Diurnal cycle of Isospora spp. oocyst shedding in Eurasian blackbirds (Turdus merula)

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Abstract: Diurnal fluctuations in the appearance of parasites have been recognized for more than 60 years but have been largely ignored in studies examining the role of parasites in connection with evolutionary aspects of behaviour, ecology, and population dynamics. The disregard of diurnal fluctuations, however, can influence the reliability and interpretation of data. I examined shedding of Isospora spp. oocysts in faeces of naturally infected, free-living Eurasian blackbirds (Turdus merula L., 1758). Adult birds and nestlings shed coccidian oocysts (Isospora spp.) predominantly in the afternoon. The results are in agreement with earlier studies on coccidian oocyst shedding in other bird species. They are discussed with regard to these studies and to practical implications for future investigators in this field.

Introduction

With their influential paper, Hamilton and Zuk (1982) brought parasites into the centre of discussion about sexual selection. They proposed that animals should choose mates for heritable disease resistance by scrutiny of characters whose full expression is dependent on health and vigour. Infections with blood parasites were used as health parameters, with the consequence that protozoan blood parasites became a favourite group for testing the Hamilton–Zuk hypothesis. Weatherhead and Bennett (1991) stated that this was done "largely in ignorance of the natural history of the parasites involved". As a consequence, tests of the predictions were based on bold assumptions, resulting in critical discussions (Endler and Lyles 1989; Moller 1990; Clayton 1991; Weatherhead and Bennett 1991, 1992). Specifically, the assumption that a bird's parasite burden could be reliably determined by the examination of a single blood smear was repeatedly criticized (Cox 1989; Pruett-Jones et al. 1991; Weatherhead and Bennett 1991, 1992; Cooper and Anwar 2001). These criticisms addressed the disregard of annual (Weatherhead and Bennett 1991, 1992; Sanz et al. 2002), seasonal (Weatherhead and Bennett 1991, 1992; Allander and Sundberg 1997; Hatchwell et al. 2000), and geographical (Merilä et al. 1995) sources of variation in parasite prevalence and intensity. Less attention, however, has been paid to shorter periodic phenomena (e.g., daily or diurnal periodicities). One such neglected phenomenon is the diurnal shedding of coccidian oocysts in the faeces of host birds. The most commonly used indirect method for the diagnosis of gastrointestinal parasitic infection is the examination of faecal material for parasitic stages (oocysts, eggs, larvae). However, if the shedding of parasitic stages underlies diurnal fluctuations, as has been demonstrated for oocysts of coccidians in sparrows (Boughton 1933; Kruszewicz 1995), house finches (Carpodacus mexicanus (Muller, 1776); Brawner and Hill 1999), pigeons (Boughton 1937), and dark-eyed juncos (Junco hyemalis (L., 1758); Hudman et al. 2000), then the likelihood of detecting an infection or the measurement of infection abundance will vary with collection time and may thus result in distorted estimates of infection frequencies and intensities. Consequently, diurnal fluctuations may play a role in all studies in which behavioural, ecological, and evolutionary aspects of parasites and their hosts are examined, namely at the levels of individuals, populations, and species. For this reason, it is surprising that the importance of considering diurnal fluctuations has only recently received attention (Brawner and Hill 1999; Hudman et al. 2000; Cooper and Anwar 2001).

In this study I examine shedding of Isospora spp. oocysts in faeces of naturally infected, free-living adults and nestlings of Eurasian blackbirds (Turdus merula L., 1758). I show that there is a diurnal periodicity of oocyst shedding...
from undisturbed, free-ranging adult birds, which has so far been shown only for dark-eyed juncos (Hudman et al. 2000). Furthermore, I show that the same diurnal periodicity in oocyst shedding is found in nestlings, which were trapped on nests. With this study I want to confirm the results of other studies and provide further evidence that the phenomena of diurnal oocyst shedding actually exists in nature and not only in the laboratory. Additionally, I want to draw attention to the risks of hasty interpretations of parasitic assays.

Methods

This study was part of an ongoing survey (1995–2000) of a free-ranging, color-banded population of Eurasian blackbirds in the Botanical Garden of Bonn (Germany). Faecal samples from adult birds were collected in 1996 (May–June), 1998 (May–August), and 2000 (June–July). They were collected by an observational method that did not require capturing of birds. Birds were observed in activities that take place preferentially on the ground (feeding, collecting nesting material, collecting food for nestlings) and were followed until they defaecated. Faeces were collected only if the source of the sample could be determined unambiguously. For 19 adult individuals, one or more samples were collected both between 0700 and 1200 (morning samples) and between 1200 and 1800 (afternoon samples). If no oocysts were detected in both the morning and afternoon samples, the respective bird was declared noninfected.

During the survey, most nestlings were weighed, measured (tarsus length, bill length, and wing length), and banded at the age of 8 to 10 days. From April to July of the years 1996, 1997, and 1998, one faecal sample from each nestling was collected when possible. Morning samples were obtained from 38 nestlings and afternoon samples from 150 nestlings. Because the sample sizes of morning and afternoon samples were so different, an additional, smaller data set was created: each of the 38 morning samples was paired with one afternoon sample with a similar collection date. Of course, the selection occurred with no knowledge of the infection status of the nestlings. Thus, a reduced data set with 76 independent samples from 76 nestlings was created, with 38 samples collected in the morning and 38 collected in the afternoon.

Faecal samples were stored at 4 °C in 2% potassium bichromate and examined using a flotation method (Bürger and Stoye 1983). After removal of large particles (seeds, grass, pebbles), samples were centrifuged for 10 min at 300g. The supernatant was removed and pellets were weighed to the nearest 0.01 mg. Each pellet was suspended in 6 mL of flotation medium (saturated NaCl–ZnCl₂ solution) with careful mixing to avoid production of air bubbles. Four compartments of McMaster counting chambers, each with a volume of 0.15 mL, were then filled. Each chamber was viewed under a microscope, the floating oocysts were counted, and a mean oocyst count per sample was calculated. This mean oocyst count was divided by the mass of the faecal sample to obtain standardized counts.

The parasites of interest, Isospora spp., belong to the family Eimeriidae. They undergo an alternating cycle of sexual and asexual reproduction. In most species the asexual phase occurs in the intestinal epithelium of the host, where one or more asexual cycles are completed before sexual development occurs (Olsen 1974). Oocysts, the products of the sexual phase, are released into the host’s intestinal tract and passed in faeces. Under the proper humidity, temperature, and oxygen conditions, oocysts sporulate and are infective upon ingestion by a host.

Parasite counts, especially microparasite counts, have a highly variable distribution in the host population (Goater and Holmes 1997). Therefore, data were analyzed with two-tailed nonparametric tests and α was set at 0.05.

Results and discussion

Ninety percent of adult birds (17 of 19) were infected with Isospora spp. In eight birds, oocysts were found in both morning and afternoon samples; in another eight birds, oocysts were found only in afternoon samples; and in a single bird, oocysts were found only in the morning sample. Thus, it was significantly more likely that oocysts would be detected in afternoon samples than in morning samples (McNemar test; df = 1, p = 0.039, n = 19). In nestlings, a similar distribution of apparently infected and noninfected birds was found. Thus, in both data sets, oocysts were more likely to be found in samples collected in the afternoon. If the complete data set was considered, oocysts were detected in 65% of afternoon samples (98 of 150) but in only 37% of morning samples (14 of 38). In the reduced (balanced) data set, 63% of afternoon samples contained isosporan oocysts (24 of 38), but only 37% of morning samples contained oocysts (14 of 38). In both cases, the difference was significant (G tests, df = 1: complete data set, G = 10.063, p = 0.002, n = 188; reduced data set, G = 5.326, p = 0.021, n = 76).

Oocyst counts were significantly different between morning and afternoon samples for both adult birds and nestlings. In both cases, mean oocyst counts in morning samples were significantly lower than counts in afternoon samples (Table 1, Fig. 1).

The greater likelihood of finding isosporan oocysts in afternoon samples (presence/absence data) and the higher oocyst counts in afternoon samples (abundance data) are in accordance with earlier studies of the diurnal excretion of Isospora spp. oocysts by dark-eyed juncos (Hudman et al. 2000), house finches (Brawner and Hill 1999), house sparrows (Passer domesticus (L., 1758)) (Boughton 1933; Kruszewicz 1995), and Eurasian tree sparrows (Passer montanus (L., 1758)) (Kruszewicz 1995). In his seminal study, Boughton (1933) reported that in house sparrows, the largest quantity of oocysts was found in faecal droppings collected from 1400 to 2000. He also sporadically examined various bird species from Milwaukee County Zoological Gardens (Boughton 1933) and found that in most bird groups, a higher percentage of Isospora spp. oocysts was shed in the afternoon. Similarly, Brawner and Hill (1999) observed that house finches shed more oocysts at 1600 and 2000 than at 0800 and 1200. Kruszewicz (1995) found isosporan oocysts in faeces of Eurasian tree sparrows between 1000 and 1800 only, and he found that the oocyst output
from house sparrows started at about 1400 and finished at midnight. Hudman et al. (2000) collected faeces from dark-eyed juncos both between 1500 and 0500 and between 0500 and 1200. Birds were kept in cages only for the collection of faeces and were otherwise free ranging. In agreement with the findings from laboratory settings, Hudman et al. (2000) were more likely to detect isosporan oocysts in faeces produced at night than in those produced during the day. The results of my study of adult free-ranging Eurasian blackbirds thus confirm that this diurnal periodicity, with oocysts being shed predominantly in the afternoon, exists in nature and not just in the laboratory. My finding that Eurasian blackbird nestlings shed oocysts more frequently and at higher abundance in the afternoon corroborates the findings of Kruszewicz (1995), who artificially infected nine house sparrow nestlings and eight Eurasian tree sparrow nestlings with isosporan oocysts. In his study, almost no oocysts were excreted in the morning hours, and the highest oocyst production took place between 1800 and 2200. Because nestlings in my study were naturally infected and trapped on nests, my results further indicate that the same pattern of diurnal oocyst shedding can be observed in nature.

The importance of considering large-scale temporal phenomena such as seasonal and annual fluctuations in infection prevalence and abundance has repeatedly been emphasized and critically discussed (Weatherhead and Bennett 1991, 1992; Allander and Bennett 1994; Allander and Sundberg 1997; Hatchwell et al. 2000; Sanz et al. 2002). The importance of diurnal phenomena, however, has only recently been addressed. Cooper and Anwar (2001) mention the possibility of failing to detect infections by blood parasites in a blood smear because of periodicities. Hudman et al. (2000), in their study of dark-eyed juncos, take account of the diurnal periodicity in the shedding of isosporan oocysts and recommend that future studies of coccidia focus on samples collected at night. Brawner and Hill (1999) devoted a whole paper to this subject. I emphatically agree that diurnal periodicities must be taken into account in studies on behavioural, ecological, and evolutionary aspects of parasite–host associations. The results of my study and that of Brawner and Hill (1999) show that the time of day at which samples are collected can have a significant effect on the reliability and validity of data. Like their findings for house finches, my results for Eurasian blackbirds indicate that it is unsuitable to collect faecal samples in the morning hours if Isospora sp. is the organism of interest. However, this is not a general pattern. Boughton (1937) found that oocysts of an

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**Table 1.** Comparison of oocyst counts from faeces of Eurasian blackbirds (*Turdus merula*) collected in the morning and in the afternoon.

<table>
<thead>
<tr>
<th></th>
<th>Morning samples</th>
<th>Afternoon samples</th>
<th>Z</th>
<th>p</th>
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<tr>
<td><strong>Adult birds</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>19</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>1.1</td>
<td>55.7</td>
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<td></td>
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<tr>
<td>25th percentile</td>
<td>0.0</td>
<td>30.6</td>
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<td></td>
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<tr>
<td>75th percentile</td>
<td>21.3</td>
<td>142.1</td>
<td></td>
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<tr>
<td>Wilcoxon’s test</td>
<td>–2.533</td>
<td>0.011</td>
<td></td>
<td></td>
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<tr>
<td><strong>Nestlings (full data set)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>38</td>
<td>144</td>
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<tr>
<td>Median</td>
<td>0.0</td>
<td>20.2</td>
<td></td>
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<tr>
<td>25th percentile</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>75th percentile</td>
<td>6.1</td>
<td>189.4</td>
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<tr>
<td>Mann–Whitney U test</td>
<td>–4.005</td>
<td>&lt;0.001</td>
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<td><strong>Nestlings (reduced data set)</strong></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>N</td>
<td>38</td>
<td>38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>0.0</td>
<td>24.2</td>
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<tr>
<td>25th percentile</td>
<td>0.0</td>
<td>0.0</td>
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<td>75th percentile</td>
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<td>163.4</td>
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<tr>
<td>Mann–Whitney U test</td>
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<td>0.002</td>
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</table>
unspecified species of the genus *Eimeria* were shed predominantly in the morning hours. Thus, as Brawner and Hill (1999) pointed out, a major step prior to any study involving parasites should be determination of the most consistent method for assessing parasite prevalence or abundance. First, a technically reliable method for detection of infections or measurement of parasite abundance in a given sample has to be developed. Then (at least in a preliminary study), repeated sampling from the same individuals within short time periods should be conducted. If this repeated sampling does not lead to repeatable results, one can assume that periodicity underlies the shedding of oocysts. In contrast to annual or seasonal periodicities, which probably represent "real" fluctuations in parasite prevalence or abundance (e.g., seasonal phenomena known as "spring rise" or "autumn rise"; Greve 1985; Atkinson and van Riper 1991), diurnal periodic phenomena, such as the one described here, probably represent fluctuations in the detectability of parasites rather than fluctuations in parasite prevalence or abundance. Cooper and Anwar (2001), relating to studies of blood parasites, pointed out that the failure to find parasites does not necessarily indicate that they are absent. Unfortunately, almost nothing is known about the underlying physiological, anatomical, or developmental mechanisms leading to a diurnal periodicity of oocyst shedding and, thus, oocyst detectability. Experiments carried out by Boughton (1933, 1988) suggest that the periodicity is dependent on the host's hours of activity and rest. However, the physiological changes in the host that trigger periodic oocyst shedding and the consequent advantages for the parasite or the host are still unclear. Furthermore, the anatomy of the host's digestive tract may play a role in the shedding periodicity. In birds, caeca, if present, are equipped with a sphincter and are evacuated separately from the rectum. The caecal evacuation rhythm ranges from 1:7 to 1:12 (ratio of caecal to rectal evacuations; Duke 1986). Eurasian blackbirds have paired caeca (McLelland 1979) and it is known that isosporans also parasitize caecal epithelial cells (Boughton 1930). Coccidians colonizing caeca would thus be dependent on the caecal evacuation rhythm to shed oocysts with faeces.

The diurnal shedding of oocysts is not the only periodic phenomenon in coccidians that can result in variable time-dependent probabilities of detecting infections or measurements of oocyst abundance. In *Eimeria* spp., oocysts from a single infection are shed over several days. The number of shed oocysts starts low, increases to a maximum, and then decreases until the infection has run its course (Fuller et al. 1995). Furthermore, several infections can occur simultaneously. Thus, an observed oocyst shedding pattern may be the product of several different simultaneously occurring periodic phenomena. In view of this potential complexity, my recommendations for future investigators are to (i) focus analyses on presence/absence data because such data are less likely to lead to distorted results; (ii) collect several samples from each individual within short time periods; and (iii) collect samples at an appropriate time, when oocyst shedding is expected to be at its peak. I believe that these considerations are also valid for other parasite–host systems, since high variability in repeated measurements of apparent parasite presence or abundance is not an exclusive phenomenon of protozoan parasites (Doster and Goater 1997).

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### References


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