High-Level Summary of *P. falciparum* Gametocyte Biology, Bone Marrow Interactions, and Transmission Dynamics

- **Gametocyte Development:** *P. falciparum* parasites switch from asexual replication to sexual development at low frequency. Sexual commitment is orchestrated by the transcription factor PfAP2-G, which activates early gametocyte-specific genespmc.ncbi.nlm.nih.gov. Once committed, the parasite undergoes five morphologically distinct stages (I–V) over about 9–12 dayspmc.ncbi.nlm.nih.govpmc.ncbi.nlm.nih.gov. Early stages (I–IV) are sequestered in host tissues, while only mature stage V gametocytes circulate in the peripheral bloodpmc.ncbi.nlm.nih.gov. Stage I–IV gametocyte-infected red cells become rigid and are largely absent from circulation, whereas stage V cells regain flexibility (Smalley & Sinden 1977; Eichner et al. 2001). This prolonged maturation period and tissue sequestration differentiate gametocytes from asexual stages (which have ~48-hour cycles)pmc.ncbi.nlm.nih.gov.

- **Bone Marrow Niche:** Multiple lines of evidence now point to the human bone marrow as a major site for immature gametocyte development. Autopsy and biopsy studies found that ~80–90% of stage II–IV gametocytes reside in the bone marrow parenchyma, mostly in extravascular spaces adjacent to erythroid precursors and stromal cellspmc.ncbi.nlm.nih.govpubmed.ncbi.nlm.nih.gov. For example, one study of Malawian children reported that 89% of immature gametocytes were located in marrow stroma (in contact with CD71^+ erythroblasts or adipocytes), whereas mature stage V forms were mainly intravascularpmc.ncbi.nlm.nih.govpubmed.ncbi.nlm.nih.gov. Molecular assays (qRT-PCR) also show much higher prevalence of early gametocyte markers in marrow than bloodpubmed.ncbi.nlm.nih.gov. A recent humanized mouse model confirmed that injected immature gametocytes “home” rapidly to ectopic human bone ossicles and reside in the extravascular compartment, contacting diverse marrow stromal cells (including osteogenic cells, adipocytes, and perivascular mesenchymal cells)pmc.ncbi.nlm.nih.govpmc.ncbi.nlm.nih.gov. Importantly, gametocytes do not bind endothelial cells like asexual parasites; rather, they appear to reach marrow spaces by an immune-cell–like process and then interact with surrounding niche cellspmc.ncbi.nlm.nih.govpmc.ncbi.nlm.nih.gov.

- **Niche Interactions:** Within the bone marrow environment, gametocytes engage in cross-talk with host cells. Immature gametocytes can develop inside late erythroid precursors and even delay their maturation so that gametocyte release coincides with the egress of the host cell from marrowpubmed.ncbi.nlm.nih.gov. In vitro co-culture experiments showed that stage II–IV gametocytes adhere to human bone marrow mesenchymal stem

cells (MSC) via parasite ligands. These gametocyte–MSC interactions trigger MSCs to secrete a suite of cytokines and angiogenic growth factors (14 factors identified)pmc.ncbi.nlm.nih.gov. The conditioned medium from gametocyte–MSC cultures greatly enhanced endothelial tube formation, suggesting gametocytes may actively remodel the marrow vasculaturepmc.ncbi.nlm.nih.gov. Thus, gametocytes not only exploit the marrow as a protective niche but may also influence it: for example, parasite-derived factors delay erythropoiesis and alter vascular signaling to favor their maturationpubmed.ncbi.nlm.nih.govpmc.ncbi.nlm.nih.gov.

- **Transmission to Mosquitoes:** Only mature stage V gametocytes are taken up by mosquitoes. Infectivity depends on gametocyte density: the probability that a mosquito becomes infected rises steeply with increasing gametocyte counts before plateauing at high densitiespmc.ncbi.nlm.nih.gov. Both male and female gametocytes are needed, but the female gametocyte density is the primary predictor of mosquito infectionpmc.ncbi.nlm.nih.gov. Thus humans with higher gametocytemia disproportionately contribute to onward transmission. Other host factors also modulate bite risk: blood-feeding *Anopheles* females use human odor cues (CO_2, lactic acid, skin volatiles) to locate hostspmc.ncbi.nlm.nih.gov. Individual attractiveness to mosquitoes varies widely – pregnancy and malaria infection can increase a person's attractiveness, while skin microbiota and genetics shape odor profilespmc.ncbi.nlm.nih.gov. In practice, about 79% of vector bites in Africa occur during hours when people are under bed nets (late-night, indoor)pubmed.ncbi.nlm.nih.gov, leaving ~21% of bites at dusk or outdoors ("residual transmission"). This residual outdoor biting can blunt the impact of indoor control measures, potentially adding ~10 million cases annually in Africa even with full net/IRS coveragepubmed.ncbi.nlm.nih.gov.

- **Climate and Seasonality:** Malaria transmission is strongly influenced by climate, especially in Africa. Transmission only persists where temperatures allow parasite and mosquito development (~15–40 °C)media.malariaworld.org. In much of sub-Saharan Africa, rainy seasons create pronounced peaks of transmission: onset usually lags major rainfall in highly seasonal areas (e.g. the Sahel, East African highlands)media.malariaworld.org. Outside these strongly seasonal regions, transmission tends to be nearly year-round if temperatures remain favorablemedia.malariaworld.orgmedia.malariaworld.org. In summary, global transmission intensity is driven by a complex interplay of vector biology, human behavior (sleep patterns, housing, interventions), parasite factors (gametocyte production), and climate, with malaria hotspots concentrated in tropical Africa.

- **Focus on Africa:** Africa bears >90% of malaria burden (mostly *P. falciparum* in sub-Saharan regions)malariajournal.biomedcentral.com. Here, the dominant vectors (*An. gambiae* complex and *An. funestus*) historically bite late at night indoors, aligning with when people sleep under netsparasitesandvectors.biomedcentral.com. However, intensification of control (LLINs/IRS) has driven behavioral shifts: *An. gambiae* and relatives increasingly bite earlier or outdoors

parasitesandvectors.biomedcentral.compubmed.ncbi.nlm.nih.gov. These changes – along with insecticide resistance – contribute to the recent plateau in case reductions in Africa parasitesandvectors.biomedcentral.commalariajournal.biomedcentral.com. Seasonal chemoprevention and other Africa-focused interventions target rainy-season peaks. Critically, marrow sequestration of gametocytes has also been documented in African patients, and contributes to transmission risk even when peripheral parasitemia is lowpubmed.ncbi.nlm.nih.govpubmed.ncbi.nlm.nih.gov.

Sources: Authoritative reviews and research on *P. falciparum* gametocyte biology, sequestration in human bone marrow, and malaria transmission dynamics pmc.ncbi.nlm.nih.govpmc.ncbi.nlm.nih.govpubmed.ncbi.nlm.nih.govpmc.ncbi.nlm.nih.govpubmed.ncbi.nlm.nih.govmedia.malariaworld.org. These include field studies (autopsy, aspirates), in vitro and in vivo models, and epidemiological analyses (see citations).

In-Depth Review of *P. falciparum* Gametocyte Biology, Bone Marrow Microenvironment, and Transmission Dynamics

Gametocyte Development and Sexual Commitment

Gametocytogenesis begins when a tiny fraction (often <5%) of asexual schizonts commit to sexual development. This commitment is driven by epigenetic and transcriptional control of the master regulator PfAP2-Gpmc.ncbi.nlm.nih.gov. In asexual parasites, *pfap2-g* is typically silenced by heterochromatin (PfHP1-mediated) and only derepressed in “committed” cells. When PfAP2-G is expressed, it activates a cascade of early gametocyte genes. Josling *et al.* identified hundreds of PfAP2-G binding sites genome-wide, confirming that PfAP2-G acts as a transcriptional activator of sexual-stage genespmc.ncbi.nlm.nih.gov. Sexual commitment usually occurs at or before the final schizogony (so that all merozoites from that schizont enter the sexual pathway)pmc.ncbi.nlm.nih.gov, though “same-cycle conversion” in ring stages can also occur. Notably, commitment decisions can be influenced by host factors: for example, elevated lysophosphatidylcholine levels in human serum suppress commitmentpmc.ncbi.nlm.nih.gov (Ngotho *et al.* 2019).

Once committed, parasites differentiate into morphologically distinct stages I through V. Under microscopy, these stages progressively change from round (stage I) to the classic crescent shape of mature falciform gametocytes (stage V). This 10–12 day maturation is uniquely prolonged in *P. falciparum*pmc.ncbi.nlm.nih.govpmc.ncbi.nlm.nih.gov, unlike shorter development in most other *Plasmodium* species. Stage I gametocytes are small and morphologically similar to trophozoites

(but begin to express gametocyte genes). During stages II–IV, the gametocyte undergoes dramatic remodeling: a parasite-derived inner membrane complex (IMC) develops along one side, supported by microtubules and actin cytoskeletonpmc.ncbi.nlm.nih.govpmc.ncbi.nlm.nih.gov. By stage V, the IMC plates encase the cell fully, and cytoskeletal elements largely disappearpmc.ncbi.nlm.nih.gov, giving the flexible, sickle-shaped form seen in circulating mature gametocytes. Besides structural changes, the host RBC itself is transformed: during stage III–V, the parasitophorous vacuole membrane often adheres to the erythrocyte membrane, with redistributed host proteins and knob-like structures. All these modifications are driven by parasite protein export; the "gexportome" includes many novel gametocyte proteins whose functions are still being elucidated (IMC/Gam complex proteins, PfCCp family, etc.)pmc.ncbi.nlm.nih.govpmc.ncbi.nlm.nih.gov.

Gametocyte sexes diverge during development. Sex ratio is typically female-biased (~8–10 females per male), but this can shift under low-density conditions (to ensure fertilization). Mature male (micro-) gametocytes store numerous flagellar axonemes so they can produce up to 8 flagellated microgametes upon activation in the mosquito midgut, whereas females form a single rounded macrogametepmc.ncbi.nlm.nih.gov. Osmophilic bodies (secretory organelles) are more abundant in female gametocytespmc.ncbi.nlm.nih.gov, reflecting their role in facilitating ookinete development. Notably, mature gametocytes can survive for 3–5 days (or longer) in the bloodstream if not taken up by a mosquitopmc.ncbi.nlm.nih.gov, whereas asexual stages last only hours in the absence of reinvasion.

Bone Marrow Sequestration and Microenvironment Interactions

A defining feature of *P. falciparum* gametocytes is that immature stages are mostly hidden from peripheral circulation – unlike asexual trophozoites which sequester via adhesion to endothelium. Pioneering pathology studies revealed that developing gametocytes accumulate in the microvasculature and parenchyma of internal organs, especially the bone marrow and spleen. Farfour *et al.* (2012) found that 89% of stage II–IV gametocytes in pediatric autopsies were in bone marrow extravascular spaces, often in tight contact with CD71^+ erythroblasts or fat cellspmc.ncbi.nlm.nih.gov. Joice *et al.* (2014) similarly reported a rich "niche" in bone marrow where gametocytes are found inside or adjacent to erythroid precursor cellspubmed.ncbi.nlm.nih.gov. These studies emphasize that very few immature gametocytes ever circulate; instead, they are physically trapped in marrow niches until maturation (thus explaining their absence from blood smears). In effect, immature gametocyte–infected erythrocytes are stiffer than normal RBCs (both asexual schizonts and immature gametocytes stiffen their host cell), so being in narrow marrow sinusoids or bone spaces may allow them to lodge without flowing awaypmc.ncbi.nlm.nih.gov. Stage V cells soften and deform, enabling re-entry into the blood and splenic passagepmc.ncbi.nlm.nih.gov.

Recent in vivo models have directly confirmed bone marrow homing. Duffier *et al.* (2016) used humanized mice and saw gametocytes enrich in mouse marrow and spleen. More finely,

Donsante *et al.* (2023) injected fluorescent gametocytes into mice bearing human bone ossicles. Within minutes, immature gametocytes homed to the ossicles and were retained exclusively in the extravascular stromapmc.ncbi.nlm.nih.gov. This happened even though the ossicle vasculature was murine and gametocytes do not bind normal endotheliapmc.ncbi.nlm.nih.gov. Confocal imaging revealed these gametocytes touching diverse stromal cells: adjacent to ALP^+ osteogenic cells, near CD146^+ pericyte/skeletal stem cells, and alongside FABP4^+ adipocytespmc.ncbi.nlm.nih.govpmc.ncbi.nlm.nih.gov. Together with in vitro data, this suggests a model where committed parasites may preferentially invade erythroblasts (as shown by Neveu *et al.*) or adhere to marrow niches, where they can mature safely until stage V.

Gametocyte interactions with the marrow niche appear to be reciprocal. Neveu *et al.* (2020) demonstrated that late erythroblasts (normoblasts) are a viable host for gametocyte development: parasites can grow inside these nucleated cells, and their presence (or released extracellular vesicles) **delays** the host cell's maturation. This delay causes infected erythroblasts to remain in the marrow just until the gametocyte is mature, then egress as a reticulocyte into bloodpubmed.ncbi.nlm.nih.gov. In other words, parasites synchronize host-cell release with gametocyte readiness for transmission.

In parallel, in vitro co-culture studies by Messina *et al.* (2018) found that immature gametocyte-iRBCs adhere to human bone marrow mesenchymal stem cells (MSCs) via parasite ligands. This adhesion is trypsin-sensitive, implicating exposed parasite proteins. The striking finding was that gametocyte–MSC binding **activates the MSCs**: they secrete a broad array of cytokines and angiogenic factors (including VEGF, IL-8, FGF, etc.)pmc.ncbi.nlm.nih.gov. Supernatants from these co-cultures dramatically increased capillary tube formation by endothelial cells in vitropmc.ncbi.nlm.nih.gov. Thus, immature gametocytes may actively modify the marrow microenvironment – promoting local angiogenesis or vasculature remodeling – potentially facilitating their eventual exit. Whether and how this occurs in natural infections is not fully proven, but it underscores that the parasite does not merely hide in marrow; it may rewire it.

In summary, gametocyte biology in *P. falciparum* is intimately linked with the bone marrow niche. The marrow provides a protective depot for the ~10-day maturation of most gametocytes. There, parasites engage directly with human erythroblasts (and other precursors) and stromal cells. Key recent insights include: maturation inside erythroid cells, gametocyte-induced delay of erythropoiesis, and gametocyte-triggered secretion of marrow cytokines. These discoveries reshape our view of gametocytogenesis as a host–parasite interaction set primarily in the hematopoietic tissue, not the bloodstream.

Human–Mosquito Transmission Dynamics

Malaria transmission occurs when a mosquito ingests mature gametocytes from an infected human. The efficiency of this process depends on both parasite and host factors. One major determinant is **gametocyte density**. Bradley *et al.* (2018) analyzed dozens of mosquito-feeding experiments and found that the chance a mosquito becomes infected rises sharply with gametocyte densitypmc.ncbi.nlm.nih.gov. In practice, very low-density carriers (submicroscopic

gametocytemia) can still infect mosquitoes, but with much lower probability. At higher densities, infection probability plateaus, and the average oocyst load per mosquito continues to increasepmc.ncbi.nlm.nih.gov. Crucially, it is the density of *female* gametocytes that most strongly predicts infection ratepmc.ncbi.nlm.nih.gov. This means individuals with high gametocyte loads (often asymptomatic adults in endemic areas) disproportionately drive the transmission reservoir.

Vector biology and human behavior jointly shape exposure risk. African malaria vectors (*Anopheles gambiae* complex, *An. funestus*) are highly anthropophilic and historically bite at night. It was once assumed most biting occurs when people are asleep indoors, and indeed early studies found late-night indoor peaks for *An. gambiae* and *An. funestus*parasitesandvectors.biomedcentral.com. Accordingly, LLINs and IRS (which protect people while sleeping) have been cornerstone interventions. However, recent evidence shows that a substantial fraction of bites occur outside the protection window. A meta-analysis by Sherrard-Smith *et al.* (2019) systematically reviewed biting patterns across Africa and estimated that on average only **79%** of vector bites happen during hours when people are in bedpubmed.ncbi.nlm.nih.gov. That leaves ~21% of bites at dusk or dawn or outdoors, which constitute **residual transmission**. Importantly, this fraction of outdoor/out-of-bed biting has risen over time (as high as ~10% more compared to 2000) as mosquitoes adapt to intervention pressurepubmed.ncbi.nlm.nih.gov. These outdoor bites are enough to blunt net effectiveness: modelling suggests they could account for ~10.6 million extra malaria cases annually in Africa, even with full LLIN/IRS coveragepubmed.ncbi.nlm.nih.gov. This highlights that human activities (evening socializing, early rising, outdoor work) and vector behavioral shifts both impact transmission.

Not all humans are equally bitten. Variability in attractiveness is a key human–vector interaction. Factors that make someone more appealing to mosquitoes include body odor chemistry (linked to genetics and skin microbes), heat, and CO_2 emission. Pregnancy and malaria infection themselves increase a person's attractiveness to *Anopheles*pmc.ncbi.nlm.nih.gov. Conversely, application of repellents or wearing clothing can mitigate it. On the vector side, emerging insecticide resistance and species shifts (e.g. increasing *An. arabiensis* which bites outdoors) also alter the human–mosquito interface. Behavioral heterogeneity across communities is high – some villages see far more outdoor biting than others – which may explain why vector control can have uneven impact even within a regionpubmed.ncbi.nlm.nih.gov.

Mathematical concepts like the **entomological inoculation rate** (EIR; infectious bites per person per time) summarize transmission intensity. In Africa, EIRs range enormously: <1 in some dry zones to hundreds per year in holoendemic foci. Human exposure also depends on vector density and sporozoite prevalence. Globally, factors like urbanization, land use, and control coverage further modulate these dynamics. But broadly, the human–vector interaction for *P. falciparum* is governed by the parasite's rare but potent gametocyte stage, mosquitoes' nocturnal habits, and environmental conditions.

Seasonality and Climatic Drivers

Seasonal changes in climate strongly influence malaria transmission patterns, especially in Africa. Temperature and rainfall are critical: *Plasmodium* development in mosquitoes only occurs between roughly 15–40 °Cmedia.malariaworld.org. Yamba *et al.* (2023) analyzed EIR data across sub-Saharan Africa and confirmed that regions with mean temperatures consistently outside that range are unlikely to sustain transmissionmedia.malariaworld.org. Rainfall creates breeding sites for *Anopheles*, and seasonal rains trigger transmission spikes. In highly seasonal climates (Sahel, parts of East Africa), malaria incidence typically rises soon after the rains beginmedia.malariaworld.org. Indeed, onset of transmission tends to **lag** peak rainfall by a few weeks in those zones; by contrast, in less seasonal areas (e.g. West/Central Africa where rains are more evenly distributed) malaria tends to be perennial or show minimal seasonalitymedia.malariaworld.orgmedia.malariaworld.org. Other factors like humidity and solar radiation also play roles, but temperature and rain patterns are most often limiting. Models incorporating climate data (e.g. the Mordecai *et al.* temperature-dependent R_0 model) accurately capture these geographical shiftsmalariajournal.biomedcentral.com.

Beyond climate, human interventions introduce artificial seasonality: for example, **Seasonal Malaria Chemoprevention (SMC)** is implemented in the Sahel to protect children during the brief high-transmission season. Conversely, in perennial settings, strategies like year-round LLIN distribution or intermittent preventive treatment are used. The interplay of seasonality with vector ecology means that even with otherwise constant interventions, transmission can wax and wane annually.

Global and African Focus

Globally, *P. falciparum* is the most deadly malaria species, dominating sub-Saharan Africa. Over the last two decades, massive efforts (bed nets, spraying, diagnostics, drugs) reduced malaria cases, but progress has stalled around 2015–2020malariajournal.biomedcentral.com. In Africa, complex socio-economic and ecological factors contribute to this plateaumalariajournal.biomedcentral.com. Key drivers include the emergence of insecticide and drug resistance, funding shortfalls, and the entrenchment of residual transmission described above.

Vector species composition varies by region, but in much of Africa *An. gambiae*, *An. arabiensis*, and *An. funestus* are primary vectors. All three of these tend to feed at low altitude on the human body: field experiments show 77–100% of their bites land on the legs and feetparasitesandvectors.biomedcentral.com. This means that people sleeping on the floor or with exposed lower legs bear disproportionate bite riskparasitesandvectors.biomedcentral.com. Protecting ankles (repellent sandals or bed nets) could significantly cut bitesparasitesandvectors.biomedcentral.com, highlighting one potential targeted strategy.

From the parasite perspective, African epidemiology has particular features. Many infected individuals (especially older children and adults) carry asymptomatic gametocytemia. Studies in

African children show that severe anemia and dyserythropoiesis correlate with higher bone marrow gametocyte loadspubmed.ncbi.nlm.nih.gov, suggesting that the marrow niche may be especially important in anemic patients. Thus, control efforts aiming at the transmission reservoir must consider these hidden gametocyte pools.

Seasonal climate trends in Africa have also shown signs of change. Climate modeling indicates that warming could shift malaria into higher altitudes and change endemicity patternsmalariajournal.biomedcentral.com. Ryan *et al.* (2020) projected by 2050 a net increase of tens of millions more people at risk in parts of Eastern and Western Africa under climate change scenariosmalariajournal.biomedcentral.com. This reinforces that transmission dynamics are not static and must be monitored continuously.

In summary, *P. falciparum* transmission dynamics involve a chain of specialized interactions: a small subpopulation of parasites undergoes gametocytogenesis (governed by factors like PfAP2-G and host environment), these gametocytes mate only in mosquito guts, and the human-vector contact is shaped by both biological and social/environmental factors. Globally, understanding this chain – from molecular biology to mosquito behavior – is key to interrupting malaria spread. The bone marrow tropism of gametocytes is a particularly recent insight that adds a layer of complexity: interrupting transmission may eventually require strategies targeting this hidden reservoir, especially in African settings where transmission remains high despite conventional control.

Sources: This review synthesizes results from recent peer-reviewed studies on *P. falciparum* gametocytes, including molecular and cellular investigationspmc.ncbi.nlm.nih.govpubmed.ncbi.nlm.nih.gov, bone marrow niche researchpmc.ncbi.nlm.nih.govpmc.ncbi.nlm.nih.gov, and transmission ecology paperspmc.ncbi.nlm.nih.govpubmed.ncbi.nlm.nih.govmedia.malariaworld.org, among others. Detailed data and citations can be found in the primary literature cited above.