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POPULATION DYNAMICS OF *PLASMODIUM FALCIPARUM* SPOROGONY IN LABORATORY-INFECTED *ANOPHELES GAMBIAE*

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ABSTRACT: The population dynamics of cultured *Plasmodium falciparum* parasites was examined during their sporogonic development in *Anopheles gambiae* mosquitoes. Estimates of absolute denisities were determined for each life stage, and life tables were contructed for each of 38 experimental infections. Macrogametocyte and ookinete mortalities contributed equally to the overall mortality. On average, there was a 40-fold decrease in parasite numbers in the transition from the macrogametocyte to the ookinete stage, a 69-fold decrease in the transition from ookinete to oocyst stages, and a total net decrease in parasite numbers from macrogametocyte to oocyst stage of 2,754-fold (i.e., multiplicative). There was no relationship between macrogametocyte and ookinete densities due to the inherent variability in fertility among different gametocyte cultures. There was a curvilinear relationship ($r^2 = 0.66$) between ookinete and oocyst densities. Above a threshold of about 30 ookinetes/mosquito, the oocyst yield per ookinete became increasingly greater with increasing ookinete density. There was a linear relationship ($r^2 = 0.73$) between oocyst and sporozoite densities, with an average of 663 salivary gland sporozoites produced per oocyst. Sporozoite production per oocyst was not affected by oocyst density and virtually all oocyst infections resulted in sporozoite infections of the salivery glands. This quantitative study indicates that the sporogony of cultured *P. falciparum* in laboratory-infected *A. gambiae* is an inefficient process and that the ookinete is the key transitional stage affecting the probability of vector infectivity.

Malaria transmission is contingent on the sporogonic development of the causative organism (*Plasmodium* sp.) within mosquitoes of the genus *Anopheles*. Plasmodial sporogony involves a complex continuum of events, and disruptions at any point in the sequence may affect sporozoite transmission potential. The generalized sequence of events has been known for almost a century (see Harrison [1978] for historical review). However with respect to quantitating parasite densities within infected mosquitoes, most studies have utilized either oocyst counts (the mature oocyst stage is the easiest stage to dissect and count) or the salivary gland index, which is subjective and lacks precision. More recent studies have provided absolute estimates of ookinete densities (Vaughan et al., 1991) and salivary gland sporozoite loads for individual mosquitoes (Beier et al., 1991). However, no study has examined the entire cycle in a rigorous quantitative fashion.

Our objective was to quantify each life stage and successive life stage mortality of laboratory-cultured *Plasmodium falciparum* parasites developing within *Anopheles gambiae* mosquitoes as a basis for understanding the determinants of infectivity. The conceptual framework viewed the sporogonic development of the parasite in terms of population ecology, with the mosquito representing the habitat. Theoretically, plasmodial sporogony is ideally suited for this type of approach because the habitat itself is stable and, during sporogony, there is no immigration or emigration of parasites into or out of the habitat. Thus, changes in population density from one stage to the next can be estimated directly. However, it is impossible to sample a population over time within a single habitat (i.e., a single mosquito) and therefore many discrete habitats must be sampled. This poses no problem however because all the parasites within an infectious feed arise from the same initial population (i.e., same membrane feeder), and, assuming a normal distribution in bloodmeal size and random mixing of gametocytes, the starting parasite densities among individual mosquitoes within a cage initially are homogenous. Stage-specific census values within an infection were tabulated in traditional life table format (Varley et al., 1973) to estimate stage-specific mortality rates. Density relationships between successive life stages also were examined.

MATERIALS AND METHODS

Anopheles gambiae (G3 strain) mosquitoes were infected with the NF54 strain (or derived clones) of *P. falciparum*. Gametocytes were cultured in vitro using modifications on the method described by Ponnudurai et al. (1982). The decision on when to feed the cultures to mosquitoes was based primarily on 2 general criteria: daily examination of Giemsa-stained thin smears of the culture material, noting the morphological maturity of gametocytes (stage IV:V ratio), percent gametocytemia, sex ratios, and the general appearance of

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the culture, and qualitative assessment of exflagellation. Generally, feeds were conducted within 15–19 days after culture initiation. Prior to mosquito feedings, cultures were diluted at various concentrations (range 1:8 to 1:500) with washed human erythrocytes mixed 1:1 with heat-inactivated human sera. Cohorts of 3–5-day-old mosquitoes were allowed to feed for 15 min on the diluted cultures via water-jacketed membrane feeders (1 per cage). Unengorged mosquitoes were removed. Mosquitoes were held at 24 C overnight and maintained thereafter at 27 C, 70% RH and with free access to sugar solution. Mosquitoes were provided with oviposition sites 3 days after the infectious feed. It was observed early on, that even within the same culture and culture dilution there sometimes were marked differences in gametocyte densities among feeders (e.g., pipetting error). Therefore to avoid confounding effects of interfeeder variability, each feeder (i.e., cage) was considered an "infection." Infections arising from the same culture flask were considered cohort infections. Estimates of absolute density for each life stage were obtained for 38 infections and from these data, life tables (macrogametocyte to oocyst) were constructed.

Mean macrogametocyte density per mosquito was estimated for each infection by multiplying mean bloodmeal volume times mean bloodmeal erythrocyte density times percent macrogametocytemia of the blood meal. Bloodmeal volume was determined gravimetrically by weighing pools of 30–40 mosquitoes before feeding and weighing pools of 6–10 engorged mosquitoes immediately after feeding. Engorged mosquitoes were collected and chilled immediately after feeding to reduce potential weight loss as the result of normal postfeeding diuresis. Blood meals were excised, diluted 1:200 in physiological saline, and standard erythrocyte counts were performed with a hemacytometer to estimate the erythrocyte density of the blood meals. To estimate the percent macrogametocytemia, 2 thin smears were prepared of the culture material from each membrane feeder. Slides were fixed in methanol, stained with Giemsa solution, and examined under 1,000× oil. The number of mature (stage V) macrogametocytes per 2–4 × 10^4 erythrocytes was counted, and percentages for the 2 slides were averaged. To ensure that percent macrogametocytemia in the feeders approximated that in the mosquito midgut, thin smears were also prepared from 6 mosquito blood meals immediately after feeding. This was done for 8 infections. The percentages between the blood-meal and feeder-derived material were virtually identical. Therefore in subsequent infections we utilized estimates from feeders because this method was less laborious than dissection.

Estimates of absolute ookinete density were obtained by emptying individual blood meals into 20 μl of a 3% acetic acid solution that lysed the erythrocytes, leaving only leukocytes and parasites intact. Samples were mixed by pipetting and examined with a hemacytometer under phase contrast microscopy (400×). Ookinetes were distinguished easily by their characteristic paisley shape. The numbers of mature ookinetes were counted per 4 0.1-μl corners, and appropriate calculations were performed to estimate the number of ookinetes per mosquito. Based on diluent volume (20 μl) and volume examined (0.4 μl), the theoretical limit of resolution was 50 ookinetes per mosquito. Six to 12 mosquitoes were sampled per cage. Plasmodial fertilization and ookinete formation are relatively ephemeral events. Therefore, prior to conducting regular sampling of ookinetes, a preliminary study was performed to determine the optimal time for sampling ookinetes. At selected intervals postingestion (PI), 6–10 mosquitoes were collected, midguts excised, and blood meals examined for ookinetes as above.

Mosquito midguts were dissected, stained with mercurochrome, and examined (400×) for oocysts on days 9–12 PI. Preliminary sampling indicated that this time period was optimal for sampling oocysts. Prior to day 9 PI, some oocysts were small and were easily overlooked. From day 13 PI onward, oocysts began releasing sporozoites, making oocysts fewer in number. A minimum of 10 infected midguts was sampled per cage, except when oocyst infection rates were very low (<10%). In such cases, dissections continued until 5 infected midguts were found or all mosquitoes within a cage were dissected.

Hemolymph sporozoites were collected by perfusing the hemocoels of individual mosquitoes with 20 μl of Medium 199 (M-199) (Vaughan et al., 1990). Upon mixing, perfusates were loaded into a hemacytometer and examined at 400× phase contrast. Sporozoites were counted and appropriate calculations were performed to estimate the number of sporozoites per mosquito. The theoretical limit of resolution was 50 sporozoites per mosquito. To ensure that a 20-μl perfusion volume was adequate to flush out most of the hemocoel sporozoites within a mosquito, preliminary studies were conducted whereby a 15-μl perfusate was collected in 3 sequential aliquots of 5 μl each. Greater than 95% (n = 13) of all sporozoites recovered were in the first 10 μl of perfusate.

Salivary glands from individual mosquitoes were excised, placed in small glass tissue grinders containing 35 μl M-199, and disrupted to release the sporozoites. Sporozoites were counted on a hemacytometer at 400× phase, and appropriate calculations were performed to estimate the number of sporozoites per pair of salivary glands. The theoretical limit of resolution was 87.5 sporozoites per pair of salivary glands. A minimum of 10 infected mosquitoes was sampled per cage. Mosquitoes were sampled for sporozoites on days 16–19 PI when most sporozoites had reached the salivary glands.

For each infection, census data for macrogametocytes, ookinetes, and oocysts were tabulated in the form of life tables (Varley et al., 1973; Southwood, 1978). Mortality from one stage to the next was expressed as killing power or k-value, which is simply the difference in population density, expressed as logarithms, between one life stage and the next. Thus, k-1 equals $\log_{10}$(macrogametocyte) minus $\log_{10}$(ookinete), k-2 equals $\log_{10}$(ookinete) minus $\log_{10}$(oocyst), and K equals total mortality from macrogametocyte to oocyst (i.e., k-1 plus k-2). In biological terms, k-1 represents the decrease in parasite numbers due to macrogametes not being fertilized and/or failing to differentiate to ookinetes and k-2 represents the decrease in parasite numbers due to ookinetes failing to penetrate the midgut and/or failing to develop into mature oocysts.

Because unfed mosquitoes always were removed from the cages on the day of the infectious feed, remaining

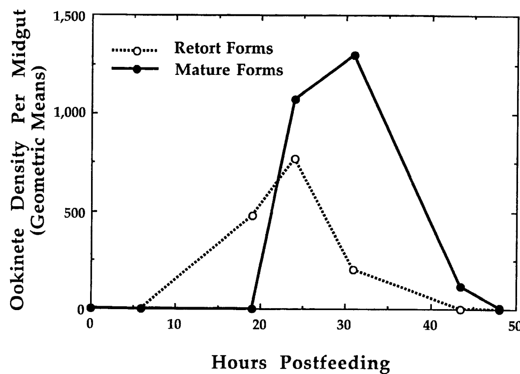

FIGURE 1. Developmental kinetics of *Plasmodium falciparum* ookinetes in the blood meals of *Anopheles gambiae* mosquitoes held at 24 C. Each time point represents the geometric mean of 6–10 mosquitoes.

TABLE I. Developmental kinetics of *Plasmodium falciparum* sporozoites in *Anopheles gambiae* mosquitoes.*

Time post ingestion (days)	Number of infected mosquitoes examined	Oocyst (%)	Hemocoel sporozoite (%)	Gland sporozoite (%)
12	8	100 (9)	0 (−)	0 (−)
13	9	100 (15)	67 (+)	44 (2,429)
14	9	100 (6)	67 (+)	67 (7,927)
16	19	58 (3)	ND†	95 (6,643)
17	18	ND	72 (477)	94 (2,428)
18	11	ND	73 (754)	100 (2,410)
19	8	ND	75 (2,056)	100 (7,153)
20	5	ND	60 (835)	100 (12,953)
27	11	ND	36 (977)	100 (9,783)

* Data are for infected mosquitoes only. Percentages indicate the proportion having infections in the designated anatomical site (midgut, hemolymph, gland). Geometric means in parentheses were calculated using only those mosquitoes having site-specified infections and do not include zero counts.

† Not determined.

mosquitoes all received gametocytes. Therefore, the computation of geometric means for ookinetes and oocysts for life tables necessarily include zero counts (i.e., uninfected mosquitoes). These zero counts represented habitats in which the parasite populations had become extinct. However, computation of sporozoite production excluded zero counts because uninfected mosquitoes cannot contribute to sporozoite production.

RESULTS

The population density of mature ookinetes peaked at 31 hr PI and was preceded by a peak in developing retort forms at 24 hr PI (Fig. 1). At 31 hr PI, most blood meals were fully enclosed in a peritrophic membrane with the beginnings of an ectoperitrophic space. Ovarian development was at Christopher stage III. By 48 hr PI, ookinetes were absent from blood meals, which themselves were in an advanced stage of digestion. The area under the curve for mature ookinetes was nearly twice that for retort forms, indicating that the retort population was undersampled. This was due to the fact that the retorts, identified by translucent cytoplasmic extensions, were more difficult to discern under phase contrast than the relatively opaque mature ookinetes. As a result, only mature forms were included in analyses.

Daily sampling of midguts, hemolymph, and salivary glands from individual mosquitoes showed that sporozoites first were released into the hemocoel on day 13 PI in 67% of 9 oocyst-positive mosquitoes sampled (Table I). Over half (4 of 6) of these hemolymph-positive mosquitoes also had infected salivary glands. By day 14 PI, 6 of 9 (67%) oocyst-positive mosquitoes had sporozoites in both hemolymph and salivary glands, indicating that sporozoite invasion of salivary glands began within a day or 2 of their release from oocysts. The oocyst infection rate and density declined substantially by day 16 PI, indicating that upon sporozoite release, empty oocysts had disappeared. Hemocoel perfusions of 42 infected mosquitoes from days 17 to 20 PI indicated that most gland-positive mosquitoes (60–75%) also contained sporozoites in the hemocoel. By day 27 PI, only 4 of 11 (37%) gland-positive mosquitoes still contained hemolymph sporozoites. Taking into account the differences in infection rates between hemolymph and gland sporozoites from days 17 to 27 PI, on average 11.3% of the total number of sporozoites produced (i.e., hemolymph plus gland sporozoites) failed to enter the salivary glands but instead remained in the hemolymph.

During the course of the study, 72 gametocyte cultures (i.e., flasks) were fed to mosquitoes, some at 3–4 culture dilutions each. Only 53% of the 72 cultures produced oocyst infection rates >30%. Regardless of infection outcome, life tables were constructed for every infection where complete data on macrogametocyte, ookinete, and oocyst densities were available. For some infections, only data on certain transitions were collected (e.g., ookinete to oocyst) and thus life tables could not be constructed for these infections. However, such data were included to analyze the density relationships between successive life stages (see below).

Life tables were constructed for each of 38

TABLE II. Life tables (means and coefficients of variation) and stage-specific mortalities (k) of *Plasmodium falciparum* populations developing within laboratory-infected *Anopheles gambiae.*

Macrogametocytes	k-1*	Ookinetes	k-2†	Oocysts	K‡
812 (63%)	1.21	49 (77%)	1.61	0.23 (324%)	2.83
1,244 (42%)	1.70	24 (95%)	1.32	0.16 (275%)	3.03
2,765 (11%)	1.46	95 (65%)	1.87	0.30 (267%)	3.33
4,340 (43%)	0.55	1,218 (65%)	1.33	56.3 (68%)	1.88
4,388 (42%)	1.50	138 (94%)	2.11	0.07 (316%)	3.61
5,254 (44%)	1.68	110 (74%)	1.86	0.51 (194%)	3.54
5,517 (11%)	1.16	382 (57%)	1.98	3.02 (96%)	3.14
6,643 (22%)	1.26	364 (60%)	1.62	7.70 (121%)	2.88
6,837 (20%)	2.85	9 (140%)	0.98	0.00 (0%)	3.83
6,997 (23%)	0.37	2,959 (20%)	1.99	29.60 (81%)	2.36
7,874 (31%)	1.15	557 (40%)	1.80	7.86 (154%)	2.95
8,074 (52%)	0.98	846 (39%)	2.24	3.85 (186%)	3.22
8,380 (34%)	1.98	86 (108%)	1.92	0.05 (447%)	3.90
8,603 (29%)	1.85	120 (83%)	1.63	1.85 (213%)	3.48
8,658 (48%)	1.76	150 (53%)	1.80	1.39 (158%)	3.56
8,903 (24%)	2.49	28 (69%)	1.46	0.00 (0%)	3.95
9,631 (57%)	1.66	210 (99%)	2.30	0.05 (448%)	3.96
9,490 (53%)	1.90	118 (59%)	2.05	0.07 (395%)	3.95
9,916 (20%)	1.37	423 (91%)	2.18	1.80 (153%)	3.55
11,047 (11%)	1.03	1,020 (46%)	2.41	2.97 (133%)	3.44
11,215 (37%)	1.16	777 (29%)	2.22	3.71 (148%)	3.38
11,495 (36%)	1.42	432 (57%)	1.79	6.02 (171%)	3.21
11,838 (30%)	1.61	291 (67%)	2.13	1.15 (122%)	3.74
12,896 (22%)	1.72	246 (65%)	1.56	5.74 (224%)	3.28
13,074 (48%)	1.33	610 (47%)	1.96	5.70 (149%)	3.29
13,296 (16%)	2.32	62 (103%)	1.71	0.22 (348%)	4.04
13,365 (11%)	1.01	1,302 (64%)	2.70	1.60 (226%)	3.71
14,113 (38%)	1.78	235 (119%)	1.47	7.00 (140%)	3.25
14,333 (31%)	1.44	517 (61%)	1.82	6.81 (199%)	3.26
14,394 (16%)	1.88	190 (58%)	1.30	8.58 (80%)	3.18
14,592 (50%)	1.76	254 (71%)	1.39	9.40 (114%)	3.15
14,726 (32%)	1.83	215 (84%)	2.09	0.74 (158%)	3.93
14,866 (19%)	1.55	419 (60%)	1.91	4.20 (193%)	3.46
18,885 (16%)	1.04	1,716 (61%)	1.78	27.12 (127%)	2.82
19,727 (24%)	2.04	180 (85%)	2.02	0.71 (227%)	4.06
19,812 (11%)	1.46	619 (68%)	2.27	2.70 (274%)	3.73
20,081 (33%)	3.52	5 (172%)	0.78	0.02 (718%)	4.30
26,115 (28%)	1.90	325 (74%)	2.51	0.00 (0%)	4.41
Average mortality	1.60 (40-fold)	+	1.84 (69-fold)	=	3.44 (2,754-fold)

* k-1, log(macrogametocyte) minus log(ookinete) and represents the mortality that occurred between these 2 life stages.
† k-2, log(ookinete) minus log(oocyst) and represents the mortality that occurred between these 2 life stages.
‡ K, k-1 plus k-2 and represents total mortality from macrogametocyte to oocyst.

mosquito infections, 26 of which yielded oocyst infection rates >30% and 12 of which were less successful (Table II). The mean densities per mosquito for macrogametocytes ranged from 812 to 26,155, ookinetes ranged from 5 to 2,959, and oocysts ranged from 0 to 56. Intrastage variability (indicated by coefficients of variation) was greatest for the oocyst stage. This large variability was reflected also in the resultant sporozoite stage. In general, data for macrogametocytes and ookinetes in each life table were normally distributed, whereas frequency distributions for oocysts and sporozoites invariably were skewed to the left (Shapiro-Wilk statistic, $P < 0.05$).

Total mortalities from macrogametocyte to oocyst (i.e., K) ranged from 1.88 (antilog = 76-fold decrease) to 4.41 (=25,704-fold decrease), with an overall average for the 38 life tables being 3.44 (=2,754-fold decrease). Macrogametocyte mortalities (i.e., k-1) ranged from 0.37 (=2-fold decrease) to 3.52 (=3,311-fold decrease), with an overall average of 1.60 (=40-fold decrease). Ookinete mortalities (i.e., k-2) ranged from 0.78 (=6-fold decrease) to 2.70 (=501-fold decrease), with an overall average of 1.84 (=69-fold decrease). Stage-specific mortalities (k-1 and k-2) for each infection were plotted to determine visually the relative contribution of each to the total mortality (K) (see Varley et al., 1973: 170). In general, k-1 and k-2 contributed equally to K.

There was no relationship between numbers of macrogametocytes and numbers of ookinetes

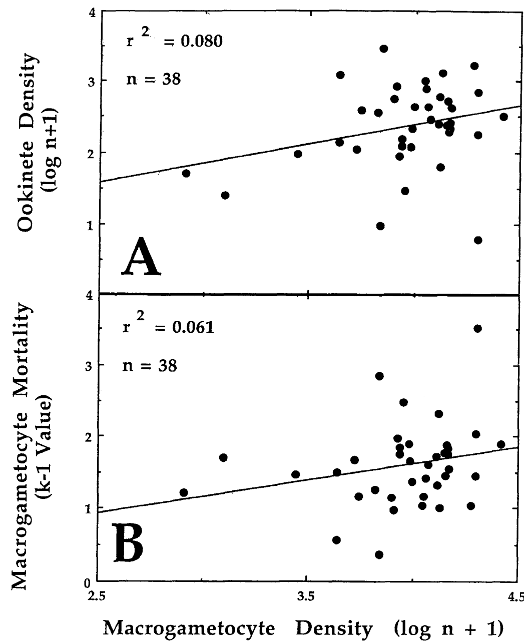

FIGURE 2. Density relationships of *Plasmodium falciparum* macrogametocytes in *Anopheles gambiae* mosquitoes. **A.** Relationship to ookinete density. **B.** Relationship to macrogametocyte mortality.

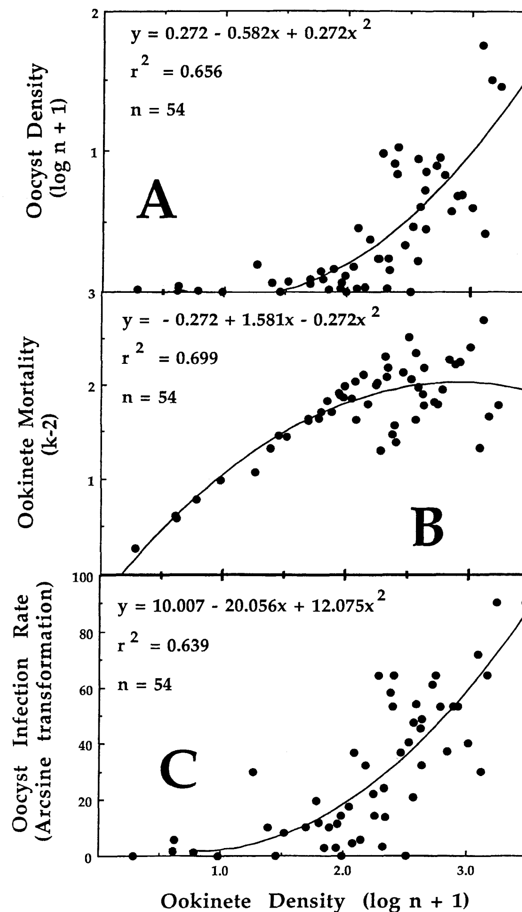

FIGURE 3. Density relationships of *Plasmodium falciparum* ookinetes in *Anopheles gambiae* mosquitoes. **A.** Relationship to oocyst density. **B.** Relationship to ookinete mortality. **C.** Relationship to oocyst infection rate.

produced (Fig. 2A), nor was there any relationship between macrogametocyte density and macrogametocyte mortality (Fig. 2B). Instead, there was a significant effect on the numbers of ookinetes produced according to the date on which an infection was conducted (i.e., the particular culture used) (ANOVA, $F = 12.1$, df = 22,305, $P < 0.0001$). This indicated that some gametocyte cultures were more fertile than others. Therefore, we analyzed separately those cohorts of infections that were infected on the same day with common cultures but at various dilutions of 3–6 dilutions each. Of the 5 cohort infections, 3 displayed a strong positive linear relationship between macrogametocyte and ookinete densities ($r^2 = 0.94$, $r^2 = 1.00$, $r^2 = 0.96$), whereas 2 of the cohort infections displayed little or no relationship ($r^2 = 0.30$, $r^2 = 0.04$).

There was a curvilinear relationship between ookinete and oocyst densities (Fig. 3A). Oocyst production was negligible at ookinete densities less than about log 1.5 (ca. 30 ookinetes/mosquito). Above this threshold, the conversion of ookinetes to oocysts became increasingly more efficient with increasing ookinete density, and ookinete mortality began to flatten out (Fig. 3B). Likewise there was a curvilinear increase in the proportion of oocyst-infected mosquitoes within a cage with increasing ookinete density (Fig. 3C).

There was a linear relationship between oocyst and sporozoite densities (Fig. 4A). If oocysts density = 1 (i.e., log[oocyst] = 0), then from the regression equation it is calculated that each oocyst produced an average of $10^{2.8216}$ or 663 salivary gland sporozoites. Sporozoite production per oocyst was not affected by oocyst density (Fig. 4B). These was no difference in overall infection rates between oocysts (70%) and salivary gland sporozoites (75%) (chi-square = 0.33, df = 1, $P = 0.57$), indicating that oocyst infections led invariably to salivary gland infections.

DISCUSSION

This study examined the population dynamics of *P. falciparum* sporogony in laboratory-infected *A. gambiae* mosquitoes. Within the sporogonic cycle, the 2 phases where mortality acts on parasite populations are the transition between gametocyte and ookinete (quantified in terms of k-1) and the transition between ookinete and mature oocyst (quantified in terms of k-2). Both transition phases have their own biological characteristics. The 2 possible mechanisms responsible for k-1 are unsuccessful macrogamete fertilization (Janse et al., 1985) and defective differentiation of zygotes to ookinetes (Gass and Yeates, 1979). Most likely, the majority of k-1 was attributable to unsuccessful gamete fertilization because the density of retort-form ookinetes did not exceed the density of mature forms (Fig. 1), indicating that most retort forms went on to form mature ookinetes.

The possible mechanisms in which k-2 may operate are failure of ookinetes to transverse the midgut and abortion of early oocysts. Mortality of early oocysts (e.g., day 2–4 PI) was not evaluated directly because regular sampling of oocysts was conducted on days 9–12 PI. Oocysts at day 5 PI were small and often difficult to distinguish from surrounding midgut tissue, but oocyst densities at days 5–6 PI were invariably less than or equal to densities on days 9–11 PI. Thus, any mortality after day 5–6 PI could not have been substantial. The "black spores" of melanized oocysts, indicative of a mosquito humoral response (Collins et al., 1986), were observed only rarely. We concluded that the majority of k-2 was attributable to ookinetes not traversing the midgut.

In general, k-1 and k-2 contributed equally to the overall mortality K among cohorts of infected mosquitoes (i.e., type II survivorship). However within a cohort, k-1 mortality acted in a relatively homogenous manner among individual mosquitoes (as indicated by the low coefficient of variation in ookinete numbers), whereas k-2 mortality was more heterogenous in its effect among individual mosquitoes (i.e., high coefficient of variation in oocyst numbers). This indicates that the intensity of k-1 from infection to infection was related more to conditions of the gametocyte cultures than to the environment of the mosquito midgut, whereas the large intermosquito variation in oocysts underscores the importance that individual variation in mosquito physiology has upon the success of ookinetes within a given mosquito. Even within a fairly inbred line of mosquitoes, some individuals provided more favorable environments for ookinete success (i.e., "susceptible" phenotypes) than others ("refractory" phenotypes).

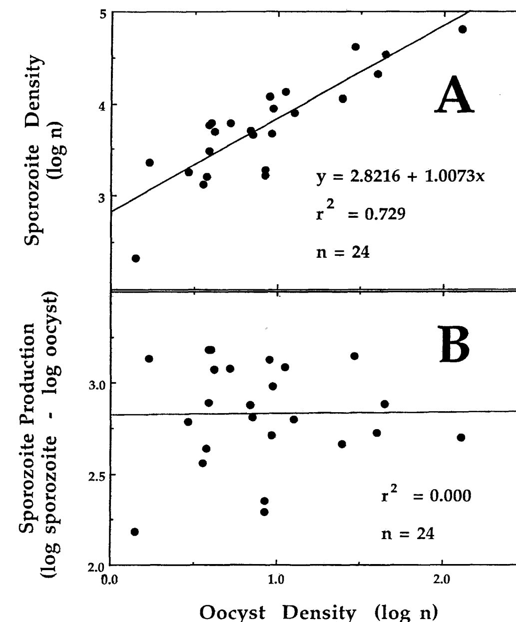

FIGURE 4. Density relationships of *Plasmodium falciparum* oocysts in *Anopheles gambiae* mosquitoes. **A.** Relationship to sporozoite density in mosquito salivary glands. **B.** Relationship to sporozoite production per oocyst.

There was no overall correlation between macrogametocyte and ookinete densities (Fig. 2A), and, even within cohort infections, correlations were not consistent from one infection to the next. Likewise, previous studies relating *P. falciparum* gametocyte densities to mosquito infectivity produced conflicting conclusions (King, 1929; Kligler and Mer, 1937; Jeffery and Eyles, 1955; Muirhead-Thomson, 1957; Ponnudurai et al., 1989). There are many factors, both extrinsic (i.e., blood factors and asexual parasitemia) and intrinsic (capacitance, sex ratios, etc.), that may act together to influence the infectivity of a gametocyte population (see Sinden [1991] for review). Thus, macrogametocyte density by itself cannot be considered a determinant of mosquito infectivity.

The ookinete stage was the first reliable determinant of infectivity. Below a threshold of about 30 ookinetes/mosquito (Fig. 2A), ookinetes failed to produce oocysts. This probably is related to

the spatial distribution of ookinetes within the clotted blood meal and the journey that ookinetes must undertake to reach the gut wall. Centrally located ookinetes must travel substantially greater distances than ookinetes formed on the periphery of the blood meal. Peripheral ookinetes probably have a better chance of success because ookinetes move rather slowly (average speed = 1 μm/min [Freyvogel, 1966]) and have but a limited time to complete the journey before being digested or voided. Thus a critical density may be required to ensure the probability of adequate numbers of peripherally located ookinetes. Above this threshold, ookinete efficiency increased with increasing density. That is, with increasing density ookinetes were able to traverse the peritrophic membrane/gut barrier more easily or, alternatively, those barriers somehow were rendered more conducive to ookinete penetration. The mechanisms underlying this increased efficiency are not known but 1 possibility may involve ookinete secretion of enzymes such as chitinase as described recently (Huber et al., 1991; Seiber et al., 1991) for *Plasmodium gallinaceum.*

The relationship between ookinete densities and oocyst infection rates (Fig. 3C) has great practical utility as it provides reasonable predictive capability for forecasting oocyst infection status (and hence sporozoite infection status) by assessing ookinetes at 31 hr PI. For example, if a cage of mosquitoes is found to have a geometric mean of 300 ookinetes/mosquito (log 300 + 1 = 2.4786), then from the equation the expected oocyst infection rate at days 9–12 PI would be arcsine(percentage) = 10.007 − 20.056(2.4786) + 12.075 (2.4786^2) or arcsine(34.478), the sine of which equals 0.5661, or about 57%. Such predictive capability could expedite scheduling of studies requiring sporozoites.

The close correspondence between oocyst and salivary gland sporozoite infection rates indicates that virtually all oocyst infections produced salivary gland infections. This differs from infections in wild-caught *A. gambiae* from Burkino-Faso (Lombardi et al., 1987) and western Kenya (Beier et al., 1990) where sporozoites failed to enter the salivary glands in 43% and 10% of infected mosquitoes, respectively. In nature there probably exist many confounding factors (e.g., genetic diversity of vectors and parasites) that may block or retard sporozoite entry into salivary glands. Even though oocyst infections in our laboratory ultimately led to salivary gland infections, not every sporozoite succeeded in entering the salivary glands. From 3 to 18% of the sporozoites produced by oocysts remained in the hemolymph (Table I). Such sporozoite loss most likely resulted from sporozoites simply being trapped in the tissues and open circulatory system of the mosquito rather than a lack of sporozoite viability as there was a general decline over time in hemolymph sporozoites, presumably as they eventually found their way to the glands. Nevertheless, the success rates of *P. falciparum* sporozoites entering *A. gambiae* salivary glands (82–97%) was decidedly greater than that calculated for *Plasmodium vivax* sporozoites in *Anopheles dirus* (23%) (Rosenberg and Rungsiwongse, 1991).

Sporozoite density in the salivary glands was related linearly to oocyst density, and, from the regression equation (Fig. 4A), 663 salivary gland sporozoites were produced per oocyst. Sporozoite production by oocysts was not affected by the density of oocysts on the midgut wall (Fig. 4B), implying that the nutritional requirements for oocyst growth and sporozoite production were essentially unlimited in our laboratory mosquitoes and there was neither an intraoocyst competition for nutrients nor a "carrying capacity" of the habitat.

These data indicate that the sporogonic cycle of *P. falciparum* in *A. gambiae* is highly inefficient. This is somewhat surprising considering that the NF54 strain (and its derived clones) are presumably of African origin and *A. gambiae* its coindigenous vector. The relative inefficiency, however, may result from the laboratory adaptation of a parasite strain to culture conditions. Early studies utilizing "natural" infections via mosquito feedings on gametocytemic humans indicate that the efficiency of *P. falciparum* sporogony is better than the laboratory infections reported here. By combining the data of Jeffrey (1956) on bloodmeal sizes and those of Jeffrey and Eyles (1955) on gametocyte infectivity of *P. falciparum* in humans to *Anopheles albimanus* and *Anopheles quadrimaculatus,* it is possible to calculate total mortality (K) in these systems. In 5 infections each, the estimated K-values for *P. falciparum* in *A. albimanus* and *A. quadrimaculatus* ranged from 1.63 to 2.18 (i.e., 43–151-fold decrease) and from 1.99 to 2.73 (i.e., 98–537-fold decreases), respectively. These studies did not measure ookinete densities and so total K cannot be partitioned into k-1 and k-2 to determine at which transition the efficiency increases. Recent studies by Beier et al. (1992) indicate that

the densities of ookinetes in wild-caught *A. gambiae* from western Kenya are so low (geometric mean <2/mosquito) that they are below what may be considered the threshold for oocyst production (Fig. 3). Yet the sporozoite rates in the same locales are among the highest in the world (4–18%). Clearly, the population dynamics of sporogony in these mosquitoes, particularly the transition between ookinete and oocyst, must be very different from that of laboratory-adapted strains.

The methods and approach developed here to describe quantitatively the sporogonic cycle of *P. falciparum* in *A. gambiae* can be applied to study the sporogonic cycle of any *Plasmodium*–vector system to identify the major developmental transitions that affect vector potential. This study indicates that the sporogony of cultured *P. falciparum* in laboratory-infected *A. gambiae* is an inefficient process and that the ookinete is the key transitional stage affecting the probability of vector infectivity.

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