Interspecific competition during transmission of two sympatric malaria parasite species to the mosquito vector

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The role of species interactions in structuring parasite communities remains controversial. Here, we show that interspecific competition between two avian malaria parasite species, *Plasmodium gallinaceum* and *P. juxtanucleare*, occurs as a result of interference during parasite fertilization within the bloodmeal of the mosquito. The significant reduction in the transmission success of *P. gallinaceum* to mosquitoes, due to the co-infecting *P. juxtanucleare*, is predicted to have compromised its colonization of regions occupied by *P. juxtanucleare* and, thus, may have contributed to the restricted global distribution of *P. gallinaceum*. Such interspecies interactions may occur between human malaria parasites and, thus, impact upon parasite species epidemiology, especially in regions of seasonal transmission.

Keywords: *Plasmodium*; fertilization; transmission-blocking; interspecific competition

1. INTRODUCTION

Establishing how species interact within a community is fundamental to understanding what factors shape community structure (Gotelli & Graves 1996). Parasite communities offer an advantageous system in which to explore community structure, primarily because of the discrete nature of habitats (i.e. individual hosts; Simberloff & Moore 1997). The majority of research has focused on helminths (reviewed in Sousa 1994; Poulin 2001), considered to be the main examples of communities where species interactions are the primary processes determining relative abundance (Cornell & Lawton 1992). However, present evidence indicates that the diversity and observed patterns of abundance reflect the individual species' life-history strategies, notably those pertaining to fecundity and transmission success. Thus, helminth parasite life histories seemingly result in aggregated species distributions that reduce the potential for species interactions (Dobson & Roberts 1994). This is not expected to be the case for vector-borne parasites, such as malaria parasites (*Plasmodium* spp.), whose distribution relies largely on the vector-vertebrate host interaction. Furthermore, the use of the same vector species by congeneric parasites will result in more overlap of the parasite species and, hence, greater potential for interaction.

Mixed-species infections of *Plasmodium* spp. occur widely throughout animal and human populations (Richie 1988). Many *Plasmodium* spp. are vertebrate host-specific and coexist in sympatry. Analysis of point prevalence data (presence-absence) in natural populations and experimental co-infections provide two alternative but complementary approaches for assessing the nature of species interactions within a host.

Epidemiological data from human populations have revealed conflicting patterns of *Plasmodium* spp. associations (Cohen 1973; Richie 1988; McKenzie & Bossert 1997a). However, such cross-sectional data reveal little about the dynamics of the interactions among the host and its parasite species community. Analysis of the long-term within-host dynamics of *Plasmodium* spp., coupled with knowledge of both the biology and the life histories of the parasite species involved, is more informative for establishing the role of species interactions in community structure. Indeed, longitudinal analyses of induced infection (Boyd & Kitchen 1937) and field studies (Maitland *et al.* 1997; Bruce *et al.* 2000) have revealed alternating patterns of species dominance, strongly indicating an important role for non-specific immune responses in regulating parasite species within-host dynamics (Bruce *et al.* 2000). Thus, by their interaction with the host, parasite species affect one another, i.e. there is apparent competition (Holt 1977). Whether such interactions have an impact on the species transmission to mosquitoes is uncertain, although the prevalence of mixed-species infections in natural mosquito populations has been found to be less than that expected if each species was transmitting independently (McKenzie & Bossert 1997b). The ability of an infectious agent to invade and persist in a host population depends on the magnitude of its basic reproductive rate, R_0 (Anderson & May 1991). The infectiousness of the malaria parasites within the vertebrate host to mosquitoes is vital for determining R_0 , therefore underlining the importance of establishing the consequences of malaria species interactions within the vertebrate host for their transmission success to mosquitoes.

The aim of this paper is to examine, in an experimental system, the effect of co-infection by two *Plasmodium* species.

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ies with different life histories on both within-host parasite dynamics and transmission success. To simulate natural systems, the effect of long-term co-species infection is examined, to address how differing strategies for long-term survival may influence the outcome of species interaction. The results are used to interpret natural prevalence data of the species concerned and, by extrapolation, to address the potential importance of such interactions for the human malaria parasite community. Thus, we consider how different adaptive life-history traits that maximize R_0 may, as in the helminth parasite communities, contribute to relative species abundance.

2. BACKGROUND BIOLOGY AND PARASITE SYSTEM

Plasmodium species are mosquito-borne protozoan parasites of vertebrates, which proliferate asexually within red blood cells (RBCs), provoking anaemia and symptoms of malaria disease. A small fraction of the asexual parasites develop into sexual male or female gametocytes (Carter & Graves 1988) that produce gametes when ingested with the bloodmeal of the mosquito in response to pH, the drop in temperature and mosquito factors (Sinden *et al.* 1996). For successful transmission to mosquitoes, the malaria parasite must achieve fertilization between its male and female gametes. The subsequent zygote transforms into a mobile ookinete, which penetrates the mosquito stomach wall, where it encysts; 8–15 days later (depending on the *Plasmodium* spp.), the mature oocyst releases several thousand sporozoites, which invade the salivary glands of the mosquito and are injected into the vertebrate host during a subsequent bloodmeal.

The course of infection of many *Plasmodium* species in the vertebrate host, notably the human lethal malaria parasite *P. falciparum* and the avian malaria *P. gallinaceum*, involves both a short acute symptomatic phase and a chronic low-density infection that persists for a considerable length of time. Such chronic infections comprise the infectious parasite reservoir (Jeffery & Eyles 1955), important for parasite survival where mosquito abundance and transmission are seasonal. In contrast to the abundance of gametocytes produced during the acute phase of infection, chronic parasite reservoirs produce very low densities of gametocytes. This study addresses the impact of co-infecting parasites on parasite transmission, with emphasis on interactions during the chronic stage of infection.

Avian malaria models offer excellent natural host–parasite systems in which to examine parasite transmission to mosquitoes under controlled laboratory conditions. *Plasmodium gallinaceum* (Brumpt 1935) and *P. juxtanucleare* (Versiani & Gomes 1941) are primarily parasites of wild and domestic fowl, and are therefore considered to have originated in Asia (Garnham 1966; Laird 1998). Both species invade mature erythrocytes and have latent exoerythrocytic forms, but have different courses of infection, which determine their infectivity to mosquitoes and hence their transmission success. *Plasmodium gallinaceum* has an intrinsically higher asexual growth rate (16–20 merozoites per RBC) and is characterized by a brief acute infection (duration of 4–5 days) when transmission to mosquitoes is highly successful. This is followed by a chronic relapsing

infection, lasting a year, when parasite occurrence in the blood is intermittent and density very low (0.01%) and, consequently, transmission is very weak and intermittent; this is the reservoir of infection. By contrast, *P. juxtanucleare* replicates slowly (two to six merozoites per RBC), parasite densities persist at relatively low (0.1–1% infected RBCs) but constant levels and transmission is sustained at intermediate levels throughout the infection. Notably, although *P. juxtanucleare* gametocyte densities are low (of the order of 0.01%), they remain highly infectious to mosquitoes for the duration of the infection: 70% or more of mosquitoes were infected after feeding on birds with very low gametocyte densities (less than 1 gametocyte per 10 000 RBCs) (Bennett & Warren 1966). A paradox that has plagued malariologists since Garnham (1969) is the failure of *P. gallinaceum* to spread despite bird host and mosquito vector availability. *Plasmodium gallinaceum* has a restricted distribution in Asia, whereas *P. juxtanucleare* has successfully spread throughout Asia (Bennett & Warren 1966; Garnham 1966, 1969; Manwell 1966; Laird 1998), the New World (Versiani & Gomes 1941; Krettli 1972; Laird 1998) and Africa (Poulsen *et al.* 2000). Although the mosquito species capable of transmitting these parasite species can differ, global vector distribution alone cannot explain the dominance of *P. juxtanucleare* over the restricted *P. gallinaceum* (Knight & Stone 1977). The natural mosquito vectors of both species are largely unknown, although both parasite species can be vectored by the same mosquito species, *Mansonia crassipes*, in Sri Lanka (Niles *et al.* 1965; Niles & Kulasiri 1967). Furthermore, *P. gallinaceum* is more oligoxenous than any other malaria parasite species, being able, under laboratory conditions, to infect at least six mosquito genera (Garnham 1966). Notably, *Aedes aegypti* appears refractory to *P. juxtanucleare* in experimental infections (Bennett *et al.* 1966).

3. MATERIAL AND METHODS

(a) *Parasite, host and mosquito species*

Plasmodium gallinaceum (Strain 8A obtained from D. Kaslow, NIH, Bethesda, MD, USA) was maintained *in vivo* by intramuscular inoculation of 1 ml of infected blood (20–45% parasitaemia) into naive chicken hosts, with frequent passage through the mosquito vector, *A. aegypti* (Liverpool Blackeye strain). Three-week-old white leghorn chickens (*Gallus gallus domesticus*) (INRA, France) were used. All experimental animals were maintained according to European Union guidelines. Parasitaemias were obtained by Giemsa staining of daily blood smears, and calculated, as percentages, from a minimum of 100 RBCs (or 20 000 RBCs for parasite detection during the chronic phase of infection); gametocytaemias were calculated from 10 000 RBCs.

A new strain of *P. juxtanucleare* was isolated in 1999 from domestic chickens in Nha Trang, Vietnam, and cloned by limiting dilution. Parasitaemias were obtained by Giemsa staining of daily blood smears, and calculated, as percentages, from a minimum of 1000 RBCs; gametocytaemias were calculated from 10 000 RBCs. *Plasmodium juxtanucleare* did not infect our *A. aegypti* mosquitoes upon repeated trials. In order to verify the functional viability of *P. juxtanucleare* male gametocytes in the absence of a laboratory vector, male gametes were prepared *in*

vitro as described previously for *P. gallinaceum* (Carter *et al.* 1979).

The two parasite species are easily distinguishable following Giemsa staining. *Plasmodium gallinaceum* are large parasites, and both schizonts (containing 16–20 merozoites) and gametocytes fill the host RBC. *Plasmodium juxtanucleare* is a 'micro'-*Plasmodium* species, and both schizonts (containing two to six merozoites) and gametocytes rarely achieve the size of the RBC nucleus. In addition, as suggested by its name, *P. juxtanucleare* is always found adjacent to the host RBC nucleus (Kissinger *et al.* 2002).

Aedes aegypti mosquitoes were maintained under standard conditions (80% humidity and 26 °C). In all transmission studies ca. 50 female mosquitoes were used to gorge on individual chickens (*in vivo* experiment) or through a membrane (*ex vivo* experiments). Transmission success was evaluated by the number of mosquitoes positive for *P. gallinaceum*; oocyst stage parasite counts were made 7 days after mosquito infection on midguts dissected from 30 gravid females and then stained with 0.5% mercurochrome in 1 × phosphate buffer solution for visualization under the microscope.

(b) *In vivo co-infection study*

Experimental infections were induced by gorging four infected mosquitoes on each chicken host. The presence of sporozoites in each of these mosquitoes was verified subsequently by dissection of the salivary glands. Twelve six-week-old chickens were thus infected with *P. gallinaceum*, of which six had been infected three weeks previously with *P. juxtanucleare* (intramuscular injection of 1 ml parasitized blood, 0.5% parasitaemia). *Aedes aegypti* mosquitoes were gorged upon infected chickens on the first day *P. gallinaceum* became patent (more than 0.1% parasitaemia) (predictably day 7 after infection by mosquitoes following the pre-erythrocytic developmental period) and for the next 3 days. Over the next three months, blood smears were taken weekly and, when these were positive for *P. gallinaceum*, mosquitoes were fed on the individual bird; four such feeds per individual were carried out over the three-month period. RBC densities (haematocrit) were measured with a haemocytometer at every blood smear.

(c) *Ex vivo infection studies*

(i) *Removal of vertebrate host immune factors*

Malaria parasite infectivity to mosquitoes can be affected by both non-specific and specific immune responses (Carter & Graves 1988; Carter & Mendis 1992). To rule out any adverse effects of immune responses induced by *P. juxtanucleare* on the viability of *P. gallinaceum* gametocytes, each species was reared in separate hosts. *Plasmodium gallinaceum*-infected blood (10 ml) was taken from an infected host, diluted five times in pre-warmed (37 °C) suspended animation (SA) solution (10 mM of Tris-HCl; 130 mM of NaCl; 10 mM of glucose; pH of 7.3) and centrifuged for 5 min at 2000 *g* (Kaushal *et al.* 1983). The supernatant (SA solution + serum) was removed, and the RBCs resuspended in naive serum and divided into two samples. Uninfected RBCs or *P. juxtanucleare*-infected RBCs from which the serum had similarly been replaced by naive serum were added to each sample. The haematocrits of the two samples were measured with a haemocytometer and equilibrated with uninfected RBCs. The samples were loaded into mosquito thistle feeders, upon which 50 mosquitoes gorged through a Baudruche membrane for 15 min. *Plasmodium gallinaceum* transmission successes (percentage of mosquitoes infected) when mixed with

P. juxtanucleare and uninfected blood were compared. This experiment was replicated three times using different infected chicken hosts.

(ii) *Vertebrate host blood quality*

Blood quality is known to influence the reproductive success of *P. gallinaceum* independently of immune factors (Rosenberg *et al.* 1984). This experiment aimed to assess the effect of *P. juxtanucleare* on *P. gallinaceum* transmission success to mosquitoes via its effect on host blood quality. *Plasmodium gallinaceum* ookinete stages, which are zygote stages infectious to mosquitoes, were prepared *in vitro* as described previously (Kaushal *et al.* 1983) and resuspended in 2 ml uninfected or *P. juxtanucleare*-infected blood from which the serum had been replaced with naive serum as in § 3c(i). The two samples of blood were loaded into thistle feeders and fed to 50 mosquitoes. This experiment was replicated three times using different infected chicken hosts.

(d) *In vitro fertilization study*

To establish at what stage *P. juxtanucleare* exerted the observed negative impact on *P. gallinaceum* transmission success, fertilization was carried out *in vitro*. *Plasmodium gallinaceum*-infected blood (10 ml, 15–36% parasitaemia) was taken from an infected host, centrifuged, resuspended in naive serum as in § 3c(i) and divided into two samples. Uninfected RBCs or *P. juxtanucleare*-infected RBCs from which the serum had been replaced by naive serum were added to each sample. Ookinetes were prepared *in vitro* from each sample as described previously (Kaushal *et al.* 1983) and counted using a haemocytometer. Although ookinetes of both species were expected in preparations combining *P. juxtanucleare* and *P. gallinaceum*-infected blood, the vastly higher gametocyte density of the acute-stage *P. gallinaceum* infections used would yield predominantly *P. gallinaceum* ookinetes. This experiment was replicated three times using different infected chicken hosts.

(e) *Statistical analyses*

Statistical analyses of parasitaemia, gametocytaemia and mosquito infection rates were conducted using the statistical package GENSTAT, v. 5.4.1. Because each individual chicken was included in the dataset many times in the *in vivo* co-infection study, we corrected for repeated measures by fitting a generalized linear mixed model with a Poisson error structure with chicken as the only term in the random model. Statistical significance was presented as Wald statistics, which are equivalent to a χ^2 . Comparisons of mosquito infection success were performed by day, specifying a binomial error structure for the percentage of mosquitoes that became infected per chicken. For the *ex vivo* and *in vitro* infection studies, mosquito infection success was compared for individual *P. gallinaceum*–*P. juxtanucleare* infected chicken combinations specifying a binomial (the percentage of mosquitoes) or Poisson (mean ookinete number) error structure.

4. RESULTS

Although co-infection with *P. juxtanucleare* did not affect the course or intensity of *P. gallinaceum* infection in the chicken hosts (parasitaemia, $\chi^2_1 = 1.9$, n.s., figure 1a; gametocytaemia, $\chi^2_1 = 1.4$, n.s., figure 1b), it significantly reduced the transmission success (proportion of mos-

Figure 1. Absence of an effect of co-infection with *P. juxtanucleare* on (a) the asexual and (b) the gametocyte parasite densities of *P. gallinaceum*: *P. gallinaceum* alone (single species, squares and solid line) and co-infection by *P. gallinaceum* and *P. juxtanucleare* (mixed species, triangles and dashed line). *Plasmodium gallinaceum* infects RBCs following a 7 day pre-erythrocytic developmental period, and therefore transmission to mosquitoes starts 7 days after infection.

quitoes that became infected) of *P. gallinaceum* during the acute phase of the infection ($\chi^2_1 = 8.24$, $p < 0.01$) and totally suppressed transmission during the chronic phase (figure 2).

In an attempt to establish the underlying cause of this negative effect of *P. juxtanucleare* on *P. gallinaceum*, a series of *ex vivo* and *in vitro* experiments were performed. First, the impact of host immune factors induced by infection was minimized by serum replacement. As in the *in vivo* infections, the presence of *P. juxtanucleare* reduced the transmission success of *P. gallinaceum*. The geometric mean percentage of mosquitoes infected with *P. gallinaceum* oocysts was greater in the absence ($89.3 \pm 3.1\%$, 99% confidence interval (CI)) than in the presence ($61.6 \pm 13.4\%$, 99% CI) of *P. juxtanucleare* ($\chi^2_1 = 9.3$, $p < 0.01$; $n = 30$ dissected mosquitoes for each of the three replicates). Second, because blood quality can affect ookinete invasion ability (Rosenberg *et al.* 1984; Vinetz *et al.* 2000), we examined the direct effect of *P. juxtanucleare*-infected RBCs on *P. gallinaceum* ookinete invasion success. There was no difference in the geometric mean percentage of mosquitoes infected following inges-

Figure 2. Effect of co-infection with *P. juxtanucleare* on the transmission success of *P. gallinaceum* from infected chicken hosts to *Aedes aegypti* mosquitoes (mean proportion of infected mosquitoes $\pm$ 95% CI): *P. gallinaceum* alone (single species, grey bars) and co-infection by *P. gallinaceum* and *P. juxtanucleare* (mixed species, white bars). No mosquitoes were infected with *P. gallinaceum* during days 28–84 post-infection in chickens co-infected with both species. Note: *A. aegypti* is refractory to *P. juxtanucleare*.

tion of *in vitro* prepared *P. gallinaceum* ookinetes in the presence ($83.4 \pm 16.9\%$, 95% CI) and absence ($87.6 \pm 11.5\%$, 95% CI) of *P. juxtanucleare* ($\chi^2_1 = 0.3$, n.s.; $n = 30$ dissected mosquitoes for each of the three replicates). Finally, to exclude the influence of mosquito factors, we studied the interference process entirely in the absence of the mosquito by performing fertilization *in vitro*. Consistently fewer ookinetes were found in *P. gallinaceum* fertilization carried out *in vitro* in the presence versus the absence of *P. juxtanucleare* ($n = 3$ different pairs of birds used infected either with *P. juxtanucleare* or *P. gallinaceum*). In the presence of *P. juxtanucleare*, *P. gallinaceum* ookinete production was $75.7 \pm 4.7\%$ (95% CI) of that in the absence of *P. juxtanucleare* ($\chi^2_1 = 5.1$, $p < 0.05$).

5. DISCUSSION

Our series of experiments demonstrate that, although there may be no apparent effect of one *Plasmodium* species on another during the course of infection within the vertebrate host, there are dramatic consequences for parasite transmission success to mosquitoes. Furthermore, we have shown that the negative effects of *P. juxtanucleare* on *P. gallinaceum* are independent of both the vertebrate immune response and the mosquito vector, strongly implicating an interspecific interaction during the process of fertilization itself. Such interspecific interference during transmission has particular consequences for *P. gallinaceum* during its chronic phase: the presence of *P. juxtanucleare* blocks transmission, inhibiting the reservoir population which is vital for parasite transmission and survival following the non-transmission season. The simplest interpretation of this negative interaction is that it is a

Figure 3. Comparative gametocyte densities (± 1 s.e.) of *P. juxtanucleare* (dashed line) and *P. gallinaceum* (solid line) during the chronic stage of the infection (days 28–84 post-infection).

consequence of the differences in the host–parasite species interactions and the differing survival strategies used by the two parasite species. *Plasmodium gallinaceum* multiplies rapidly during the initial stages of infection, resulting in a very high transmission rate. However, its long-term survival relies upon re-infection of the blood from latent tissue stages, and the relapsing parasitaemia is maintained at very low levels, reflecting the development of a strong host immune response. The source of transmission stages is therefore very low, but, as observed here, sufficient to infect mosquitoes. *Plasmodium juxtanucleare*, by contrast, replicates very slowly, and an equilibrium parasite density is maintained throughout the infection. Thus, although *P. gallinaceum* dominates in its initial phase of infection, *P. juxtanucleare* is the dominant parasite over the long term. Negative effects on *P. juxtanucleare* transmission are expected, but, because gametocyte density is a function of overall parasite density, *P. juxtanucleare* will maintain some transmission success because it produces more gametocytes than *P. gallinaceum* during the chronic stage of the infection (figure 3).

The experiments performed used only one isolate of each species, one of which has been in the laboratory for decades. Caution in extrapolating laboratory studies to explain natural species distributions is clearly required. However, the preliminary data implicating the process of fertilization and excluding host factors in the negative interaction are encouraging. Parasite adaptations to the laboratory chicken and mosquito hosts are unlikely to include changes in fertilization molecules that are largely independent of either host. Thus, if, as we believe, such negative interactions occur widely in natural populations, they would have serious consequences for the ability of *P. gallinaceum* to invade and establish itself successfully in foci occupied by *P. juxtanucleare*. *Plasmodium juxtanucleare* prevalence rates are generally high in domestic fowl populations (mean of 35%, range of 4–100%) across all continents (Garnham 1966; Bennett *et al.* 1966; Kretzli 1972; Poulsen *et al.* 2000). *Plasmodium gallinaceum*, as expected, is more difficult to detect, but its presence is revealed during epizootics (e.g. Colombo (Garnham 1966) and Thai-

land (R. E. L. Paul and S. Nithuani, personal communication)). No such epizootics have occurred in the New World or Africa, except as a result of accidental experimental release (Garnham 1966). This supports the idea that *P. gallinaceum* is indeed absent from the New World and Africa but, if present, could be transmitted by the local mosquito populations. The role of an unidentified natural host reservoir restricted to Asia necessary for *P. gallinaceum* persistence cannot be ruled out. However, we propose that the interspecific competition during transmission identified in our experimental system may have contributed to the restricted global distribution of *P. gallinaceum*, especially in regions where the abundance of competent mosquito species may be limiting or seasonal: i.e. the presence of *P. juxtanucleare* reduces the realized R_0 of *P. gallinaceum* sufficiently to inhibit its invasion and establishment.

Interspecific interference competition has been previously observed between sea-urchin species, where male sperm of one species inhibit female egg receptivity of the other species by inducing an egg anti-polyspermy response (Lambert 2000). Polyspermy has not been observed in malaria parasite fertilization (Marchoux 1899; Carter & Graves 1988), and it is likely that anti-polyspermy mechanisms exist in *Plasmodium*. Gamete surface proteins involved in mate recognition and fertilization in eukaryotes are highly diverse even between closely related species (Vacquier 1998). By contrast, malaria parasite gamete proteins involved in fertilization are remarkably conserved within the *Plasmodium* genus (Van Dijk *et al.* 2001), and, thus, may allow interspecific gamete interaction, which could explain the interference phenomenon observed in this study.

Whatever the mechanism, such interspecific gamete interference may occur between co-infecting *Plasmodium* species in animals and humans, and could explain the lower than expected prevalence of mixed infections in mosquitoes (McKenzie & Bossert 1997b). *Plasmodium falciparum* reduces *P. vivax* transmission success in co-infected individuals (Graves *et al.* 1988), but categorical evidence is difficult to obtain because many immune-related factors can severely affect transmission success. Any negative interactions would be most evident when parasite densities of one of the species are very low. Current evidence indicates that *P. vivax* is suppressed during an acute *P. falciparum* infection, but re-emerges once *P. falciparum* has subsided and become chronic (Boyd & Kitchen 1937; Maitland *et al.* 1996; Bruce *et al.* 2000). Such intra-host dynamics would create a situation comparable with that studied here, with alternating patterns of species dominance within the vertebrate host. Such reciprocal dominance may be of considerable importance for *P. falciparum* transmission from chronic infections. Individuals without apparent *P. falciparum* gametocytes (thus at a very low density) can infect mosquitoes (Boudin *et al.* 1993) and may comprise a significant proportion of the human infectious reservoir. Relapsing *P. vivax* asexual and, hence, gametocyte densities may be sufficient to interfere with *P. falciparum* transmission from such chronic infections. This negative interaction may, furthermore, partially explain why, in regions where both species occur, *P. vivax* appears to be the more transmissible (the peak in the incidence of *P. vivax* occurs in younger chil-

dren than that of *P. falciparum*) (Luxemburger *et al.* 1996; Maitland *et al.* 1997). Analogous to the bird model, despite short-term inferiority, *P. vivax* long-term transmission success may result in a more transmissible parasite, i.e. a parasite with a higher realized R_0 .

Establishing the epidemiological significance of species interactions in the human population has clear important consequences for control strategies. If negative interactions occur between sympatric human malaria species during transmission, control measures that inadvertently affect less-malignant species may lead to an increase in *P. falciparum* transmission success and, hence, exacerbate population morbidity. Conversely, interspecific interference during mating would be expected to reinforce transmission reduction achieved by transmission-blocking vaccines (Carter *et al.* 2000), thereby relaxing the strict requirements for vaccine efficacy.

We thank V. Robert and J. Koella for critical reading of the manuscript, and especially the three reviewers whose comments greatly improved this paper. This work was supported by the Institut Pasteur Paris (R.E.L.P. and P.T.B.), an EC Training and Mobility of Researchers Marie Curie Research Training grant to R.E.L.P., Institut Pasteur Nha Trang and Le réseau International des Instituts Pasteur et Instituts associés to V.A.T.N., the National Research Council in Brazil and a Manlio Cantarini post-doctoral grant to A.U.K.

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As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.