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ORIGINAL PAPER

Size variation in chewing lice *Docophorulus coarctatus*: how host size and louse population density vary together

Piotr Tryjanowski · Anetta Szczykutowicz · Zbigniew Adamski

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Abstract Chewing lice of the species *Docophorulus coarctatus* were extracted from museum specimens of their host, the great grey shrike *Lanius excubitor*, by combing feathers from 36 freshly shot birds (shot between 1962 and 1974), and samples of ten individual lice (five female, five male) were randomly collected for measurements from each bird. Female lice were bigger than males for all studied measurements ($P < 0.001$ in all cases), although the size of both sexes obtained from individual hosts was positively correlated. The overall size of lice (derived from a principal components analysis) was positively correlated with the overall size of the avian host, and also with the population density of lice on the individual host. We suggest that variation in louse morphology is due to differences in selection pressure exerted by each host and by intraspecific competition due to conspecifics. This is, to the best of our knowledge, the first evidence that Harrison's rule (parasites on larger host species are often bigger than those on smaller hosts) not only works in a multispecies comparison but also within a single host–single parasite system as well.

Keywords Body size · Coevolution · Density dependence · Harrison's rule · Parasites

P. Tryjanowski (✉)
Department of Behavioural Ecology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznan, Poland
e-mail: ptasiek@amu.edu.pl

A. Szczykutowicz
Department of Animal Taxonomy and Ecology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznan, Poland

Z. Adamski
Electron and Confocal Microscope Laboratory, Adam Mickiewicz University, Umultowska 89, 61-614 Poznan, Poland

Introduction

Chewing lice are obligate ectoparasites, completing their whole life on a single host (Barker 1994; Złotorzycka 1997). These insects do not possess wings, and therefore can only be transmitted to another host when hosts come into physical contact with one another, such as during copulation, incubation and other physical contacts (Barker 1994; Złotorzycka 1997). Although there are reported cases of hippoboscid flies carrying lice to a new host through the process known as phoresy (Baum 1968; Keirans 1975), this mechanism is exceptional. If lice spend their entire life span on one host, it is possible that host quality will influence their life history traits and morphology. For instance, host effects on louse size were tested at a multispecies level in bird–lice systems with patterns following Harrison's (1915) rule stating that parasites on large-bodied species of hosts are often bigger than those on small-bodied hosts (Johnson et al. 2005). A strong positive correlation was shown only for wing lice, whereas the correlation was very low for body lice (Johnson et al. 2005). Harrison's rule applies to closely related parasite species (Clayton et al. 2003) as well as across avian lice in general (Johnson et al. 2005). However, neither of these relationships have ever, to the best of our knowledge, been tested across individual host species, although such a test could provide a better understanding of the general results obtained from interspecific patterns. Factors other than host size and/or quality can effect ectoparasite size (e.g. Potti and Merino 1995; Poulin 1996). It is well known that population density may affect the size of different organisms because of conspecific competition for available food resources (Pianka 1983). For example, an inhibition of development due to accumulation of waste in crowded environments has been reported for *Drosophila melanogaster* (Mensua and Moya 1983) and a mite species *Caloglyphus berlesei* (Radwan 1992). Therefore, we predict that even those lice living on an avian host with different louse populations on their bodies should be affected by conspecifics, with individuals in more dense populations being smaller. Moreover, both host quality and conspecific density may interact to affect the size of lice.

Generally lice have been considered to be an organism with relatively little intraspecific variation in size (Modrzejewska 1989; Lonc and Modrzejewska 1991; Złotorzycka 1997; Ramli et al. 2000; Sychra 2005), and size was widely used to establish phylogenetics and lice taxonomies (Złotorzycka 1985; Banks and Paterson 2004). However, the technique used to obtain parasite samples was not always appropriate. For example, taking a large number of parasites from one (or even several) individual hosts could reduce the natural variation in the size of the parasites. The reason is that parasites were collected from the same environment; an individual host with a given body condition, anti-parasitic reaction and feather quality (Potti and Merino 1995; Clayton et al. 1999; Møller and Rózsa 2005). Therefore, to reduce potential sampling errors, parasites should be sampled in equal numbers for both sexes from a number of host individuals. Moreover, to avoid pseudo-replication and/or work on marginal measurements, only average values for local lice populations (separately for females and males) should be correlated with phenotypic traits of the host. For the present paper we collected data to this strict protocol.

We investigated to what extent the conditions of individual hosts affect the size of the two sexes of lice, we linked their body size to the size of the avian host and the population density of lice living on an individual host. We describe morphological variation in *Docophorulus coarctatus* (Scopoli, 1763) collected from their avian host, the great grey shrike *Lanius excubitor*. *D. coarctatus* is the most numerous species in the louse community of the great grey shrike (Szczykutowicz et al. 2006). We paid special attention to variation in louse size between the sexes, and we investigated mean louse size in relation to avian host size and louse population density.

Materials and methods

Host specimens were collected between 1962 and 1974 in the region of NE Slovakia and immediately transported to the Šarišské Museum, Bardejov, Slovakia (Hromada et al. 2003a, b; Szczykutowicz et al. 2006). Although 665 great grey shrikes were caught, parasites were only obtained from a smaller number of individuals (Hromada et al. 2003a, b). Lice were collected by T. Weisz and/or by taxidermists from recently killed birds by direct inspection for ectoparasites, without using special methods such as fumigation. All lice were preserved in 75% ethyl alcohol (more details in Hromada et al. 2003a). After removal from alcohol, all lice were subsequently cleaned and mounted on microscope slides for examination and identification. For this study we chose the most numerous species (present in 37% of hosts) of lice in the great grey shrike—*D. coarctatus* (Phthiraptera: Ischnocera) (Szczykutowicz et al. 2006). Only well preserved lice (with all the characters required for measurement) were used in morphometric analyses. We measured lice from 36 shrike individuals (22 females, 12 males, 2 undetermined) from which a minimum of 5 males and 5 females of *D. coarctatus* in good condition could be sampled at random by a laboratory assistant who had no knowledge of the hypotheses to be tested. All lice from one bird host were stored in the same tube. However, for species and sex diagnosis lice were divided according to species and sex (Szczykutowicz et al. 2006) and the laboratory assistant therefore had to pick out lice at random, directly from the tube without being able to see the size of the lice. Sometimes, after examination by microscope, lice were found in poor condition (for example, the measured part of body was destroyed) and so the laboratory assistant had to repeat the procedure of taking out lice randomly from the tube.

Five characters were measured for the study of size: total body length, abdomen length, head length, abdomen width (taken at the widest point) and head width (taken at the widest point), see Fig. 1. These characters follow Lonc and Złotorzycka (1983), Złotorzycka (1985), Ramli et al. (2000) and were chosen for the reproducibility of measurement and overall morphological representation. Louse specimens were mounted on stubs with double sticky tabs. Then they were coated with gold in a Balzers SPC 050 ion coater and were observed, analysed and measured in Zeiss Evo 40 XVP scanning electron microscopes. To reduce potential measurement error all measurements were made by the same person (ZA).

The distributions of morphometric variables were not significantly different from normal (Kolmogorov–Smirnov test, $P > 0.2$ in all cases) and all were highly significantly intercorrelated ($r > 0.55$, $P < 0.001$ in all cases). Consequently, to analyse overall body size, we used values obtained from a principal components analysis with VARIMAX rotation of all five log-transformed measurements (Piersma and Davidson 1991). All morphometric variables had positive loadings of similar magnitude (0.83–0.94) on the first component (PC1) which explained 59.5% of the

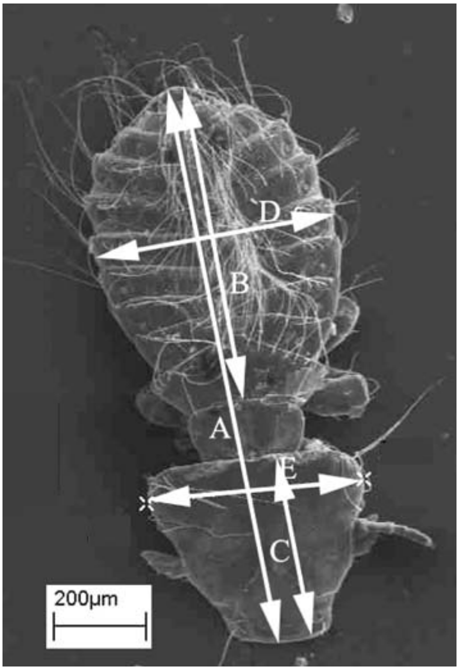

Fig. 1 Microscope picture of male *Docophorulus coarctatus* with marked morphological measurements. Explanations: (**A**) total body length; (**B**) abdomen length; (**C**) head length; (**D**) abdomen width, taken at the widest point; and (**E**) head width, taken at the widest point

morphological variation in female lice and 74.4% of the morphological variation in males.

Similarly, to present the overall body size of the host, we used the first principal component obtained from great grey shrike body measurements: wing length, tail length, tarsus length, bill length and bill height. All measurements were taken post mortem on the fresh individuals by the same person (T. Weisz, for details see Hromada et al. 2003a, b). All morphometric variables had positive loadings of similar magnitude (0.51–0.82) on the first component which explained 38.0% of the variation in shrike morphology.

To reduce variance and to obtain data approximating to a normal distribution, parasite density (number of lice per individual host) was log-transformed before analysis. The effects of lice density, host size and their interaction on the size of female and male *D. coarctatus* were tested using multiple regression. In graphs, only simple factor effects are shown to present the full information. Statistical analyses were performed using SPSS 11.0 for Windows following recommendations in Zar (1999).

Results

A total of 360 adult *D. coarctatus* from 36 shrike individuals was measured. Descriptive statistics based on averages for individual hosts (see Sect. ''Methods'') show that the two sexes of the lice species are very distinct in terms of size and shape (Table 1). All morphological variables were characterised by relatively low variability (ratio of SD to mean), with a coefficient of variation always below 17%.

Female lice were generally bigger than males (Table 1) and, interestingly, measurements of females and males from the same host were positively correlated (Fig. 2), with Pearson *r*-values between 0.441 and 0.721 ($P < 0.006$ for all tested morphological traits).

We did not find a significant influence of avian host sex on any louse size measurements (*t*-test, $P > 0.3$ in all comparisons) or louse population density ($t = 0.10$, $P > 0.9$). The mean *D. coarctatus* population density per host was 70.5 ± 40.9 individuals (range 13–215). We did not find a significant correlation between louse population density and avian host size ($r = 0.234$, $P = 0.18$).

The overall size of lice was positively related to overall host size (Fig. 3a, b), to louse population density (Fig. 4a, b) and there was also a significant interaction between host size and louse population density with a relatively smaller increase in louse size at higher densities and larger hosts sizes (Table 2). We did not find significant differences between sexes in the response of louse size to both host size and louse population density.

Because some authors suggested that bill size affects the ability of avian hosts to control their louse populations (Clayton et al. 1999; Johnson et al. 2005), we undertook a separate analysis on this trait. Although bill size was positively related to overall shrike size (in the PCA presented above), we did not find a relationship between bill width or height and louse size or the density of lice on individual hosts ($r < 0.29$ and $P > 0.1$ in all comparisons; after Bonferroni correction for multiple tests $P > 0.2$ in all comparisons).

Discussion

The species *D. coarctatus* is probably the most common louse of the great grey shrike (Szczykutowicz et al. 2006), and probably in other shrikes as well (Złotorzycka 1976). Our recorded louse body size measurements are within species limits provided by general references on the genus *Docophorulus* and also by identification keys (Złotorzycka 1980).

The variation in parasite body measurements and ratios is generally low in comparison to other insects (Złotorzycka 1994; Poulin 1996) as also found in our study. Therefore, the low variation in our samples is unlikely to be an artefact of sampling bias. The relative consistency of louse measurements may be due to the stability of the environment (body feathers of the great grey shrike as the host), and the low probability of horizontal transfer (Baum 1968; Marshall 1981; Potti and Merino 1995; Złotorzycka 1997). Alternatively, small variation in louse size could be a consequence of avian preening (Clayton et al. 1999), however this is unlikely to be important in our study species, because *D. coarctatus* is a head louse and only a small proportion live on the feathers susceptible to preening pressure (Złotorzycka 1997). Similar to the general patterns of louse size, we also found that females were bigger than males (Złotorzycka 1997; Ramli et al. 2000; Sychra 2005), although few studies have tested this explicitly (cf. Baum 1968; Zakir and Iqbal 2002; Sychra 2005). Differences in size or shape of particular louse structures are at least potentially

Table 1 Summary of morphometric data for *Docophorolus coarctatus* (in µm), derived ratios and body size obtained from the first axis of a principal components analysis

Characters	Female			Male			*t*-Value[a]	*P*
	Mean	SD	Range	Mean	SD	Range		
Total body length	1,528.1	97.5	1,336.4–1,680.7	1,148.1	55.9	1,129.3–1,373.0	23.68	<0.001
Abdomen length	977.1	166.1	756.0–1,596.2	712.7	47.2	619.5–802.8	10.19	<0.001
Head length	452.9	28.5	352.5–505.8	415.2	15.4	383.1–448.8	9.24	<0.001
Abdomen width, taken at the widest point	576.9	65.1	432.4–679.9	501.7	57.6	374.0–615.7	14.20	<0.001
Head width, taken at the widest point	510.0	28.2	442.6–561.2	458.9	23.9	400.7–511.4	15.32	<0.001
Ratio of body length to head length	3.38	0.20	3.02–3.90	3.00	0.12	2.72–3.28	13.99	<0.001
Ratio of head length to head width	0.89	0.06	0.74–1.05	0.91	0.05	0.79–1.03	–2.14	0.040
Ratio of abdomen length to abdomen width	0.60	0.08	0.34–0.74	0.70	0.07	0.55–0.83	–8.13	<0.001
Overall body size—PC1	0.75	0.70	–0.83–1.94	–0.75	0.63	–1.98–1.93	9.71	<0.001

The final two columns summarise tests of differences between female and male lice

[a] $df = 35$, paired *t*-test (see Sect. ''Methods'')

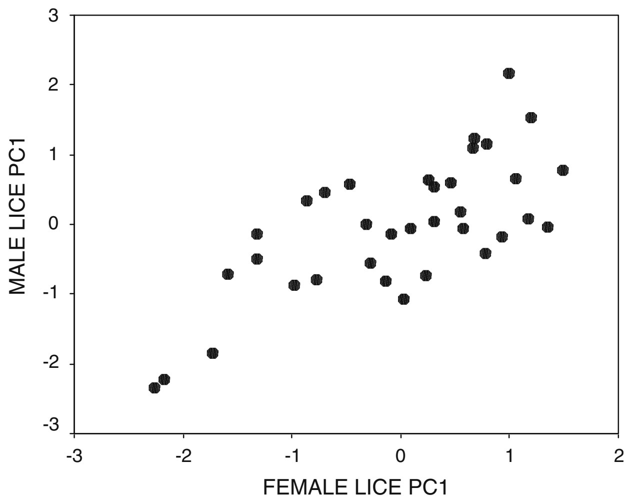

Fig. 2 Relationship between male and female *Docophorulus coarctatus* overall body size obtained from the same 36 avian host individuals. Overall size was estimated from the first axis of a principal components analysis (*PC1*)

affected by different host defences, for instance by their immunological system (Clayton et al. 1999, 2003; Ramli et al. 2000; Møller and Rózsa 2005).

Interestingly, we found that the louse size is related to host size and local louse population density on individual hosts. To our knowledge, this is the first test of Harrison's rule on the relationship between size of parasites and size of their hosts in a single species. We confirmed the general pattern that larger hosts have larger parasites (Harrison 1915; Kirk 1991; Johnson et al. 2005). Moreover, we show negative interactions between host size and louse population density, which may be interpreted as conspecific competition for available resources as space and/or food (Pianka 1983). It has been suggested that bill size might affect the ability of avian hosts to control their louse populations (Clayton et al. 1999; Johnson et al. 2005), but we did not find a relationship between bill size and either louse size or the number of lice on individual hosts. This suggests that the body size of parasitic lice (at least in parasites like *D. coaractus* generally confined to the head and neck regions of the host) were not affected by preening efficiency (Clayton et al. 1999). Hence, the important question is—which potential mechanism can explain the positive association between parasite population density and their body size? When lice are transferred to a new host individual they encounter the new environmental conditions offered by that individual, where they develop, reproduce and age. If conditions are beneficial (expressed for example as a large overall host body size, where large hosts are superior competitors and thus gain more resources which can be exploited by parasites), lice may achieve larger size. Because fecundity is positively correlated with female body size in insects (Sibly and Calow 1986; cf. also suggestions in Clayton et al. 1999), large female lice produce more juveniles and consequently the number of chewing lice found on a large host is greater than on small hosts. Moreover, louse size may be density dependent, as suggested by the negative interaction between host size and louse population density. Therefore, the increase in louse size with increased louse population density was smaller for large than for small hosts.

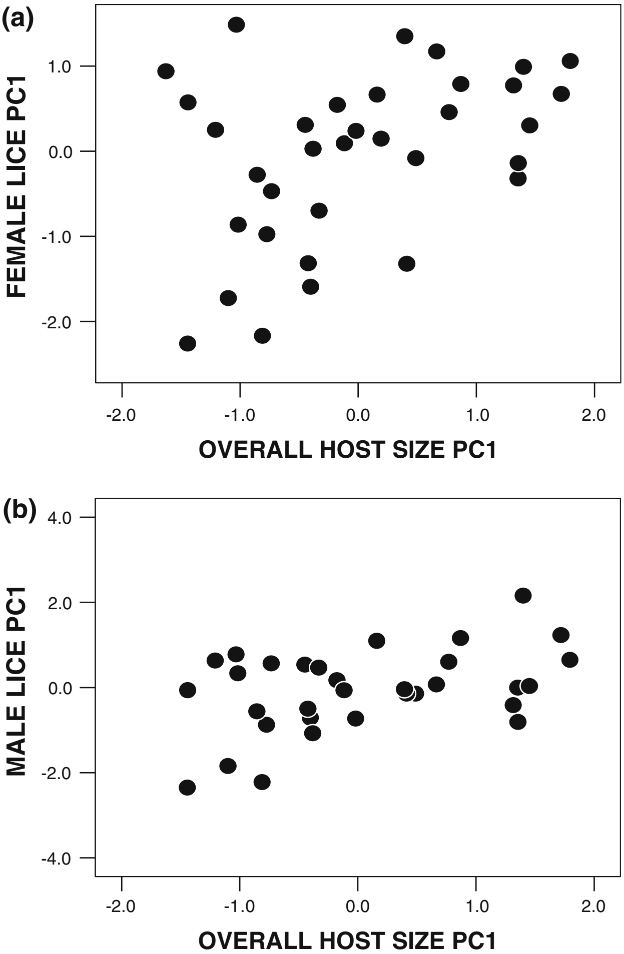

Fig. 3 Relationship between overall body size of (**a**) female and (**b**) male lice and overall size of the avian host. Overall size was estimated from the first axis of a principal components analysis (*PC1*)

To conclude, both intraspecific competition and selection for optimal size in relation to that of the host were important determinants of the size of chewing lice of shrikes. In the study of coevolutionary processes between parasite and host not only host quality and size is important, but so is the local density of lice living on an individual host and its interaction with host size.

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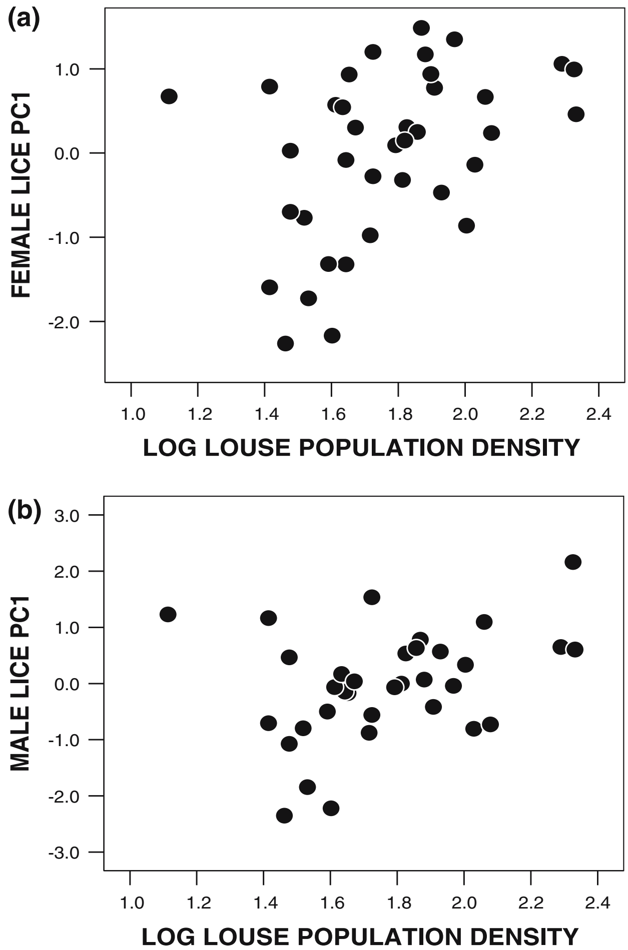

Fig. 4 Relationship between overall body size of (**a**) *Docophorulus coarctatus* females and (**b**) *D. coarctatus* males and louse population density on individual avian hosts. Overall size was estimated from the first axis of a principal components analysis (*PC1*)

Table 2 The relationship between overall body size of female and male *Docophorolus coarctatus* (obtained from principal components analysis) and host size and population density and their interaction in a multiple regression analysis

Variable	$\beta \pm$ SE	*t*-Value	*P*
D. coarctatus female			
Overall host size	2.61 ± 0.89	2.92	0.006
Log lice po pulation density	0.59 ± 0.17	3.57	<0.001
Interaction between above factors	–2.33 ± 0.91	–2.56	0.015
D. coarctatus male			
Overall host size	3.09 ± 0.91	3.41	0.002
Log lice population density	0.60 ± 0.17	3.55	0.001
Interaction between above factors	–2.90 ± 0.93	–3.13	0.003

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