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Evolution

Sex proportions of *Haemoproteus* blood parasites and local mate competition

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ABSTRACT **Recent genetic evidence suggests that parasitic protozoa often reproduce by "selfing," defined as sexual stages from a single, clonal lineage fertilizing each other. Selfing favors production of an excess of female over male progeny. We tested whether the proportion of male gametocytes of blood parasites of the genus *Haemoproteus* was affected by variables that could influence the probability of selfing. Proportions of male *Haemoproteus* gametocytes from 11 passerine host populations were not affected by the age of the parasites' avian hosts, date in season, sex of host, intensity of host's infection, or prevalence of parasites within host populations.**

Ecological conditions promoting inbreeding also favor female-biased sex proportions in offspring (1–5). In the case of parasitoid wasps, brothers fertilize their sisters before they leave the larva from which they emerge (in essence, "selfing"). Because only daughters produce offspring, more grandchildren are left by adult wasps producing the minimum number of sons necessary to fertilize all of the daughters. This strategy eliminates local mate competition between male siblings (1). In contrast, where multiple females lay eggs on a larva, probability of outbreeding increases, and a wasp's optimum strategy is to produce offspring in a more even sex proportion (6). Compelling empirical evidence has emerged from a diversity of taxa in support of Hamilton's (1) prediction that optimum sex proportion is a function of the probability of outbreeding (reviewed in ref. 5).

Tibayrenc and colleagues have suggested that many parasites of humans reproduce by selfing (7–11), where selfing is defined as sexual stages from a single, clonal lineage fertilizing each other. The conclusion that parasites reproduce frequently by this kind of selfing has significant importance for modeling disease population dynamics and for disease treatment. Although the conclusion of selfing has been challenged for *Plasmodium* (12–16), additional support for selfing comes from the finding of Read *et al.* (17) that human *Plasmodium* gametocytes had female-biased sex proportions (0.18 male). Working backwards from Hamilton's (1) equation for optimum sex proportion, Read *et al.* (17) calculated that the sex proportion they observed was consistent with human hosts transmitting on average 1.2 *Plasmodium* genetic (selfing) lineages to each vector blood meal (also see ref. 18). In this paper, we test for sex proportion adjustment in a related genus, *Haemoproteus*, that parasitizes birds.

Each species of blood parasite of the order Haemosporidia (which includes *Plasmodium, Haemoproteus*, and *Leucocytozoon*) tends to be specific to a limited number of vertebrate hosts (in which it reproduces asexually only) and of biting fly (Diptera) vectors (in which they reproduce sexually) (19–21) (additional asexual stages may be ignored for our purposes). In as few as 6 days after being infected, vertebrate hosts carry parasite gametocytes in their blood. Vectors taking blood meals from infected vertebrates can themselves become infected. Within infected flies, each male gametocyte divides to produce eight microgametes, each capable of fertilizing a single macrogamete; a single macrogamete arises from each female gametocyte (19–21). Hence, a completely inbreeding haemosporidian population need only produce one male and eight female gametocytes in vertebrate hosts to ensure fertilization of all macrogametes in fly hosts (i.e., a gametocyte sex proportion of 0.11 male). In contrast, completely outbreeding populations should produce equal numbers of male and female gametocytes (1, 17, 22).

Gametes fuse and form zygotes in the gut of fly vectors in a matter of seconds after a blood meal. This is rarely as long as intervals between vector feedings; thus, separate hosts almost never provide different genetic lineages to individual vectors. Hence, parasite outbreeding can occur only if vertebrate hosts become infected from one or more flies carrying separate parasite genetic lineages. Subsequently, different lineages can interbreed in the next vector, as has been demonstrated experimentally for *Plasmodium* (see references in ref. 14). There are numerous cues that a parasite could use to gauge its allocation to the sexes of its gametocytes. Because younger birds have had less exposure time, they are more likely to carry fewer species of parasite (23, 24); hence, they may be more likely to carry fewer genetic lineages of individual parasite species. Thus, it might benefit parasites to produce a more female-biased gametocyte sex proportion inside younger hosts. However, in temperate systems, the proportion of vectors carrying haemosporidians increases through the breeding season (25–28). Because the young of most bird species hatch when parasite prevalence peaks in vector populations, we might instead expect younger birds to rapidly acquire numerous parasite lineages. This leads to the prediction that young birds will have more even gametocyte sex proportions than adults have. These competing hypotheses predict a relationship between host age and parasite sex proportion.

Over winter, birds in temperate zones are no longer exposed to *Haemoproteus* vectors. As host birds combat infections, they may eliminate some parasite lineages. Relapses of *Leucocytozoon* (29, 30) and *Haemoproteus* (27, 28, 31, 32) in adult hosts in spring enable parasites to infect insect vectors and subsequently to find young or new vertebrate hosts. Concomitantly, adults that had reduced their infections over winter to single-parasite lineages may become infected with new parasite lineages. This would lead us to expect that avian hosts early in the season would carry more female-biased gametocyte sex proportions than birds sampled later.

Another potential proximate cue that parasites could use is the sex of their avian host. Differences in biology (e.g., time spent incubating) may expose each host sex to conditions that differentially affect opportunities for infection, and sex-

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specific hormones could provide necessary cues to parasites for sex allocation (30).

The number of parasite lineages within a host is an ideal proximate cue for parasites seeking to optimize sex proportion of their progeny. If hosts carrying multiple parasite lineages develop more intense infections than when carrying single lineages (D.S., unpublished observations for *Leucocytozoon*), we would expect more even gametocyte sex proportions with high-intensity infections. On the other hand, if single lineages do not pay the costs of interlineage competition, they may be able to reach higher infection intensities than multiple, competing lineages are able to do (see ref. 33). These competing possibilities predict a relationship between infection intensity and parasite sex proportion.

Finally, prevalence of parasites within host populations could act as an ultimate cue indicative of outbreeding potential (17, 22). Five percent of the human hosts described by Read *et al.* (17) carried *Plasmodium* gametocytes in their blood, reducing the parasite's probability of outbreeding and providing a potential explanation for observed female-biased gametocyte sex proportions. In contrast, *Leucocytozoon* prevalence of 100% in ducklings (*Anas platyrhynchos* and *A. rubripes*) was associated with 46% male gametocytes. Additional observations (22) provided strong evidence that gametocyte sex proportions are less female-biased when parasite prevalence is high, despite substantial differences in biology of hosts, vectors, and parasites.

We examined gametocyte sex proportions of five species of *Haemoproteus* from 11 avian host populations. *Haemoproteus* is transmitted by ceratopogonid midges (20, 21). In captivity, ceratopogonids can live up to 35 days (34), but most undoubtedly survive for less time than this, and individual midges probably do not travel very far during their lives. If their feeding cycle is similar to that of *Simulium rugglesi* (black fly vector of *Leucocytozoon simondi*), individual midges surviving this long would feed a maximum of seven times. Thus, they have limited opportunity to transmit or obtain parasite lineages between hosts, and this would enhance the possibility of parasite inbreeding. We predicted that gametocyte sex proportions within each avian population would (*i*) vary with host age, (*ii*) become less female-biased later in their hosts' breeding season, (*iii*) vary with host sex, (*iv*) vary with host infection intensity, and (*v*) be less female-biased in host populations with higher *Haemoproteus* prevalence.

METHODS

From the blood smear collection of the International Reference Centre for Avian Haematozoa, we selected 11 avian populations from geographically separated locales (Table 1). Some populations were of the same host species, but distances between locales made it highly likely that these samples were independent. In some cases, data were gathered from populations that had been sampled for several years. For populations with sufficient data, we found no differences in population sex proportions between years (analyses of variance, all P values > 0.10). Hence, we pooled years for tests.

Prevalence (percentage of hosts in which parasites were detected) was the proportion of hosts that had *Haemoproteus* in blood smears (35). Prevalence data are published for some locales (25, 36–38). Sex proportions were computed from a minimum sample of 100 fully developed gametocytes from each avian host (sexing criteria are described in refs. 39 and 40). Gametocyte samples of 100 are only obtainable from relatively intense infections (41), so we were able to determine sex proportions only at infection intensities usually > 0.1 gametocyte per microscope field. Because of this and because we did not attempt to sex young parasites, samples used to obtain gametocyte sex proportions within a population are much smaller than samples used to obtain population infection prevalence.

Infection intensities were the number of parasites (at all stages of development) per 100 microscope fields. Intensities were measured under oil at ×1000 magnification with sections of smears with fairly uniform blood cell densities; each field may contain 100–150 blood and parasite cells. Variation in the density of blood cells in smears argues for caution in interpreting intensity data. Data analyses were performed on a microcomputer package (42).

RESULTS

Gametocyte sex proportions were normally distributed when hosts were pooled or considered independently; results were not affected by arcsine transformation. We compared gametocyte sex proportions in birds that were <1 year old with birds that were >1 year old. For three populations for which we had data, no significant differences emerged (Fig. 1). Hence, host age did not appear to influence gametocyte sex proportions.

We then tested whether gametocyte sex proportions became less female-biased later in the season. For seven host populations for which we had data, only the gametocyte sex proportion of Konnevesi pied flycatchers responded in this fashion (Table 2). Populations for which we had the largest samples (black-headed grosbeaks and great tits) had gametocyte sex proportions that did not change over the course of the season. Because the black-headed grosbeak population is nonmigratory and inhabits a less seasonal latitude than the other hosts, these conditions may alter aspects of parasite transmission and outbreeding dynamics (43, 44). We cannot make the same arguments for the great tit population because, although it is nonmigratory, it is not found in a nonseasonal habitat. Bivariate scatterplots of sex proportion versus Julian date for each species revealed no nonlinear relationships. In general, we do not have consistent evidence that gametocyte sex proportions

Table 1. Species of avian host with associated *Haemoproteus* parasite and population sources for data used in this study

Host species	*Haemoproteus* species	Locale	Latitude and longitude	Year(s)
White-throated sparrow, *Zonotrichia albicollis*	*coatneyi*	Algonquin Park	45°N 77°W	1958–59
		Tantramar	46°N 66°W	1972–74
American robin, *Turdus migratorius*	*fallisi*	Newfoundland	54°N 62°W	1969–72
		Tantramar	46°N 66°W	1972–74
Black-headed grosbeak, *Pheucticus melanocephalus*	*coatneyi*	New Mexico	35°N 105°W	1985
Pied flycatcher, *Ficedula hypoleuca*	*balmorali*	Uppsala	60°N 18°E	1989
		Konnevesi	63°N 26°E	1991–92
		Meltaus	67°N 25°E	1992
Great tit, *Parus major*	*majoris*	Gotland	57°N 19°E	1990–92
Barn swallow, *Hirundo rustica*	*prognei*	Kiev	51°N 31°E	1991
		Denmark	56°N 10°E	1992

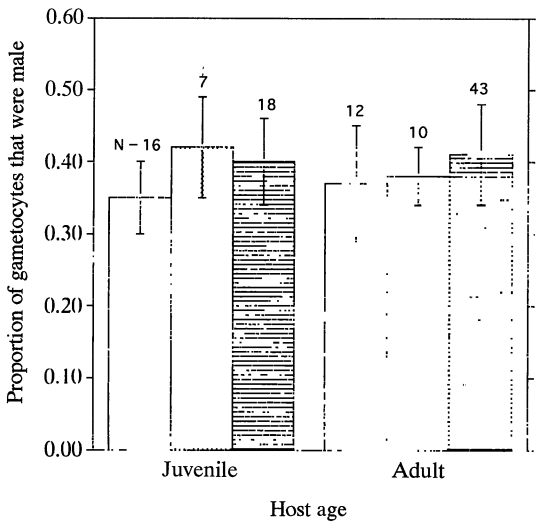

FIG. 1. Gametocyte sex proportions ± SD by host age for Uppsala pied flycatchers (open bars), Konnevesi pied flycatchers (stippled bars), and Gotland great tits (striped bars). Variances were homogeneous between age groups (Bartlett's tests; all P values > 0.20). Differences were not significant (two-tailed t-tests; all P values > 0.10).

become less female-biased as conditions for lineage mixing would appear to improve.

Because of differences in biology, we predicted different gametocyte sex proportions within male versus female avian hosts. Two of eight populations had gametocyte sex proportions that were significantly more female-biased in female than male birds (Table 3). Of these populations, Tantramar white-throated sparrows had the greatest skew in gametocyte sex proportions, but this was based on a sample of five individuals only. The other significant result was for Konnevesi pied flycatchers, but two other pied flycatcher populations had less female-biased sex proportions in female than in male hosts. In sum, two significant results do not appear to be general or consistent among or within species.

The fourth prediction we tested was that *Haemoproteus* sex proportions would vary with infection intensity. Infection intensities ranged from 0.10 to 45.00 gametocytes per field with >70% of the infections having <5.00 gametocytes per field. Infection data were logarithmically transformed (45, 46). Of nine populations, only Tantramar white-throated sparrows had gametocyte sex proportions that increased significantly with infection intensity (Table 4), and six populations had nonsignificant increases. Again, black-headed grosbeaks opposed this trend. For each host population, bivariate scatterplots suggested no nonlinear relationships between sex proportion and infection intensity. Overall, results suggest that *Haemoproteus* sex proportions are unrelated to the intensity of their host's infection.

Table 2. Relationship between Julian date and gametocyte sex proportions

Host species	Locale	n	Pearson r	One-tailed P
American robin	Newfoundland	8	−0.60	0.94
American robin	Tantramar	12	−0.08	0.90
Black-headed grosbeak	New Mexico	67	−0.04	0.87
Pied flycatcher	Konnevesi	17	0.56	0.01
Pied flycatcher	Meltaus	4	0.30	0.35
Great tit	Gotland	30	−0.08	0.84
Barn swallow	Kiev	5	0.05	0.47
Barn swallow	Denmark	8	0.49	0.11

Sample sizes (n) differ from those reported elsewhere because dates for some blood smears were unavailable.

Our final prediction was that gametocyte sex proportions would be less female-biased in host populations with higher *Haemoproteus* prevalence. Significant differences between average gametocyte sex proportions among avian host populations (Table 5) did not fit the prediction (Fig. 2). Furthermore, gametocyte sex proportions were not consistent among different host populations of the same species. The most conspicuous contradiction to our prediction was the black-headed grosbeak population, with 100% *Haemoproteus* prevalence and one of the most female-biased gametocyte sex proportions (Fig. 2, Table 5). In sum, *Haemoproteus* gametocyte sex proportions did not appear to be influenced by prevalence.

DISCUSSION

Adaptive adjustment of gametocyte sex proportion in a species requires genetic variation. This condition is met in *Plasmodium* because cultured clones differ in the gametocyte sex proportions they produce (17, 47). Potential for genetic variation in sex proportions is enhanced by the haploid life cycle of *Plasmodium* and therefore is not constrained by Mendelian segregation of sex chromosomes. Thus, it seems likely that sex proportions could adapt to local conditions in *Haemoproteus*, at least in the long term.

Haemoproteus gametocyte sex proportions were more or less invariant with respect to variables we measured. Perhaps there is no relationship between the proximate variables we chose and sex proportion because the variables are unreliable predictors of outbreeding potential or because *Haemoproteus* does not have sensory or behavioral apparatus necessary to respond to proximate cues. We begin with the former possibility. First, although prevalence would seem to be a likely predictor of outbreeding, Julian date does not consistently predict prevalence (23, 24), possibly because of climatic influences on vector emergence dates. Second, flies may have no preference for the

Table 3. Effect of sex of host on gametocyte sex proportions

Host species	Locale	Gametocyte sex proportion (n), mean ± SD: Male hosts	Gametocyte sex proportion (n), mean ± SD: Female hosts	t	Two-tailed P
White-throated sparrow	Tantramar	0.19 ± 0.02 (2)	0.49 ± 0.03 (3)	−12.38	0.001
American robin	Tantramar	0.30 ± 0.07 (5)	0.30 ± 0.06 (7)	0.02	0.98
Black-headed grosbeak	New Mexico	0.33 ± 0.06 (39)	0.35 ± 0.06 (28)	−1.03	0.31
Pied flycatcher	Uppsala	0.40 ± 0.07 (5)	0.35 ± 0.09 (7)	1.01	0.34
Pied flycatcher	Konnevesi	0.37 ± 0.04 (10)	0.44 ± 0.05 (7)	−3.02	0.009
Pied flycatcher	Meltaus	0.42 ± 0.07 (9)	0.36 ± 0.01 (2)	1.20	0.26
Great tit	Gotland	0.41 ± 0.07 (29)	0.40 ± 0.07 (24)	0.66	0.51
Barn swallow	Denmark	0.34 ± 0.04 (6)	0.37 ± 0.08 (2)	−0.67	0.53

Sample sizes (n) may vary from those reported elsewhere because the sex of some hosts was not determined. For each species, variances were homogeneous between sexes (Bartlett's tests; all P values > 0.08).

Table 4. Effect of intensity of host infection (number of parasites per 100 microscope fields at ×100 magnification) on parasite gametocyte sex proportion

Host species	Locale	*n*	Pearson *r*	Two-tailed *P*
White-throated sparrow	Tantramar	16	0.56	0.03
American robin	Tantramar	12	0.13	0.81
Black-headed grosbeak	New Mexico	67	−0.02	0.86
Pied flycatcher	Uppsala	28	0.14	0.48
Pied flycatcher	Konnevesi	17	0.37	0.14
Pied flycatcher	Meltaus	11	−0.09	0.80
Great tit	Gotland	53	0.11	0.45
Barn swallow	Kiev	5	0.13	0.83
Barn swallow	Denmark	8	0.35	0.40

Infection intensities were logarithmically transformed. *n*, Sample size.

sex of the host they feed from in successive meals. Hence, parasites would not be able to use these two cues to predict outbreeding potential. Also, we detected no relationship between host infection intensity and gametocyte sex proportion. This suggests that infection intensity is unrelated to the number of parasite lineages infecting a vertebrate host or that *Haemoproteus* cannot respond to this cue. With regard to this latter possibility, Read *et al.* (17) also failed to find any sex proportion adjustment by *Plasmodium* to proximate cues. This would mean that any adaptive variation in sex proportion would be the outcome of long-term rather than dynamic influences.

The long-term cue we measured was prevalence. However, *Haemoproteus* prevalence within populations can vary substantially from year to year [e.g., in one locality in insular Newfoundland, *Haemoproteus* prevalence ranged from 26% to 56% in a 4-year study (36)]. This variation could easily arise because of variation in the density of vectors and suitable hosts. Density of suitable hosts may vary in a complex fashion because each *Haemoproteus* species can infect multiple host species (19, 20). For example *Haemoproteus fallisi* can infect five bird species in Newfoundland (36). Moreover, prevalence can vary substantially at sites located a few hundred kilometers apart (27, 36, 48). In short, there is substantial uncertainty associated with prevalence, and this may result in long-term sex proportion optima that are suboptimal in the short term or may result in sex proportions that are unable to change fast enough to keep up with environmental optima. Nonetheless, we would expect long-term optima to differ between different locales and, as a result, differences between gametocyte sex proportions. However, we observed only minor variation in gametocyte sex proportions at different locales, and no clear adaptive explanation emerged for the few differences we did observe. If the similar sex proportions we observed in our *Haemoproteus* samples reflect adaptive optima for outbreeding, this would suggest that outbreeding occurs equally often regardless of prevalence. This could occur if multiple genetic lineages of *Haemoproteus* are more common within hosts than would be expected by chance in populations with low parasite prevalence. A test of this idea awaits genetic analyses.

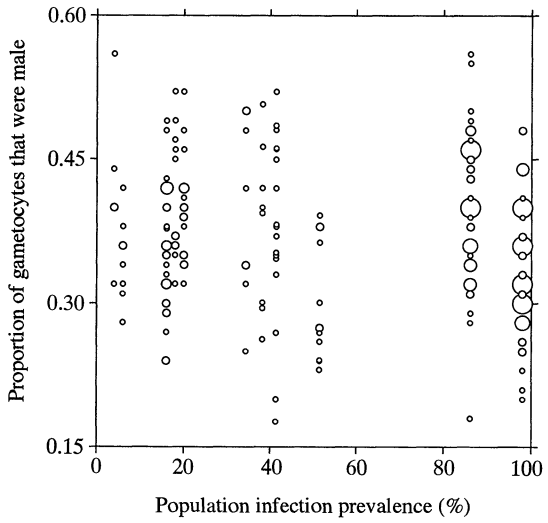

FIG. 2. Gametocyte sex proportions relative to prevalence (%) of infected hosts within the population. Statistics are given in Table 5. Circles in order of increasing size represent one, two, three, four, five, and six or more (maximum nine) overlapping points.

Another potentially important influence on sex proportions that we have not considered is different costs for each sex of offspring (5). All else being equal, a mother cell (schizont) should invest equally in each sex of offspring (her merozoites). Larger, male merozoites could select for female-biased sex proportions. However, no morphological differences have been detected that allow one to determine the ultimate sex of a merozoite. Although *Haemoproteus* microgametocytes of various avian host species are 10% smaller than macrogametocytes (40, 49, 50), it is unlikely that gametocyte sizes are any indication of initial parental investment. At any rate, these differences should produce male-biased sex proportions. In fact, we observed female-biased sex proportions for each *Haemoproteus* population we sampled, as has been reported

Table 5. Relationship between prevalence of infected hosts and gametocyte sex proportion

Host species	Locale	Hosts sampled, no.	Hosts with parasites (prevalence), %	Hosts with intense infections, no.	Proportion of male gametocytes, mean ± SD
American robin	Tantramar	130	51.5	12	0.30 ± 0.06 A
Black-headed grosbeak	New Mexico	100	100.0	67	0.34 ± 0.06 B
Barn swallow	Denmark	243	4.9	8	0.35 ± 0.04
Pied flycatcher	Uppsala	87	17.2	28	0.36 ± 0.07
White-throated sparrow	Tantramar	210	41.4	17	0.38 ± 0.10
American robin	Newfoundland	199	38.2	8	0.38 ± 0.09
White-throated sparrow	Algonquin	423	34.3	8	0.39 ± 0.10
Pied flycatcher	Konnevesi	559	18.8	17	0.40 ± 0.05 C, D
Great tit	Gotland	403	86.1	53	0.41 ± 0.07 C, D
Pied flycatcher	Meltaus	141	18.4	11	0.41 ± 0.07 C, D
Barn swallow	Kiev	137	4.4	5	0.42 ± 0.09 C

Variances were homogeneous between populations (Bartlett test, $\chi^2 = 14.5, P = 0.15$). The mean gametocyte sex proportions differed significantly (Tukey test, $P < 0.05$) between the population labeled A and populations labeled C and between the population labeled B and populations labeled D. See Fig. 2.

for various populations and species of *Plasmodium* (11, 17, 41, 47, 51, 52).

It is significant that all of our sex proportions were female-biased. This could occur because sex proportions are somehow genetically fixed for the genus. Higher mortality of male gametocytes could also skew sex proportion at maturity. Or, as suggested above, the number of *Haemoproteus* lineages within hosts is constant for each host population sampled, and local mate competition selects for female-biased sex proportions. We have no data to test or eliminate any of these hypotheses. On the other hand, we have no direct evidence for local mate competition in *Haemoproteus.* Local mate competition theory may be a useful predictor of gametocyte sex proportion for *Plasmodium* and *Leucocytozoon* (11, 17, 18, 22), and sex proportion may be an important indicator of the frequency of selfing, but we have no evidence that sex proportion variation in *Haemoproteus* is clearly adaptive. Although sex proportions may be diagnostic of outbreeding frequency in some parasite taxa, each may have to be considered separately.

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