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Sex allocation and population structure in apicomplexan (protozoa) parasites

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Establishing the selfing rate of parasites is important for studies in clinical and epidemiological medicine as well as evolutionary biology. Sex allocation theory offers a relatively cheap and easy way to estimate selfing rates in natural parasite populations. Local mate competition (LMC) theory predicts that the optimal sex ratio (r^*; defined as proportion males) is related to the selfing rate (s) by the equation $r^* = (1-s)/2$. In this paper, we generalize the application of sex allocation theory across parasitic protozoa in the phylum Apicomplexa. This cosmopolitan phylum consists entirely of parasites, and includes a number of species of medical and veterinary importance. We suggest that LMC theory should apply to eimeriorin intestinal parasites. As predicted, data from 13 eimeriorin species showed a female-biased sex ratio, with the sex ratios suggesting high levels of selfing (0.8–1.0). Importantly, our estimate of the selfing rate in one of these species, *Toxoplasma gondii*, is in agreement with previous genetic analyses. In contrast, we predict that LMC theory will not apply to the groups in which syzygy occurs (adeleorins, gregarines and piroplasms). Syzygy occurs when a single male gametocyte and a single female gametocyte pair together physically or in close proximity, just prior to fertilization. As predicted, data from four adeleorin species showed sex ratios not significantly different from 0.5.

Keywords: gametocytes; *Plasmodium*; selfing; syzygy; *Toxoplasma*; virulence

1. INTRODUCTION

Aspects of parasite population structure, such as selfing rate and the number of genotypes infecting each host, have consequences for the evolution of drug resistance (Mackinnon & Hastings 1998), parasite virulence (Herre 1993; Frank 1996), disease diagnosis (Tibayrenc *et al.* 1990; Hastings & Wedgwood-Oppenheim 1997), and the development and assessment of vaccines and curative drugs (Dye 1992; Gupta *et al.* 1997). Despite considerable recent attention, the population structure of parasitic protozoa species, and in particular estimates of selfing rates, have remained highly controversial (Tibayrenc 1995). This controversy has arisen in part because much previous evidence has come from indirect genetic measures such as linkage disequilibrium, which are open to multiple explanations (Paul & Day 1998). However, direct genetic measures are extremely laborious and expensive to obtain (Paul *et al.* 1995), and so there is a need for more accessible indirect methods.

Sex allocation theory offers a tool for inferring selfing rates in natural populations of parasites (Read *et al.* 1992). If mating takes place between the offspring of one or a few mothers (a subdivided population), then a female-biased sex ratio (where sex ratio is defined as the proportion of males) is favoured by a process termed local mate competition (LMC; Hamilton 1967). This female bias arises because it reduces competition among brothers for mates, and because it increases the number of mates for each of the mother's sons (Taylor 1981). The optimal sex ratio (r^*) can be shown to depend upon the selfing rate (s; defined as the proportion of a mother's daughters that are fertilized by her sons) by the equation $r^* = (1-s)/2$ (Hamilton 1967). The optimal sex ratio favoured by natural selection should thus decline from 0.5 for complete outcrossing ($s = 0$) to 0 for complete selfing ($s = 1$), the latter interpreted as meaning that a female should produce the minimum number of sons required to fertilize all of her daughters. This is one of the most quantitatively verified aspects of evolutionary biology (Charnov 1982; Godfray 1994; Herre *et al.* 2000). Several recent studies have applied LMC theory successfully to malaria and other closely related blood parasites (Read *et al.* 1992, 1995; Pickering *et al.* 2000). However, the evidence linking the selfing rate and sex ratio is not perfect (Schall 1989; Paperna & Landau 1991; Shutler *et al.* 1995) and so it is still not clear how widely sex-ratio theory can be used to estimate population structure in parasites.

In this paper, we generalize the application of sex allocation theory across parasitic protozoa in the phylum Apicomplexa. This cosmopolitan phylum consists entirely of parasites, and includes a number of species of medical and veterinary importance such as malaria parasites (*Plasmodium* spp.), piroplasms (*Babesia* spp. cause babesiosis in humans and red water fever in cattle, and *Theileria* spp. cause East Coast fever in cattle), adeleorins (*Hepatozoon* spp. cause an often fatal hepatozoonosis in dogs) and coccidia (various species of *Cryptosporidium*, *Eimeria*, *Isospora*, *Neospora*, *Sarcocystis* and *Toxoplasma* are pathogenic to immunocompromised humans or are the causative agents of veterinary coccidiosis). Our specific aims are to: (i) make theoretical predictions for when selfing should (and should not) lead to biased sex ratios in apicomplexan species; (ii) test these theoretical predictions; and (iii) use the sex-ratio data to estimate selfing levels in apicomplexan species.

2. BACKGROUND BIOLOGY

The phylum Apicomplexa, class Sporozoasida, can be divided into five taxonomic groups following Roberts & Janovy (1996). The gregarines (subclass Gregarinasina) are generally one-host parasites of invertebrates. The

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adeleorins (subclass Coccidiasina, suborder Adeleorina) are one-host parasites of invertebrates or vertebrates, or two-host parasites that alternately infect haematophagous (blood-feeding) invertebrates and the blood of vertebrates. All species of piroplasms (subclass Piroplasmasina) are two-host parasites infecting ticks and vertebrates. Haemospororins (subclass Coccidiasina, suborder Haemospororina), often known as the malaria parasites, are two-host Apicomplexa that parasitize blood-feeding dipteran flies and the blood of various tetrapod vertebrates. Finally, the eimeriorins (subclass Coccidiasina, suborder Eimeriorina), frequently called the coccidia (although this term is often used to include the adeleorins), are a diverse group that includes one-host species of invertebrates, two-host species of invertebrates, one-host species of vertebrates and two-host species of vertebrates.

Apicomplexan life histories involve the alternation of sexual and asexual reproduction, and the features relevant to understanding their sex allocation can be summarized as follows (Roberts & Janovy 1996). Haploid infective stages called sporozoites infect host tissues to 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. For the purposes of consistency among groups of Apicomplexa and also with previous malaria parasite sex-ratio papers, we refer to gamonts as gametocytes. In the gregarines and piroplasms, gametocytes are thought to be isogamous, and so we cannot present sex allocation data. However, in most species of the other three groups, the gametocytes are sexually dimorphic.

In these three groups (adeleorins, eimeriorins and haemospororins), 'male' microgametocytes rupture to release a number of male gametes, while 'female' macrogametocytes give rise to a single female gamete. We will refer to micro- or macrogametocytes and micro- or macrogametes as male or female gametocytes and male or female gametes, respectively. The male gametes fertilize the larger female gametes to form diploid zygotes. In terms of sex allocation, male gametocytes are functionally equivalent to males, and female gametocytes are equivalent to females. Similarly, the different male gametes arising from a single male gametocyte are equivalent to multiple matings by a male gametocyte, with each being able to fertilize a single female gamete. When we discuss the sex ratio we mean the gametocyte sex ratio, defined as the proportion of gametocytes that are male. In all cases we assume that male and female gametocytes are equally costly to produce, as observed in *Plasmodium falciparum* (T. G. Smith, P. Laurenco, R. Carter, D. Walliker and L. C. Ranford-Cartwright, unpublished data). The diploid zygotes undergo meiosis, including genetic recombination involving random segregation of chromosomes and crossing over of homologous regions of DNA, which restores the haploid state. Zygotes then divide mitotically to form spores containing the infective stages that initiate the period of asexual proliferation once again.

The production of gametocytes and the process of fertilization occur by different means in a variety of host tissues depending on which of the five major groups of Apicomplexa a particular species belongs to. For the purposes of this paper, there is an important distinction between the groups in which syzygy occurs (gregarines, adeleorins and piroplasms) and those in which it does not (haemospororins and eimeriorins). Syzygy is defined as the process whereby 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 prior to gametogenesis (Barta 1999). The crucial consequence of syzygy is that gametes from a single male gametocyte are only able to fertilize the gamete from a single female gametocyte. The excess of male gametes die. In contrast, in species where syzygy does not occur, sexual development involves gametes pairing. In this case, different gametes arising from a single male gametocyte are able to fertilize gametes arising from a number of female gametocytes.

3. SEX ALLOCATION IN THE APICOMPLEXA: THEORETICAL PREDICTIONS

(a) *Species without syzygy*

Sex-ratio models have been developed previously for malaria and related haemospororin parasites where fertilization occurs in the blood meal of the invertebrate host (Read *et al.* 1992, 1995; Dye & Godfray 1993; Pickering *et al.* 2000). In these species, gametes competing for matings will generally be those found in a single blood meal, and so mating will occur between the different parasite genotypes (clones) that are in a single host (and producing gametocytes) rather than those found in many hosts. This leads to the potential for LMC and selfing if there are low numbers of parasite genotypes infecting each host.

We suggest that the natural history of eimeriorin species where fertilization occurs in the intestines of the host should also lead to LMC and appreciable levels of selfing. As with the species where fertilization occurs in the blood, this should occur when low numbers of parasite genotypes infect each host (Shirley & Harvey 1996). However, the extent of LMC and selfing may be increased further in some eimeriorins because (i) sexual development and fertilization occur on a very localized scale in a small portion of the intestinal cells of infected hosts (Marquardt 1973; Long 1993), and (ii) male gametes only disperse a short distance to fertilize female gametes (Hammond 1973; Dubey 1993). Consequently, if different genotypes infect different areas of the intestine, then high levels of LMC and selfing may occur even if the host is infected by a large number of genotypes (Johnson 1997). As with blood parasites, enough male gametes must be produced to fertilize the female gametes, and so a lower bound is placed on the sex ratio of $1/(1+c)$, where c is the average number of viable gametes released from a male gametocyte after exflagellation (figure 1). This lower bound may be increased by mortality or if gametes have trouble locating mates.

(b) *Species in which syzygy occurs*

Syzygy occurs in the gregarines, adeleorins and piroplasms. These groups consist of species in which

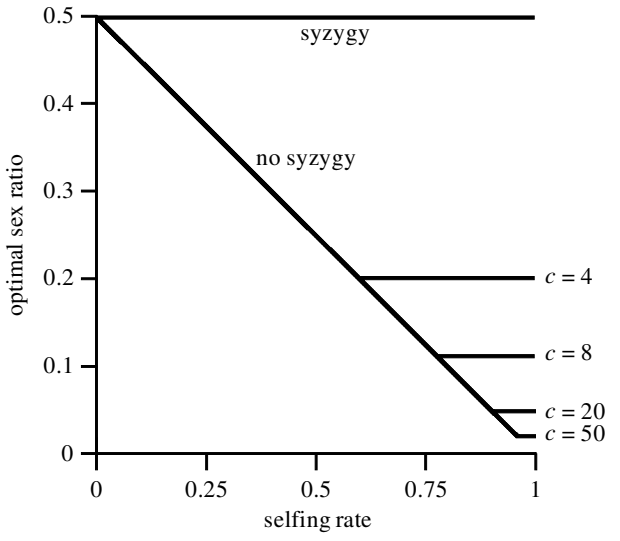

Figure 1. The optimal sex ratio (r^*) plotted against the selfing rate (s) for apicomplexan species in which syzygy occurs (gregarines, adeleorins and piroplasms), and syzygy does not occur (eimeriorins and haemospororins). The relationship for species in which syzygy does not occur is plotted for various values of c, the average number of viable gametes released from a male gametocyte after exflagellation.

fertilization takes place in the tissues of blood-feeding invertebrate hosts (two-host adeleorins and piroplasms) and in the intestines of invertebrate hosts (most gregarines and one-host adeleorins), and so appreciable levels of selfing may occur for the same reasons as in the species where syzygy does not occur.

However, we argue that female-biased sex ratios will not be selected for species where syzygy occurs. The crucial consequences of syzygy for sex allocation are that (i) an increase in the proportion of female gametocytes would not decrease competition between male gametes from the same male gametocyte, and (ii) an increase in the proportion of female gametocytes would not allow gametes from the same male gametocyte to fertilize additional female gametes. This means that syzygy removes the two factors that favour female-biased sex ratios under conditions of LMC (Taylor 1981). Consequently, even when selfing occurs, LMC of a form that favours female-biased sex ratios does not occur. Instead, the reproductive success of a parasite is always optimized by ensuring that there are enough male gametocytes to form pairs with the female gametocytes. A sex ratio of 0.5, equal numbers of male and female gametocytes, is therefore always favoured (figure 1). We show this more formally in Appendix A.

To conclude, we make two predictions. First, in species where syzygy does not occur (haemospororins and eimeriorins), the sex ratio should be equal (0.5) or female biased (<0.5), and will provide information on selfing rates (figure 1). Second, in species where syzygy occurs (adeleorins, gregarines and piroplasms), the sex ratio should be equal (0.5) and not provide any information on selfing rates (figure 1).

4. METHODS

We obtained data in three ways: (i) from the literature; (ii) we made counts from samples, in the form of slides provided by other workers, and (iii) we made counts from samples, in the form of slides that we had prepared ourselves. Blood films were stained with Giemsa or Diff-Quik® (Dade Diagnostics, Aguada, Puerto Rico), whereas tissue sections were stained variously with iron haematoxylin, haematoxylin and eosin, or periodic acid–Schiff reaction, depending on the source of the material (see references in table 1). Samples were examined under a light microscope, with gametocytes sexed according to Gardiner *et al.* (1988). The sources for all data and type of infection (natural or experimental) are given in table 1. We have not included data from haemospororin species, because these have already been analysed elsewhere from an LMC perspective (Read *et al.* 1992, 1995; Shutler *et al.* 1995; Pickering *et al.* 2000).

We first tested for biased sex ratios, defined as a significant deviation from a sex ratio of 0.5, in each species. Ideally, we would test within each species by using samples from different hosts as independent data points. However, biological (rarity) and ethical considerations mean that generally only a single or small number of hosts has been sampled for each parasite species. We therefore used a G-test to look for biased sex ratios within each individual host, using the total counts made from a number of samples on slides. We then examined the average sex ratios of the species in which syzygy did and did not occur, testing for a significant deviation from 0.5. Proportion data such as sex ratio usually have non-normally distributed error variance and unequal sample sizes. To avoid these problems, we initially analysed the data with a general linear model analysis of deviance, assuming binomial errors, and a logit link function in the GLIM statistical package (Crawley 1993). However, after fitting the explanatory variables, the ratio of the residual deviance to the residual degrees of freedom was greater than 10, showing considerable over-dispersion, suggesting that the data did not fit the assumption of binomial errors (McCullagh & Nelder 1983). Consequently, the sex ratios were arcsine square-root transformed and used as the response variable assuming normal errors.

5. RESULTS

We obtained sex-ratio data (proportion of gametocytes that were male) on 13 eimeriorin species, representing seven genera in which syzygy does not occur (table 1). In all of these cases, a female bias was recorded. In five species the sex ratio was not quantified and merely recorded as female biased, whereas for eight species we have quantitative estimates of the sex ratio. In all of these cases, the sex ratio was significantly female biased (table 1). The average sex ratio of these species was 0.11 and significantly different from 0.5 ($t_7 = 9.20$, $p < 0.01$).

We obtained sex-ratio data on four adeleorin species, representing four genera where syzygy occurs (table 1). In one species the sex ratio was not quantified, and merely recorded as equal numbers of male and female gametocytes. In the other three species the sex ratio was quantified and not significantly different from 0.5 (table 1). Data are lacking on species where syzygy occurs because sexual dimorphism of the gametocyte stage at the light microscope level is rare among adeleorins, and absent altogether in the piroplasms. The average sex ratio of the species where syzygy occurs was not significantly different from 0.5 ($t_2 = 0.51$, $p > 0.2$).

Our sex-ratio data estimated selfing rates of 0.44 to 1.0 (mean = 0.79) in the eimeriorin species (table 1). The estimates of selfing rates in table 1 have been given ranges to

Table 1. *Characteristics of 19 eimeriorin species, in which syzygy does not occur, and 15 adeleorin species, in which syzygy does occur*

(The data were obtained from (n_g, number of gametocytes counted which, for literature data, is only given when reported in the original source): (Bonnin *et al.* 1995, personal communication)[1]; (Hammond *et al.* 1961)[2]; (Pakandl *et al.* 1996)[3]; (Haberkorn 1971)[4]; (Chauve *et al.* 1994)[5]; (this study, slides provided by S. Desser; $n_g = 72$[6]; $n_g = 96$[7]; $n_g = 102$[8]; $n_g = 110$[9])[6–9]; (this study, slides provided by M. Cameron; $n_g = 225$[10]; $n_g = 109$[23])[10,23,24]; (Dubey 1993)[11]; (Dubey & Fayer 1976)[12]; (Dubey 1978; $n_g = 2256$[13]; $n_g 527$[14])[13,14]; (Dubey 1979; $n_g = 21$)[15]; (Baker *et al.* 1996)[16]; (Dubey 1982)[17]; (Dubey *et al.* 1982; $n_g = 300$)[18]; (Bristovetsky & Paperna 1990; $n_g = 143$)[19]; (Paperna & Finkelman 1996*a*,*b*)[20]; (Dubey & Frenkel 1972)[21]; (Omata *et al.* 1997)[22]; (Lainson 1981, 1992; $n_g = 300$)[25]; (Minchin & Woodcock 1910)[26]; (Siddall & Desser 1992)[27]; (Siddall & Desser 1990)[28]; (this study, slide provided by M. Siddall; $n_g = 235$)[29]; (references in Smith & Desser 1997)[30]; (Smith 1996; $n_g = 228$)[31]. The second column shows infection type, i.e. whether the data were taken from experimental or natural infections. The majority of the experimental infections were initiated with spores from natural infections. The following columns give the average number of gametes observed per male gametocyte, the average sex ratio (proportion of male gametocytes), a *G*-test on the hypothesis that there are equal numbers of male and female gametocytes (a sex ratio of 0.5), and estimated selfing rate given the observed sex ratio.)

species	infection type	gametes per male gametocyte	sex ratio	*G*-test	estimated selfing-rate
Eimeriorin species (no syzygy)					
Cryptosporidium parvum[1]	experimental	16	<0.50	—	>0
Eimeria auburnensis[2]	experimental	>1000	—	—	—
Eimeria coecicola[3]	experimental	>1	<0.50	—	>0
Eimeria contorta[4]	natural	40–60	—	—	—
Eimeria mulardi[5]	natural	—	<0.50	—	>0
Eimeria perforans[6]	natural	133	0.13	133.40[b]	0.75
Eimeria stiedae[7]	natural	317	0.04	317.33[b]	0.92–1.0
Eimeria stiedae[8]	natural	271	0.14	271.00[b]	0.73
Eimeria tenella[9]	experimental	97	0.05	97.33[b]	0.89–1.0
Eimeria tenella[10]	natural	97	0.11	159.10[b]	0.78–1.0
Frankelia buteonis[11]	natural	12	—	—	—
Isospora bigemina[12]	experimental	6–12	—	—	—
Isospora ohioensis[13]	experimental	20–50	0.07	1977.00[b]	0.86–1.0
Isospora ohioensis[14]	experimental	—	0.05	535.40[b]	0.91–1.0
Isospora rivolta[15]	experimental	$\leqslant 70$	0.19	8.66[a]	0.62
Lankesterella sp.[16]	natural	$\leqslant 16$	<0.50	—	>0
Sarcocystis cruzi[17]	experimental	3–11	<0.05	—	0.90–1.0
Sarcocystis tenella[18]	experimental	8–11	0.01	382.30[b]	0.99–1.0
Schellackia agame[19]	experimental	—	0.28	28.73[b]	0.44
Schellackia ptyodactyli[20]	natural	—	<0.50	—	>0
Schellackia muriviperae[20]	natural	5–12	—	—	—
Toxoplasma gondii[21]	experimental	12 (6–21)	0.02–0.04	—	0.92–1.0
Toxoplasma gondii[22]	experimental	—	<0.50	—	>0
Toxoplasma gondii[23]	natural	30	0.06	75.52[b]	0.89–1.0
Toxoplasma gondii[24]	natural	41	—	—	—
Adeleorin species (syzygy)					
Cyrilia lignieresi[25]	natural	—	0.50	0	—
Desseria rovigensis[26]	natural		*ca.* 0.5	—	—
Desseria myxocephali[27]	natural	4	—	—	—
Haemogregarina balli[28]	natural	4	—	—	—
Haemogregarina balli[29]	natural	—	0.49	0.21	—
Hepatozoon aegypti[30]	natural	4	—	—	—
Hepatozoon breinli[30]	natural	4	—	—	—
Hepatozoon catesbianae[30]	natural	2	—	—	—
Hepatozoon erhardovae[30]	natural	2	—	—	—
Hepatozoon gracilis[30]	natural	4	—	—	—
Hepatozoon mauritanicum[30]	natural	4	—	—	—
Hepatozoon mocassini[30]	natural	4	—	—	—
Hepatozoon rarefaciens[30]	natural	4	—	—	—
Hepatozoon sipedon[30]	natural	2	—	—	—
Hepatozoon sp.[31]	natural	—	0.53	0.90	—

[a] $p < 0.005$.
[b] $p < 0.001$.

include 1.0 in cases where the sex ratios are sufficiently biased that complete sib mating occurs, and that the species is merely producing enough male gametocytes to fertilize the female gametocytes (see § 3(a)).

We obtained counts of the number of gametes per male gametocyte in 16 eimeriorin species (table 1). Across species, the number of gametes per male gametocyte, ranged from 12 in *Frankelia buteonis* to greater than 1000 in

Eimeria auburnensis. In addition, we obtained counts of the number of gametes per male gametocyte in 12 adeleorin species (table 1). Across species, the number of gametes per male gametocyte, ranged from two to four.

6. DISCUSSION

It has previously been argued that LMC theory should apply to species where fertilization occurs in the blood, as with *Plasmodium*, and other haemospororins. We have suggested that LMC theory should apply to other apicomplexan parasites, the eimeriorins, where fertilization occurs in the intestine of the host. As predicted, a female-biased sex ratio was observed in 13 eimeorin species (table 1). In contrast, we predicted that, independent of the selfing rate, LMC theory would not apply to species in which syzygy occurs, the adeleorins, gregarines and piroplasms. As predicted, unbiased sex ratios were observed in four adeleorin species (table 1).

The phylogeny of the Apicomplexa is currently a matter of much debate (Barta 1989; Barta *et al*. 1991; Allsopp *et al*. 1994; Escalante & Ayala 1995; Lang-Unnasch *et al*. 1998). In all resolutions, syzygy is the ancestral condition for the phylum. However, the number of times that syzygy has been lost is unclear, with some studies suggesting only once (e.g. Barta 1989), and others more than once (e.g. Lang-Unnasch *et al*. 1998). By allowing for LMC of a form that favours female-biased sex ratios we suggest that the loss of syzygy has allowed biased sex ratios to evolve in species where high levels of selfing occur. The evolution of female-biased sex ratios could then have favoured the production of more gametes per male gametocyte, which are observed for haemospororins and eimeriorins, especially if there are high mortality rates in the intestine. Given high enough selfing rates, this could have allowed more female-biased sex ratios to evolve. This potential for coevolution between sex ratio and gamete numbers could have resulted in the large numbers of gametes per male gametocyte that are observed in the eimeriorin species which have female-biased sex ratios (table 1). However, it should also be stressed that because syzygy may have only been lost once or twice, our ability to test such scenarios with formal comparative methods will be limited by a lack of degrees of freedom (Harvey & Pagel 1991; Read & Nee 1991, 1993, 1995). Note that in other contexts, some authors have felt comfortable applying formal tests to analogous situations (e.g. Brookfield 1993; Orr 1998). In this case, such tests would demonstrate highly significant associations.

The sex ratios of the eimeriorin species suggest high levels of selfing, averaging 0.8–1.0 (table 1). The extremely female-biased sex ratios observed in these parasite species are similar to those observed in insect species when total selfing ($s=1$) occurs (Green *et al*. 1982; Hardy & Cook 1995; West & Herre 1998). The number of gametes produced per male gametocyte (c) is much greater in the eimerion species ($c=12$–1000; table 1) than in haemospororins ($c=4$–8; Read *et al*. 1995), allowing more female-biased sex ratios and a greater accuracy when estimating high selfing rates (figure 1).

How do our data contribute to the debate on the population structure of parasitic protozoa species? Our sex-ratio data predict extremely high selfing rates in eimeriorin species. Importantly, our estimates of selfing rates from sex-ratio data include a species for which genetic analyses of population structure have been carried out, *Toxoplasma gondii*. The estimates from both the genetic analyses (Tibayrenc *et al*. 1991; Sibley & Boothroyd 1992) and the sex-ratio data (table 1) are in agreement, with effective clonality predicted. Such selfing is probably a consequence of matings occurring on a very local scale within intestinal tissue (see § 3). In contrast, the life histories of other apicomplexan species (two-host adeleorins, piroplasms and haemospororins) provide a means for gametocytes and gametocytes from different genotypes (clones) to mix before mating occurs. This will lead to selfing levels that vary enormously, depending upon other biological variables, such as infection and transmission rates and the feeding habits of the invertebrate hosts (Read *et al*. 1995; Shutler & Read 1998). The sex-ratio data from haemospororin species are consistent with this prediction, showing a range of sex ratios from extremely female biased to 50% males (Schall 1989; Read *et al*. 1992, 1995; Shutler *et al*. 1995; Pickering *et al*. 2000).

To conclude, our results illustrate two general points about the pros and cons of applying optimality models and an evolutionary biology approach to infectious disease research (Stearns 1999). First, our data show that, despite assuming equilibrium states which are not an obvious feature of microparasite populations (Anderson & May 1991), the optimality approach is able to explain variation in a life-history trait (sex ratio) across a taxonomically diverse range of microparasites. In particular, sex-ratio data provide the clearest demonstration of the importance of population structure as a determinant of the direction of natural selection. Theory suggests that population structure is one of the key determinants of parasite virulence (Frank 1996). Second, the consequences of syzygy provides an example of when a basic assumption of models developed for eukaryotes does not apply, emphasizing the importance of checking basic assumptions when applying such models to microparasites.

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APPENDIX A

In this section we show that syzygy leads to equality (0.5) being the optimal sex ratio independent of selfing rates. Assume that mating occurs between n parasites in a host and that all parasites are equally abundant in the host. Assume syzygy occurs. In order to determine the optimal strategy we consider the fitness of a mutant producing a sex ratio r' in a population of individuals who produce a sex ratio r. The equation for the fitness of the mutant (W') is given by $W' = r' z_{\mathrm{m}} + (1 - r')z_{\mathrm{f}}$, where z_{m} and z_{f} are the

probability of male and female gametocytes being mated, respectively. The equations for z_m and z_f depend upon whether the sex ratio in the patch is male or female biased. If there is a female-biased sex ratio then $z_m = 1$, and $z_f = r' + (n-1)r/((n-1)(1-r) - r')$. If there is a male-biased sex ratio then $z_m = (1 - r' + (n-1)(1-r)/(r' + (n-1)r)$, and $z_f = 1$. The optimal sex ratio, r^*, is that which cannot be beaten by any other strategy. This can be determined through iterative simulations (see West & Godfray 1997) or graphically. In all cases it can be shown that $r^* = 0.5$, independent of the value of n (figure 1). Note that this theoretical argument could apply to other life histories, such as when males and females form long-term pair bonds and in which extra pair copulations do not take place.

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