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Are Lice Associated with Ring-billed Gull Chick Immune Responses?

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Abstract.—Little is known about whether chewing lice (Insecta: Phthiraptera) suppress or activate immunity in birds. Here, relationships between lice (*Quadraceps punctatus*, *Saemundsonnia lari* and *Austromenopon transversum*) and immune parameters were evaluated in Ring-billed Gull (*Larus delawarensis*) chicks. Eosinophils made up greater proportions of leukocytes in chicks with more lice (partial $r = 0.64$, $P < 0.001$), suggesting louse-induced immunoactivation via either eosinophil production or redistribution to peripheral blood. However, there were no significant relationships between lice and other immune parameters (proportion heterophils, proportion lymphocytes, heterophil:lymphocyte ratio, response to injection with phytohaemagglutinin). Collectively, results suggest only minor immune responses to lice, but experimental manipulations of louse infestations are needed to better quantify these responses and evaluate their potential consequences for fitness. *Received 17 June 2011, accepted 16 December 2011.*

Key words.—Amblycera, chewing lice, immunity, Ischnocera, *Larus delawarensis*, Phthiraptera, phytohaemagglutinin, Ring-billed Gull.

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By definition, parasites reduce host fitness (Anderson and May 1979; Clayton and Moore 1997). One way in which hosts' fitness can be reduced is via diversion of energy from, for example, growth into immune function to fight parasites (Sheldon and Verhulst 1996; Lochmiller and Deerenberg 2000; Costantini and Møller 2009). Another is if parasites suppress immune function, making hosts susceptible to other parasites (Wikel *et al.* 1994; Jackson *et al.* 2009). Effects of lice (Insecta: Phthiraptera) on avian host immune functions are poorly studied. Here we evaluated whether lice are associated with upregulated (immunoactivated) or downregulated (immunosuppressed) immune functions in Ring-billed Gull (*Larus delawarensis*) chicks.

Immunoactivation may indicate either pre-emptive immune investment against future parasites or active attempts to combat current parasites (Norris and Evans 2000). Immunoactivation may be reflected in larger immune organs, higher concentrations of immune components in blood, more robust responses to experimental immune challenge, and altered leukocyte profiles (Shutler *et al.* 1999; Norris and Evans 2000; Ardia 2005). Positive correlations between host immune function and parasites would be consistent with immunoactivation. Negative correlations would be consistent with either immunoactivation having successfully defended against previous parasites, or with current parasites causing immunosuppression.

Birds can be hosts to chewing lice of the Phthiraptera suborders Amblycera and Ischnocera (Price *et al.* 2003). Both immunoactivation (Wikel 1996; Owen *et al.* 2010) and immunosuppression (e.g. Wikel *et al.* 1994) have been reported for various external (ecto-) parasites of vertebrates but little is known about whether lice have these effects on birds. Intuitively, immunoactivation may only be provoked by amblycerans because they feed on blood, bringing them into direct contact with host immune systems (e.g. Møller and Rózsa 2005; Whiteman *et al.* 2006; Moreno-

Rueda 2010). In contrast, ishnocerans are suspected of feeding only on feathers and dander, presumably out of reach of host immune systems. However, louse diets on birds have not been well studied. Moreover, direct contact between ectoparasites and host immune systems is not required for immunoactivation in mammals (James 1999). Evidence is equivocal for relationships between louse parasitism and avian immune function (Saino *et al.* 1995; Møller *et al.* 1996; Eens *et al.* 2000; Blanco *et al.* 2001; Prelezov *et al.* 2002, 2006; Møller and Rózsa 2005; Whiteman *et al.* 2006), indicating that further data are needed.

We quantified chewing louse abundance (number of parasites per host including uninfected hosts; Bush *et al.* 1997) and measured immune function in Ring-billed Gull chicks to test whether lice are immunoactivational or immunosuppressive. Immunoactivation would suggest that lice impose fitness costs directly, whereas immunosuppression would suggest that costs may arise indirectly, predicting that birds with lice will have higher abundances of other parasites.

STUDY AREA AND METHODS

Ring-billed Gull chicks, estimated to be between 20 and 35 d of age (Ryder 1993), were captured by hand from a colony of <100 pairs at Indian Point Sandhills West on Prince Edward Island, Canada between 1 and 15 July 2003. Head-bill length (Dzubin and Cooch 1992) was measured to the nearest 0.01 cm using calipers, and chicks were banded with numbered Canadian Wildlife Service bands.

To evaluate relative immune investment in leucocytes, a sterile 25-gauge needle was used to puncture the meta-tarsal vein, and a single drop (<50 µl) of blood was transferred to a microscope slide. A sterile piece of cotton was applied to the puncture site until bleeding stopped. Blood smears were stained using Hema 3 (Biomedical Sciences, USA). At 1000x magnification, we classified the first 200 leukocytes (excluding thrombocytes) encountered as basophils, eosinophils, heterophils, lymphocytes, or monocytes. Leukocyte proportions were the number of a leukocyte type counted divided by 200. H/L ratios were numbers of heterophils divided by numbers of lymphocytes. Repeatabilities (Moreno *et al.* 1998) on a subsample of 21 blood smears indicated that heterophils, lymphocytes, and eosinophils were repeatable ($P < 0.004$) whereas monocytes and basophils were not (Ps ≥ 0.22); the latter were excluded from analyses.

Approximately 1.0 x 0.5 cm of the middle of leading edges of right patagia were plucked clean of feathers so that patagial thickness could be measured (nearest 0.01 mm) with pressure-sensitive calipers (Mitutoyo, Japan). Centers of plucked areas were swabbed with 70% ethanol, allowed to dry, and then injected with 0.2 µg of phytohemagglutinin (PHA) in 0.2 ml of phosphate-buffered saline (Smits *et al.* 1999). We returned 24 h later to measure patagial thickness at injection sites three to six times; means (of the three largest if >three measurements; Brinkhof *et al.* 1999) were used in analyses. PHA responses were differences between pre- and post-injection patagial thickness.

Lice were sampled by dust-ruffling (Walther and Clayton 1997) wherein one massages plumage with flea powder (Puroguard Insecticides Ltd., Dorion, Quebec) for 5 min. over a collecting tray. All chicks were returned to the colony following dust-ruffling. Contents of collecting trays were stored in 70% methanol in sealed containers until counted under a dissecting microscope in the lab after the field season. Specimens were sent to T. Galloway, University of Manitoba, for identification.

Forty-one chicks were dust-ruffled but twelve were excluded due either to chick feces in samples that impaired louse counts, or due to missing head-bill length measurements. Head-bill length was included as an independent variable in all models to control for chick age and body size. Except where indicated, variables were transformed to produced normal distributions (Shapiro-Wilk tests, Ps ≥ 0.09); in the text we refer only to original variable names. To determine if total louse abundance was associated with immune parameters, we used multiple regression with square root total louse abundance as the dependent variable and a single immune parameter (arcsine square root eosinophil proportion, $\log_{10}$ heterophil proportion, arcsine square root lymphocyte proportion, $\log_{10}$ H/L ratio, or PHA response) and head-bill length as independent variables.

Relationships were also tested within each suborder of lice. Square-root ischnoceran abundance was normally distributed (W = 0.96, P = 0. 25) and relationships were tested as above. Amblyceran abundance fit a negative binomial distribution ($\chi^2_5 = 4.2$, $P = 0.53$); thus with amblyceran abundance as the dependent variable, we used generalized linear models (GLMS) with negative binomial error distribution and log link function. For immune parameters correlated with both louse suborders, we determined whether relationships with amblyceran abundance were independent of ischnoceran abundance using a GLM (negative binomial error distribution and log link function) with amblyceran abundance as the dependent variable and an immune parameter, square root ischnoceran abundance, and head-bill length as independent variables. We tested whether the relationship with ischnoceran abundance was independent of

Table 1. Louse infestation parameters for 29 Ring-billed Gull chicks (Min = minimum, Max = maximum).

Parasites	Abundance					Prevalence (%)	Proportion of total lice
	Min	Max	Median	Lower limit of interquartile range	Upper limit of interquartile range		
Quadraceps punctatus	1	35	11	4	20	100.0	77.7
Saemundsonnia lari	0	5	1	0	3	65.5	9.8
Austromenopon transversum	0	17	0	0	2	41.4	12.5
Total lice	1	48	13	8	24	100.0	

Table 2. Descriptive statistics for immune parameters and head-bill length for 29 Ring-billed Gull chicks (Min = minimum, Max = maximum).

Trait	Min	Max	Median	Lower limit of interquartile range	Upper limit of interquartile range
Eosinophil proportion	0.00	0.13	0.04	0.02	0.06
Heterophil proportion	0.15	0.70	0.32	0.24	0.38
Lymphocyte proportion	0.23	0.78	0.61	0.49	0.71
H/L ratio	0.19	3.0	0.52	0.34	0.79
PHA response (mm)	0.003	0.049	0.020	0.014	0.027
Head-bill length (cm)	7.10	9.14	8.14	7.79	8.43

amblyceran abundance using a multiple regression model with an immune parameter as the dependent variable and square root ischnoceran abundance, amblyceran abundance, and head-bill length as independent variables.

RESULTS AND DISCUSSION

Descriptive statistics for variables are in Tables 1 and 2. After controlling for head-bill length, proportions of white blood cells that were eosinophils were positively correlated with total louse abundance, and with abundance of each suborder of lice (ishnocerans: Table 3, Fig. 1a and b; amblycerans: Table 4, Fig. 1c). After controlling for ischnoceran abundance, the correlation between amblyceran abundance and eosinophil proportion remained significant (estimate = 12.0, SE = 4.6, $z = 2.6$, $P = 0.009$) and after controlling for amblyceran abundance the correlation between ischnoceran abundance and eosinophil proportion remained significant (partial $r = 0.43$, $t = 2.4$, $P = 0.03$). Parasitism by amblyceran and ischnoceran lice thus appeared to cause immunoactivation that included increases in, or redistribution of, eosinophils. Although avian eosinophils are poorly understood (Maxwell 1987), they appear to be involved in responses to chewing lice and other ectoparasites (Saino *et al.* 1995, 1998; Prelezov *et al.* 2002, 2006). Alternatively, chicks with low eosinophil proportions may have had better primary defenses (e.g. grooming) or decreased exposure to lice.

There were no correlations between any of our other immune measures and total, is-

Table 3. Results from multiple regression models with (a) total louse abundance and head-bill length, or (b) ischnoceran abundance and head-bill length as independent variables and an immune parameter as the dependent variable. Chicks (N = 29) with high abundances of total lice and ischnoceran lice had a higher proportion of leukocytes that were eosinophils.

	Eosinophil %		Heterophil %		Lymphocyte %		H/L ratio		PHA response	
	Partial r	P	Partial r	P	Partial r	P	Partial r	P	Partial r	P
(a) Total louse model										
Total louse abundance	0.64	<0.001	-0.03	0.87	-0.13	0.50	0.03	0.89	-0.13	0.50
Head-bill length	-0.42	0.03	-0.15	0.43	0.15	0.44	-0.12	0.55	0.35	0.07
(b) Ischnoceran model										
Ischnoceran abundance	0.57	0.002	-0.03	0.90	-0.14	0.47	0.04	0.83	-0.15	0.45
Head-bill length	-0.39	0.04	-0.15	0.43	0.16	0.43	-0.12	0.53	0.35	0.07

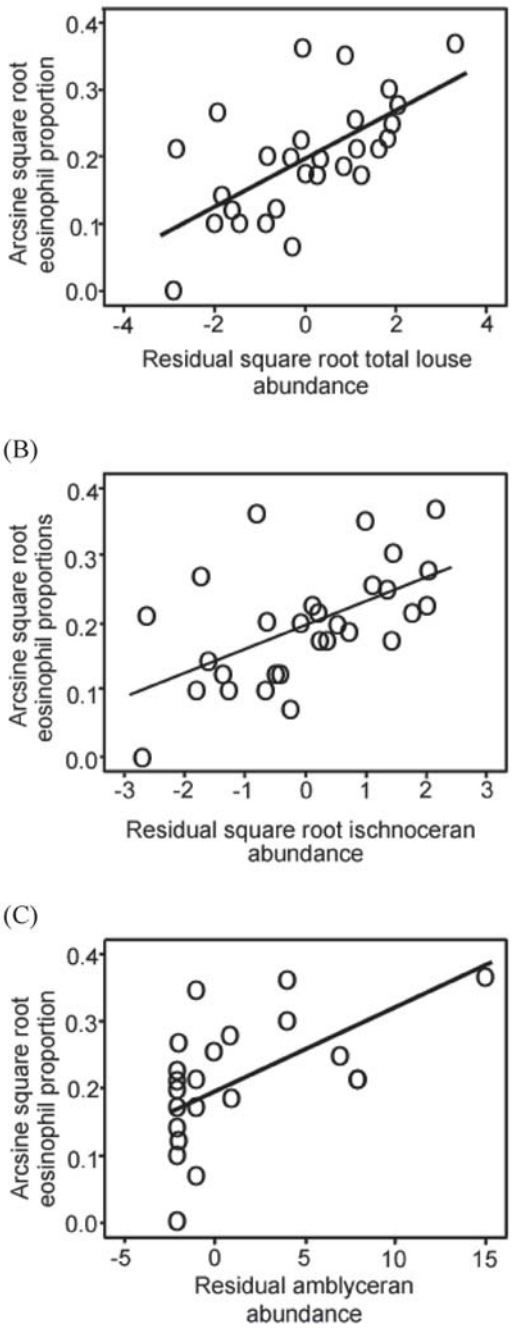

Figure 1. Relationship between proportion eosinophils and (a) total louse abundance, (b) ischnoceran abundance, and (c) amblyceran abundance when head-bill length was controlled for. N=29.

chnoceran (Table 3), or amblyceran (Table 4) louse abundance. Immune variables we measured can be associated with parasitism in other systems (Saino *et al.* 1998; Prelezov *et al.* 2002; Boughton *et al.* 2006; references in Owen and Clayton 2009; Pap *et al.* 2011; but see Saino *et al.* 1998; Morales *et al.* 2004; Pap *et al.* 2011; Jacquin *et al.* 2011 who found no relationships). Some aspects of immunoactivation may not be detected if an initial host response successfully removes lice; this would be detected as the opposite pattern where enhanced immune responses would be associated with lower parasite loads; note that none of our results were consistent with this. If louse infestations were fairly recent, there may have been insufficient time for immunoactivation or immunosuppression; this would produce non-significant associations, as we observed. However, it is unlikely that eosinophils would react faster than other aspects of immunity that we measured (Sorci and Faivre 2005). Other unresolved issues are whether avian immune systems can control lice (e.g. for PHA response, see Owen and Clayton 2009), or whether there are threshold lice infestations that affect immune investment. Finally, several other factors could influence immune function, such as unmeasured parasites, nutrition, or social interactions, and these could have overwhelmed the associations we measured. The fact that we detected immunoactivation of eosinophils is thus perhaps telling.

In conclusion, our results provide some evidence that lice may be immunoactivational in Ring-billed Gull chicks, but no evidence that lice are immunosuppressive. These results suggest that further evaluation of effects of lice on both wild and domestic birds, particularly using manipulations of louse infestation or host immune function, may reveal important fitness costs to wild and domestic birds from lice that heretofore have received little attention.

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Table 4. Results of generalized linear models (negative binomial error distribution and log link function) with a single immune parameter and head-bill length as independent variables and amblyceran abundance as the dependent variable. Chicks (N = 29) with high abundance of amblyceran lice had a higher proportion of leukocytes that were eosinophils.

	Estimate	SE	*z*	*P*
Eosinophil model				
Eosinophil proportion	15.9	4.1	3.8	<0.001
Head-bill length	1.0	0.7	1.5	0.14
Heterophil model				
Heterophil proportion	-1.8	2.4	-0.7	0.47
Head-bill length	-0.2	0.8	-0.3	0.78
Lymphocyte model				
Lymphocyte proportion	-0.2	2.8	-0.1	0.95
Head-bill length	<0.1	0.8	<0.1	>0.99
H/L ratio model				
H/L ratio	-1.1	1.4	-0.7	0.47
Head-bill length	-0.2	0.8	-0.3	0.79
PHA response model				
PHA response	-15.4	36.1	-0.4	0.67
Head-bill length	0.0	0.9	0.0	0.91

Two anonymous reviewers improved the manuscript. Procedures were approved by the Acadia Animal Care Committee (Approval #17-03) and Canadian Wildlife Service Permits (10681 E and SC2243).

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