Nematode parasite species richness and the evolution of spleen size in birds

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Abstract: Some of the interspecific variation in spleen sizes among bird species can be explained by interspecific variation in the proportion of birds infected by parasitic nematodes. Because prevalences of parasitic infections vary considerably in space and time within a host species, other variables may provide better measures of the selective pressure exerted by parasites on their hosts. For instance, the number of parasite species (species richness) exploiting a host population or species provides a more reliable index of the pressure exerted by parasites across generations. Among bird species, relative spleen size correlated positively with the species richness of nematode parasites exploiting a host species. This relationship was found after correcting for avian body mass (g), avian phylogeny, and sampling effort. A possible trade-off between investment in resistance against parasites and investment in reproduction was highlighted by a negative relationship between relative spleen size and relative testis mass. Parasitic nematodes could influence the trade-off, increasingly favoring investment in resistance as their species richness increases. The results of this comparative analysis and of previous ones suggest a causative role for parasitic nematodes in the evolution of avian spleen size.

Résumé : Une partie de la variation interspécifique de la taille de la rate chez les oiseaux peut s'expliquer par la variation interspécifique de la proportion des oiseaux infectés par des nématodes parasites. Comme la fréquence des parasitémies varie considérablement dans le temps et dans l'espace chez une espèce hôte, d'autres variables peuvent fournir de meilleures mesures de la pression de sélection qu'exercent les parasites sur leurs hôtes. Par exemple, le nombre d'espèces parasites (la richesse en espèces) qui exploitent une population ou une espèce hôte donne un indice plus juste de la pression de sélection exercée pendant plusieurs générations. Chez les oiseaux, la taille relative de la rate est en corrélation positive avec la richesse en espèces des nématodes parasites qui exploitent une espèce hôte. Cette relation a été démontrée après avoir tenu compte de la masse (g) des oiseaux, de leur phylogénie et de l'effort d'échantillonnage. Une corrélation négative entre la taille relative de la rate et la taille relative des testicules a mis en lumière l'existence possible d'un compromis entre l'énergie investie dans la résistance aux parasites et l'énergie investie dans la reproduction. Il se peut que les nématodes parasites influencent ce compromis en favorisant de plus en plus l'investissement dans la résistance à mesure que leur richesse en espèces augmente. Les résultats de cette analyse et d'autres analyses antérieures semblent indiquer que les nématodes parasites jouent un rôle de cause à effet dans l'évolution de la taille de la rate chez les oiseaux.

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Introduction

Parasites meet all the criteria necessary to be direct agents of selection, i.e., they cause reductions in fitness and the influence they exert varies both within and across species (Goater and Holmes 1997). There is now considerable empirical evidence that parasites have influenced the evolution of host behaviour, life-history traits, and morphology (e.g., Ruiz 1991; Zuk 1992; Lafferty 1993). Host characteristics that play a direct role in avoiding or resisting parasites are particularly interesting in this context. Because the production of any antiparasite organ or behaviour is costly, the selective pressure exerted on hosts by parasites should influence the trade-off between investment in resistance against parasites and investments in other functions.

Recent comparative studies have shown that the relative level of development of some organs involved in immunity against parasites is proportional to the selective pressure exerted by parasites. Møller and his colleagues have shown that bird species that are likely to be exposed to more intense parasitism, such as dichromatic species, migratory species, or those living in the tropics, have relatively larger organs of immune defense (spleen and (or) Bursa of Fabricius) than related species exposed to fewer parasites (Møller 1998; Møller and Erritzøe 1998; Møller et al. 1998). These results suggest indirectly that the action of parasites can select for investment in organs of resistance to infection. In a more direct test with respect to helminth parasites, John (1995) found a positive relationship between a measure equivalent to the prevalence of nematode parasites (the proportion of birds infected by nematodes in a sample) and the relative spleen mass of bird species. Prevalence of other helminths, i.e., cestodes and trematodes, did not covary with relative spleen

mass (John 1995). This comparative evidence is supported by an intraspecific study of wild geese in which smaller spleen size was associated with more severe nematode infections (Shutler et al. 1999). In mammals, the spleen is a major lymphoid organ and is prominent in its action against nematode parasites (John 1994*a*). The function of the avian spleen is not yet fully understood, but it appears to play an even greater role in systemic immunity than its mammalian counterpart because of the scarcity of lymphatic vessels and nodes in birds (John 1994*b*). Swelling of the spleen is a possible pathological response to infection by certain helminths (e.g., splenomegaly in human schistosomiasis; Warren 1978), but changes in spleen size usually appear adaptive rather than merely pathological (John 1994*b*). In many bird species, lymphocytes account for most of the volume of the spleen (Toivanen and Toivanen 1987), suggesting that the size of the spleen may be a good indicator of the strength of the immune response mounted against invading parasites and of its capacity to deal with potential infections.

The most interesting but not the only explanation for the relationship between spleen mass and nematode prevalence is that bird species in which a greater proportion of individuals incur nematode infections have been selected for greater investment in immune defense via a larger spleen. Because of the likely trade-offs between spleen mass and the size or function of other organs, large spleen sizes should only be selected in bird species consistently exposed to intense parasitism. For instance, circulating testosterone levels correlate negatively with relative spleen size across bird species (A.P. Møller and J. Wingfield, unpublished data), suggesting that there are costs associated with large investments in spleen development that only receive compensation in species exposed to intense parasitism. Following from the likelihood of a trade-off and the observed relationship with testosterone levels, one would predict that the relative spleen size (investment in immune defense) should be negatively correlated with the size of gonads, particularly testes in males (investment in current reproduction).

Prevalences of nematodes and other helminths, however, are known to vary greatly in space and time, i.e., from season to season and from one population to the next (Rausch 1983). Therefore, prevalence may not be the ideal measure of the selective pressure exerted by nematodes because it fluctuates too much in response to environmental conditions. The number of nematode species exploiting a host species (nematode species richness) may be a better index of selective pressure. Parasite species richness can vary in space, i.e., among populations (e.g., Edwards and Bush 1989), but usually not as widely as prevalence. However, parasite species richness rarely varies in time, i.e., across seasons or generations. In addition, parasite species richness provides a more explicit measure of the diversity of organs and tissues that are likely to be attacked by nematodes. Here we investigate the relationship between relative spleen mass and nematode species richness across bird species. We also look for a trade-off between spleen and testis sizes to illustrate the relative cost of developing a large spleen. Our analysis controlled for sampling effort, bird body mass, and phylogenetic influences, and provides further support for a role of nematode parasites in the evolution of avian spleens.

Methods

Data on avian body and spleen masses were obtained from the same bird individuals and for a range of species by de la Riboisière (1910). All birds were from the Holarctic region, mostly from Europe. They were caught in the wild and held in captivity for a few days before the measurements were taken and none of them died from disease during that time. Body mass (g) was obtained after plucking, and thus excludes plumage mass. Body and spleen masses were averaged across conspecific birds to obtain species values. Earlier studies (Møller 1998; Møller and Erritzøe 1998) have shown that seasonal and other intraspecific variation in spleen sizes are generally not as pronounced as interspecific variation, making the use of mean specific values obtained from several conspecifics adequate for the purposes of a comparative analysis. Testis mass for different bird species were obtained from Møller (1991).

Information on nematode parasite species richness was obtained from several different studies (see Table 1) performed on bird populations from either the same or a different geographical area as the ones studied for body and spleen masses by de la Riboisière (1910). Richness measures represent parasite richness at the host-population level and not the full list of nematode species infecting a bird species across its geographical range; the latter measure would overestimate the actual parasite pressure incurred by a bird population. Bird species were only included if at least five individual birds had been examined for nematode parasites and if all visceral organs had been searched. We recorded the actual number of birds examined to control for sampling effort (see below), a variable which always explains much of the interspecific variation in parasite species richness (Walther et al. 1995).

All variables were ln-transformed to achieve homogeneity of variances (Harvey 1982). We used the independent contrasts method to control for phylogenetic effects (Felsenstein 1985). Phylogenetic contrasts were derived from the molecular phylogeny of birds of Sibley and Ahlquist (1991) and computed using the CAIC software, version 2.0 (Purvis and Rambaut 1995). To correct for the effect of body mass, this variable was included as a covariate in multiple regressions. To illustrate pairwise relationships between variables while accounting for potential confounding variables, we also used the residuals of a regression of the variable of interest on the confounding variable. All regressions and correlations were forced through the origin (see Garland et al. 1992 for details).

Results

Data on spleen sizes and nematode species richness could be obtained for 24 bird species only (Table 1). Given the complete resolution of their phylogenetic relationships (Sibley and Ahlquist 1991), it was possible to derive 23 sets of independent contrasts from these species. Data on testis sizes were available for 13 of these species (i.e., 12 sets of contrasts).

Contrasts in spleen mass correlated positively with contrasts in body mass ($r = 0.907$, $n = 23$, $P < 0.0001$; Fig. 1). Contrasts in testis mass also correlated positively with contrasts in body mass ($r = 0.860$, $n = 12$, $P = 0.0002$). In a multiple regression, with contrasts in testis mass as the dependent variable and contrasts in body mass as a covariate, a negative relationship was found between contrasts in spleen mass and contrasts in testis mass ($P = 0.024$), suggesting that there is a trade-off between investments in the two organs (Fig. 2).

Contrasts in nematode species richness were corrected for contrasts in sampling effort by taking the residuals of the

Table 1. Summary of the data set used in the comparative analysis.

Bird species	Body mass (g)	Spleen mass (g)	Nematode species richness (no. of nematode species)	No. of birds examined	References
Aythya ferina	832	0.25	8	12	Borgarenko (1984)
Alauda arvensis	31	0.04	3	7	Borgarenko (1984)
Alca torda	608	0.20	3	49	Threlfall (1971)
Ardea cinerea	1214	0.50	4	9	Borgarenko (1984)
Columba livia	287	0.14	0	21	Borgarenko (1984)
Corvus corone	435	1.00	7	11	Borgarenko (1984)
Cuculus canorus	97	0.06	0	7	Borgarenko (1984)
Fratercula arctica	343	0.09	1	160	Threlfall (1971)
Fulica atra	518	0.36	7	63	Borgarenko (1984)
Galerida cristata	21	0.01	1	5	Borgarenko (1984)
Gallinula chloropus	234	0.10	4	9	Borgarenko (1984)
Lanius excubitor	34	0.06	4	18	Borgarenko (1984)
Larus argentatus	749	0.29	7	42	Buck et al. (1976); Pemberton (1963)
Lophortyx californicus	176	0.10	1	137	Chandler (1970)
Merops apiaster	67	0.09	11	19	Borgarenko (1984)
Motacilla alba	22	0.03	4	44	Borgarenko (1984)
Passer montanus	23	0.02	3	48	Borgarenko (1984)
Phasianus colchicus	1290	0.77	9	39	Borgarenko (1984)
Phylloscopus trochilus	8	0.01	1	14	Borgarenko (1984)
Rallus aquaticus	69	0.06	4	8	Borgarenko (1984)
Sturnus vulgaris	74	0.05	7	14	James and Llewellyn (1967)
Turdus merula	83	0.09	5	21	Borgarenko (1984)
Turdus viscivorus	115	0.09	7	23	Borgarenko (1984)
Upupa epops	70	0.07	8	38	Borgarenko (1984)

linear regression between these two variables. This adjusted measure of nematode species richness correlated positively with contrasts in avian body mass ($r = 0.439$, $n = 23$, $P = 0.031$; Fig. 1). In a multiple regression, with contrasts in spleen mass as the dependent variable and contrasts in body mass as a covariate, a positive relationship was found between contrasts in nematode species richness (corrected for sampling effort) and contrasts in spleen mass ($P = 0.034$; Fig. 3). In other words, after controlling for avian body mass and sampling effort, bird species with larger spleens tend to harbour more species of parasitic nematodes than related species with smaller spleens.
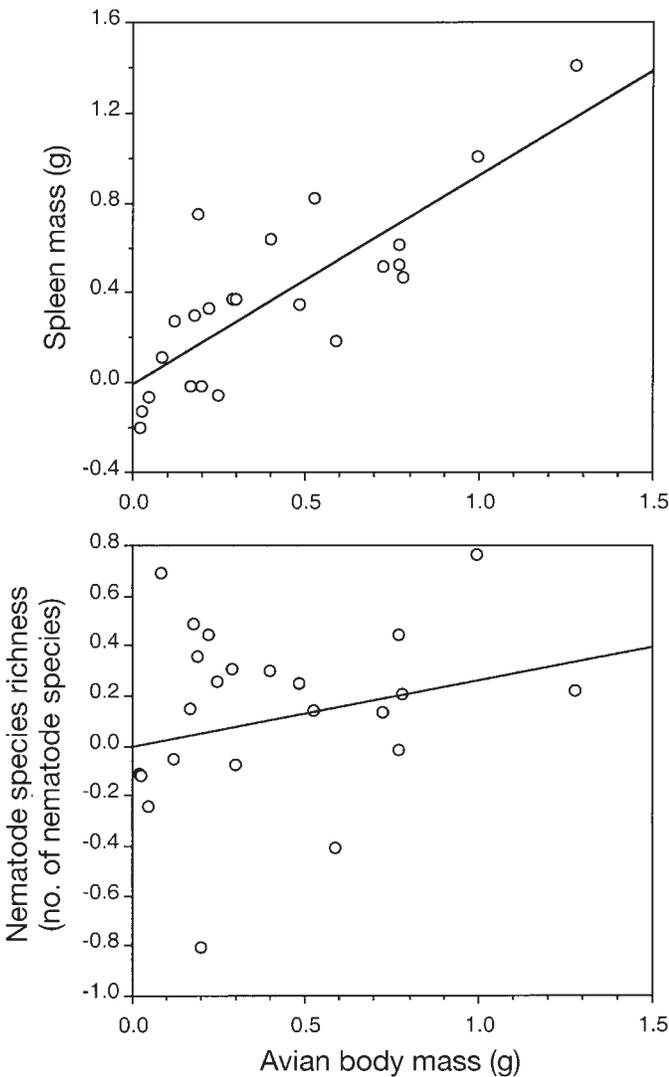
Discussion

Little is known about the operation of the avian immune system against helminth parasites (Danforth and Augustine 1989). Although it has other functions, it appears that the spleen may play an important role in avian immunity (Toivanen and Toivanen 1987; John 1994a, 1994b). In a study of covariation between spleen size and helminth infections among geese, Shutler et al. (1999) found that individuals with smaller spleens harboured greater numbers of nematodes than individuals with larger spleens, after controlling for seasonal effects and geese body size. This suggests that individuals with lower investment in immunity are more susceptible to nematode infections and that higher investment should be favoured in bird species exposed to higher infection levels. An earlier

comparative study showed that bird species in which a high proportion of individuals harbour nematode parasites have relatively larger spleens than related species in which fewer individuals harbour nematodes (John 1995). Here, using the same spleen size data as John (1995), we show that bird species that are hosts to many nematode species have relatively larger spleens than related species that are hosts to fewer nematode species. Nematode prevalence and nematode species richness may correlate independently with spleen size, however, these two measures of the selective pressure exerted by nematodes may covary across bird species, so that their separate effects could be difficult to distinguish. Because parasite species richness does not fluctuate as widely over time as parasite prevalence does, it may prove a more reliable indicator of the importance of parasites as agents of selection. In any event, these results, combined with the few experimental studies using splenectomy or other methods (see John 1994b), suggest a causative role for nematodes in the evolution of relative spleen size in birds.

The link between nematode parasitism and spleen size must be relatively strong to emerge from a comparative analysis. Avian spleen mass and morphology are known to show seasonal changes in some bird species, often associated with either migrations or reproduction (e.g., Oakeson 1953; Heitmeyer 1988). These cyclical fluctuations could perhaps mirror seasonal variation in exposure to parasitism (John 1994b). Whatever the reasons for these changes, they may cause background noise and imprecision in the estimation of

Fig. 1. Relationship between avian body mass and both spleen mass ($y = 0.917x$) and nematode species richness, corrected for host sampling effort ($y = 0.309x$). Data are from 23 sets of phylogenetically independent contrasts.

Fig. 2. Relationship between avian testis mass and spleen mass. Data are from 12 sets of phylogenetically independent contrasts. Here both measures are relative and not absolute contrasts and are corrected for body mass using residuals.
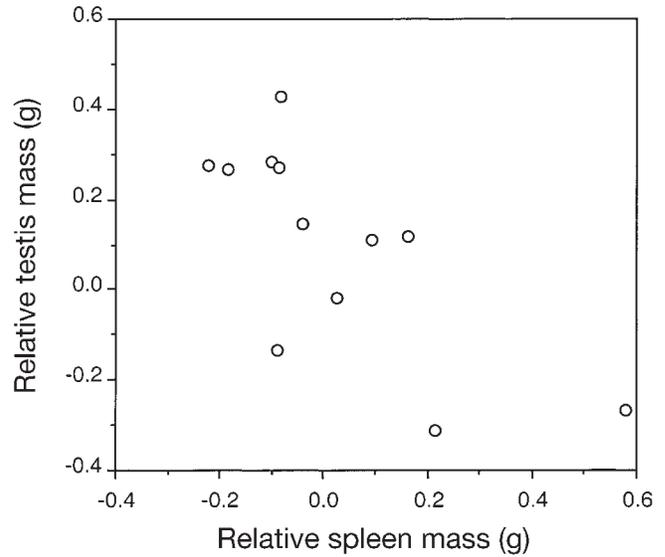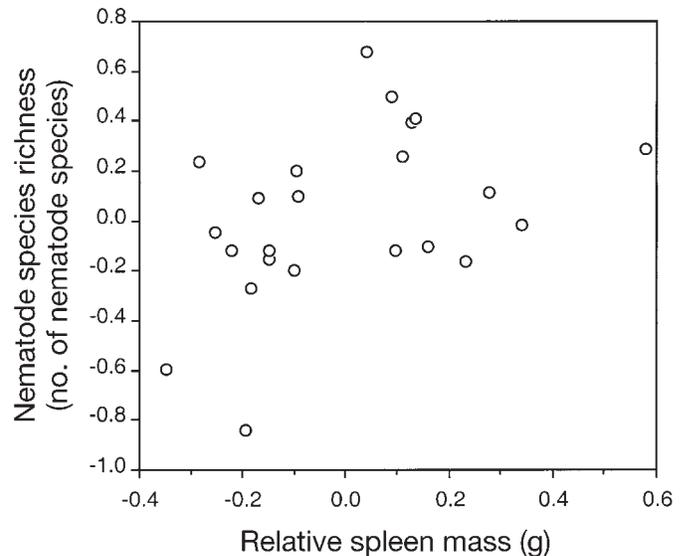
Fig. 3. Relationship between nematode species richness and avian spleen mass. Data are from 23 sets of phylogenetically independent contrasts. Here both measures are relative and not absolute contrasts and are corrected for body mass and sampling effort using residuals.

species-specific values for spleen mass. Despite this, and the fact that our spleen and parasite data had different origins, we still found a significant association between spleen size and nematode species richness. It would now be interesting to verify whether similar relationships exist between parasitism and the development of defense organs in other groups of hosts, such as mammals.

According to the arguments presented above, infections by many different species of nematode parasites could select for large spleens in bird hosts. At the same time, other constraints will limit any increases in spleen size. The negative correlations between relative spleen size and either levels of testosterone in the blood (A.P. Møller and J. Wingfield, unpublished data) or relative testis mass (present study) suggest that investment in spleen development are made at the expense of investments in testis growth or other functions. Large testes are beneficial to males in many avian taxa where sperm competition is intense (Møller 1991). The optimal

spleen size is therefore the product of a trade-off between resistance against parasites and sperm production. Nematode species richness influences the cost of not investing in defense against parasites, and thus pushes the trade-off one way or the other. Trade-offs between reproductive investment and spleen development have not been investigated among female birds in this study, but we expect them to mirror those observed for males.

Is avian spleen size related only to nematode parasite species richness or would other helminths also influence it?

John (1995) found no relationship between the prevalence of cestodes and trematodes and relative spleen size. He argued that the adult cestodes found in birds may not be as invasive as nematodes and that estimates of trematode prevalence are more likely to be imprecise. In a preliminary analysis not reported here, we found no link between species richness of either cestodes or trematodes and avian spleen size (all $P >$ 0.5; S. Morand, unpublished data). Although the reasons are not yet clear, the similarity between the results of the analyses using species richness as a measure of parasitism and John's (1995) results based on studies of prevalence suggests that the relative importance of nematodes is greater than that of other helminths. This is supported by the recent intraspecific study of Shutler et al. (1999) on geese in which only nematode infections were related to spleen size and not cestode or trematode infections.

Most previous comparative studies of parasite species richness have uncovered relationships with host traits that, unlike a relatively large spleen, may promote the acquisition and accumulation of parasite species over evolutionary time (see Poulin 1997 for a review). These host features include body size, geographical range, population density, longevity, and diet. Clearly, large-bodied, long-lived, widespread host species occurring at high densities and having a broad diet encounter more species of parasites and provide them with more opportunities and space for colonization. These relationships indicate that parasite species richness is highest in host species with features that facilitate the establishment of new parasite species. The results of the present study, however, show that the accumulation of many parasite species may in turn select for adaptations against parasites, such as a large spleen, in those hosts. This causal loop could create patterns of covariation between host characteristics that would otherwise be more or less independent of one another.

Acknowledgements

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