SUPPLEMENTARY INFORMATION: DATA

Parasite genotypes. Clonal *Plasmodium chabaudi* genotypes were isolated from independent infected *Thamnomys rutilans* (thicket rats) caught in different locations in the Central African Republic and Congo-Brazzaville in the late 1960s and early 1970s. To isolate different genotypes, samples from these wild caught infections were diluted to an average of 1 parasite per inoculum and administered to mice, and parasites from the resulting infections stored as frozen stabilites (Beale et al 1978; Mackinnon & Read 1999). These genotypes were confirmed as *P. chabaudi* by morphology (Carter & Walliker 1975) and electrophoretic enzyme analysis used to show they are genetically distinct (Carter 1978). Most of these wild caught infected rodents harboured mixed infections of 2 or 3 *P. chabaudi* genotypes (Carter 1978). These genotypes form part of the WHO Registry of Standard Malaria Parasites, held at The University of Edinburgh, UK.

Estimating relative male fecundity. Read et al's (1992) model incorporates limited male fecundity into the classical model of LMC. If a proportion z of individuals in a mating group are male, and a proportion $1-z$ are female, then the relative numbers of viable male and female gametes are zc and $1-z$ where c is the relative fecundity of males. The number of ookinetes produced by the mating group is determined by the limiting sex, i.e. it is proportional to $\min(zc, 1-z)$ and the unbeatable sex ratio (z^*) equalises the number of viable male and female gametes, $z^*c = 1-z^*$. Our data suggest that this maximum occurs at $z^* = 0.33$ [0.20, 0.39], and rearranging obtains $c = 2.03$ [1.56, 4.00].

Genetic variation in patterns of sex allocation. In this analysis it was necessary to control for all possible sources of variation to test for an effect of genotype identity, so we fitted infection parameters as covariates and day post infection as a factor. The only infection parameter remaining in the minimal model was the density of parasites, which correlated positively with sex ratio ($b = 27.35 \times 10^6$/ml ± 13.41). Because some genotypes appeared to follow the same sex allocation pattern throughout infections we tested which could be grouped together without causing significant change in model deviance. Genotypes DK, CW and CR all followed significantly different sex allocation patters but AS, AJ and ER could be grouped together ($\chi^2_{24} = 20.48$; P = 0.669).

Table S1: Analysis of sex ratios produced by six genotypes throughout infections.

Minimal model	**LRT (χ^2)**	**P**
Genotype	NA	
Day post infection	NA	
Genotype:day post infection	$\chi^2_{55} = 159.55$	< 0.0001
Parasite density	$\chi^2_1 = 5.25$	0.022
Non significant terms deleted from maximal model		
Reticulocyte density	$\chi^2_1 = 0.01$	0.913
Red blood cell density	$\chi^2_1 = 0.15$	0.696
Host mass	$\chi^2_1 = 0.53$	0.467
Gametocyte density	$\chi^2_1 = 1.14$	0.286

If there is genetic variation for the number of gametes produced by male gametocytes (*c*), this could also explain some of the observed variation in patterns of sex allocation. In this case, Read et al's (1992) model can be used to estimate *c* (see above). For example, estimating *c* from sex ratios on day 5 post infection gives: CR = 2.39 [1.67, 3.67]; DK = 3.80 [3.40, 4.28]; CW = 7.89 [6.52, 9.86]; and ER, AS, AJ = 12.28 [10.40, 14.90].

Explaining sex ratio variation throughout infections. To specifically investigate how infection parameters co-vary with sex ratio across infections we excluded day post infection from the models. Data suggest that sex is determined early in gametocyte development and maturation time of rodent malaria gametocytes is thought to take 24-48hours. If our assays detect mature gametocytes there could be a temporal mismatch of up to 48hours between observed sex ratio and any environmental cues involved. To test for this possibility we ran three analyses in which infection parameters were observed at the same time, 24 hours and 48 hours before sex ratios. The minimal model in which infection parameters were observed 48 hours before sex ratios, explained significantly more deviance than the other two minimal models (log-likelihoods for 0, 24 and 48 hours before: 229.10, 185.69 and 168.50 respectively; LRT for 0 versus 24 hours: $\chi^2_1 = 86.83$; $P < 0.0001$; and 0 versus 48 hours; $\chi^2_3 = 121.21$; $P < 0.0001$).

Table S2: Sex ratios vary throughout infections and correlate with infection and host parameters observed 48 hours previously.

Minimal model	**LRT (χ^2)**	**P**
Genotype group	NA	
Red blood cell density	NA	
Gametocyte density	NA	
Parasite density	NA	
Genotype group:red blood cell density	$\chi^2_3 = 15.86$	0.0012
Genotype group:gametocyte density	$\chi^2_3 = 22.11$	< 0.0001
Genotype group:parasite density	$\chi^2_3 = 35.35$	< 0.0001
Non significant terms deleted from maximal model		
Mass	$\chi^2_1 = 3.26$	0.071
Reticulocyte density	$\chi^2_1 = 0.19$	0.663
Genotype group:mass	$\chi^2_3 = 0.20$	0.977
Genotype group:reticulocyte density	$\chi^2_3 = 7.30$	0.063

Infection genetic diversity and facultative sex allocation.

Here, we compared the sex ratios produced by single-genotype infections of each of our six genotypes with those produced by six-genotype infections. It should be noted that in the single infections, genotype DK produced the least female-biased sex allocation pattern. This genotype produces the lowest parasite density of our panel and is a poor competitor (Bell et al 2006), so would not have been disproportionately represented in the six-genotype infections.

Table S3: Analysis of sex ratios produced by infections differing in genetic diversity during their growth phase.

Minimal model	LRT (χ^2)	P
Genetic diversity	NA	
Day post infection	NA	
Genetic diversity:day post infection	$\chi^2_2 = 19.93$	< 0.0001
Gametocyte density	$\chi^2_1 = 11.94$	< 0.0001
Non significant terms deleted from maximal model		
Reticulocyte density	$\chi^2_1 = 0.20$	0.654
Red blood cell density	$\chi^2_1 = 0.02$	0.881
Host mass	$\chi^2_1 = 3.44$	0.064
Parasite density	$\chi^2_1 = 1.06$	0.304

Facultative sex allocation of focal genotypes.

Table S4a: Sex ratios of focal genotypes in single and two-genotype infections during the growth phase of infections.

Minimal model	LRT (χ^2)	P
Focal genotype	NA	
Genetic diversity	NA	
Day post infection	NA	
Focal:diversity	$\chi^2_2 = 9.98$	0.007
Diversity:day	$\chi^2_2 = 12.52$	0.002
Focal:day	$\chi^2_4 = 11.27$	0.024
Non significant terms deleted from maximal model		
Reticulocyte density	$\chi^2_1 = 0.94$	0.333
Red blood cell density	$\chi^2_1 = 3.47$	0.062
Host mass	$\chi^2_1 = 1.03$	0.310
Infection parasite density	$\chi^2_1 = 0.02$	0.886
Infection gametocyte density	$\chi^2_1 = 1.03$	0.310
Focal parasite density	$\chi^2_1 = 0.27$	0.604
Focal gametocyte density	$\chi^2_1 = 0.16$	0.693

Table S4b: Sex ratios and proportional representation of focal genotypes during the growth phase of infections.

Minimal model	LRT (χ^2)	P
Focal genotype	NA	
Day post infection	NA	
Proportion of focal parasites	NA	
Proportion of focal gametocytes	$\chi^2_1 = 16.08$	< 0.0001
Red blood cell density	$\chi^2_1 = 10.61$	0.001
Focal genotype:day	$\chi^2_4 = 21.23$	< 0.0001
Proportion of focal parasites:focal genotype	$\chi^2_2 = 10.44$	0.005
Non significant terms deleted from maximal model		
Focal gametocyte density	$\chi^2_1 = 0.16$	0.876
Focal parasite density	$\chi^2_1 = 0.91$	0.339

Infection parasite density	$\chi^2_1 = 1.03$	0.311
Infection gametocyte density	$\chi^2_1 = 0.22$	0.642
Reticulocyte density	$\chi^2_1 = 0.47$	0.493
Host mass	$\chi^2_1 = 0.49$	0.486
Proportion of focal gametocytes:focal genotype	$\chi^2_2 = 0.26$	0.876

Table S5a: Sex ratios of focal genotypes in single and two-genotype infections during the post-peak phase of infections.

Minimal model	**LRT (χ^2)**	**P**
Focal genotype	NA	
Genetic diversity	NA	
Day post infection	$\chi^2_4 = 129.14$	< 0.0001
Focal:diversity	$\chi^2_2 = 27.33$	< 0.0001
Red blood cell density	$\chi^2_1 = 6.89$	0.009
Non significant terms deleted from maximal model		
Diversity:day	$\chi^2_4 = 5.80$	0.215
Focal:day	$\chi^{28} = 4.42$	0.817
Reticulocyte density	$\chi^2_1 = 3.33$	0.068
Host mass	$\chi^2_1 = 0.42$	0.515
Infection parasite density	$\chi^2_1 = 0.01$	0.922
Infection gametocyte density	$\chi^2_1 = 1.59$	0.208
Focal parasite density	$\chi^2_1 = 0.70$	0.401
Focal gametocyte density	$\chi^2_1 = 0.37$	0.543

Table S5b: Sex ratios and proportional representation of focal genotypes during the post-peak phase of infections.

Minimal model	**LRT (χ^2)**	**P**
Focal genotype	$\chi^2_2 = 24.83$	< 0.0001
Day post infection	$\chi^2_4 = 47.92$	< 0.0001
Red blood cell density	$\chi^2_1 = 10.04$	0.002
Non significant terms deleted from maximal model		
Host mass	$\chi^2_1 = 0.15$	0.695
Reticulocyte density	$\chi^2_1 = 2.84$	0.092
Infection parasite density	$\chi^2_1 = 0.23$	0.632
Infection gametocyte density	$\chi^2_1 = 0.16$	0.685
Focal gametocyte density	$\chi^2_1 = 3.01$	0.083
Focal parasite density	$\chi^2_1 = 2.51$	0.113
Proportion of focal parasites	$\chi^2_1 = 2.85$	0.091
Proportion of focal gametocytes	$\chi^2_1 = 2.68$	0.102
Focal genotype:day	$\chi^2_8 = 13.12$	0.108
Proportion of focal parasites:focal genotype	$\chi^2_2 = 1.44$	0.486
Proportion of focal gametocytes:focal genotype	$\chi^2_2 = 3.00$	0.223

References.

Beale, G.H., Carter, R. and Walliker D. (1978) Genetics. Pages 213–245 *in* R. Killick Kendrick and W. Peters, eds. Rodent malaria. Academic Press, London.

Bell, A.S., *et al*. (2006) Within-host competition in genetically diverse malaria infections: Parasite virulence and competitive success. *Evolution* 60, 1358-1371

Carter, R. (1978) Studies on enzyme variation in murine malaria parasites *Plasmodium-berghei, P-yoelii, Plasmodium-vinckei* and *Plasmodium-chabaudi* by Starch-Gel Electrophoresis. *Parasitology* 76, 241-267

Carter, R. and Walliker, D. (1975) New observations on the malaria parasites of rodents of the Central African Republic—*Plasmodium vinckei* petted subsp. nov. and *Plasmodium chabaudi* Landau, 1965, *Ann. Trop. Med. Parasitol*. 69, 187–196.

Mackinnon, M.J., and Read, A.F. (1999) Genetic relationships between parasite virulence and transmission in the rodent malaria *Plasmodium chabaudi*. *Evolution* 53, 689-703

Read, A.F. *et al.* Gametocyte sex-ratios as indirect measures of outcrossing rates in malaria. *Parasitology* 104, 387-395 (1992).