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Anders Pape Møller · Lajos Rózsa

Parasite biodiversity and host defenses: chewing lice and immune response of their avian hosts

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Abstract Antagonistic host-parasite interactions lead to coevolution of host defenses and parasite virulence. Such adaptation by parasites to host defenses may occur to the detriment of the ability of parasites to exploit alternative hosts, causing parasite specialization and speciation. We investigated the relationship between level of anti-parasite defense in hosts and taxonomic richness of two chewing louse suborders (Phthiraptera: Amblycera, Ischnocera) on birds. While Amblyceran lice tend to occur in contact with host skin, feed on host skin and chew emerging tips of developing feathers to obtain blood, Ischnoceran lice live on feathers and feed on the non-living keratin of feather barbules. We hypothesized that Amblyceran abundance and richness would have evolved in response to interaction with the immune system of the host, while Ischnoceran taxonomic richness would have evolved independently of immunological constraints. In an interspecific comparison, the abundance of Ischnocerans was positively related to host body size, while host body mass and Ischnoceran taxonomic richness accounted for the abundance of Amblycerans. Amblyceran taxonomic richness was predicted by the intensity of T-cell mediated immune response of nestling hosts, while the T-cell response of adults had no significant effect. In contrast, Ischnoceran taxonomic richness was not predicted by host T-cell responses. These results suggest that the taxonomic richness of different parasite taxa is influenced by different host defenses, and they are consistent with the hypothesis that increasing host allocation to immune defense increases Amblyceran biodiversity.

Keywords Amblycera · Immunity · Ischnocera · Parasites · Species diversity

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A. P. Møller (✉)
Laboratoire de Parasitologie Evolutive, CNRS UMR 7103,
Université Pierre et Marie Curie, Bât. A,
7ème étage, 7 quai St. Bernard, Case 237,
75252 Paris Cedex 05, France
E-mail: amoller@snv.jussieu.fr
Tel.: +33-1-44272594
Fax: +33-1-44273516

L. Rózsa
Animal Ecology Research Group,
Hungarian Academy of Sciences,
Hungarian Natural History Museum,
Budapest, Ludovika t. 2, 1083, Hungary

Introduction

Parasites constitute a very large proportion of extant species (Price 1980). A major factor contributing to this explanation is that parasites by definition acquire some or all of their resources from living organisms. Common means of avoiding or reducing parasitism include behavioral, mechanical and physiological defenses (e.g. Hart 1990, 1997; Klein 1990; Wakelin 1996). Why has the ability to exploit the resources of other individuals become the main means of resource acquisition among living organisms? The main answer lies in the observation that parasite exploitation, host defense and new means of parasite attack are the outcome of aggressive coevolutionary interactions. Strict coevolution between two or few interacting players is more likely to result in specialization and speciation than diffuse coevolution among many different species (Thompson 1994). Thus, coevolutionary theory predicts positive covariation between levels of defense, specialization and speciation.

Lice (Phthiraptera) are the only parasitic insects that complete their entire life cycle upon the body surface of birds showing low levels of pathogenicity (Clayton and Tompkins 1994, 1995). However, lice still influence major aspects of avian life history such as flight performance (Barbosa et al. 2002), metabolism (Booth et al. 1993), life expectancy (Brown et al. 1995; Clayton et al. 1999) and sexual selection (Clayton 1990; Kose and Møller 1999; Kose et al. 2000). It still seems likely that

parasites other than lice have imposed more important selection pressures on birds than have lice.

Species richness of avian parasites is known to covary with host body size, range size, habitat and phylogeny (Gregory 1997). However, there is much less knowledge about factors influencing species richness of avian lice in particular. Host body size tended to covary positively with louse species richness across host species (Rothschild and Clay 1952). Moreover, past bottlenecks in host population size are known to cause long-lasting decreases in louse richness (Rózsa 1993a; Paterson et al. 1999). Clayton and Walther (2001) showed an interaction between the fine structure of bill tip and louse abundance. Since preening by the bill tip plays a major role in avian defense against lice (Clayton 1991), this result indicates that measures of host defenses may covary with measures of lousiness in birds.

Here, we test whether the richness and abundance of avian lice have evolved in response to interaction with host immune system. We differentiate between two suborders of lice occurring on birds: Ischnocera and Amblycera. As indicated by recent molecular analyses (Johnson and Whiting 2002; Barker et al. 2002), "chewing lice (Mallophaga)" is a paraphyletic taxon, consisting of two major taxa (Johnson and Clayton 2003). Ischnoceran lice live on feather surfaces and tend to graze the non-living keratin of feather barbules. Typically, they have no direct contact with living host tissues, with the exceptions of the preening bill and grooming foot, as host mechanical defenses. In contrast, most Amblycerans tend to walk directly on the host skin, often feed on the excretions and fragments of the skin, and also chew the emerging tips of developing feathers to obtain blood. They may even serve as intermediate hosts of avian microfilarial worms indicating a high frequency of blood feeding (Cohen et al. 1991), which is exceptional among Ischnocerans (but see Barlett 1993). Amblycerans are also more likely to defecate directly onto host skin, while the feces of Ischnoceran lice is less likely to get in contact with host skin. Thus, we expect the avian immune response to interact with Amblyceran infections, while Ischnocerans should evolve more independently of immunological constraints.

As a measure of immune response, we used a standard measure of T-cell mediated immune response to subcutaneous challenge of nestlings at a standardized age with a novel mitogen (phytohemagglutinin). Bird species that suffer from intense parasite-induced nestling mortality have evolved stronger cell-mediated immune responses than species with little or no mortality (Martin et al. 2001). T-cell response based on this test is also a reliable predictor of survival in several studies of birds (Møller and Saino 2004). We used mean abundance (the number of lice divided by the number of birds) and taxonomic richness as measures to quantify "typical levels" of Amblyceran and Ischnoceran burdens of different bird species (Rózsa et al. 2000). These measures are not fully independent; taxonomically richer louse burdens tend to be more abundant as well (Clayton and Walther 2001).

Materials and methods

Host species included

We included all species for which information on T-cell mediated immune response had been published by 31 December 2003 (Casto et al. 2001; Ewenson et al. 2001; Hoi-Leitner et al. 2001; Johnsen et al. 2000; Smits et al. 1999; Soler et al. 1999; Tella et al. 2000, 2002), or for which we or our collaborators had recorded responses to the phytohemagglutinin test (Blount et al. 2003 and the remaining species). We only included altricial host species.

T-cell mediated immune response

During 2000–2002, the first author spent large parts of April–June for searching nests of birds, in which nestlings could be tested for cell-mediated immune response in Southern Spain around Granada and Sierra Nevada and in Northern Denmark. We used the T-cell mediated immune response to a challenge with phytohemagglutinin (Goto et al. 1978; McCorkle et al. 1980; Parmentier et al. 1993; Dietert et al. 1996). Injection with phytohemagglutinin results in local activation and proliferation of T-cells, followed by local recruitment of inflammatory cells and increased expression of major histocompatibility complex molecules (Goto et al. 1978; Abbas et al. 1994; Parmentier et al. 1998). Before injection, we removed the feathers from a small spot of skin on the wing web (patagium) of the right and the left wings and marked the sites of injection with a permanent, water-resistant color marker. Then, we measured the thickness of the skin to the nearest 0.01 mm with a pressure-sensitive caliper (Teclock SM112). For each wing web we made three measurements to quantify measurement error. We found repeatabilities above 0.95. Subsequently, we injected 0.02 mg phytohemagglutinin dissolved in 0.04 ml saline in one wing web, and 0.04 ml physiological water in the other wing web. Approximately 24 h later, we remeasured the thickness of the skin at the two sites of injection, as described above. The index of cell-mediated immune response was simply calculated as the difference in thickness of the wing web injected with phytohemagglutinin 24 h after and just before injection, minus the difference in thickness of the wing web injected with physiological water. We calculated mean responses for each brood and then calculated an overall mean based on these brood mean values. The full data set is provided in the electronic appendix.

We have found highly significant, consistent differences in cell-mediated immune response in nestlings among species (63.4% of the variance among species: $F = 18.55$, df = 41, 371, $P < 0.001$, data from Denmark 2001, Møller et al. 2003). Furthermore, we have found for 18 species with mean estimates available from both Spain and Denmark that there is significantly more variation among than within species in mean

cell-mediated immune response (82.7% of the variance among species: one-way ANOVA: $F = 11.94$, df = 17, 18, $P < 0.001$, Møller et al. 2003). Nestlings were injected at a standard relative age during their ontogeny (when they were two-thirds through their normal nestling period) rather than at a similar absolute age. This procedure ensured that nestlings were tested at a similar developmental stage, which excludes the possibility that the recorded responses are dependent on developmental age. Studies of age-dependent cell-mediated immunity in barn swallow *Hirundo rustica* nestlings have shown little variation in intensity of response during the period 10–16 days (in a species with a 20 days nestling period) (Møller et al. 2003).

A thorough characterization of immunocompetence (the ability to raise an efficient response to a parasite attack) requires that both T- and B-cell-mediated and innate immunity are quantified (National Research Council 1992). However, we suggest that a single measure recorded in a standardized way across a range of species is superior to no measure at all. Furthermore, we note that at the interspecific level T-cell mediated immune response is positively correlated with antibody production to a challenge with sheep red blood cells (Møller et al. 2001).

Mean abundance and taxonomic richness of Amblycerans and Ischnocerans

Mean abundance of the two louse suborders were quantified by adding the number of lice belonging to different species and dividing it by the number of hosts examined. This approach means that we treat louse suborders as different ecological guilds and we calculate the mean abundance of these two guilds rather than that of each louse species. Sample size does not bias the expected value of mean abundance, although its variability increases dramatically at low sample sizes due to random noise (Rózsa et al. 2000). To reduce noise, we only used species with 35 or more individuals (an arbitrary limit) sampled quantitatively for lice. Abundance data were available for 23 bird species from Balát (1966), Blagoveshchensky (1951), Cerny (1970), Fowler and Wlilliams (1985), Lee and Clayton (1995), Rózsa (1990), Rózsa et al. (1996), and Shumilo and Lunkashu (1972).

Widely distributed bird species often host congeneric louse species each restricted to different non-overlapping parts of the host distribution thus exhibiting an allopatric distribution. Thus, parasite species richness of widely distributed bird species would overestimate the true parasite richness that each local bird population has to face (Clay 1964). Therefore, we used genera richness rather than species richness to quantify the taxonomic richness of Amblycerans and Ischnocerans harbored by different bird species. This procedure also partly resolves the problem that species richness would provide an inflated estimate due to uncertainty of the status of morpho-species. The number of louse genera was obtained from Price et al. (2003), Hackmann (1994) and Burley et al. (1991) for 80 bird species.

Host sampling intensity affects number of known parasite taxa, but not mean abundance (Gregory 1990; Walther et al. 1995). Therefore, we also quantified the intensity of parasitological surveys focused on different bird species. For this purpose, we used a computerized database on biological literature published by the Centre for Agriculture and Biosciences International (CABI). The number of hits on host scientific name mentioned with any of the terms "parasit*", "pathogen*", "helminth*", "mite*", "louse", "lice" was used to assess parasitological study intensity (where "*" acts as a truncation sign). Only hits from the titles and abstracts were recorded, and the time range was limited to January 1984–2002. The number of hits ranged from 0 to 72. Body mass for hosts was obtained from our own field measurements or from Dunning (1993).

Statistical methods

Information on abundance and genera richness of lice, and on T-cell response of nestling and adult hosts was not available for all bird species, and sample sizes therefore differ among analyses. Abundance of Amblycera and Ischnocera, cell-mediated immune response and body mass were $\log_{10}$-transformed and study intensity was $\log_{10}(x+1)$-transformed before analysis to achieve distributions that did not differ significantly from normal distributions. We set the significance level at 5%. All values reported are means (SE in parentheses).

Using log-transformed body mass as a covariate in the analyses, we controlled for allometry effects of cell-mediated immune responses. Across species we found a significant positive relationship between $\log_{10}$-transformed immune response and $\log_{10}$-transformed body mass ($F = 25.03$, df = 1, 60, $r^2 = 0.29$, $P < 0.0001$, slope (SE) = 0.26 (0.05)). However, a similar regression between $\log_{10}$-transformed immune response and $\log_{10}$-transformed skin thickness before injection was not significant ($F = 2.25$, df = 1, 60, $r^2 = 0.05$, $P = 0.08$, slope (SE) = 0.20 (0.13)). A multiple linear regression with $\log_{10}$-transformed immune response as the dependent variable and $\log_{10}$-transformed body mass and $\log_{10}$-transformed skin thickness before injection as independent variables revealed a significant partial regression coefficient only for body mass, but not for skin thickness ($F = 11.14$, df = 2, 59, $r^2 = 0.35$, $P < 0.0001$, slope (SE) for body mass = 0.35 (0.08), $t = 4.37$, $P < 0.0001$; slope (SE) for skin thickness = 0.04 (0.12), $t = 0.37$, $P = 0.72$). Use of $\log_{10}$-transformed body mass thus corrects efficiently for interspecific differences in body size without causing any bias due to initial thickness of skin among species.

Phenotypic mean values for species cannot be considered statistically independent observations because cases of convergent evolution are mixed with cases of

similarity due to common ancestry, and, therefore, we calculated statistically independent linear contrasts for each variable according to the method developed by Felsenstein (1985).

We used a composite phylogeny based on information in Sibley and Ahlquist (1990) and Barker et al. (2001) for families and orders, combined with information in Badyaev (1997), Blondel et al. (1996), Cibois and Pasquet (1999), Leisler et al. (1997), Martin and Clobert (1996), Møller et al. (2001), Sheldon and Winkler (1993) and Seibold and Helbig (1995).

We adopted the software CAIC to make the calculations of contrasts (Purvis and Rambaut 1995). All branches were assigned the same length, although a second set of analyses based on uneven branch lengths, assuming a gradual evolution model as implemented in the software by Purvis and Rambaut (1995), produced qualitatively similar results (details not shown). We tested for violations of statistical assumptions by regressing standardized contrasts against their standard deviations (Garland et al. 1992). None of these tests revealed any significant deviations, after Bonferroni adjustment for multiple tests. Contrasts with extreme residuals were deleted from analyses to test for robustness of results, and this did not change any of the conclusions presented here. Similarly, tests using ranked independent variables in cases with extreme residuals did not produce qualitatively different results. Contrasts were analyzed by forcing regressions through the origin, as recommended by Purvis and Rambaut (1995).

Results

Mean abundance and genera richness in the two sub-orders of lice

Amblycerans were almost three times more abundant than Ischnocerans) (Table 1,). Most bird species harbor a very few louse genera with the median being 2 for both Amblycerans and Ischnocerans. The number of Amblyceran genera was positively correlated with the number of Ischnoceran genera across host species (Pearson $r = 0.37$, $t = 3.49$, df = 78, $P = 0.0008$), also when the analysis was based on contrasts ($F = 7.42$, df = 1,77, $r^2 = 0.09$, $P = 0.008$). Excluding extreme values exceeding ±1.96 SD did not change this conclusion ($F = 5.14$, df = 1,72, $r^2 = 0.07$, $P = 0.03$). Host species with high mean abundance of Amblycerans also had high mean abundance of Ischnocerans (Pearson $r = 0.64$, $t = 3.81$, df = 21, $P = 0.001$; contrasts: $F = 5.56$, df = 1,21, $r^2 = 0.21$, $P = 0.03$). Excluding extreme values exceeding ±1.96 SD did not change this conclusion ($F = 21.70$, df = 1,19, $r^2 = 0.53$, $P = 0.0002$).

The numbers of Amblyceran and Ischnoceran genera were only weakly related to study intensity, accounting for a maximum of 13% of the variance (Amblyceran genera richness and study intensity: Pearson $r = 0.35$, $t = 3.35$, df = 78, $P = 0.001$; Ischnoceran genera richness: Pearson $r = 0.34$, $t = 3.24$, df = 78, $P = 0.002$).

Mean abundance of Ischnocerans was not significantly correlated with Ischnoceran genera richness (after controlling for host body mass, study intensity and sample size: Pearson $r = -0.09$, $t = 0.50$, df = 21, $P = 0.63$), also when the analyses were based on contrasts ($F = 5.05$, df = 4,18, $r^2 = 0.53$, $P = 0.007$; partial regression for Ischnoceran genera richness: slope (SE) = −0.04 (0.15), $t = 0.27$, $P = 0.79$). Including Amblyceran genera richness as yet another independent variable revealed a stepwise linear regression model that only included host body mass as a significant predictor of the abundance of Ischnocerans (Fig. 1a; analysis based on

Table 1 Descriptive statistics of mean abundances of Amblyceran and Ischnoceran lice measured across bird species

Variable	Mean	SE	Median	Range	*N*
Mean abundance of Amblycera	2.28	0.90	0.41	0–18.56	23
Mean abundance of Ischnocera	6.54	1.65	1.37	0–27.39	23
No. of genera of Amblycera	1.71	0.11	2	0–4	80
No. of genera of Ischnocera	1.71	0.09	2	0–4	80

N is number of host species

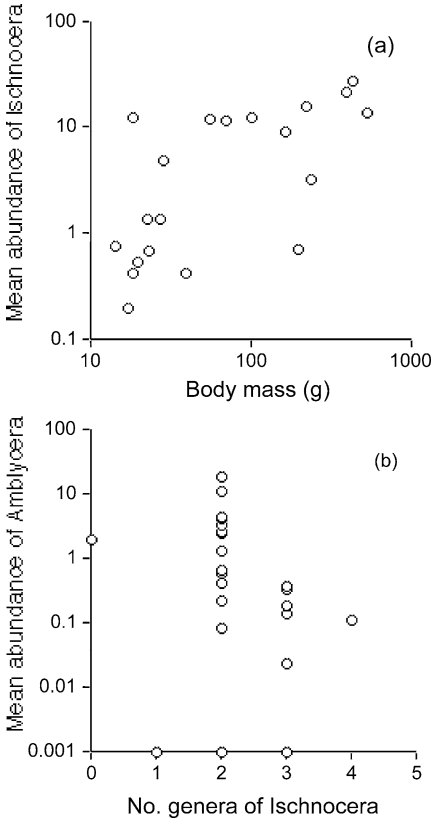

Fig. 1 **a** Mean abundance of Ischnoceran lice in relation to body mass of the host and **b** mean abundance of Amblyceran lice in relation to number of genera of Ischnocera. *Each data point* represents a host species. Note that the *y*-axis in **a** and **b** and the *x*-axis in **a** are logarithmic

contrasts: $F = 19.72$, df = 1,21, $r^2 = 0.48$, $P = 0.0002$, slope (SE) = 1.32 (0.30)). Mean abundance of Amblycerans was not significantly correlated with Amblyceran genera richness (after controlling for host body mass, study intensity and sample size: Pearson $r = -0.20$, $t = 1.05$, df = 21, $P = 0.31$), and this correlation was not significant when based on contrasts ($F = 1.99$, df = 4,18, $r^2 = 0.31$, $P = 0.14$; partial regression for Amblyceran genera richness: slope (SE) = 0.12 (0.08), $t = 1.48$, $P = 0.16$)). Including Ischnoceran genera richness as yet another independent variable revealed a linear regression model that only included Ischnoceran genera richness and host body mass as significant predictors of abundance of Amblycerans (Fig. 1b; analysis based on contrasts: $F = 4.47$, df = 5,17, $r^2 = 0.57$, $P = 0.009$, Ischnoceran genera richness: slope (SE) = −0.30 (0.09), $t = 3.20$, $P = 0.005$; body mass: slope (SE) = 0.99 (0.31), $t = 3.21$, $P = 0.005$).

Richness of louse genera and host T-cell response

Amblyceran genera richness increased significantly with increasing magnitude of nestling T-cell mediated immunity (Fig. 2a; $F = 12.36$, df = 1,56, $r^2 = 0.18$, $P = 0.0009$, slope (SE) = 1.60 (0.46)), while there was no positive relationship for Ischnoceran genera richness (Fig. 2b; $F = 1.53$, df = 1,56, $r^2 = 0.03$, $P = 0.22$). Statistical analyses based on standardized linear contrasts revealed that Amblyceran genera richness also increased significantly with nestling T-cell response (Fig. 2c; linear regression: $F = 13.30$, df = 1,55, $r^2 = 0.18$, $P = 0.0006$, slope (SE) = 2.37 (0.65)). This relationship was not confounded by any extreme values since regressions based on ranks provided very similar results. Exclusion of an extreme negative value for number of Amblyceran genera (Fig. 2c, bottom left) only strengthened the relationship ($F = 15.54$, df = 1,54, $r^2 = 0.22$, $P = 0.0002$, slope (SE) = 2.45 (0.62)). Similarly, the lack of a significant relationship between Ischnoceran genera richness and nestling T-cell response was confirmed by the analysis of linear contrasts (Fig. 2d; linear regression forced through the origin: $F = 0.01$, df = 1,55, $r^2 = 0.00$, $P = 0.91$).

We included the measure of study intensity, the origin of the T-cell data (whether the data originated from wild or captive birds), and body mass as potentially confounding variables in a multiple linear regression based on linear contrasts. The overall multiple regression model for contrasts of Amblyceran genera richness was statistically significant ($F = 9.78$, df = 3,57, $r^2 = 0.34$, $P < 0.0001$). Amblyceran genera richness was still significantly positively correlated with nestling T-cell response (slope (SE) = 1.15 (0.49), $t = 2.35$, $P = 0.02$).

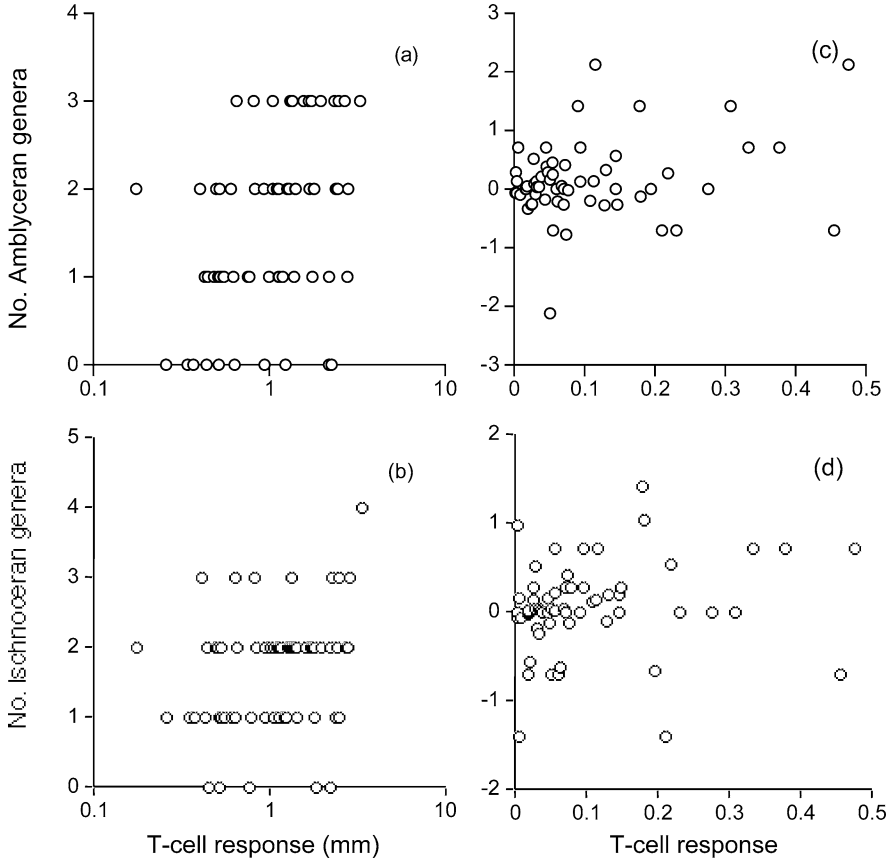

Fig. 2 Number of genera of Amblycera (**a**) and Ischnocera (**b**) in relation to T-cell mediated immune response (mm) of nestling birds. Contrasts in the number of genera of Amblycera (**c**) and Ischnocera (**d**) in relation to contrasts in T-cell mediated immune response (mm) of nestling birds. Note that the *x*-axes in **a** and **b** are logarithmic

Likewise the overall multiple regression model for contrasts of Ischnoceran genera richness was statistically significant ($F = 4.92$, df = 3,57, $r^2 = 0.21$, $P = 0.004$). However, Ischnoceran genera richness was not significantly correlated with nestling T-cell response (slope (SE) = 0.45 (0.41), $t = 1.09$, $P = 0.279$).

Although nestling and adult T-cell response were positively correlated (Pearson $r = 0.60$, $t = 4.06$, df = 30, $P = 0.003$), we determined the importance of age-specific immunity by determining the relationship between louse genera richness (dependent variable) and nestling and adult T-cell response. The overall multiple regression model for Amblycerans was statistically significant (linear regression forced through the origin: $F = 5.54$, df = 4,40, $r^2 = 0.35$, $P = 0.001$). Amblyceran genera richness was still significantly positively correlated with nestling T-cell response (Fig. 3a; slope (SE) = 1.21 (0.62), $t = 2.00$, $P = 0.04$), while the relationship with adult T-cell response was not significant (slope (SE) = −0.72 (0.80), $t = 0.90$, $P = 0.37$). This relationship was not confounded by any extreme values since regressions based on ranks provided very similar results. Exclusion of an extreme negative value for number of Amblyceran genera (Fig. 3a, bottom left) did not affect the relationship ($F = 3.01$, df = 4,39, $r^2 = 0.24$, $P = 0.003$). The overall multiple regression model for Ischnoceran genera richness was statistically significant ($F = 4.42$, df = 4,40, $r^2 = 0.31$, $P = 0.005$). Amblyceran genera richness was still significantly positively correlated with nestling T-cell response (slope (SE) = 1.21 (0.60), $t = 2.01$, $P = 0.04$), while the relationship with adult T-cell response was not significant (slope (SE) = −0.92 (0.81), $t = 1.15$, $P = 0.26$). Ischnoceran genera richness was not significantly correlated with nestling T-cell response (Fig. 3b; slope (SE) = −0.17 (0.45), $t = 0.38$, $P = 0.70$), or with adult T-cell response (slope (SE) = 0.32 (0.58), $t = 0.56$, $P = 0.61$). Thus, the number of genera of Amblyceran lice increased with increasing T-cell response of nestlings, but showed no significant relationship with T-cell response of adult hosts.

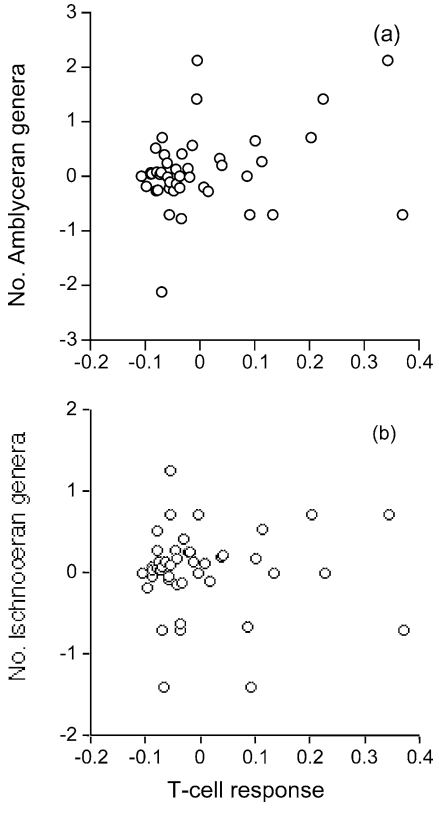

Fig. 3 Contrasts in number of genera of Amblycerans (**a**) and number of genera of Ischnocerans (**b**) in relation to contrasts in T-cell mediated immune response of nestlings after controlling for contrasts in adult T-cell mediated immune response

Discussion

Amblyceran genera richness increased with the strength of cell-mediated immune response of their nestling hosts, while that was not the case for Ischnoceran lice. This study is the first to suggest a relationship between Amblyceran louse richness and avian immune response. This raises the question whether birds control the Amblyceran lice by means of T-cell mediated immune responses. It is also the first study of any group of parasitic organisms showing a relationship between taxonomic richness and levels of host defense. In our study Ischnocerans served as a natural control group because this sub-order does not interact with hosts at the sub-epidermal level, contrary to what is the case for Amblycerans. We found no relationship between host immune response and richness of this control group, as predicted. Presuming that the relationship between Amblyceran genera richness and T-cell mediated immune response is due to a direct interaction between hosts and parasites, one can argue that large Amblyceran richness also select for increased investment in T-cell mediated immune responses of avian hosts. However, an opposite direction of causality cannot be excluded; we hypothesize that strong host immune responses select for specialization and hence diversification in Amblyceran lice. Hosts may have evolved strong immune responses due to interactions with other, more virulent parasites, and subsequently acquired a more diverse assemblage of Amblycerans. These two hypotheses are not mutually exclusive, and our results do not allow us to test the direction of causality. However, we hypothesize that the latter direction of causality appears to be more likely; i.e. Amblyceran richness evolved in response to efficient host defenses that had evolved during interactions with other, more virulent parasites.

Factors other than host immunity also account for interspecific differences in parasite taxonomic diversity. Previous studies have suggested that size, density and age of the host population can be important determinants of parasite richness (Dritschilo et al. 1975; Strong et al. 1977; Gregory 1990; Ebert et al. 2001). This possibility does not detract from the general interest of our

study since we found clear differences in patterns of richness of Ischnoceran and Amblyceran lice that cannot be accounted for by any of these factors, since the same assemblage of hosts was investigated for the two suborders of parasites.

Not only the magnitude, but also the timing of particular immune reactions within the host life cycle is relevant for their parasites. Lice are often claimed to be more heavily dependent on the parent-offspring transmission route than other avian ectoparasites (see e.g. Clayton and Tompkins 1994, 1995 and references therein). Since parasites are most vulnerable at the invasion of a new host individual and the foundation of a new population there, we propose that the above claim is further supported by our present results. We demonstrated a positive covariation of Amblyceran richness with the nestling immune responses, while no similar relationship appears to exist with the adult immune responses.

The positive correlation between louse abundance and taxonomic richness was already known from previous studies (Clayton and Walther 2001). However, our present results suggest that it is not abundance and richness of lice in general, but Amblyceran abundance that correlates positively with Ischnoceran taxonomic richness. This finding points at a particular shortcoming of most former ecological analyses on louse assemblages; authors typically do not differentiate between suborders of lice, rather they treat lice as a homogeneous ecological guild (but see Clayton et al. (1992) for an exception).

Previous experimental studies repeatedly showed that the size of avian louse burdens is effectively limited by host mechanical defenses (Clayton 1991; Rózsa 1993b; Clayton et al. 1999). While we do not doubt that mechanical defenses also affect Amblyceran lice, we note that these experiments were focused on Ischnocerans, and it remains unknown to what extent this result is valid for Amblycerans. Our results indicate that Amblycerans coevolve with the avian immune system.

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