PERIODIC INFECTIVITY OF *PLASMODIUM* GAMETOCYTES TO THE VECTOR. A REVIEW

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Summary :

Frank Hawking, in 1966 postulated that in synchronous malaria infections, the brief period of infectivity of gametocytes was timed to occur when the vector bites. Since this early work, numerous studies had contributed to confirm and explain this phenomenon with bird, rodent and primate *Plasmodium*. Data on the periodic production of gametocytes, the duration of their maturation, the effect of the schizogony on the infectivity and the circadian bioavailability of gametocytes provide some more informations on the periodic *Plasmodium* gametocyte infectivity to the vector. This paper is intended to be a review of contributions on the "Hawking phenomenon" and to summarize the principal causal hypotheses. The conclusion stresses the practical consequences for experimental studies and epidemiological surveys.

KEY WORDS : *Plasmodium*, *Leucocytozoon*, gametocytes, infectivity, transmission, sequestration, periodicity.

Résumé : INFECTIVITÉ PÉRIODIQUE DES GAMÉTOCYTES DE *PLASMODIUM* POUR LE VECTEUR. MISE AU POINT

Franck Hawking en 1966 énonce et confirme l'hypothèse suivante : dans les infections palustres synchrones, la période d'infectivité maximale des gamétocytes coincide avec le pic d'activité des moustiques vecteurs. Depuis, de nombreux travaux ont confirmé ses observations et expliqué ce phénomène pour certains Plasmodium *d'oiseaux, de Rongeurs et de Primates. Les observations sur la production périodique des gamétocytes, la durée de leur maturation, l'effet de la rupture des schizontes sur leur infectivité et les variations circadiennes de leur biodisponibilité pour le moustique ont permis des avancées dans la compréhension du mécanisme de périodicité d'infectivité des gamétocytes de* Plasmodium. *Cet article a pour objectif de présenter une compilation des données disponibles sur le "phénomène de Hawking" et d'en résumer les principales hypothèses explicatives. La conclusion indique les conséquences pratiques pour les travaux expérimentaux et les études épidémiologiques.*

MOTS CLÉS : Plasmodium, Leucocytozoon, *gamétocytes, infectivité, transmission, séquestration, périodicité.*

A remarkable feature of most of bird, rodent and primate malaria is the precise timing of its recurrent attacks which are generally at some multiple of 24 hours. This implies that the duration of the erythrocytic asexual cycle is stable and that all the parasites behave synchronously, reaching schizogony at the same time. Cell division takes place at an hour of the day constant for each species of malaria parasite which depends on the location of the host or for some species mostly on the time of inoculation of the parasite. The biological purpose of such an accurate timing in the cycle of *Plasmodium* was shown by Hawking (1970) to assist them to present infective gametocytes at the time mosquitoes bite and so rendering the transmission more efficient. In other terms, Hawking showed that the production of short-lived gametocytes was cyclic, leading to circadian variations in the ability of *Plasmodium* to infect the mosquito vector and it was called by Garnham & Powers (1974), "the Hawking phenomenon". Since Hawking's work, new data on periodic infectivity of *Plasmodium* and other Haemosporidia to the vector have been published and experimental work has been done in order to determine its mechanism. This periodic infectivity depends on the duration of gametocyte maturation, periodic sequestration of gametocytes and periodic release of parasite and/or vertebrate host compounds that regulate the gametocyte infectivity. This paper is intended to be a review of contributions on the periodic infectivity of the gametocytes of the Haemosporidia to their vectors and to summarize the principal causal hypotheses. The conclusion stresses the practical consequences of the Hawking phenomenon for experimental studies and epidemiological surveys.

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PERIODIC PRODUCTION OF GAMETOCYTES

The periodicity of the production of gametocytes can be assessed in different ways (Table I). Total gametocyte counts can be made at short timed intervals over a period of several days, exflagellation rate can be observed at regular intervals and morphological analysis of the gametocytes can be made over time. Gametocyte morphology undergoes age-related transformations so that different morphological stages can be defined, although all intermediates can be observed. During the course of normal ageing, the gametocyte diameter increases at first and becomes then smaller while its chromophilic character increases. The nucleus is at firts relatively small, then enlarges before becoming condensed and progressively smaller. The nucleus is granular at the begining of its evolution and colloidal at the end (Landau *et al.*, 1979).

AVIAN *PLASMODIUM*

Periodic production of sexual stages was first demonstraded with avian *Plasmodium*. In 1934, Shah claimed that the number of gametocytes of *P. cathemerium* (24 hour cycle) in the peripheral circulation of *Serinus canarius* varied at different periods during the day, and showed a daily increase around 6 p. m. This was the first report of a periodicity in the production of *Plasmodium* sexual stages. In a continuation of that earlier work, Gambrell (1937) also observed a peak in the number of gametocytes at 6 p. m. Hawking *et al.* (1968*a*) confirmed the periodical character of the gametocytogenesis of *P. cathemerium* and showed a nocturnal peak of exflagellating microgametocytes at 4-6 a. m. A corresponding cycle was demonstrated in the morphology of the gametocytes which undergo six maturation stages.

P. relictum matutinum was studied by Gambrel (1937) and was shown to present a definite increase in numbers of gametocytes around the time of segmentation which takes place daily at 8 a.m. *P. gallinaceum* gametocytes were shown by Lumsden & Bertram (1940) to be produced in broods and to peak at about the same time as the percentage of schizonts increased (i. e. alternatively at midnight and at midday in relation to the 36 hour cycle). Similarly, Hawking *et al.* (1972) described a peak of exflagellation of *P. gallinaceum* microgametocytes which developped in four maturation stages. Roller & Desser (1973) demonstrated that the numbers of *Leucocytozoon simondi* gametocytes in ducks peaked during day time when the activity of the vector *Simulium rugglesi* was maximum. Similar fluctuations of the numbers of gametocytes in the peripheral blood were seen in *L. smithi* infected fowls. The diurnal peaks occured at the time when *S. slossonae,* the vector, bites (Noblet & Noblet, 1976). The inversion of the photo-period shifts the peaks by 12 hours (Noblet & Noblet, 1977) and continued exposure of fowls to light suppressed the periodicity of gametocytes in the peripheral blood (Gore & Noblet, 1977). Pinealectomy, however did not have any effects on this periodicity (Gore *et al.*, 1982).

RODENT *PLASMODIUM*

With rodent malaria, the first observation of a rhythm in the production of gametocytes was that of Hawking *et al.* (1972) who showed that the exflagellating *P. cha-*

	Time of		Number of gametocyte stages described	Interval between two gametocytes broods (hours)
	Total gametocyte peak	Exflagellation peak		
P. cathemerium	6 p.m.	4-6 p.m.	6 stages	24
P. relictum	8 a.m.	NS	NS	24
P. gallinaceum	noon/midnight	noon/midnight	4 stages	36
L. simondi	day time	NS	NS	24
L. smithi	day time	NS	NS	24
P. chabaudi	NS	midnight-4 p.m.	4 stages	24
P. vinckei	NS	NS	4 stages	24
P. berghei	*	NS	NS	NS
P. yoelii	NS	NS	4 stages	24
P. cynomolgi	NS	midnight (Langur) 6 p.m. (Nilgiri)	8 stages	48
P. knowlesi	midnight	midnight	4 stages	24
P. falciparum	NS	night time	5 stages	48
P. vivax	NS	NS	7 stages	48

NS = not studied; * = Depending to the time of parasite inoculation.

Table I. – Periodic production of Haemosporidia gametocytes.

baudi gametocytes were in great number at 0-4 a. m at the time of schizogony. Later, a 24 hour rhythm of production of gametocytes was confirmed by Gautret *et al.* (1996*c*) with *P. chabaudi* as well as with *P. vinckei vinckei* and *P. v. petteri* (Gautret *et al.*, 1995; 1996*a*). Landau *et al.* (1979) described four evolutionary stages (0, I, II and III) in rodent *Plasmodium* gametocytes. In parasites like *P. berghei* and *P. yoelii,* a periodic production of gametocytes can also be shown after synchronization. *P. berghei* was synchronized by Hawking *et al.* (1972) in *Thamnomys surdaster* by repetitive passages from *Thamnomys* to *Thamnomys* and showed a peak of exflagellation of microgametocytes at a time depending of the time of inoculation of the parasite to the rodent. *P. y. yoelii* and *P. y. nigeriensis* were synchronized by Percoll-glucose concentration of young stages for inoculation (Deharo *et al.*, 1994) and a 24 hour rhythm of production of gametocytes was demonstrated (Gautret *et al.* 1995; 1996*b*).

PRIMATE *PLASMODIUM*

Work was also performed with primate *Plasmodium.* Eight stages of maturation were identified with *P. cynomolgi ceylonensis,* Langur strain (C strain) gametocytes in rhesus monkeys (Hawking *et al.*, 1968*a*) with a peak of exflagellation occuring at midnight every 48 hours. Rao *et al* (1971) also observed a peak of exflagellation but it occured at 6 a. m. probably because they used the Nilgiri strain and worked in Delhi whereas Hawking worked in London. Similarly, five stages of maturation were demonstrated with *P. knowlesi* in rhesus monkeys and the total numbers of gametocytes increased at midnight every 24 hours as well as the numbers of exflagellating gametocytes (Hawking *et al.*, 1968*a*). *P. vivax* has been less studied. Boyd (1935) estimated the duration of the maturation of morphologically infective gametocytes to be 48 hours and described seven evolutive stages.

P. FALCIPARUM

P. falciparum gametocytes undergo five maturation stages in man (Field & Shute, 1956), in splenectomized *Aotus* (Hawking *et al.*, 1971) and *in vitro* (Smalley, 1976). Stage V needs nine days to be produced but three more days are required for the gametocytes to be able to exflagellate (Jeffery & Eyles, 1955). Observation with *P. falciparum* in man showed more exflagellations at night than during day time and, exflagellation peaks were observed at 48 hour intervals (Hawking *et al.*, 1971). The authors considered this observation to be related to a circadian tendency in the production of infective sexual stages. However, the difficulty to evidence this periodicity, due to both the approximate 48 hour rhythm of *P. falciparum* asexual stage development and the long time of gametocyte maturation led Hawking to conclude that his results required futher investigations to be confirmed.

Thus, in all the species studied, new gametocytes are produced following successive schizogonies and undergo morphological maturation stages leading to the formation of gametocytes infective to the vector at a precise timing. The life cycle of the parasites is carefully timed so that they are most ready for transfer from the vertebrate host to mosquito during the hours when the mosquitoes are most likely to be feeding (Hawking, 1975). The case of *P. gallinaceum* with an alternative production of infective gametocytes at midnight and at noon, in relation with its 36 hour schizogonic rhythm could be the consequence of an adaptation to the bitting habits of *Aedes* or *Culex* vectors in Asia.

INFECTIVE STAGES OF GAMETOCYTES AND DURATION OF THEIR MATURATIONS

The chronology of gametocyte development (i. e. the time required by a merozoite to undergo the different evolutionary stages of gametocytogenesis) can be assessed by evaluating the relative proportion of each stages at short timed interval during the course of gametocytaemia (Table II). The scattering of the pigment or the exflagellation ability of male gametocytes are used by several authors as an indicator of the maturity/infectivity of sexual stages. We consider, however, correlation analysis between the proportion of each stage at the time of vector feeding and the number of oocyst in insect midgut to be a much more accurate and reliable parameter for the identification of the infective gametocyte stage.

	Infective morphological stage	**Duration of maturation from merozoite to infective stage**	**Mean life span of infective stage**
P. cathemerium	III (F)-IV (M)	24 h/30-36 h/26-29 h	
P. gallinaceum	IV	60/64 h	17 h
P. chabaudi	II	48 h	3-6 h
P. vinckei	II	36 h	3-6 h
P. berghei	NS	26 h	7 h
P. yoelii	0	24 h	5 h
P. cynomolgi	IV/V (F)-VII (M)	58 h	12 h
P. knowlesi	IV	31 h	5 h
P. falciparum	V	12 days	2.5 days

NS = not studied ; h = hours; F = female ; M = male.

Table II. – Infective stage of *Plasmodium* gametocytes and duration of their maturation.

Bird *Plasmodium*

Shah by studing the morphology of *P. cathemerium* gametocytes concluded that they reached their maturity after about 24 hours, at the time of schizogony. Gambrel (1937), by contrast, considered the lenght of the maturation of a merozoite into mature gametocyte to be 30-36 hours, and the gametocytes ageing 24 hours to be premature gametocytes. Both authors considered the scattering of the pigment as a criterion for maturity. Hawking *et al.* (1968*a*), identified type III and type IV gametocytes to be the mature stages respectively for macrogametocytes and microgametocytes and the time required was 26-29 hours. They considered the exflagellation to be the indication of the full maturity of the microgametocytes. In this latter work, the authors used an Italian strain isolated from a sparrow near Rome in 1966 and the time of schizogony was 11 p. m. Shah used a strain from the University of Syracuse whose origin was not mentioned and Gambrell used the D strain from Rome. Both strains underwent schizogony at 6 p. m. The discrepancies in the duration of the gametocyte maturation and in the time of schizogony may be due to the use of different strains called *P. cathemerium*. *P. gallinaceum* gametocyte maturity was considered by Lumsden and Bertram (1940) to be achieved in 60 hours. This was confirmed by Hawking *et al.* (1969; 1972) who demonstrated a relatively brief period (17 hours) of exflagellation of male gametocytes and evaluated the duration of the maturation to reach type IV gametocytes to be 64 hours.

Rodent *Plasmodium*

With *P. chabaudi, P. v. vinckei* and *P. v. petteri*, the time of maturation of merozoites to type II infective gametocytes is 48, 48 and 36 hours and they degenerate in six to three hours (Gautret *et al.* 1995; 1996*a*; 1996*c*). *P. chabaudi* microgametocytes were shown by Hawking *et al.* (1972) to be able to exflagellate during seven hours. Mons *et al.* (1985) synchronized the development of *P. berghei* gametocytes *in vitro* and *in vivo* in rats and estimated the time of maturation to be 26 hours (confirming work by Hawking *et al.* (1972) in *Thamnomys*) and the mean duration of infectivity was shown to be about 13 hours. *P. y. yoelii* and *P. y. nigeriensis,* when synchronized by the Percoll-glucose technic, develop their merozoites into mature type 0 gametocytes in 24 hours and degenerate in three hours. This is consistent with the observation by Killick-Kendrick & Warren (1968) who showed that the first infective gametocytes appeared 24 hours after the rupture of hepatic meronts. The discrepancy between the infective stages of the different rodent *Plasmodium* (stage II *for P. vinckei* and *P. chabaudi* and stage 0 or *P. yoelii*) is surprising but correlates with a similar discrepancy in the sequestration of gametocytes in capillaries (see below).

Primate *Plasmodium*

P. cynomolgi ceylonensis (Langur C strain) merozoites transform into mature female (type IV and IV) and male (type VII) gametocytes in 58 hours and degenerate in about 12 hours (Hawking *et al.*, 1968*a*). *P. knowlesi* mature type IV male and female gametocytes need 31 hours to be produced and degenerate in no more than five hours (Hawking *et al.*, 1968*a*; 1968*b*).

P. falciparum

The life span of *P. falciparum* mature stage V gametocyte is longer than that of other mammalian *Plasmodium* but relatively short. Smalley & Sinden (1977) observed the presence of exflagellating stage V gametocytes in the blood smears obtained from patients following radical chloroquine treatment during a mean time of 2.4 days. *P. falciparum* gametocytes can remain infective to the vector for many days as demonstrated by Jeffery, Young & Eyles (1956) and Smalley & Sinden (1977) who successfully infected mosquitoes from gametocyte carriers up to 11-12 days following radical chloroquine treatment. It must be pointed out however that these infections led to considerably reduced oocyst burden per infected mosquito.

Thus, with the exception of *P. falciparum*, mature infective gametocytes of the different species of *Plasmodium* have a short life span which does not exceed 17 hours and is most of the time, only between three and seven hours.

TEMPORARY LOSS OF GAMETOCYTE INFECTIVITY DURING SCHIZOGONY

Independently of cyclical maturation of infective gametocytes, several authors recently described mechanisms of periodic production of factors inhibiting gametocyte infectivity.

Rodent *Plasmodium*

Motard *et al.* (1990) showed that gametocytes in mice infected with *P. v. petteri* loose temporarily their infectivity for *Anopheles stephensi* at the time of schizogony. This temporary loss of gametocyte infectivity was demonstrated to be linked with a peak of production of nitric oxide during the schizogony (Motard *et al.*, 1993). The responsability of antibodies in this phenomenon is very unlikely as gametocyte infectivity inhibition occurs in *P. berghei* infected SCID mice for *A. stephensi* (Sinden *et al.*, 1993; Fleck *et al.*, 1994).

PRIMATE *PLASMODIUM*

A transcient inhibition of *P. vivax* gametocyte infectivity was observed in man at the time of clinical paroxisms (Mendis *et al.* 1990). Tumour necrosis factor and gamma interferon levels are increased when *P. vivax* and *P. cynomolgi* gametocytes infectivity for *A. tessellatus* is reduced during clinical paroxisms (Karunaweera *et al.*, 1992; Naotune *et al.*, 1991). White blood cells and reactive nitrogen intermediates are necessary to obtain an inhibition of gametocyte infectivity by the supernatant of cultured peripheral blood mononuclear cells stimulated by frozen-thawed blood stages of *Plasmodium*. This is the case for *P. vivax*/*A. tessellatus* in man and for *P. falciparum* (3D7)/*A. stephensi, in vitro* (Naotunne *et al.*, 1993).

In conclusion, inhibition of *Plasmodium* gametocyte infectivity at the time of schizogony may be the consequence of the activation of cellular immunity effectors via the secretion of cytokines and production of nitric oxides. It is very likely that parasite origin factors are released at the time of schizogony also and play a role of initial activators of the white cells.

SEQUESTRATION AND CIRCADIAN BIOAVAILABILITY OF GAMETOCYTES

An alternative explanation to the Hawking phenomenon is the existence of a circadian preferential distribution (sequestration, retention or release) of infective gametocytes in the capillaries, rendering them available to the mosquito vector. Sequestration of asexual blood stages of *Plasmodium* in deep capillaries is very well known and hundreds of publications have been produced since the first observation with *P. falciparum* by Marchiafava & Bignami (1894). Sequestration of other Haemosporidia gametocytes by contrast have been less studied.

BIRD *PLASMODIUM*

Missiroli (1939) made the first report of sequestration of *P. praecox* gametocytes in sparrows by demonstrating an increase of their numbers in the blood of birds submited to mosquito *Culex* bites. Roller & Desser (1973) showed that gametocytes of *L. simondi* were in greater numbers in hepatic blood than in cardiac blood of ducks when the peripheral parasitemia was low at night.

RODENT *PLASMODIUM*

Experiments with rodent *Plasmodium* are very numerous and signifiant. Comparison between gametocyte composition of blood from the tail vein of *P. y. yoelii* infected mice and blood from mosquito midgut immediately after engorgement showed an enrichment of type 0 and I microgametocytes in the latter when the infectivity to the vector is maximal (Landau *et al.*, 1979; Gautret *et al*, 1996*b*). This phenomenon was neither seen by Janse *et al.* (1985) when comparing *P. berghei* gametocyte composition of blood from mice tail vein, mice heart blood and mosquito blood meal nor by Mons *et al.* (1985) who observed the same numbers of exflagellating gametocytes *in vitro* and *in vivo*. This is probably because it only occurs at the time of maximum infectivity and affects the infective stage only. With *P. v. petteri* and *P. chabaudi*, stage II infective gametocytes are found in greater numbers in mosquito blood meal than in mice tail vein blood (Gautret *et al.*, 1996*a;* Gautret *et al.*, 1996*c*). When experiments were performed with *P. chabaudi* at the time type 0 gametocytes peak in mice blood, no sequestration was observed indicating that the retention of gametocytes in deep capillaries affects only the infective stage and therefore occurs at a precise time in the cycle of maturation.

PRIMATE *PLASMODIUM*

With primates, Dei-Cas *et al.* (1980*a*; 1980*b*) also observed a difference in the counts of various stages of *P. inui* macrogametocytes when comparing peripheral monkey blood and mosquito meals and concluded that a sequestration of certain types of gametocytes in monkey capillaries occurs.

P. FALCIPARUM

P. falciparum mature gametocytes are detectable in human peripheral blood around 10 days after asexual stages (Ross & Thomson, 1911), corresponding to 10 days after the first peak of fever producing schizonts (Bray, personal communication). Immature gametocytes are sequestered in spleen capillaries and in bone marrow (De Beaurepaire Arago, 1930; Garnham, 1931; Thomson & Robertson, 1935; Smalley *et al.*, 1980) but not in placental vessels (Blacklock & Gordon, 1924; Bray & Sinden, 1979; Desowitz & Buchbinder, 1992). In splenectomized *Aotus* young *P. falciparum* gametocytes are not sequestered anymore, indicating that the spleen plays a major role in this phenomenon (Hawking *et al.*, 1971; Ward *et al.*, 1972). Similar results were obtained by Bray (1958) in chimpanzees. Van den Berghe & Chardome (1951) showed that *P. falciparum* gametocytes in man were more numerous in smears made of cutaneous scarifications than in peripheral blood smears. The changes of shape of erythrocytes parasitized by *P. falciparum* gametocytes is associated with the development of microtubules under the parasite membrane (Sinden *et al.*, 1978)

which may make the infected cell more rigid, leading to a mechanical sequestration in the capillaries. Recently, specific cytoadherence to C32 cells of the red blood cells parasitized *in vitro* by stages I and II *P. falciparum* gametocytes was demonstrated (Rogers *et al.*, 1996*a*). This adherence was inhibited by anti-CD36 antibodies and by anti-ICAM I antibodies. Infected red cells use the modified band 3 to adhere to C32 and CD36-transfected CHO cells (Rogers *et al.*, 1996*b*). This adherence to CD36 was confirmed by Day *et al.* (1998) and demonstrated to be linked with knob presence and HRP1 expression in parasitized red blood cells.

In conclusion, phenomenon of sequestration/release of infective gametocytes in the fine capillaries is cyclical and probably results from the cyclical production of mature gametocytes. By favoring the absorption of the gametocytes by the vector it facilitates the transmission of the sexual stages from the mamalian to the insect.

PERIODIC TRANSMISSION OF *PLASMODIUM* TO THE VECTOR

The logical consequence of the above reported observations should be a circadian cycle in the infectivity of *Plasmodium* to the mosquito. Transmission experiments have been therefore conducted by several authors in order to investigate variations in the production of oocysts in insects according to the time of feeding.

BIRD *PLASMODIUM*

A periodical variation in the numbers of avian *Plasmodium* oocysts obtained in mosquitoes fed on the vertebrate host at different times of the day was demonstrated with *P. cathemerium* (Shah *et al.* 1934; Hawking *et al.*, 1968*a*), *P. gallinaceum* (Lumsden & Bertram, 1940), *P. praecox* (Missiroli, 1937). In all instances, the peak of oocysts was found to be between schizogonic peaks.

RODENT *PLASMODIUM*

With rodent malaria, it was demonstrated for *P. v. petteri* only (Motard *et al.* 1990; Gautret *et al.*, 1996*a*). By contrast, *P. chabaudi* does not show any variation in its ability to infect *A. stephensi* mosquitoes throughout the day despite a circadian variation in the numbers of type II infective gametocytes. This was considered to be due to the coexistence of the peak of type II gametocytes and of the schizogony in this particular species (Gautret *et al.*, 1996*c*). A circadian tendency in the infectivity of *P. y. nigeriensis* gametocytes to the mosquito was observed by Motard during asynchronous infections; the infectivity beeing greater when the proportion of mature schizonts and rings was the lowest (unpublished data). No variation however, was observed over time, in the infectivity of *P. y. yoelii* gametocytes because it develop asynchronously with different gametocyte stages present at all the times (Gautret *et al.*, 1996*b*).

PRIMATE *PLASMODIUM*

With *P. cynomolgy ceylonensis* (Langur C strain), it was found by Hawking *et al.* (1966-1968*a*) that infectivity of gametocytes to *A. stephensi* increased at midnight every 48 hours. This results were confirmed with *P. cynomolgi cynomolgi* (M strain) and *A. maculatus* (Coatney *et al.*, 1971) and with *P. cynomolgi ceylonensis* (Langur C strain) and *A. stephensi* (Garnham & Powers, 1974). With *P. knowlesi*, oocyst numbers in *A. stephensi* midguts were higher when feeding at midnight than at mid-day (Hawking *et al.* 1968*b*). *P. coatneyi* was shown to have a 48-hour rhythm of infectivity for *A. freeborni* (Coatney *et al*, 1971). Yang *et al.* (1984) and Yang (1996) demonstrated that *P. vivax* gametocyte infectivity to *A. sinensis* showed a 48 hour pattern in patients naturally infected with malaria.

P. FALCIPARUM

With *P. falciparum*, neither experiments by Bray *et al.* (1976), or by Githeko *et al.* (1993) showed a greater infectivity of young gametocyte carriers to mosquitoes during night-time. However, in Bray's experiment, mosquitoes were successively fed at 10 a. m. and 10 p. m. and in Githeko's study, at 4 p. m. and 7 p. m. Therefore, these results do not exclude a variation of *P. falciparum* gametocyte infectivity during the day or over a 48-hour cycle as transmission experiments were performed at 12 and seven hour intervals. The 2.4 day life span of *P. falciparum* infective gametocytes cannot account however, for an impairment of periodical infectivity in fully synchronous infections. Mature gametocytes are produced at each schizogony (i. e. at 48 hourly intervals) and released in the peripheral circulation some nine days after. If the great majority of gametocytes remain infective for 2.4 days, infective gametocytes from two successive broods should overlap during a brief period of 0.4 days (10 hours). Between these overlaping period, the remaining infective population should be therefore significantly reduced. In natural infections. *P. falciparum* can develop synchronously in relatively non-immune children in endemic areas (Bray, personal communication) but this is not the common feature, particularly during the primary attack and in semi-immune patients, contrary to the other human plasmodia. Furthermore, in most natural *P. falciparum* infections, two broods of parasites are present, cycling 24 hours out of phase (White *et al.*, 1992). It is probable that variations in the

infectivity of *P. falciparum* gametocytes has not been shown, partly by reason of the weak synchronicity of its schizogonic development. It should be of interest to study during two 48 hour cycles, the effect of schizogony on transmission in this particular species, in relation with the degree of synchronicity of the parasitemia and gametocytemia and the circadian bioavailability of the gametocytes for the mosquito.

In conclusion, in all fully synchronous *Plasmodium* species, (with the exception of *P. chabaudi*) a periodicity of infectivity of gametocytes to mosquitoes can be evidenced with an inter-schizogonic peak of oocysts.

GENERAL CONCLUSION

In all the *Plasmodium* species, a periodic production of mature gametocytes can be demonstrated, frequently associated with a sequestration/release phenomenon of infective gametocytes. In most of the cases, the kinetic of morphologically mature gametocytes in the vertebrate host and/or the resulting infectivity to the vector shows an accurate circadian rhythm adapted to the bitting habits of the insects. It has been demonstrated for *Leucocytozoon* in ducks (Roller & Desser, 1973) and for the plasmodia of *Thamnomys rutilans* (Gautret *et al.*, 1998). Three parasites fail to show circadian variations in their infectivity to *Anopheles*. *P. chabaudi* because schizogony and production of mature gametocytes coincide, does not show any circadian variation of gametocyte infectivity. *P. falciparum* gametocytes were not demonstrated to show a circadian rhythm in their ability to infect mosquitoes because infections in human are frequently asynchronous. *P. berghei* because of its spontaneous asynchronous development, produces mature gametocytes independently of the time. With synchronous species of *Plasmodium*, when experimental or natural infectivity is studied, it is strongly recommended that the experiments are performed at the same time of the day if a valuable comparison is to be made. It is important to perform the experiment at the time of maximum infectivity if one wants to study the influence of the immune system or of drugs on transmission. During epidemiological records, the identification of gametocyte carriers should consider infective gametocytes while taking the morphological and chronobiological data into account.

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REFERENCES

BLACKLOCK B. & GORDON R.M. Malaria parasites in the placental blood. *Annals of Tropical Medicine and Parasitology*, 1924, *19*, 35-45.

BOYD M.F. On the schizogonous cycle of *Plasmodium vivax*, Grassi and Feletti. *American Journal of Tropical Medicine*, 1935, *15*, 605-615.

BRAY R.S. Studies on malaria in chimpanzees. VI *Laveriana falciparum*. *American Journal of Tropical Medicine and Hygiene*, 1958, *7*, 20-24.

BRAY R.S., MCCRAE A.W.R. & SMALLEY M.E. Lack of a circadian rhythm in the ability of the gametocytes of *Plasmodium falciparum* to infect *Anopheles gambiae*. *International Journal for Parasitology*, 1976, *6*, 399-401.

BRAY R.S. & SINDEN R.E. The sequestration of *Plasmodium falciparum* infected erythrocytes in the placenta. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1979, *73*, 716-719.

COATNEY G.R., COLLINS W.E., MCWARREN W. & CONTACOS P.G. The primate malarias, 1971, US Government Printing Office, Washington.

DAY K.P., HAYWARD R.E., SMITH D. & CULVENOR J.G. CD36-dependent and knob expression of the transmission stages of *Plasmodium falciparum* is stage-specific. *Molecular and Biochemical Parasitology*, 1998, *93*, 167-177.

DE BEAUREPAIRE ARAGAO H. Evolution of the gametes of *Plasmodium falciparum*. *Memorias do Instituto Oswaldo Cruz*, 1930, *14*, 41-56.

DEHARO E., GAUTRET P., GINSBURG H., CHABAUD A.G. & LANDAU I. Synchnonization of *Plasmodium yoelii nigeriensis* and *P. y. killicki* infection in the mouse by means of Percoll-glucose gradient stage fractionation: determination of the duration of the schizogonic cycle. *Parasitology Research*, 1994, *80*, 159-164.

DEI-CAS E., MAUROIS P., DUTOIT E., LANDAU I. & MILTGEN F. Étude sur les gamétocytes des *Plasmodium* de mammifères: Morphologie et infectivité de *Plasmodium inui*. *Cahiers de l'ORSTOM, Série Entomologie Médicale et Parasitologie*, 1980*a*, *18*, 111-112.

DEI-CAS E., MAUROIS P., LANDAU I., DUTOIT E., MILTGEN F., VERNES A. & BIGUET J. Morphologie et infectivité des gamétocytes de *Plasmodium inui*. *Annales de Parasitologie Humaine et Comparée*, 1980*b*, *55*, 621-633.

DESOWITZ R.S. & BUCHBINDER G. The absence of *Plasmodium falciparum* gametocytes in the placental blood of a woman with a peripheral parasitaemia. *Annals of Tropical Medicine and Parasitology*, 1992, *86*, 191-192.

FIELD J.W. & SHUTE P.G. A morphological study of the erythrocytic parasites. *In* The microscopic diagnosis of human malaria II, 1956, Kuala Lumpur Government Press.

FLECK S.L., BUTCHER G.A. & SINDEN R.E. *Plasmodium berghei*: serum-mediated inhibition of infectivity of infected mice to *Anopheles stephensi* mosquitoes. *Experimental Parasitology*, 1994, *78*, 20-27

GAMBREL W.E. Variations in gametocyte production in avian malaria. *American Journal of Tropical Medicine*, 1937, *17*, 689-727.

GARNHAM P.C.C. Observations on *Plasmodium falciparum* with special reference to the production of crescents. *Kenya and East African Medical Journal*, 1931, *8*, 2-21.

GARNHAM P.C.C. *Malaria parasites and other haemosporidia.* Blackwell scientific publications Oxford, 1966.

GARNHAM P.C.C. & POWERS K.G. Periodicity of infectivity of plasmodial gametocytes: the "Hawking phenomenon". *International Journal for Parasitology*, 1974, *4*, 103-106.

GAUTRET P., CHABAUD A.G. & LANDAU I. *Plasmodium vinckei vinckei* and *P. yoelii nigeriensis*: pattern of gametocyte production and development. *Parassitologia*, 1995, *37*, 17-24.

GAUTRET P., CHABAUD A.G. & LANDAU I. Chronosexuality of murid rodent plasmodia in the Central African Republic. *Parassitologia*, 1998 (in press).

GAUTRET P., GANTIER J.C., BACCAM D., MILTGEN F., SAULAI M., CHABAUD A.G. & LANDAU I. The gametocytes of *Plasmodium vinckei petteri*, their morphological stages, periodicity and infectivity. *International Journal for Parasitology*, 1996*a*, *26*, 1095-1101.

GAUTRET P., MILTGEN F., CHABAUD A.G. & LANDAU I. Synchronized *Plasmodium yoelii yoelii:* pattern of gametocyte production, sequestration and infectivity. *Parassitologia*, 1996*b*, *38*, 575-577.

GAUTRET P., MILTGEN F., GANTIER J.C., CHABAUD A.G. & LANDAU I. Enhanced gametocyte formation by *Plasmodium chabaudi* in immature erythrocytes: pattern of production and infectivity to mosquitoes. *Journal of Parasitology*, 1996*c*, *82*, 900-906.

GITHEKO A.K., BRANDLING-BENNET A.D., BEIR M., MBOGO C.M., ATIELI F.K., OWAGA M.L., JUMA F. & COLLINS F.H. Confirmation that *Plasmodium falciparum* has aperiodic infectivity to *Anopheles gambiae*. *Medical and Veterinary Entomology*, 1993, *7*, 373-376.

GORE T.C. & NOBLET G.P. The effect of photoperiod on the deep body temperature of domestic turkeys and its relationship to the diurnal periodicity of *Leucocytozoon smithi* gametocytes in the peripheral blood of turkeys. *Poultry Science*, 1978, *57*, 603-607.

GORE T.C., NOBLET G.P. & NOBLET R. Effects of pinealectomy and occular enucleation on diurnal periodicity of *Leucocytozoon smithi* Haemosporina gametocytes in the peripheral blood of domestic turkeys. *Journal of Protozoology*, 1982, *29*, 415-420.

HAWKING F. The clock of malaria parasites. *Scientific American*, 1970, *222*, 123-132.

HAWKING F. Circadian and other rhythm of parasites. *Advances in Parasitology*, 1975, *13*, 123-182.

HAWKING F., GAMMAGE K. & WORMS M.E. The asexual and sexual circadian rhythms of *Plasmodium vinckei chabaudi*, of *P. berghei* and of *P. gallinaceum*. *Parasitology*, 1972, *65*, 189-201.

HAWKING F., WILSON M.E. & GAMMAGE K. Evidence for cyclic development and short-lived maturity in the gametocytes of *Plasmodium falciparum*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1971, *65*, 549-559.

HAWKING F., WORMS M.E. & GAMMAGE K. 24 and 48 hours cycles of malaria parasites in the blood; their purpose, production and control. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1968*a*, *62*, 731-760.

HAWKING F., WORMS M.E. & GAMMAGE K. Host temperature and control of 24-hours and 48-hours cycles in malaria parasites. *The Lancet*, 1968*b*, *i*, 506-509.

HAWKING F., WORMS M.E. & GAMMAGE K. Duration of the mature gametocytes of *P. gallinaceum*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1969, *63*, 421.

HAWKING F., WORMS M.E., GAMMAGE K. & GODDARD P.A. The biological purpose of the blood-cycle of the malaria parasite *Plasmodium cynomolgi*. *The Lancet*, 1966, *ii*, 422-424.

JANSE C.J., MONS B., ROUWENHORST R.J., VAN DER KLOOSTER P.F.J., OVERDULVE J.P. & VAN DER KAAY H.J. *In vitro* formation of ookinetes and functional maturity of *Plasmodium berghei* gametocytes. *Parasitology*, 1985, *91*, 19-29.

JEFFERY G.M. & EYLES D.E. Infectivity to mosquitoes of *Plasmodium falciparum* as related to gametocyte density and duration of infection. *American Journal of Tropical Medicine and Hygiene*, 1955, *4*, 781-789.

JEFFERY G.M., YOUNG M.D. & EYLES D.E. The treatment of *Plasmodium falciparum* infection with chloroquine, with a note on infectivity to mosquitoes of primaquine and pyrimethamine-treated cases. *American Journal of Hygiene*, 1956, *64*, 1-11.

KARUNAWEERA N.D., CARTER R., GRAU G.E., KWIATKOWSKI D., DEL GIUDICE G. & MENDIS K.N. Tumour Necrosis Factor-dependent parasite-killing effects during paroxysms in non-immune *Plasmodium vivax* malaria patients. *Clinical and Experimental Immunology*, 1992, *88*, 499-505.

KILLICK-KENDRICK R. & WARREN M. Primary exoerythrocytic schizonts of mammalian *Plasmodium* as a source of gametocytes. *Nature*, 1968, *220*, 191-192.

LANDAU I., MILTGEN F., BOULARD Y., CHABAUD A. G. & BACCAM D. Études sur les gamétocytes des *Plasmodium* du groupe "*vivax*" : morphologie, évolution, prise par les Anophèles et infectivité des microgamétocytes de *Plasmodium yoelii*. *Annales de Parasitologie Humaine et Comparée* , 1979, *54*, 145-161.

LUMSDEN W.H.R. & BERTRAM D.S. Observations on the biology of *Plasmodium gallinaceum* Brumpt, 1935, in the domestic fowl, with special reference to the production of gametocytes and their development in *Aedes aegypti* (L). *Annals of Tropical Medicine and Parasitology*, 1940, *34*, 135-160.

MARCHIAFAVA E. & BIGNAMI A. On summer, autumn malarial fevers. Translated from the I[st] Italian edition by J. H. Thompson, 1894, London, The New Sydenham Society.

MENDIS K.N., NAOTUNNE T.D.S., KARUNAWEERA N.D., DEL GUIDICE G., GRAU G.E. & CARTER R. Anti-parasite effects of cytokines in malaria. *Immunology Letters*, 1990, *25*, 217-220.

MISSIROLI A. Modificazioni periodiche del numero di gametociti di *Plasmodium praecox*. *Rivista di Parassitologia* 1939, *4*, 279-285.

MONS B., JANSE C.J., BOORSMA E.G. & VAN DER KAAY H.J. Synchronized erythrocytic schizogony and gametocytogenesis of *Plasmodium berghei in vivo* and *in vitro*. *Parasitology*, 1985, *91*, 423-430.

MOTARD A., BACCAM D. & LANDAU I. Temporary loss of *Plasmodium* gametocytes infectivity during schizogony. *Annales de Parasitologie Humaine et Comparée*, 1990, *65*, 218-220.

MOTARD A., LANDAU I., NUSSLER A., GRAU G., BACCAM D., MAZIER D. & TARGETT G.A.T. The role of reactive nitrogen intermediates in modulation of gametocyte infectivity of rodent malaria parasites. *Parasite Immunology*, 1993, *15*, 21-26.

NAOTUNE T DE S., KARUNAWEERA N.D., DEL GUIDICE G., KULARATNE M.U., GRAU G., CARTER R. & MENDIS K.N. Cytokines kill malaria parasites during infection crisis: extracellular complementary factors are essential. *Journal of Experimental Medicine*, 1991, *173*, 523-529.

NAOTUNE T DE S., KARUNAWEERA N.D., MENDIS K.N. & CARTER R. Cytokine-mediated inactivation of malarial gametocytes is dependent on the presence of white blood cells and involves reactive nitrogen intermediates. *Immunology*, 1993, *78*, 555-562.

NOBLET G.P. & NOBLET R. Periodicity of *Leucocytozoon smithi* gametocytes in the peripheral blood of domestic turkeys. *Poultry Science*, 1976, *55*, 1088-1093.

NOBLET G.P. & NOBLET R. Effect of photoperiod on the diurnal periodicity of *Leucocytozoon smithi* gametocytes in the peripheral blood of domestic turkeys. *Poultry Science*, 1977, *56*, 1206-1208.

RAO C.K., GHOSE J.N., KRISHNASWAMI A.K. & RAGHAVAN N.G.S. Gametocyte cycle in *P. cynomolgi* Nilgiri strain infection in rhesus monkeys. *Journal of Communicable Disease*, 1971, *3*, 190-194.

ROGERS N.J., DARAMOLA O., TARGETT G.AT. & HALL B.S. CD36 and Intercellular Adhesion Molecule 1 mediate adhesion of developing *Plasmodium falciparum* gametocytes. *Infection and Immunity*, 1996*a*, *64*, 1480-1483.

ROGERS N.J., TARGETT G.A. T. & HALL B.S. *Plasmodium falciparum* gametocytes adhesion to C32 cells via CD36 is inhibited by antibodies to modified band 3. *Infection and Immunity*, 1996*b*, *64*, 4261-4268.

ROLLER N.F. & DESSER S.S. Diurnal periodicity in peripheral parasitemias in ducklings *Anas boschas* infected with *Leucocytozoon simondi* Mathis and Leger. *Canadian Journal of Zoology*, 1973, *51*, 1-9.

ROSS R. & THOMSON D. Some enumerative studies on malarial fever. *Annals of Tropical Medicine and Parasitology*, 1911, *4*, 267-306.

SHAH K.S. The periodic development of sexual forms of *Plasmodium cathemerium* in the peripheral circulation of canaries. *American Journal of Hygiene*, 1934, *19*, 392-403.

SHAH K.S., ROZEBOOM L.E. & DEL ROSARIO F. Studies on the infectivity of *Plasmodium cathemerium* of canaries for mosquitoes. *American Journal of Hygiene*, 1934, *20*, 502-507.

SINDEN R., CANNING E.U., BRAY R.S. & SMALLEY R.E. Gametocyte and gamete development in *Plasmodium falciparum*. *Proceedings of the Royal Society of London B Biological Sciences*, *201*, 375-399.

SINDEN R.E., BARKER G.C., PATON M.J., FLECK S.L., BUTCHER G.A., WATERS A., JANSE C.J. & RODRIGUEZ M.H. Factors regulating natural transmission of *Plasmodium berghei* to the mosquito vector, and the cloning of a transmission-blocking immunogen. *Parassitologia*, 1993, *35*, *Suppl*, 107-112.

SMALLEY M.E. *Plasmodium falciparum* gametocytogenesis *in vitro*. *Nature*, 1976, *264*, 271-272.

SMALLEY M.E., ABDALLA S. & BROWN J. The distribution of *Plasmodium falciparum* in the peripheral blood and bone marrow of Gambian children. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1980, *75*, 103-105.

SMALLEY M.E. & SINDEN R.E. *Plasmodium falciparum* gametocytes: their longevity and infectivity. *Parasitology*, 1977, *74*, 1-8.

THOMSON J.G. & ROBERTSON A. The structure and development of *Plasmodium falciparum* gametocytes in the internal organs and peripheral circulation. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1935, *29*, 31-40.

VAN DEN BERGHE L. & CHARDOME M. An easier and more accurate diagnosis of malaria and filariasis through the use of the skin scarification smear. *American Journal of Tropical Medicine*, 1951, *31*, 411-413.

WARD R.A., HAYES D.E., HEMBREE S.C., RUTLEDGE L.C. ANDERSON S.J. & JOHNSON A.J. Infectivity of *Plasmodium falciparum* gametocytes from *Aotus trivirgatus* to anopheline mosquitoes. *Proceedings of the Helminthological Society of Washington*, 1972, *39*, *special issue*, 33-46.

WHITE N.J., CHAPMAN D. & WATT G. The effects of multiplication and synchronicity on the vascular distribution of parasites in *falciparum* malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1992, *86*, 590-597.

YANG B. Experimental and field studies on some biological characteristics of *Plasmodium vivax* isolated from tropical and temperate zones of China. *Chinese Medical Journal*, 1996, *109*, 266-271.

YANG B.L., WAN W.J., WANG W.R., HU H.X., LI H.X., WANG X.A., LI X.L., LI C.Z. & HUANG G.Z. Experimental studies on the periodical viability of gametocytes of *Plasmodium vivax*. *Chi Sheng Chung Hsueh Yu Chi Sheng Chung Ping Tsa Chih*, 1984, *2*, 238-242.

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