DO BLOOD PARASITES AFFECT PATERNITY, PROVISIONING AND MATE-GUARDING IN PURPLE MARTINS?[1]

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Abstract. We examined hypothetical relationships between blood parasite infection in Purple Martins *Progne subis* and extra-pair paternity of males, feeding performance of parents of both sexes, and mate-guarding intensity of males. We found no evidence that infection with either of two parasites, *Haemoproteus prognei* and an unspecified microfilariad, affected extra-pair paternity. Infected males were no more likely than uninfected males to be cuckolded, and infection did not preclude extrapair fertilization success. Infection also was unrelated to provisioning rates by adult males and females and to mate-guarding intensity by males. However, subadult females provisioned significantly less when infected with *Haemoproteus*, suggesting that young females have a lower resistance to parasites than adult females, or males.

Key words: parasite, sexual selection, Haemoproteus, *microfilaria, extra-pair fertilization,* Progne subis.

In Purple Martins (*Progne subis*), extra-pair fertilizations (EPFs) occur frequently and are strongly associated with male age-class (Morton et al. 1990, Wagner et al. 1996a). Males in the adult age class (two years old or more) possess bright indigo plumage, whereas yearling males retain a dull brown and white subadult plumage, despite undergoing a complete molt (Stutchbury 1991). Adult males are rarely cuckolded and achieve 96% paternity, whereas subadult males lose 43–61% of paternity through extra-pair fertilizations obtained by adult males (Morton et al. 1990, Wagner et al. 1996a). Females were found to follow a simple mating strategy: pair with an adult male and avoid extra-pair copulations (EPCs), or pair with a subadult and obtain EPCs from an adult male (Wagner et al. 1996a). By mating with older males, females may obtain genes for their offspring from males whose longevity might indicate parasite resistance (Hamilton 1990). Davidar

and Morton (1992) found that a haematozoan, *Haemoproteus prognei*, is prevalent in Purple Martins. Our first goal was to examine whether a relationship exists between extra-pair paternity and the frequency of *Haemoproteus*. Specifically, we asked whether infected males were cuckolded more often than uninfected males, and whether males that obtained extra-pair fertilizations were less likely to be infected than those that did not obtain them.

Our second goal was to determine whether the vigor of males and females, as measured by the provisioning of nestlings, was associated with infection. Feeding is limiting in Purple Martins, which have an exceptionally long nestling period of 28 days (Allen and Nice 1952). We estimate that an average parent catches flying insects and delivers them back to the nest approximately 1,700 times before molting and migrating thousands of kilometers to South America (Wagner et al. 1996b). Parents provisioned equally on average and apparently often were unable to feed their entire brood because at least one chick starved in 20% of 80 nests (Wagner et al. 1996b). If parasites detrimentally affect the performance of martins, the result may be evident in the form of lower feeding rates.

Whereas feeding rate is a measure of the vigor of both sexes, a potential additional measure of male vigor is mate-guarding intensity. Males guard by escorting their nest-building mates from the colony and sometimes attacking other males in flight or on the ground. There is marked variation in mate-guarding intensity among males, with some males escorting the female on virtually every foray and others rarely escorting their mates (Morton 1987, Wagner et al. 1996a). The source of this variation is unknown. Because more energy is required for males to fly after the female than remain perched at the colony, we examined whether mate-guarding intensity was lower in infected males.

In addition to *H. prognei*, a second blood parasite, an unspecified microfilariad, also occurs in substantial frequencies (Davidar and Morton, unpubl. data). Whereas *Haemoproteus* did not affect the survival of subadult Purple Martins (Davidar and Morton 1992),

microfilaria is associated with reduced survival in sub-adults (Davidar and Morton, unpubl. data). The apparent virulent nature of this parasite on subadults provided us with the additional goal of examining whether microfilaria-infected individuals reduced their feeding rates, and whether infected males had lower paternity and mate-guarding intensities.

METHODS

We studied Purple Martins in Severna Park, Maryland from April–July, 1991–1993. The study colony consisted of three 24-compartment aluminum martin houses which can be lowered and raised on 4.3 m poles (Morton 1987, Morton et al. 1990). In each of the three years, we trapped adults in a single night (3, 8, and 3 July, respectively) during the period when most eggs had hatched but few chicks had fledged. We used custom-made traps that simultaneously drop over the entrances of all the cavities (Morton and Paterson 1983). We obtained two blood smears from breeding martins by clipping a toe-nail and squeezing a drop of blood onto a slide. The slide was fixed in methanol and stained. A 100× oil-immersion lens was used to detect intraerythrocytic stages of *H. prognei*. Several microscopic fields were selected at random, and the detection of at least one *H. prognei* per 600 red blood cells resulted in our declaring the sample positive. About 50 microscopic fields were scanned per slide before a negative sample was declared. For detecting a blood-borne microfilariad, which are relatively large and very easy to identify, the slide was scanned once under a 10× microscopic field. The presence of a microfilaria was scored as a positive smear.

Paternity of nestlings in 1992 and 1993 was determined using multi-locus DNA fingerprinting by the methods in Wagner et al. (1996a). We recorded feeding rates during 3-hr observation periods in May–July of each year, which began at 07:00–07:30. Because of the close proximity of nests, we were able to accurately watch two to eight nests simultaneously and record which parent delivered food to the nestlings. We observed 69 nests for a mean of 11.3 hr (range 6–21) and a total of 780 nest-hours. We measured feeding rate as the number of times during a 3-hr observation period that an individual delivered food to the nest. Brood size (the number of chicks that fledged) differed significantly between adult males (mean = 3.88) and subadult males (mean = 3.17) (Wagner et al. 1996b) and between female age classes (adult mean ± SD = 3.79 ± 1.03, n = 48; subadult = 3.32 ± 0.79, n = 31, Mann-Whitney U-test, z = 2.10, P = 0.04). There also was a negative relationship between brood size and feeding rate per nestling (Wagner et al. 1996b). Therefore, in addition to analyzing feedings per nestling/3 hr, we also analyzed feedings per brood/3 hr. Feeding rates did not change with age of nestlings, as determined by regressing nestling age for each 3-hr watch with the number of feedings delivered to each nest (r^2 = 0.0005, n = 259 watches, P > 0.90; Wagner, et al. 1996b); thus, we included feeding rates for nestlings of all ages combined (mean age ± SD = 12 ± 6 days). Mate-guarding intensity was calculated as the percentage of female departures in the period of nest-building during which the male escorted the female to the ground (Morton 1987). We analyzed pairs that had a minimum of 10 observations (mean = 35 ± 20). Because some individuals were observed breeding again in a subsequent year, we analyzed individuals only during their first year of observation to avoid the statistical problem of nonindependence.

RESULTS

Of 21 adult males with known paternity, 6 were infected with *Haemoproteus* and 10 with microfilaria. Two adult males were cuckolded and neither were infected with either parasite. Of four adult males that were infected with both parasites, none were cuckolded and none obtained EPFs. Only 1 of 16 subadult males were infected by *Haemoproteus*, and 1 of 15 with microfilaria. None of 9 cuckolded subadult males were infected with *Haemoproteus* compared to 1 of 7 noncuckolded subadults. One of 7 cuckolded subadults was infected with microfilaria compared to 0 of 8 subadults that were not cuckolded. None of the subadult males were infected with both parasites.

We also detected no relationship between infection by either blood parasite and the paternity achieved by males through extra-pair fertilizations. One of the four adult males that obtained EPFs was infected by *Haemoproteus*, whereas six of the 21 adult males that did not obtain EPFs were infected (Fisher's exact test, ns). One of the four adult males that obtained EPFs was infected with microfilaria, whereas 10 of 21 adult males that did not obtain EPFs were infected with microfilaria (Fisher's exact test, ns). No subadult males obtained EPFs.

To appraise whether infection may have affected the performance of males and females, we examined two variables: feeding rates of both sexes and mate-guarding intensity of males. There were no significant differences in feeding rates between infected and uninfected males (Table 1; Mann-Whitney U-tests: Ps > 0.14). There also were no differences in the feeding rates of adult females as a function of infection by either parasite (Table 1; Ps > 0.3). In contrast to adult females, subadult females infected with *Haemoproteus* delivered significantly fewer feedings per nestling (z = 2.18, P < 0.04) and per brood (z = 2.11, P < 0.04) than uninfected subadult females. There were no significant differences for subadult females according to infection with microfilaria (Ps > 0.5).

We analyzed mate-guarding separately for each male age class because subadult males guarded at significantly higher intensities than adult males (Wagner et al. 1996a). Table 2 shows that infection with either parasite was unrelated to mate-guarding intensity for both adult and subadult males.

DISCUSSION

The first question we asked was whether parasites affected paternity in Purple Martins. Our sample sizes of infected males were very small, but they suggested that females were not more likely to obtain extra-pair fertilizations if their mates were infected and that infection did not reduce a male's chances of obtaining EPFs. If sexual selection via extra-pair copulation is mediated by parasites, then we may expect one of two female strategies. First, females might attempt to obtain EPCs when they are paired to infected males and

TABLE 1. The mean rate ($\pm$ SD) at which adult and subadult male and female Purple Martins delivered food to nestlings (per 3 hours) according to whether they were infected or uninfected by blood parasites.

	Feeding rate		
	Per nestling	Per brood	
Adult males (*n*)			
Haemoproteus			
Uninfected	4.47 $\pm$ 1.38	16.56 $\pm$ 3.79	(14)
Infected	5.22 $\pm$ 2.62	15.84 $\pm$ 4.86	(6)
Microfilaria			
Uninfected	4.69 $\pm$ 1.71	15.64 $\pm$ 3.52	(12)
Infected	4.70 $\pm$ 2.05	17.40 $\pm$ 4.71	(8)
Subadult males (*n*)			
Haemoproteus			
Uninfected	3.85 $\pm$ 1.22	12.25 $\pm$ 3.78	(20)
Infected	6.02 $\pm$ 3.37	15.50 $\pm$ 7.72	(4)
Microfilaria			
Uninfected	4.18 $\pm$ 1.97	12.85 $\pm$ 4.98	(20)
Infected	4.40 $\pm$ 1.08	11.83 $\pm$ 2.46	(4)
Adult females (*n*)			
Haemoproteus			
Uninfected	4.10 $\pm$ 1.55	14.87 $\pm$ 4.50	(11)
Infected	4.49 $\pm$ 1.81	14.94 $\pm$ 4.30	(15)
Microfilaria			
Uninfected	4.22 $\pm$ 1.24	14.76 $\pm$ 4.18	(18)
Infected	3.96 $\pm$ 1.98	14.92 $\pm$ 5.09	(7)
Subadult females (*n*)			
Haemoproteus			
Uninfected	5.64 $\pm$ 2.03 a	18.08 $\pm$ 4.75 b	(19)
Infected	4.20 $\pm$ 0.46 a	13.78 $\pm$ 2.23 b	(7)
Microfilaria			
Uninfected	5.34 $\pm$ 1.97	17.17 $\pm$ 4.79	(22)
Infected	4.82 $\pm$ 1.11	15.58 $\pm$ 3.66	(4)

Values with the same letter within a column were significantly different from each other. ($P < 0.04$).

avoid EPCs when paired to uninfected males. If this is the case, then females also are expected to obtain EPCs from uninfected males. Both predictions assume that females can determine whether males are infected. This could be accomplished if infected males had duller plumage (which we did not measure) or if they were physically less vigorous as indicated by their behavior. We found no differences in either the feeding rates or mate-guarding intensities of infected and uninfected males. This suggests that infection does not affect the vigor of males, and therefore females may not be able to determine the infection status of males based on these behaviors. This view is consistent with the simple female strategy of avoiding subadult males and preferring adults as mates or EPC partners (Wagner et al. 1996a). If parasites do not affect performance during the breeding season (despite having potentially deleterious effects, see Davidar and Morton 1992), then females may seek EPCs from adult males because

adults have proven their resistance, rather than because they are less likely to be infected. In fact, adult males had significantly higher frequencies of infection of *Haemoproteus* than subadult males (Davidar and Morton 1992). Thus, female preference for adult males is clearly not because fewer older males are infected.

The marked relationship between paternity and male age class, and male age class and plumage coloration in Purple Martins (Stutchbury, 1991) suggests that plumage coloration might be selected via female preference for adult males as mates and EPC partners (Hamilton and Zuk 1982, Hamilton 1990). The female strategy of accepting EPCs when paired to a subadult but not an adult male suggests that females may not discriminate whether individual adult males are infected. This assumption is consistent with the finding that infection did not preclude EPF success by adult males. However, the question remains whether plumage brightness among adult males affects paternity via female preferences.

The other issue we addressed was whether parasites affected provisioning rates of females. Adult females fed at similar rates, regardless of infection by either parasite. In contrast, subadult females provisioned less when infected by *Haemoproteus*. This finding is consistent with Davidar and Morton's (1992) suggestion that older females may be less vulnerable to the effects of parasites because they have survived previous exposure, whereas younger females may be more at risk of suffering deleterious effects. The sex ratio of subadults in our study population has been consistently male-biased (Wagner et al. 1996a), apparently due to higher mortality of young females (Morton and Wagner, unpubl. data). This raises the interesting question of whether young females have a lower resistance to the effects of parasites than adults and whether this difference exists only for females and not males.

TABLE 2. Mate-guarding intensity by males according to age class and parasite infection. Mate-guarding intensity is the percentage (mean $\pm$ SD) of female departures during which the male escorted his mate (sample sizes in parentheses).

	Adult males	Subadult males
Haemoproteus		
Uninfected	45.0 $\pm$ 24.7 (13)	68.3 $\pm$ 19.1 (21)
Infected	50.6 $\pm$ 16.8 (7)	65.0 $\pm$ 21.3 (4)
Microfilaria		
Uninfected	49.9 $\pm$ 21.3 (11)	66.7 $\pm$ 20.8 (19)
Infected	45.8 $\pm$ 23.9 (8)	74.5 $\pm$ 11.0 (5)

There were no significant differences in mate-guarding intensity between infected and uninfected males within age class (Mann Whitney U-tests: all $Ps > 0.30$).

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COOPERATIVE BREEDING IN GRAY JAYS: PHILOPATRIC OFFSPRING PROVISION JUVENILE SIBLINGS[1]

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Abstract. We present evidence for cooperative breeding in Gray Jays (*Perisoreus canadensis*), a species characterized by delayed dispersal but long thought not to exhibit the life-history tactic of helping. Our findings to date suggest that the alloparental care of younger siblings by philopatric yearlings is confined to the post-fledging period of nutritional dependence. We encourage other workers to investigate whether helping may be confined to this period in other semi-social species as well. Ongoing work is aimed at evaluating the fitness consequences of helping in our study population.

Key words: cooperative breeding, delayed dispersal, helping, Gray Jay, Perisoreus canadensis.

The Gray Jay (*Perisoreus canadensis*) is conspicuous among the Corvidae for its apparent lack of cooperative breeding (Strickland 1991, Strickland and Ouellet 1993). The failure to detect cooperative breeding in this species, and in its Eurasian congener (Siberian Jay, *P. infaustus*; Blomgren 1971, Lindgren 1975, Ekman et al. 1994), is unexpected because these jays are characterized by delayed dispersal (Strickland and Ouellet 1993), the necessary but not sufficient precursor of cooperative breeding (Koenig et al. 1992), and because cooperative breeding is thought to be a phylogenetically conserved trait in corvids (Edwards and Naeem 1993, Cockburn 1996).

We were prompted to renew our search for cooperative breeding in Gray Jays by the apparently skeptical suggestion that alloparental care of younger siblings may occur during the post-fledging period of nutritional dependence (Ekman et al. 1994). Here, we document the discovery of helping during that period and encourage workers to search for, and examine the fitness consequences of, this form of cooperation in other semi-social species.