Malaria sex ratios

Can sex-allocation theory account for the sex ratios of malaria parasites? The sex ratios of many metazoans change in response to environmental cues, often as predicted by theory, providing some of the best evidence that adaptationist thinking actually works[1]. Now, Paul *et al.*[2] have reported that environmental conditions also affect the sex ratio of malaria parasites. This discovery allows a fuller analysis of the adaptive basis of sex allocation in these protozoans. More generally, it provides a model for testing the use of optimality theory when applied to infectious disease research – a key component of the much hyped 'Darwinian Medicine'[3].

Transmission of *Plasmodium* occurs when a mosquito has a bloodmeal containing the dioecious haploid stages known as gametocytes. Gametocytes rupture within the vector, releasing either one female gamete or up to eight male gametes. Zygotes formed following fertilization are briefly diploid before haploid asexual replication is resumed, first in the mosquito and subsequently back in a vertebrate host. Individual haploid lineages are capable of generating gametocytes of both sexes and there is no evidence of sex differences in gametocyte mortality[2,4]. In the peripheral blood of infected humans, birds, lizards and rodents, gametocyte sex ratios are almost always female biased, but are often more variable than expected by chance[2,4–9]. Variability occurs between hosts within a population and during the course of single infections, which led to the suggestion that sex allocation might partially be a response to environmental conditions[4,7,8]. The experiments recently reported in *Science*[2] are the first to demonstrate this response.

Linking erythropoiesis and the sex ratio

Paul *et al.*'s study[2] was triggered by their earlier observations of gametocyte sex ratios in chickens experimentally infected with *Plasmodium gallinaceum*[9]. In lethal infections, the gametocyte sex ratio remained female biased throughout; however, in infections successfully contained by protective immune responses, sex ratios became progressively less female biased[9]. An important symptom of malaria is anaemia, which occurs largely as a result of red blood cell destruction by replicating parasites. Anaemia induces erythropoiesis, the release of reticulocytes (immature red blood cells) into the bloodstream. Among surviving chickens, Paul *et al.*[2] observed that gametocyte sex ratio became less female biased as reticulocyte concentration increased. To determine whether this correlation was causal, Paul *et al.*[2] induced erythropoiesis by exposing chickens to hypoxic conditions or by removing 25% of total blood volume. Both treatments generated less female-biased sex ratios than observed in control infections, even though there were no differences in parasite numbers. However, the shift in sex ratio was not a response to the presence of reticulocytes: the replacement of 20% of the blood volume with reticulocyte-rich blood had no effect on the sex ratio. Thus, erythropoiesis, rather than the inducing anaemia or the resulting reticulocytes, apparently affects the sex ratio.

Erythropoiesis is induced by the endogenous hormone erythropoietin (Epo) and, as demonstrated by numerous top athletes and cyclists, by exogenous Epo. Paul *et al.*[2] found that injections with mouse recombinant Epo shifted gametocyte sex ratios in the rodent malaria *Plasmodium vinckei* closer to 1:1, apparently without any other effects on the parasites. This shift was indistinguishable from the shift induced by hypoxia. Combining these results, Paul *et al.*[2] concluded that the parasite sex ratio is altered as a direct response to Epo concentrations.

Rigorously controlled testing of this conclusion should be possible *in vitro*, at least for *Plasmodium falciparum* – the most virulent of the human malarias and the only malaria parasite that can be reliably cultured. Recent *in vitro* experiments have demonstrated that all the progeny of a sexually committed parental parasite are the same sex[10]. By extending this approach to include a wide variety of Epo treatments, it should be possible to resolve whether sex is irrevocably determined by the parent (facultative sex allocation[1]) or whether the environment directly determines the sex of developing gametocytes (environmental sex determination[11]). In addition, comparisons of gene-expression patterns in otherwise identical parasites induced to develop into different sexes could be used to determine the elusive molecular genetic mechanism that allows individual haploid lineages to produce both males and females.

Adaptive explanations?

Is a shift of gametocyte sex ratio in response to Epo adaptive? Paul *et al.*[2] argue that it is, because they found that reproductive success was greatest when the sex ratio shifted in response to natural Epo levels, but was lower when sex ratio shifts are induced by experimentally altered erythropoiesis.

In single clone infections, where all zygotes will be the result of self fertilizations, the optimal sex ratio is one that results in just enough male gametes to fertilize all the available female gametes. Because one male gametocyte can produce up to eight male gametes, while a female gametocyte produces just one gamete, a sex ratio of up to 8:1 should be favoured. Why should erythropoiesis trigger a shift from female to male sex allocation?

Transmission-blocking immunity is well known in malaria infections. A variety of evidence demonstrates that the viability of male gametes (but not gametocytes) is disproportionately reduced by vertebrate effector mechanisms acting in the midgut of the mosquito after a bloodmeal[2,12]. In these circumstances, natural selection should favour a shift from females to males to ensure the number of zygotes is maximized. Paul *et al.*[2] assume that rising Epo concentrations coincide with the period when sex-biased antigamete immunity begins to impact on fertilization success.

Just so stories?

Like the best adaptationist hypotheses – and characteristic of those concerned with sex allocation – Paul *et al.*'s explanation provokes several tractable questions. First, it requires that Epo level is a reliable indicator of the onset of significant reductions in male gamete viability. Why should Epo concentrations be more informative than the effector molecules themselves? Second, antibodies play a key, although not necessarily unique, role in antigamete immunity. Immunological orthodoxy states that from antigen presentation it takes a naïve host about two weeks to mount an effective T-cell-dependent antibody response[13]. However, the sex-ratio shifts observed by Paul *et al.*[2] occurred only a few days after the first gamete antigens could have been presented to the host immune system; therefore, perhaps a T-cell-independent sex-biased effector mechanism is involved? Third, what happens in chronic infections where gametocytes are present for extended periods of time? What happens when gametocytes are produced in semi-immune hosts who are not anaemic?

There might also be other adaptive explanations for Epo-induced sex ratio alterations. For instance, bloodmeals from anaemic hosts might contain fewer red blood cells, and thus fewer gametocytes and gametes; hence, less female-biased

 0169-5347/00/$ – see front matter PII: S0169-5347(00)01893-0

sex ratios are required to ensure sufficient males enter the mating pool. Lack of data on gamete motility and location efficiency means that we cannot yet incorporate the effect of these parameters into models to predict the gametocyte sex ratio. Nevertheless, comparative work on the sex ratios of *Plasmodium* species, transmitted by vectors that do or do not concentrate blood during feeding might be informative.

Future directions

The discovery that malaria sex ratios are altered in response to environmental cues opens up a range of new experimental options for analysing the evolution of sex ratios in these protozoa. First, experimentally generated sex ratios could be used to directly test the fitness consequences of sex-ratio variation. An unexpected finding, reported in human and lizard malarias[5,6], is that sex ratios closer to 1:1 generate more zygotes than the more female-biased sex ratios that predominate in nature. If this correlation proves to be causal, the assumptions of several current models might be violated. Second, there is no reason to expect that only one environmental cue will influence the malaria sex ratio – identifying other cues would enable testing of other sex-allocation theories. For instance, the role of local mate competition (LMC) (Ref. 14) in shaping malaria sex ratios has been controversial[4,7,8,15,16], but evidence of facultative sex-ratio adjustment in the presence of co-infecting clones would be compelling evidence that LMC plays an important part. Detecting the presence of unrelated clones might seem unlikely, but the specificity of antibody binding to polymorphic epitopes might be easily assayed, even by a single-celled organism. More generally, a combination of environmental and genetic factors might explain the variable sex ratios observed in malaria parasites[2,4–8]. Using animal models it should be possible to experimentally alter factors such as Epo concentrations, the strength of sex-biased antigamete immunity and genetic relatedness within infections to test this proposition.

Much of what is optimistically known as darwinian medicine involves adaptationist arguments[3]. Many hypotheses, particularly those concerned with inherited human disease, will be hard to advance beyond *Just So Stories*. Those concerning infectious diseases might be more vulnerable to quantitative experiments because relevant animal models are often well established. Theories of the evolution of virulence bear much in common with models underpinning sex-ratio evolution[7]. Simple optimality arguments underlie work in both areas but typically these assume population dynamic equilibrium – not an obvious feature of many medically relevant diseases. How successful can virulence theory be? Rigorous testing requires quantitative measures of the fitness tradeoffs associated with different levels of pathogen virulence; however, obtaining such data is a major experimental challenge. The beauty of sex-allocation theory – and possibly a reason for its quantitative success – is that the relevant tradeoffs are usually obvious. If we cannot understand gametocyte sex ratios for malaria, there is little reason to think we can understand more complex phenotypes, such as virulence. Paul *et al.*'s[2] discovery provides a new way to move forward our rudimentary understanding of malaria sex ratios.

Acknowledgements

We thank S. West and R. Paul for comments. S.R. and A.R. are funded by the NERC, BBSRC and Leverhulme Trust.

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