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

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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 proportion of male gametocytes 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 ratios higher than 0.5 predicted a proportion of infected mosquitoes 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 gametes was the primary determinant of fertilization success. These results raise two 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 sexes, sex determination cannot be due to segregation of chromosomes (Carter & Graves, 1988). Several factors have been identified as influencing the success of the infection of mosquitoes. Considering only Plasmodium falciparum, both gametocyte density (Boyd, 1949) and the sickle cell trait status of gametocyte carriers (Robert et al., 1996) increase infectivity; those acting negatively include specific and non-specific transmission blocking factors (Sinden & Smalley, 1976; Mulder et al., 1994). Despite the sexual reproduction of P. falciparum in the midgut of mosquitoes, the sex ratio of gametocytes has generally not been considered a factor influencing infectivity to mosquitoes (Boudin et al., 1989a; Read et al., 1992; Noden et al., 1994; Boyd et al., 1995). They are apparently alone in suggesting that variations in sex ratio, perhaps due to variations in microgametocyte densities, might affect the infectivity of gametocyte carriers. We studied the effects of the gametocyte sex ratio on the infectivity of naturally infected carriers of P. falciparum gametocytes to anophelines by means of experimental infections conducted in the town of Yaoundé, Cameroon.

Materials and Methods

Experimental infections

Thick blood films were prepared from patients with malaria-like complaints and stained with Giemsa's stain. Gametocyte density was based on a count against 1000 leucocytes, assuming an average number of 8000 leucocytes/μL of blood. Gametocyte sex was determined based on the 5 classical criteria (Carter & Graves, 1988): (i) females are larger than males, (ii) the ends of the cells are angular in females and round in males, (iii) the nucleus is smaller in females than in males, (iv) the granules of malaria pigment are centrally located in females and more widely scattered in males, and (v) the cytoplasm stains deep blue in females and pale purple in males. Throughout, we defined sex ratio as the ratio of males to females, but most of the analyses were done using the proportion of gametocytes that were male. Venous blood was collected and immediately placed in a Parafilm™ membrane feeder and maintained at 37°C. Batches of a local strain of Anopheles gambiae s.s. were allowed to feed for 15 min. Midguts were dissected and examined for oocysts after 7 d as described previously (Tchuinkam et al., 1993).

Enrolment criteria were: at least 20 mosquitoes dissected for each experimental feed, with volunteers being at least 4 years old, not infected with species of Plasmodium other than P. falciparum and with a gametocyte density ≥55 μL (at least 7 gametocytes observed in the thick blood film).

Data analysis

When the dependent variable was a proportion (sex ratio, or proportion of mosquitoes infected), logistic regression models with Williams' correction for over-dispersion (Collet, 1991) were used. Models were fitted to the data using the GLIM® statistical package (Crawley, 1993). Parameter estimates (with twice the associated standard errors as the approximate 95% confidence intervals) were determined by maximum likelihood; statistical significance was tested using change in deviance, which approximates a χ² distribution with the corresponding degrees of freedom. Other regressions were simple least squares. Data on mean cell load (total oocysts/number of dissected mosquitoes) and densities of trophozoites (+) and gametocytes were log-transformed.

When sex ratio was an independent variable, it was subject to angular transformation. This has the disadvantage that all sex ratios were weighted equally, even though some are based on considerably larger gametocyte counts than others (see below). Consequently, several analyses were repeated, excluding experiments in which sex ratios were based on gametocyte counts of less than 15 or 30.

All the analyses of the effects of sex ratio on infectivity were repeated, controlling statistically for the effects of total gametocyte density (i.e., asking, for a given gametocyte density, whether sex ratio has an effect on infectivity...
Sex ratio and the sex ratio (AD=0.88, d.f.=1, NS; Fig. 2), even considering only those gametocyte carriers whose sex 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=99, P<0.05; 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. 5).

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. Analysed 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 wasunaltered 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 excluded sex ratios of sex ratio on the proportion of mosquitoes that became infected 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/mL, the probability that a gametocyte carrier was infective (i.e., infected at least one mosquito) 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 estimates were based on 15 or more gametocytes (AD=1=24, d.f.=1, NS).
Discussion

From our results there is no evidence that variation in 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 linked with both 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 among 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.

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 ratios present in our study, models assuming a gamete sex ratio of 1:1 conspicuously failed to fit the data (Figs 3 and 4). One possible implication is that more male gametes are required for successful fertilization than would be expected from random mating of gametes, at least given what is currently believed to be the number of viable gametes released per male gamete. Second, the most common sex ratios in *P. falciparum* isolates from Cameroon were associated with lower transmission success. This is somewhat paradoxical: variants producing less female-biased sex ratios, as are evidently found in Cameroon, should be favoured by natural selection since these would maximize the probability of infecting a mosquito. One possibility is that some unrecognized selection pressure may maintain more female-biased sex ratios. Perhaps the benefit of increased infectivity is lessened if the associated higher oocyst loads reduce vectorial capacity (survival, activity, etc.); evidence for that is well established for various *Plasmodium* species (Maier et al., 1987), but remains...
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 found in representative samples of gametocyte carriers and/or in other areas.

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References


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Announcement

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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.

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