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Toxoplasma gondii, sex and premature rejection

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Adaptive sex ratio theory explains why gametocyte sex ratios are female-biased in many populations of apicomplexan parasites such as *Plasmodium* and *Toxoplasma*. Recently, Ferguson has criticized this framework and proposed two alternative explanations – one for vector-borne parasites (e.g. *Plasmodium*) and one for *Toxoplasma*. Ferguson raises some interesting issues that certainly deserve more empirical attention. However, it should be pointed out that: (1) there are theoretical and empirical problems for his alternative hypotheses; and (2) existing empirical data support the application of sex ratio theory to these parasites, not its rejection.

Ferguson suggests that natural selection has favoured female-biased sex ratios in *Plasmodium* and related Haemospororin parasites to promote inefficient fertilization as a form of reproductive constraint [1]. He argues that this is advantageous because it reduces the number of oocysts formed, which avoids compromising vector fitness. Even if there is selection to reduce parasite burdens within mosquitoes (a possibility that remains to be theoretically or empirically demonstrated), there are two problems with Ferguson's suggestion. First, it would be much easier and less wasteful to constrain oocyst numbers by decreasing the number of gametocytes produced. There is abundant evidence that *Plasmodium* parasites are able to adjust their rate of gametocytogenesis adaptively in response to environmental conditions [2–4]. Second, reproductive restraint achieved through sex ratio adjustment is not necessarily evolutionarily stable. A population minimizing fertilization success with a female-biased sex ratio could easily be invaded by a less-female-biased mutant. This is because the increased mating success of the mutant would

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more than compensate for the reduction in total transmission from the host.

Ferguson made an analogy with the problem of how many eggs an insect should lay per 'patch' (the clutch size), but this analogy illustrates the problems with his suggestion. First, insects do appear to adjust their clutch size so as not to overexploit local resources [5]. However, they do so by adjusting the number of eggs that they lay (analogous to the number of gametocytes produced) and not by adjusting embryo mortality (analogous to Ferguson's idea of reproductive constraint through female-biased sex ratios). Second, Ferguson's argument appears to rely on the assumption that if natural selection is acting on the clutch size, then it cannot simultaneously act upon the sex ratio. However, there is abundant evidence suggesting that insects adjust clutch size and sex ratio simultaneously, with the sex ratio conforming to sex allocation theory [5].

The idea that female-biased sex ratios are required to keep vectors alive cannot apply to apicomplexans that are not vector-borne. Instead, Ferguson argues that, in *Toxoplasma gondii*, the female bias also results in inefficient fertilization, but the unfertilized female gametes reproduce parthenogenetically. There is no empirical evidence for this novel prediction; indeed, as far as we are aware, sexual reproduction characterized by meiosis and syngamy is an obligate part of the life cycle of all apicomplexans. But, even if it is not, the existence of parthenogenetic reproduction provides no explanation of how selection has generated the observed sex ratios.

By contrast, the sex ratio hypothesis is well supported by theory and can, without invoking any undiscovered life cycles, explain a wide range of sex ratio patterns observed in Apicomplexa [6,7]. Ferguson rejects the conventional sex ratio theory because he states that it always assumes that there are enough male gametes to fertilize all the female gametes. This is not true. Elsewhere, we have incorporated this fertilization problem into sex ratio theory. We demonstrated that if there are not enough male gametes (as, for instance, there might not be enough male gametes in small bloodmeals or at low gametocyte densities), then less-female-biased sex ratios are favoured by natural selection [6,8,9] (Gardner, A. *et al.*, unpublished). However, to account for the female-biased sex ratios seen in many apicomplexan populations, we contend that usually there must be enough males to fertilize all the female gametes. This is because when mating takes place among clone mates (extreme inbreeding), natural selection will favour the sex ratio that maximizes zygote number. Ferguson contends instead that males are normally limiting, so that observed sex ratios are not maximizing oocyst number. In fact, experimental data from *Plasmodium* shows the opposite pattern, consistent with adaptive sex ratio theory: experimental manipulation giving a less-female-biased sex ratio led to a decrease in oocyst load [10]. More generally, data on fertilization efficiency, or zygote, gamete or gametocyte death merely show that, as with free-living organisms, not all apicomplexan gametes produce reproductive offspring. Such data most assuredly do not demonstrate that males are selectively limited.

Nonetheless, we are only too aware that data in this area are woefully inadequate [6,7]. For instance, Ferguson asserts that, in *T. gondii*, observed sex ratios would lead to female gametes outnumbering male gametes, and so large numbers of unfertilized female gametes remain, irrespective of the efficiency of fertilization. We are unconvinced. When surveying the literature, we found that most studies did not quantify the sex ratio or number of microgametes per microgametocyte (a tradition Ferguson maintains). However, our own counts suggested 1.9–2.6 male gametes per female gamete [11]. It is also worth noting that such data can overestimate the female bias in the sex ratio [12], and hence underestimate the ratio of male to female gametes. Based on these data, the highly inbred nature of *T. gondii* population [13] and our theoretical arguments, we assert that the number of male gametes must be equal to or greater than the number of female gametes in *T. gondii*. Otherwise, clones producing a less-female-biased sex ratio would have invaded the population. Actually, the crucial point is not whether there are unfertilized females, but rather whether sex ratios less-female-biased than those found naturally would result in more zygotes. If they did, the sex allocation theory would have some problems [7]. But until that is demonstrated, rejection is premature. Again, comparison with insects illustrates this point, where it is well understood that adaptive female-biased sex ratios can lead to high levels ($>30\%$) of females remaining unfertilized (virgins) [14].

Nonetheless, Ferguson is right that most free-living organisms make use of a vast excess of male gametes in the mating arena. If we are right in that there are sufficient males to fertilize all the females gametes in apicomplexan mating arenas, despite the female-biased gametocyte sex ratios we see in Nature, then apicomplexan gametes must have some very interesting ways of locating each other and for male gametes to move on from already mated (or mating) female gametes. The sex allocation view is well-founded in theory, does not require us to invoke novel life cycles, and has made several novel predictions that have subsequently proved to be quantitatively correct [6,7]. We make another prediction: the biology of gamete–gamete interactions in Apicomplexa will be more fascinating and possibly more complex than anything seen in species that apparently require vast numbers of male gametes to get the job done.

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Research Focus Response

More on *Toxoplasma gondii*, sex and premature rejection

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In the Scottish legal system, there is the verdict of 'not proven' when neither innocence nor guilt can be clearly established; and this is where I would place the sex allocation theory as applied to the Apicomplexa. I am not proposing rejection of the sex allocation theory, just that it is premature to consider basing treatment or control strategies on population structures calculated from sex ratios obtained from single blood-smears.

I would like to thank West and colleagues for their comments above. Any theory used to explain the obvious female-biased sex ratio should be based on solid foundations. This is a complex issue and I am not the first to question the sex allocation theory [1]. It is difficult to answer succinctly all the points raised by West *et al.* I agree with their point (1), but am not convinced on point (2).

The impression given is that there are unusual or novel biological assumptions in the alternative explanations I proposed [2]. However, every assumption is based on extrapolation from well proven data. First, I am not promoting inefficient fertilization as a form of 'reproductive constraint' – just stating that it is a simple fact of life. One of the most consistent observations within the plant and animal kingdoms is that there is massive overproduction (wastage) of male gametes. These are not minor differences: in humans, a normal sperm count is 100 million to 700 million per ejaculate, with counts below 50 million considered, clinically, unlikely to result in the successful fertilization of a single egg [3]. The novel idea is efficient fertilization. It is undeniable that, because the female-biased sex ratio exists, it is assumed there must be sufficient microgametes to fertilize all of the macrogametes, otherwise any precocious mutations that produce more microgametes or microgametocytes would be selected for. However, this *per se* is not proof that the assumption is correct – there could be other explanations. There is supporting evidence in that less-female-biased sex ratios led to a decrease in oocyst load [4]. However, there are data showing the exact opposite [5]. Second, a reduction in oocyst load is not consistent with the theory – it should remain unchanged. A number of possible confounding factors have been identified (reviewed in Ref. [6]). I agree that the crucial point is, would less-female-biased sex ratios result in more zygotes, but this has been shown to be the case in certain studies [5]. The real problem is the mass of conflicting results.

The comment that data on gamete formation, fertilization efficiency or zygote death in relation to oocyst formation and infectivity can be ignored is surprising. For example, Read *et al.* use the figure of up to eight microgamete being produced by each microgametocyte in their calculations for *Plasmodium* spp. [7], which I assume is based on the detailed studies of Sinden and colleagues [8]. However, if this is the case, it is only fair to point out that these studies also showed that 25–40% of the microgametes were anucleate and others had poor motility – in other words, only about half of the microgametes produced were viable [8]. Surely, studies identifying physiological [9] and immunological [10] changes in the host serum that can affect the number of viable microgametes produced are relevant?

Concerning clutch size and sex ratio: the known biological limitations of both apicomplexan parasites and insects should be applied within the model. First, as described previously, due to basic differences in their reproductive biology, care has to be taken when extrapolating from metazoan to protozoan species [2]. Second, insects can choose when, where and how many eggs they lay, unlike the malaria parasite that has no direct control over the number of gametocytes ingested by the mosquito. In addition, it might be premature to dismiss the idea of embryo (zygote) deletion as a possible mechanism because it has recently been shown that a high proportion of ookinetes can undergo apoptosis (programmed cell death) [11]. For organisms, such as *Plasmodium* spp., which

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