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Short Communication

Double gametocyte infections in apicomplexan parasites of birds and reptiles

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Abstract The simultaneous occurrence of male and female gametocytes inside a single host blood cell has been suggested to enhance apicomplexan transmission ["double gametocyte infection (DGI) hypothesis"]. We did a bibliographic search and a direct screen of blood smears from wild birds and reptiles to answer, for the first time, how common are these infections in the wild. Taking these two approaches together, we report here cases of DGIs in *Plasmodium*, *Haemoproteus*, *Leucocytozoon* and *Hepatozoon*, and cases of male–female DGIs in *Haemoproteus* of birds and reptiles and in *Leucocytozoon*

of birds. Thus, we suggest that DGIs and male–female DGIs are more widespread than previously thought, opening a new research avenue on apicomplexan transmission.

In *Plasmodium* and related apicomplexan parasites, the occurrence of two or more gametocytes inside a single host blood cell recently attracted the attention of researchers for their potential to enhance parasite transmission between vertebrate hosts (Jovani 2002). In these microparasites, transmission occurs when the gametocytes contained in the host blood cells are ingested within the bloodmeal of a suitable vector. Once in the vector, male and female gametes differentiate from male and female gametocytes, respectively, and at least one of the mobile male gametes must search for and fertilize one of the immobile female gametes contained in the same bloodmeal. Gametocytes are normally alone within single host blood cells (e.g. erythrocytes), but they are also found in couples (double gametocyte infections, DGIs) or in trios (TGIs) within a given blood cell.

Female gametes are very small compared with the bloodmeal volume ingested by the vector; and male gametes do not seem to have any searching mechanism to find the female gamete (Gaillarg et al. 2003). Thus, it seems logical that some mechanism operating while in the vertebrate host could have evolved to increase the proximity of gametocytes and thus gametes when on the bloodmeal; and two non-mutually exclusive hypotheses have been proposed. Jovani (2002) proposed that male–female DGIs (i.e. one blood cell infected by one male and one female gametocyte) might reduce the time needed for male gametes to encounter female gametes, facilitating parasite transmission between vertebrate hosts. Gaillard et al. (2003) recently proposed that erythrocytes infected by gametocytes could adhere among them in peripheral host capillaries, enhancing the probability of gamete encounter once in the vector bloodmeal.

The hypothesis proposed by Gaillard et al. (2003) agrees with the discovered pattern of aggregated distribution of erythrocytes infected by malaria gametocytes, both in human blood (Gaillard et al. 2003) and in the vector bloodmeal (Pichon et al. 2000). The "DGI hypothesis" relies on the assumption that DGIs, and especially male–female DGIs, are widespread in nature, but this is currently unconfirmed (Jovani 2002). The goal of this note is to show that DGIs are widespread among in vivo apicomplexan parasites of wild populations of birds and reptiles. Moreover, we also report the occurrence of other multiple gametocyte infections (MGIs, i.e. more than one gametocyte inside a single erythrocyte), because they inform us that the needed process to produce a DGI does occur in a given host–parasite system, i.e. the entrance and growth into a given erythrocyte of more than one gametocyte.

We first systematically searched in the parasite literature for cases of MGIs. Information regarding this kind of infection is little likely to appear in the title, abstract, or keywords of a publication. Consequently, we were forced to search our files for papers on apicomplexan infections; and then we accurately revised each paper for any mention of MGIs. Our survey found any reference on the possible relevance of MGIs for any aspect of the biology of the parasite. Indeed, some papers even contained photographs of DGIs, but without any mention in the text (e.g. Mushi et al. 1999). Despite this evident lack of attention of researchers for DGIs and MGIs as a whole, we found eight works with information on DGIs and TGIs (Table 1).

Table 1 Some examples of published data on the occurrence of multiple gametocyte infections in apicomplexan parasites. *DGI* Double gametocyte infection

Host	Parasite	Observation	Reference
Columba livia	*Haemoproteus columbae*	DGIs in 17 of 30 host individuals infected, with 14–97 DGIs in each individual host	Graczyk et al. (*1994*)
	H. columbae	A photograph of a male–female DGI	Bennett and Peirce (*1990*)
	H. columbae	A photograph of a male–female DGI, a photograph of a TGI	Mushi et al. (*1999*)
	Haemoproteus spp	A photograph of a female–female DGI	Tsai et al. (*1986*)
Geochelone denticulate	*H. geochelonis*	Drawings and photographs of male–female DGIs; text: "quite common" occurrence of DGIs	Lainson and Naiff (*1998*)
Birds	*Haemoproteus*, *Plasmodium*, *Leucocytozoon* spp	Drawings of: immature DGIs from 20 *Haemoproteus* spp, one immature TGI in *H. kairullaevi*, one male–female DGI in *H. bennetti*, one male–male DGI in *H. ortalidum*, two immature DGIs in *P. octamerium* and *P. columbae*, one immature DGI and one male–female DGI in *L. caulleryi*	Valkiunas (*1997*)
Caiman c. crocodiles	*Hepatozoon caimani*	Photographs of DGIs and TGIs, sex unknown	Lainson et al. (*2003*)
Homalopsis buccata	*Hepatozoon* spp	Photograph of a DGI, sex unknown	Salakij et al. (*2002*)

Moreover, from our own blood smear collection, we selected those blood samples of 11 bird and four reptile species infected by some apicomplexan parasites. These blood smears were collected from wild hosts in Germany and Spain during the period 1996–2002 (see Table 1). On each smear, we actively searched for MGIs on a portion equivalent to ca. 2,000 erythrocytes for *Haemoproteus* and *Hepatozoon* infections and ca. 25,000 erythrocytes for *Leucocytozoon* infections, recording when possible the sex for mature gametocytes involved in a MGI. MGIs were found in most of the parasite–host associations we examined (Table 2).

Table 2 Occurrence of DGIs, male–female (m-f)DGIs, and triple gametocyte infections (TGIs) in blood smears inspected for this study. *N* Number of infected individual hosts inspected, or number of individual hosts with at least one

D/TGI. Numbers in parentheses indicate the number of D/TGIs in each infected host. – Sex of parasite could not be determined

Host	Country	*N*	*N* DGIs	*N* m-f DGIs	*N* TGIs
Haemoproteus parasites					
Buteo buteo	Germany	8	3 (1, 1, 2)	0	0
Falco tinnunculus	Germany	3	0	–	0
F. subbuteo	Germany	7	0	–	0
Tyto alba	Spain	4	1 (3)	0	0
Bubo bubo	Spain	21	3 (1, 2, 2)	0	0
Strix aluco	Germany, Spain	21	10 (1, 2, 2, 2, 3, 4, 10, 11, 13, 27)	1 (1)	4 (1, 1, 3, 15)
Parus caeruleus	Spain	46	10 (1, 1, 1, 1, 1, 2, 2, 3, 4, 6)	0	0
Delichon urbica	Spain	57	7 (1, 1, 1, 1, 1, 1, 2)	1 (1)	0
Lanius collurio	Spain	11	0	–	1 (1)
Passer domesticus	Spain	39	11 (1, 1, 1, 1, 2, 2, 2, 2, 2, 5, 6)	4 (1, 1, 1, 1)	0
Leucocytozoon parasites					
B. bubo	Spain	19	0	–	0
Athene noctua	Spain	2	0	–	0
Strix aluco	Spain	5	0	–	0
Parus caeruleus	Spain	15	2 (1, 2)	–	0
Hepatozoon parasites					
P. caeruleus	Spain	9	0	–	0
Lacerta lepida	Spain	38	0	–	0
L. monticola	Spain	105	5 (1, 1, 1, 2, 3)	–	0
Podarcis muralis	Spain	28	0	–	0
P. hispanica	Spain	19	0	–	0

Moreover, from a parasite survey conducted by Shurulinkov and Golemansky (2002, 2003), P.S. revised their own notes taken during blood smear inspection in a search for records of MGIs. Although not comparable with the other samples because it was not a systematic search for MGIs, it is of qualitative importance and also denotes the low reporting of these infections in publications, although present in the researchers' notes. A total of 15 DGIs were recorded from 11 birds infected by *Haemoproteus* of five bird–parasite assemblages (bird–parasite [number of birds with DGIs, total number of DGIs]: *Sylvia borin–H. belopolskyi* [1, 3], *Lanius minor–H. lanii* [2, 2], *Lanius collurio–H. lanii* [2, 2], *Acrocephalus arundinaceus–H. payevskyi* [2, 3], *Muscicapa striata–H. pallidus* [4, 5]). Most of these DGIs were constituted by female gametocytes. However, *H. payevskyi* infecting *A. arundinaceus* showed a case of a male–female DGI. Moreover, two cases of DGIs were recorded in *Plasmodium polare* infecting one individual of *Hirundo rustica*. See Shurulinkov and Golemansky (2002, 2003) for the total numbers of species and individuals examined.

Our results support the view that DGIs are widespread in a variety of host–parasite associations, including *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* infections of birds and *Haemoproteus* and *Hepatozoon* parasites of reptiles. Moreover, we found male–female DGIs in *Haemoproteus* parasites of birds and reptiles and in *Leucocytozoon* of birds, validating the major assumption of the “DGI hypothesis”. The widespread occurrence of DGIs, and particularly of male-female DGIs, opens a promising direction for future research on the transmission strategies of apicomplexan parasites.

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