Evolutionary Ecology Research, 2000, **2**: 171–184

Sex ratio and virulence in two species of lizard malaria parasites

John Pickering,[1,2] Andrew F. Read,[3] Stella Guerrero[1] and Stuart A. West[1,3]

[1]*Smithsonian Tropical Research Institute, Apartado 2072, Balboa, Republic of Panama,* [2]*Institute of Ecology, University of Georgia, Athens, GA 30602-2602, USA and* [3]*Institute of Cell, Animal and Population Biology, University of Edinburgh, Edinburgh EH9 3JT, UK*

ABSTRACT

Evolutionary theory predicts that both the virulence and the sex ratio of a parasite can depend upon its population structure, and be positively correlated. With only one or a low number of strains within a host, a low sex ratio and a relatively low virulence are predicted. With high numbers of strains within a host, a more even sex ratio and a high parasite virulence are predicted. We examined gametocyte sex ratio and a possible correlate of virulence, parasite density (parasitaemia), in natural populations of two species causing lizard malaria, *Plasmodium* '*tropiduri*' and *P. balli*. The mean sex ratios of both species were female-biased, consistent with estimate selfing rates of 0.36 and 0.48 respectively. In *P.* '*tropiduri*', as we predicted, a positive correlation was also observed between our measure of virulence, parasitaemia and the gametocyte sex ratio. Furthermore, the gametocyte sex ratio was positively correlated with gametocyte density (gametocytaemia). This is consistent with facultative sex allocation in response to variable population structure if gametocytaemia is an indicator of the number of clones within a host. These relationships were not observed in *P. balli*.

Keywords: gametocytes, parasitaemia, *Plasmodium*, population structure, selfing, virulence.

INTRODUCTION

The study of population structure in parasites is potentially important for a number of evolutionary, epidemiological and clinical reasons. For example, considerable attention has recently been paid to how parasite virulence (defined here as the reduction in lifetime reproductive success of a host following parasite infection) should depend upon population structure within hosts (Herre, 1993; Bull, 1994; Ewald, 1994; Read, 1994; Ebert and Herre, 1996; Frank, 1996). In particular, a number of theoretical models have been developed which suggest that increased numbers of unrelated strains (increased genetic variability) within a host generates natural selection in favour of higher virulence (Bremermann and Pickering, 1983; Frank, 1992, 1996; Nowak and May, 1994; Herre, 1995; May and Nowak, 1995). This occurs because the increased competition favours rapid growth in order to achieve greater relative success within the host, and that virulence is assumed to correlate

* Address all correspondence to Stuart A. West, Institute of Cell, Animal and Population Biology, University of Edinburgh, Edinburgh EH9 3JT, UK. e-mail: stu.west@ed.ac.uk

with parasite growth rate. However, there are very few empirical data to support this hypothesis, mainly due to the difficulty of measuring population structure and virulence in natural populations (Herre, 1993).

Sex allocation theory offers an excellent tool for inferring population structure in natural populations of parasites (Read *et al.*, 1992, 1995; West *et al.*, in press). If mating takes place in a subdivided population, where the offspring of one or a few mothers mate in a patch, then a female-biased sex ratio (where sex ratio is defined as the proportion of males) is favoured by a process termed 'local mate competition' (Hamilton, 1967). This female bias arises because it reduces competition among brothers for mates, and because it increases the number of mates for each of the mother's sons (Taylor, 1981). For the purpose of our study, a useful prediction of theory is that the optimal sex ratio, r^*, is $(1 - s)/2$, where s is the selfing rate, defined as the proportion of a mother's daughters that are fertilized by her sons (Read *et al.*, 1992, 1995). The optimal sex ratio favoured by natural selection should thus decline from 0.5 for complete outcrossing ($s = 0$) to 0 for complete selfing ($s = 1$), the latter interpreted as meaning that a female should produce the minimum number of sons required to fertilize all her daughters. This is one of the most well-verified areas in evolutionary biology. It is able to explain considerable variation in sex ratio across populations and species (Hamilton, 1967; Charnov, 1982; Waage, 1982; Herre, 1985, 1987; Wrensch and Ebbert, 1993; Herre *et al.*, 1997; West and Herre, 1998a). Furthermore, individuals of numerous species have been shown to facultatively adjust the sex ratio of their offspring in response to variable local mate competition (Charnov, 1982; Werren, 1987; Godfray, 1994; Herre *et al.*, 1997).

Evolutionary theory thus predicts that both the sex ratio and virulence of a parasite species will respond to the same change in population structure. They can even be theoretically modelled in the same way (Frank, 1992, 1998; Herre, 1993). In the Appendix, we show that this leads to the readily testable prediction that the sex ratio and virulence of a parasite should be positively correlated (Fig. 1). With only one or a low number of strains within a host (extremely subdivided population structures and large amounts of selfing), an extremely low sex ratio and a relatively low virulence are predicted. With high numbers of strains within a host (a more panmictic mating structure and low amounts of selfing), a more even sex ratio and a high parasite virulence are predicted. This predicted correlation between sex ratio and virulence could be tested across parasite species or between different populations of the same species. In addition, if parasites can facultatively alter both sex ratio and virulence in response to local conditions, the relationship would also be found across hosts within a single population. This provides a relatively simple opportunity to test how virulence evolves, because sex ratios can be substantially cheaper and easier to obtain than genetic data on the population structure.

We test our prediction in two species causing lizard malaria, *Plasmodium* '*tropiduri*', and *P. balli*. Both local mate competition and virulence theory have been applied to *Plasmodium.* The life history of *Plasmodium* and related parasites is expected to lead to local mate competition, and previous studies have shown that the average gametocyte sex ratios of natural populations are broadly consistent with the predictions of local mate competition theory (see background biology; Schall, 1989, 1996; Read *et al.*, 1992, 1995; Boudin *et al.*, 1993; Paul *et al.*, 1995; Shutler *et al.*, 1995; Robert *et al.*, 1996; West *et al.*, in press). The applicability of virulence theory to malaria, and specifically the consequences of parasite population structure, has been discussed recently by Read and colleagues (Taylor *et al.*, 1998; Mackinnon and Read, 1999a,b; Read *et al.*, in press). We use

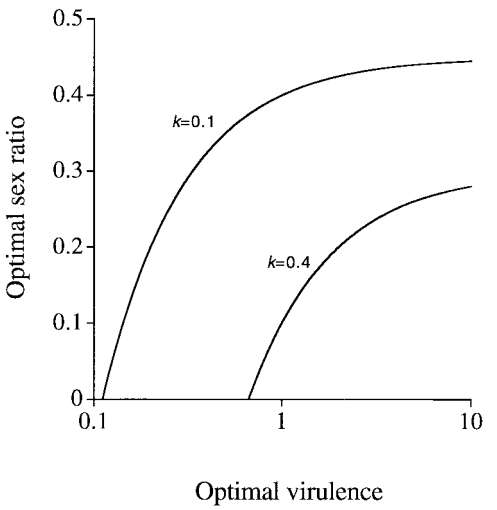

Fig. 1. Predicted relationship between sex ratio and virulence. The figure is based upon equation (A3), with $(d+\gamma)=1$. k is the exponent in the relationship between transmission and virulence.

the density of circulating parasites (parasitaemia) as a measure of virulence. This is based on the assumptions that, all other things being equal, parasitaemia reflects parasite growth rates, not just the number of parasites that infect an individual, and that host fitness is inversely related to parasitaemia. In the Discussion, we present evidence for these assumptions, both in the context of lizard malaria and, more generally, human and rodent malaria.

MATERIALS AND METHODS

Background biology

We studied two malaria parasite species, *P.* '*tropiduri*' and *P. balli*, in natural populations of the lizard *Anolis limifrons* (Sauria, Iguanidae) in the Republic of Panama. The natural history of these parasites, and their effect on *A. limifrons* populations, are described elsewhere (Guerrero *et al.*, 1977; Rand *et al.*, 1983). For a description and additional information on *P. balli*, see Telford (1969, 1974). For taxonomic considerations concerning *P.* '*tropiduri*', we follow Rand *et al.* (1983), who treat this taxon as a single species closely related to *P. floridense*, *P. tropiduri* and *P. minasense* (see Telford, 1974, 1979). The ecology of this population of *A. limifrons* has been described in further detail by Andrews (Andrews *et al.*, 1983; Andrews, 1991).

The biology of malaria parasites relevant to sex ratio evolution is discussed *ad nauseum* by Read *et al.* (1992). Briefly, within vertebrate hosts, clonally proliferating haploid parasites produce haploid gametocytes. Inside the vector, macro- (male) or micro- (female) gametocytes rupture to release either a single macrogamete (functionally equivalent to a single egg) or up to eight microgametes (functionally equivalent to sperm) respectively. Fertilization between these gametes leads to the formation of a diploid zygote. Gametes from the same clone are self-compatible, and random mating between gametes occurs (Ranford-Cartwright *et al.*, 1993; Babiker *et al.*, 1994; Taylor *et al.*, 1997a). The probability

of self-fertilization is determined by the frequency of a clone in the gametocyte population in a blood meal; all else being equal, this is the inverse of the number of clones in the blood meal. Thus, the potential for local mate competition arises because malaria populations are structured: gametes competing for matings are those found in a single blood meal, rather than the gametocyte population in many hosts. More formal proofs of this verbal argument are given by Read *et al.* (1992, 1995) and Dye and Godfray (1993). In this paper, we examine the gametocyte sex ratio, defined as the proportion of gametocytes in the peripheral blood of lizards that were microgametocytes (male).

Methods

Blood samples were collected between May 1976 and December 1977 from wild-caught lizards in the Panama Canal Zone. Blood was drawn from a clipped toe onto a slide, fixed with methanol and stained with geimsa. Each sample was then examined under a microscope at 1000×, and 1000 erythrocytes were counted recording the species, number and type of parasites in these cells. We calculated the parasitaemia (proportion of blood cells infected with parasites). This can be further broken down into gametocytaemia (proportion of blood cells infected with gametocytes) and asexual parasitaemia (proportion of blood cells infected with asexual parasites). The sex ratio of gametocytes was estimated by counting males and females on the slide. The number of gametocytes counted per sample ranged from 1 to 200, and this variation was weighted for in the sex ratio analyses (see below). Male and female gametocytes are approximately equal in size and can be distinguished by colour: males stain pink and females blue. Ten of the lizards infected with *P*. '*tropiduri*' were kept in captivity in cages outside, and further blood samples were taken at periods for up to 4 months.

Statistical analysis

All analyses were carried out using the GLIM statistical package (Crawley, 1993). Proportion data such as sex ratio usually have non-normally distributed error variance and unequal sample sizes. To avoid these problems while retaining maximum power, we analysed the data with a general linear model analysis of deviance, assuming binomial errors, and a logit link function. The number of male gametocytes in a sample was used as the response variable and the total number of gametocytes in a sample as the binomial denominator. Importantly, this form of analysis weights each data point according to its sample size (number of gametocytes in the sample) and so controls for the fact that different numbers of gametocytes were counted from different samples, and that the error variance is greater with small samples.

Initially, a full model was fitted to the data, including all explanatory variables and their interactions. All continuous explanatory variables were assessed for non-linearity by fitting quadratic terms. Terms were then removed from the full model by stepwise deletion (Crawley, 1993). Whether the removal of a term caused a significant increase in deviance was assessed with a χ^2-test. The appropriateness of the assumption of binomial errors was checked by comparing the residual deviance with the residual degrees of freedom after fitting the explanatory variable. Large relative values of the residual deviance indicate overdispersion, which may result in overestimation of significance levels. To account for this, we rescaled the deviance by the heterogeneity factor (HF), the ratio of the residual deviance to

the degrees of freedom (McCullagh and Nelder, 1983). After correcting for overdispersion, the significance of a term was tested for using an *F*-test (Crawley, 1993).

RESULTS

P. '*tropiduri*' was found in 36 lizards and *P. balli* in 22 lizards. *P.* '*tropiduri*' was more common than *P. balli*: *P.* '*tropiduri*' occurred in 73% ($n = 16$) of the lizards that contained *P. balli*, and *P. balli* occurred in 44% ($n = 16$) of the lizards that contained *P.* '*tropiduri*'. The mean, 95% confidence intervals, and percentile limits of parasitaemia, gametocytaemia and gametocyte sex ratio are given in Table 1 for both species. The proportion of parasites present as gametocytes is comparable to that seen in other species of lizard malaria (Schall, 1989, 1996), but substantially higher than levels observed in mammalian hosts (Taylor and Read, 1997).

In both species, the gametocyte sex ratio was female-biased, and showed more variation than was expected given a binomial distribution (*P.* '*tropiduri*': HF = 4.19, Fig. 2a; *P. balli*: HF = 3.26, Fig. 2b). The overall population gametocyte sex ratio showed a greater female bias in *P. balli* (0.26) than in *P.* '*tropiduri*' (0.32), a difference that was almost significant ($F_{1,56} = 3.74$, $0.10 > P > 0.05$, HF = 3.85). In both species, the gametocyte sex ratio did not differ significantly between hosts that only contained that species (single-species infections) and hosts that also contained the other species (mixed-species infections) (*P.* '*tropiduri*': $F_{1,34} = 0.00004$, $P > 0.1$, HF = 3.00; *P. balli*: $F_{1,20} = 1.22$, $P > 0.1$, HF = 3.26).

We examined whether the gametocyte sex ratio differed between the two *Plasmodium* species when they co-occurred in the same host. There where five hosts with mixed-species infections where the number of gametocytes counted were >10 for both species. For four of these cases, the female bias was greater in *P. balli*. In these four cases, the sex ratio difference was significant twice ($\chi^2_1 = 8.40$, $P < 0.005$; $\chi^2_1 = 6.72$, $P < 0.01$), verging on significance once ($\chi^2_1 = 3.74$, $0.10 > P > 0.05$), and not significant once ($\chi^2_1 = 0.37$, $P > 0.10$). In the fifth host, the female bias was greater in *P.* '*tropiduri*', a difference that was not significant ($\chi^2_1 = 0.18$, $P > 0.10$).

We used regression analyses to determine whether the gametocyte sex ratio was positively correlated with parasitaemia, as we predicted. In *P.* '*tropiduri*', there was a significant positive correlation between gametocyte sex ratio and parasitaemia across individuals

Table 1. The characteristics of natural infections of *P.* '*tropiduri*' and *P. balli**

Species	Variable	Mean	95% CI	5% Perc.	95% Perc.
P. '*tropiduri*'	Parasitaemia	0.037	0.021–0.052	0.001	0.159
P. '*tropiduri*'	Gametocytaemia	0.015	0.008–0.021	0.003	0.064
P. '*tropiduri*'	Gametocyte sex ratio	0.32	0.28–0.36	0.00	0.53
P. balli	Parasitaemia	0.010	0.003–0.018	0.001	0.036
P. balli	Gametocytaemia	0.005	0.003–0.008	0.001	0.020
P. balli	Gametocyte sex ratio	0.26	0.22–0.31	0.00	0.37

* Shown are the mean, 95% confidence intervals, 5% and 95% percentiles of parasitaemia (proportion of red blood cells containing parasites), gametocytaemia (proportion of red blood cells containing gametocytes) and the gametocyte sex ratio. The data represent 36 lizards infected with *P.* '*tropiduri*' and 22 infected with *P. balli*. In cases where individual lizards were sampled on multiple days, only the first sample was included.

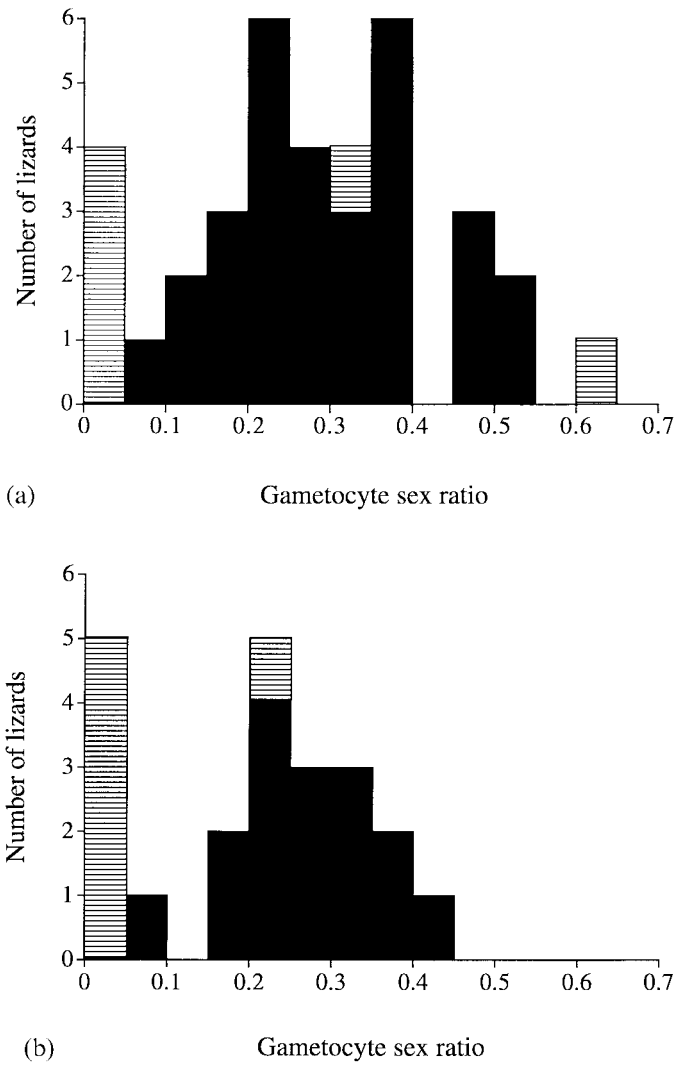

Fig. 2. Frequency distribution of gametocyte sex ratios (proportion of micro-gametocytes (males)). (a) *P.* '*tropiduri*', (b) *P. balli*. The solid bars represent samples with >15 gametocytes counted, while the striped bars represent smaller samples.

($F_{1,34} = 11.12$, $P < 0.01$, $r^2 = 0.25$, HF = 3.25; Fig. 3a). In *P. balli*, this relationship was also positive, but non-significant ($F_{1,20} = 1.29$, $P > 0.1$, $r^2 = 0.06$, HF = 3.38; Fig. 3b).

We then examined the variation in the gametocyte sex ratio data in more detail, to examine whether parasitaemia was the only and best correlate. In *P.* '*tropiduri*', the gametocyte sex ratio of a sample was also positively correlated with gametocytaemia ($F_{1,34} = 14.46$, $P < 0.01$, $r^2 = 0.30$, HF = 3.00; Fig. 4a). Gametocytaemia explained the greatest percentage of the variation in gametocyte sex ratio, and when they were all included in a multiple regression analysis, both parasitaemia and asexual parasitaemia were non-significant (both gave $F_{1,20} = 1.23$, $P > 0.1$). The correlation with parasitaemia when it was the only variable in

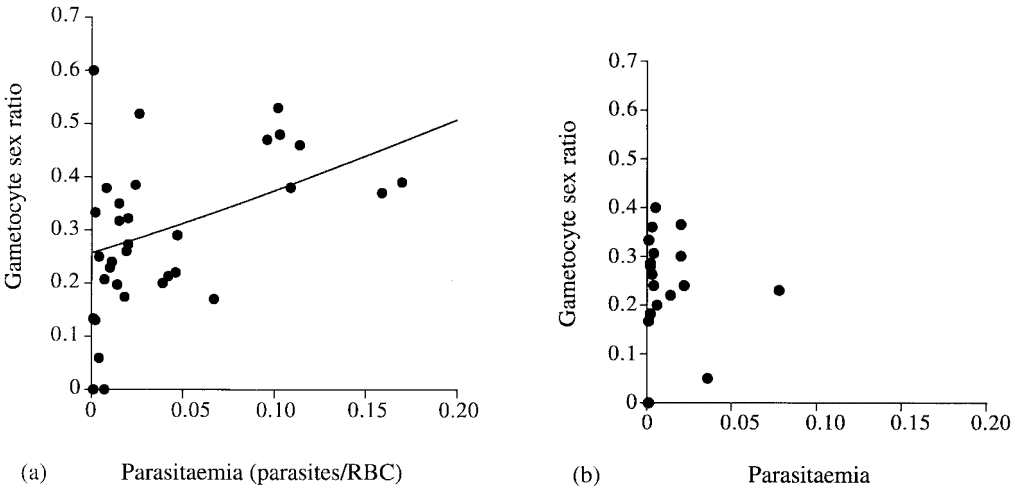

Fig. 3. The relationship between gametocyte sex ratio and parasitaemia (proportion of red blood cells containing parasites). (a) *P.* '*tropiduri*', (b) *P. balli*.

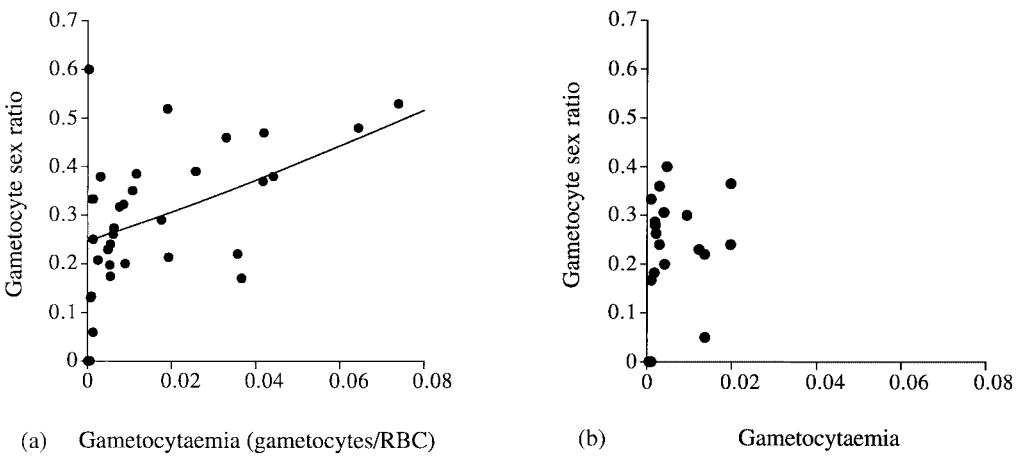

Fig. 4. The relationship between gametocyte sex ratio and gametocytaemia (proportion of red blood cells containing gametocytes). (a) *P.* '*tropiduri*', (b) *P. balli*.

the analysis arises, therefore, because parasitaemia is correlated with gametocytaemia ($F_{1,35} = 12.25$, $P < 0.01$, $r^2 = 0.66$). In *P. balli*, the gametocyte sex ratio of a sample was also positively correlated to gametocytaemia, but the relationship was non-significant ($\chi^2_1 = 0.02$, $P > 0.1$, $r^2 < 0.01$; Fig. 4b).

We also examined whether sex ratio changed within individual infections during the course of an infection. We collected data on *P.* '*tropiduri*' from 10 individuals that were sampled more than once. For each lizard, we calculated the slopes of the relationships between sex ratio, parasitaemia and gametocytaemia. These slopes show the correlations within a host during the course of an infection and were the data points used in subsequent analyses. This method meant that each individual lizard supplied only a single data point to

the analyses, thus avoiding pseudoreplication (Hurlbert, 1984). Within individuals, there was a consistent trend for the sex ratio to be positively correlated with both parasitaemia (7 of 9 individuals that had a non-zero slope; $P = 0.037$, Wilcoxon signed rank test) and gametocytaemia (9 of 10 individuals; $P = 0.002$, Wilcoxon signed rank test).

DISCUSSION

The average and overall gametocyte sex ratios of both *P.* '*tropiduri*' and *P. balli* were female-biased (Table 1), as predicted by sex ratio theory under conditions of local mate competition. In addition, the gametocyte sex ratio was more female-biased in the rarer species, although this difference only verged on significance. Rarer species should have a greater female bias because lower prevalence is likely to lead to less mixed infections (Read *et al.*, 1995; West and Herre, 1998a). Local mate competition theory predicts that, if the sex ratio in a population is r, then the selfing rate s is given by the equation $s = 1 - 2r$ (Read *et al.*, 1992). Our data estimate the mean selfing rates (and 95% confidence intervals) of *P.* '*tropiduri*' and *P. balli* to be 0.36 (0.27–0.43) and 0.48 (0.38–0.57) respectively. Note that these, and in particular the estimate for *P. balli*, are lower bounds. This is because a minimum proportion of male gametocytes is required to ensure that all the female gametes are fertilized (Green *et al.*, 1982; Read *et al.*, 1992, 1995; Nagelkerke and Hardy, 1994; West *et al.*, 1997; West and Herre, 1998a,b). Nonetheless, our results provide further support for the notion that high levels of selfing occur in some populations of malaria (Read *et al.*, 1992, 1995; Hill *et al.*, 1995; Paul *et al.,* 1995).

The results for *P.* '*tropiduri*' provide evidence of facultative changes of gametocyte sex ratio in response to variable levels of selfing (local mate competition). Gametocyte sex ratios were less female-biased in hosts with higher parasitaemia and gametocytaemia (Figs 3a and 4a). Multiple-regression analyses showed that gametocytaemia was the most important correlate of gametocyte sex ratio, and that when it was included in the analysis, the effect of parasitaemia and asexual parasitaemia were not significant. A similar correlation between gametocyte sex ratio and gametocytaemia was reported in the rodent malaria *P. chabaudi* in laboratory mice (Taylor, 1997). These correlations are predicted if a facultative change of the gametocyte sex ratio occurs in response to variable levels of selfing (local mate competition). If mixed-clone infections contain more parasites, and in particular higher gametocytaemia, parasites could be adaptively adjusting their sex ratio in response to local conditions. Evidence for this assumption comes from work on *P. chabaudi*, in which mixed-clone infections have significantly higher gametocytaemias, and in the final stages of an infection, higher parasitaemias (Taylor *et al.*, 1997a,b, 1998; but see Read *et al.*, in press).

We also observed a similar gametocyte sex ratio pattern within hosts during the course of an infection: gametocyte sex ratios were more female-biased when there was less parasitaemia and gametocytaemia. The number of strains infecting an individual can increase during the course of an infection due to new infections, or decrease through strains having different reproductive strategies or mortality rates over time.

To be able to facultatively shift the gametocyte sex ratio in response to variable levels of selfing (local mate competition), parasites must be able to determine the number of clones and their relative frequency in an individual. In principle, there are a number of mechanisms by which they could do this. Gametocyte densities *per se*, or a correlate of gametocyte density, may be an indirect cue (Taylor *et al.*, 1997a,b). Another possibility is

that parasites could use the specificity of the antibodies produced by the immune system to detect the presence and densities of antigenically distinct clones. Although our '*tropiduri*' and Taylor's (1997) data are consistent with facultative shifts in gametocyte sex ratio in response to local gametocyte densities, we also note that correlations between sex ratio and measures of parasite or gametocyte densities were not found in *P. balli* (this study), other lizard malarias (Schall, 1989), *P. falciparum* in humans (Robert *et al.*, 1996), or *Haemoproteus* populations in birds (Shutler *et al.*, 1995; Shutler and Read, 1998). A possible explanation for the cases in which predicted correlations were not found is that ensuring there are male gametocytes in a blood meal is a limiting factor (see below).

We predicted a positive relationship between sex ratio and our measure of virulence, parasitaemia (Appendix; Fig. 1). This prediction arises because virulence and gametocyte sex ratio are both predicted to increase facultatively with the number of strains in a host. The data from *P.* '*tropiduri*' support this prediction, both across individuals (Fig. 3a), and within individuals during the course of an infection. Although data showing facultative alteration of virulence in response to the strains in a host is lacking (Read *et al.*, in press), there is evidence for facultative variation in other life-history traits of malaria parasites (Buckling *et al.*, 1997). Our use of parasitaemia as a measure of virulence was based on two assumptions. First, we assumed that high parasitaemias reflect greater growth rates and not just infection levels. Experimental work with rodent malaria supports this prediction: peak parasitaemia levels are determined by growth rates and not just the number of parasites used to infect an individual (Grovenor *et al.*, 1995; Mackinnon and Read, 1999a).

Second, we assumed that, all other things being equal, host fitness is inversely related to parasitaemia. In animal (rodent) models under controlled experimental conditions, measures of virulence (weight loss and probability of death) are strongly correlated with parasitaemia (Taylor *et al.*, 1998; Mackinnon and Read, 1999a,b). In experimental human infections, there is also a strong correlation between the severity of symptoms and parasitemia with, for example, fever apparently triggered by high parasitaemias (Kwiatkowski and Greenwood, 1989). In natural human infections, parasitaemia is clearly not the sole cause of disease; other factors must also play a role (e.g. varying levels of acquired immunity). Indeed, Taylor *et al.* (1998) found that even when there were non-significant differences in the parasitaemia of rodent malaria infections, virulence was still significantly higher in mixed-clone infections than single-clone infections. Nevertheless, parasitaemias are almost always higher in symptomatic than asymptomatic infections (e.g. Englebrecht *et al.*, 1995; Contamin *et al.*, 1996; Beck *et al.*, 1997), and parasitaemias above a threshold often form part of the definition of a clinical case of malaria (e.g. Contamin *et al.*, 1996).

In the context of lizard malaria, there is also evidence that parasite densities and virulence are correlated. Higher parasitaemia is associated with reduced success in competitive interactions between males (Schall and Dearing, 1987), and with higher densities of immature erythrocytes (Schall *et al.*, 1982; Rand *et al.*, 1983; Schall, 1983), which trigger the principal physiological consequences of *Plasmodium* infection (Schall *et al.*, 1982). These relationships are relatively weak, perhaps because of time lags between high parasitaemia and its consequences (Schall, 1983). In addition, some components contributing to virulence, such as the destruction of non-infected blood cells by the host's immune system, may be density-independent (Schall, 1996). Such effects, together with factors such as age, previous exposure, innoculating dose, host genotype, and so on (Greenwood *et al.*, 1991) will, of course, weaken the statistical relationship between virulence and parasitaemia.

Nevertheless, such noise should obscure any associations between sex ratio and our measure of virulence (parasitaemia), rather than generate artefactual relationships.

Why did the patterns differ in *P. balli*? Given our assumptions, this result may indicate that *P. balli* clones do not facultatively adjust gametocyte sex ratio and/or virulence. However, the *P. balli* data set was smaller, showed less variation in all variables and gametocytes were very rare (Table 1; Figs 3b and 4b). In particular, the gametocyte density in the blood is low enough that ensuring there are male gametes in a blood meal may become the limiting factor. In this case, natural selection favours a less female-biased sex ratio, and the sex ratio is predicted to vary little with selfing rates. Similar arguments are thought to explain variation in sex ratio in invertebrate species that produce small clutch sizes (Nagelkerke and Hardy, 1994; Nagelkerke, 1996; West *et al.*, 1997; West and Herre, 1998b). Two other possible important factors with *P. balli* are that: (1) the low numbers of gametocytes used to estimate the sex ratio would have limited our statistical power to detect any relationship (type II error), and (2) the low variability in all variables suggests that the number of clones does not vary much between natural infections in hosts, and so that either there is an insufficiently strong pattern to detect, or there has been very little selection for facultative strategies (Herre, 1987; Herre *et al.*, 1997). With regards to all of these possibilities, it is worth noting that, if we analyse the *P.* '*tropiduri*' data using only points that occur in the same range of parasitaemia levels as that observed in *P. balli*, then we find no significant relationships with sex ratio.

Finally, we are well aware of the limitations of our data. Although one advantage is that they are natural field data, our findings are observational and so open to multiple explanations. For example, if female gametocyte mortality increases at higher parasitaemia, our interpretations are suspect (we note, however, that at least in *P. falciparum* (Smalley and Sinden, 1977) and other lizard malaria parasites (Schall, 1989), there is no evidence of differential mortality, and that the two species we examined had significantly different sex ratios within the same host). Nonetheless, the predicted relationship between sex ratio and virulence provides a relatively easy way to test theory in natural populations. Our data are the first test of this relationship, and are consistent with our prediction. These data suggest several areas that should be examined experimentally and through the collection of more field data. More generally, this also shows how evolutionary biology and optimality models can be used to provide insights into matters of clinical and epidemiological importance, such as disease virulence.

ACKNOWLEDGEMENTS

We thank A. Buckling, M. Mackinnon, J. Schall, B. Shapiro, D. Shutler and L. Taylor for comments on the manuscript. Funding was provided by STRI (J.P., S.G. & S.A.W.) and the BBSRC (A.F.R. & S.A.W.).

REFERENCES

Andrews, R.M. 1991. Population stability of a tropical lizard. *Ecology*, **91**: 1204–1217.

Andrews, R.M., Rand, A.S. and Guerrero, S. 1983. Seasonal and spatial variation in the annual cycle of a tropical lizard. In *Advances in Herpetology and Evolutionary Biology: Essays in Honor of Ernest E. Williams* (A.G.J. Rhodin and K. Miyata, eds), pp. 441–454. Cambridge: Museum of Comparative Zoology.

Babiker, H.A., Ranford-Cartwright, L., Currie, D., Charlwood, J.D., Billingsley, P., Teuscher, T. and Walliker, D. 1994. Random mating in a natural population of the malaria parasite *Plasmodium falciparum*. *Parasitology*, **109**: 413–421.

Beck, H.-P., Felger, I., Huber, W., Steiger, S., Smith, T., Weiss, N., Alonso, P. and Tanner, M. 1997. Analysis of multiple *Plasmodium falciparum* infections in Tanzanian children during the Phase III trial of malaria vaccine Spf66. *J. Infect. Dis.,* **175**: 921–926.

Boudin, C., Olivier, M., Molez, J., Chiron, J. and Ambroise-Thomas, P. 1993. High human malarial infectivity to laboratory-bred *Anopheles gambiae* in a village in Burkina Faso. *Am. J. Trop. Med. Hyg*., **48**: 700–706.

Bremermann, H.J. and Pickering, J. 1983. A game-theoretical model of parasite virulence. *J. Theor. Biol.*, **100**: 411–426.

Buckling, A.G.L., Taylor, L.H., Carlton, J.M.-R. and Read, A.F. 1997. Adaptive changes in *Plasmodium* transmission strategies following chloroquine chemotherapy. *Proc. R. Soc. Lond. B*, **264**: 553–559.

Bull, J.J. 1994. Virulence. *Evolution*, **48**: 1423–1437.

Charnov, E.L 1982. *The Theory of Sex Allocation*. Princeton, NJ: Princeton University Press.

Contamin, H., Fandeur, T., Rogier, C., Bonnefoy, S., Konate, L., Trape, J.-F. and Mercereau-Puijalon, O. 1996. Different genetic characteristics of *Plasmodium falciparum* isolate collected during successive clinical episodes in Senegalese children. *Am. J. Trop. Med. Hyg.*, **54**: 632–643.

Crawley, M. 1993. *GLIM for Ecologists*. Oxford: Blackwell Scientific.

Dye, C. and Godfray, H.C.J. 1993. On sex ratio and inbreeding in malaria parasite populations. *J. Theor. Biol.*, **161**: 131–134.

Ebert, D. and Herre, E.A 1996. The evolution of parasitic diseases. *Parasitol. Today*, **12**: 96–101.

Englebrecht, F., Felger, I., Genton, B., Alpers, M. and Beck, H.-P. 1995. *Plasmodium falciparum*: Malaria morbidity is associated with specific Merozoite Surface Antigen 2 Genotypes. *Exp. Parasitol.*, **81**: 90–96.

Ewald, P.W. 1994. *Evolution of Infectious Diseases*. Oxford: Oxford University Press.

Frank, S.A. 1992. A kin selection model for the evolution of virulence. *Proc. R. Soc. Lond. B*, **250**: 195–197.

Frank, S.A. 1996. Models of parasite virulence. *Q. Rev. Biol.*, **71**: 37–78.

Frank, S.A. 1998. *Foundations of Social Evolution.* Princeton, NJ: Princeton University Press.

Godfray, H.C.J. 1994. *Parasitoids: Behavioural and Evolutionary Ecology*. Princeton, NJ: Princeton University Press.

Green, R.E., Gordh, G. and Hawkins, B. 1982. Precise sex ratios in highly inbred parasitic wasps. *Am. Nat.*, **120**: 653–665.

Greenwood, B., Marsh, K. and Snow, R. 1991. Why do some African children develop severe malaria? *Parasitol. Today*, **7**: 277–281.

Grovenor, M.B., McLean, A.R. and Kwiatkowski, D. 1995. The regulation of malaria parasitaemia: Parameter estimations for a population model. *Parasitology*, **110**: 115–122.

Guerrero, S., Rodriguez, C. and Ayala, S.C. 1977. Prevalencia de hemoparasitos en legartijas de la Isla Barro Colorado, Panama. *Biotropica*, **9**: 118–123.

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

Herre, E.A. 1985. Sex ratio adjustment in fig wasps. *Science*, **228**: 896–898.

Herre, E.A. 1987. Optimality, plasticity and selective regime in fig wasp sex ratios. *Nature*, **329**: 627–629.

Herre, E.A. 1993. Population structure and the evolution of virulence in nematode parasites of fig wasps. *Science*, **259**: 1442–1445.

Herre, E.A. 1995. Factors influencing the evolution of virulence: Nematode parasites of fig wasps as a case study. *Parasitology*, **111**: S179–S191.

Herre, E.A., West, S.A., Cook, J.M., Compton, S.G. and Kjellberg, F. 1997. Fig wasp mating systems: Pollinators and parasites, sex ratio adjustment and male polymorphism, population

structure and its consequences. In *Social Competition and Cooperation in Insects and Arachnids*, *Vol. I: The Evolution of Mating Systems* (J. Choe and B. Crespi, eds), pp. 226–239. Princeton, NJ: Princeton University Press.

Hill, W.G., Babiker, H.A., Ranford-Cartwright, L.C. and Walliker, D. 1995. Estimation of inbreeding coefficients from genotypic data on multiple alleles, and application to estimation of clonality in malaria parasites. *Genet. Res.*, **65**: 53–61.

Hurlbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. *Ecol. Monogr.*, **54**: 187–211.

Kwiatkowski, D. and Greenwood, B.M. 1989. Why is malaria fever periodic? A hypothesis. *Parasitol. Today*, **5**: 264–266.

Mackinnon, M.J. and Read, A.F. 1999a. Genetic relationship between virulence and transmissibility in the rodent malaria *Plasmodium chabaudi. Evolution*, **53**: 689–703.

Mackinnon, M.J. and Read, A.F. 1999b. Selection for high and low virulence in the malaria parasite *Plasmodium chabaudi*. *Proc. R. Soc. Lond. B*, **266**: 741–748.

May, R.M. and Nowak, M.A. 1995. Coinfection and the evolution of parasite virulence. *Proc. R. Soc. Lond. B*, **261**: 209–215.

McCullagh, P. and Nelder, J.A. 1983. *Generalized Linear Models*. London: Chapman & Hall.

Nagelkerke, C.J. 1996. Discrete clutch sizes, local mate competition, and the evolution of precise sex allocation. *Theor. Pop. Biol.*, **49**: 314–343.

Nagelkerke, C.J. and Hardy, I.C.W. 1994. The influence of developmental mortality on optimal sex allocation under local mate competition. *Behav. Ecol.*, **5**: 401–411.

Nowak, M. and May, R.M. 1994. Superinfection and the evolution of parasite virulence. *Proc. R. Soc. Lond. B*, **255**: 81–89.

Paul, R.E.L., Packer, M.J., Walmsley, M., Lagog, M., Ranford-Cartwright, L.C., Paru, R. and Day, K.P. 1995. Mating patterns in malaria parasite populations of Papua New Guinea. *Science*, **269**: 1709–1711.

Rand, A.S., Guerrero, S. and Andrews, R.M. 1983. The ecological effects of malaria on populations of the lizard *Anolis limifrons* on Barro Colorado Island, Panama. In *Advances in Herpetology and Evolutionary Biology: Essays in Honor of Ernest E. Williams* (A.G.J. Rhodin and K. Miyata, eds), pp. 455–471. Cambridge: Museum of Comparative Zoology.

Ranford-Cartwright, L.C., Balfe, P., Carter, R. and Walliker, D. 1993. Frequency of cross-fertilisation in the human malaria parasite *Plasmodium falciparum*. *Parasitology*, **107**: 11–18.

Read, A.F. 1994. The evolution of virulence. *Trends Microbiol.*, **2**: 73–76.

Read, A.F., Narara, A., Nee, S., Keymer, A.E. and Day, K.P. 1992. Gametocyte sex ratios as indirect measures of outcrossing rates in malaria. *Parasitology*, **104**: 387–395.

Read, A.F., Anwar, M., Shutler, D. and Nee, S. 1995. Sex allocation and population structure in malaria and related parasitic protozoa. *Proc. R. Soc. Lond. B*, **260**: 359–363.

Read, A.F., Mackinnon, M.J., Anwar, M.A. and Taylor, L.H. in press. Kin selection models as evolutionary explanations of malaria. In *Virulence Management: The Adaptive Dynamics of Pathogen–Host Interactions* (U. Dieckmann, J.A.J. Metz, M.W. Sabelis and K. Sigmund, eds). Cambridge: Cambridge University Press.

Robert, V., Read, A.F., Essong, J., Tchuinkam, T., Mulder, B., Verhave, J.P. and Carnevale, P. 1996. Effect of gametocyte sex ratio on infectivity of *Plasmodium falciparum* to *Anopheles gambiae*. *Trans. R. Soc. Trop. Med. Hyg.*, **90**: 621–624.

Schall, J.J. 1983. Lizard malaria: Host–parasite ecology. In *Lizard Ecology: Studies of a Model Organism* (R.B. Huey, E.R. Pianka and T.W. Schoener, eds), pp. 84–100. Cambridge, MA: Harvard University Press.

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

Schall, J.J. 1996. Malarial parasites of lizards: Diversity and ecology. *Adv. Parasitol.*, **37**: 255–333.

Schall, J.J. and Dearing, M.D. 1987. Malaria parasitism and male competition for mates in the western fence lizard *Sceloporus occidentalis*. *Oecologia*, **73**: 389–392.

Schall, J.J., Bennett, A.F. and Pulham, R.W. 1982. Lizards infected with malaria: Physiological and behavioural consequences. *Science*, **217**: 1057–1059.

Shutler, D. and Read, A.F. 1998. Extraordinary and ordinary blood parasite sex ratios. *Oikos*, **82**: 417–424.

Shutler, D., Bennett, G.F. and Mullie, A. 1995. Sex proportions of *Haemoproteus* blood parasites and local mate competition. *Proc. Nat. Acad. Sci. USA*, **92**: 6748–6752.

Smalley, M.E. and Sinden, R.E. 1977. *Plasmodium falciparum* gametocytes: Their longevity and infectivity. *Parasitology*, **74**: 1–8.

Taylor, L.H. 1997. *Epidemiological and Evolutionary Consequences of Mixed-genotype Infections of Malaria Parasites*. Edinburgh: University of Edinburgh.

Taylor, L.H. and Read, A.F. 1997. Why so few transmission stages? Reproductive restraint by malaria parasites. *Parasitol. Today*, **13**: 135–140.

Taylor, L.H., Walliker, D. and Read, A.F. 1997a. Mixed-genotype infections of malaria parasites: Within-host dynamics and transmission success of competing clones. *Proc. R. Soc. Lond. B*, **264**: 927–935.

Taylor, L.H., Walliker, D. and Read, A.F. 1997b. Mixed-genotype infections of the rodent malaria *Plasmodium chabaudi* are more infectious to mosquitoes than single-genotype infections. *Parasitology*, **115**: 121–132.

Taylor, L.H., Mackinnon, M.J. and Read, A.F. 1998. Virulence of mixed-clone and single-clone infections of the rodent malaria *Plasmodium chabaudi. Evolution*, **52**: 583–591.

Taylor, P.D. 1981. Intra-sex and inter-sex sibling interactions as sex determinants. *Nature*, **291**: 64–66.

Telford, S.R. 1969. A new malarial parasite *Plasmodium balli* from Panama. *J. Protozool.*, **16**: 431–437.

Telford, S.R. 1974. The malarial parasites of *Anolis* species (Sauria: Iguanidae) in Panama. *Int. J. Parasitol.*, **4**: 91–102.

Telford, S.R. 1979. A taxonomic reconsideration of some *Plasmodium* species from iguanid lizards. *Ann. Parasitol.*, **54**: 129–144.

Waage, J.K. 1982. Sib-mating and sex-ratio strategies in Scelionid wasps. *Ecol. Ent.*, **7**: 103–112.

Werren, J.H. 1987. Labile sex ratios in wasps and bees. *Bioscience*, **37**: 498–506.

West, S.A. and Herre, E.A. 1998a. Partial local mate competition and the sex ratio: A study on non-pollinating fig wasps. *J. Evol. Biol.*, **11**: 531–548.

West, S.A. and Herre, E.A. 1998b. Stabilizing selection and variance in fig wasp sex ratios. *Evolution*, **52**: 475–485.

West, S.A., Herre, E.A., Compton, S.G., Godfray, H.C.J. and Cook, J.M. 1997. A comparative study of virginity in fig wasps. *Anim. Behav.*, **54**: 437–450.

West, S.A., Smith, T.G. and Read, A.F. in press. Sex allocation and population structure in Apicomplexan (Protozoa) parasites. *Proc. R. Soc. Lond. B*.

Wrensch, D.L. and Ebbert, M.A. 1993. *Evolution and Diversity of Sex Ratio in Insects and Mites*. New York: Chapman & Hall.

APPENDIX

Here we formalize the general prediction that the virulence and sex ratio of a parasite subject to local mate competition should be positively correlated. Assume that there are a number of parasite strains (clones) in a host with a coefficient of relatedness. If n co-infecting strains are unrelated and equally abundant, then the coefficient of relatedness equals $1/n$ (Frank, 1992). Several studies have shown that the optimal level of virulence increases with higher n (Bremermann and Pickering, 1983; Frank, 1992, 1996). For example, Frank (1992, 1996) showed that, assuming the only trade-off was between transmission and virulence, the optimal level of virulence (α^*, the disease induced mortality rate) is given by the equation

$$\alpha^* = k(d + \gamma)/(1/n - k) \tag{A1}$$

where d is the disease-free mortality rate, γ is the rate at which hosts clear the parasite, and k is the exponent in the relationship between transmission ($\beta(\alpha)$) and virulence, $\beta(\alpha) = b\alpha^k$ (for $k < 1/n$).

If we assume that mating occurs randomly between the strains that infect a host, then sex ratio theory predicts that the optimal sex ratio is given by

$$r^* = (n - 1)/2n \tag{A2}$$

(Hamilton, 1967; Read *et al.*, 1992). By rearranging equation (A1) and substituting it into equation (A2), it can be shown that

$$r^* = \frac{\alpha^*(1 - k) - k(d + \gamma)}{2\alpha^*} \tag{A3}$$

which predicts a positive correlation between sex ratio and virulence (Fig. 1). Note that as $\alpha^* \to \infty$, $r^* = 0.5(1 - k)$. This does not asymptote necessarily at 0.5 because $n < 1/k$ to give equation (A1) (Frank, 1992, 1996). If mating only takes place between a subsample of the strains in a host that are producing gametocytes at any particular time, then the same relationship would be predicted, but with a lower (more female-biased) sex ratio for a given level of virulence.