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THE PRESENCE OF *PLASMODIUM FALCIPARUM* GAMETOCYTES IN HUMAN BLOOD INCREASES THE GRAVIDITY OF *ANOPHELES GAMBIAE* MOSQUITOES

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Abstract. We conducted a field study in an area of endemic malaria transmission in western Kenya to determine whether mosquitoes that feed on gametocyte-infected blood but do not become infected have reduced or enhanced fecundity in comparison to mosquitoes fed on uninfected blood. Fifteen paired membrane-feeding experiments were conducted in which two strains of *Anopheles gambiae* mosquitoes were simultaneously fed on either *Plasmodium falciparum*–infected blood from children or uninfected control blood from adults. The presence of noninfecting gametocytes in blood increased the probability that *An. gambiae* would produce eggs after one blood meal by sixfold (odds ratio for control relative to infected blood group 0.16; 95% CI 0.10–0.23). This result could not be explained by variation in blood meal size or hemoglobin content between hosts. When children cleared their infections, the difference in gravidity between mosquitoes fed on their blood and uninfected adults disappeared, suggesting this phenomenon is due to the presence of *Plasmodium* gametocytes in blood and not to host-specific factors such as age. This result was observed in two mosquito strains that differ in their innate fecundity, suggesting it may apply generally. To our knowledge, this is the first time that *Plasmodium* has been implicated as enhancing vector gravidity.

INTRODUCTION

Several laboratory studies and some field studies have shown that malaria parasites (*Plasmodium* spp.) can reduce both the survival and fecundity of their mosquito vector.[1–9] The detrimental impact of malaria parasites on mosquito fecundity has been more consistently observed than reduced survival. For example, a meta-analysis of laboratory studies of malaria-vector interactions suggests that whereas *Plasmodium* can reduce mosquito survival, the effect is generally small and varies with experimental design and species combination.[8] In contrast, the negative impact of *Plasmodium* on vector fecundity is much more ubiquitous and has been observed in a variety of laboratory (e.g., Refs. 1–5,10–13) and field experiments.[6]

Despite the negative effects that *Plasmodium* imposes on its vectors, it is generally assumed that malaria parasites have no regulatory effect on mosquito populations because the proportion of mosquitoes that become infected is low. For example, in many endemic areas, oocyst and sporozoite infection rates do not exceed 1–2%,[14] although it can reach almost 10% in some locations.[15] Occurring at such low frequency, it is unlikely that parasites could substantially reduce vector population growth rates even if they imposed a high fitness cost on mosquitoes. However, the fact that few mosquitoes become infected with oocysts does not necessarily mean that few experience fitness costs due to *Plasmodium.* For example, simple exposure to parasitized blood in itself, regardless of whether it leads to infection or not, may have consequences for vector fitness. *Anopheles gambiae,* the most important vector of the deadliest of human malaria parasites, *Plasmodium falciparum,* may encounter parasites in 70% or more of their blood meals in some malaria endemic regions (e.g., Refs. 16,17). If mosquito fitness is indeed reduced, or conversely enhanced, simply by imbibing infected blood, encountering parasites at this frequency could influence the growth rate of vector populations. Examination of the full range of outcomes accruing from the interaction between malaria parasites and their vectors is crucial to understand the nature of selective forces acting on this transmission cycle and may provide clues as to how it could be destabilized.

Whereas several studies both in the laboratory and in the field have tested for a direct effect of *Plasmodium* development on mosquito fitness, none have examined the possibility that infected blood is harmful, or indeed beneficial, in the absence of successful parasite development. Here we conducted a series of experiments in an area of endemic malaria transmission in western Kenya to determine whether *An. gambiae* mosquitoes that fed on human blood infected with *P. falciparum* gametocytes had altered fecundity relative to those fed on uninfected controls, even when successful parasitism did not occur (no development of oocysts). The Mbita Point region where these experiments were conducted is an ideal location for this study because prior studies have shown that although the rate of *P. falciparum* infection in humans is moderate (34%), only a small percentage of *An. gambiae* mosquitoes that feed on gametocyte carriers become infected to the oocyst stage and beyond (0.6–12%).[18] Such low infection probabilities ensured an adequate sample of "infected but not infectious" blood samples could be obtained to test for exposure-related fitness impacts on mosquitoes. In addition to gametoctye presence in the blood meal, other proximate measures of blood meal quality (size and hemoglobin content) were measured and their association with mosquito fecundity examined.

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MATERIALS AND METHODS

Study site. This study was conducted at the Mbita Point Research and Training Center of the International Center of

Insect Physiology and Ecology (ICIPE), located in Suba District, western Kenya. The major malaria vectors in the region are *Anopheles funestus*, *Anopheles gambiae,* and *Anopheles arabiensis.*[19,20] Experiments described below took place between May and August 2003.

Recruitment of gametocyte carriers and controls. The Kenyan National and University of Miami ethical review committees approved the recruitment procedures reported in this study. *Plasmodium falciparum* gametocyte carriers were detected during parasitological surveys of healthy children. Prior to surveys, school head teachers were informed of the purpose of the study, and information was passed onto to parents verbally and in writing. On the day of each parasitological survey, thick blood film smears, biodata (name, age, gender), and filter paper blood samples were collected from all participating children. After staining with Giemsa, slides were examined microscopically for the presence of both the asexual and gametocyte stages of *P. falciparum*. Gametocytes were quantified as the number occurring in 200 fields of the thick blood film.

Individuals who were found to carry gametocytes were asked to donate a 3–4 mL venous blood sample and became enrolled upon giving written consent. Children unable to give consent because they were too young were volunteered to participate by parents or guardians. Exclusion criteria included individuals with mixed-species infections, any symptoms indicating severe clinical malaria, anemia, and other concomitant diseases requiring hospitalisation or follow-up, and children less than 3 years old. All individuals with gametocytes and high asexual parasitemia (> 1,000 parasites/μL) were referred to the local health center and treated with Fansidar.

On the day of parasitological surveys, one uninfected volunteer from our program staff was recruited to act as the uninfected control. Controls were individuals who had no malaria parasites in their thick blood smear on the day of the experiment and no reports of fever or illness in the prior 2 weeks. All control individuals were Kenyan males between the ages of 20 and 40, and none were taking malaria prophylaxis and/or treatment at the time of blood collection. Control individuals were asked to provide a 3–4 mL venous blood sample after giving written consent. Prior to withdrawing their blood, infected and control volunteers were taken to the ICIPE Clinic for a health check by the attending physician. Only those deemed in good health status were allowed to participate.

Hematology and experimental infection of mosquitoes. Two laboratory strains of *An. gambiae* s.s. mosquitoes were used in these experiments, one originating from the Mbita area (MB) and one from Ifakara (IF) in southern Tanzania. On the day of blood feeding, screened pots containing 50 females of either the IF or MB strain were taken to the laboratory (5–6 pots per strain). Adult mosquitoes were 3–5 days old at the time of each experiment and had been starved of glucose solution (10%) for 6–8 hours prior to blood feeding. Mosquitoes used in each feeding trial were drawn from the same larval cohort and were randomly allocated to infection treatments after they had been allowed to mate in cages. On the day of each experiment, a gametocyte carrier was randomly paired with an uninfected control adult. A fingerprick blood sample was taken from a subsample of participants and used to calculate their blood hemoglobin content (HemoCue Ltd., Derbyshire, UK). A 3–4 mL blood sample was taken from each member of the pair and immediately transferred into 5–6 prewarmed membrane mini-feeders (approximately 0.5 mL/feeder). Half the mosquitoes feeding on each blood type (control or infected) were from the MB strain, and half were IF (2–3 pots of each mosquito strain per blood treatment). Mosquitoes were exposed to the membrane feeders for 30 minutes, during which blood was maintained at 37°C.

Mosquito fecundity measurement. After their first blood feed, all nonengorged mosquitoes were removed from pots by aspiration and killed. Of the mosquitoes that did blood feed, 10–20 from each strain and parasite treatment (C or I) were removed and transferred individually into 30-mL plastic tubes, with the rest being kept in pots. All mosquitoes were then transferred into a 27°C incubator where they remained until the end of each experiment, being maintained on a 10% glucose solution. After 3 days, all mosquitoes kept in tubes were transferred to new tubes. Hematin that had accumulated within the first holding tubes was quantified using a standard photometric assay as described in Refs. 13 and 21 to provide an estimate of blood meal size.

Seven days after blood feeding, all mosquitoes from the control and infected groups were killed with chloroform and dissected. Mosquitoes had no access to an oviposition substrate prior to death. On dissection, the number of eggs found in each mosquito was counted, and one of their wings was removed and measured as an index of body size. The midguts of all those in the infected group were stained with 2% mercurochrome and examined under a microscope for the presence of oocysts.

Separating the effects of human host age and gametocyte infection. In the feeding trials described above, treatment effects due to *P. falciparum* could have been confounded by age differences between control and gametocyte-positive hosts. In all our experiments, gametocyte carriers were children (< 10 years old), and uninfected controls were adults (20–40 years old). This situation was a necessary limitation of our experimental design and ethical protocol: Children were recruited only if they possessed gametocytes (thus no comparisons of infected and uninfected children were possible), and our project encompassed intensive parasitological survey of children only (thus no comparison of infected and uninfected adults was possible). Human age is indirectly related to several hematological and physiologic properties including immunity to *Plasmodium,*[22] exposure to mosquito bites and thus development of anti-mosquito antibodies,[23] hemoglobin content,[24] and blood amino acid composition,[25,26] any of which could influence mosquito fecundity independently of parasite presence.

To disentangle the effects of human age and infection status, a subset of gametocyte carriers from our initial experiments were asked to volunteer a second venous blood sample (3–4 mL) when they were deemed parasite-free (by microscopy), as were the uninfected control individuals they were initially paired with. After consent was obtained from both parties, a second round of membrane feeding experiments was conducted as described above, except in this case both the child and the adult were uninfected. A filter paper blood sample was taken from both parties immediately prior to these experiments to allow for retrospective molecular evaluation of parasite presence.

Detection of subpatent parasites in follow-up experiments. Molecular analysis was conducted on all filter paper blood samples taken from the subsample of initially infected children and their uninfected control who were selected for follow-up experiments. To test for the presence of subpatent infection in these individuals, *P. falciparum* DNA extraction was performed on filter paper blood samples.[27] The resultant sample was tested for the presence of the *P. falciparum pfg377* gene after two rounds of amplification by polymerase chain reaction (as described in Ref. 28).

Statistical analysis. Two measures of mosquito fecundity were examined in these experiments: 1) the presence of eggs in mosquitoes after one blood meal and 2) the number of eggs produced by mosquitoes that became gravid after one blood meal (gravid mosquito = produced at least one egg). Logistic regression analysis was carried out on data collected at the individual mosquito level to assess whether the prevalence of eggs in mosquitoes differed between infection and/or age treatment groups.[29] By treating individual mosquitoes as data points, we were able to incorporate a potentially critical explanatory variable measured only at the individual level, body size, into our statistical analysis. Body size is an important determinant of mosquito fecundity, and in particular gravidity,[30] and thus it was essential to take it into account when testing for any effect of infection treatment. Experimental trial was included as additional, fixed explanatory variable in all analyses so that the effect of treatment could be evaluated while controlling for between-replicate variation in mosquito fecundity.

Egg prevalence, the dependent variable in these analyses of individual mosquitoes, was treated as a binary variable, with mosquitoes with eggs assigned a value of 1, and those without given 0. In all cases, the test statistic was the χ^2 value for likelihood ratio of each explanatory variable. In these analyses, experimental trial (coded as 1–15 for the 15 feeding trials), mosquito strain (IF or MB), and wing size (continuous variable) were fit as explanatory variables in addition to treatment group (gametocyte-infected or uninfected blood). The maximal statistical model included all explanatory variables and their treatment group interactions. All non-significant terms were sequentially dropped to yield a minimum model. A total of 856 and 347 mosquitoes were included in analyses of egg prevalence in experiments of gametocyte-infected children and adult controls and recovered children and adult controls, respectively

A similar approach was taken to analyze treatment differences in the number of eggs produced by gravid mosquitoes. In these analyses, the response variable was egg number, which was analyzed using general linear models (GLM).[31] A total of 256 and 189 mosquitoes were included in analyses of egg number in experiments of gametocyte-infected children and adult controls and recovered children and adult controls, respectively. Explanatory variables were the same as for logistic regression analyses: infection treatment, experimental trial, mosquito strain, and wing size. Interaction effects between infection group and all other explanatory variables were also fit. General linear models were also used to test for treatment differences in hemoglobin content and blood meal size taken from infected and uninfected donors. The test statistic for all general linear models analyses was the F-value, and the significance level (α) for all statistical tests was set at 0.05.

RESULTS

A total of 15-paired feeding trials using gametocyte-infected and control blood were conducted, during which the fecundity of 931 mosquitoes was assessed (IF = 601, MB = 330; difference in numbers between strains is because fewer MB took full blood meals from the membrane feeder than IF). Of those 15 trials, only one yielded oocysts in mosquitoes. Within this sole infection-generating trial, the prevalence of eggs in mosquitoes that developed oocysts was no different from those that fed on infectious blood but did not (χ_1^2 = 0.91, P = 0.34, N = 32). Restricting analysis to the remaining 14 trials in which no oocyst infections were observed (N = 856), the prevalence of eggs after blood feeding varied between trials (χ_{13}^2 = 54.57, $P < 0.01$, range 10–76%) and in response to host infection status (χ_1^2 = 84.16, $P < 0.01$). Mosquitoes that had fed on infected blood were almost six times more likely to produce eggs after their first blood meal than those that fed on uninfected blood (Figure 1; odds ratio [OR] for egg prevalence in controls relative to infected group, 0.16; 95% confidence interval [CI], 0.10–0.23). Although IF mosquitoes tended to produce more eggs than MB, the relationship between gravidity and host blood infection status did not vary between strains (infection status × mosquito strain: χ_1^2 = 0.21, P = 0.64). Differences in gravidity observed in these experiments were unrelated to variation in mosquito wing size (χ_1^2 = 0.81, P = 0.37, average [+ SEM] = 4.15 mm [0.01], range 3.6–4.8 mm). When data were pooled so that each trial represented one data point giving the proportion of mosquitoes with eggs in a given treatment combination (14 trials × 2 mosquito strains × 2 treatment categories: N = 52), similar results were obtained as from the analysis of individual mosquitoes presented above: the proportion of mosquitoes producing eggs was much greater if their blood meal contained gametocytes (χ_1^2 = 49.54, $P < 0.01$).

Restricting analysis to gravid mosquitoes, the number of eggs they produced was not related to host blood infection status (mean ± SE: control, 65.8 ± 12.4; infected blood, 64.8 ± 3.6), with the difference in egg number between mosquitoes fed on infected versus uninfected blood varying inconsistently between experimental trials (treatment × experimental trial: $F_{7,255}$ = 2.31, P = 0.03, Figure 2).

The average blood meal size taken by mosquitoes varied between feedings trials ($F_{12,213}$ = 2.64, $P < 0.01$), mosquito strains ($F_{1,213}$ = 10.76, $P < 0.01$, IF > MB), and with wing size ($F_{1,213}$ = 12.19, $P < 0.01$, positive association). Controlling for variation in these parameters (by retaining them as explanatory variables in the GLM model), an additional effect of infection treatment on blood meal size was detected: the average meal taken from uninfected adult blood was larger than from gametocyte-infected blood ($F_{1,213}$ = 5.33, P = 0.02; mean ± SE: infected blood = 14.16 ± 0.82 μg, N = 364, uninfected blood = 16.78 ± 0.88 μg, N = 521). Uninfected adults tended to have a higher hemoglobin content than gametocyte-carrying children but not significantly so ($F_{1,8}$ = 3.89, P = 0.08; mean ± SE: infected child's blood = 11.5 ± 0.5 g/dL, control adult's blood = 14.1 ± 1.2 g/dL).

As all infected volunteers in this study had gametocytes, it was not possible to test whether the fecundity effects we report were gametocyte-specific or would have arisen also from blood infected with the asexual stage of the parasite alone. However, among gametocyte carriers, there was variation in

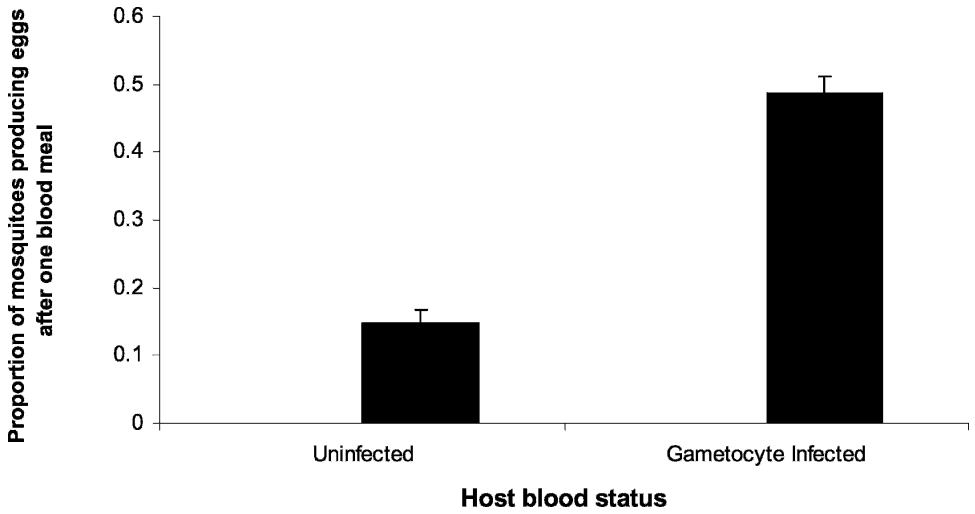

FIGURE 1. The prevalence of eggs in *An. gambiae* mosquitoes 7 days after feeding on blood from gametocyte-infected children ($n_{mosquitoes}$ = 454) or uninfected adults ($n_{mosquitoes}$ = 305). Prevalences are the average of 14 infection trials and 2 mosquito strains. Error bars give the binomial standard error.

both the presence and density of asexual parasites (6 of 14 noninfectious carriers had asexuals in addition to gametocytes). Restricting analysis only to gametocyte carriers, we found that the gravidity of mosquitoes tended to be lower if asexuals were in the blood as well as gametocytes, although this effect was not statistically significant ($\chi_1^2 = 3.25$, $P = 0.07$; OR for egg productions from gametocytes only versus gametocyte + asexual infected blood = 2.32; 95% CI 0.93–5.77). The effect of asexual parasites was more pronounced when their abundance was investigated. Both the density of asexual parasites ($\chi_1^2 = 4.63$, $P = 0.03$) and gametocytes ($\chi_1^2 = 5.07$, $P = 0.02$) influenced the probability that mosquitoes would produce eggs after feeding on noninfectious gametocytemic blood. Interestingly, these two parasite stages had opposing effects on mosquito fecundity, with gravidity rising in response to gametocyte density and falling with increasing asexual density (Figures 3A and 3B). There was no correlation between asexual and gametocyte density within our gametocyte carriers ($F_{1,11} = 0.62$, $P = 0.42$).

Seven of the initially *P. falciparum*–infected children who participated in infection experiments were followed up to test whether the effect of their blood on mosquito fecundity changed when they cleared their infection. Retrospective molecular analysis (*pfg377*) indicated that two of these seven

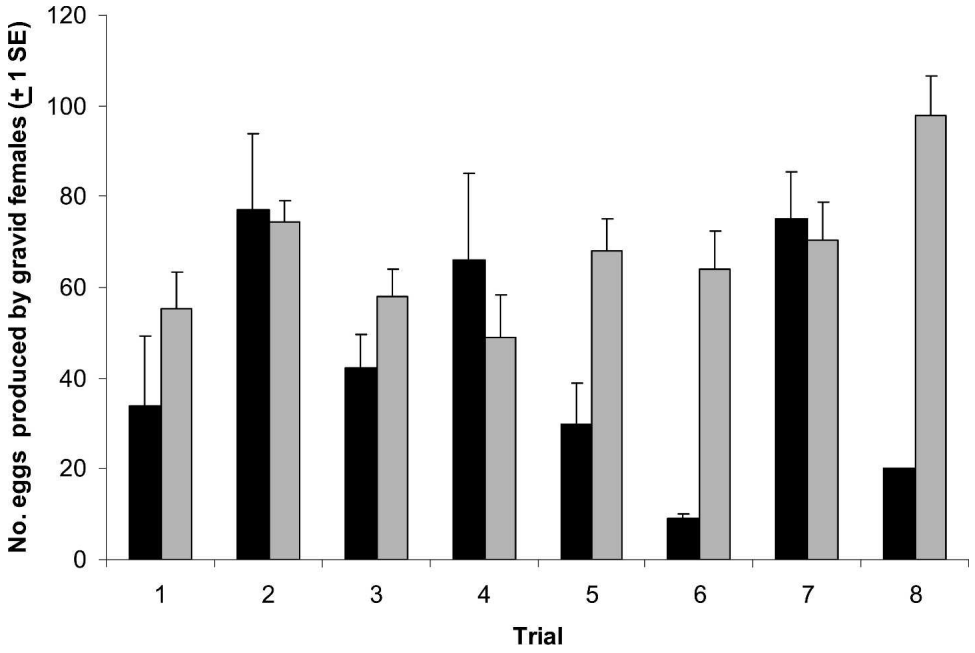

FIGURE 2. Number of eggs in gravid *An. gambiae* mosquitoes 7 days after feeding on either *P. falciparum* gametocyte-infected or control blood. Data is presented separately for each of the 8 trials in which at least one mosquito in each treatment group produced eggs after feeding. Black bars are for mosquitoes fed control blood from adults and gray bars for mosquitoes fed gametocyte-infected blood from children. Bars represent one standard error.

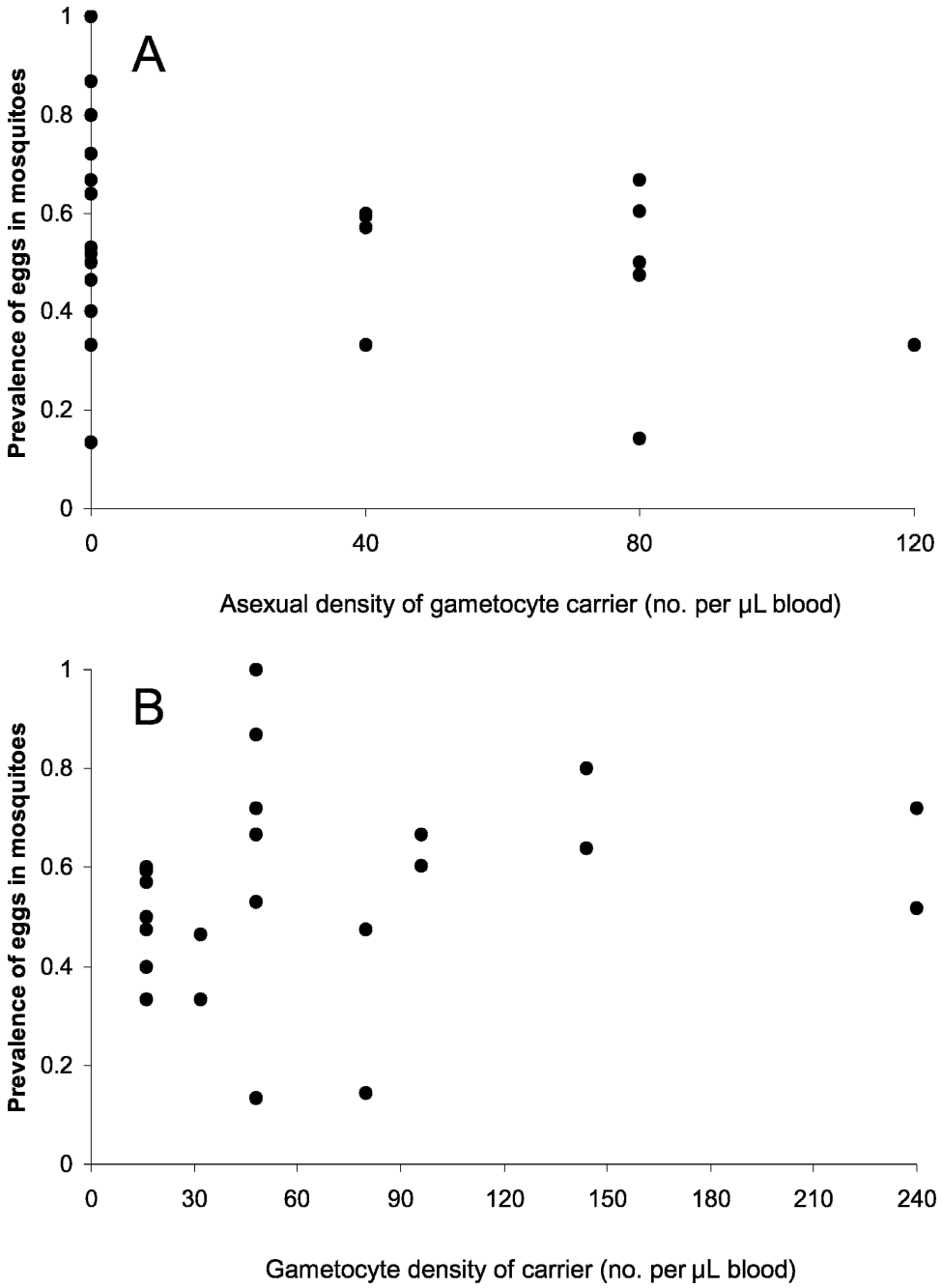

FIGURE 3. Relationships between the gravidity of noninfected mosquitoes in relation to the density of asexual (**A**) and gametocyte stage parasites (**B**) in their blood meal. Only mosquitoes fed gametocytemic blood were included in this analysis. Both asexual and gametocyte density were significant predictors of mosquito gravidity when included as explanatory variables in a logistic regression analysis. When both factors were included in the model (in addition to mosquito strain and trial), there was evidence that gravidity fell with asexual density (A: $\beta = -0.86$, SE = 0.40) and rose with gametocyte density (B: $\beta = 0.89$, SE = 0.39).

were still infected when we deemed them parasite-free by microscopy (4–10 weeks after Fansidar treatment), but none of the adults who acted as uninfected controls had submicroscopic infections. All further analysis was restricted to the five follow-up trials in which both the child (initially infected) and adult (uninfected control) were parasite-free at the time of second examination. This subset of five pairs accurately represented the larger group of children assayed in earlier experiments in that when they had gametocytes, their blood gave rise to substantially higher egg prevalence in mosquitoes than blood from the uninfected controls (Table 1).

The difference in egg prevalence between mosquitoes fed on children's blood and those fed on an uninfected adult control depended on whether the child had *P. falciparum* gametocytes or not (Host type × child infection status: $\chi_1^2 = 7.72$, $P < 0.01$). When children had *P. falciparum* infections, their blood consistently gave rise to higher egg prevalence in *An. gambiae* than did the uninfected controls ($\chi_1^2 = 22.49$, $P < 0.01$; Figure 4A). In contrast, when these same children had cleared their *P. falciparum* infections, there was no significant difference in the prevalence of eggs produced from their blood and that of the uninfected adult controls ($\chi_1^2 = 1.99$, P

TABLE 1

Egg prevalence in *Anopheles gambiae* mosquitoes 7 days after being membrane-fed blood infected with *P. falciparum* gametocytes (from children) or uninfected control blood from adults

	Egg prevalence in *An. gambiae* after blood-feeding	
Trial number	Uninfected control	Gametocyte infected
1	0.00 (17)	0.44 (34)
2	0.00 (22)	0.33 (27)
3	0.00 (32)	0.48 (23)
4	0.44 (9)	0.83 (42)
5	0.33 (18)	0.38 (34)
Mean (±SE)	0.15 ± 0.10	0.49 ± 0.09

Data are for five pairs of individuals that later participated in follow-up trials when the gametocyte carriers had cleared their infections (data for follow-ups shown in Fig. 3B). Numbers in brackets represent the number of mosquitoes used in each trial. The mean gives the average prevalence of eggs in mosquitoes in different treatment groups across five trials.

= 0.16; Figure 4B). Although IF mosquitoes were generally more likely to have eggs than the MB strain, the relationship between host type (adult versus child) and child infection status did not vary between mosquito strains (host type × child infection status × mosquito strain: $c_1^2 = 0.39$, $P = 0.53$). Differences in egg prevalence between mosquito strains could not be explained by variation in body size (wing length NS in all analyses).

Restricting analysis to gravid mosquitoes, there was no relation between the number of eggs they produced and host type either when children had parasites ($F_{1,82} = 0.34$, $P = 0.56$, mean egg number ± SE from infected child's blood = 56.0 ± 3.4, uninfected adult blood = 70.4 ± 12.8), and when they had cleared them ($F_{1,89} = 1.73$, $P = 0.19$, mean egg number ± SE from recovered child's blood = 51.8 ± 4.4, uninfected adult blood = 66.5 ± 6.5).

Blood meal size is one factor that could explain the change in mosquito gravidity as parasites cleared. However, across the five trials of this follow-up experiment, there were no systematic differences in the blood meal size taken from uninfected adults and children, either at the time when children were infected ($F_{1,95} = 0.38$, $P = 0.54$, mean ± SE for infected child's blood = 15.5 ± 1.4 μg, for adult's blood = 16.7 ± 1.3 μg) or when they had recovered ($F_{1,65} = 2.05$, $P = 0.16$, mean ± SE for recovered child's blood = 11.6 ± 1.2 μg, for adult's blood = 14.3 ± 1.5 μg). Similarly, there was no difference in the blood hemoglobin concentration of children used in these experiments at the time they had gametocytes and when they had recovered ($F_{1,5} = 1.25$, $P = 0.31$, mean Hb when infected [± SE] = 12.3 [1.2] g/dL, when recovered = 11.3 [0.4] g/dL). Furthermore, at no time point (either when children were infected or after they had recovered) did children have a higher value of hemoglobin than the uninfected control adults. Indeed, the only detectable hematological difference between children and adults was a higher hemoglobin concentration in adults when compared with recovered children (13.90 g/dL versus 11.28 g/dL, $F_{1,8} = 8.21$, $P = 0.02$).

DISCUSSION

Here we have shown for the first time that the presence of *P. falciparum* gametocytes in human blood has a large effect on the gravidity of *An. gambiae* mosquitoes. We observed that the proportion of *An. gambiae* mosquitoes that produced eggs after one blood meal was almost six times greater when given children's blood infected with *P. falciparum* gametocytes than when given uninfected adult blood; provided that these gametocytes did not successfully infect the mosquito. Critically, this difference in gravidity between children's and adult's blood disappeared when children cleared their parasites, suggesting the difference was specifically due to *Plasmodium* in the blood and not other factors that differed between controls and gametocyte-infected hosts, such as age. Furthermore, the increased gravidity of mosquitoes that fed on gametocytemic blood was sensitive both to gametocyte and asexual density, rising in with former and falling with the latter. This suggests fecundity enhancement occurs particularly in response to the presence of transmission stages in blood and is not a general response to asexual infection.

Our results are from trials in which gametocytes did not infect mosquitoes. As commonly observed in other studies,[18,32–35] the majority of mosquitoes that fed on infected human blood in this study did not become infected (< 7% of trials yielded oocyst-infected mosquitoes). Mosquitoes that did become infected with oocysts had a similar rate of egg prevalence than the uninfected controls. It is likely that *An. gambiae* only occasionally become infected after feeding on malaria-infected humans in nature (rate of mosquito infection from infected blood is generally < 20%[18,32–35]). Thus, the fecundity effects we report here may be indicative of the general gravidity outcome to vectors when they encounter gametocyte-infected hosts.

To further lend weight to the generality of our observations, the effect of gametocytemic blood on gravidity was stable across two *An. gambiae* strains that differed in their innate ability to produce eggs. Additionally, the increased gravidity of mosquitoes fed gametocyte-infected blood was remarkably consistent between trials of different infected human hosts, whom almost certainly had genetically distinct *P. falciparum* infections. Thus, we hypothesize this effect could apply widely throughout a range of mosquito, host, and parasite populations.

The mechanistic basis for this phenomenon is unclear, as the higher rate of gravidity in mosquitoes fed infected blood could not be related to blood meal size or haemoglobin content (these measures were generally similar or higher in uninfected blood). It is possible, however, that infected (but noninfectious) blood is of higher quality to *An. gambiae* in terms of other types of nutrients. For example, *Plasmodium* is known to alter the amino acid composition of human blood,[36] and the amino acid composition of infused diets is known to influence mosquito fecundity.[37–41] It is possible that changes in blood amino acid composition due to *Plasmodium* are conducive to oogenesis, an effect that is not observed when parasites successfully infect mosquitoes because their growth is detrimental. Studies of the rodent malaria parasite *P. chabaudi* have shown that the blood of mice who undergo severe infection gives rise to the highest fecundity in mosquitoes,[42] thus it is possible that some infection-related changes in blood chemistry can increase blood quality to mosquitoes. Detailed biochemical studies of host blood composition should be undertaken to resolve whether the changes in mosquito gravidity we report can best be explained by variation in host blood quality, or a mosquito response to the threat of parasitism.

It is also possible that there is an adaptive explanation for higher egg prevalence in mosquitoes exposed to parasitized blood. Evolutionary theory predicts that an organism should

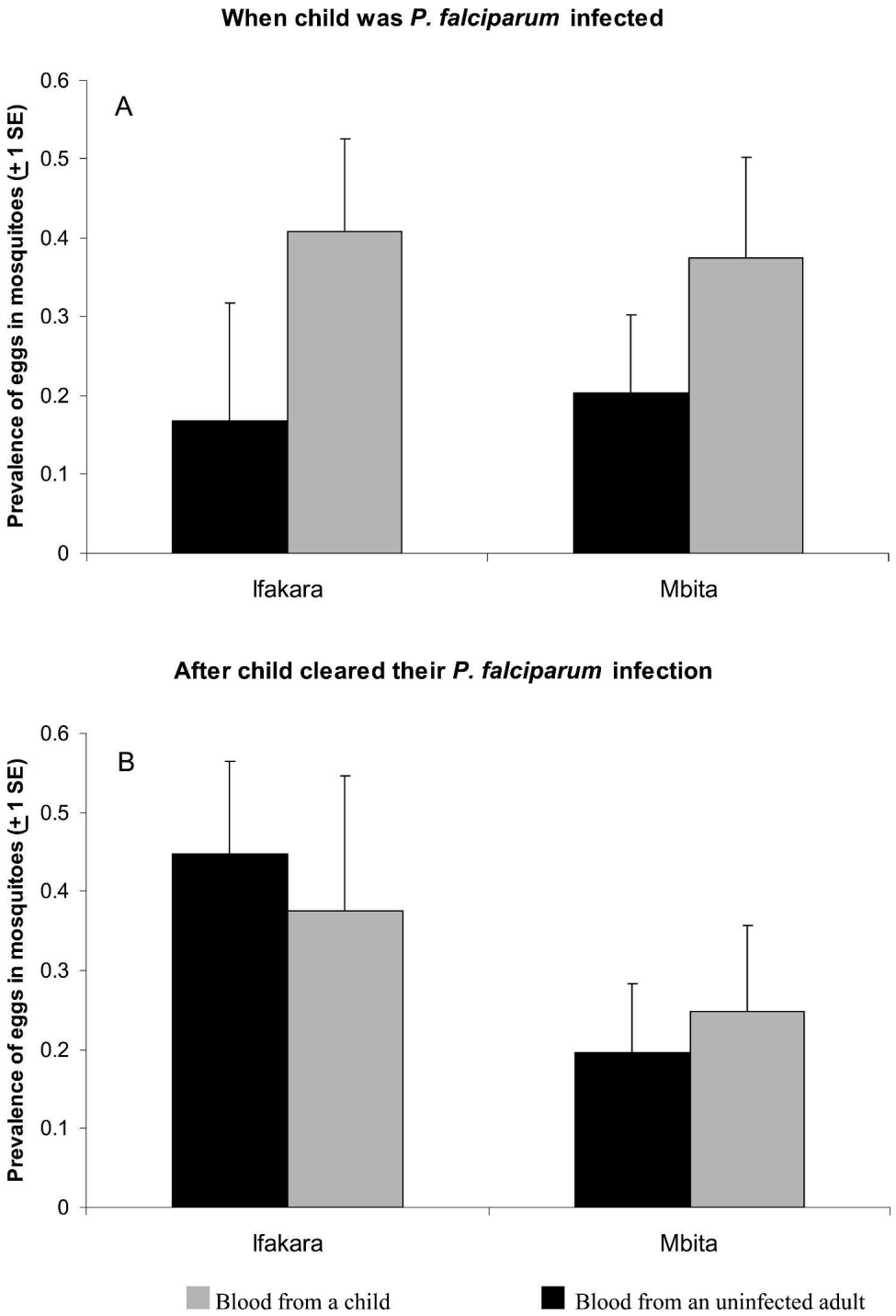

FIGURE 4. Comparisons of egg prevalence in two strains of *An. gambiae* 7 days after feeding on blood from uninfected adults and *P. falciparum*–infected children (**A**) and uninfected adults and the same children after they had cleared their parasites (**B**). Data are the mean values obtained from 5 pairs of individuals (adult and child) assayed both when the children had malaria and after they had recovered. Bars are standard errors.

shift its reproductive schedule forward when under threat of parasitism that will cut its longevity and/or reproductive success at a later age,[43] a phenomenon that has been observed in several invertebrates.[44] *Plasmodium* can reduce vector longevity,[8] and infection is known to have long-lasting detrimental effects on fecundity.[5] It is possible that the presence of parasites in the blood meal is sufficient to induce mosquitoes to divert energy that would be otherwise used for longevity into producing an early clutch. This effect might be obscured when mosquitoes actually go on to develop oocysts if active parasite growth interferes with the energy budget of the mosquito. It is also possible that the enhancement of gravidity accompanying gametocyte infection is the result of coevolution between the parasite and vector, with the parasite evolving a benefit to entice mosquitoes to bite gametocyte-infected hosts. Such an exposure-related benefit could perhaps select

for increased biting on infective hosts, even if the minority of mosquitoes that go on to become infected have reduced fecundity (as shown in Refs. 13, 45). Notably, we found that the presence and density of asexuals concomitant with gametocytes was associated with reduced mosquito gravidity, with egg prevalence in mosquitoes being highest after the consumption of noninfectious blood meals with high gametocyte density and no asexuals. Thus, if enhanced gravidity after one blood meal is indeed a fitness benefit to *Anopheles*, it appears to be maximized at the point where the infection is most transmissible. Further investigation of mosquito fecundity as they feed on hosts at different stages of infection is required to test this hypothesis, as is elucidation of the influence of early reproduction on mosquito lifetime reproductive fitness.

Certainly, the parasite treatment we administered had a large influence on mosquito fecundity. Whether it could influence mosquito population dynamics in nature depends on whether our estimates of fecundity as assayed by dissection can be translated into actual oviposition rates. We chose to assay mosquito fecundity by dissection instead of oviposition because we wished to identify the innate capacity for reproduction on different blood sources, in isolation of extraneous variation due to oviposition behavior and mortality. By not providing an oviposition substrate for mosquitoes, some may have resorbed all or part of their follicles before dissection, a phenomenon that could lead to an underestimation of fecundity. Further, our results could be misrepresentative of fecundity under natural conditions if mosquitoes fed uninfected blood are more likely to oviposit their eggs than those feed gametocytemic blood. Investigation of both the rate of resorption and oviposition of eggs following ingestion of gametocytemic and uninfected blood will clarify whether our results can be extrapolated to free-living mosquitoes that have unrestricted opportunities to lay their eggs.

If the enhanced gravidity on gametocytemic blood reported here occurs under natural conditions, it could impact both mosquito fitness and population dynamics. Age-at-first-reproduction is a pivotal determinant of fitness, and studies of a wide range of taxa have shown that this measure has larger effects on lifetime reproductive fitness than any other life history trait.[44] In mosquitoes, age-at-first reproduction will be determined by the number of blood meals required before eggs are produced, and one nonproductive meal could substantially reduce lifetime reproductive capacity. For example, the daily survival of female *An. gambiae* mosquitoes in the field has been estimated at 80–85%.[46,47] At this rate, if a mosquito delays producing eggs until it has had two blood meals (assuming a second meal is taken 1–3 days after the first), its chance of surviving to reproductive age is cut by 30–60%.

The requirement for numerous blood meals before eggs can be produced is not unusual in *Anopheles* mosquitoes.[48–51] Laboratory studies have shown that up to 30% of *An. gambiae* will fail to produce eggs after one blood meal,[49] with field studies indicating that 20% to > 50% of mosquitoes caught in houses are in a pregravid state (have blood fed but did not produce eggs).[50,51] The cause of pregravidity is usually thought to be small body size,[30,51] with multiple blood meals prior to reproduction being required by small individuals to compensate for nutritional deficits.[49] To our knowledge, this is the first time that factors associated with host blood and/or the presence of parasites have been shown to influence pregravidity. If increased gravidity after biting gametocyte-infected humans is a stable outcome of vector-parasite interactions, we speculate it could explain why there appears to have been no selection on mosquitoes to avoid infected people. Further examination of the generality of this phenomenon and its underlying mechanism will substantially increase our understanding of how small-scale interactions between vectors and parasites can influence malaria epidemiology.

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