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Size matters for lice on birds: Coevolutionary allometry of host and parasite body size

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Body size is one of the most fundamental characteristics of all organisms. It influences physiology, morphology, behavior, and even interspecific interactions such as those between parasites and their hosts. Host body size influences the magnitude and variability of parasite size according to Harrison's rule (HR: positive relationship between host and parasite body sizes) and Poulin's Increasing Variance Hypothesis (PIVH: positive relationship between host body size and the variability of parasite body size). We analyzed parasite–host body size allometry for 581 species of avian lice (~15% of known diversity) and their hosts. We applied phylogenetic generalized least squares (PGLS) methods to account for phylogenetic nonindependence controlling for host and parasite phylogenies separately and variance heterogeneity. We tested HR and PIVH for the major families of avian lice (Ricinidae, Menoponidae, Philopteridae), and for distinct ecological guilds within Philopteridae. Our data indicate that most families and guilds of avian lice follow both HR and PIVH; however, ricinids did not follow PIVH and the "body lice" guild of philopterid lice did not follow HR or PIVH. We discuss mathematical and ecological factors that may be responsible for these patterns, and we discuss the potential pervasiveness of these relationships among all parasites on Earth.

KEY WORDS: Harrison's rule, Phthiraptera, phylogenetic generalized least squares models, Poulin's increasing variance hypothesis.

> Yet, however much we celebrate diversity and revel in the peculiarities of animals, we must also acknowledge a striking "lawfulness" in the basic design of organisms. This regularity is most strongly evident in the correlation of size and shape.
> Gould (1974)

A century ago Harrison (1915) published a study on kiwi lice and concluded that species of lice specific to large-bodied host species tend to be large themselves. This hypothesis, often called Harrison's rule (HR), was originally proposed only for congeneric louse species, that is, in comparisons of closely related parasites sharing similar morphologies and life histories. Since then, HR has been documented in a wide variety of parasitic organisms including nematodes (Kirchner et al. 1980; Harvey and Keymer 1991; Morand et al. 1996; Morand and Sorci 1998), rhizocephalan barnacles (Poulin and Hamilton 1997), fleas, flies, lice, ticks, mites, as well as herbivorous insects associated with specific host plants (Harvey and Keymer 1991; Kirk 1991; Morand et al. 2000; Johnson et al. 2005).

More recently Poulin noted that, while large parasite species inhabit large-bodied host species, small parasite species can infest both small and large-bodied hosts (2007). Consequently, Poulin hypothesized that in comparisons across host species, not only the mean, but also the variance in the body size of parasite species should increase with increasing host body size. In addition to the ecological mechanism described by Poulin (2007), we also expect that there will be greater variation in parasite body size on larger hosts due to a self-evident allometric relationship (a scaling effect due to an allometric "biological power law"; Xiao et al. 2011). Indeed, authors routinely apply log transformations when illustrating allometric relationships to remove this effect. These transformations allow the investigation of size-independent relative variability. We refer to the increase in relative variability with increasing host body size (independent of the scaling effect) as Poulin's Increasing Variance Hypothesis (PIVH).

Biological principles are known to govern body size. For example, body size is directly related to fecundity: all else being equal, larger organisms bear more offspring (Honěk 1993). In parasites, an increase in body size associated with increased fecundity may also drastically influence parasite virulence (Poulin 2007). A thorough understanding of the predictive value and domain of validity of HR and PIVH is therefore crucial to our understanding of host–parasite interactions and parasite ecology in general.

Former authors have tested for HR among mammals and their lice. These studies found relationships consistent with HR for both chewing lice (Trichodectids; Harvey and Keymer 1991) and sucking lice (Anoplurans; Cannon 2010). Birds are also infested with chewing lice. Studies of louse and host body size among birds and chewing lice have supported HR among relatively small clades of lice, for example, species of lice in the genus *Actornithophilus* (Kirk 1991) and species of lice in the genus *Columbicola* (Johnson et al. 2005). Johnson et al. (2005) also sampled 78 species of lice from a wide variety of avian orders and found that, as a group, avian lice adhered to HR.

There are, however, a few known exceptions to HR, such as Monogeneans (Simková et al. 2001). In case of avian lice, different ecological guilds within philopterids exhibit different parasite–host body size relationships. Johnson et al. (2005) showed that HR was valid for *Columbicola* wing lice but not for Physconelloidinae body lice, and Yamagishi et al. (2014) found that HR was valid for *Quadraceps* wing lice but not for *Saemundssonia* head lice parasitizing two species of *Larus* gulls. Among published studies, lice seem to adhere to HR in most cases; however, we do not know whether a publication bias (failure to publish nonsignificant or negative results) distorts this view.

Avian lice (Phthiraptera: Amblycera, Ischnocera) are optimal candidates for studying host–parasite body size allometries because they lack free-living stages; they spend their whole life cycle attached to the host body. Moreover, they constitute a species-rich and intensively studied taxon thus enabling large sample sizes and appropriate statistical power for analyses. Four families infest avian hosts. Menoponids and philopterids are diverse and widely distributed across the whole class of birds. Contrarily, laemobothriids and ricinids are relatively homogenous groups. The former one is restricted to a few large-bodied orders of birds, whereas ricinids parasitize only small-bodied Passerines and hummingbirds.

There are four common "guilds" or "ectomorphs" of lice within the feather louse family Philopteridae: "body lice,", "generalist lice," "head lice,", and "wing lice." Although lice in these guilds do not represent monophyletic clades (Johnson et al. 2012, Bush et al. 2016), they do share distinct morphological and behavioral characteristics that appear to be adaptations for particular microhabitats on the body of their hosts. Head lice have plump bodies and large triangular heads, with which they grasp feathers to avoid being detached when the host scratches. Wing lice have long slender bodies and they can insert their bodies in the spaces between adjacent feather barbs to avoid being removed during preening, which is the bird's principle defense against ectoparasites. Body lice have round bodies and heads and live in the fluffy parts of abdominal contour feathers where they burrow into the downy feather matrix to avoid preening. In contrast, generalist lice have intermediate body shapes and appear to escape from preening by running quickly (Clayton et al. 2016).

Our aim is to test HR and PIVH across a large sample of avian louse species while controlling for both host and parasite phylogenies with advanced biostatistical methodologies. Because topologies of bird and louse phylogenetic trees tend to differ considerably (Clayton et al. 2003), we controlled for their presumed effects separately. We take a comprehensive approach, using data from 581 louse species and 678 louse lineages, which is roughly 15% of the known diversity of avian lice (Price et al. 2003) representing three louse families, and all four major guilds of philopterid lice.

Materials and Methods

DATA COLLECTION

Louse body sizes were obtained from published species and subspecies descriptions (for a complete list of taxonomic literature used in this study see Supporting Information), mostly from species descriptions that were freely available for us during the data gathering period of the present work (mostly 1998–2008). Note that there is generally a sampling bias in taxonomic publications. For most organisms, large species are typically described first. Lice are no exception. The lice of small-bodied bird species are clearly undersampled and underdescribed (Price et al. 2003). We did, however, obtain a large proportion of all existing species descriptions (see next).

We used only one measure of louse body size: the log-transformed total body length of adult females. Male body size was not used because it is more strongly subjected to sexual selection (Tryjanowski et al. 2009), a phenomenon outside the focus of our present study. Whenever we had more than one source of data for the body length of the same lice from the same host species, we averaged values. Most of the data for parasite body size is at the scale of louse species, however, when available, data regarding distinct louse subspecies or populations associated with particular host species were included as separate louse lineages.

When possible, we linked the measurements of each louse species or lineage to the species of bird from which they were collected. However, this was not always possible. Some species of lice are associated with multiple bird species, and the taxonomic literature does not always provide distinct measurements of lice based on each host association. In such cases, we linked parasite data to the taxonomical "type host" (Price et al. 2003). For mathematical necessities, whenever a bird species was linked to two or more louse species in a particular analysis, we randomly selected one of the lice and we repeated the same process 20 times to measure the sensitivity of the parameters of the fitted models to the random selection. We give the relative SDs as percentages of the estimated values as the measure of sensitivity.

Our first analysis focused on louse families. The family Laemobothriidae is a small family (20 known species, Price et al. 2003), and was represented by few samples in our dataset (17 lineages of two species); therefore, we excluded this family from all analyses. Our final dataset was composed of 221 menoponid species, 317 philopterid species, and 43 ricinid species. Overall, a total of 581 louse species were represented in our study that comprise roughly 15% of all known species of avian lice (Price et al. 2003). Many of these species were further subdivided into subspecies and host-specific populations, thus samples involved 375 menoponid, 514 philopterid, and 100 ricinid lineages. Actual sample sizes in analyses are lower (and differ across different analyses) because species of lice associated with multiple host species were subsampled (as described above). In the sensitivity analyses the sample sizes varied between 240 and 263, 331 and 352, 82 and 93 in case of menoponids, philopterids, and ricinids, respectively.

Our second analysis focused on the ecological guilds within philopterid lice. In this family, markedly different body shapes characterize lice that inhabit different anatomic locations in the plumage, as described above. We followed Johnson et al. (2012) in grouping philopterid genera into four distinct ecomorphs or guilds: body lice, generalists, head lice, and wing lice, represented in our dataset by 44, 127, 123, and 107 lineages, respectively. These groups are not monophyletic units; they are ecological guilds including distantly related genera that exploit the same class of environmental resources in a similar way (Simberloff and Dayan 1991). In these analyses, we excluded genera that were not classified into any of these guilds by Johnson et al. (2012). In the sensitivity analyses, the sample sizes varied between 106 and 113, 103 and 113, 93 and 99 for generalists, head lice, and wing lice, respectively.

Avian body size was expressed as log-transformed body mass obtained from Dunning (2008). If the bird species was sexually dimorphic, a mean of male and female body mass was used.

ANALYSES WITH AND WITHOUT PHYLOGENETIC CONTROLS

Previous studies of HR that have applied a statistical control for phylogenetic artifacts raise an important question: should we control phylogenetic effects using the parasite phylogeny, the host phylogeny, or both? Some authors have contended that is best to compare only regions of host–parasite phylogenies that are cophylogenetically congruent (Harvey and Keymer 1991, Morand et al. 2000), restricting datasets in this way ensures that comparisons are simultaneously controlling for both the phylogenetic history of both the host and the parasite. However, given that the bird and louse phylogenies are seldom strongly congruent (Johnson et al. 2002, Clayton et al. 2003, 2016, Bush et al. 2016), a vast proportion of louse diversity would be excluded if only taxa associated with cospeciation events were considered. Moreover, louse body size may exhibit drastic morphological changes within a few generations (Clayton et al. 1999). Is it relevant to incorporate host phylogenetic effects into statistical models when studying a labile trait? Taking this into consideration some studies of HR in avian lice have conducted phylogenetically independent comparisons using only the parasite phylogeny. From a mathematical point of view, this approach seems reasonable because it controls for phylogenetic dependence among the response species. However, because correlations among the parasite lineages may ultimately originate from phylogenetic dependence of the hosts, models fitted to host phylogeny capture a different aspect of phylogentic dependence than models controlled for parasite phylogeny. Therefore, we conducted analyses in four ways: (1) without any phylogenetic control, (2 and 3) with a control for avian phylogenies based on Hackett et al. (2008) and Ericson et al. (2006), and (4) a control for parasite phylogeny.

AVIAN PHYLOGENY

We applied PGLSs model (Garamszegi 2014) to control for host phylogenetic effects in tests of HR and PIVH. We used the phylogeny of birds in nexus format extracted from the BirdTree database (http://www.birdtree.org) that was derived from the global avian phylogeny of Jetz et al. (2012). As suggested by Rubolini et al. (2015), 1000 trees for Hackett et al. (2008), and 1000 trees for Ericson et al. (2006) were downloaded to represent possible phylogenetic affinities for the host birds. We carried out this procedure for the three louse families taken together, and also for the hosts of those philopterids that were classified into guilds. DendroPy Python library (Sukumaran and Holder 2010) was used to calculate 50% majority-rule consensus (MRC) trees. We applied these MRC trees during the further computations. All avian phylogenetic trees were fully resolved (i.e., had no polytomy) and included branch lengths. In each analysis, we used all data from birds that hosted only one louse species, and we randomly sampled one louse species from those species of birds that hosted more than one species of louse.

PARASITE PHYLOGENY

We compiled molecular phylogenies of lice using genetic sequences available in GenBank. Relatively few of the genera and species of lice in our study were represented by any genetic sequences, and the available sequences were relatively short, and thus carried little phylogenetic information. Partial segments of coding mitochondrial COI (Cytochrome c oxidase subunit I) and nuclear elongation factor alfa and nuclear elongation factor alfa (EF1-α) genes were the sequences best represented (both shorter than 400 bp). From these data, we built Bayesian inference phylograms based on either or both of these sequences in MrBayes 3.1 (Ronquist and Huelsenbeck, 2003), using substitution models estimated by BIC model selection in the Partition Finder 1.1 (Lanfear et al. 2012). In each dataset (COI, EF, and COI + EF concatenated), two independent Metropolis-coupled Markov chain Monte Carlo analyses, each with six incrementally heated chains, were run in MrBayes for 10 million generations, sampling every 1000 generations with the first 25% of samples excluded as burn-in. Convergence was assessed through examination of the SD of split frequencies, consensus phylograms were computed after discarding burn-in period trees. All the resulted trees had a high uncertainty, with high proportion of polytomies and low PP (posterior probabilities) supports, even after several changes in the parameters, thus they were basically unsuitable for our purposes (details not shown).

Therefore, instead of relying exclusively on molecular phylogenies, we constructed an estimation of louse phylogeny that is partially based on the taxonomic hierarchy of the lice, particularly at lower taxonomical levels. Naturally, louse taxonomy itself is also based on molecular phylogenies to a certain degree; however, it also incorporates information from many other aspects of louse biology, and morphology in particular. The phylogeny of major Amblyceran groups, and especially that of menoponid genera, follows Marshall's (2003) phenotypic classification. Our ricinid tree follows Lonc's (1990) cladistic Wagner tree, published as a collateral result of her studies on the phenetic relationships. Note that this tree differs significantly from the species group concept of Nelson (1972), Rheinwald (1968), and Price et al. (2003), and instead follows the phylogenetic relationships among Ischnoceran genera that were proposed by Smith (2000, 2001). In addition to this, Cruickshank et al.'s (2001) molecular data were used to join the *Penenirmus* + *Pseudolipeurus* clade as sister clade of *Strigiphilus* + *Philopterus* complex, the (*Austrophilopterus* + *Cuculicola*) + *Picicola* clade as a sister clade of *Cuclotogaster*, and the genus *Formicaricola* as a sister clade of *Paragoniocotes*. The *Formicaricola* + *Paragoniocotes* clade is a sister of the genus *Psittaconirmus*, whereas *Forficuloecus* was joined as a sister clade of *Neopsittaconirmus* (as in Price and Johnson 2007). Genera within the *Philopterus* complex follows systematics of Mey (2004). The genera *Acidoproctus*, *Ornithobius*, and *Bothriometopus* were united into a monophyletic clade following Cicchino and Mey (2007). Finally, Page et al. (2003) was followed to resolve relationships within the *Philoceanus* complex. Within *Pectinopygus*, cormorant (*Phalacrocorax*) lice were considered to be more closely related to each other, and darter (*Anhinga*) lice were considered to be relatively closer to them than to the gannet (*Morus*) lice (Hughes et al. 2007). Within each genus, subgenera (if any), species, and subspecies (if any) of Price et al. (2003) were accepted as relevant phylogenetic branches. Finally, host-specific populations were considered phylogenetic lineages, with the assumption that these populations are more-or-less isolated on particular host species. The majority of branching points occurred below the genus level, and thus were mostly based on systematical decisions using classical taxonomy rather than molecular studies. Compared to our avian phylogenies, the louse tree carried less phylogenetic information because it had no branch length values and polytomies were common.

The datasets (as "comma-separated value" files) and phylogenies including all birds (in nexus format) and lice (in CAIC format) used in the present study, from which the trees for the different datasets were derived, are available in the Supporting Information.

STATISTICAL METHODS

All statistical analyses were performed in R 3.3.1 (R Core Team 2016). To account for phylogenetic nonindependence and variance heterogeneity, PGLSs models were applied using the package "nlme" (Pinheiro et al 2014). The log-transformed female louse body length was the response variable in all cases and we used the log-transformed host body masses and the grouping factor (either louse families or guilds) and their interactions as explanatory variables. The models were fitted with (PGLS) and without (GLS) a phylogenetic correlation structure (corPagel in package "ape"; Paradis et al. 2004). The λ parameter of the Pagel model characterizing the phylogenetic signal was estimated through maximum likelihood estimation within the PGLS model. The models contained a power variance function structure (Pinheiro and Bates 2000) allowing for changing variance in function of the logarithm of host body mass. The models were fitted using different variance structures: constant variance and different variances in groups. We made the model selection using both Akaike's and Bayesian information criterions (AIC and BIC) to search for the best fitted models in term of correlation and variance structure.

The models were checked via visual inspection of diagnostic plots (residuals vs. fitted values, and QQ plots to check normality) as proposed by Pinheriro and Bates (2000).

To test HR, we examined the slopes of the fitted lines, and to test PIVH, we examined the power exponents of the fitted variance functions in the different louse families and philopterid guilds.

We fitted reparameterized models enabling a direct estimation of the fitted lines' coefficients. The slope of the lines (identical to the allometric exponent on the original measurement scales) were compared with the "lsmeans" function of the "lsmeans" R package using Tukey corrections (Lenth and Hervé 2015).

We report the results of one sample (see third paragraph of Materials and Methods) analyzed using both Hackett et al. (2008) and Ericson et al. (2006) bird backbones and our louse phylogeny. To estimate the effect of random sampling of one louse from the lice species parasitizing the same host, we carried out a sensitivity analysis. We repeated the sampling and model fitting 20 times and calculated the relative SDs of the estimated parameters using the 20 random samples.

All of the analyses detailed below were also carried out without any phylogenetic controls to determine whether phylogenetic history influences the fitted models. All the R scripts and the parameter estimates as "comma-separated value" file are available in the Supporting Information.

Results

Across different phylogenies, female louse body length generally exhibits a strong phylogenetic signal (Hackett et al. 2008, phylogeny: $\lambda = 0.69$, 95% CI: 0.60, 0.78; Ericson et al. 2006, phylogeny: $\lambda = 0.69$, 95% CI: 0.60, 0.78; louse phylogeny: $\lambda = 0.97$, 95% CI: 0.96, 0.98), this holds for both louse families, and also for philopterid guilds (Hackett et al. 2008, phylogeny: $\lambda = 0.75$, 95% CI: 0.64, 0.86; Ericson et al. 2006, phylogeny: $\lambda = 0.76$, 95% CI: 0.65, 0.87; louse phylogeny: $\lambda = 0.94$, 95% CI: 0.9–0.98). The relative SDs of the estimates were below 5%.

The models fitted with and without phylogenetic correlation structures were significantly different (likelihood ratio test: $P < 0.0001$) in both analyses. The AIC value was −52 in case of the GLS analysis of the louse families, and −276, −276, −516 for the Hackett and Ericson backbone bird phylogenies, and the parasite phylogeny, respectively, in the PGLS analysis. In case of the philopterid guilds, the AIC value was −275 for the GLS analysis, and −361, −362, −377 for the Hackett and Ericson backbone bird phylogenies, and the louse phylogeny, respectively, in the PGLS analysis.

The phylogenetically controlled (PGLS) models provided better fit to the data than models without phylogenetic controls (GLS) in case of avian phylogenies, but not in case of the louse phylogeny, based on a visual examination of diagnostic plots.

FAMILIES

The phylogenetically controlled analyses produced roughly similar results to the analyses that did not control for phylogeny considering HR and PIVH (Table 1).

HR

Without phylogenetic controls, the slopes of allometric relationships between log(female louse body length) and log(host body mass) differed significantly from 0 ($P < 0.0001$) and also from each other in all families (Table 1, Figure 1). PGLS models using avian phylogenies showed that allometric exponents (slopes) differed significantly from 0 ($P < 0.0001$) and also from each other. However, when controlling for the parasite phylogeny the allometric relationships between female louse size and host body mass differed significantly between the Menoponidae and Ricinidae families, and a similar, though nonsignificant, difference was found between the Philopteridae and Ricinidae families (Table 2). The relative SD of slopes were less than 16%.

PIVH

Either with or without phylogenetic controls, the estimated variance power exponents were positive and similar for the Menoponidae and Philopteridae families, meaning an increase of relative variance with increasing host body size. In the case of Ricinidae, the power exponent was considerably smaller, not distinguishable from 0 in all models (Table 1).

The relative SD of the estimates was less than 20% in menoponids and philopterids, while much larger, 110%, in louse phylogeny in case of ricinids. Thus, PIVH appears mostly as a weak tendency, but not a statistically verifiable trend in ricinids.

PHILOPTERID GUILDS

In case of the body lice guild, the regression slope did not differ significantly from 0 ($P = 0.44$), and the difference of the AIC values between the model with and without body lice was negligible (only 2), thus we excluded the body louse guild from the subsequent analyses.

The PGSL analyses produced roughly similar results to the analysis without controlling for phylogenetic dependence in case of the regression parameters. The slopes of generalist lice were the smallest and the slope of wing lice was the largest (except in case of louse phylogeny, where the slopes of head and wing lice were similar). The variance power parameters with phylogenetic control were similar in all analyses (Table 3). The relative SD of the slopes in the 20 random samples were less than 14%, and the relative SDs of the estimated power exponents were less than 16%.

Table 1. Best model regression coefficients (intercepts and slopes with 95% confidence intervals) predicting log(female louse body length) in families as calculated from generalized least squares analyses without and with phylogenetic information using Hackett et al. (2008), Ericson et al. (2006), avian phylogenies, and louse phylogeny using the same dataset.

Model	Family	Intercept	Slope (95% CI)	Power exponent (95% CI)	λ (95% CI)
GLS	Menoponidae	7.36	0.047 (0.03, 0.064)	0.33 (0.18, 0.48)	
	Philopteridae	7.03	0.119 (0.103, 0.136)	0.28 (0.15, 0.4)	
	Ricinidae	7.56	0.21 (0.17, 0.25)	−0.03 (−0.31, 0.25)	
PGLS, Hackett bird phylogeny	Menoponidae	7.34	0.049 (0.035, 0.072)	0.45 (0.32, 0.58)	
	Philopteridae	7.2	0.092 (0.107, 0.141)	0.42 (0.3, 0.53)	0.69 (0.6, 0.78)
	Ricinidae	7.73	0.19 (0.168, 0.249)	0.35 (0.1, 0.61)	
PGLS, Ericson bird phylogeny	Menoponidae	7.34	0.05 (0.038, 0.076)	0.45 (0.31, 0.58)	
	Philopteridae	7.2	0.093 (0.104, 0.139)	0.42 (0.3, 0.53)	0.69 (0.6, 0.78)
	Ricinidae	7.73	0.191 (0.176, 0.258)	0.34 (0.09, 0.6)	
PGLS, louse phylogeny	Menoponidae	7.21	0.073 (0.015, 0.13)	0.33 (0.2, 0.46)	
	Philopteridae	7.33	0.078 (0.021, 0.134)	0.35 (0.23, 0.46)	0.97 (0.96, 0.98)
	Ricinidae	7.51	0.157 (0.118, 0.197)	0.1 (−0.14, 0.33)	

We present the estimated power exponents of the variance functions with 95% CIs, and the estimated λ parameters with 95% CIs of Pagel models. Estimations were made with the Hacckett and Ericson avian MRC and the louse trees. The actual sample sizes are 247 (Menoponidae), 347 (Philopteridae), and 84 (Ricinidae).

Table 2. Paired comparisons between louse families.

Model	Comparison	Estimated difference	SE	*P*-value
GLS	M-P	−0.072	0.012	<0.0001
	M-R	−0.163	0.022	<0.0001
	P-R	−0.091	0.022	0.0001
PGLS, Hackett bird phylogeny	M-P	−0.043	0.011	0.0004
	M-R	−0.141	0.025	<0.0001
	P-R	−0.098	0.026	0.0004
PGLS, Ericson bird phylogeny	M-P	−0.043	0.011	0.0003
	M-R	−0.141	0.025	<0.0001
	P-R	−0.097	0.026	0.0004
PGLS, louse phylogeny	M-P	−0.005	0.041	0.9917
	M-R	−0.085	0.03	0.0147
	P-R	−0.08	0.035	0.0623

Estimated differences with SEs and *P* values between slopes in louse families (M: Menoponidae; P: Philopteridae; R: Ricinidae).

Table 3. Best model regression coefficients (intercepts and slopes with 95% confidence intervals) predicting log(female lice body length) in philopterid guilds as calculated from generalized least squares analyses without and with phylogenetic information using Hackett et al. (2008), Ericson et al. (2006), avian phylogenies, and louse phylogeny using the same dataset.

Model	Guild	Intercept	Slope	Power exponent	λ
			(95% CI)	(95% CI)	(95% CI)
GLS	Generalist	7.29	0.061	0.85	
			(0.044, 0.077)	(0.6, 1.11)	
	Head	7.08	0.106	0.8	
			(0.087, 0.126)	(0.52, 1.08)	
	Wing	7	0.149	1.04	
			(0.124, 0.174)	(0.79, 1.28)	
PGLS, Hackett bird phylogeny	Generalist	7.4	0.041	0.78	
			(0.014, 0.064)	(0.51, 1.05)	
	Head	7.16	0.092	0.77	0.75
			(0.065, 0.118)	(0.47, 1.06)	(0.64, 0.86)
	Wing	7.09	0.131	0.86	
			(0.099, 0.164)	(0.6, 1.11)	
PGLS, Ericson bird phylogeny	Generalist	7.4	0.041	0.78	
			(0.016, 0.065)	(0.51, 1.05)	
	Head	7.16	0.093	0.75	0.76
			(0.067, 0.12)	(0.46, 1.05)	(0.65, 0.87)
	Wing	7.09	0.134	0.84	
			(0.101, 0.167)	(0.59, 1.1)	
PGLS, louse phylogeny	Generalist	7.42	0.042	0.9	
			(−0.004, 0.089)	(0.66, 1.14)	
	Head	7.13	0.116	0.88	0.94
			(0.065, 0.168)	(0.62, 1.14)	(0.9, 0.98)
	Wing	7.2	0.102	0.99	
			(0.038, 0.166)	(0.77, 1.22)	

We present the estimated power exponents of the variance functions with 95% CIs, and the estimated λ parameters with 95% CIs of Pagel models. The actual sample sizes are 109 (generalists), 111 (head lice), and 91 (wing lice).

Table 4. Paired comparisons between philopterid guilds.

Model	Comparison	Estimated difference	SE	*P*-value
GLS	G-H	−0.045	0.013	0.0016
	G-W	−0.088	0.015	<0.0001
	H-W	−0.043	0.016	0.0212
PGLS, Hackett bird phylogeny	G-H	−0.052	0.013	0.0003
	G-W	−0.092	0.017	<0.0001
	H-W	−0.04	0.016	0.0411
PGLS, Ericson bird phylogeny	G-H	−0.053	0.013	0.0003
	G-W	−0.093	0.017	<0.0001
	H-W	−0.041	0.016	0.0357
PGLS louse phylogeny	G-H	−0.074	0.016	<0.0001
	G-W	−0.06	0.018	0.0022
	H-W	0.014	0.019	0.7474

Estimated differences with SEs and *P* values between slopes in guilds (G: generalist, H: head, W: wing).

HR

Allometric relationships between log(female louse body length) and log(host body mass) were significant ($P < 0.0001$) in all guilds (Table 3, Figure 2). PGLS models showed that allometric exponents (slopes) varied between guilds as a function of log(host body mass) and the slope of generalists proved to be significantly smaller than that of the other two guilds (Table 4).

PIVH

The estimated variance power parameters were around 0.8 indicating that the relative variance significantly increases in all cases.

Discussion

We explored how parasite body size (HR) and its variability (PIVH) increases with host body size, using a large dataset of chewing lice associated with birds of the world applying PGLS models to account for phylogenetic nonindependence and variance heterogeneity. All the above results appear to be robust in case of using the well-resolved avian phylogenies and not controlling for phylogeny. The results are a bit less convincing in case of louse phylogeny, probably due its les precise estimation.

To the best of our knowledge, it is the first study to use such models fitted with different variance structures to test HR and PIVH. We based our analyses on a dataset of 581 species and several intraspecific lineages (subspecies, populations) of avian lice, thus comprising the broadest based test for HR to date. Moreover, the present study is the first empirical test for PIVH.

Our data support HR for the menoponid, philopterid, and ricinid families of avian lice; however the slope of these relationships were markedly different independently from the model used except in case of louse phylogeny. Ricinids exhibit, by far, the greatest slope, whatever phylogenetic model we apply. Similarly, philopterids exhibit a slope that is steeper than that of the menoponids. Morand and Poulin (2002) suggested that the slopes of host–parasite body size relationships are influenced by parasite abundance. Whatever the reason for these differences, they indicate formerly unknown characters of louse families that deserve further ecological and evolutionary consideration.

Our data also support PIVH in menoponid and philopterid lice, but this pattern is not supported in ricinid lice. These observed patterns make biological sense in light of the different natural histories of these louse families. Ricinid lice are widespread on hummingbirds and small-bodied passerines, occurring only scarcely on medium-sized passerines (Rheinwald 1968, 2007). Compared to host body size, they are the largest of avian lice and their joint allometry to host body size is the steepest. The variability of their size apparently does not follow PHIV, either with or without phylogenetic control. This corresponds well to the biological impression that this is a homogenous group of parasites restricted to small birds.

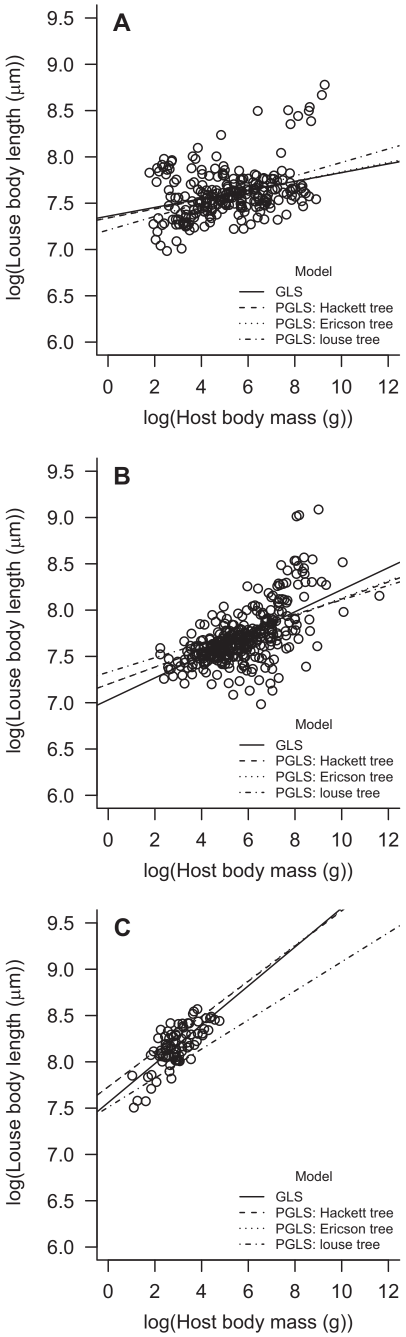

Figure 1. Host–parasite body size allometries for three major families of avian lice: Menoponidae A Philopteridae B and Ricinidae C. Louse body length and host body mass are given on log–log scale. The raw data are plotted with the generalized least squares (GLS) and the three phylogenetic generalized least squares (PGLS: Hackett, Ericson, and louse phylogeny) regression lines.

When different guilds of philopterids were analyzed separately, all of the guilds except body lice exhibited positive

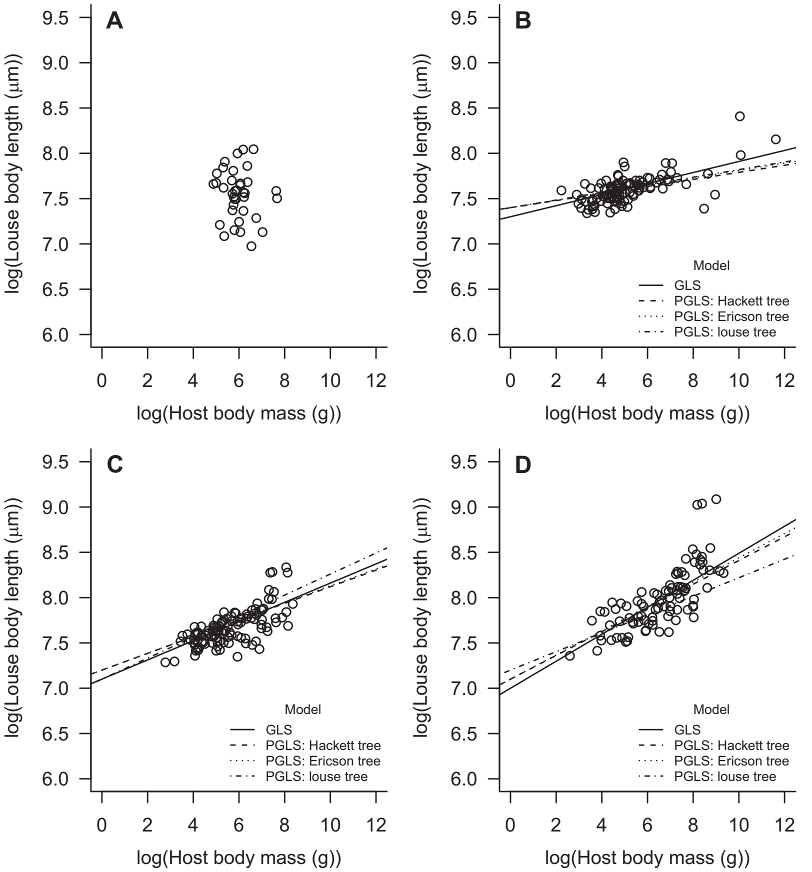

Figure 2. Host–parasite body size allometries for the four body shape guilds of philopterid avian lice: body lice A generalists B head lice C and wing lice D. Louse body length and host body mass are given on log–log scale. The raw data are plotted with the generalized least squares (GLS) and the three phylogenetic generalized least squares (PGLS: Hackett, Ericson, and louse phylogeny) regression lines except in case of body lice A where the slope was not significantly different from zero.

host–parasite body size allometries. The body sizes of the wing lice guild exhibited the steepest allometric relationship to host body size. This relationship was less steep, but still significant in head lice and generalist lice, except in case of louse phylogeny. In contrast, louse body size was not related significantly to host size among the body lice, which is consistent with the results of Johnson et al. (2005).

Parasite body size variability increased with host body size in wing lice, head lice, and generalist lice as predicted by PIVH. In contrast, the body size of the body lice guild did not follow PIVH. It is not entirely clear why body lice do not follow HR or PIVH. One possibility is that the body lice may be interacting with an aspect of bird morphology that does not correlate with overall host body size. Body lice inhabit the fluffy layer of the avian plumage, and the structural properties of this layer have been shown to be independent of avian body size in the order Columbiformes (Johnson et al. 2005). Additional studies are necessary to determine if this could explain why this major group of avian parasites does not adhere to HR or PIVH across all avian orders.

Poulin (2007) suggested that the increased variance in parasite size on large hosts is a consequence of relaxed spatial constraints on large bodied hosts. On large hosts, there may be enough niche space for parasites to effectively occupy very different aspects of an adaptive landscape. Given that large hosts tend to harbor multiple species of parasites, and the species in each assemblage tend to represent distinct guilds (e.g., wing and body lice on Columbiformes, or head lice, wing lice, and body lice on Psittaciformes, Johnson et al 2012), we suggest that interspecific competition could also be a factor leading to PHIV. Competition for food or space is known to occur among different species of feather lice (Bush and Malenke 2008, Clayton et al. 2016). Interspecific competition between lice could lead to behavioral and morphological character-displacement that would increase the variability of lice on larger hosts.

Second, larger bodied parasites may be an easier target for hosts to remove as they defend themselves (e.g., "predation pressure" as in Blanckenhorn 2000). Experiments have shown that host defensive systems, such as avian preening and grooming behaviors, can effectively localize and destroy larger bodied parasites (Clayton et al. 1999). Thus being small is a low-risk, low-benefit strategy in terms of mortality and fecundity, whereas large body size is a high-risk, high-benefit strategy for parasites. Large-bodied hosts may harbor parasite species of different body sizes that represent different risk-taking strategies and thus coexist stably in the absence of spatial constraints.

It is unlikely that a discovery bias (the assumption that a large proportion of lice on small bodied species remain unknown relative to that on larger hosts) has led to spurious analyses of allometry between host and parasite body sizes. HR is probably not affected by this discovery bias because many lice from small hosts are represented in our study, and the estimation of the slope is not particularly sensitive to additional points in this size range. We cannot exclude the possibility, however, that our tests of PIVH might have been affected by a discovery bias. Although our dataset includes many small hosts, our estimation of the body size variability of the parasites hosted by small-sized hosts may be relatively poor, especially because small bird species constitute the majority of the global avifauna. However, it is most likely that this sampling bias arbitrarily increased parasite body size variability on small hosts and, thus, the effect according to PIVH might have been underestimated in our study.

Our results show that both HR and PHIV capture important and general aspects of body size evolution in lice, and our results suggest that these may be general patterns influencing the evolutionary ecology of other parasites as well. The mathematical characteristics of these allometric relationships differ among taxa and also among ecological guilds, and in a few groups the patterns are practically absent. More research is needed comparing related taxa where parasites adhere to HR and PIVH and where they fail to adhere to HR and PIVH. Perhaps, in a comparative context, these rare exceptions can provide key information and improve our understanding of why these patterns appear to apply to so many different parasite taxa.

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DATA ARCHIVING

All data, bird and louse trees and R scripts of this study are published as Supporting Information. The louse and bird data used are also archived at http://datadryad.org/ under the doi:10.5061/dryad.t9t0v.

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