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Local mate competition and transmission bottlenecks: A new model for understanding malaria parasite and other sex ratios

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HIGHLIGHTS

- Local mate competition assumes females increase transmission to the next generation.
- Malaria female production and transmission success may not be linearly related.
- We model the effect of a non-linear female-transmission relationship on sex ratio.
- Decreasing fitness returns on female production favors less investment in females.
- More offspring per patch also favors more equal investment in males and females.

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ABSTRACT

The local mate competition model from sex ratio theory predicts female-biased sex ratios in populations that are highly subdivided during mating, and is thought to accord well with the population structure of malaria parasites. However, the selective advantage of female-biased sex ratios comes from the resulting increase in total reproductive output, an advantage the transmission biology of malaria parasite likely reduces. We develop a mathematical model to determine how bottlenecks in transmission that cause diminishing fitness returns from female production affect sex ratio evolution. We develop four variations of this model that incorporate whether or not parasite clones have the ability to detect others that occupy the same host and whether or not the number of clones affects the total mating population size. Our model indicates that transmission bottlenecks favor less female-biased sex ratios than those predicted under LMC. This effect is particularly pronounced if clones have no information about the presence of coexisting clones and the number of mating individuals per patch is fixed. The model could extend our understanding of malaria parasite sex ratios in three main ways. First, it identifies inconsistencies between the theoretical predictions and the data presented in a previous study, and proposes revised predictions that are more consistent with underlying biology of the parasite. Second, it may account for the positive association between parasite density and sex ratio observed within and between some species. Third, it predicts a relationship between mortality rates in the vector and sex ratios, which appears to be supported by the little existing data we have. While the inspiration for this model came from malaria parasites, it should apply to any system in which per capita dispersal success diminishes with increasing numbers of females in a patch.

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1. Introduction

Evolutionary models rest on their underlying assumptions, with some models more robust than others to deviations from those assumptions. Stressing a model by altering its assumptions is often very informative, and can lead to intriguing and unexpected

insights. We focus on one example from sex ratio theory, a prominent focus in evolutionary biology that makes quantitative predictions about how natural selection will shape the ratio of males to females in populations (West, 2009). The early models in sex ratio theory, which were conceived by Düsing (1884, translation in Edwards (2000)) and popularized by Fisher (1930), considered large, randomly mating populations. They concluded that equal investment in males and females would be favored because parents producing the less common sex would receive a fitness advantage due to the greater mating success of their offspring. The first major extension of the theory came from Hamilton (1967),

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who showed that modifying certain key assumptions of the Fisherian model could favor strongly biased sex ratios. For example, if, during mating, a population is highly subdivided into patches containing the offspring of one or a few mothers and these offspring compete with one another for breeding opportunities, then female-biased sex ratios can be favored by the resulting increase in the reproductive value of the offspring or, equivalently, in the total number of grand-offspring produced.

This model, termed local mate competition (LMC), has received support from studies of a wide range of arthropods whose population structure matches this model well (reviewed by West, 2009). LMC has also been tested for malaria parasites (*Plasmodium* and related genera sensu Martinsen et al., 2008) with more limited success (reviewed by Schall, 2009). Deviations from the predictions of LMC have often been attributed to adaptive sex ratio adjustments to compensate for limited male fecundity or mating group size within a patch (Fertility Insurance, Shutler and Read, 1998; West et al., 2001, 2002). Here we consider another possibility – that the life cycle of malaria parasites deviates from the standard assumptions of LMC in a way that may alter sex ratio predictions – and we determine what sex ratios would be favored if such deviations were accounted for.

Malaria parasites have a two-host life cycle (Fig. 1). Asexual replication in a vertebrate host culminates in the production of male and female sexual cells, the gametocytes, which are the only parasite stage to survive transmission to the second host, a blood-feeding insect vector. Mating takes place within minutes of entering the vector, with female gametocytes producing one female gamete and males producing up to 8 flagellated male gametes. Following mating, zygotes undergo further asexual replication and become oocysts, with each oocyst containing 1000s of transmission stage parasites, the sporozoites (Rosenberg and Rungsiwongse, 1991).

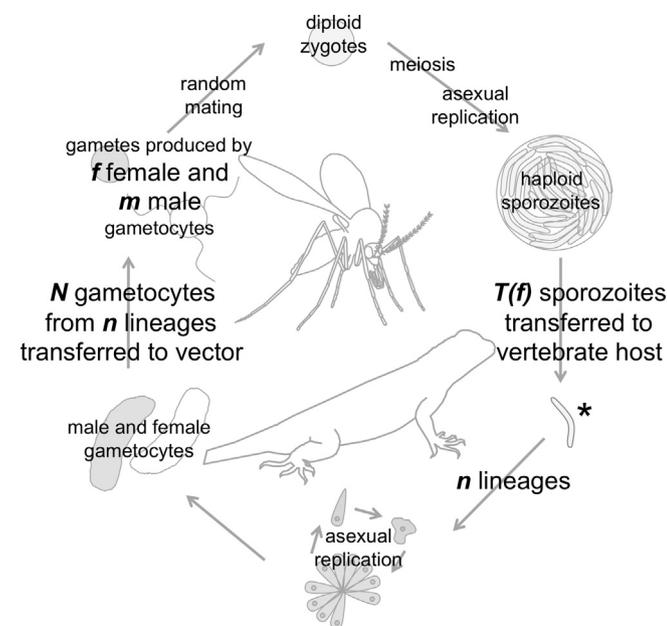


Fig. 1. Starting at the asterisk (bottom right), a vertebrate host harbors n clonal parasite lineages one of which is mutant. These lineages each reproduce asexually and ultimately produce male and female sexual cells, termed gametocytes. Transmission through a blood meal provides f female and m male gametocytes to the vector, where $N=f+m$ is regarded as a function of n . Gamete production and random mating produce diploid zygotes in numbers proportional to f and these eventually produce a large number of haploid sporozoites. These are transmitted to a new vertebrate host in numbers $T(f)$ written as function of f but with diminishing returns (Fig. 2). These start the cycle again. This life cycle is common to all malaria parasites, though the specific hosts pictured are those of the lizard malaria parasite *Plasmodium mexicanum*.

The sporozoites travel to the insect's salivary glands from whence they will be transmitted the next time the insect feeds.

The division of malaria parasites in separate insect vectors during mating coupled with the presence of many parasites deriving from only one or a few clonal lines (we refer to these as clones or lineages; they are the result of asexual replication in both insect and vertebrate host; Paul and Day, 1998; Read and Day, 1992) apparently match the population structure assumed by LMC well (Read et al., 1992). However, despite the similarities of the malaria life cycle to the standard LMC model, the advantage of producing a female-biased sex ratio could be reduced if the number of female gametocytes ingested isn't linearly related to ultimate transmission success. If increased female production does not increase patch productivity at all, no female bias is favored (Colwell, 1981). Intermediate situations in which producing more female gametocytes increases transmission somewhat, but not proportionally, are also possible and are explored here (Table 1). For malaria parasites, having a linear relationship between female production and transmission success would require the number of female gametocytes ingested to be directly proportional to the number of zygotes, oocysts and sporozoites produced and for the number of sporozoites produced to be directly proportional to the probability of initiating a new infection.

Several characteristics of malaria parasite transmission biology make it possible that increased female production does not linearly increase transmission success. For example, there is evidence of density-dependent mortality during zygote development (Zollner et al., 2006); even if more zygotes are formed when sex ratios are female-biased, higher mortality rates may prevent a proportional increase in oocyst and sporozoite production. Furthermore, increased sporozoite production itself may not greatly affect transmission success. Sporozoites are passed to a new vertebrate host from the salivary glands of the insect vector, which may become saturated. Also, transmission is relatively efficient even with very few sporozoites (Klein et al., 1987; Ungureanu et al., 1976). One study found that doses of 10 to 10,000 sporozoites were all equally capable of inducing infection, with only a slight reduction in time to patency between the highest and lowest dose (12 days for 10,000, 16–17 days for 10; Ungureanu et al., 1976). These traits almost certainly complicate the relationship between the number of females present in the mating population and transmission success, a relationship on which the selective advantage of female-biased sex ratios depends.

Here we develop a mathematical model to explore the consequences of a non-linear female-transmission relationship on the evolutionarily stable sex ratio. We compare four variations of this model that incorporate differences in the information available to the clones and the effects of multiple co-occurring lineages on the total number of offspring in the patch. These variations are: (1) plastic/additive—clones are able to detect and respond to the presence of other lineages occupying the same patch (i.e. sex ratios display phenotypic plasticity sensu Schall, 2009) and each additional clone increases the total mating population by a set amount

Table 1
The strength of the $T(f)$ bottleneck.

	Fitness $w(n)$ (Eq. (5))	ES sex ratio ($n=4$)
No bottleneck pure LMC on males	$w(n)=pf$	$r^* = \frac{n-1}{2n} = 0.375$
Partial bottleneck (Fig. 1) LMC on males, partial LRC on females	$w(n)=pT(f)$	$r^*=0.440$
Complete bottleneck Male LMC and female LRC balanced	$w(n)=p$	$r^*=0.500$

Tabulated values of r^* belong to the plastic additive model.

(additive), (2) plastic/fixed—sex ratios are plastic but the total mating population size does not depend on the number of lineages, (3) uniform/additive—clones are not able to detect or respond to the presence of co-occurring lineages or the size of the mating population (i.e. sex ratios in all patches are uniform regardless of the number of lineages and are locally adapted to maximize fitness given the distribution of patch types in the overall population, as described in Schall, 2009) and mating population size is additive, and (4) uniform/fixed—sex ratios are uniform across patch types and offspring numbers per patch are fixed. We compare the sex ratio predictions derived from this model with those of standard LMC for both individual infections and population-wide patterns, including the patterns predicted by Read et al. (1995)’s derivation relating parasite prevalence in a host population with predicted sex ratio (see Section 3.4). In Sections 3.5 and 3.6, we discuss how these predictions relate to existing data on malaria parasite sex ratios and how the model may apply to other biological systems.

2. Methods

2.1. General model

The general parasite life cycle is diagrammed in Fig. 1. By analogy with the standard LMC model (Hamilton, 1967), we interpret this as an infinite island model in which the patch is the insect vector, the mated females who disperse are the diploid zygotes, and their offspring are the sporozoites, which multiply and ‘mature’ into male and female gametocytes in a vertebrate host. N gametocytes are passed to the insect vector: these derive equally from the n clones that colonized the vertebrate host and mate at random to produce the next generation of zygotes.

The two transmission episodes in Fig. 1 life cycle provide two potential ‘bottlenecks’ and we treat each of them differently. The transmission of the sporozoites down to the vertebrate is modeled with diminishing returns using the functional form:

$$T(f) = 1 - e^{-af} \tag{1}$$

Fig. 2 with a parameter a , which regulates the strength of the effect. This will indeed have an effect on the sex ratio dynamics as, for a particular N , a greater female bias will increase f and thereby decrease the marginal transmission rate.

On the other hand, the transmission of the gametocytes to the vector is modeled through the effect of n on N , and we use the

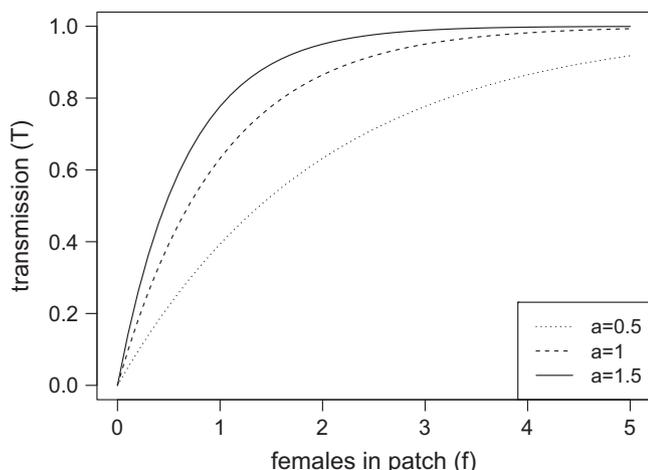


Fig. 2. A plot of Eq. 3 showing the effect of a on the relationship between female production (f) and transmission success (T).

general form

$$N = \hat{n} \left(\frac{n}{\hat{n}} \right)^\beta \tag{2}$$

here β is a parameter ($0 \leq \beta \leq 1$) measuring the extent to which N responds to variations in n . With $\beta=0$ there is no response and N has the ‘fixed’ value \hat{n} ; with $\beta=1$ there is an ‘additive’ response and N takes the value n . Thus in the fixed case, the N transmitted gametocytes comprise n clones of equal size but N is the number that would be transmitted in the additive case when there are \hat{n} clones.

Our analysis will keep track of the change in the number of mutant zygotes from one generation to the next, that is, over a complete cycle (Fig. 1). Before we turn to that it is worth mentioning that in a single cycle the parasite will in general encounter two values of the lineage number n : values n_0 in the vertebrate and n_1 in the vector. Generally there is a single blood meal between the two hosts and the n -values are equal or at least highly correlated, and we will in fact work with a single n . But for clarity we observe that n_0 carries the information used by the sporozoite in determining the sex ratio (in the plastic case) whereas n_1 determines the size of f which feeds into the second bottleneck $T(f)$.

We track mutant fitness beginning with a mutant zygote and its clonal derivatives in the vertebrate. Our assumption of $n-1$ resident and 1 mutant clone (with sex ratios r and s , respectively, as in Taylor and Bulmer (1980)) gives us an overall sex ratio of $[(n-1)r+s]/n$ and this determines the numbers of male and female gametocytes in the vector as:

$$\begin{aligned} m &= N \left(\frac{(n-1)r+s}{n} \right) \\ f &= N \left(\frac{(n-1)(1-r)+(1-s)}{n} \right) \end{aligned} \tag{3}$$

The mutant genetic representation in these male and female gametocytes is

$$\begin{aligned} p_M &= \frac{s}{(n-1)r+s} \\ p_F &= \frac{1-s}{(n-1)(1-r)+1-s} \end{aligned}$$

so that after mating the overall proportion of mutant genes in the diploid zygotes will be

$$p = \frac{p_M + p_F}{2} \tag{4}$$

For each value of n , mutant fitness can be measured as the mutant’s share of sporozoites transmitted to the vertebrate:

$$w(n) = w(s, r; n) = pT(f) \tag{5}$$

2.2. Equilibrium equations

We now formulate the equations for evolutionary equilibrium of the sex ratio for each of our four variations.

2.2.1. Variations 1 and 2: Plastic

The parasite can determine and respond to the value of n . The N -bottleneck is additive ($\beta=1$) or fixed ($\beta=0$). In both cases, the sex ratio equilibrium is attained at the value $r=r^*$ at which

$$\left. \frac{\partial w}{\partial s} \right|_{s=r=r^*} = 0. \tag{6}$$

2.2.2. Variations 3 and 4: Uniform

The parasite is unable to determine the value of n , and evolution responds to a suitable average value of n . We describe the distribution of n among patches with the negative binomial (B) using the

parameterization given in [Bliss and Fisher \(1953\)](#). The two necessary parameters are μ , the mean number of lineages per patch, and k , which is inversely related to the degree of aggregation of lineages among patches. When k is high, the negative binomial closely approximates a Poisson distribution. When k is low, the variance of the distribution is high relative to its mean because lineages tend to clump together, creating more patches with many or few lineages than expected by chance (i.e. under a Poisson distribution). This makes the negative binomial useful for describing the distribution of parasites in a population of hosts because a small proportion of hosts often harbors the majority of parasites ([Anderson and May, 1991](#); [Crofton, 1971](#)). In fact, many plants and animals show significant aggregation ([Bliss and Fisher, 1953](#)) so the distribution should be generally applicable. Taking B_n to be the frequency of patches of type n , the probability of a clone being in a patch with n clones is

$$Q_n = \frac{nB_n}{\sum_i iB_i} \tag{7}$$

where the sum runs over the range of possible patch types. Mutant fitness is then measured as an average taken across all patch types:

$$W = \sum_n w(n)Q_n \tag{8}$$

using the fitness function of Eq. (5). For most runs we used an n -range between 1 and 1000. The high maximum n is only necessary when μ is high (e.g. for high prevalence- see [Section 3.4](#)) but was used elsewhere for consistency.

The N -bottleneck is again additive ($\beta=1$) or fixed ($\beta=0$) and in each case the sex ratio equilibrium is attained at the value $r=r^*$ at which

$$\left. \frac{\partial W}{\partial s} \right|_{s=r^*} = 0. \tag{9}$$

Our equilibrium Eqs. (6) and (9) cannot be solved analytically and numerical solutions for a variety of parameter combinations are presented in the results section.

2.3. Population-wide sex ratios

Some studies of malaria parasite sex ratios have compared the average population sex ratios (across multiple patches) of different populations. It may therefore be useful to know what average population sex ratio is predicted by different variations of the model. Average population sex ratios are also helpful for comparing the predictions of different model variants because the predicted sex ratios of some variants depend on n (both plastic variants) and some do not (both uniform variants).

For both of the uniform variations of the model ([Section 2.2.2](#)), the same sex ratio is expected in all patch types, and this will be the average population sex ratio. For both plastic variations ([Section 2.2.1](#)), the sex ratios predicted will vary based on n , and the average sex ratio across all patches will depend on the frequency of patch types in the population. In this case, the average clone sex ratio is a weighted average of the sex ratios predicted in each patch type:

$$r^*_{pop} = \sum_n r^*_n Q_n \tag{10}$$

with Q_n given in Eq. (7).

The average patch sex ratio, which does not take into account how many lineages may share a patch and is therefore more closely comparable to existing data for malaria parasites, is:

$$r^*_{patch} = \sum_n r^*_n B_n \tag{11}$$

3. Results and discussion

3.1. The $T(f)$ bottleneck and LRC

We begin with some general remarks about sex ratio theory, designed to help readers put our results in context. Our model represents a particular case of local resource competition (LRC), the idea that the sex ratio of a population should be biased toward the sex that competes for resources less intensely with relatives ([Clark, 1978](#)). In this sense, the $T(f)$ bottleneck creates competition among females for a share of the transmission success (see [Appendix A](#) for mathematical support of this claim). Of course females are themselves a significant reproductive resource for males and thus LMC is an additional source of LRC acting on males ([Taylor, 1981](#); [West, 2009](#)).

To better understand the interaction between LRC among males and among females, we look at two extreme cases. At one end of the spectrum we have no bottleneck and transmission is proportional to production so $T(f)=f$. In this case the only source of LRC is the standard LMC among males ([Hamilton, 1967](#)). At the other extreme is the complete bottleneck case in which $T(f)$ is independent of f and transmission is fixed. In this case males and females are faced with exactly the same level of local competition and the patch behaves like a finite random mating population for which the sex ratio is known to be unbiased ([Fisher, 1930](#)). Our model lies between these two extremes; $T(f)$ behaves like f for small f and like a constant for large f ([Table 1](#)).

The additive variations of our model also have ties to the Trivers–Willard hypothesis (T–W), which proposes that progeny sex ratios should be shifted in response to conditions (environmental or parental) that alter the relative reproductive value of male vs. female offspring ([Trivers and Willard, 1973](#)). In our model an increase in n decreases per capita transmission success for both sexes (an effect of crowding), but the effect of this can be alleviated by a reduction in the number of female offspring leading to an increase in fitness per daughter (see [Appendix A](#)). This provides an example of the interaction between LRC and T–W.

3.2. Sex ratios among patches

When mothers are able to detect and respond to the number of lineages present in a patch, the unbeatable sex ratios identified by our model are higher (less female-biased) than those predicted by LMC for all values of n and a we tested ([Fig. 3](#)). This result is

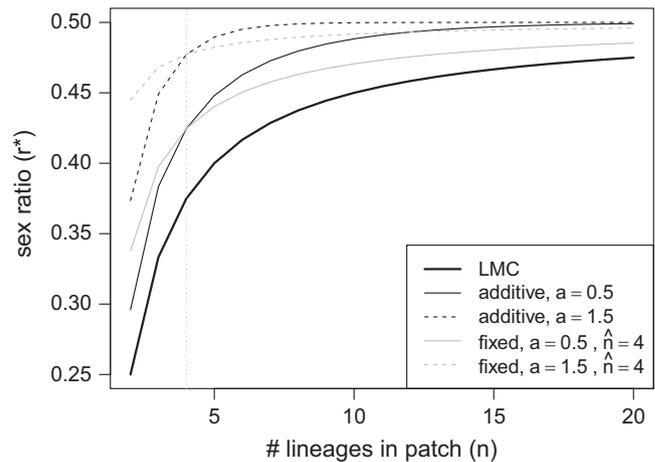


Fig. 3. Model results showing the sex ratios predicted for patches with different numbers of lineages (n) under different variations and parameterizations of the model (see legend). The vertical dashed line shows $n = \hat{n} = 4$. Only the first two model variations are shown here (plastic/additive and plastic/fixed) because the predictions of the uniform model variations do not vary with n .

consistent with our intuition. The concave-down shape of the bottleneck $T(f)$ gives a higher female marginal return when fewer females are produced, thus decreasing the female-bias of the sex ratio. This effect is more pronounced for higher a (Fig. 3).

Comparing the plastic/fixed and plastic/additive cases, sex ratios in the additive case are lower when $n < \hat{n}$ and higher when $n > \hat{n}$ (Fig. 3). This result is also consistent with our expectation. The number of females in the patch at equilibrium is represented by $f = n(1 - r^*)$ (Eqs. (2) and (3)) in the additive case ($\beta = 1$) and by $f = \hat{n}(1 - r^*)$ in the fixed case ($\beta = 0$). When $n > \hat{n}$, the additive f is the larger of the two, and therefore more influenced by the bottleneck, favoring a less female-biased sex ratio.

Overall, the relationship between the number of lineages in a patch and the patch sex ratio predicted by our model shows a similar trend to that predicted by LMC, though the specific sex ratios predicted may be substantially higher depending on the strength of the vector-to-vertebrate bottleneck.

3.3. Population-wide sex ratios

Exploring the effect of transmission bottlenecks on the population-wide sex ratio (i.e. average across all clones or patches) is useful for a number of reasons. First, if mothers are unable to detect or respond to the number of co-occurring lineages in their patches, they should produce a sex ratio that is adapted to the distribution of patch types in the population. In this case, infection and population sex ratios would be the same. Second, researchers may not have the ability to assess the numbers of lineages in individual patches but may have information about average population sex ratios and numbers of occupied patches (see Section 3.4 for an example). Third, population-

wide sex ratios provide a useful way of comparing the predictions of the uniform vs. plastic variations of our models.

For all four variations of our model, sex ratios generally increase with increasing bottleneck strength (i.e. higher a) and are higher than the predictions of LMC (Fig. 4). There are two exceptions, both relating to the uniform/additive case: first, our model predicts sex ratios that are slightly lower than the corresponding LMC model under some population structures (μ and k) and second, it shows a very slight decrease in r^* over some ranges of a (e.g. see Fig. 4 when $\mu = 5$ and $k = 0.5$: the black dotted LMC line is perfectly horizontal, while the black solid model line dips slightly). We are at a loss to explain this behavior, though we note that both deviations from the general pattern are slight, occur for only a limited range of parameter values, and would be indistinguishable in a biological system.

While it is true that predicted sex ratios generally increase with increasing bottleneck strength, some variations of our model are more sensitive to these shifts in a than others. The uniform/fixed case appears to be the most sensitive to small changes in a (Fig. 4). Population sex ratios also increase with increasing μ , as in LMC, and are bounded by 0 and 0.5. Sex ratios appear to decrease slightly with increasing k , though overall predicted sex ratios are relatively insensitive to increases in k over multiple orders of magnitude, especially for lower μ (Fig. 4). The relationship between sex ratio and both μ and k is likely due to their effect on the overall population structure. As can be seen in the inset plots in Fig. 4, increasing both μ and k increases the proportion of infections that have multiple clones, thereby favoring less-biased sex ratios (as under standard LMC).

It may also be worth noting that without the vector-to-vertebrate transmission bottleneck included in the model, two

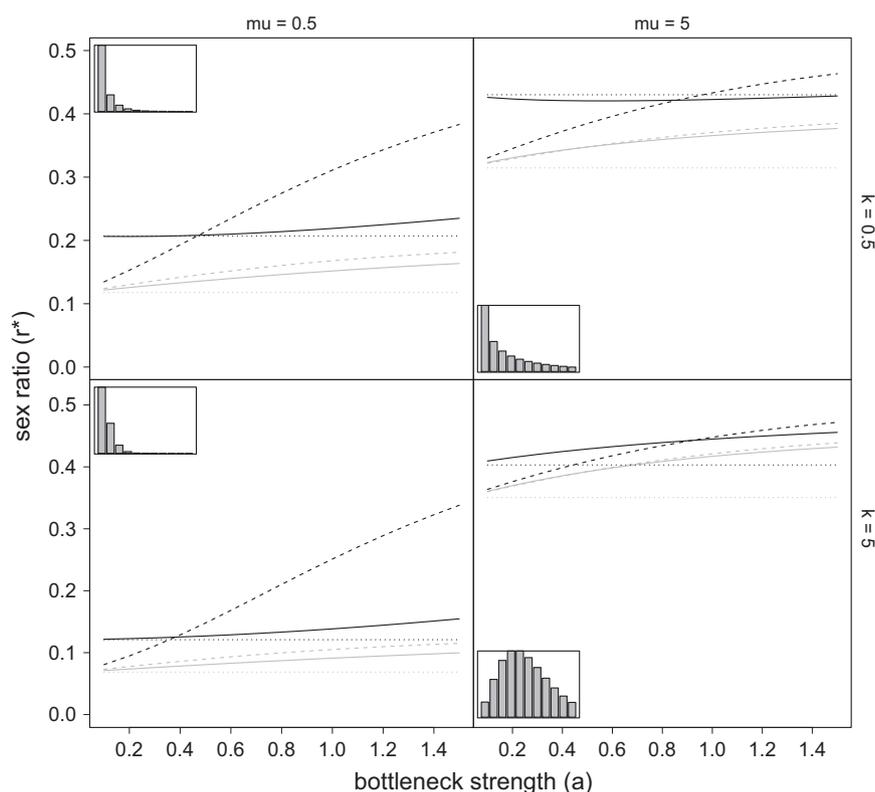


Fig. 4. The effect of bottleneck strength (a) on the average population sex ratio (proportion male, r^*) for different population structures (plots) and model variations (line types). μ (μ) is the average number of lineages per patch and k is inversely related to the degree of aggregation. The small graph inset in each panel shows proportion of infections (y -axis) with each number of lineages (x -axis, ranging from 0 to 20) based on μ and k . The line types correspond to variations of the model (and match those used in Fig. 5, which provides a legend) as follows: black, solid lines are uniform/additive; black, large-dashed lines are uniform/fixed; black, small-dashed lines are LMC uniform/additive; gray solid lines are plastic/additive; gray large-dashed lines are plastic/fixed; gray small-dashed lines are LMC plastic/additive, LMC plastic/fixed and LMC uniform/fixed (all give the same prediction). All sex ratios shown for plastic model variations are averaged over patches; sex ratios averaged over all clones show the same shape relative to a but are higher. Results are from a run of the model with $\hat{n} = 4$.

different sex ratios are predicted depending on the other model assumptions. While only the plastic/additive assumptions are actually consistent with standard LMC, it is worthwhile to compare sex ratios of our model with those predicted in the absence of the bottleneck (which we refer to collectively as the LMC variations). In the absence of a bottleneck, the sex ratios predicted under the uniform/fixed assumptions are lower than those predicted under the uniform/additive assumptions (Figs. 4 and 5). Both plastic variations of the LMC model predict the same sex ratios, which interestingly match the uniform/additive sex ratios if averaged over all clones and match the uniform/fixed sex ratios if averaged over all patches (Figs. 4 and 5).

3.4. Transmission bottlenecks, prevalence and malaria parasite sex ratios

Empirical studies of malaria parasite sex ratios in natural populations often lack developed methods for measuring the number of parasite clones present in individual infections (e.g. genetic testing; Schall, 2009). Some researchers have instead used the proportion of hosts infected (also referred to as parasite prevalence, P) to estimate the average number of clones per infection in a population (Read et al., 1995; Shutler et al., 1995). If clones are distributed among hosts following a negative binomial distribution, a relatively straightforward relationship exists between prevalence and the mean number of clones per infection: $P = 1 - (1 + \mu/k)^{-k}$ (Anderson and May, 1991). This expression can be rearranged to give the mean number of clones per infection based on prevalence, provided a reasonable estimate of k is available. Using this strategy, Read et al. (1995) derive an expression for predicting sex ratio directly from prevalence data (hereafter we refer to it as the “prevalence derivation” of LMC). We plotted the predictions of our model against P (using $\mu = k[(1 - P)^{-1/k} - 1]$ and $k = 1$; Read et al., 1995) to provide a useful framework for comparing the predictions of our model and the prevalence derivation as well as to illustrate the predicted population-wide effects of our model in a form more easily compared with existing empirical data (Fig. 5).

Read et al. (1995) present data on the average sex ratios for natural populations of malaria parasites in the genus *Leucocytozoon* (and one population of *Plasmodium falciparum*) that appear to match their predictions well. However, if sex ratios are averaged over all patches (which they are), the prevalence derivation's predictions match the predictions we obtained for LMC only in the uniform/additive case (Figs. 5 and 6)¹. We believe that the assumptions of the uniform/additive case are unlikely to be accurate for malaria parasites. Sex ratio data from multiple species suggest that malaria parasites adjust their sex ratio relative to the number of clones present (i.e. they are not uniform; e.g. *Plasmodium chabaudi*, Reece et al., 2008; *Plasmodium mexicanum*, Neal and Schall, 2014b; *P. falciparum*, Sowunmi et al., 2009b), and while there is evidence that infections with more lineages produce more sexual cells (Taylor et al., 1997), this effect is not always strong (i.e. they are not additive; Vardo-Zalik, 2009) or indeed observed for all species (Read and Taylor, 2001). If we assume that either of these assumptions (uniform sex ratios or additive parasite numbers) is inaccurate, the LMC model does not account for the data presented in Read et al. (1995) as well (Fig. 6, “LMC all other variations”). However, the sex ratios predicted by both plastic

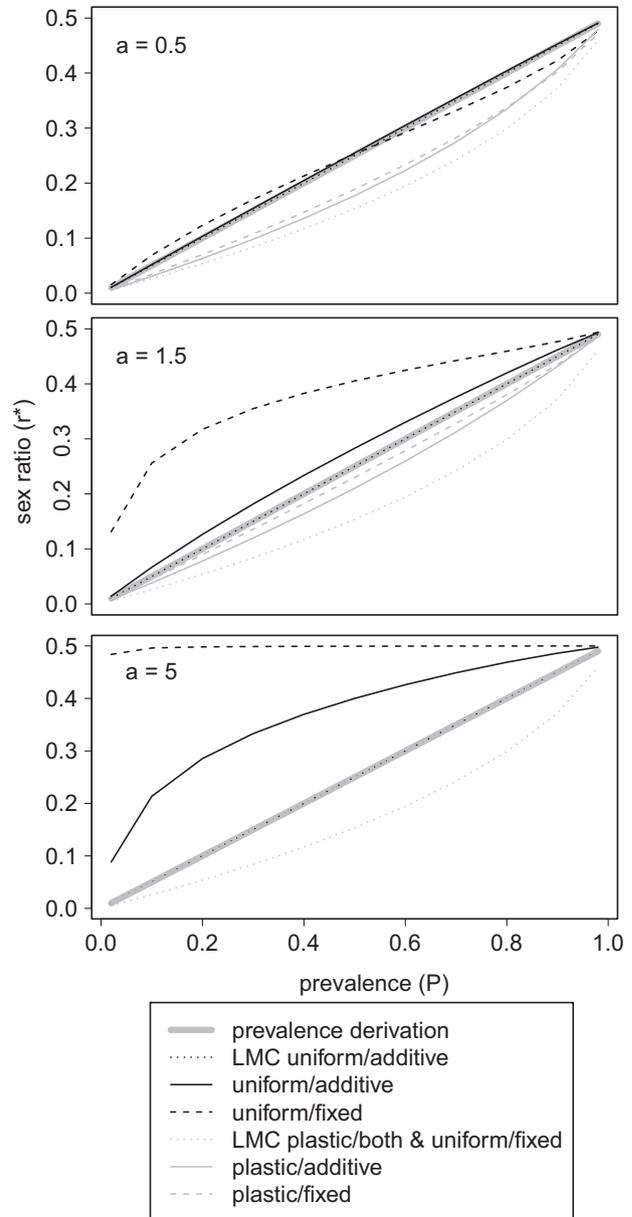


Fig. 5. The predicted relationship between sex ratio (r^*) and the prevalence of a parasite in a host population (i.e. proportion of hosts infected, P). The relationship between P , μ , and k is given in Section 3.4 of the text. Model variations (line types) are summarized in the legend, which also corresponds to Fig. 4. Results shown are from a run with $\hat{n} = 4$ and $k = 1$. Sex ratios from the plastic model variations are averaged over all patches. Note that in the bottom panel, the plastic/additive and plastic/fixed model variations predict the same sex ratios as the prevalence derivation, so they are not readily visible on the plot.

variations of our model produce sex ratio predictions very similar to the prevalence derivation, especially when bottlenecks are strong (Fig. 5). Our model is therefore as capable as the prevalence derivation of explaining the observed relationship between prevalence and sex ratio, but our model may more closely approximate the underlying selective forces. Our model may also account for the sex ratios observed for a similar study in avian *Haemoproetus* in which observed sex ratios were uniformly just below 0.5 proportion male and not related to prevalence (Shutler et al., 1995). Such a pattern is consistent with our model, particularly if the parasite is unable to detect and respond to cues in its host or if bottlenecks are particularly strong (e.g. ‘uniform/fixed’, $a = 5$, Fig. 5).

¹ Note that the prevalence derivation matches the predictions of our plastic/additive model variation (which incorporates assumptions consistent with the original LMC) if sex ratios are averaged over all clones. Our model is therefore consistent with their derivation, but highlights the conflict between their derivation and the data presented.

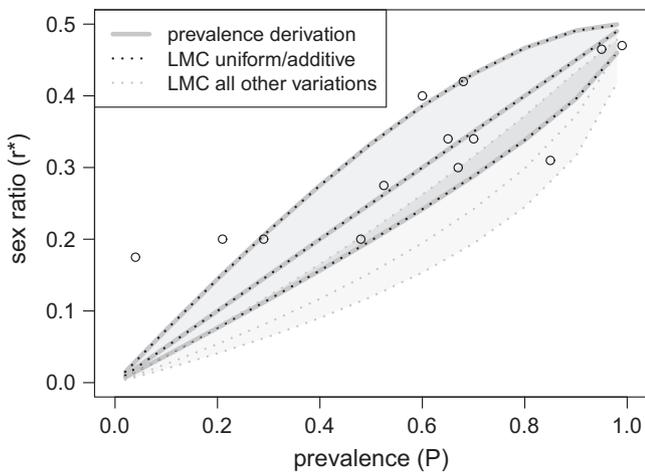


Fig. 6. Comparison of data taken from Read et al. (1995) (points) with model predictions from the same paper ('prevalence derivation') and different variations of LMC. As in Read et al. (1995), three lines are provided for each model which correspond to $k=0.5$ (top), $k=1$ (middle) and $k=2$ (bottom). The area encompassed by these lines is shaded to highlight the area of the graph that is consistent with each model given the range in k assumed by Read et al. (1995). Note that male fecundity sets a minimum on sex ratio predictions that is not included here but is likely important, especially for infections at low prevalence sites.

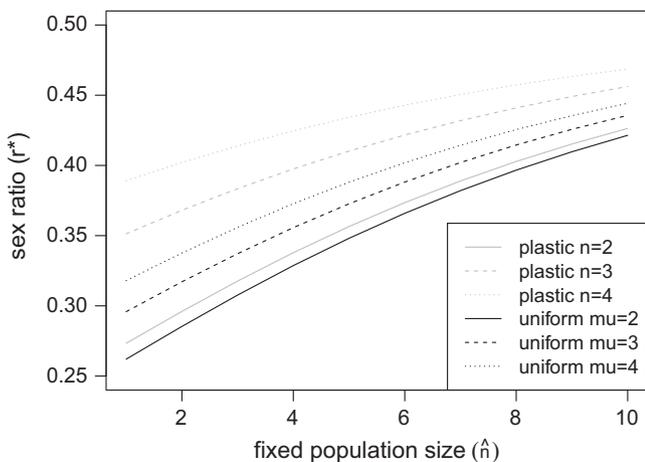


Fig. 7. Predicted relationship between fixed patch size (\hat{n}) and sex ratio (r^*). Only fixed model variations are shown because \hat{n} cancels out in additive models (see Eq. (2)). For the plastic/fixed variation, predicted sex ratios are for 3 different values of n ($n=2, 3, 4$). For the uniform variation, predicted sex ratios are for three different populations with different mean number of clones per infection ($\mu=2, 3, 4$) and $k=0.5$.

3.5. Comparison of model with other existing data

In addition to explaining patterns in existing data on prevalence and sex ratio with more realistic assumptions than the prevalence derivation, transmission bottlenecks may also account for some previously unexplained patterns of malaria parasite sex ratios. For example, a positive relationship is sometimes observed between parasite density and sex ratio (e.g. *Plasmodium tropiduri*: Pickering et al., 2000; *P. mexicanum*: Neal and Schall, 2014b; Schall, 2000; *P. chabaudi*: Taylor, 1997), which, at least for *P. mexicanum* and *P. chabaudi*, cannot be attributed to a link between the number of clones in an infection and gametocyte density (Neal and Schall, 2014b; Taylor, 1997). Our model shows that if patch size is fixed, increases in \hat{n} increase r^* (Fig. 7). In other words, if gametocyte density varies but does not depend on the number of clones in an infection (i.e. it is fixed by some other variable like immune

competency or resource availability), infections with higher densities of gametocytes are predicted to produce higher sex ratios. Models currently applied to malaria parasite sex ratios cannot account for a positive relationship between gametocyte density and sex ratio in the absence of a link between gametocyte density and clonal diversity.

Even though this positive relationship has been observed among infections for relatively few species (perhaps because few species have high enough average gametocyte densities to overcome the selective force of Fertility Insurance, which favors a negative relationship), the trend may also exist among species: *P. falciparum* infections typically have no more than 5% of erythrocytes infected (Mackinnon and Read, 2004) and have relatively low sex ratios, generally between 10 and 20% (Read et al., 1992; Robert et al., 1996; Sowunmi et al., 2009a and b). *P. chabaudi* generally has a higher rate of infected erythrocytes, perhaps 5–30% (Mackinnon and Read, 2004), and somewhat higher sex ratios (15–45% (Reece et al., 2008, 2003)). *P. mexicanum* infections can have up to 90% of erythrocytes infected (Vardo-Zalik and Schall, 2009), sometimes with predominantly gametocytes, and has average sex ratios around 40–45% male (Neal, 2011; Neal and Schall, 2014a, 2014b, 2010; Osgood and Schall, 2004; Osgood et al., 2003, 2002). More detailed data from additional species would help determine whether this pattern holds. Our model is the first to predict this relationship within or across species.

Additionally, a positive relationship between the strength of transmission bottlenecks and sex ratio would also support our model's predictions. Ideally, bottleneck strength would relate female production to ultimate transmission success (Fig. 2), but unfortunately, we know of no such data for any species. There are, however, data from a limited number of species on the proportion of females that successfully form oocysts, which is at least a part of the path from production to transmission. *P. falciparum* appears to generally have a lower proportion of females fail to reach the oocyst stage than *P. chabaudi* (Vaughn, 2007) and has correspondingly lower sex ratios (see previous paragraph). *Plasmodium vivax* has a similar or slightly higher failure rate compared with *P. falciparum* and the average sex ratio reported by one study (the only one we know of with detailed data) is 27% male (Zollner et al., 2006), which falls somewhere between *P. falciparum* and *P. chabaudi*. These data are far from conclusive—they only measure part of the relevant bottleneck and don't control for other factors that likely influence sex ratios, like the number of clones per infection, but they are suggestive of the predicted pattern and emphasize the need for more detailed within and cross species data on development in the vector and transmission.

Most other existing data on sex ratios in malaria parasites describe general patterns. These patterns, such as higher sex ratios in infections with more clones (Neal and Schall, 2014b; Reece et al., 2008), are consistent with LMC whether or not transmission bottlenecks are accounted for, and are therefore not specific enough to distinguish between the two models. Data suggesting sex ratio adjustments in response to low fecundity or small mating groups are also consistent with our model. Fertility Insurance predicts an increased investment in males under certain conditions, and transmission bottlenecks would not disrupt this trend.

3.6. Extensions

Even though our focus has been on malaria parasites, our goal was to develop a model that could apply generally to different organisms. While 'transmission bottlenecks' sound particularly suited for a host-parasite system, by transmission we simply mean the transmission of genes from one generation to the next. Organisms could experience bottlenecks due to many different biological factors; low resource availability, predation and disease

all reduce the probability that genes will be transmitted from one generation to the next. The intensity of each of these transmission blockers may also depend on the number of females in a patch, especially if females use proportionally more resources or are more attractive to predators or parasites.

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Appendix A

The standard LMC model. We work with an infinite island model with demes occupied by a fixed number n of mated females. We assume that each female has a large number of male and female offspring and then dies. These offspring mate at random on the deme. Following this, the males die and the mated female offspring disperse randomly to form new demes of size n . The problem is to calculate the evolutionarily stable sex ratio. This is the standard local mate competition (LMC) model and we will work with that here. A “partial dispersal” variation of this that we will not consider allows a fraction of the mated female offspring to remain on their native deme and compete with one another and with any immigrant females, for the n breeding slots.

Consider a focal mated female and suppose she has x daughters and y sons. For this standard LMC model her fitness w is completely determined, on average, by x and y : $w = w(x, y)$. Now let D be her fitness per daughter through daughters and let S be her fitness per son through sons. Then her fitness has the form:

$$w(x, y) = D(x, y)x + S(x, y)y. \tag{A.1}$$

Note: Her fitness is calculated as the number of copies of her genes projected into the future. Now any such copies will either descend through a daughter or a son, but not both, so her fitness is indeed a sum of two terms as above.

If we let the sex ratio of her offspring be r , then with a fixed number of offspring, x and y are both functions of r , and we differentiate her fitness with respect to r :

$$\frac{dw}{dr} = \frac{\partial w}{\partial x} \frac{dx}{dr} + \frac{\partial w}{\partial y} \frac{dy}{dr} \tag{A.2}$$

where

$$\frac{\partial w}{\partial x} = \frac{\partial D}{\partial x}x + D + \frac{\partial S}{\partial x}y \text{ and } \frac{\partial w}{\partial y} = \frac{\partial D}{\partial y}x + \frac{\partial S}{\partial y}y + S. \tag{A.3}$$

Now with a fixed number K of offspring, $dy/dr = K$ and $dx/dr = -K$ and normalizing K to be 1, we can write (Taylor, 1981):

$$\frac{dw}{dr} = \frac{\partial w}{\partial y} - \frac{\partial w}{\partial x} = (S - D) + \left(\frac{\partial D}{\partial y} - \frac{\partial D}{\partial x}\right)x + \left(\frac{\partial S}{\partial y} - \frac{\partial S}{\partial x}\right)y. \tag{A.4}$$

The three brackets on the right display the different ways through which a change in sex ratio affects fitness. The first bracket provides the obvious effect on changes in the number of sons and daughters (e.g. an additional son adds S to her fitness). The second bracket provides the effect of an increase in the number of sons or daughters on her fitness per daughter and finally the third bracket does the same for fitness per son.

We now calculate D and S . Since all the daughters disperse, neither x nor y have any effect on fitness per daughter and D is a constant which we take to be 1. If there were partial dispersal of females, so that some daughters remain and compete with one another for spots on the natal deme, then we would expect $\partial D/\partial x$ to be negative. Turning to S , since all mating takes place on the deme, male fitness is entirely provided through the females on the deme so that:

$$S = \frac{(n-1)\hat{x} + x}{(n-1)\hat{y} + y} \tag{A.5}$$

where we let \hat{x} and \hat{y} be the average number of female and male offspring of the other $n-1$ breeding females. Then:

$$\frac{\partial S}{\partial x} = \frac{1}{ny^*} \text{ and } \frac{\partial S}{\partial y} = -\frac{1}{ny^*} \frac{x^*}{y^*} \tag{A.6}$$

where the derivatives are evaluated at the population-wide equilibrium values x^* and y^* . As expected, an increase in the number of daughters has a positive effect on the fitness of a son (more mating opportunities) whereas an increase in the number of sons has a negative effect on the fitness of a son (increased competition for matings). If we put these expressions into Eq. (A.4) we get

$$\frac{dw}{dr} = \left(\frac{x^*}{y^*} - 1\right) - \frac{1}{ny^*} \left(\frac{x^*}{y^*} + 1\right)y^*. \tag{A.7}$$

Taking $y^* = r^*$ and $x^* = 1 - r^*$ and simplifying gives us:

$$\frac{dw}{dr} = \frac{1}{r^*} \left(\frac{n-1}{n}\right) - 2. \tag{A.8}$$

Setting this to zero gives us an equilibrium at

$$r^* = \frac{n-1}{2n}. \tag{A.9}$$

and this is convergence stable (Christiansen, 1991) as, from Eq. (A.8) an increase in r^* will decrease dw/dr , making it negative, thereby encouraging the spread of sex ratio variants with smaller r . This is the standard diploid LMC result (Hamilton, 1967; Taylor and Bulmer, 1980), the female bias resulting from the competitive pressure among brothers for mates.

The effect of the bottleneck. Let $F = (n-1)\hat{x} + x$ be the total number of female offspring born on the deme. Under the standard LMC model, these F females all survive and disperse. The effect of the bottleneck is to let the actual number L of females who survive and disperse be somewhat less than F such that the probability of

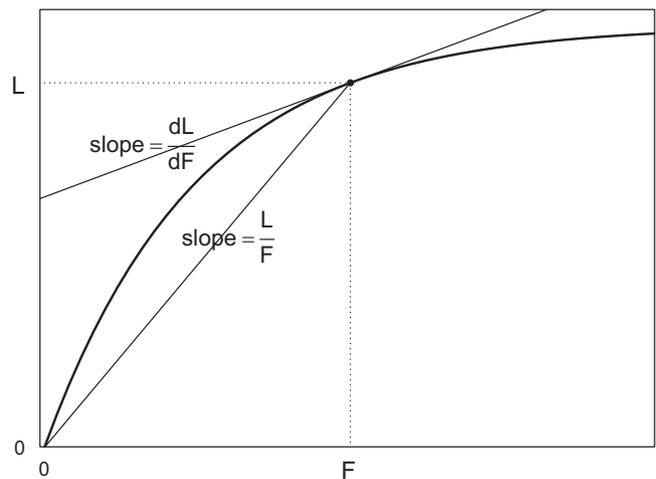


Fig. A.1. If the graph of L against F is concave-down, the slope dL/dF of the graph at any point will be less than the slope L/F of the line from the origin to the point, and from Eq. (A.10) this makes $\partial D/\partial x$ negative. Note that if L were proportional to F , the graph would be linear and the two slopes would be the same making $\partial D/\partial x$ zero.

dispersal decreases with F . In fact we assume that the graph of L against F is increasing but with decreasing slope (Fig. A.1).

Now the focal breeder will get her fair share x/F of the dispersing females, so that her fitness through daughters is xL/F and her fitness per daughter is $D=L/F$. Then:

$$\frac{\partial D}{\partial x} = \left(\frac{L'(F)}{F} - \frac{L(F)}{F^2} \right) \frac{dF}{dx} = \frac{1}{F} \left(L'(F) - \frac{L(F)}{F} \right) \quad (\text{A.10})$$

since $dF/dx=1$. The concave-down form of the graph of L against F makes $\partial D/\partial x$ negative (Fig. A.1) and from Eq. (A.4) this increases dw/dr at the LMC equilibrium (Eq. (A.9)), making it positive and thereby encouraging the spread of sex ratio variants with larger r leading to a less female-biased sex ratio.

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