Evolution of gametocyte sex ratios in malaria and related apicomplexan (protozoan) parasites

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'Survival of the fittest' is usually interpreted to mean that natural selection favours genes that maximize their transmission to the next generation. Here, we discuss recent applications of this principle to the study of gametocyte sex ratios in malaria and other apicomplexan parasites. Sex ratios matter because they are an important determinant of fitness and transmission success – and hence of disease epidemiology and evolution. Moreover, inbreeding rates can be estimated from gametocyte sex ratios. The sex ratio is also an excellent model trait for testing the validity of important components of what is being marketed as 'Darwinian medicine'.

The study of offspring SEX RATIOS (see Glossary) has provided one of the greatest success stories for the evolutionary (ADAPTATIONIST or Darwinian) approach to biology[1–3]. Theoretical models that predict the best or UNBEATABLE SEX RATIO for a given situation can be constructed relatively easily. These models have been remarkably successful in explaining why the sex ratio is approximately 1:1 in many species, as well as when it should deviate from that. Indeed, the close, sometimes quantitative, fit between theoretical predictions and observational or experimental data gives sex ratio theory a predictive power almost comparable to that of the 'hard' sciences of chemistry and physics[4].

This evolutionary approach to sex ratio differs from the mechanistic approach described elsewhere[5,6]. It attempts to explain and predict the sex ratio in terms of what would be favoured by natural selection; that is, why has natural selection favoured the sex ratios that we observe in nature? By contrast, a mechanistic approach attempts to explain how certain sex ratios are achieved by describing sex-determining mechanisms. On their own, neither approach provides a complete account of sex ratios. Moreover, each can assist the other. Knowledge of the evolutionary forces shaping sex ratio evolution can suggest, for instance, what kind of environmental cues might influence sex determination, and also explain when natural selection favours variable sex ratios.

Sex ratio theory applied to apicomplexans

This section introduces the predictions that theoretical models make for apicomplexan sex ratios. We use the term 'sex ratio' to refer to the GAMETOCYTE SEX RATIO, which we define as the proportion of gametocytes that are male. The relevant natural history for those unfamiliar with the group is given in Box 1. What is the unbeatable sex ratio if there is only one distinct clonal lineage per host (hereafter referred to as clone or genotype) and total INBREEDING (selfing)? In this case, the unbeatable sex ratio will be the one that maximizes successful transmission (i.e. number of zygotes)[7]. Given that one male gametocyte can produce several gametes that could fertilize the gametes produced by several female gametocytes, the best sex ratio will therefore be very female biased. Specifically, if c is the mean number of viable gametes released by a male gametocyte then the best sex ratio to produce will be $1 \div (1 + c)$.

However, if there is a chance that some OUTCROSSING will occur, then a less-female-biased sex ratio will be favoured by natural selection[7]. This is because mutant clones that produce more males will be present at a higher frequency among the mating males and hence obtain a disproportionate share of the mates (and hence make a greater genetic contribution to the next generation). In the extreme, with no inbreeding, a sex ratio of 0.5 (50% males) will be favoured, because at this point, FITNESS through males will balance fitness obtained through females. (This is the reason that a sex ratio of 50% males dominates in humans and other populations with negligible inbreeding[8].)

Glossary

Sex ratio: The proportion of individuals that are male.
Adaptationist approach: The attempt to explain trait values in terms of the process of adaptation.
Unbeatable sex ratio: The sex ratio with the highest fitness (which can therefore not be beaten by any other sex ratio) for a given set of conditions. This sex ratio is often termed an evolutionary stable strategy: a population of individuals playing this strategy cannot be invaded by a mutant that produces a different sex ratio.
Gametocyte sex ratio: The proportion of gametocytes that are male (microgametocytes).
Inbreeding: Mating between related gametes. The level of inbreeding is defined as Wright's coefficient of inbreeding (F), the probability that two homologous genes in two mating gametes are identical by descent.
Outcrossing: Mating between unrelated gametes.
Fitness: Genetic representation in future generations.
Darwinian medicine: The application of the adaptationist approach to matters of medical importance.

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Box 1. Cast

The Apicomplexa form a large and cosmopolitan phylum that consists entirely of parasites, including a number of species of medical and veterinary importance, and can be split into five taxonomic groups[a].

Adeleorins (subclass Coccidiasina, suborder Adeleorina)
These are one-host parasites of invertebrates or vertebrates, or two-host parasites that alternately infect haematophagous (blood feeding) invertebrates and vertebrates (e.g. *Cyrilia*, *Desseria*, *Haemogregarina* and *Hepatozoon*).

Eimeriorins (subclass Coccidiasina, suborder Eimeriorina)
These are often called the coccidia (although this term is also used to include the adeleorins) and are a diverse group that include one-host parasites of invertebrates, two-host parasites of invertebrates, one-host parasites of vertebrates and two-host parasites of vertebrates (e.g. *Cryptosporidium*, *Cyclospora*, *Eimeria*, *Isospora*, *Neospora*, *Sarcosystis* and *Toxoplasma*).

Gregarines (subclass Gregarinasina)
These are generally one-host parasites of invertebrates (e.g. *Gregarina*, *Lankesteria* and *Mattesia*).

Haemospororins (subclass Coccidiasina, suborder Haemospororina)
These are often known as the malaria parasites and are two-host parasites of blood-feeding dipteran flies and various tetrapod vertebrates (e.g. *Haemoproteus*, *Leucocytozoon* and *Plasmodium*).

Piroplasms (subclass Piroplasmasina)
These are two-host parasites infecting ticks and vertebrates (e.g. *Babesia* and *Theileria*).

We are concerned only with the three groups in which sexually dimorphic stages have been observed, the adeleorins, eimeriorins and haemospororins (although our ideas would apply to any species in which dimorphic sexual stages can be found). The basic life history of all these species involves an alteration of sexual and asexual reproduction, and the features relevant to understanding their sex allocation can be summarized as follows[a]. Haploid infective stages called sporozoites infect host tissues and form feeding stages called trophozoites. These stages undergo a period of asexual proliferation and become multicelled stages called meronts (or schizonts). These rupture to produce merozoites, some or all of which transform into sexual stages, which are termed gametocytes for the haemospororins and eimeriorins, and gamonts for the gregarines, adeleorins and piroplasms[b]. For the purposes of consistency we refer to gamonts as gametocytes.

'Male' gametocytes (microgametocytes) rupture to release a number of male gametes (~4–8 in haemospororins[c] and ~5–1000 in eimeriorins[d]), whereas 'female' gametocytes (macrogametocytes) give rise to a single female gamete. The male gamete fertilizes the larger female gamete to form a diploid zygote. The diploid zygote undergoes meiosis, including genetic recombination involving random segregation of chromosomes and crossing-over of homologous regions of DNA, which restores the haploid state. This immature oocyst then divides mitotically to form spores containing the infective stages (sporozoites), which initiate the period of asexual proliferation once again.

Fundamental to applying sex ratio theory to these groups is the possibility that inbreeding can occur[c]. In haemospororins and adeleorins, this arises because fertilization occurs in the blood meal of the invertebrate host, so mating will commonly occur between the gametes from parasites infecting a single vertebrate host. When few parasite genotypes infect a host, this will lead to inbreeding (for example, when single clones self-fertilize). In eimeriorin species, fertilization occurs in the intestines of the host and so, if few genotypes (clones) infect a host, there will be large amounts of inbreeding. In addition, the extent of inbreeding might be increased further in some eimeriorin species because: (1) sexual development and fertilization occur on a very localized scale in a small portion of the intestinal cells of infected hosts[e,f], and (2) male gametes only disperse a short distance to fertilize female gametes[g,h]. Consequently, if different genotypes infect different areas of the intestine, they will not cross fertilize, in which case, high levels of selfing would occur even when the host is infected by many genotypes[i].

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The idea that the sex ratio favoured by natural selection depends on inbreeding rates can be quantified theoretically, and the unbeatable sex ratio (r^*; defined as the sex ratio strategy with the highest fitness) can be shown (Fig. 1) to be related to the inbreeding rate by a pleasingly simple equation (Eqn 1):

$$r^* = (1 - F) \div 2 \qquad [1]$$

where F is Wright's coefficient of inbreeding (the probability that two homologous genes in two mating gametes are identical by descent). Eqn 1 is remarkably robust, and can be shown to hold with very general models as well as those specifically based on the life history of parasites such as *Plasmodium*[7,9–11]. The way in which the population is structured at various levels (e.g. host, house, village

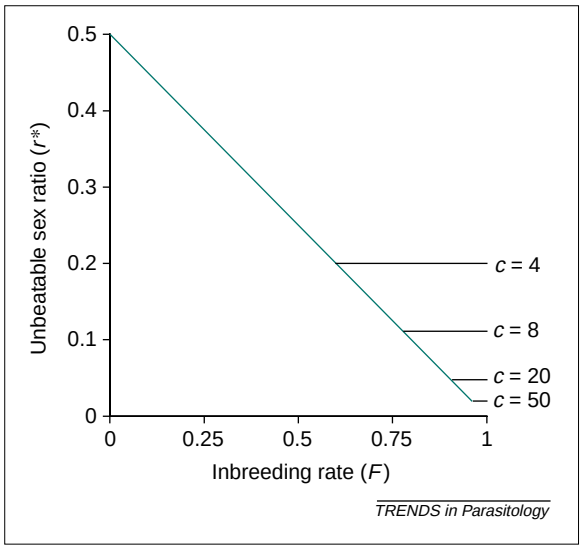

Fig. 1. The unbeatable sex ratio (r^*) plotted against the inbreeding rate (F). When the rate of inbreeding is high, the extent of female bias in the sex ratio is constrained by the need to produce enough male gametes to fertilize the female gametes. This constraint is determined by c, the mean number of viable gametes released by a male gametocyte[7].

and region) can affect F, but Eqn 1 holds nonetheless (S. Nee *et al.*, unpublished). However, a lower limit is put on the expected sex ratio of $1 \div (1 + c)$ by the need to produce enough male gametes to fertilize the female gametes, as described above (c is the mean number of viable gametes released by a male gametocyte).

For multicellular organisms, Eqn 1 is one of the best-verified predictions of evolutionary theory, with substantial quantitative support coming from a wide range of organisms, including wasps, ants, beetles, mites, spiders, snakes and a variety of flowering plants[1,3,12–14].

Predictions

The theory described above make several testable predictions for the sex ratios of haemospororin and eimeriorin parasites. (1) The sex ratio should be 0.5 or female biased, and never male biased. (2) The extent of female bias observed in the sex ratio (r) of a population or species should be related to the inbreeding rate (F) by Eqn 1. (3) Across species and populations, the sex ratio should be negatively related to correlates of the inbreeding rate. For example, higher gametocyte prevalence (that is, the proportion of hosts that are infectious) is likely to lead to lower levels of inbreeding, and so the sex ratio should be positively correlated with gametocyte prevalence[11].

These three predictions require that natural selection act merely on the average sex ratio produced in a population or species. However, conditional adjustment of the offspring sex ratio in response to local conditions has been observed in many organisms (especially ants, bees and parasitic wasps, whose haplodiploid sex-determining mechanism readily enables a mother to control the sex of her offspring[1–3]), and malaria parasites have been shown conditionally to alter another aspect of reproduction (the commitment to asexual or sexual stages) in response to environmental conditions[15–17]. If apicomplexan parasites can detect the likelihood with which they will inbreed and adjust their sex ratio accordingly then we would make one more prediction. (4) More female-biased sex ratios should occur during infections in which there is a higher probability of inbreeding (i.e. fewer clones)[7,18]. This predicts variation in the sex ratio: (1) across infections with different numbers of genotypes producing gametocytes, and (2) within infections over time as the number of genotypes producing gametocytes changes.

This final prediction provides a clear example of the need to understand both the mechanistic and the evolutionary approach, a point that we shall return to when discussing future directions. The evolutionary approach predicts when sex ratios should be adjusted conditionally (Why?) and in what direction. However, an understanding of mechanism is required to determine whether this shift in sex ratio is expected (if sex ratios are genetically fixed and cannot be adjusted conditionally then we should not expect shifts) and, if so, in response to what cues (How?). Consequently, an understanding from both approaches is required to interpret sex ratio data fully.

Supporting evidence

Several recent studies of apicomplexan sex ratios have provided support for these predictions. First, the sex ratios observed in natural populations of haemospororin and eimeriorins are generally female biased (Fig. 2). Second, for cases in which we have sex ratio data and direct genetic estimates of the selfing rate, they are in quantitative agreement. (1) The mean sex ratio observed (0.18) in human malaria infections in Papua New Guinea predicted an inbreeding rate of 0.64–1.0, which was confirmed by molecular genetic analyses that showed an inbreeding rate of 0.9 (Refs 19,20). (2) Molecular genetic analyses of *Toxoplasma gondii* show extremely high levels of inbreeding[21,22] and, as expected, *T. gondii* sex ratios are extremely female biased (mean sex ratio of 0.02–0.06)[23].

Third, variation in the sex ratio across species and populations are consistent with sex ratio theory. The mean sex ratio (0.22) observed in human malaria in Cameroon is less female biased than that observed in Papua New Guinea (0.18), as predicted by a greater potential for outcrossing in Cameroon, where transmission intensity, and hence the number of clones per host, is greater[24]. Across *Leucocytozoon* populations in birds, more-female-biased sex ratios are observed for populations in which gametocyte prevalence is lower, as would be predicted by the fact that inbreeding rates are likely to be higher in these populations (Fig. 3)[11]. In addition, the sex ratios of eimeriorin species are more female biased and less variable than those of haemospororin species (Fig. 2), which is expected because eimeriorin species mate on a very local scale within intestinal tissue, and so inbreeding rates are expected to be consistently high, even when multiple genotypes infect a host (Box 1)[23].

Fourth, there is evidence that some apicomplexan species show conditional sex ratio strategies,

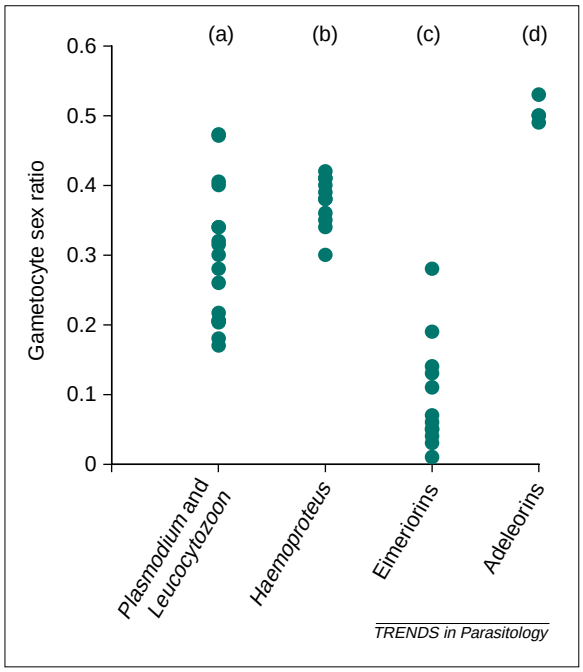

Fig. 2. The mean sex ratios observed in populations of: (a) *Plasmodium* and *Leucocytozoon*; (b) *Haemoproteus*; (c) Eimeriorins (*Eimeria*, *Isospora*, *Sarcocystis*, *Schellackia* and *Toxoplasma*); (d) Adeleorins (*Cyrilia*, *Desseria*, *Haemogregarina* and *Hepatozoon*). Data are from Refs 7,11,18,23,24,29,30 and 54.

producing less female-biased sex ratios in hosts infected with more clones, when inbreeding rates will be lower. Studies on lizard[18,25] and rodent (L.H. Taylor, PhD thesis, University of Edinburgh, 1997) malarias have shown a positive relationship between gametocyte densities and the sex ratio, both across and within infections (Fig. 4). This relationship is predicted if gametocyte densities are higher in infections containing more clones, and there is some evidence for this[26,27].

More support: species with syzygy

Some apicomplexans (adeleorins, gregarines and piroplasms) infringe a necessary assumption of Eqn 1 in such a way that unbiased sex ratios are predicted. 'Syzygy' occurs when a single male gametocyte and a single female gametocyte pair together physically or in close proximity, either in host cells or in the lumen of host organs, just before gametogenesis[28]. The crucial consequence of syzygy is that gametes from a single male gametocyte can only fertilize the gamete from a single female gametocyte (excess gametes die). Consequently, the reproductive success of a parasite is always maximized by ensuring there are enough male gametocytes to form pairs with female gametocytes, and so a sex ratio of 0.5 (50% males) is favoured[23]. Data from four species of adeleorins support this prediction (Fig. 2)[23]. Unfortunately, sexual dimorphism of the gametocyte stage at the light microscope level is rare in adeleorins, and is completely absent from piroplasms and gregarines, which also have syzygy. Further work looking for sexually dimorphic species in these groups, and measuring their sex ratios, would be extremely useful.

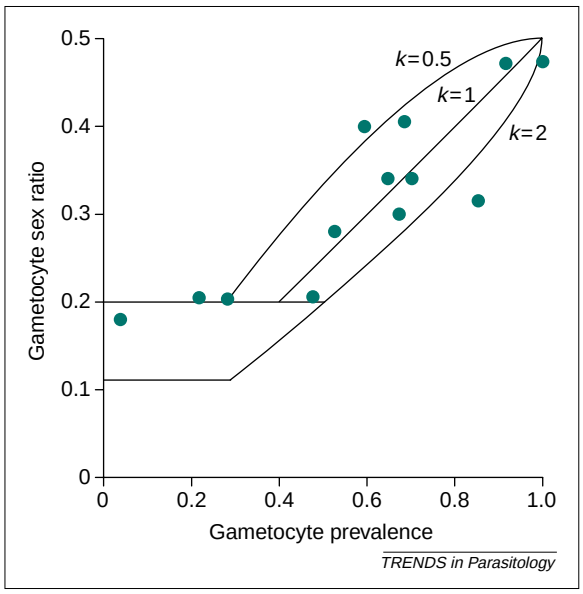

Fig. 3. The observed relationship between the sex ratio and gametocyte prevalence across populations of *Leucocytozoon* and *Plasmodium* parasites. This positive relationship is predicted because when fewer hosts are infectious transmission rates will be lower and mixed infections rarer, and so the rate of inbreeding will be higher. The solid lines show the predicted relationships for various degrees of parasite genotype (clone) aggregation. The variable *k* represents the aggregation parameter from the negative binomial equation[11].

Unpredictable sex ratios and fertility insurance

The above data provide strong support for the application of sex ratio theory to apicomplexans, but some data are contradictory. (1) Across *Haemoproteus* populations in birds, there is no evidence of the predicted relationship between sex ratio and gametocyte prevalence[29] that was observed across *Leucocytozoon* populations (Fig. 3)[11]. (2) Data from within populations of human malaria, some lizard malarias and *Haemoproteus* fail to show the relationship between gametocyte density and sex ratio[24,29,30] that was observed in other lizard and rodent malarias (Fig. 4)[18,25] (L.H. Taylor, op. cit.). (3) Data from *Haemoproteus* of lizards show no consistent trend towards female-biased sex ratios and possibly even some male-biased sex ratios[31]. (4) Data from several *Plasmodium* species show that estimates of the sex ratio taken at different stages from the same infection can be extremely different[30,32,33] (L.H. Taylor, op. cit.).

One factor that might play an important role in explaining these discrepancies is 'fertility insurance'[33–35]. Consider a situation in which there is a danger that female gametes might not encounter any male gametes. This could occur for a number of non-exclusive reasons, including: (1) small blood meals and/or low gametocyte densities lead to an appreciable chance that a blood meal contains no male gametocytes; (2) high mortality of male gametocytes or gametes; (3) low mobility of gametes. Under these conditions, natural selection would favour a less-female-biased sex ratio in order to increase the frequency of female gametes being fertilized[32,35]. Fertility insurance can easily be incorporated into sex ratio models and quantitative predictions made for the favoured sex ratio[35]. Importantly, the logic of this idea has already been supported from data on highly inbred parasitic wasps in which brood sizes are small and the production of extra males is favoured as an insurance against the possibility that all the males might die during development[36–39].

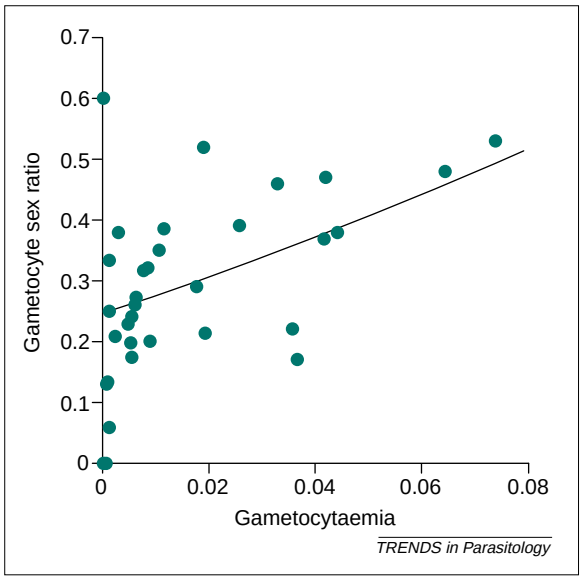

Fig. 4. The observed relationship between the sex ratio and gametocytaemia (proportion of red blood cells containing gametocytes) in the lizard malaria *Plasmodium tropiduri*. This positive relationship is predicted when a lower gametocyte density reflects fewer clones per host, and the parasites are adjusting their sex ratio conditionally based on this higher probability of inbreeding[18].

Fertility insurance is not an alternative to (or in conflict with) the sex ratio theory described above. Fertility insurance should be regarded as an additional factor that can be incorporated into more complicated sex ratio models [indeed, the lower limit of $1 \div (1 + c)$ discussed above reflects the simplest possible case of fertility insurance][35]. Specifically, fertility insurance can only have an effect on the sex ratio when female-biased sex ratios are being favoured by inbreeding – in this case, fertility insurance favours a less-female-biased sex ratio. In a population with no inbreeding, the sex ratio favoured would be 0.5, regardless of the probability that female gametes encounter male gametes.

An enhanced sex ratio model that incorporates fertility insurance could explain some of the data that apparently contradict the predictions of Eqn 1 (Refs 33–35). For example, if fertility insurance was particularly important for *Haemoproteus* species then we would not expect to observe extremely female-biased sex ratios or a relationship between sex ratio and gametocyte prevalence (and indeed we do not). This might be due to small vector size, leading to low numbers of gametocytes in a blood meal, and/or the concentration of blood by their vectors, reducing gamete mobility[34]. Similar ideas could be applied to lizard malaria parasites with small vectors. Clearly, much further work is required on the dynamics of mating within vectors to test this possibility.

Fertility insurance could also explain why sex ratios are variable within infections[33–35]. For example, less-female-biased sex ratios would be predicted at times when immune pressure reduces gamete survival or mobility[33]. This could provide an explanation for the observations in at least some *Plasmodium* infections that the sex ratio becomes less female biased during the course of infection, correlating with an increase in immune pressure[32,33], and, across infections, when the sex ratio is negatively correlated with gametocyte density[5]. Detailed discussion of this idea applied to *Plasmodium* is provided in an accompanying paper[5], in which it is suggested that the hormone that induces red blood cell production (erythropoietin) is used as a cue of host immune status[33]. Clearly, this is another area in which there is considerable scope for further work[40]. For example, is gametocyte or gamete survival and/or mobility reduced sufficiently to make fertility insurance important? Why should erythropoietin be more informative than an assay of more direct cues such as effector molecules? Are there other, more direct, environmental cues that *Plasmodium* responds to and, if so, how do they interact to determine the sex ratio?

Future directions

We hope that this article has emphasized the enormous potential for future research on apicomplexan sex ratios. There are six particularly important areas. First, direct molecular genetic estimates of population structure and inbreeding rates open up several exciting avenues[20,41–46]. (1) Do the mean sex ratios of populations correspond quantitatively to the mean inbreeding rate as predicted by Eqn 1? Such data would be extremely useful from populations in which the inbreeding rate (F) is considerably lower than 1.0, because lower confidence intervals will be placed on estimates of F from sex ratio data owing to the need to produce enough male gametes to fertilize the female gametes (Fig. 1). (2) Across populations and species, is the sex ratio positively correlated with the inbreeding rate? (3) Measures of clonal diversity across individual hosts within a population would allow testing for conditional sex ratio shifts in response to the likelihood of inbreeding. Usefully, molecular data are accumulating rapidly on many *Plasmodium* populations and so, in many cases, all that is required is the relatively easy task of collecting sex ratio data. (4) Measures of both sex ratios and relative abundance of different genotypes within single hosts will allow testing for conditional sex ratio shifts in response to relative abundance within a host. Theory predicts that genotypes that are producing more gametocytes will experience a higher level of inbreeding and should produce more-female-biased sex ratios; genotypes producing relatively few gametocytes will experience a lower level of inbreeding and should produce less-female-biased sex ratios. There is evidence for such sex ratio shifts in parasitic wasps[47].

Second, several areas of basic biology need further investigation, especially those suggested by the possible importance of the fertility insurance hypothesis[33–35]. In particular, very little is known about: (1) parasite–vector interactions – in many cases, the vector has not even been identified[34], still less have there been estimates of how many gametocytes there are in a blood meal or the likelihood of female gametes being fertilized[35]; (2) the consequences of the vertebrate immune response for gametocyte and gamete survival and mobility[33]; (3) the extent to which gametocyte aggregation in the blood[48]

Box 2. What do sex ratios tell us about the inbreeding rate of protozoan parasites?

The rate of inbreeding and extent (or not) of clonality in protozoan parasites, especially *Plasmodium*, has been the subject of much debate[a–d]. This controversy has arisen in part because much previous evidence has come from indirect genetic measures such as linkage disequilibrium (non-independent segregation of alleles at pairs of loci), which are open to multiple explanations and cannot be used easily to estimate the inbreeding rate quantitatively[a,e]. More recently, molecular genetic analyses of the diploid products of sex (oocysts) have been used to provide direct estimates of the inbreeding rate[f]. However, this method is extremely laborious and expensive, and so has only been applied to two populations[f,g]. In addition, interpretation of this data is also proving problematic – estimates of the inbreeding coefficient from human malaria in Papua New Guinea vary from 0.48 to 0.90, depending upon how one accounts for the existence of null alleles in the data[e]. Also, this method could not be applied to all populations – the prevalence of infection in mosquitoes from low-transmission areas (often <1 in 1000) makes this method impractical[h].

Sex ratio data provide a relatively cheap and easy indirect method with which to estimate the population structure of a species[i]. Specifically, the inbreeding rate (F) can be estimated from the observed sex ratio (r) using Eqn I:

$$F = 1 - 2r \qquad [I]$$

The validity of this approach is supported by the fact that, in cases for which we have molecular genetic and sex ratio data, they are in agreement[f,i,j].

A major advantage of the sex ratio approach is that sex ratio data are relatively easy to obtain and so can be collected from many species and populations, allowing generalizations to be made. The sex ratio data from haemospororin species show a range of sex ratios from extremely female biased to 50% males, suggesting that the rate of inbreeding varies enormously between populations and species, from highly inbred to highly outbred (see Fig. 2 in main text), depending upon transmission rates[k] (see Fig. 3 in main text). By contrast, the data from eimeriorin species are highly female biased, predicting consistently high inbreeding rates (see Fig. 2 in main text), as expected from the fact that mating occurs on a very local scale within the intestine[j] (see Box 1 in main text).

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reduces the need for fertility insurance; (4) cues that might allow parasites to shift their sex ratios conditionally; (5) the mean number of viable gametes released by a male gametocyte (c). In *Plasmodium mexicanum*, a lizard malaria, the modal value of c is around 2 (Ref. 25), and so the maximum female bias that could be favoured by natural selection is 0.33, which happens to be that observed. Similarly low values of c across *Haemoproteus* species could explain their lack of sex ratio variation in response to a correlate of inbreeding[29], and the variation in C as a result of immune-induced mortality could explain variation in the sex ratio during infections[33,35].

Third, almost nothing is known about mechanism of sex determination within the Apicomplexa[6,33,49,50], despite its fundamental importance for determining the extent to which parasites can adjust their sex ratio in response to environmental conditions (as opposed to having genetically fixed strategies) and whether male and female gametocytes are equally costly to produce[49]. Is sex determination a response to one or more environmental cues that are related to inbreeding rates and/or the need for fertility insurance? How linked are the cellular and molecular mechanisms involved in sex determination to those in commitment to asexual or sexual stages[15,49,51,52] (e.g. 'stressful' conditions might favour production of gametocytes[15–17] and the need for fertility insurance[33])?

Fourth, more sex ratio data are required from species in which syzygy occurs; a sex ratio of 0.5 is then predicted irrespective of the inbreeding rate[23].

Fifth, many fundamental assumptions that can alter the predictions of sex ratio theory need to be tested. Is gametocyte mortality sex biased? Current evidence suggests that it is not[7,53] but this conclusion is based on perilously low sample sizes (seven infections) given the importance of the issue. Are estimates of the sex ratio biased by sampling from hosts with high gametocyte densities and/or by being taken from relatively large blood vessels (vectors typically feed from very small vessels)? How frequently is the sex ratio underestimated from Giemsa-stained blood films owing to male gametocytes being incorrectly identified as immature gametocytes (compared with estimates based on mRNA probes or stage- and sex-specific antibodies)[49]?

Sixth, much of the data and background biology presented in this article are drawn from studies of human and laboratory *Plasmodium*. Expanding research of all forms to a wider range of species might turn up novel aspects of biology, with fundamental consequences for sex allocation, as is the case with

syzygy[23] (e.g. explaining why the sex ratio pattern is so different in *Haemoproteus*[34]).

Conclusions

Existing data suggest that apicomplexan sex ratios are broadly consistent with the predictions of sex ratio theory. In addition to providing an explanation for patterns of variation in the sex ratio per se, this work is important because it provides an excellent model trait[11,23] with which to test the usefulness of 'DARWINIAN MEDICINE' – if we cannot use the adaptationist approach to understand sex ratios then we cannot use it to understand more complex traits that are of direct medical importance, such as virulence. It also provides a relatively easy method for estimating the inbreeding rate in natural populations, a parameter that is currently the subject of much debate (Box 2). However, work on the sex ratios of apicomplexans is still in its infancy compared with studies of other taxa, and there is much to catch up on – even in the realms of human malaria and associated laboratory models.

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