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Effect of gametocyte sex ratio on infectivity of *Plasmodium falciparum* to *Anopheles gambiae*

V. Robert1*, A. F. Read2, J. Essong3, T. Tchuinkam1, B. Mulder1, J.-P. Verhave4 and P. Carnevale1

Institut Français de Recherche Scientifique pour le Développement en Coopération/Organisation de Coordination pour la Lutte contre les Épidémies en Afrique Centrale, P.O. Box 288, Yaoundé, Cameroon; 1Institute of Cell, Animal and Population Biology, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JY, UK; 2Faculty of Sciences, P.O. Box 812, University of Yaoundé 1, Cameroon; 3Department of Medical Parasitology, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

Abstract

Insectary-reared *Anopheles gambiae* were experimentally fed with the blood of 90 naturally infected human volunteers carrying gametocytes of *Plasmodium falciparum*. At least one mosquito was successfully infected in 74% of experiments. The probability that a gametocyte carrier was infective, the probability that a mosquito became infected, and the number of oocysts harboured were related to gametocyte density. The mean proportion of male gametocytes was 0.217 (i.e., 3.6 females for every male). Sex ratios differed significantly between gametocyte carriers. Variation in sex ratio was not related to the probability that a gametocyte carrier was infective. Among infective people whose sex ratio estimates were based on a reasonable number of gametocytes, sex ratio significantly predicted the proportion of infected mosquitoes and mean oocyst load, with infectivity rising as the proportion of male gametocytes increased towards 50%. There was no indication that infectivity reached a peak at some intermediate sex ratio, as would be expected if random mating of gametocytes was the primary determinant of fertilization success. These results raise 2 interesting questions: why should higher sex ratios be more infective, and why is the observed population sex ratio lower than that which produces the greatest infectivity?

Keywords: malaria, *Plasmodium falciparum*, gametocyte sex ratio, infectivity to anophelines

Introduction

The change from a human to an anopheline environment is a critical phase in the life cycle of malaria parasites. Mature gametocytes are the only stage able to initiate this transition. Gametocytes are morphologically and physiologically distinguishable as males or females. As single haploid asexual parasites can give rise to both physiologically distinguishable as males or females. As single haploid asexual parasites can give rise to both

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Results

Ninety experiments were included in the analysis; 3921 gametocytes were observed and 3162 mosquitoes were dissected. The mean gametocyte density was 247.4 ± 0.5 (range 56–1416/μL, median 100/μL). Gametocytes were sexed as 824 males (21%) and 2968 (76%) females, with 129 (3%) of indeterminate sex. The overall mean proportion of males was 0.217 (95% Cl 0.196–0.231), which is about 3.6 females for every male. The total number of males plus females ranged between 3 and 288 (mean 42.1, median 23). All but 2 sex ratios were female-biased (i.e., with more females than males); the highest observed proportion of males was 0.54 (95% CI 0.33–0.74) (Fig. 1); there were significant differences

between the sex ratios of the 90 gametocyte carriers (AD = 171, d.f. = 89, P < 0.001). Sex ratio was unrelated to host sex (AD = 0.33, d.f. = 1, not significant [NS]) and age (AD = 0.60, d.f. = 1, NS). Sex ratio was not correlated with the density of trophozoites (AD = 0.11, d.f. = 1, NS) or with the density of gametocytes (AD = 0.06, d.f. = 1, NS). This latter relationship was not improved by fitting an appropriately scaled quadratic term, so there was no indication that sex ratio was maximal at some intermediate gametocyte density.

People with higher gametocyte densities were more likely to be infective (i.e., to infect at least one mosquito; AD = 8.78, d.f. = 1, P < 0.005). The proportion of infected mosquitoes varied significantly between gametocyte carriers (AD = 796.2, d.f. = 89, P < 0.0001) around an overall average of 14.9% (95% CI 13.7–16.2%). Sixty-seven gametocyte carriers (74%) gave rise to at least one infected mosquito. The maximum proportion of infected mosquitoes was 72%. The mean oocyst density (number of oocysts per dissected mosquito) was 0.81 (range 0.2–63). Similarly, the percentage of infected mosquitoes was strongly correlated with the density of all gametocytes (AD = 15.14, d.f. = 1, P < 0.001) and with the density of either sex (males, AD = 14.77, d.f. = 1, P < 0.001; females, AD = 11.79, d.f. = 1, P < 0.001). Male and female gametocyte densities were highly correlated with each other (r = 0.70, P < 0.0001), and consequently neither sex predicted the proportion of infected mosquitoes significantly better than the other.

Sex ratio and proportion of infected mosquitoes

The probability that a gametocyte carrier was infective (i.e., infected at least one mosquito) was unrelated to the sex ratio (AD = 0.88, d.f. = 1, NS). Gametocyte sex ratio in carriers was unrelated to the proportion of infected mosquitoes (AD = 1.92, d.f. = 1, NS; Fig. 2), even considering only those gametocyte carriers whose sex ratio estimates were based on 15 or more gametocytes (AD = 1.24, d.f. = 1, NS). Spearman's rank correlations on all data, or on sex ratios based on 15 or more gametocytes, led to the same conclusions (r = 0.19, n = 90, P = 0.08; r = 0.18, n = 46, P = 0.20). However, on theoretical grounds, a linear relationship was not expected: all other things being equal, fertilization rates should reach a peak at the female-biased gametocyte sex ratio which results in a 1:1 gamete sex ratio, and decline at more or less biased ratios as one of the sexes becomes limiting among the gametes. The number of viable gametes per male gametocyte has not been determined; at most, 8 are produced, but morphological evidence suggests that only 4–6 of these are viable (reviewed by Read et al., 1992, p. 391). Given this uncertainty, we examined whether infectivity reached a peak at some sex ratio, as follows. For each gametocyte carrier, we calculated the deviation of the observed gametocyte sex ratio from either (i) the average gametocyte sex ratio observed in the population (1:3.6), (ii) a 1:5 sex ratio, or (iii) a 1:8 sex ratio. Each of these values was fitted to the relevant statistical model as a squared term. Should infectivity reach a peak around one of these values, the parameter estimate for the squared deviation term should be negative and significant, so that smaller and larger values would be less infective. Analyzed in this way, there was no evidence that the proportion of mosquitoes infected was greatest at (i) the observed mean gametocyte sex ratio, (ii) the sex ratio of 1:5, or (iii) the sex ratio of 1:8 which would be expected to result in most zygotes if all male gametes were viable (AD = 0.37, AD = 0.07, AD = 0.20, respectively, d.f. = 1, NS in each case). This qualitative picture was unaltered when sex ratios based on a count of fewer than 15 or 30 gametocytes were excluded from the analysis.

Given that sex ratio is unrelated to the probability that a person is infective, gametocyte carriers that are uninfected for other reasons may introduce unnecessary 'noise' into the analyses. We therefore analysed the effects of sex ratio on the proportion of mosquitoes that became infective in feeds only on people who were infective. Again, there was no effect of sex ratio (r = 0.13, AD = 1.16, d.f. = 1, n = 67, NS). However, this analysis included people with fewer than 15 gametocytes counted. Excluding these less reliable estimates, gametocyte sex ratio was positively correlated with the proportion of infected mosquitoes (r = 0.31, AD = 4.69, d.f. = 1, n = 46, P < 0.05; Fig. 3). This was also apparent in the further reduced data set involving sex ratios based only on counts of 30 or more gametocytes (r = 0.33, AD = 4.33, d.f. = 1, n = 37, P < 0.05); in this reduced data set, gametocyte density did not correlate with the proportion of infected mosquitoes (AD = 1.86, d.f. = 1, n = 46, NS), indicating that at gametocytaemia higher than 240/μL, infected mosquitoes were more likely to infect a new mosquito.
Sex ratio and oocyst loads did not predict the proportion of infected/(mosquitoes uninfected) = a^2 (which in this data set was the best fit logistic probability curve assuming infectivity on a reason­ why should oocyst loads continue to increase as sex ratios increase? If this relationship is causal, it is puzzling for 2 reasons. First, in theory, all other things being equal, maximum infectivity should occur at the sex ratio at which there are just sufficient male gametes to fertilize all the female gametes. At least across the range of gametocyte sex ratio of *P. falciparum* gametocytes contributes to heterogeneity in the probability of a gametocyte carrier being infective. However, we believe this is the first report of a weak but significant relationship between sex ratio and infectivity. Among infective gametocyte carriers, sex ratio was positively related with infectivity, with oocyst prevalence and oocyst load. Sex ratio explained around 10% of the variance in mean oocyst load, about the same or more as is (independently) explained by gametocyte density in the same data set. This sex ratio effect was not detectable amongst all gametocyte carriers in this study (i.e., the infective and uninfected people), presumably because the sex ratio estimates based on very low gametocyte densities were necessarily less reliable, and carriers not infective for some reason obscured the pattern. These reasons could include incompetence or inefficiency of gametocytes, or the presence of transmission-blocking immunity.

**Discussion**

From our results there is no evidence that variation in the sex ratio of *P. falciparum* gametocytes contributes to heterogeneity in the probability of a gametocyte carrier being infective. However, we believe this is the first report of a weak but significant relationship between sex ratio and infectivity. Among infective gametocyte carriers, sex ratio was positively related with infectivity, with oocyst prevalence and oocyst load. Sex ratio explained around 10% of the variance in mean oocyst load, about the same or more as is (independently) explained by gametocyte density in the same data set. This sex ratio effect was not detectable amongst all gametocyte carriers in this study (i.e., the infective and uninfected people), presumably because the sex ratio estimates based on very low gametocyte densities were necessarily less reliable, and carriers not infective for some reason obscured the pattern. These reasons could include incompetence or inefficiency of gametocytes, or the presence of transmission-blocking immunity.
controversial for *P. falciparum* (see Chege & Beier, 1990; Robert et al., 1990). Gametocyte carriers with gametocyte sex ratios very close to 1 may shed light on these issues, especially if they show reduced infectivity; unfortunately, such carriers were rare among our subjects (Fig. 1).

Our results were obtained from gametocyte carriers presenting 2 characteristics: (i) they harboured high gametocytaemia (>55/µL of blood), well known to represent a small part of the general population of the gametocyte carriers in endemic zones, and (ii) many of them were recruited at the beginning of a simple malaria attack. Garnham (1966) suggested that the gametocyte sex ratio decreased during the course of the infection, though we know of no supporting data. It would be interesting to know whether the patterns reported here are interesting to know whether the patterns reported here are found in representative samples of gametocyte carriers and/or in other areas.

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**Announcement**

Fifth International Conference on Travel Medicine
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This conference is organized by the International Society of Travel Medicine and co-sponsored by the World Health Organization, Geneva University Faculty of Medicine, University of Toronto, World Tourism Organization, Centers for Disease Control and Prevention (USA), and the Swiss Society of Tropical Medicine and Parasitology.

Abstracts must be submitted by 1 November 1996. The conference fees are Swiss francs 480 before 1 November 1996 and Swiss francs 600 after that date (reduced fees of Swiss francs 270 and 370, respectively, for students and nurses; ISTM members may deduct Swiss francs 20 from all fees).

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