J. Parasitol., 88(2), 2002, pp. 258–263

FERTILITY INSURANCE AND THE SEX RATIOS OF MALARIA AND RELATED HEMOSPORORIN BLOOD PARASITES

Stuart A. West, Todd G. Smith, Sean Nee, and Andrew F. Read
Institute of Cell, Animal and Population Biology, University of Edinburgh, Edinburgh EH9 3JT, U.K. *e-mail: stu.west@ed.ac.uk*

ABSTRACT: The sex ratio (z^*; proportion of gametocytes that are male) of malaria and related hemospororin blood parasites has been predicted to be related to the inbreeding rate (f) by the simple equation $z^* = (1 - f)/2$. Although there is some empirical support for this prediction, there are several cases where the sex ratio is less female biased or more variable than expected. Here, we present a theoretical model that may be able to explain some of these discrepancies. We show that if low gametocyte densities lead to a danger that female gametes may not encounter any male gametes, then natural selection favors a less female-biased sex ratio as a form of 'fertility insurance' to ensure that female gametes are mated. This model can be applied to a number of situations. In particular, (1) empirical data suggest that the number of gametocyes per blood meal can be low enough to favor fertility insurance in some *Plasmodium* infections in humans and (2) our model predicts facultative shifting toward less-biased sex ratios in response to immune pressure that reduces gametocyte or gamete survival or mobility, consistent with some recent experimental data from *Plasmodium* species of birds and mice.

In recent years there has been an increasing interest in the study of gametocyte sex ratios (defined as the proportion of gametocytes that are male) in *Plasmodium* (malaria) and related hemospororin blood parasites (West et al., 2001; Read et al., 2002). In these species, after a period of haploid asexual proliferation in a vertebrate host, transmission to a dipteran vector occurs via dioecious haploid sexual stages, the gametocytes. Within the gut of the dipteran host, female (macro-) gametocytes give rise to a single female gamete, whereas male (micro-) gametocytes release up to 8 viable male gametes. Random mating occurs among gametes within 20 min of a blood meal (Ranford-Cartwright et al., 1993; Babiker et al., 1994; Taylor et al., 1997). Importantly, this can lead to inbreeding because malaria populations are structured, with the gametes competing for matings being those found in a single blood meal (and, therefore, from a single host), rather than the gametocyte populations in many hosts.

Research has focused on applying a classic result from evolutionary theory (Hamilton, 1967), which predicts that the unbeatable gametocyte sex ratio (defined as the gametocyte sex ratio that leads to the highest fitness and cannot be beaten by any other strategy, z^*) should be correlated to the inbreeding rate (f) by the equation $z^* = (1 - f)/2$ (Fig. 1; Read et al., 1992). This simple equation has been relatively successful in explaining the gametocyte sex ratios observed in natural populations of *Plasmodium* and *Leucocytozoon* species (West et al., 2001; Read et al., 2002). Specifically, (1) the sex ratios observed in natural populations are generally female-biased; (2) more female-biased sex ratios are observed in populations where parasite prevalence is lower and inbreeding rates are expected to be lower; and (3) in cases where we have sex ratio data and direct genetic estimates of the inbreeding rate, they are in quantitative agreement.

However, this simple equation has not been able to explain all the patterns observed, especially those from species of the closely related hemospororin genus *Haemoproteus*. Specifically, (1) across *Haemoproteus* populations in birds there is no relationship between sex ratio and parasite prevalence (Shutler et al., 1995; Shutler and Read, 1998) and (2) data from several *Plasmodium* species show that estimates of the sex ratio taken at different stages from the same infection can be extremely variable (Schall, 1989; Taylor, 1997; Paul et al., 1999, 2000, 2002).

One possible explanation for these discrepancies is 'fertility insurance' (West et al., 2001). If there is a danger that female gametes may not encounter any male gametes, then natural selection would favor a less female-biased sex ratio as a form of fertility insurance to ensure that female gametes are mated. Shutler and Read (1998) suggested that this could explain the pattern in *Haemoproteus* parasites, where the small vectors take small blood meals, because it could lead to low numbers of gametocytes per blood meal (termed the 'vector size hypothesis'). Analogously, Paul et al. (1999, 2000, 2002) argued that this could explain the variable sex ratios within infections of *Plasmodium* species if less female-biased sex ratios occurred at times when immune pressure reduces gametocyte survival or gamete survival or mobility. Here we formalize this intuitive argument and discuss whether fertility insurance can account for previously inexplicable sex ratio variation.

A FERTILITY INSURANCE MODEL

The basic prediction of sex ratio theory applied to hemospororin blood parasites is that the unbeatable gametocyte sex ratio (z^*) should be correlated to the inbreeding rate (f) by the equation $z^* = (1 - f)/2$ (Fig. 1; Read et al., 1992). The unbeatable sex ratio should therefore decline from 0.5 for complete outcrossing ($f = 0$) to 0 for complete inbreeding ($f = 1$), the latter interpreted as meaning that a lineage (clone) should produce the minimum number of male gametocytes needed to ensure fertilization of the female gametes it produces. Specifically, if c is the mean number of viable gametes released by a male gametocyte, then the most female biased that the unbeatable sex ratio is predicted to ever become is $1/(1 + c)$ (Fig. 1; Read et al., 1992). This predicted lower limit on the sex ratio, set by the number of viable gametes, represents a form of fertility insurance. However, it implicitly assumes that the gametes from a very large (effectively infinite) number of gametocytes are able to interact in a blood meal—our aim here is to relax this assumption and consider a more complicated form of fertility insurance.

Here, we consider how the unbeatable sex ratio is influenced by low gametocyte densities, leading to a danger that female gametes may not encounter any male gametes and hence remain

Received 11 April 2001; revised 10 October 2001; accepted 10 October 2001.

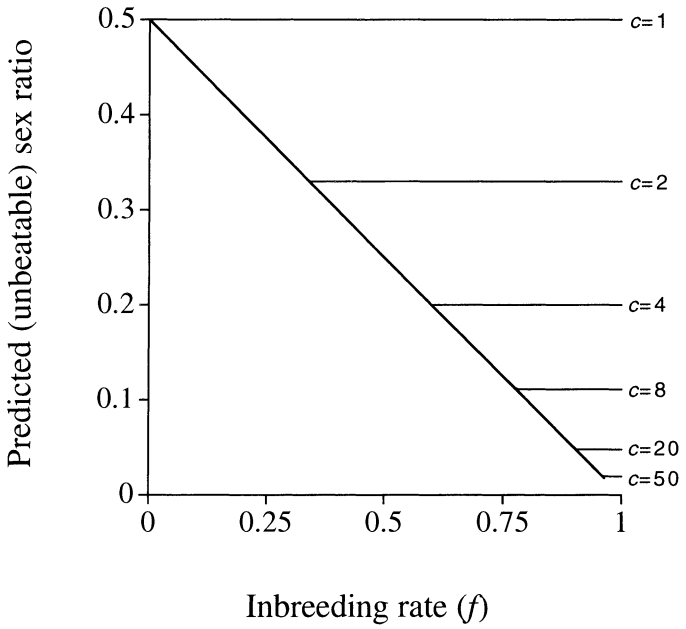

FIGURE 1. The unbeatable sex ratio (z^*) plotted against the inbreeding rate (f) for various values of c, the average number of viable gametes released from a male gametocyte after exflagellation. This figure assumes that the gametes from a very large number (effectively infinite) of gametocytes are able to interact.

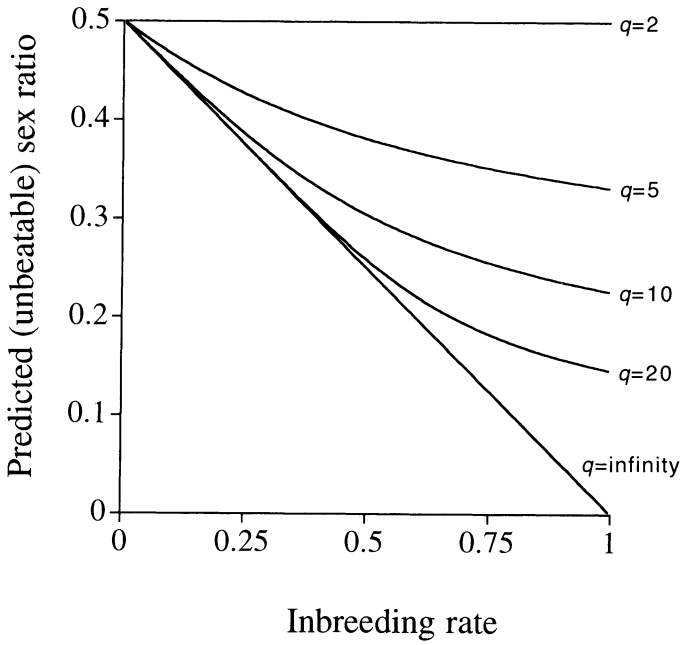

FIGURE 2. The optimal sex ratio (z^*) plotted against the inbreeding rate (f) for various numbers of gametocytes whose gametes are able to interact (q). Note that as q becomes smaller (especially <20), a less female-biased sex ratio is favored as a form of fertility insurance.

unfertilized. There is good evidence, at least for *Plasmodium* spp., that fertilization is an important bottleneck (Vaughan et al., 1992; Gouagna et al., 1998). Fertilization leads to a spherical zygote that protrudes into a retort form before becoming an elongated motile ookinete. The number of retort forms of *Plasmodium falciparum* in *Anopheles gambiae* are some 40-fold lower than the number of macrogametocytes in a blood meal (range, 2- to 3,000-fold). Much of this reduction is probably because of unsuccessful fertilization. Female gametocytes can be unmated for a number of nonexclusive reasons, including (1) small blood meals or low gametocyte densities (or both) leading to an appreciable chance that a blood meal does not contain any male gametocytes (Shutler and Read, 1998); (2) high mortality of male gametocytes or gametes (Paul et al., 2000); and (3) low mobility of gametes.

One possible way to examine this scenario would be to make assumptions about how gametes move and then model the probabilities of gametes interacting (Paul et al., 1999). Unfortunately, this approach is hampered by our lack of knowledge of exactly how gametes move within a blood meal. It is known that flagellated male gametes are motile and are likely to be attracted to nonmotile female gametes, but gamete-specific fertilization receptors have not yet been identified (Sinden, 1998).

The lack of knowledge of how gametes move has led us to take a more heuristic approach. We assume that each blood meal is made up of l groups of q gametocytes, with a total of ql gametocytes in the blood meal. These groups represent areas within which gametes are able to interact. We assume that within each group, the gametes can only interact with gametes from the other $q - 1$ gametocytes in their group. If all the gametes within a blood meal can interact, then $l = 1$. Our model assumes that each male gametocyte can produce a large number of gametes (c) and hence examines how the possibility that there will be no male gametocytes in a blood meal (or in a group of gametocyctes that can interact) influences the unbeatable sex ratio. The model we present here ignores the need for fertility insurance caused by a low c value (Fig. 1), which has already been discussed in detail by Read et al. (1992).

In the appendix, we show that, as expected from verbal arguments, for a given inbreeding rate, the unbeatable sex ratio becomes less female-biased with decreasing numbers of gametocytes able to interact. Specifically, the unbeatable sex ratio (z^*) is related to the inbreeding rate (f) and the average number of gametocytes whose gametes are able to interact in a blood meal (q) by the equation

$$f = \frac{(1 - 2z^*)(z^* - 1 + (1 - z^*)^q)}{z^* - 1 + (1 - z^*)^q(1 + 2z^*(q - 1))}. \quad (1)$$

This presentation of this equation is slightly confusing because the inbreeding rate (f) is given as the subject on the left hand side, yet we are interested in how the inbreeding rate influences the unbeatable sex ratio (z^*)—however, it must be written in this form because it cannot be solved with z^* as the subject. Nonetheless, Equation 1 can be solved numerically to predict the unbeatable sex ratio for a given inbreeding rate and number of gametocytes whose gametes can interact (Fig. 2). For a given inbreeding rate, the optimal sex ratio becomes less female-biased with decreasing numbers of gametocytes. Note that as $q \to \infty$, Equation 1 reduces to $z^* (1 - f)/2$, the classic local mate competition (LMC) model of Hamilton (1967) and that as $q \to 2$, Equation 1 reduces to $z^* = 0.5$. Aside from its importance for the purposes of this paper, this result provides another way of showing that a sex ratio of 0.5 is favored when syzygy occurs, as in that case $q = 2$ (see West, Smith, and Read, 2000).

This favoring of a less female-biased sex ratio with decreasing q, to produce 'insurance' males, is qualitatively similar to

that predicted and observed with invertebrate species that produce small clutch sizes (Griffiths and Godfray, 1988; Nagelkerke and Hardy, 1994; Nagelkerke, 1996; West et al., 1997; Hardy et al., 1998; West and Herre, 1998). Equation 1 predicts the average sex ratio of a population, and if lineages are able to assess the importance of fertility insurance (for example, through gametocyte densities or immune response related factors), facultative adjustment of the sex ratio.

DISCUSSION

Fertility insurance

Inbreeding leads to selection for female-biased sex ratios (Hamilton, 1967). If there is a danger that female gametes may not get mated by male gametes, then a less female-biased sex ratio is favored by natural selection as a form of fertility insurance. We distinguish between two forms of fertility insurance, depending upon whether the danger that a female gamete does not get fertilized is because of (1) low numbers of viable gametes being released per male gametocyte (modeled by Read et al., 1992; Fig. 1) or (2) low gametocyte densities (modeled in this paper; Fig. 2). The effect of low gametocyte densities arises because it introduces a (stochastic) chance that there will be no male gametocytes in a group of gametocytes whose gametes will mate. Our model formalizes this idea with a parameter that represents the average number of gametocytes whose gametes are able to interact in a blood meal (q)—each blood meal is assumed to consist of l such groups. If all the gametes in a blood meal can interact, then $l = 1$, and q is the total number of gametocytes in the blood meal.

How low must q be before fertility insurance significantly alters the unbeatable sex ratio? This depends on 2 things: f, the inbreeding rate, and c, the mean number of viable gametes released by a male gametocyte. When the inbreeding is sufficiently high, fertility insurance will alter the unbeatable sex ratio if q is sufficiently low, e.g., when $f > 0.25$ or 0.50 and $q = 10$ or 20, respectively (Fig. 2). But, when c is low, the extent of female bias favored by natural selection is bounded by the need to produce enough males to fertilize the females (Fig. 1), and so even lower values of q are required to alter the unbeatable sex ratio; e.g., if $c = 2$, then $q < 5$–10 would be required to change the unbeatable sex ratio when $f > 0.5$ (compare Fig. 1 with Fig. 2).

How important is fertility insurance?

The number of gametes released from a male gametocyte (c) is highly variable within the Apicomplexa. In some eimeriorin species, 100s or 1000s of gametes are produced (West, Smith, and Read, 2000), in which case fertility insurance because of a low q definitely could influence the unbeatable sex ratio. In *Plasmodium*, *Haemoproteus*, and *Leuctozoon* species, a maximum of 8 male gametes are released per male gametocyte (although this estimate is based on data from a perilously low number of species), so that the maximum female bias that selection could favor, even at extremely highly levels of selfing, is 0.11 (= 1/9; Read et al., 1992; Fig. 1). However, many of the 8 gametes are thought to be inviable on the basis of morphology. For example, in *Plasmodium mexicanum*, a lizard malaria, the number of flagella seen on the male gametocyte suggests that the modal number of viable gametes is around 2 (Schall, 2000). In these cirucumstances, the maximum female bias that could be favored by natural selection is 0.33. Consequently, fertility insurance because of a low q would only influence the unbeatable sex ratio at intermediate levels of inbreeding ($0.25 < f < 0.75$) and when q is very low (<5). Although data on this species suggest that there are many more gametocytes per blood meal than this (Schall, 2000), it is not known what fraction of these will be able to interact, i.e., the value of l is not known. Nonetheless, in this species, the mean sex ratio is approximately equal to 0.33 (mean = 0.372), suggesting that $f \geq 0.33$ and that $q \geq 5$.

For *P. falciparum*, the number of viable gametes per gametocyte is in the region of 4 (Read et al., 1992). This would place a lower bound on the optimal sex ratio of around 0.20 (Fig. 1), so that fertility insurance because of a low q could play a role in reducing the female bias for selfing rates over about 0.5 and when q is less than approximately 15. Is q in this region? The number of gametocytes in a blood meal provides an upper limit on q, i.e., assuming $l = 1$. Within human populations, gametocyte densities are highly variable, with maxima in excess of 100 or even 1,000 gametocytes per blood meal (Tchuinkam et al., 1993; Robert, Read, et al., 1996; Robert, Tchuinkam, et al., 1996; Taylor and Read, 1997). Taking blood meal size as 1–3 μl and using measurements of gametocyte density in blood, it is clear that (1) in some patients at least, densities in the region of <20 gametocytes per blood meal can still result in infected mosquitoes and (2) some human populations have mean gametocyte densities in infected people that would lead to <10 gametocytes per blood meal (Boudin et al., 1993; Tchuinkam et al., 1993; Robert et al., 1995). In these cases, the unbeatable sex ratio will be less female biased. We note, however, that gametocytes can aggregate in the blood (Pichon et al., 2000) and such overdispersion will increase the average number of gametocytes per blood meal in those meals that do contain gametocytes. This would counteract the need for fertility insurance because of a low q and emphasizes the importance of studying gametocyte distributions in blood meals and not just the average density in blood sampled by researchers.

How does fertility insurance because of a low q influence our ability to use sex ratio data to estimate inbreeding rates (Read et al., 1992; West, Herre, and Sheldon, 2000; West, Smith, and Read, 2000; Nee et al., 2002)? Whether the selection on sex ratio imposed by low gametocyte densities in some people will have a detectable effect of observed sex ratios will depend on the exact details of the distribution of gametocyte densities per host in a population and how this frequency distribution relates to total transmission. In particular, fertility insurance because of low q will not be a problem for estimating inbreeding rates if most transmission and most sex ratio data come from hosts with high gametocyte densities. In this case, we would still expect facultative sex ratio shifts in response to low gametocyte densities, but the cases where this occurs would have a relatively weak influence on the mean population sex ratio. In the 2 *P. falciparum* populations for which we have the best sex ratio data, Papua New Guinea (Read et al., 1992) and Cameroon (Robert, Read, et al., 1996), mean population sex ratios are around 0.2, the maximum degree of female bias expected if $c = 4$, implying that fertility insurance because of a

TABLE I. Estimates of the number of gametocytes in a blood meal from *Haemoproteus* species in birds. Ceratopogonidae (*Culicoides* spp.), the biting midges, are the vectors for *Haemoproteus* species that infect birds in the northern hemisphere. We obtained an estimate of their blood size meal by averaging that taken by *Culicoides austeni* (0.20 μl) and *Culicoides grahami* (0.07 μl), 2 species with comparable wing lengths that bite humans in Africa (Hopkins and Nicholas, 1953). Gametocytaemia data were obtained from this study, slides provided by S. Desser, Ontario, Canada*, and Shutler et al. (1995)†. The number of erythrocytes per microliter of bird blood was assumed to be 3.5×10^6 (Andrew, 1965).

Species	Size of blood meal (μl)	Gametocytaemia (%)	Gametocytes per microliter of blood	Gametocytes per blood meal
Haemoproteus nettionis	0.14	0.43*	15,050	2,100
Haemoproteus smithi	0.14	2.55*	89,250	12,495
Haemoproteus simondi	0.14	1.16*	40,600	5,684
Various	0.14	<5.00†	<175,000	<24,500

low q does not impact at a population-wide level on the favored sex ratio.

Can fertility insurance explain the inexplicable data?

Data on the number of gametocytes per blood meal allow us to test the vector size hypothesis of Shutler and Read (1998). In *Haemoproteus* populations with putatively high levels of selfing, sex ratios are not as female biased as expected. Fertility insurance because of a low q could account for this (Fig. 2) if, because of small vector size, the number of gametocytes per blood meal is low enough that providing enough male gametocytes to fertilize the females becomes important, i.e., $q < 20$. However, the number of gametocytes per blood meal is well in excess of this figure (Table I). Thus, in its strictest sense, the vector size hypothesis is falsified. However, fertility insurance could still be the factor explaining the disparate results observed in *Haemoproteus*. For example, a characteristic of the gut of vectors of these parasites could make it more difficult for male gametes to locate female gametes (termed the vector environment hypothesis by Shutler and Read [1998]), possibly because of the smaller vectors concentrating blood as they feed or the male gametes of *Haemoproteus* species being less efficient at locating female gametes. In these examples, the effective consequence, a higher l and a lower q, would be the same as with the vector size hypothesis. Alternatively, the pattern in *Haemoproteus* could be explained if the number of viable gametes per male gametocyte (c) was sufficiently low—for example, if the mean value of c was approximately 2, as is the case in *P. mexicanum* described previously.

Our model also predicts facultative shifts in the sex ratio. For example, a less female-biased sex ratio is favored when (1) gametocyte densities are lower; (2) gametocytes suffer higher mortality; and (3) male gametes are less able to move, find, and fertilize female gametes, i.e., q is lower, because of a higher l. Work on the avian malaria parasite *Plasmodium gallinaceum* and the murine malaria parasite *Plasmodium vinckei* are consistent with this prediction (Paul et al., 1999, 2000, 2001). In *P. gallinaceum*, the sex ratio was observed to become less female biased during the course of an infection, correlating with an increase in both hematologic and immune factors that may adversely affect gamete fertilization efficiency of the malaria parasite in the mosquito midgut. However, as with the *Haemoproteus* data, it should be noted that this pattern could also be explained by fertility insurance in response to a variable number of viable gametes per male gametocyte (c), with a less female-biased sex ratio being favored if the mortality of male gametocytes or gametes is increased relative to that of female gametes or gametocytes. Indeed, this emphasizes how the same factors could potentially lead to fertility insurance in response to both low q and low c.

If parasites are adjusting their gametocyte sex ratios facultatively in response to the problem of fertility insurance, then we might expect to observe, across infections, the sex ratio to be negatively correlated with gametocyte density, as has been observed in *Plasmodium* in humans (Paul et al., 2001). However, a number of other studies have reported positive correlations (Shutler et al., 1995; Taylor, 1997; Pickering et al., 2000; Schall, 2000), and in other populations, no relationships have been found (Shutler et al., 1995; Robert, Read, et al., 1996; reanalysis of data collected by Read et al. [1992] gives $r_s = -0.32$, $P = 0.21$, n = 14). These discrepancies may be accounted for by the contrasting effects of inbreeding and fertility insurance if sex allocation is facultative: gametocyte densities can increase with the number of clones per host (Taylor et al., 1997), but more clones per host reduces selfing and hence favors *less* female-biased sex ratios. An important consideration, and one that requires future work, is that most sex ratio estimates from natural populations are derived from infections with high gametocyte densities, e.g., >100 gametocytes per blood smear. The volume of blood in a blood smear is of the same order of magnitude as that in a blood meal, and so we may not yet be estimating sex ratios from hosts who have gametocyte density levels low enough to favor facultative sex ratio shifts in response to fertility insurance because of a low q.

Conclusions and future directions

More generally, we conclude by emphasizing the enormous potential for future research in this area (Paul et al., 2002; West et al., 2001). Our theoretical model provides a framework with which empirical work could be used to test the potential role of fertility insurance because of low gametocyte densities. In particular, further progress in this area requires more detailed knowledge of (1) parasite–vector interactions; (2) how gametes locate each other within the blood meal; (3) the consequences of the vertebrate immune response for gametocyte or gamete survival and mobility; (4) the environmental cues influencing sex determination; (5) the effects of vectors concentrating blood on fertilization efficiency; and (6) the extent to which gametocyte aggregation in the blood reduces the problem of fertility insurance. For example, anecdotal evidence on the production of zygotes even when gametocytes are at extremely low densities in *Plasmodium* species suggests that male gametes are able to locate female gametes highly efficiently (Carter and Graves, 1988; Tchuinkam et al., 1993). Once we understand how this happens for the different parasite genera, in different vectors, then we can estimate l and hence determine the extent to which male gametes are a limiting factor for the different parasite species.

ACKNOWLEDGMENTS

We thank R. Carter, S. Desser, R. Lainson, I. Paperna, R. Paul, J. Schall, D. Shutler, D. Walliker, and especially L. Ranford-Cartwright for useful discussion and comments on the manuscript. Funding was provided by the BBSRC and NERC (S.A.W. and A.F.R.) and NSERC of Canada (T.G.S.).

LITERATURE CITED

ANDREW, W. 1965. Comparative hematology. Grune and Stratton, New York, New York, 188 p.

BABIKER, H. A., L. RANFORD-CARTWRIGHT, D. CURRIE, J. D. CHARLWOOD, P. BILLINGSLEY, T. TEUSCHER, AND D. WALLIKER. 1994. Random mating in a natural population of the malaria parasite *Plasmodium falciparum*. Parasitology **109:** 413–421.

BOUDIN, C., M. OLIVIER, J. M. MOLEZ, J. P. CHIRON, AND P. A. THOMAS. 1993. High human malarial infectivity to laboratory-bred *Anopheles gambiae* in a village in Burkina Faso. American Journal of Tropical Medicine and Hygiene **48:** 700–706.

CARTER, R., AND P. M. GRAVES. 1988. Gametocytes. *In* Malaria. Principles and practice of malariology, W. H. Wernsdorfer and I. McGregor (eds.). Churchill Livingstone, Edinburgh, U.K., p. 253–305.

DYE, C., AND H. C. F. (*Sic*) GODFRAY. 1993. On sex ratio and inbreeding in malaria parasite populations. Journal of Theoretical Biology **161:** 131–134.

FRANK, S. A. 1998. Foundations of social evolution. Princeton University Press, Princeton, New Jersey.

GOUAGNA, L. C., B. MULDER, E. NOUBISSI, T. TCHUINKAM, J. P. VERHAVE, AND C. BOUDIN. 1998. The early sporogonic cycle of *Plasmodium falciparum* in laboratory-infected *Anopheles gambiae*: An estimation of parasite efficiency. Tropical Medicine and International Health **3:** 21–28.

GRIFFITHS, N. T., AND H. C. J. GODFRAY. 1988. Local mate competition, sex ratio and clutch size in bethylid wasps. Behavioural Ecology and Sociobiology **22:** 211–217.

HAMILTON, W. D. 1967. Extraordinary sex ratios. Science **156:** 477–488.

HARDY, I. C. W., L. J. DIJKSTRA, J. E. M. GILLIS, AND P. A. LUFT. 1998. The influence of developmental mortality on optimal sex allocation under local mate competition. Biological Journal of the Linnean Society **64:** 239–270.

HOPKINS, C. A., AND W. L. NICHOLAS. 1953. *Culicoides austeni*, the vector of *Acanthocheilonema perstans*. Annals of Tropical Medicine and Parasitology **47:** 276–283.

NAGELKERKE, C. J. 1996. Discrete clutch sizes, local mate competition, and the evolution of precise sex allocation. Theoretical Population Biology **49:** 314–343.

———, AND I. C. W. HARDY. 1994. The influence of developmental mortality on optimal sex allocation under local mate competition. Behavioral Ecology **5:** 401–411.

NEE, S., S. A. WEST, AND A. F. READ. 2002. Inbreeding and parasite sex ratios. Proceedings of the Royal Society London Series B. (in press)

PAUL, R. E. L., P. T. BREY, AND V. ROBERT. 2002. *Plasmodium* sex determination and transmission to mosquitoes. Parasitology Today **18:** 32–38.

———, T. N. COULSON, A. RAIBAUD, AND P. T. BREY. 2000. Sex determination in malaria parasites. Science **287:** 128–131.

———, A. RAIBAUD, AND P. T. BREY. 1999. Sex ratio adjustment in *Plasmodium gallinaceum*. Parasitologica **41:** 153–158.

PICHON, G., H. P. AWONO-AMBENE, AND V. ROBERT. 2000. High heterogeneity in the number of *Plasmodium falciparum* gametocytes in the bloodmeal of mosquitoes fed on the same host. Parasitology **121:** 115–120.

PICKERING, J., A. F. READ, S. GUERRERO, AND S. A. WEST. 2000. Sex ratio and virulence in two species of lizard malaria parasites. Evolutionary Ecology Research **2:** 171–184.

RANFORD-CARTWRIGHT, L. C., P. BALFE, R. CARTER, AND D. WALLIKER. 1993. Frequency of cross-fertilisation in the human malaria parasite *Plasmodium falciparum*. Parasitology **107:** 11–18.

READ, A. F., M. ANWAR, D. SHUTLER, AND S. NEE. 1995. Sex allocation and population structure in malaria and related parasitic protozoa. Proceedings of the Royal Society London Series B **260:** 359–363.

———, A. NARARA, S. NEE, A. E. KEYMER, AND K. P. DAY. 1992. Gametocyte sex ratios as indirect measures of outcrossing rates in malaria. Parasitology **104:** 387–395.

———, T. G. SMITH, S. NEE, AND S. A. WEST. 2002. Sex ratios of malaria parasites and related protozoa. *In* Sex ratios: Concepts and research methods, I. C. W. Hardy (ed.). Cambridge University Press, Cambridge, U.K. (in press)

REECE, S., AND A. F. READ. 2000. Malaria sex ratios. Trends in Ecology and Evolution **15:** 259–260.

ROBERT, V., G. L. GOFF, J. ESSONG, T. TCHUINKAM, B. FAAS, AND J. P. VERHAVE. 1995. Detection of *falciparum* malarial forms in naturally infected Anophelines in Cameroon using a fluorescent anti-25-kD monoclonal antibody. American Journal of Tropical Medicine and Hygiene **52:** 366–369.

———, A. F. READ, J. ESSONG, T. TCHUINKAM, B. MULDER, J. P. VERHAVE, AND P. CARNEVALE. 1996. Effect of gametocyte sex ratio on infectivity of *Plasmodium falciparum* to *Anopheles gambiae*. Transactions of the Royal Society of Tropical Medicine and Hygiene **90:** 621–624.

———, T. TCHUINKAM, B. MULDER, J.-M. BODD, J.-P. VERHAVE, P. CARNEVALE, AND R. L. NAGEL. 1996. Effect of the sickle cell trait status of gametocyte carriers of *Plasmodium falciparum* on infectivity to Anophelines. American Journal of Tropical Medicine and Hygiene **54:** 111–113.

SCHALL, J. J. 1989. The sex ratio of *Plasmodium* gametocytes. Parasitology **98:** 343–350.

———. 2000. Transmission success of the malaria parasite *Plasmodium mexicanum* into its vector: Role of gametocyte density and sex ratio. Parasitology **121:** 575–580.

SHUTLER, D., G. F. BENNETT, AND A. MULLIE. 1995. Sex proportions of *Haemoproteus* blood parasites and local mate competition. Proceedings of the National Acadamy of Sciences, USA **92:** 6748–6752.

———, AND A. F. READ. 1998. Extraordinary and ordinary blood parasite sex ratios. Oikos **82:** 417–424.

SINDEN, R. E. 1998. Gametocytes and sexual development. *In* Malaria: Parasite biology, pathogenesis and protection, I. W. Sherman (ed.). American Society for Microbiology, Washington, D.C., p. 25–48.

SMITH, T. G., P. LOURENCO, R. CARTER, D. WALLIKER, AND L. C. RANFORD-CARTWRIGHT. 2000. Commitment to sexual differentiation in the human malaria parasite, *Plasmodium falciparum*. Parasitology **121:** 127–133.

TAYLOR, L. H. 1997. Epidemiological and evolutionary consequences of mixed-genotype infections of malaria parasites. Ph.D. Disseration. University of Edinburgh, Edinburgh, U.K.

———, AND A. F. READ. 1997. Why so few transmission stages? Reproductive restraint by malaria parasites. Parasitology Today **13:** 135–140.

———, D. WALLIKER, AND A. F. READ. 1997. Mixed-genotype infections of malaria parasites: Within-host dynamics and transmission success of competing clones. Proceedings of the Royal Society London Series B **264:** 927–935.

TAYLOR, P. D., AND M. G. BULMER. 1980. Local mate competition and the sex ratio. Journal of Theoretical Biology **86:** 409–419.

———, AND S. A. FRANK. 1996. How to make a kin selection model. Journal of Theoretical Biology **180:** 27–37.

TCHUINKAM, T., B. MULDER, K. DECHERING, H. STOFFELS, J. P. VERHAVE, M. COT, P. CARNEVALE, J. H. E. T. MEUWISSEN, AND V. ROBERT. 1993. Experimental infections of Anopheles gambiae with Plasmodium falciparum of naturally infected gametocyte carriers in Cameroon: Factors influencing the infectivity to mosquitoes. Tropical Medicine and Parasitology **44:** 271–276.

VAUGHAN, J. A., B. H. NODEN, AND J. C. BEIER. 1992. Population dynamics of *Plasmodium falciparum* sporogony in laboratory-infected *Anopheles gambiae*. Journal of Parasitology **78:** 716–724.

WEST, S. A., AND E. A. HERRE. 1998. Stabilizing selection and variance in fig wasp sex ratios. Evolution **52:** 475–485.

———, ———, S. G. COMPTON, H. C. J. GODFRAY, AND J. M. COOK. 1997. A comparative study of virginity in fig wasps. Animal Behaviour **54:** 437–450.

———, ———, AND B. C. SHELDON. 2000. The benefits of allocating sex. Science **290:** 288–290.

———, S. E. Reece, and A. F. Read. 2001. Gametocyte sex ratios of malaria and related apicomplexan (protozoa) parasites. Trends in Parasitology **17:** 525–531.

———, T. G. Smith, and A. F. Read. 2000. Sex allocation and population structure in Apicomplexan (Protozoa) parasites. Proceedings of the Royal Society London Series B **267:** 257–263.

APPENDIX

In this section, we determine how the number of gametocytes whose gametes are able to interact within a blood meal influences the unbeatable sex ratio. Our starting point is the canonical model of LMC (Hamilton, 1967; Taylor and Bulmer, 1980), which has been discussed previously as a model for malaria and related apicomplexan parasites (Read et al., 1992; Dye and Godfray, 1993; Read et al., 1995; Pickering et al., 2000; Reece and Read, 2000; West, Herre, and Sheldon, 2000; West, Smith, and Read, 2000; West et al. 2001; Read et al., 2002; Nee et al., 2002). We modify this model of competition among the $2n$ haploid lineages in an infection (which arise from n diploid zygotes initiating the infection) by including the following term:

$$g(y,z) = \Pr\{\text{a female is mated: a function of } y, \text{ the sex ratio played by a focal (clonal) lineage and } z, \text{ the sex ratio played by the others in the group}\},$$

where sex ratio is the proportion of male gametocytes produced by a lineage. Note that the sex ratio is decided by the haploid lineages, after several generations of asexual proliferation in the haploid state, and not by the diploid zygote "parent" (a single haploid stage can give rise to male and female gametocytes, with sex being determined just before the production of gametocytes—before the nuclear division of the sexually-committed schizont; Smith et al. [2000]).

Fitness, w, is then given by

$$w = (1 - y)g(y, z) + \frac{y}{(2n - 1)z + y}((2n - 1)(1 - z) + (1 - y))g(y, z) \quad \text{(A1)}$$

Following the procedure of Taylor and Frank (1996) and Frank (1998), we find the ESS (unbeatable) sex ratio, z^*, by solving

$$\left.\frac{\partial w}{\partial y}\right|_{y=z=z^*} + \phi\left.\frac{\partial w}{\partial y}\right|_{y=z=z^*} = 0, \quad \text{(A2)}$$

which gives

$$(2n - 1 - 4nz^*)g + 4nz^*(1 - z^*)g_y + \phi(4nz^*(1 - z^*)g_z - (2n - 1)g) = 0, \quad \text{(A3)}$$

where g_i denotes the partial derivative of the function g. ϕ is the correlation between the sex ratio played by the focal lineage and a randomly chosen member of the group *excluding* the focal lineage. ϕ is related to f, the correlation between the sex ratio played by the focal lineage and a randomly chosen member of the group *including* the focal lineage by the following relationship:

$$\phi = \left(f - \frac{1}{2n}\right)\frac{2n}{2n - 1}. \quad \text{(A4)}$$

This can be derived from the decomposition of f into its component correlation coefficients, which is discussed in detail by Nee et al. (2002). f is Wright's coefficient of inbreeding and can also be interpreted as the average relatedness of 2 randomly chosen members of the group. Note that ϕ is nonzero even if the n diploid "parents" are independent, because a lineage will be related to the other lineage that arises from the same diploid zygote. Readers familiar with LMC theory as presented by, for example, Frank (1998), may wonder why our basic model does not simply use group average sex ratio instead of the terms that are a sum of the sex ratio played by the focal lineage and the sex ratio played by the *other* members of the group (which is why ϕ rather than f enters Eq. A3). The reason is that we need to keep these separate in order to have the function g behave sensibly.

In order to make further progress we need to specify a function for the probability that a female is mated (g). In order to do so we assume that the gametes of q gametocytes are able to interact in a blood meal. In this case, it can be shown that

$$g(y, z) = 1 - \left(1 - \frac{y + (2n - 1)z}{2n}\right)^{q-1}. \quad \text{(A5)}$$

Using this definition for g, we solve Equation A3 to find the following implicit formula for z^*:

$$f = \frac{(1 - 2z^*)(z^* - 1 + (1 - z^*)^q)}{z^* - 1 + (1 - z^*)^q(1 + 2z^*(q - 1))}. \quad \text{(A6)}$$

If all female gametocytes are mated ($q \to \infty$), this equation simplifies to the classical Hamilton (1967) model ($f = [1 - 2z^*]$), whose relevance to apicomplexan parasites is discussed in detail elsewhere (Nee et al., 2002).