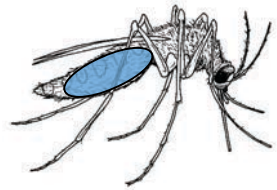





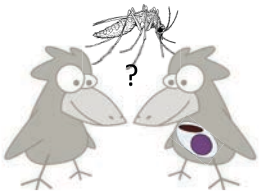


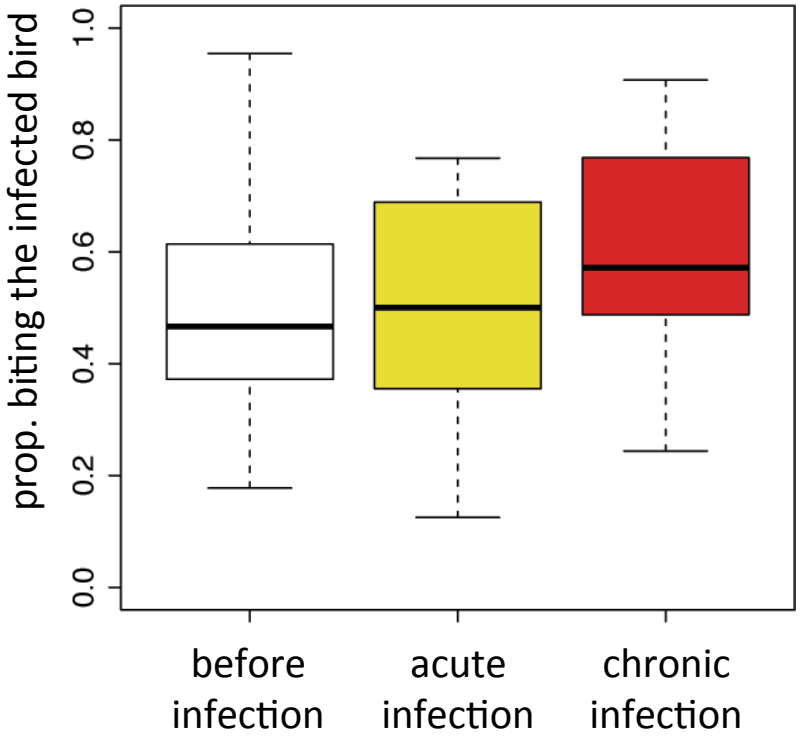



microsat profiles



# *Results - Infection and mosquito behaviour*

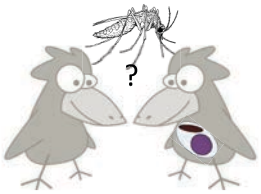



- Before: no overall preference (49%, $p$=0.96) but substantial variation (up to 95% of attracted mosquitoes)

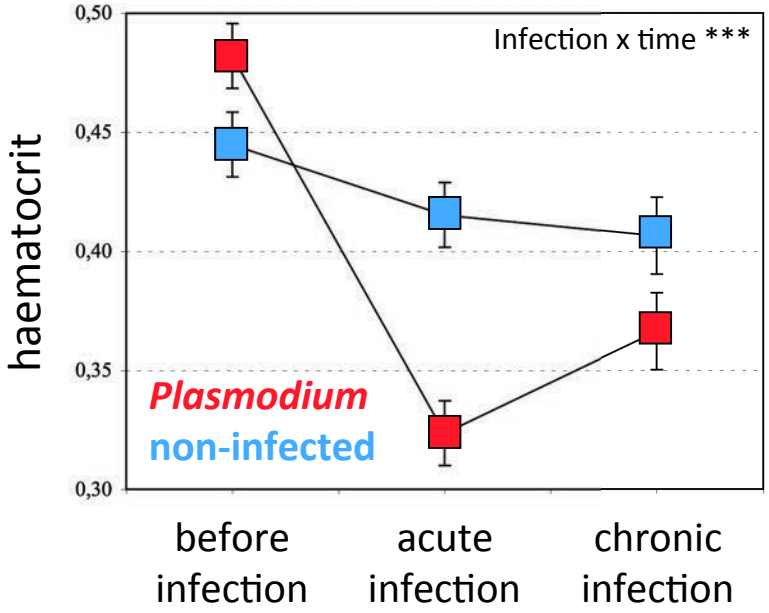
- Acute infection: no detectable change (54%, $p$=0.21)

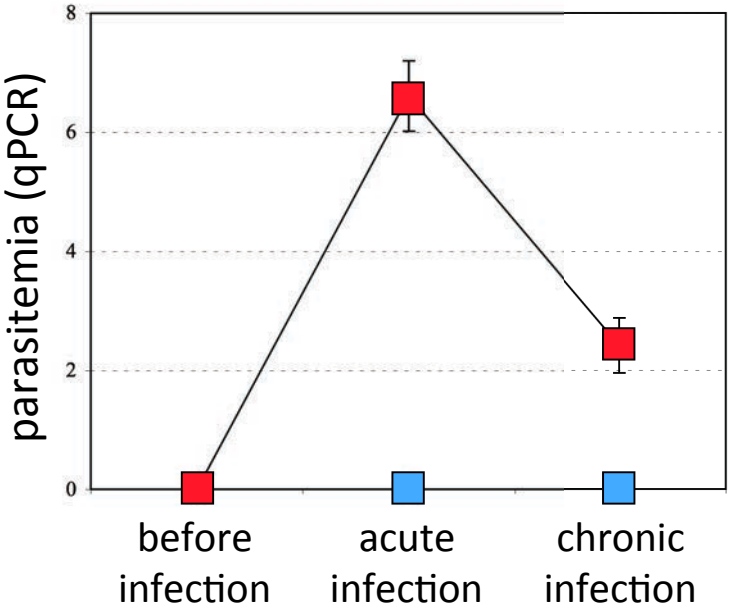
- Chronic infection: more mosquitoes have fed on the infected hosts (60%, $p$<0.0001)

- Difference acute *vs.* chronic?



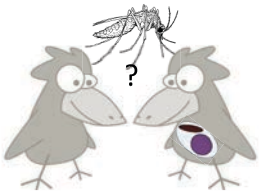
# *Results* - Plasmodium *effects on bird condition*



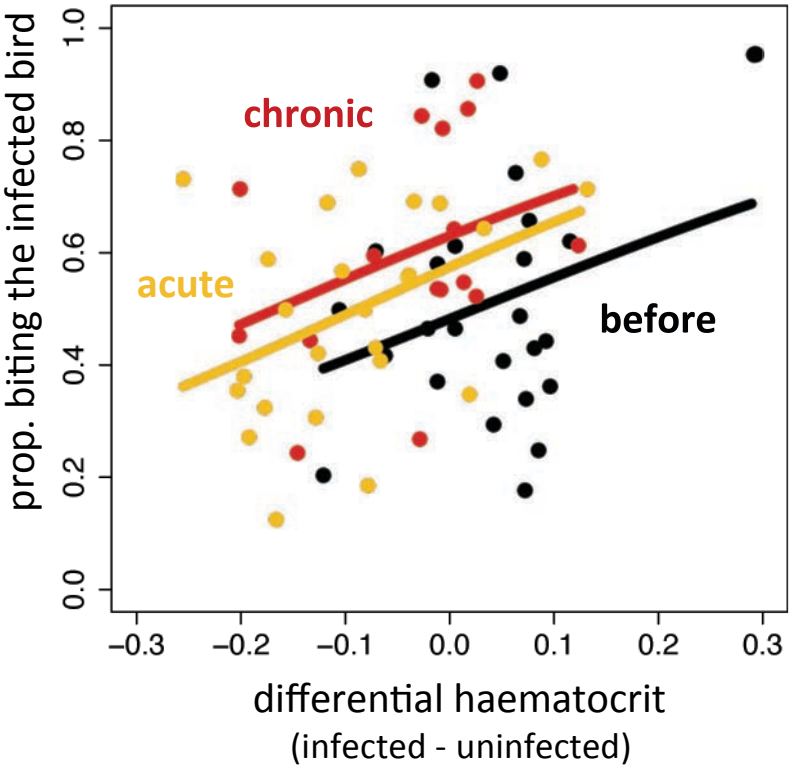




- Acute infection: severe anemia, a consequence of high parasitemia
- Chronic infection: lower parasitemia, haematocrit recovers

- RBC = resource for egg production - Anaemia should affect mosquito feeding behaviour



# *Results - Infection, haematocrit and mosquito behaviour*

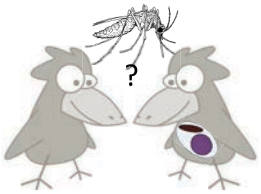



- Δ haematocrit (ΔH) ($\chi^2_1$=3.9, $p$=0.04)
  - positively related to choice
  - constant across timepoints

- Global infection ($\chi^2_2$=17.8, $p$<0.0001)
  - contrast 0-10 ($\chi^2_1$=1.5, $p$=0.22)
  - contrast 24-(0+10) ($\chi^2_1$=15.2, $p$<0.0001)

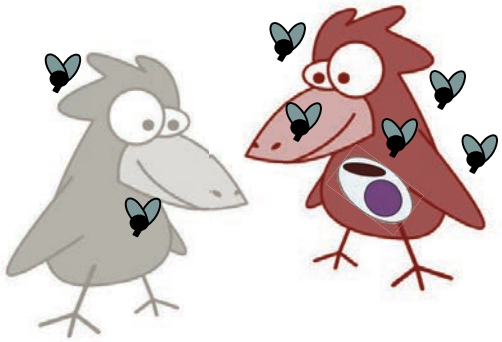
- Shift in ΔH towards lower values in acute infection (anaemic infected birds)

- Comparison before *vs.* acute only
  - ΔH ($\chi^2_1$=8.1, $p$=0.004)
  - infection ($\chi^2_1$=4.4, $p$=0.035)



# *Manipulation of host attractiveness - Conclusions*

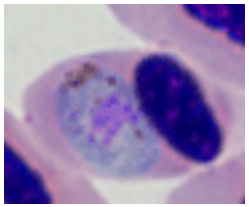
**_Plasmodium relictum_ increases the attractiveness of infected hosts**



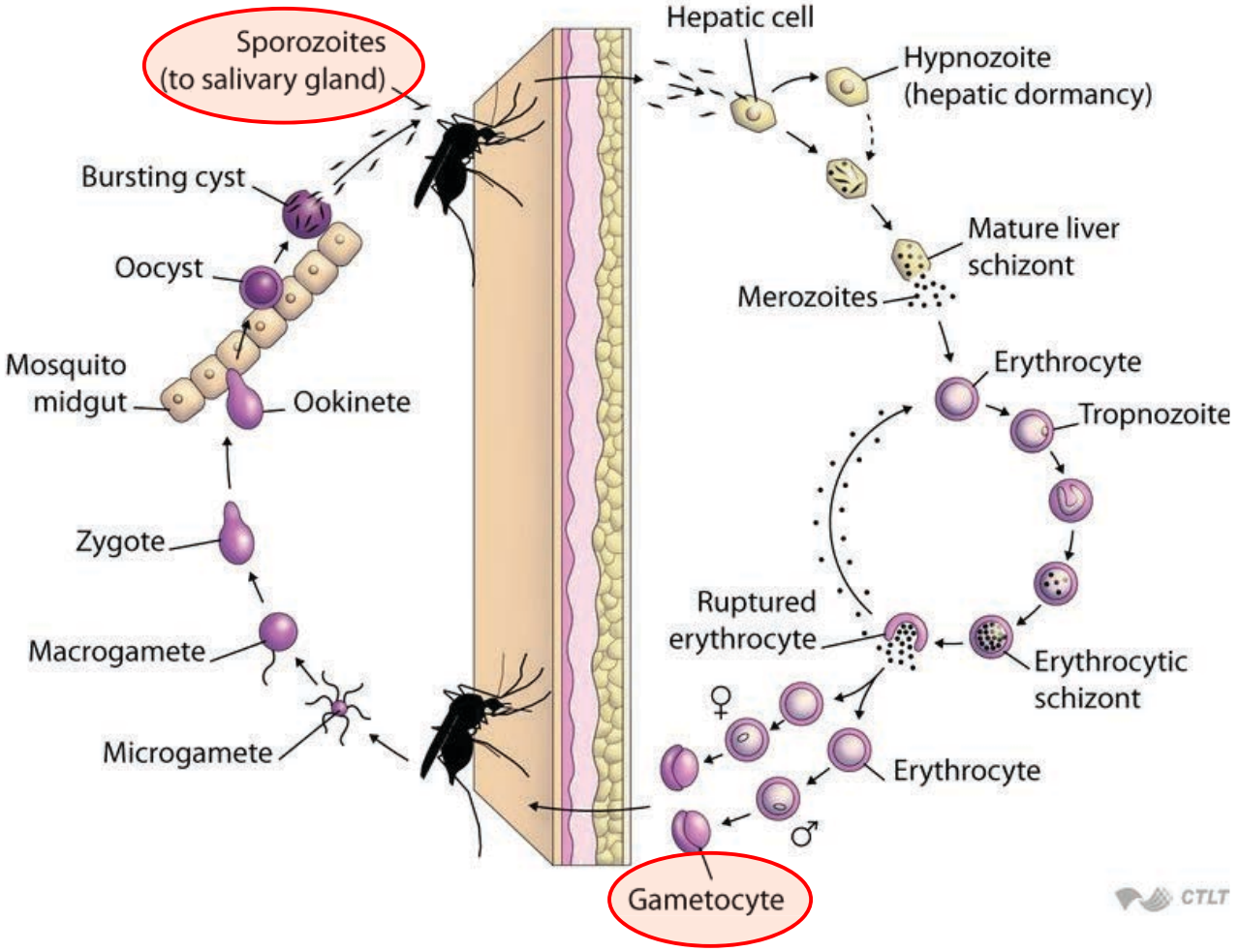
- **Strong effect in chronic infection:**
  - despite a preference for birds with high haematocrit, mosquitoes choose to feed on anaemic infected birds

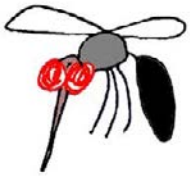
- **No clear detectable effect in acute infection:**
  - high anaemia may have hidden any parasite-induced manipulation
  - nevertheless suggests a parasite effect: infected hosts found as attractive as uninfected birds, whereas should have repeled mosquitoes
  - attractiveness prevented because of high costs of infection in mosquitoes?

- **Assessment of bird condition? / Factors driving mosquito behaviour?**
  - haematocrit partly involved, but also other factors ($CO_2$, odours, …)
  - capture and analysis by GC-MS of bird body odours (inter-individual variation, effects of infection, …)



# *Malaria: the life cycle*



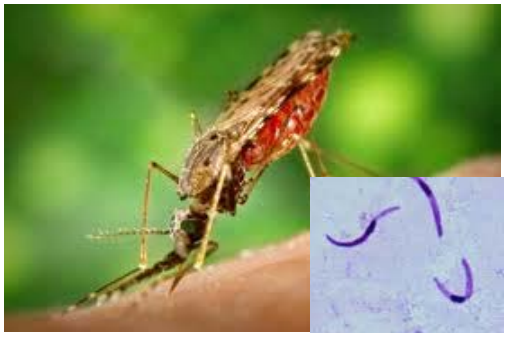


# *Alteration of the biting behaviour (quantitative manipulation)*

Change in saliva composition (anti-haemostatic molecules, apyrase) and obstruction of alimentary tract associated with infection

- Prolonged feeding time / persistence
- Difficulty to obtain full blood meal > promoting host seeking behaviour
- Multi-host feeding

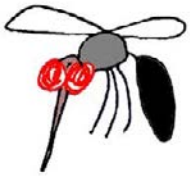
>> Alteration of host-choice behaviour?



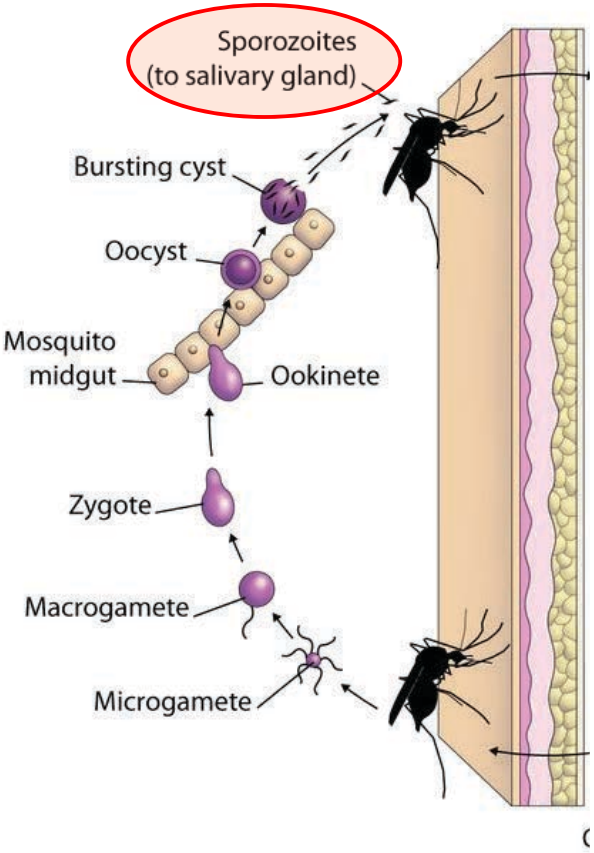
*Plasmodium* & mosquito
(Koella *et al.* 1998 *PRSLB*)

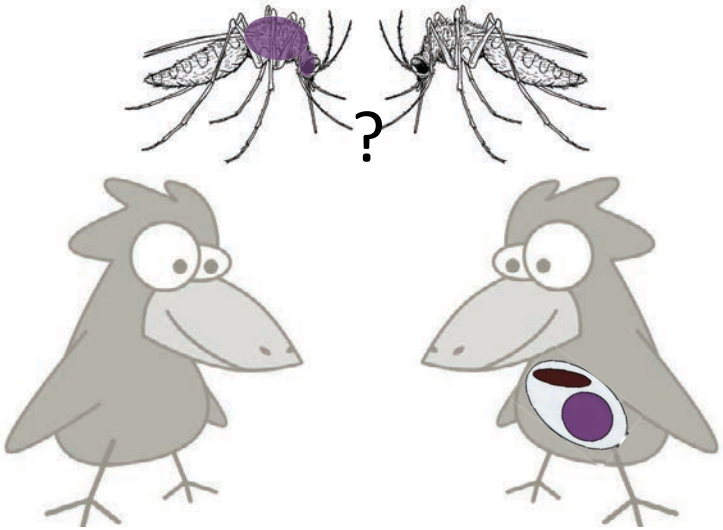
*Trypanosoma* & tsetse fly
(Van den Abeele *et al.* 2010 *PLoS Pathog*)

*Leishmania* & sand fly
(Roger & Bates 2007 *PLoS Pathog*)

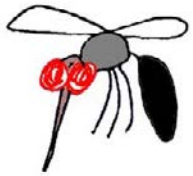


# *Behaviour of infected mosquitoes* (*qualititative manipulation*)

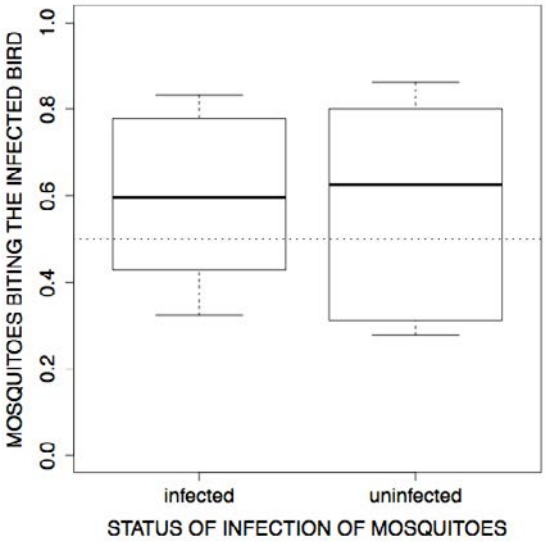


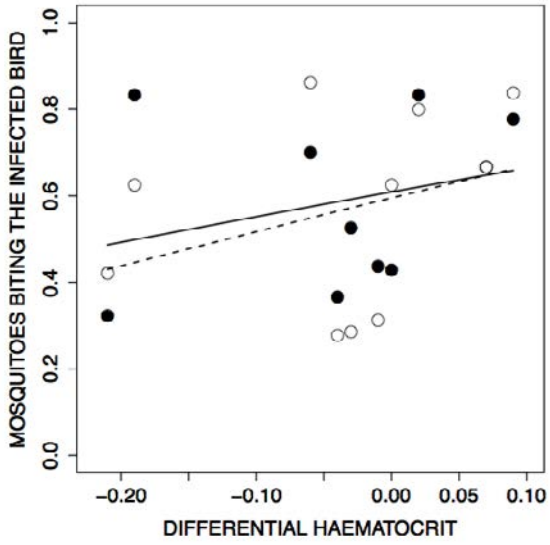



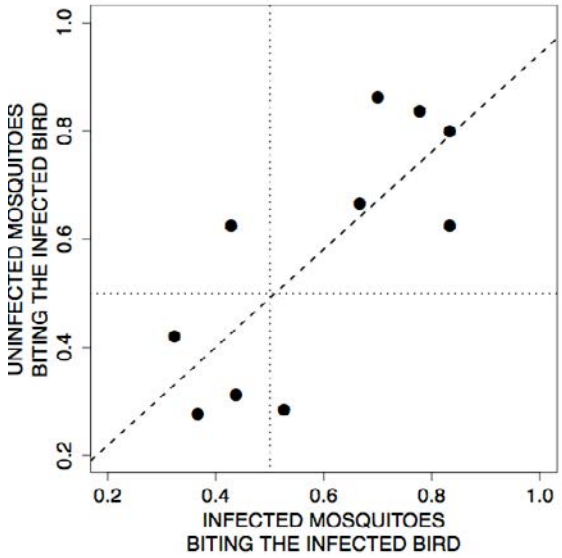

- Choice experiment
  - mosquitoes uninfected *vs* infected by sporozoites
  - birds uninfected *vs* chronic infection (55 dpi)
  - same parasite in birds and vectors
- 10 different pairs of birds
- 80 mosquitoes (40/40) allowed to feed for 2 hrs



# *Feeding behaviour of infected mosquitoes*

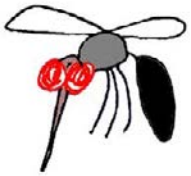



- Choice toward infected hosts (~60%)
- No overall difference in feeding preference between infected and uninfected mosquitoes
- Within a pair, infected and non-infected made similar choice (independant of the relative attractivness of the infected bird)

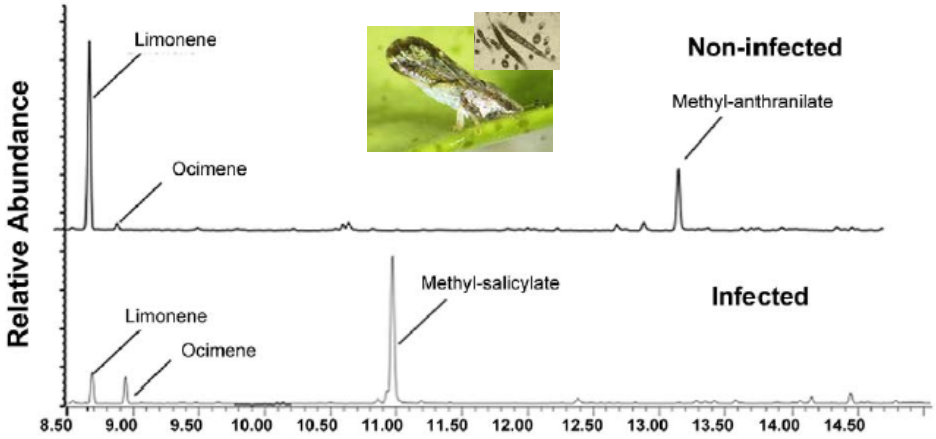
- The malaria parasite within the bird is driving the choice of infected mosquitoes
- Same parasites in birds and vectors / What if different parasite stocks/lineages?

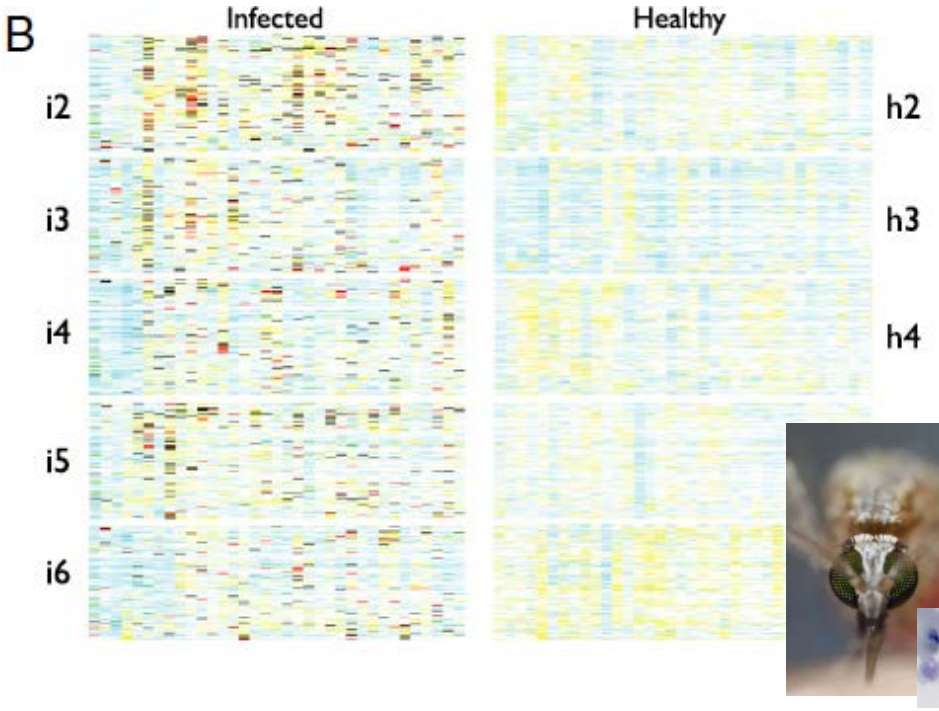


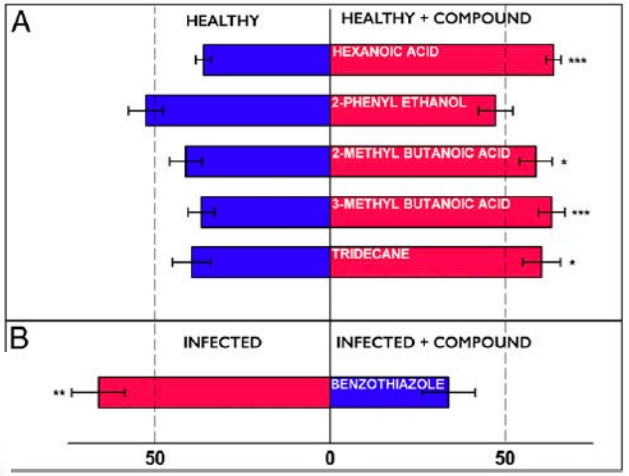
# *Manipulation of vector behaviour - Perspectives*

What makes infected birds more attractive?

Malaria: Exageration of a molecule targeted by mosquitoes for host seeking behaviour



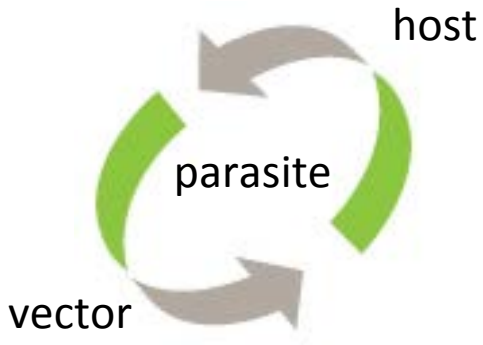



- PART 3 -

# Can malaria react plastically to mosquito biting?

## *Vector-transmitted parasites*



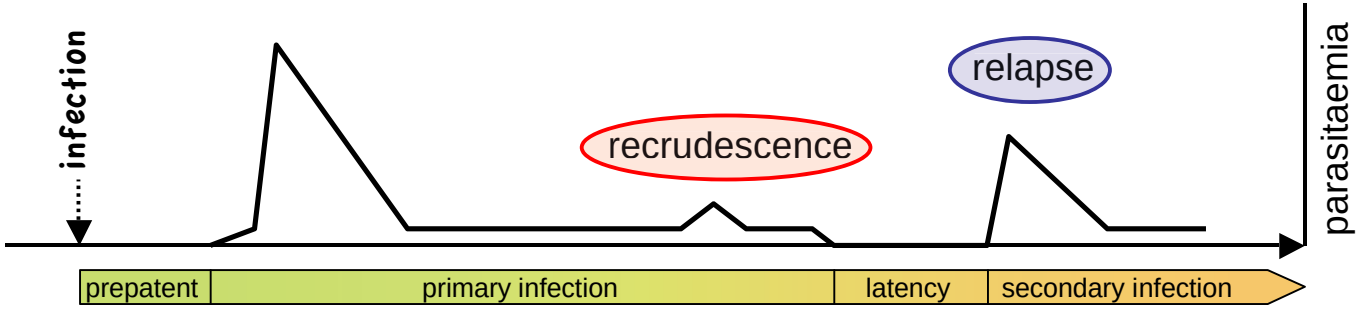

Better understanding of the specific relationships between *Plasmodium* and its vector to control malaria epidemiology

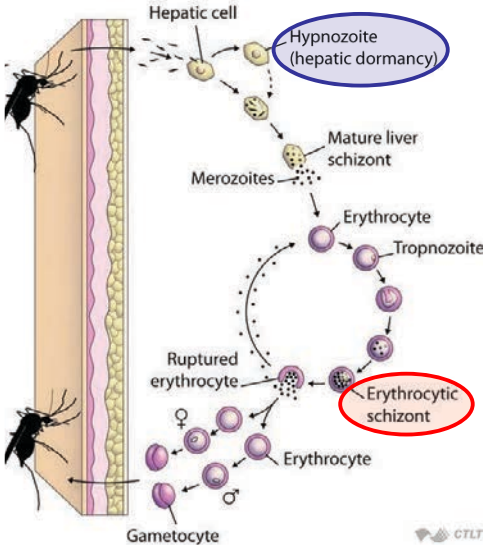
Parasite density is key in transmission

Parasite density varies

- in time and space at the population level
- during the course of infection at the individual level

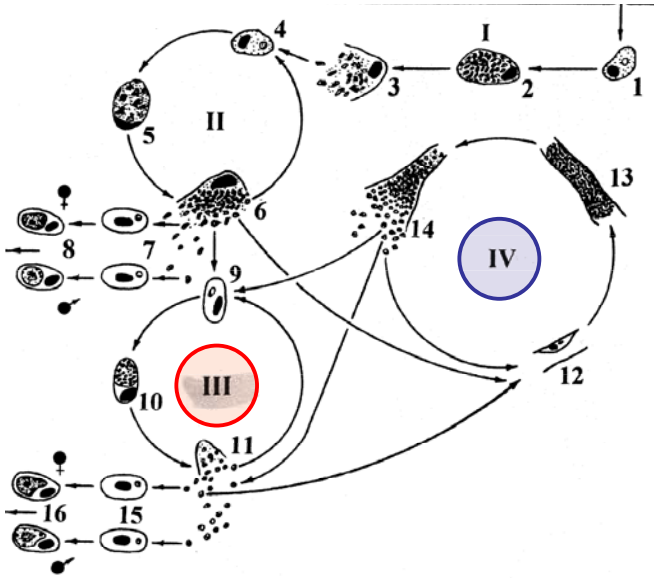
# *Relapses: a plastic parasite strategy?*




**[ EXOERYTHROCYTIC ]**

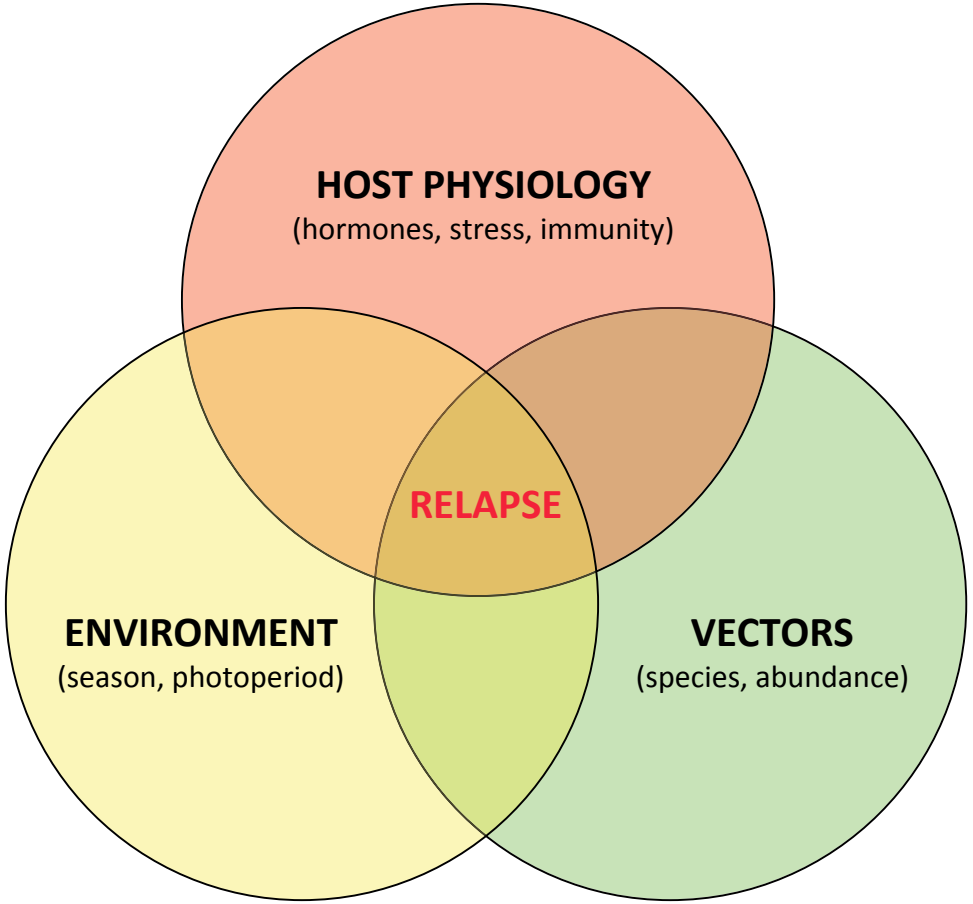
**[ ERYTHROCYTIC ]**




Human malaria (*P. vivax*)

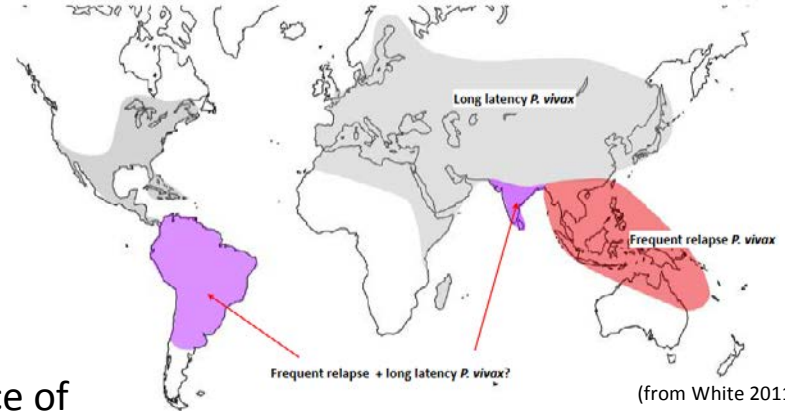
Bird malaria (*P. relictum*)
(modified from Valkiunas 2005)

# *Relapses: a plastic parasite strategy?*



# *Relapses: evidence for a role of vectors?*

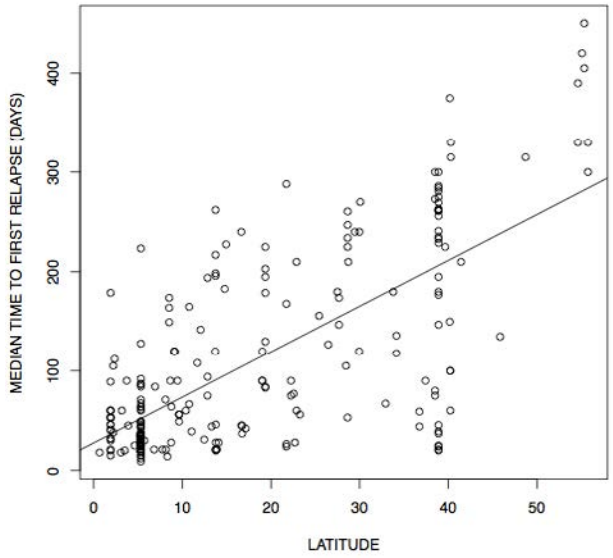
**--> Obvious benefit for transmission if the up-regulation of parasite in-host growth and gametocytogenesis does not happen at random but in response to the appearance of vectors**




(from White 2011)

• Adaptation to the (seasonal) fluctuations in the abundance of vectors:

- pulse *vs.* non-pulse vectors (Allan & Mahrt 1989)
- long patency *vs.* frequent relapse in *P. vivax* (White 2011)

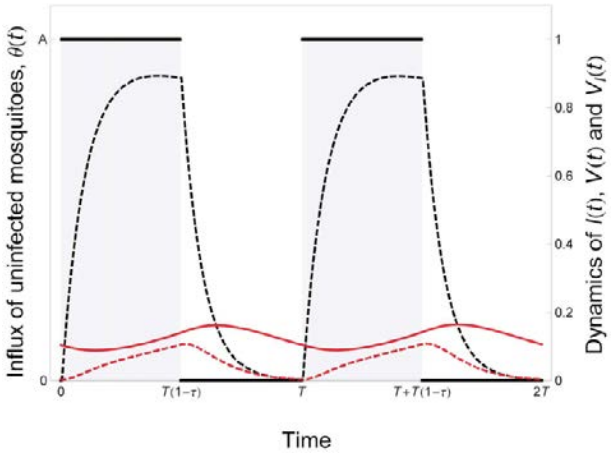


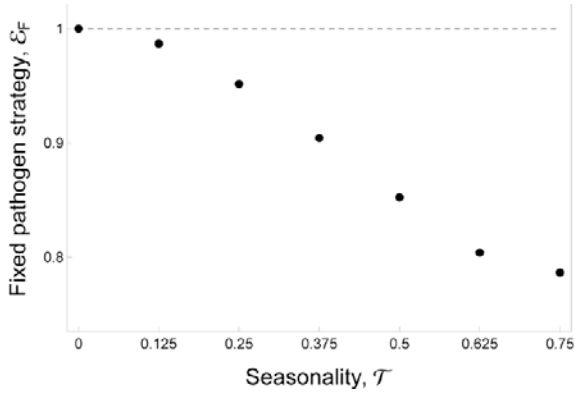
## *Relapses: what does theory tell us?*

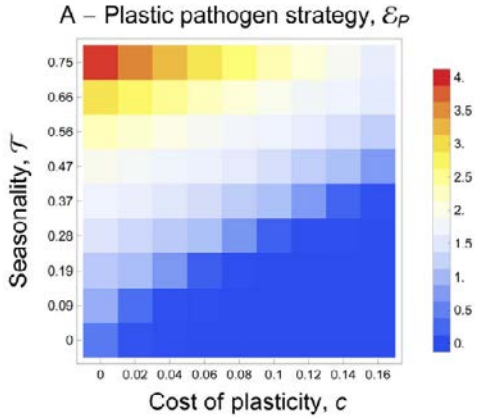
$$s_M = \underbrace{\frac{\beta_2}{m m_I}\widetilde{S\theta}\,\Delta\beta_1}_{\text{Benefit of transmission}} \underbrace{-\Delta\alpha}_{\text{Cost of virulence}} + \underbrace{\frac{\beta_2}{m m_I}\Delta COV}_{\text{Benefit of plasticity}} > 0 \qquad (4)$$

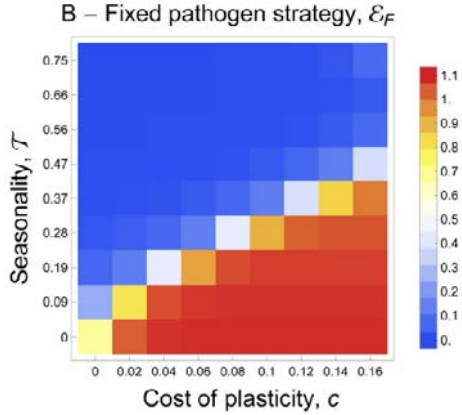
where $\widetilde{S\theta} = \frac{1}{T}\int_0^T S(t)\theta(t)dt$, $\Delta\beta_1 = \frac{1}{T}\int_0^T (\beta_{1M}(t) - \beta_1(t))dt$,

$\Delta\alpha = \frac{1}{T}\int_0^T (\alpha_M(t) - \alpha(t))dt$ and $\Delta COV = COV_t(S\theta, \beta_{1M}) - COV_t(S\theta, \beta_1)$.



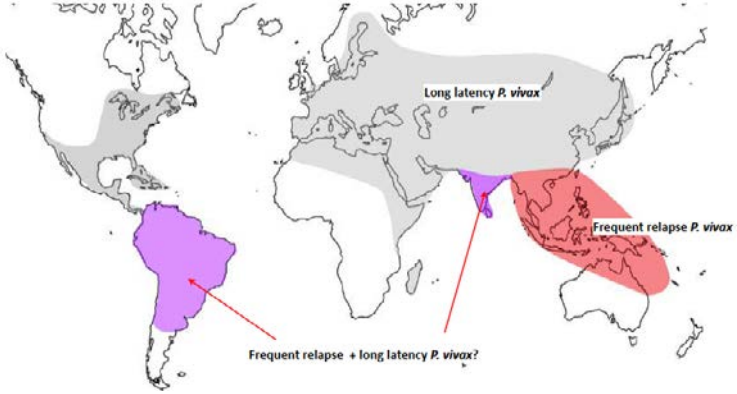




Plastic transmission strategies can evolve in response to vector density fluctuations

# *Relapses: evidence for a role of vectors?*

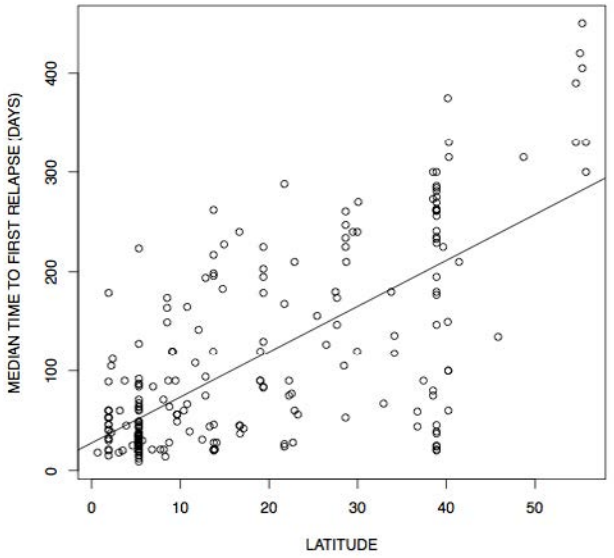
**--> Obvious benefit for transmission if the up-regulation of parasite in-host growth and gametocytogenesis does not happen at random but in response to the appearance of vectors**




(from White 2011)

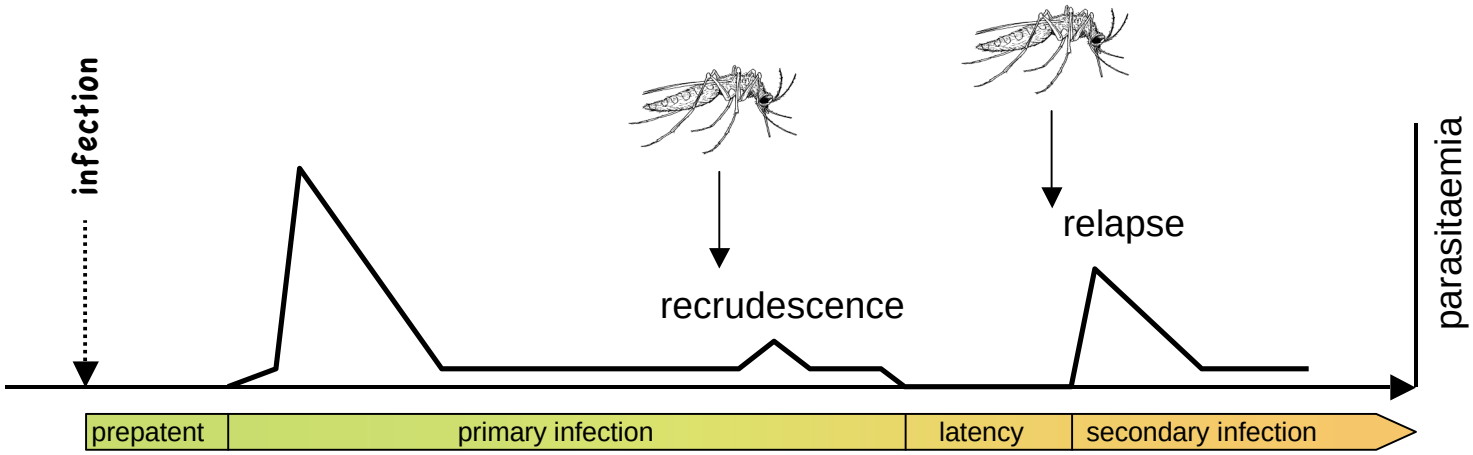
• Adaptation to the (seasonal) fluctuations in the abundance of vectors:
- pulse *vs.* non-pulse vectors (Allan & Mahrt 1989)
- long patency *vs.* frequent relapse in *P. vivax* (White 2011)

• Relapse triggered by the biting of uninfected vectors:
- match between disease prevalence and vector abundance
(longitudinal cohort studies; Paul et al. 2004; Hulden et al. 2008)
- enhanced asexual replication and gametocytogenesis as a consequence of mosquito biting
(rodent malaria; Billingsley et al. 2005, but not in Shutler et al. 2005)

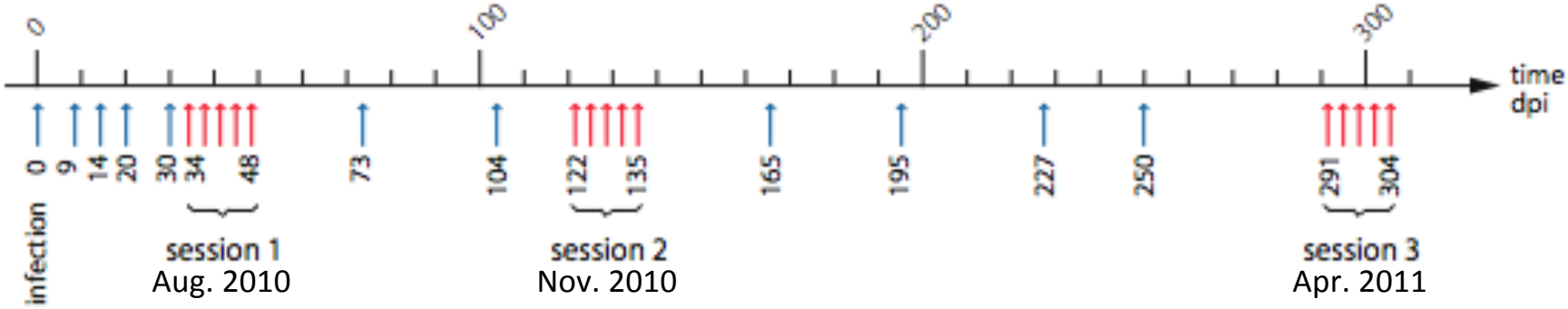


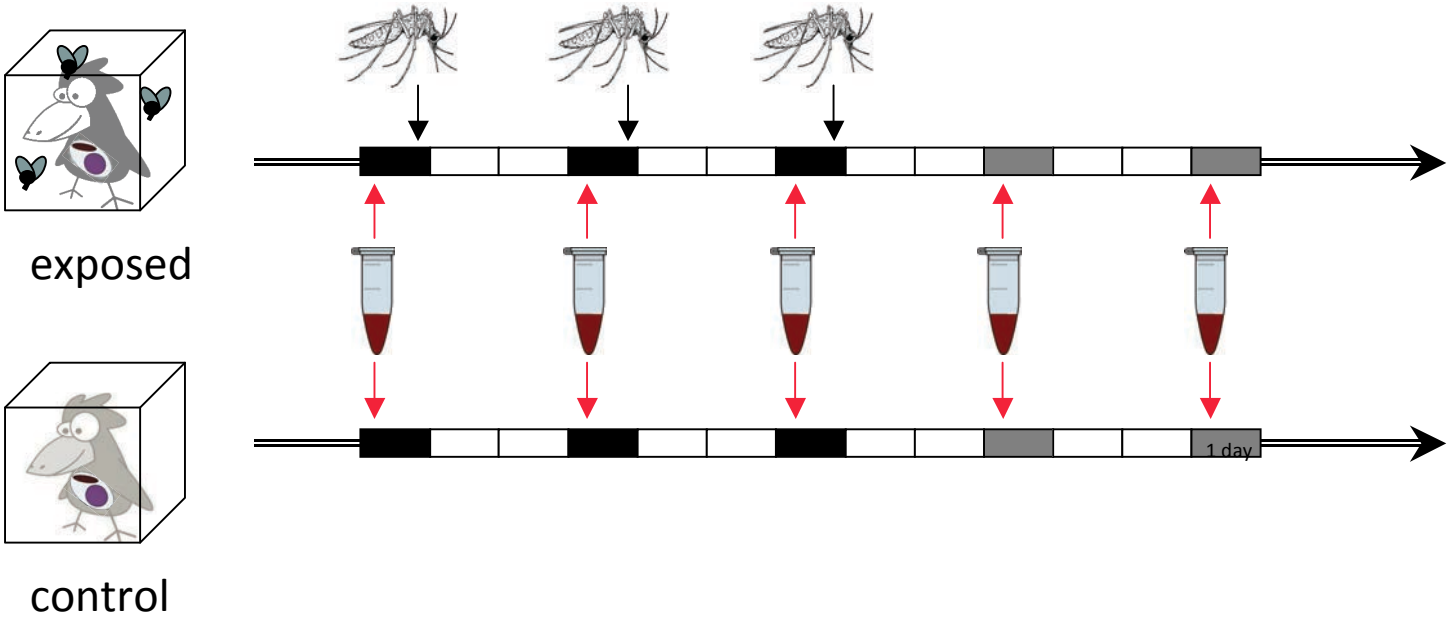


**Can mosquito bites affect parasite in-host replication and *Plasmodium* transmission?**

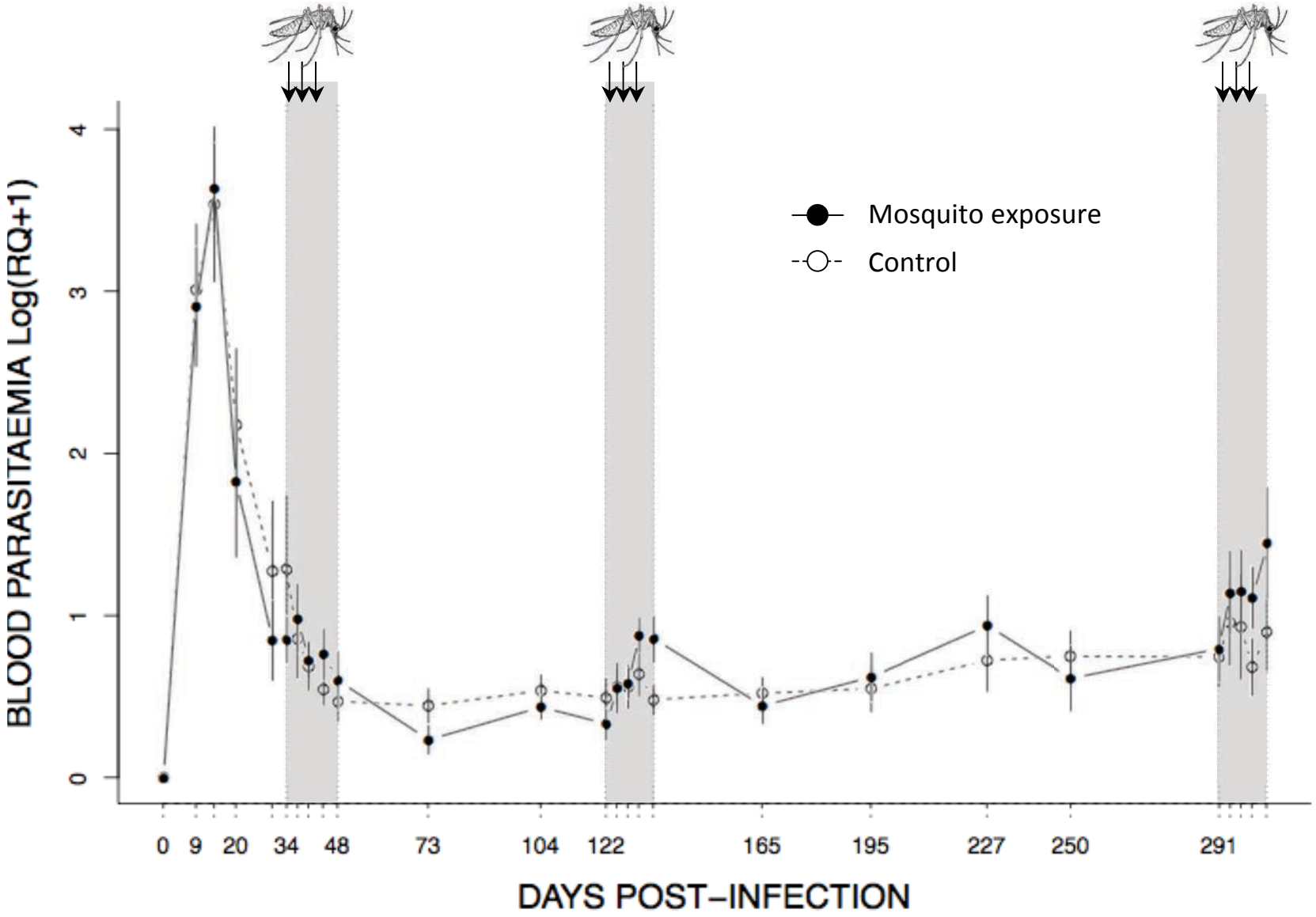


# *Relapses: an experimental test*

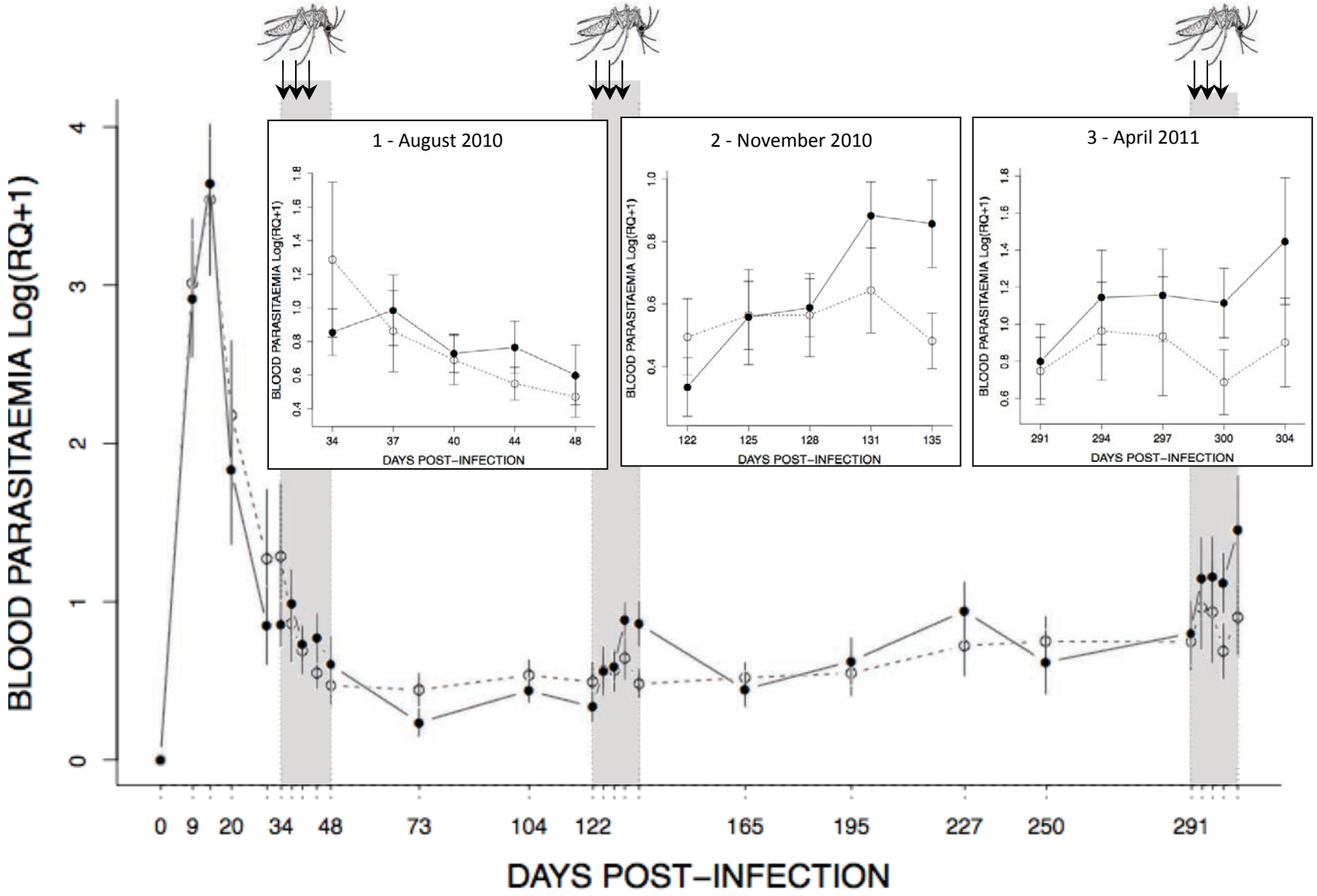


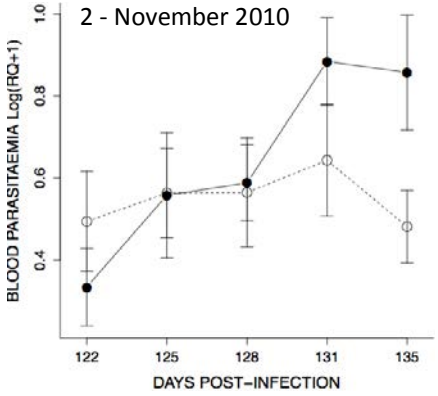
## *Vector bites, parasite dynamics*

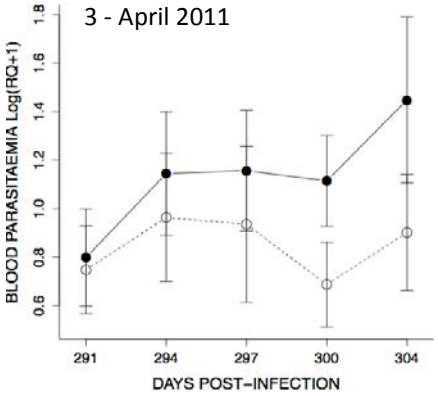


# *Vector bites, parasite dynamics*

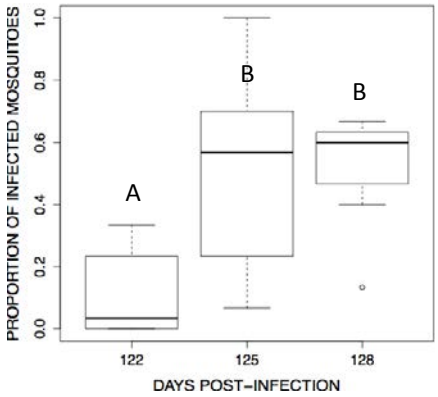


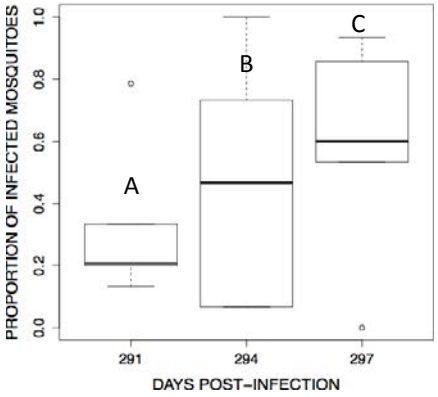
# *Vector bites, parasite dynamics & transmission*



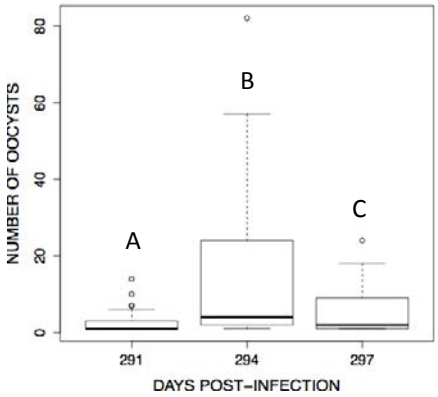



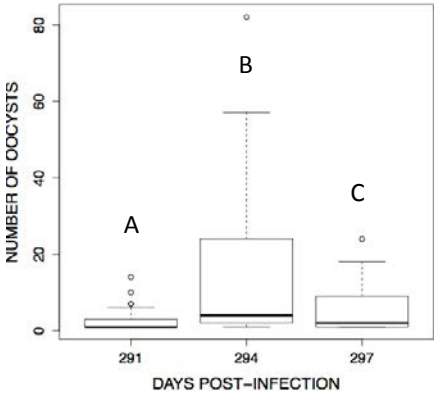

Higher parasitaemia after bites




Higher infection rate when birds had been previously exposed

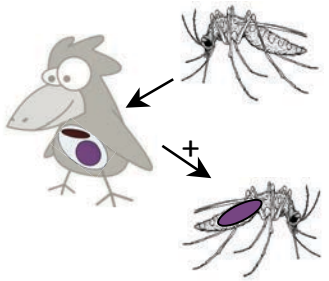




Higher oocyst burden when birds had been previously exposed

# *Vector bites, parasite dynamics & transmission - Summary*

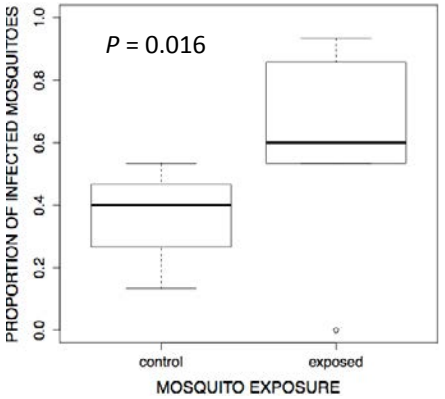
- Birds never cleared *Plasmodium* infection (no true relapse in this system)



- Change in parasite replication as a response to mosquito bites during the **chronic** stage of infection

- Mirrored in transmission to mosquitoes (higher infection rate/oocyst burden)

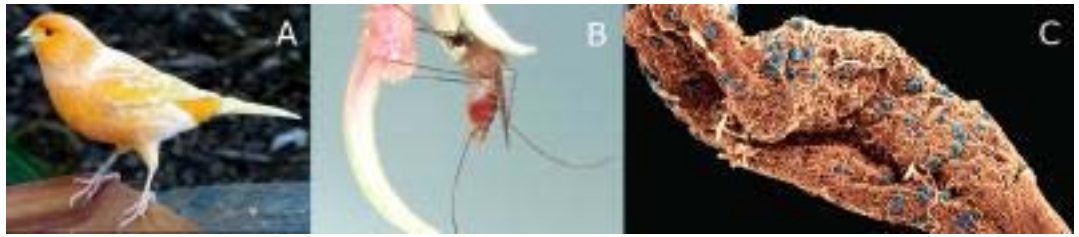
- Control (unexposed) birds infective for mosquitoes at 307 dpi
  > Difference in transmission success between control and exposed birds?




- Bites of uninfected vectors can have a strong impact on malaria epidemiology

- Relapse: interaction between different synergetic stimuli (vector, host physiology, environment)

- Avian malaria system offers interesting perspectives to investigate the underlying mechanisms of relapses (stimuli perceived parasites, specificity, parasite strains…)




Avian malaria:  experimental and field work on various aspects of host-parasite interactions

Besides its divergence with human malaria, still gives valuable inputs for understanding the ecology, evolution and epidemiology of malaria

Still a lot to do using this experimental system

For more details

- Malaria infection increases bird attractiveness to uninfected mosquitoes. *Ecology Letters*, 16: 323-329
- Both infected and uninfected mosquitoes are attracted toward malaria infected birds. *Malaria Journal*, 12: 79
- Evolution of plastic transmission strategies in avian malaria. *PLoS Pathogens*, 10: e1004308

- Alterations of mosquito feeding behaviour by the avian malaria *Plasmodium relictum* (to come)
- From bird to mosquito: within-host dynamics and transmission of avian malaria (R. Pingeault et al., to come)