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Splenic Mass of Masked Shrews, *Sorex cinereus*, in Relation to Body Mass, Sex, Age, Day of the Year, and Bladder Nematode, *Liniscus* (=*Capillaria*) *maseri*, Infection

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ABSTRACT: The spleen is an important organ of vertebrates. Splenic mass can change in response to a variety of factors. We tested whether splenic mass of masked shrews, *Sorex cinereus*, was related to sex, age, time of the year, or intensity of bladder nematode (*Liniscus* [=*Capillaria*] *maseri*) infection, after controlling for host body mass. For females, body mass was a strong predictor of splenic mass. For males, splenic masses were greater later in the year and in more heavily infected males. The latter appeared to represent a threshold response wherein only the most heavily infected individuals had enlarged spleens.

Organisms face a complex array of competing demands that influence their ability, or need, to invest in maintenance of their organs (Nordling et al., 1998; Coop and Kyriazakis, 2001; Rigby et al., 2002). An important function of the vertebrate spleen includes production of lymphocytes that are involved in fighting infections (John, 1994a). Immune investment varies by sex (Grossman, 1989; Klein, 2004); this may be related to pregnancy (Roberts et al., 1996), mating systems (Folstad and Karter, 1992), or differential exposure to parasites (Zuk and McKean, 1996). In many taxa, males may be more intensely infected with parasites (Poulin, 1996; Zuk, 1996; Shalk and Forbes, 1997; Klein, 2000), but see McCurdy et al. (1998) who found more intense hematozoan infections in female birds. Immune investment can also vary with age (Ottinger and Lavoie, 2007), as immune systems mature or as energy is directed to potentially more rewarding activities, such as reproduction (Folstad and Karter, 1992), or in response to seasonal changes in the structure or size of parasite communities (Forbes et al., 1999; Møller et al., 2003).

The importance of the spleen to vertebrate immunity is reflected by more intense infections with various parasite taxa in both splenectomized (Ali and Behnke, 1985; Hillgarth and Wingfield, 1997) and splenomegalous individuals of various bird and mammal species in the lab (Vincent and Ash, 1978; Watkins et al., 1991; Giacomo et al., 1997; Garside et al., 1989; Kristan and Hammond, 2000). The relationship between splenic mass and parasitism in wild populations has not been as well studied (Shutler et al., 1999; Morand and Poulin, 2000; Brown and Brown, 2002; Kristan and Hammond, 2003; de Bellocq et al., 2007). Splenomegaly is more common with nematode than with trematode or cestode infection (John, 1994b), possibly because the latter more often remain in the digestive tract and cause minimal internal trauma (Haukisalmi et al., 1994), whereas nematodes more often migrate through tissue, potentially causing significant trauma (Read and Skorping, 1995).

We recently reported sex-biased prevalences of adult bladder nematodes (*Liniscus* [=*Capillaria*] *maseri*) of 21% in female versus 75% in male masked shrews, *Sorex cinereus*, (Cowan et al., 2007), a widely distributed and abundant insectivore in North America (Banfield, 1974). Although the specific life cycle of this nematode is undescribed, based on life cycles of related nematode taxa, *L. maseri* is likely ingested as either eggs or early-stage larvae. These migrate as later-stage larvae through the host gut wall and eventually reach the bladder, where mature, mated females begin passing eggs with urine. Splenomegaly can persist for several weeks in some helminth infections (Ali and Behnke, 1985; Garside et al., 1989). Several other kinds of infections can cause splenomegaly, as can trauma (Watkins et al., 1991; Runyan et al., 2008). We tested for associations between splenic mass and host sex, age, time of the year, and current infection intensity by bladder nematodes, after controlling for host body mass. The variety of other factors that can cause splenic mass to increase make it less likely that we would observe significant associations with the variables we had available.

Details on study methods and study locations are provided in Cowan et al. (2007). Briefly, shrews were captured in pitfall traps in 8 separate samples in southern Nova Scotia in May, June, and August 2005, and in May and June 2006. Most shrews were found dead in the trap; live shrews were humanely killed by thoracic compression. All shrews were dissected within 12 hr of capture; sex was determined in all cases (Rogers Brambell, 1935). Bladders were compressed between 2 microscope slides and viewed using a compound microscope to enumerate bladder nematodes. Infection intensity was scored as 0 if there were 0 nematodes, 1 if there were 1–2, 2 if there were 3–4, 3 if there were 5–6, 4 if there were 7–10, and 5 if there were more nematodes than could be counted. Spleens were removed and weighed to the nearest 0.01 g. Carcasses were frozen at −40 C and later cleaned by dermestid beetles to enable us to age shrews based on tooth wear. Shrews were assigned to 1 of 5 ordinal age categories based on tooth wear, with a value of 1 including younger, pre-breeding animals with no wear on teeth and a value of 5 including older reproductive animals with extensive wear (Cowan et al., 2007).

Statistical analyses were carried out in SAS Version 9.1 (SAS Institute, Cary, North Carolina). Because of previously observed sex biases in infection intensity (Cowan et al., 2007), we analyzed each sex separately. We first ran univariate analyses to reveal simple data structure and then used general linear models (GLMs) where $\log_{10}$(splenic mass) was the response variable, and body mass, age, time of the year, and infection intensity were explanatory variables. We sequentially dropped the least-important variables (highest *P*s) from GLMs until only significant ($P < 0.05$) variables remained (Sorci et al., 1996). Means are reported ±SE. Sample sizes vary because some variables were unrecorded in some shrews.

Splenic masses were similar in females (n = 52, mean = 0.034 ± 0.003 g) and males (n = 51, mean = 0.038 ± 0.006 g) ($F_{1,101} = 1.0$, $P = 0.32$). For females, univariate analyses revealed significant increases in splenic mass with body mass and age and a less significant decrease with time of the year, but no association with infection intensity (Table I). For males, the same univariate analyses revealed significant associations between body mass and infection intensity (Table I). The relationship between splenic mass and infection intensity (Fig. 1) suggested a curvilinear association for males, but not for females. Thus, a curvilinear term (the square of infection intensity) was entered into GLMs for males.

In the GLM analyses for females, splenic mass was unrelated to age, time of the year, or infection intensity; after dropping these variables, only a significant positive relationship between spleen and body mass remained ($R^2 = 0.14$, $F_{1,50} = 8.1$, $P = 0.007$). In GLM analyses for males, splenic mass was greater later in the year ($F_{1,47} = 4.7$, $P = 0.03$) and with higher infection intensity (squared) ($F_{1,47} = 12.5$, $P = 0.001$; overall model $R^2 = 0.23$, $F_{2,47} = 6.9$, $P = 0.002$). This latter significant association appeared to be driven by a small number of intensely infected males (Fig. 1), consistent with a threshold response.

In female shrews, we found some evidence that splenic mass increases with age and decreases with time of the year (possibly because of changing parasite communities), but these relationships were overwhelmed by a positive association with body mass. In male shrews, we also found evidence of greater splenic mass with greater body mass, but this was overwhelmed by the association with bladder nematodes. Only males appeared to face infections sufficiently intense to lead to splenomegaly. The fact that we obtained a significant association despite the many other factors that can influence splenic mass, particularly in an observational study, thus provides compelling evidence of an association with bladder nematodes.

Splenomegaly was not evident at low-intensity infections, and no females in the samples were intensely infected (classes 4 and 5). In general, infection intensities were significantly higher for males (Cowan et al., 2007), despite the short life span of both males and females (usually less than 14 mo for *Sorex* spp. shrews) (Pruitt, 1954; van Zyll de Jong, 1983) and the consequent short exposure time to parasites. Higher infection intensities in males likely reflect greater exposure to

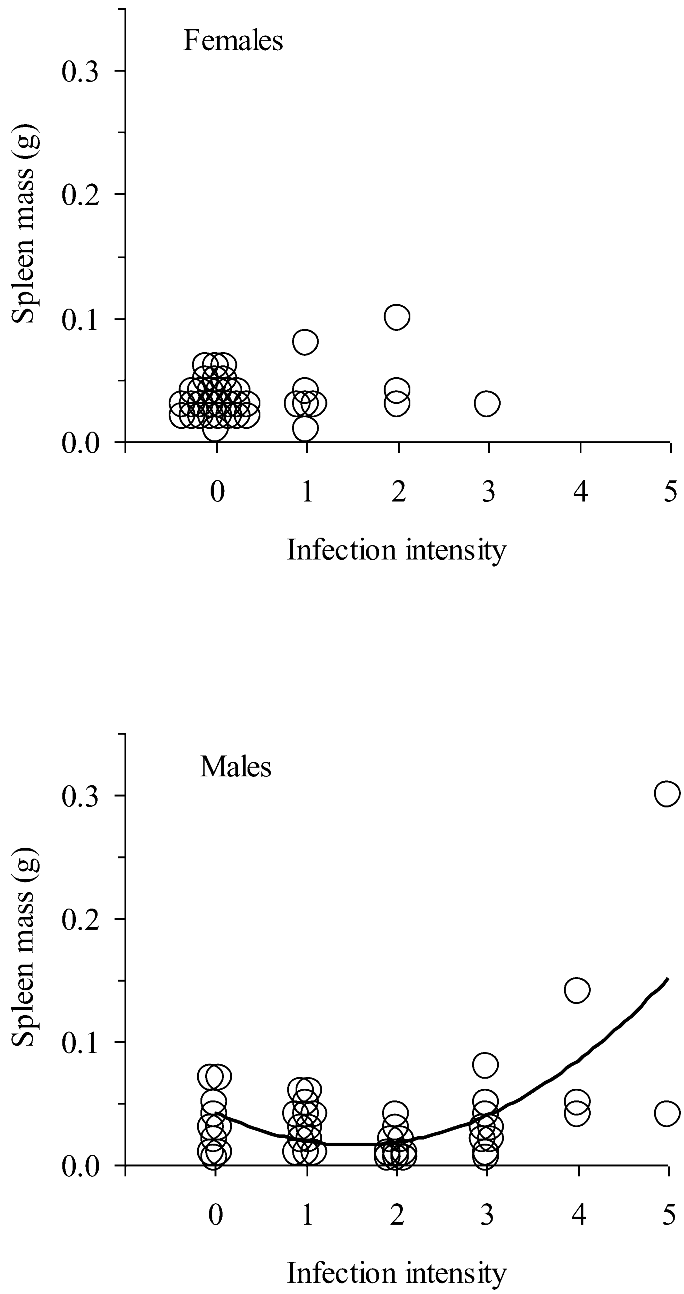

FIGURE 1. (Top) Female splenic masses were not greater for females with more-intense infections. (Bottom) Spleens were larger in males with more-intense infections (solid line depicts second-order polynomial). In both figures, points are laterally offset to reveal overlapping observations.

parasites and not lesser investments in immunity due to endocrine inhibition (Grossman, 1989; Folstad and Karter, 1992). Although the life history of masked shrews is poorly understood because of high capture and handling mortality, data from other shrew species suggest that home ranges of males are larger than those of females (Hawes, 1977; Cantoni, 1993). Larger ranges would potentially expose males to more parasite infective stages.

Splenomegaly in this species appeared to be a threshold response that was provoked only at extreme-infection intensities. Our results contribute to a growing body of evidence that splenomegaly is associated with high helminth, especially nematode, infection intensities in wild populations (Shutler et al., 1999; Morand and Poulin, 2000; Kristan and Hammond, 2003; Brown and Brown, 2002; de Bellocq et al., 2007). Such responses by shrews, given their short life spans, are surprising. Controlled tests of this association will be difficult with masked shrews because individuals rarely survive capture.

TABLE I. Pearson correlations between infection intensities of bladder nematodes in masked shrews and several explanatory variables according to sex. Sample sizes vary because not all data were collected from all shrews.

	Females			Males		
Explanatory variable	*N*	*r*	*P*	*N*	*r*	*P*
Body mass	52	0.37	0.007	50	0.31	0.03
Age	47	0.31	0.03	38	0.01	0.97
Time of the year	52	−0.26	0.06	51	0.15	0.28
Infection intensity	48	0.15	0.32	50	0.29	0.04

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