Sex allocation and population structure in malaria and related parasitic protozoa

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SUMMARY

Here we demonstrate how sex allocation theory, one of the best verified areas of metazoan evolutionary biology, can be successfully applied to microparasitic organisms, by relating parasite prevalence and sex ratio in the Haemosporina. Members of this taxon, which includes *Plasmodium*, are parasitic protozoa with obligate sexual cycles in which dioecious haploid gametes drawn from the peripheral blood of a vertebrate host fuse within a dipteran vector. Consequently mating takes place within a highly subdivided population, a condition known to promote local mate competition and inbreeding and hence the evolution of female-biased sex ratios. We used an epidemiological framework to investigate mating patterns and sex ratio evolution within natural populations of these parasites. This phenotypic approach compliments more conventional biochemical approaches to the population genetics of parasitic protozoa. Data are presented which support a theoretical relation between transmission-stage sex ratio and prevalence across parasite populations. These results are consistent with a large inter-population variation in genetic structure and argue against sweeping generalizations about the clonality or otherwise of populations of these parasitic protozoa.

1. INTRODUCTION

Recent enthusiasm that the adaptationist programme can contribute to biomedical research (Williams & Nesse 1991, 1994; Ewald 1994; Westoby 1994; Futuyma 1995) could spread beyond evolutionary biology if novel and successful quantitative predictions about biomedical phenomena were made from evolutionary theory. Here we demonstrate a successful application of sex allocation theory, one of the quantitatively best verified areas of metazoan evolutionary biology (see, for example, Werren 1980; Charnov 1982; Herre 1985, 1987, Frank 1990; Antolin 1993; Wrench & Ebbert 1993), to infectious disease organisms. In so doing, we investigate the population structure of malaria parasites and related organisms, a contentious issue otherwise accessible only by conventional biochemical analysis (see, for example, Tibayrenc *et al.* 1990; Babiker *et al.* 1994).

Parasitic protozoa of the suborder Haemosporina, which includes *Plasmodium* and a number of related genera including *Leucocytozoon*, have obligate sexual cycles which share the following biological details (Sinden 1983; Carter & Graves 1988; Desser & Bennett 1993). After a period of asexual proliferation in a vertebrate host, transmission to a dipteran vector occurs via dioecious haploid sexual stages, the gametocytes. Female gametocytes give rise to a single female gamete, whereas male gametocytes rupture and release up to eight viable male gametes. A single clone can produce self-compatible male and female gametes, and clones differ in the gametocyte sex ratios produced. Mating occurs amongst gametes present in up to 3 μl of peripheral blood drawn by a vector; it occurs within 20 mins of a blood meal, so that cross-fertilization of genotypes derived from different hosts is extremely unlikely. Consequently, mating takes place within a highly subdivided population, a condition known to promote local mate competition and inbreeding, and hence the evolution of biased sex ratios (Hamilton 1967; Charnov 1982; Antolin 1993). Several authors have suggested that sex allocation theory should apply to malaria (Ghiselin 1974; Pickering 1980; Schall 1989; Read *et al.* 1992); for those unfamiliar with the ideas, Read *et al.* (1992) provide a gentle introduction to the relevant theory in the context of *Plasmodium*.

The biology of malaria parasites suggest that gametocyte sex ratios will vary with selfing rates in natural parasite populations (Read *et al.* 1992). Where relatively few clones contribute to the gamete pool within a blood meal, local mate competition should select for female-biased sex ratios. In the extreme case, where only a single genotype is present, selection will favour clones which produce just enough male gametes to fertilize all female gametes. However, where many genotypes are present, clones producing fewer female-biased sex ratios will obtain a disproportionate share of matings and so be favoured by natural selection. Thus, gametocyte sex ratio should reflect the average selfing rate in a population.

More formally, it can be shown (Read *et al.* 1992;

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Dye & Godfray 1993) that the selfing rate, s (the proportion of a rare mutant's female gametes fertilized by her male gametes), is related to r^*, the ESS sex ratio (proportion of gametocytes in the peripheral blood that are male), as

$$s = 1-2r^*, \tag{1}$$

subject to the condition that there are sufficient male gametes to fertilize all female gametes in a blood meal. Equation 1 is simply an inversion of Hamilton's classic formula (Hamilton 1967). Dye & Godfray (1993) observe that s is Wright's coefficient of inbreeding. Standard assumptions underlie the model leading to equation 1; Read *et al.* (1992) make these explicit and review the evidence for them in *Plasmodium*. In the Appendix we show that equation 1 is robust in the face of biological complications, such as variable population structures, which determine the probabilities with which different selfing rates occur in blood meals in a population.

The idea that gametocyte sex ratios will vary with selfing rates in natural parasite populations is clearly vulnerable to falsification by molecular genetic data. For example, using equation 1 and an estimate of the average sex ratio of the *Plasmodium falciparum* population in humans in the Madang Region of Papua New Guinea, Read *et al.* 1992 predicted that between 64–100% of malaria zygotes in that population were the result of self fertilization. That estimate has recently been confirmed by molecular analysis of malaria oocysts from wild caught mosquitos (R. E. L. Paul *et al.*, personal communication). Molecular data also exist for a malaria population in Tanzania: the selfing rate is about 0.33 (Babiker *et al.* 1994; Hill *et al.* 1995). However, the average gametocyte sex ratio in this population is unknown. From equation 1, Read *et al.* (1992) would predict this to be about 0.33.

However general conclusions drawn from estimates of average selfing rates and sex ratios in a single population are likely to be uncompelling. Ideally, genetic and sex ratio data from a number of populations are needed, but are likely to be prohibitively expensive to obtain. We therefore investigated the theoretical effects of different levels of transmission. This generates a novel and more readily testable prediction relating prevalence to sex ratio.

2. MODEL

Here we formalize the following idea. When many hosts are infectious, transmission rates will be high and mixed infections (and hence outcrossing) common. In such circumstances, selection will favour fewer female-biased sex ratios. In contrast, when few hosts are infectious, transmission rates will be lower, outcrossing less common and selection will favour more female-biased sex ratios.

Calculating probabilities from the point of view of a mutant, the probability of a rare mutant self-fertilizing in a blood meal containing exactly i clones is

$$(p_i\, i/m)(1/i), \tag{2}$$

where p_i is the probability that a blood meal will contain i clones and m is the mean number of clones per person in the population. Thus the selfing rate of the mutant is given by

$$(1/m)\sum_{i=1}^{\infty} p_i = (1/m)P, \tag{3}$$

where P is the prevalence of infectious vertebrate hosts in a population (which we assume can be approximated as the proportion of vertebrate hosts with gametocytes in the peripheral blood). Typically, parasite distributions are overdispersed in host populations as a consequence of heterogeneities in exposure, susceptibilities to infection or parasite survival within hosts. Frequently, such distributions are well described by the negative binomial (Anderson & May 1991), in which case we can write, $m = k[(1-P)^{-1/k}-1]$, and

$$r^* = (1/2)\langle 1-\{P/[k\,(1/1-P)^{1/k}-k]\}\rangle, \tag{4}$$

where k is a parameter which varies inversely with the degree of clonal aggregation ($k \to 0$ as all the clones in a population are concentrated in fewer and fewer hosts). More elaborate theory could easily be constructed, but is probably unwarranted given the resolution of the data.

From equation 4, it can be seen that the sex ratio favoured by natural selection should become less female-biased as gametocyte prevalence increases. This is because as more hosts become infectious, mixed infections (and hence outcrossing) will become more common. The exact form of the relation between prevalence and the sex ratio favoured by natural selection depends on the degree to which parasite clones are aggregated in hosts. As far as we are aware, no estimates of k have been made for genotypes of haemosporidians in vertebrate hosts, but by way of comparison we note that values of k for helminth infections in human populations typically lie between 0.1–1.0 and in non-human animals between 0.1–3.0 (Anderson & May 1991). Equations 1 and 4 are both subject to the condition that there are sufficient male gametes to fertilize all female gametes in a blood meal, that is, that $r^*c \geqslant 1-r^*$, where c is the average number of viable gametes released from a male gametocyte after exflagellation. Morphological evidence indicates c is at most eight and probably closer to four (Desser & Bennett 1993; Sinden 1983).

So far as we are aware, the relation between gametocyte prevalence and sex ratio described by equation 4 has not been previously predicted or observed. To test the model we assayed Haemosporidian parasites from a number of populations.

3. METHODS

Thin films of peripheral blood from a range of bird species at different locations were Geimsa-stained using standard methods (Schall 1989; Read *et al.* 1992). Blood films were examined for the Haemosporidian genus *Leucocytozoon* and sex ratios were estimated as described by Schall (1989). Birds were caught by a variety of means, including mist nets, nest traps and shooting; sampling was during breeding or late

summer. Details of some of these studies are published (Norris *et al.* 1994; Shutler *et al.* 1995). Evidence that gametocyte sex ratios in peripheral blood represent primary sex ratios unaffected by sex-biased mortality is reviewed elsewhere (Read *et al.* 1992). Throughout, sex ratios are given as a proportion of gametocytes that were male.

4. RESULTS

The bird parasite populations sampled, and the number of hosts and parasites assayed were as follows. Blackbirds, *Turdus merula*, Oxfordshire, U.K., n_p (number hosts sampled for parasites) = 191, n_r (number of hosts assayed for sex ratios) = 5, n_g (number of gametocytes counted) = 386; white-throated sparrow, *Zonotrichia albicollis*, Newfoundland, Canada, n_p = 225, n_r = 3, n_g = 300; *Great tits*, *Parus major*, Oxfordshire, U.K., n_p = 107, n_r = 9, n_g = 606; ptarmigan, *Lagopus mutus*, Troms County, North Norway, n_p = 55, n_r = 2, n_g = 132; willow grouse, *L. lagopus*, Troms County, North Norway, n_p = 84, n_r = 20, N_g = 880; tawny owls, *Strix aluco*, Oxfordshire, U.K., n_p = 45, n_r = 6, n_g = 849; mallard ducks, *Anas platyrhynchos*, black ducks *A. rubripes*, and hybrids, Kennisis Lake, Canada, n_p = 46, n_r = 17, n_g = 1700; American robin, *Turdus migratorius*, Labrador, Canada, n_p = 72, n_r = 10, n_g = 1000; American robin, Tantramar, New Brunswick, Canada, n_p = 143, n_r = 6, n_g = 600; American robin, Newfoundland, Canada, n_p = 171, n_r = 17, n_g = 1700; mallards, black ducks and hybrids, Lake Sasajewan, Canada, n_p = 84, n_r = 62, n_g = 6200; mallards, black ducks and hybrids, Swan Lake, Canada, n_p = 35, n_r = 33, n_g = 3300.

Leucocytozoon taxonomy is largely based on the host (Desser & Bennett 1993); on that basis the species represented in this study are probably *L. shaartusicum* (in blackbirds), *L. cambournaci* (in white-throated sparrows), *L. majoris* (in great tits), *L. lovati* (in *Lagopus* spp.), *L. ziemanni* (in tawny owls), *L. simondi* (in ducks) and *L. dubreuili* (in American robins).

Gametocyte sex ratio in the only *Plasmodium falciparum* population for which we are aware of relevant data (Read *et al.* 1992) and from the populations of *Leucocytozoon* spp. in birds are shown together with the theoretical curves derived from equation 4 in figure 1. As predicted, there is an absence of unbiased sex ratios when prevalence is low and an absence of extremely female-biased sex ratios when prevalence is high.

5. DISCUSSION

The relation between ESS sex ratio and prevalence predicted by simple theory accords with that found across *Leucocytozoon* populations. That this pattern is sufficiently robust to be detectable is encouraging,

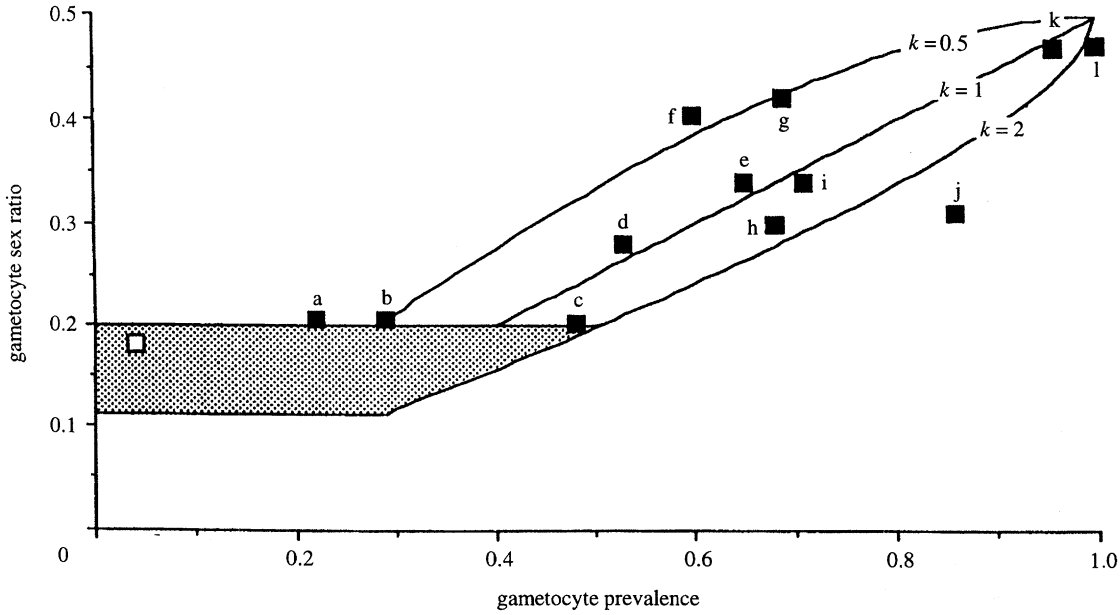

Figure 1. Prevalence and sex ratio (proportion male) of gametocytes in the peripheral blood of various host populations, together with theoretical expectation (from equation 4) for various degrees of clonal aggregation (k). Equation 4 is subject to the condition that there are sufficient male gametes to fertilize all female gametes in a blood meal; this depends on the average number of viable gametes released per male gametocyte, which is uncertain (see text) leading to a region of uncertainty (shown as shaded area). Open square, *Plasmodium falciparum* in Madang region, Papua New Guinea (Read *et al.* 1992); filled squares, *Leucocytozoon* spp. from the following bird populations (see text for latin binomials) (*a*) blackbirds, Oxfordshire, U.K.; (*b*) white-throated sparrow, Newfoundland, Canada; (*c*) great tits, Oxfordshire, U.K.; (*d*) ptarmigan, Troms County, North Norway; (*e*) willow grouse, Troms County; (*f*) tawny owls, Oxfordshire, U.K.; (*g*) mallard ducks, black ducks, and hybrids, Kennisis Lake, Canada; (*h*) American robin, Labrador, Canada; (*i*) American robin, Tantramar, New Brunswick, Canada; (*j*) American robin, Newfoundland, Canada; (*k*) mallards, black ducks and hybrids, Lake Sasajewan, Canada; (*l*) mallards, black ducks and hybrids, Swan Lake, Canada.

given the differences in host and vector biology in the populations studied, and the notorious variation in haematozoon prevalences through time and across populations (see, for example, Cox 1989; Loye & Zuk 1991; Weatherhead & Bennett 1991).

Interestingly, a different haemosporidian genus, *Haemoproteus*, exhibits female-biased sex ratios closer to unity even at low prevalences (Shutler *et al.* 1995). Across eleven populations, gametocyte prevalences ranged from 0.044–1.0 but sex ratios fell in a relatively narrow band between 0.30–0.42 and were unrelated to population prevalence. There are a number of possible explanations why our model accords with the pattern seen across *Leucocytozoon* but apparently not *Haemoproteus* populations. One is that the main vectors of these two genera differ. The former are mainly transmitted by blackflies (Simuliidae), the latter by biting midges (*Culicoides*). These vectors take up about their own mass in blood (Crosskey 1962), but midges are somewhat smaller. A midge blood meal may therefore contain on average fewer gametocytes. If so, sex ratios less female-biased than expected for a given prevalence may be maintained by selection in *Haemoproteus* populations to ensure sufficient male gametes to fertilize all females (Herre 1985). This speculation could be tested by examining sex ratios and oocyst densities of *Leucocytozoon* and *Haemoproteus* in the same host populations; direct inferences from vector body size are rather difficult given species differences in the ability of vectors to concentrate blood cells (Vaughan *et al.* 1991).

The lively and ongoing controversy (see, for example, Dye 1991; Walliker 1991; Day *et al.* 1992; Read & Day 1992; Maynard Smith *et al.* 1993; Babiker *et al.* 1994; Tibayrenc 1994; Hill *et al.* 1995) surrounding the idea (Tibayrenc *et al.* 1990) that parasitic protozoa with obligate sexual cycles such as *Plasmodium* have a clonal population structure coincides with recent enthusiasm that the adaptationist programme might contribute to biomedical research (Williams & Nesse 1991, 1994; Ewald 1994; Westoby 1994; Futuyma 1995). Our analysis and data provide a quantitative example. The genetic structure of Haemosporidian populations depends critically on the self fertilization rate (Day *et al.* 1992, Read & Day 1992). As our model makes explicit, general statements about population structure of a particular protozoan species will be possible only if the determinants of the distribution of clones per blood meal are relatively invariant across populations. From the figure it is obvious that there are no cross-population generalities about mating patterns. Sex ratio varies widely, consistent with large variation in genetic structure and inconsistent with either widespread clonality or widespread panmixia. For instance, gametocyte sex ratios in *P. falciparum* populations in Papua New Guinea are consistent with selfing rates of more than 64% (Read *et al.* 1992), whereas sex ratios of *Leucocytozoon* populations infecting ducks at Lakes Swan and Sasajewan in Canada are those favoured by natural selection in panmictic populations (see figure 1 and equation 1). Thus, generalizations about the genetic structure – clonal or otherwise – of Haemosporidian species are unwarranted.

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APPENDIX

Read *et al.* (1992) used a very simple model to derive a theoretical relation between selfing probability and sex ratio for malaria and, on the basis of that model, estimated selfing probability from sex ratio data. Dye & Godfray (1993) analysed a more biologically explicit model and established that the simple model is robust to the complexities with which they were concerned, in particular inbreeding. Their paper raises the interesting question of why so many biological details, which might naturally be thought important, are apparently not so. Here we attempt to shed some light on that question by generalizing the basic sex ratio model.

If there are always exactly n clones in a 'patch' (i.e. in the gametocyte population in a blood meal), standard arguments, such as used by Read *et al.* (1992), lead to the following equation defining the ESS sex ratio, r^*:

$$r^* - \tfrac{1}{2} + s/2 = 0, \qquad \text{(A 1)}$$

where the selfing probability, s, is simply $1/n$.

From this equation follows the familiar result, $r^* = (1-s)/2$. We now wish to derive the ESS sex ratio for the more realistic situation in which there is not always exactly n clones in a patch, but there are various selfing probabilities, s_i, each experienced with probability q_i. Because the reproductive output of a strategy, w, is proportional to the sum of the $q_i w_i$, with w_i being the reproductive output when there is a selfing probability s_i, we can generalize the standard arguments in an obvious way and arrive at the following equation defining the ESS sex ratio:

$$\Sigma\, q_i(r^* - \tfrac{1}{2} + s_i/2) = 0. \qquad \text{(A 2)}$$

The solution to this equation is

$$r^* = \Sigma\, q_i r_i^* \equiv \tfrac{1}{2}(1 - \Sigma\, q_i s_i), \qquad \text{(A 3)}$$

where r_i^* is the ESS sex ratio if the selfing probability is always exactly s_i. Hence we have the result that $r^* = (1-\bar{s})/2$ and we can infer $\bar{s}$, the mean selfing probability, from sex ratio data, as was done by Read *et al.* (1992). Notice that the biological mechanisms determining the p_i do not affect this result, which addresses the question raised at the beginning of this Appendix. (This more explicit analysis reveals that the unspecified 'average' number of clones used by Read *et al.* (1992) is actually the harmonic mean number, as in Herre (1985).)

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