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ARE MULTIPLE INFECTIONS MORE SEVERE FOR PURPLE MARTINS (*PROGNE SUBIS*) THAN SINGLE INFECTIONS?

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Abstract.—Between 1986 and 1993, we studied a breeding colony of Purple Martins (*Progne subis*) in Maryland that were chronically infected with two blood parasites: *Haemoproteus prognei*, a haematozoan, and an unidentified filarial nematode. We assessed whether cross-infections are more severe than single infections by comparing the return rates of birds infected with either or both parasites and the return rates of uninfected controls. Birds were captured every year and banded, and blood smears were taken for parasite screening. Average prevalence of filaria among the 400 birds screened during this period was 22%. Birds usually became infected by their second year (SY), and infected SY birds had significantly lower return rates than older birds. Cross-infections were rare (8%) and were fatal in 90% of cases. With one exception, birds infected with *H. prognei* acquired filaria as a second infection, which suggests that although both blood parasites are relatively benign for older Purple Martins, co-infection with a second parasite (in this case, *H. prognei*) almost always results in death. *Received 1 May 2003, accepted 26 June 2005.*

Key words: avian blood parasites, cross-infections, disease severity, *Haemoproteus prognei*, *Progne subis*, Purple Martins.

Est-ce que les Infections Multiples Sont Plus Sévères que les Infections Simples chez *Progne subis*?

Résumé.—Entre 1986 et 1993, nous avons étudié une colonie reproductrice de *Progne subis* du Maryland qui a été infectée de manière chronique par deux parasites du sang : *Haemoproteus prognei*, un hématozoaire, et un nématode filaire non identifié. Nous avons évalué si les infections multiples sont plus sévères que les infections simples en comparant le taux de retour des oiseaux infectés avec chacun des parasites ou les deux et le taux de retour de témoins non infectés. Les oiseaux étaient capturés chaque année et bagués, et des frottis sanguins ont été prélevés pour un dépistage parasitaire. La prévalence moyenne des filaires parmi les 400 oiseaux dépistés au cours de cette période était de 22%. Les oiseaux devenaient habituellement infectés au cours de leur seconde année (SA), et les oiseaux SA infectés ont eu un taux de retour significativement plus faible que les plus vieux oiseaux. Les infections multiples étaient plutôt rares (8%) et étaient fatales dans 90% des cas. Avec une exception, les oiseaux infectés avec *H. prognei* ont été infectés une seconde fois par un filaire, ce qui suggère que malgér le fait que les deux parasites soient relativement bénins chez les plus vieux *Progne subis*, la co-infection par un second parasite (dans ce cas, *H. prognei*) conduit presque toujours dans la mort.

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IDENTIFYING SOURCES OF parasite-mediated selection on host traits is important for evaluating the effects of parasites on host fitness. Arthropod-transmitted blood parasites are common in birds, and haematozoan parasites occur in a wide variety of bird families (Bennett et al. 1982, 1988). Whereas some parasitic infections are virulent, others are relatively benign (Bennett et al. 1993, Davidar and Morton 1993, Wagner et al. 1997). Effects of parasites on host populations can also differ, depending on host age and sex (Thomas et al. 1995, Moore and Wilson 2002). Although birds suffering from haematozoan infections can recover and become resistant to further re-infections through acquired immunity (Ahmed and Mohammed 1978, Atkinson 1991, Atkinson and Van Riper 1991, Yorinks and Atkinson 2000, Atkinson et al. 2001), parasites, such as *Plasmodium*, can remain in the host tissues or blood for a long time. During periods of stress, these latent infections can re-occur (Applegate and Beaudoin 1970). Although infection with one parasite can provide cross-immunity against other parasites (Cohen 1973, Nacher et al. 2000), malaria–filaria co-infections can be more severe than single infections (Nacher et al. 2001, Spiegel et al. 2003, Graham et al. 2005). Free-living animals are known to have multiple infections (Schmid-Hempel 2003). The importance of multiple infections as sources of selection on host populations has not been adequately explored.

Comprehensive studies on the effects of parasites on avian populations can shed light on their selective role on host traits. We describe the results of a long-term study on the prevalence of *Haemoproteus prognei*, a haematozoan parasite, and a filaria nematode on a colony of Purple Martins (*Progne subis*) in Maryland. We have shown that birds infected with *H. prognei* do not differ from uninfected birds in survivorship or in measures of reproductive success (Davidar and Morton 1993, Wagner et al. 1997). Here, we examine the effects of cross-infections with *H. prognei* and the filarial nematode on the survivorship of Purple Martins and determine whether cross-infections are more severe than single infections.

METHODS

The study was conducted on a colony of Purple Martins founded in 1976 in Severna Park, Anne Arundel County, Maryland. Breeding birds were captured each year in early July, between 2200 and 0300 hours, and banded with federal plastic and metal leg bands that permitted recognition of individual birds from one year to the next. Parasitological screening of birds in the colony started in 1986. From 1986 to 1993, one or two thin blood smears on glass slides were obtained from each breeding adult by squeezing the bird's clipped toenail. Sampling was done overnight when parasites circulate in the peripheral blood. Each slide was labeled with the bird's band number and sex, fixed in methanol, and stained (see Davidar and Morton 1993 for details).

The whole slide was scanned thoroughly under a 10× microscopic field to detect the microfilaria, the transmission stage of the filarial nematode. Blood smears obtained for the years 1988–1993 were used. The presence of a microfilaria was scored as a positive. A single species of filarial parasite infected this colony (confirmed by G. F. Bennett). However, without the older worms, it was not possible to definitively identify the nematode. *Haemoproteus prognei* was the only other blood parasite that infected the colony. Blood smears obtained for the years 1986–1993 were scanned for *H. prognei*. The parasite was identified by its intra-erythrocytic gametocytic stage (see Davidar and Morton 1993). We used a 100× oil immersion lens to detect intraerythrocytic stages. We scanned ~50 microscopic fields before declaring a slide negative for *H. prognei*. One bird had vent flukes, but no intestinal parasites were found despite extensive sampling (P. Davidar and E. S. Morton unpubl. data). We used prevalence and incidence as a measure of disease in the population. Prevalence is defined as the number and proportion of infections in the sample, and incidence is defined as the number and proportion of birds that had newly acquired infections since a previous sampling date.

Birds were initially screened at the second-year (SY) stage, the first calendar year of life when birds in their juvenal plumage start their first breeding cycle. Birds older than the SY stage with definitive plumage were termed ASY. Older ages were further classified as three-year-old (TY) and four-year-old birds (4Y). Most older birds screened were recaptures and a smaller proportion were immigrants to the colony (see Davidar and Morton 1993). To determine whether the filarial infection was initially contracted at the younger age classes or whether all age classes were equally susceptible, we looked at the age of the first infection in birds of known ages. Each bird was an independent sample, and the same bird did not appear in two different age classes. Fifty-four banded birds of known ages were compared. Thirty-five nestlings near fledging at 25–28 days posthatching were also screened. In addition, 12 nestlings were hand-raised and maintained in outdoor cages after fledging for eight weeks to see whether they acquired the infection near the breeding colony.

Purple Martins traditionally return to their breeding colonies after overwintering in the Neotropics, which makes it possible to follow the infection status and return rates for individual birds (Morton and Derrickson 1990). Because adult Purple Martins return to prior breeding sites, those that were not recaptured probably died (Morton and Derrickson 1990). Return rates can be used to test whether infected birds differ from uninfected controls in survivorship. Return rates of SY and ASY birds of known infection status were scored the following year. To determine whether initial infections were more virulent in SY than in ASY birds, return rates of all infected birds were pooled and compared with those of birds infected only at the post-SY stage. A chi-square or a *G* test was used to test whether infected and uninfected birds had similar return rates (Sokal and Rohlf 1981).

Results

Average prevalence of filaria from 1988 to 1993 was 22%. Sixteen percent of the birds sampled were infected in 1988, 24% in 1989, 19% in 1990, 21% in 1991, 30% in 1992, and 21% in 1993. Of the 400 birds sampled between 1988 and 1993, only 32 (8%) were cross-infected with *H. prognei*. Of them, 23 were ASY birds (10 males and 13 females) and 9 were SY birds (6 males and 3 females).

Table 1. Number and percentage of male and female second-year (SY) and after-second-year (ASY) Purple Martins infected with filaria.

Age classes		Infected	Uninfected
SY	Male	22 (19%)	93 (81%)
	Female	11 (14%)	65 (86%)
ASY	Male	23 (22%)	80 (78%)
	Female	30 (28%)	76 (72%)

There were significant differences in prevalence of the filarial infection between SY and ASY birds ($\chi^2 = 3.86$, df = 1, $P < 0.05$), but there was no difference in infection status between males and females in either age class (male and female birds: $\chi^2 = 0.21$, df = 1, NS; SY males and females: $\chi^2 = 0.69$, df = 1, NS; ASY males and females: $\chi^2 = 0.98$, df = 1, NS; Table 1).

Birds had equal probability of becoming infected at the SY and TY stages, but older birds tended to have a higher incidence (onset of initial infection) of filaria (Table 2). However, 35 nestlings and 12 hand-raised nestlings held in outdoor cages did not acquire any blood parasite, which suggests either that initial infections were at the SY age or that birds died if they were infected as yearlings (hatching year [HY]).

The return rate of SY birds infected with *H. prognei* was not significantly different from that of uninfected birds, whereas the return rate of filaria-infected SY birds was significantly lower (Table 3). Of nine SY birds cross-infected with both filaria and *H. prognei*, only two were subsequently recaptured. The return rate of cross-infected SY birds was not significantly different from that of filaria-infected birds ($G^2 = 0.62$, NS). Cross-infections were not recorded in TY birds. The return rate of TY birds infected with either parasite was not significantly different from that of uninfected birds (Table 4).

To determine whether birds of all age classes newly infected with either parasite had similar

Table 2. Incidence (number of newly acquired infections) of filaria in Purple Martins of different age classes.

Age classes	Infected	Uninfected	Total number of birds
Second year (SY)	37 (19%)	158 (81%)	195
Third year (TY)	13 (19%)	56 (81%)	69
Fourth year (4Y)	4 (33%)	8 (67%)	12

TABLE 3. Recapture rates of infected and uninfected SY Purple Martins.

Infection	Total number of birds	Recaptured	χ^2/G test [a]	P
Haemoproteus prognei	34	14 (41%)	0.023	NS
Filaria	23	2 (9%)	6.78	0.01
Cross-infections	11	2 (18%)	11.41	<0.001
Uninfected	149	47 (32%)		

[a] Comparison with uninfected controls. NS = nonsignificant.

TABLE 4. Number of infected and uninfected TY Purple Martins recaptured the following year. There were no cross-infected birds in the sample.

Infection	Total number of birds	Recaptured	χ^2/G test [a]	P
Haemoproteus prognei	10	8 (80%)	2.45	NS
Filaria	24	11 (46%)	0.023	NS
Uninfected	57	29 (51%)		

[a] Comparison with uninfected controls. NS = nonsignificant.

probability of being recaptured, return rates of newly infected birds were compared with those of uninfected controls. Among 46 newly infected SY birds, the return rate (14; 30%) did not differ significantly from that of 131 uninfected SY birds (43; 32%) ($\chi^2 = 0.09$, df = 1, NS). Similarly, the return rate of 19 infected ASY birds (13; 68%) was higher than that of 27 uninfected ASY birds (13; 48%), but not significantly so ($\chi^2 = 1.87$, df = 1, NS). However, when SY and ASY birds were compared, 13 of 19 ASY birds were recaptured, compared with 14 of 46 SY birds ($\chi^2 = 7.99$, df = 1, $P < 0.005$), which indicates that initial infections are more severe for SY than for ASY birds.

Ten ASY birds acquired cross-infections at the post-SY stage, of which only one five-year-old was recaptured the next year. Nine of those birds were first infected with filaria and acquired *H. prognei* as a later infection, whereas only one bird showed the reverse pattern. Of the nine, six were four years and older, and one uninfected bird acquired both infections when it was seven years old.

DISCUSSION

Our study demonstrates that the severity of parasitic infections differs with the parasite, with age of the host, and with multiple infections. Because transmission and prevalence of both parasites in Purple Martins is remarkably stable, these parasites probably play an important role in host fitness. Here, we show the importance of multiple or co-infection on fitness as shown in return rates in Purple Martins.

Cross-infections with multiple parasites are common in human and animal populations (Buck et al. 1978, Tshikuka et al. 1996, Fellowes and Kraaijeveld 1998) and can be benign or severe (Cohen 1973; Nacher et al. 2000, 2001; Spiegel et al. 2003; Graham et al. 2005). Our study suggests that co-infections with filaria and *H. prognei* in this population of Purple Martins are nearly always fatal, because although both parasites each infect approximately a quarter of the host population, almost no birds have both parasites simultaneously. Graham et al. (2005) have shown that filariasis, especially in the prepatency stage, exacerbates malarial infections in mice (*Mus musculus*). Malaria requires a delicate immunological balance with its rodent host (Li et al. 2001, Artavanis-Tsakonas et al. 2003) that can be upset by filaria co-infection. By contrast, the anti-inflammatory properties of pre-existing filarial infections may modify the severity of malarial infections (Lwin et al. 1982, Yan et al. 1997, Yoshida et al. 2000). Therefore, the timing of multiple infections may affect the outcome (Graham et al. 2005). Possible interactions between parasites are suggested, because in almost all cases of co-infection, *H. prognei* was acquired after filarial infection. This suggests that birds infected with *H. prognei* may be resistant to filarial infections.

The prevalence of filaria (22%) and *H. prognei* (28%; Davidar and Morton 1993) infections is fairly stable over the years, which is suggestive of an evolutionary equilibrium between these specialized parasites and their Purple Martin host. But the filarial parasites are more costly to Purple Martins when first acquired, and these costs are higher at some ages than at others. For example, *H. prognei* is more virulent to Purple Martins when infection occurs during a bird's hatching year, on its first southward migration. We inferred this because no infected yearlings (HY) were found among birds wintering in Brazil and because birds that had successfully migrated back from Brazil to breed, as SY birds, had higher parasitemia than infected adults. Such higher parasitemia levels are indicative of birds infected for the first time (Davidar and Morton 1993). Birds infected by filaria, even as SY birds, had lower survival, as shown by their lower return rates. For filarial infection, initially acquired infections are more severe in SY birds than in adults, whereas *H. prognei*-infected SY birds survived as well as infected older birds. Thus, both HY and SY birds pass through a window of vulnerability to parasitic infections, but birds infected for the first time when older are less vulnerable to parasites. Therefore, there must be strong selection on the SY stage, and birds passing through this window of vulnerability retain chronic infections that do not appear to affect their survivorship and reproduction (Davidar and Morton 1993, Wagner et al. 1997), unless they become co-infected.

Studies on the effect of parasites on avian mate choice have produced mixed results (Møller 1990, Hamilton and Poulin 1997). This could be because many individual birds do not show debilitating effects of parasites (Bennett et al. 1988, Weatherhead and Bennett 1991) or because parasites differ in their degree of severity, depending on host age. Both blood parasites are relatively benign for older Purple Martins that are able to forage and reproduce as well as uninfected birds of the same age (Davidar and Morton 1993, Wagner et al. 1997). This difference is related to the immunocompetence of the host, with immunologically naïve younger birds being less resistant to parasitic infections (Davidar and Morton 1993). Infections are more severe in the immunologically naïve younger age classes, and initial infections are more virulent than subsequent infections (Atkinson 1991, Atkinson and Van Riper 1991, Davidar and Morton 1993, Yorinks and Atkinson 2000, Atkinson et al. 2001). But co-infection with a second parasite, particularly the filaria, appears to extend the age-dependent window of susceptibility described above.

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