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Toxoplasma gondii and sex: essential or optional extra?

David J.P. Ferguson

The evolutionary and biological significance of a female-biased sex ratio within apicomplexan parasites has been the subject of much discussion. It is proposed that the sex allocation theory, as applied to inbreeding populations, can explain the sex ratios observed for this diverse group of parasites. This is based on a mathematical model, which assumes that the majority of microgametes will succeed in fertilizing macrogametes. Is this a realistic assumption? It is possible, for different reasons, that the theory may not to be applicable to either malaria parasites or *Toxoplasma gondii*.

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In malaria parasites (*Plasmodium* spp.), vector fitness will be of primary importance and may be based on reproductive restraint with few macrogametes being fertilized (due to limited numbers of microgametes) and developing into oocysts. The low number of oocysts results in infectious mosquitoes without compromising their fitness. By contrast, transmission in *T. gondii* and other coccidian parasites requires maximum oocyst output and they may have evolved a strategy wherein sexual reproduction is possible but incorporates a default pathway in which unfertilized macrogametes are capable of development into viable oocysts. This would maximize their reproductive potential while retaining the possibility of cross fertilization.

Toxoplasma gondii is probably the most successful protozoan parasite of vertebrates, based on the number of infections. It is a member of the phylum Apicomplexa and is a coccidian parasite with the cat as the definitive host but any warm-blooded animal, including humans, can act as intermediate hosts. This parasite has a worldwide distribution, with a high incidence of infection in humans and domestic animals. A review of the incidence of human infections revealed that approximately a third (in the range of 12–80%, depending on the country) of the adult population is serologically positive (chronically infected) for *T. gondii*. This means that there are over one billion chronically infected humans worldwide. Fortunately, *T. gondii* is a well-adapted parasite, which generally causes little disease unless the host immune system is compromised in situations such as AIDS [1]. However, human infections represent a dead-end for the parasite with no prospect of transmission. In modern times, the fact that humans are not a food source, except for rare individuals who may be taken by wild carnivores, and cultural and religious practices (burial or cremation) means that the parasite dies with the host. The high incidence of infection suggests that the parasite must have evolved efficient, if indiscriminate, methods for infecting new hosts. In fact, the parasite has evolved a complex life cycle (Fig. 1) with two transmission mechanisms; one involves ingestion of oocysts produced by coccidian development in cats and the other uses the tissue cyst stage present in chronically infected intermediate hosts [2].

One aspect of the *T. gondii* life cycle has intrigued me since my original studies of the sexual stages

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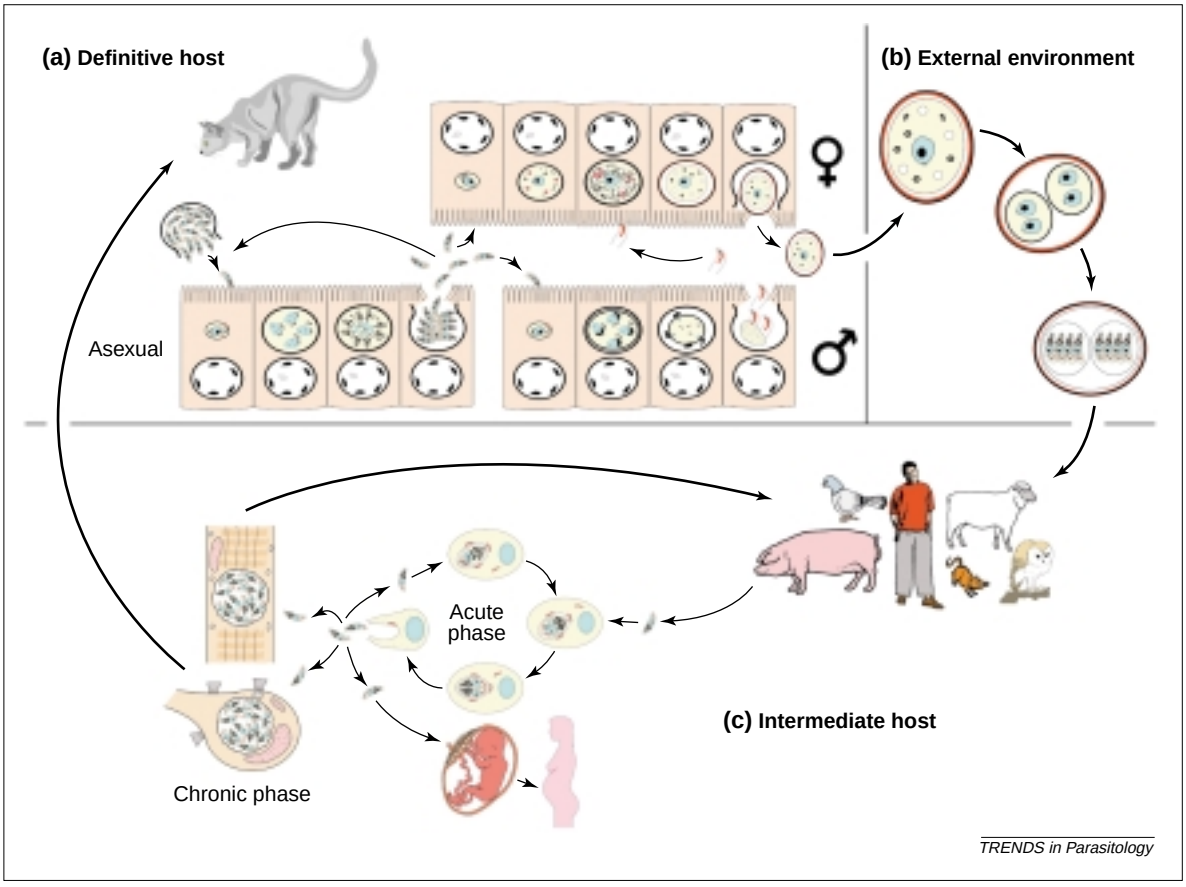

Fig. 1. Diagram representing the life cycle of *Toxoplasma gondii*. (a) The cat is the definitive host with sexual development occurring in the small intestine giving rise to the oocysts. These contaminate the external environment (b) and, after sporulation, can infect a wide range of intermediate hosts when ingested with food or water (faecal contamination). (c) Within the intermediate host, the parasite undergoes only asexual development. An acute phase is followed by a chronic phase, when the parasite is present in tissue cysts, predominately located in the brain or musculature. These tissue cysts can transmit the infection to other intermediate hosts or to the cat when ingested (carnivorism). In addition, if the acute phase occurs during pregnancy, the parasite can cross the placenta and infect the foetus (congenital transmission). Key: bold arrows, parasite movements between hosts; thin arrows, parasite developmental stages within host.

~30 years ago [3,4] – the unequal sex ratio. Very few males (microgametocytes) (only 2–5% of total gametocytes), producing low numbers (20–30) of male gametes (microgametes) are formed compared with the large number of females (macrogametocytes). There appears to be little or no allowance for wastage of male gametes during the fertilization process. This did not make sense. However, it has recently been proposed that the sex allocation theory, in a situation where there is likely to be inbreeding, could explain the female-biased sex ratios [5]. This appeared to be the long-awaited answer. However, when applying the theory to apicomplexan parasites, the assumption seemed to be that the majority of microgametes would succeed in fertilizing different macrogametes [5]. This is different from the normal biological situation where male gametes are produced in vast excess, and forced a re-examination of the basis for this hypothesis.

Recently, there have been much debate on the biological and evolutionary significance of female-biased sex ratio within apicomplexan parasites [6–8]. However, the original sex allocation theory, whose success is well documented for certain insect species, is still controversial even in its application to *Plasmodium* spp. [7,9]. The theory has been discussed in detail [10–12], but can be summarized as follows; in a situation where inbreeding is likely, the daughters of a mother are likely to be fertilized by one of her sons (sib-mating, self-fertilization or selfing). There is no biological or evolutionary benefit to be gained from competition between her sons to fertilize her daughters. It is thus biologically wasteful to produce equal numbers of males and females – only sufficient sons to fertilize all her daughters are required. There is no doubt that the lifestyle of many apicomplexans is likely to result in inbreeding, and this is particularly true for *T. gondii* [13,14]. There is also evidence of extremely female-biased sex ratios [5]. Therefore, the Apicomplexa would appear to fulfil the basic criteria, but there are distinct biological differences between the life cycles of insects and apicomplexans, which need to be taken into consideration.

The theory was developed for metazoan organisms in which zygotes can give rise to either only males or only females. It is based on two fundamental assumptions: (1) there must be sufficient males to mate with all the females and (2) they must produce sufficient male gametes to fertilize all the eggs. Unless these criteria are met, the theory will be invalid. By contrast, the zygotes

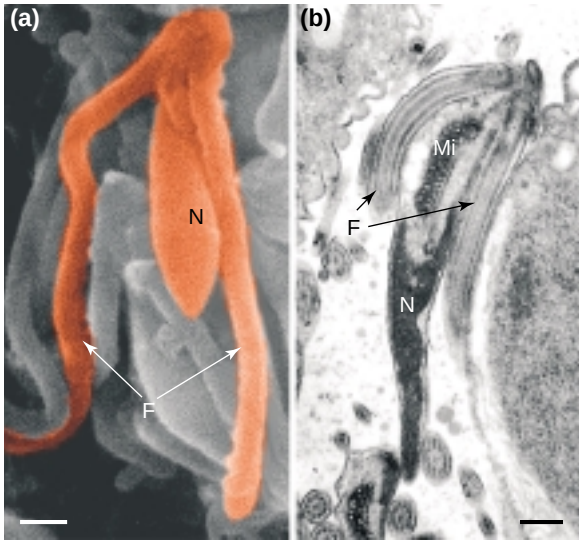

Fig. 2. Scanning (a) and transmission (b) electron micrographs of the microgamete of *Toxoplasma gondii*. Abbreviations: F, flagella; Mi, mitochondrion; N, electron-dense nucleus. Scale bars = 200 nm.

of apicomplexans develop into asexual stages that undergo multiplication before giving rise to both male and female gametocytes. A single infective sporozoite can give rise to both sexes, although the mechanism controlling the process and numbers of each sex is under investigation [7,8]. The rationale for applying the sex allocation theory to the Apicomplexa based on the number of microgametocytes (males) and macrogametocytes (females) is well founded. My only concern is certain assumptions associated with the way in which the optimal sex ratio is calculated. The optimal sex ratio is based on the minimum number of males required to mate with all of the females and fertilize all eggs – maximize zygote production. In the Apicomplexa, this is the number of microgametocytes required to produce sufficient microgametes to fertilize all the macrogametes. The males, in metazoan organisms, are considered capable of producing infinitely large numbers of viable spermatozoa and thus the number of spermatozoa is not used in calculating the optimal sex ratio. By contrast, the microgametocytes of apicomplexans produce a single batch of relatively few microgametes. To calculate the optimal sex ratio in *Plasmodium* spp., the number of viable microgametes produced by a microgametocyte was used to define the lower limit of the optimal sex ratio [15]. The authors emphasized that this is based on two assumptions: (1) most microgametes will fertilize a macrogamete (highly efficient fertilization) and (2) there must be sufficient microgametes to fertilize all the macrogametes [8,15]. It is the assumption of highly efficient fertilization and whether there are sufficient microgametes to ensure fertilization of all the macrogametes that I question from a practical point of view. The microgamete, similar to the spermatozoon, consists of nucleus, mitochondrion and flagella for locomotion (Fig. 2). It cannot replenish its energy supplies and therefore has a short active period in which to find a receptive macrogamete.

There are certain groups of apicomplexans, adeleorin spp., which have a life cycle that maximizes the chances of efficient fertilization. In these parasites, the immature gametocytes pair before maturation (syzygy) [8]. Interestingly, even these parasites, with gametocytes developing close together in the same cell, allow for 50–75% male gamete wastage (microgametocytes produce two or four microgametes) [6]. Therefore, is there any situation when it is biologically reasonable to assume that all or even the majority of microgametes will succeed in fertilizing a macrogamete?

Is fertilization highly efficient?

It is proposed that asexual multiplication before the development of the sexual stages will result in the gametocytes being relatively close together and this will allow all the microgametes to find and fertilize macrogametes [5,6]. However, a coagulating half-digested bloodmeal or a gut lumen filled with food debris cannot be considered a hospitable environment that allows easy and efficient movement of microgametes.

The majority of studies, to date, have been on malaria parasites and involved examining sex ratios in the blood of the host and then extrapolating this to what may be happening within the vector [15]. It is proposed that the optimal sex ratio in *Plasmodium* spp. is up to 8:1 (female to male) because the microgametocyte can produce eight microgametes and, in theory, the eight microgametes should be able to fertilize eight macrogametes [16]. To the best of my knowledge, there is no data to support this hypothesis. However, there are studies that have shown the exact opposite. There is convincing evidence that the process of macrogamete fertilization through the ookinete to oocyst formation is inefficient [17]. In *Plasmodium falciparum*, there is a 40- to 1000-fold reduction in the number of macrogametes ingested compared with the number of ookinetes formed (on average, only one ookinete was found for every 40 macrogametes ingested) [18]. This was due to the lack of fertilization, and the efficiency of fertilization could be directly related to the consistency of the bloodmeal [19]. This means, assuming a moderately female-biased sex ratio of 0.3, that only one in 96–2400 microgametes succeeds in fertilizing a macrogamete. In addition, there is a further 69-fold reduction between the number of ookinetes and oocysts subsequently formed (on average, only one oocyst was found for every 69 ookinetes). At best, only one in ~3000 macrogametes reaches the oocyst stage [18,19].

In *T. gondii*, there are no data on the fertilization process. However, at least two reasons mitigate against highly efficient fertilization. The first is the journey involved because, even in high-density infections, the mature microgametocytes and macrogametes are significantly separated (Fig. 3). Because oocyst-wall formation occurs within the host cell [4], it has to be assumed that fertilization occurs while the macrogamete is within the host cell. In this situation, the successful microgamete is required to escape from the host cell and parasitophorous vacuole, enter the gut lumen, swim through the debris of the luminal contents, find a host cell infected with a mature

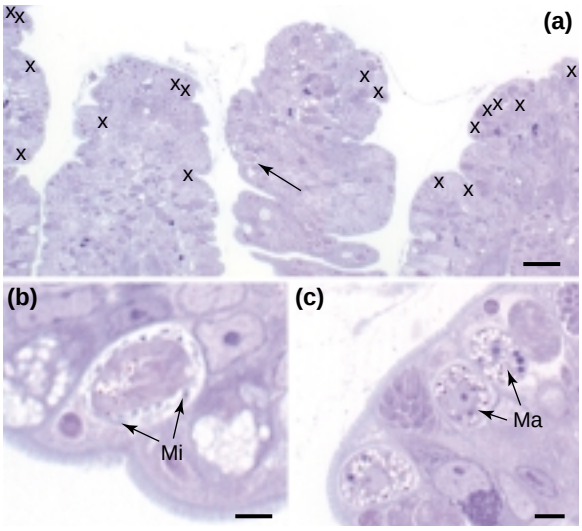

Fig. 3. (a) Light micrograph of the small intestine of a cat infected with *Toxoplasma gondii* showing the relative distances between a mature microgametocyte (arrow) and the numerous macrogametocytes (X). (b) Microgametocyte (arrow in a) with relatively few microgametes (Mi). (c) Mature macrogametes (Ma) from upper right villus in (a). Scale bar = 50 μm (a); 5 μm (b,c).

(receptive) macrogamete, penetrate the host cell plasmalemma, pass through the host cell cytoplasm, penetrate the wall of the parasitophorous vacuole before finally fertilizing the macrogamete. This would be an incredibly difficult journey. A second reason is that the theory requires the microgametes to have a co-ordinated strategy of fertilization. That is, each microgamete would have to target a different macrogamete. For the microgametes to find receptive macrogametes efficiently, it needs to be assumed that some chemo-attractant mechanism is operating. In which case, the majority of microgametes released from a microgametocyte would be attracted to the nearest receptive macrogamete (see Fig. 3). This would result in several microgametes associating with one macrogamete (Fig. 4), reducing the numbers available to fertilize the more distant macrogametes. I can think of no mechanism where it would be possible to target each microgamete to a different macrogamete.

Given the above limitations, it is unlikely that either *Plasmodium* spp. or *T. gondii* can meet the basic criteria of the sex allocation theory. The use of the number of viable microgametes produced by a microgametocyte to calculate the optimal sex ratio could be unwarranted.

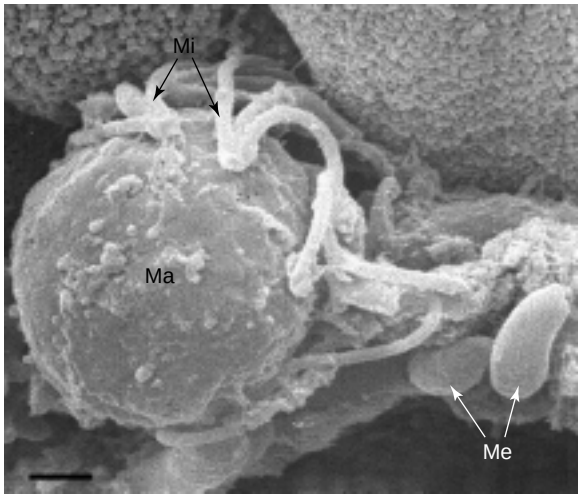

Fig. 4. Scanning electron micrograph of the small intestine of an infected cat showing a macrogamete (Ma) surrounded by several microgametes (Mi). Abbreviation: Me, merozoite. Scale bar = 1 μm.

Seeking alternative explanations

For parasites, the real measure of reproductive success is the ability to infect new hosts – the more efficient the transmission mechanism, the more successful the parasite. In parasites using environmental contamination such as the Coccidia, the larger the number of oocysts secreted, the greater the chance of being ingested by a new host. However, in parasites using an insect vector, the efficiency of transmission will be directly related to vector fitness. In *Plasmodium* spp., the question has been asked: 'why the reproductive restraint?' [20]. The simple answer must be, 'because it is the most successful strategy'. It is proposed that mosquito survival or transmission-blocking immunity could be responsible [20]. Although transmission-blocking immunity will play a role, there is strong circumstantial evidence that excess oocyst formation is detrimental because mosquitoes with high oocyst burdens are never observed in wild caught mosquitoes [21], yet are often seen in protected laboratory-fed mosquitoes [22]. In addition, low numbers of oocysts produce infectious mosquitoes with no apparent reduction in their fitness or survival. The important selection factor may not be the sex ratio but the 'clutch size' (number of oocysts). Going back to the insect model, the easiest way to increase offspring numbers would be to increase the number of eggs laid, but this must be within the limits that the host (or environment) can support. This will exhibit the strongest selection pressure because any precocious mutation that results in the production of an unsustainable number of offspring will result in the loss of the complete clutch. The oocyst number in *Plasmodium* spp. could be considered as the 'clutch size' and have more important survival implications than that of the sex ratio. It is possible that *Plasmodium* spp. have evolved a relationship with its vector which is based on an inefficient fertilization process with massive wastage of female gametes, but results in the development of low numbers of oocysts that does not compromise vector fitness or infectivity. The female-biased sex ratio may be nothing to do with inbreeding but is an adaptation to prevent overproduction of oocysts. This proposal would be consistent with the observations of the apparent ability of *Plasmodium* spp. to vary its sex ratios during an infection in response to host factors. There would be selective advantages in being able to control the relative number of each sex because the parasite could compensate for any adverse changes by increasing the numbers of males if any fertilization-blocking immunity developed [7,9,23].

However, the above explanation cannot be applied to *T. gondii*, where there must be selective advantage in maximizing the production of viable oocysts. In *T. gondii*, an alternative hypothesis is that many macrogametes remain unfertilized but are capable of forming oocysts and undergoing sporulation; a form of parthenogenesis. At first, this may seem controversial, but I can think of no alternative explanation. These parasites are normally haploid and have no sex chromosomes, which has important implications for their gametes. It means

that all macrogametocytes and microgametocytes of a strain will be genetically identical (clonal) and all of the microgametes produced by a microgametocyte will also be clonal. The only stage that is diploid is the fertilized macrogamete, and this undergoes a reduction division to produce haploid sporozoites. In other words, all of the offspring from inbreeding in a haploid organism will be clonal. This could settle the controversy over whether parasites such as *T. gondii* undergo clonal or sexual development [24]; the processes are not mutually exclusive in an inbreeding population of haploid organisms. There is no biological necessity for fertilization to provide a complete genome in *T. gondii* or other haploid organisms. In addition, it is not unprecedented in that certain insect species have evolved a life cycle in which only unfertilized eggs develop into males (haploid males) [10].

Why are sexual stages retained?

First, faecal transmission is only possible because the sporozoites are protected in the external environment by the formation of the resistant oocyst wall. Therefore, the macrogamete, which produces the oocyst wall, would need to be retained irrespective of sex. Also, *T. gondii* is capable of sex and can undergo cross-fertilization [25]. Indeed, previous studies had shown predicted levels of cross-fertilization with no evidence of a predominance of self-fertilized oocysts [26,27]. These observations are not consistent with the idea of parthenogenesis, but the results are limited to chemically induced mutants of a single strain of *T. gondii* and no data are available on the sex ratio for this strain. If parthenogenesis is not possible and unfertilized macrogametes are thus infertile, then there should be evidence of either the degeneration of a large number of unfertilized macrogametes in the gut or a large proportion of oocysts would fail to sporulate. However, no such evidence could be found (D.J.P. Ferguson, unpublished).

There is no evidence to support the parthenogenic hypothesis, although *T. gondii* has already evolved one form of parthenogenic reproduction with the development of bradyzoites within tissue cysts in the intermediate hosts. It has been shown that the parasite can be maintained for many years by passage via the tissue cyst without loss of its ability to subsequently infect cats and undergo coccidian development.

Concluding remarks

The application of mathematical modelling to biological problems has added greatly to our understanding of many complex interactions. However, it is important that the assumptions programmed into the models have biological validity. Just because certain criteria are met and the observed numbers fit the model, this should not immediately be accepted as definitive proof – other models may be equally valid. In *Plasmodium* spp., selection may favour reproductive restraint by improving vector fitness and this could be accomplished by having insufficient microgametes to fertilize all of the macrogametes. In this situation, there would be no selective advantage in maximizing zygote formation. *Toxoplasma gondii* and other coccidian parasites could have evolved a strategy wherein sexual reproduction is possible, but incorporates a default pathway in which unfertilized macrogametes are capable of development into viable oocysts. This would maximize their reproductive potential while retaining the possibility of cross-fertilization.

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