Adaptive plasticity in insect parasites – Philopterus lice and their accentor passerine hosts

Article · September 2018

1 author:

Marian Janiga
University of Žilina
194 PUBLICATIONS   563 CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Project   Analysis of mitochondrial DNA of the tatra chamois and comparative analysis of microsatelite loci   View project

Project   Laboratory of the molecular biology at the Institute of High Mountain Biology   View project

Adaptive plasticity in insect parasites – *Philopterus* lice and their accentor passerine hosts

Marián JANIGA

059 56 Slovak Republic, Institute of High Mountain Biology, Žilina University, Tatranská Javorina 7, SK, e-mail: janiga@uniza.sk

ARTICLE INFO	ABSTRACT
	One hundred and five alpine accentors *Prunella collaris* (Scopoli, 1786) and ninety four dunnocks *Prunella modularis* (Linnaeus, 1758) were captured in the West Carpathian Mountains of Slovakia in order to compare the morphology of their Ischnoceran parasites *Philopterus emiliae* Balát 1985 and *Philopterus modularis* (Denny, 1842), respectively. In the case of both host species, the largest bodied lice occurred in winter, before the beginning of the host breeding season. Lice collected during the host breeding season were smaller in size than those collected in winter. Lice collected in the autumn, following postnuptial molt were even smaller in size. The largest lice were found on the bodies of host individuals with relatively long wings. The lice *Ph. emiliae* also differed in shape. In winter, the relative size of the head was large, while in the autumn, the head was proportionately smaller in size, compared to other body regions. Adult lice *Ph. modularis* also tended to have larger heads in April than in July. Measurements of lice over an eleven year period from the same site yielded differences in shape, but not in size. This study is one of the first assessments of seasonal variation in morphology of lice.

INTRODUCTION

Throughout the evolutionary history of insect parasites, several groups have specialized as parasites of birds. Among these groups, lice (Phthiraptera) are unique in that they are permanent parasites that spend their entire life cycle on the body of the host. For this reason, lice have become a model system for the study of cophylogenetic and ecological relationships between hosts and parasites (Hafner *et al.* 1994, Johnson *et al.* 2001, 2005, Janiga 2018). However, variation in the morphological and physiological features of lice living on hosts in different habitats remains poorly understood. Environmental stress, *i.e.* limited food or susceptibility to predation usually decreases body size and influences body shape because selection enhances immature survival at the expense of smaller body size at maturity. This level of phenotypic plasticity of adult body size can be derived from the adaptive canalization hypothesis. The traits under high past selection pressure are usually conservative and less environmentally variable while traits under low past selection are variable, plastic and more sensitive to environmental factors (Stillwell *et al.* 2010). Because smaller lice may mature faster (Stockdale and Raun 1960) the shorter cycle may cause there is less time for the host to preen. According to this theory, smaller lice are probably more tolerant of preening than are bigger lice. The activity of preening increases in passerine birds during and after moulting, in accentors mainly in September (Janiga and Romanová 1997). The behaviour is known to be critical for defence against lice (Clayton *et al.* 2005). Are small lice less susceptible to preening in the main preening periods than large ones? If so, then perhaps small size louse populations living in the critical periods of preening will do better than large size lice.

Do small lice of accentors occur in autumn? If larger lice are less tolerant of preening than are smaller lice then they should occur in winter. In winter, when host defences are impaired, lice populations often increase dramatically. For example, Stockdale and Raun (1960) showed that in just 16 weeks the chicken louse *Menacanthus stramineus* is capable of increasing from three individuals to thousands. Wintering passerine birds spend most of the short daylight period feeding and resting. The abundance of accentor lice is the highest during the winter period (Janiga and Mičková 2004). Analysis of morphological divergence of lice in different seasons should reveal if raising bigger lice is the answer to less frequent controlling lice by host. Because the surface area-to-volume ratio of similar-shaped objects changes with the overall size, I also tested if there is a seasonal variation in louse shape. Given a constant shape, the surface area will decrease as the size gets bigger, and increase as the size gets smaller. The present study also reveals if there is a seasonal polymorphism concerning body shape in investigated species of parasites.

Most empirical studies have focused on parasite species-specific effects only during some life history stages. Little attention has been given to the ecomorphology of lice because generally lice are considered to be organism with relatively little intraspecific variation (Ramli *et al.* 2000, Tryjanowski *et al.* 2007). Lice have coevolved adaptations for escape from host defences. Apart from work with poultry, there are few studies documenting temporal variation in population sizes of lice, and no study has documented temporal changes in their morphology (cf. Marshall 1981, Clayton *et al.* 1999, Møller and Rózsa 2005). *Philopterus emiliae* BALÁT 1955 and *Ph. modularis* (DENNY, 1842) are host-specific ectoparasites of the alpine accentors and dunnocks, respectively (Balát 1955). They move onto new hosts mainly during periods of direct contact between host individuals, such as between parent birds and their offspring in a nest (Janiga and Mičková 2004). In this study, a long-term survey of the louse species *Ph. emiliae* and *modularis* was conducted and investigated whether the ecology and behaviour of their host species influences the morphological variability of lice living in harsh and extreme alpine and supramontane environments.

There is a particular lack of data from extreme alpine environments. The focal host species in this paper, the alpine accentor *Prunella collaris* (Scopoli 1786)*,* is a species of passerine bird that lives in high alpine regions, such as alpine meadows and rocky outcrops, above the timberline. In Central Asia, the alpine accentor breeds at 4000–5000 meters, and it has been observed at nearly 8000 meters on Mount Everest (Cramp 1988). In the West Carpathian Mountains of central Europe, the species breeds between 1600 and 2600 meters (Dyrcz and Janiga 1997). Alpine accentors have a polygynandrous mating system in which a dominant male, and a few unrelated and usually younger males, share a group territory within which a few unrelated females have overlapping ranges. However, the species is not typically social because the members of a group do not move around together within their territory (Davies *et al.* 1995, Nakamura 1995, Heer 1996). Dunnock is a very common species of the family Prunellidae. In the Carpathians, dunnocks mainly live at lower elevations than alpine accentors in the dwarf mountain pine forest below the alpine meadows.

For both host species, the results are discussed in relation to their polygynandrous mating system and suggest that ectoparasites might synchronize their life cycles with those of their hosts. Variation in lice morphology of two closely living species of accentors is addressed in relation to seasonal variation, and the sex of the parasite and host. This study is probably the first in-depth assessment of seasonal effects on morphology of lice, particularly those living on hosts in the high alpine habitats. Given the range of research on louse shape and size variability, I hypothesize that *Ph. emiliae* and *Ph. modularis* populations show some kind of species diversification but their seasonal morphological variability dependent on external conditions may be comparable between both species. Therefore, I performed a comprehensive multivariate morphometric analysis of lice populations from different seasons, first, to access the degree of size and shape variation among different seasons and second, to infer evolutionary patterns between two host species.

STUDY AREA

This study was conducted in the High Tatra, Low Tatra, Great Fatra and Choč Hills mountains (West Carpathians) of Slovakia, area was limited by borders: 49.06N × 19.29E, 49.23N × 20.27E, 48.91N × 19.53E and 48.88N × 20.14E. Summer field sites of dunnocks laid in the dwarf pine (*Pinus mugo*) line. The habitat of alpine accentors was dominated by alpine meadows and rocky outcrops.

MATERIAL AND METHODS

For the purpose of this study, a total of 105 alpine accentors were mist-netted or caught with walk-in traps between 1988 and 2003. Adult birds were sexed using the shape of the cloacal protuberance (Nakamura 1990), tarsus width, or through the use of multivariate discriminant function analysis (Heer 1999). One-year old birds were aged by the colouring of the middle and greater wing-coverts. Some of the birds were ringed as nestlings. Measured lice (*Ph. emiliae)* were collected from 40 alpine accentors by exposing live birds to chloroform fumes in a glass fumigation chamber (Permission No. 4638/2016-2.3 of the Ministry of Environment, Slovak Republic for the Institute of High Mountain Biology of the Žilina University), while keeping the head of the bird outside the chamber (Bear 1995). This method allows several birds to be sampled for lice simultaneously in windy alpine conditions (cf. Walther and Clayton 1997). Chloroform vapours in the jar kill the parasites, which fall to the bottom of the jar and can be collected. Some of the lice on the body of birds are missed using this method, but the method is a reasonably accurate predictor of overall lice abundance (Clayton and Drown 2001). Birds were kept in the fumigation chambers for approximately 15 minutes. Lice were preserved in 70% alcohol. The species, life stage and sex of lice were determined using an Olympus B 201 microscope connected to a computer running image analysis software (Media Cybernetics) (Denny 1842, Balát 1959, Zlotorzycka 1964). Louse nomenclature follows Price *et al.* (2003). For the purpose of this study we use data on 196 individuals of *Ph. emiliae*, 163 of them were

measured. Collected lice were then mounted in Canada balsam on microslides. After identification and measuring, the specimens were deposited in the collection of the Institute of High Mountain Biology of Žilina University. Differences between individual hosts were analysed in terms of the number of louse species per individual bird and the prevalence of lice (cf. Janiga and Mičková 2004). Only well preserved lice were used in morphometric analyses. Seven characters were measured: BL – total body length, AL – abdomen and thorax length, AW – abdomen width at level of segment three, HL – head length at midline, HW – head width taken at widest point, AFW – width of arcus frontalis between two frontal setae, PW – prothorax width.

Between 2007 and 2011, lice were also collected from dunnocks. Like alpine accentors, dunnocks are members of the family *Prunellidae*. Dunnocks were mist-netted at lower elevations than alpine accentors in the dwarf mountain pine forest below the alpine meadows and rocks where accentors were captured. In total, 94 specimen of dunnocks were netted; *Philopterus modularis* lice were measured from 46 of these birds. A total of 122 individual lice were measured.

STATISTICS (STATIC ALLOMETRY)

The principal component analysis (PCA) is widely used in morphological studies (Bartholomew 2007). It is a variable reduction technique which maximizes the amount of variance accounted for in the observed variables by a smaller group of variables called components. In morphological studies, PCA is a purely morphological technique of static allometry and takes into account the measures of size and shape (Jolicoeur and Mosimann 1960, Mosimann and James 1979, Rohlf and Bookstein 1987, Klembara and Janiga 1993). Principal component 1 (PC1) of a set of linear skeletal measurements provides an appropriate structural size measure. PC2 describes the largest variance in shape. Other components follow similarly but in other directions of data variation than PC2. Researchers differ with regard to how many PCs should be interpreted from a given data set. In static allometry, the procedure does not depend on a file

of limiting assumptions (Jolicoeur 1984). An implicit assumption in the use of ordination methods is that the experienced ecologist can separate meaningful patterns from random noise. An ability to distinguish 'signal' from 'noise' is essential. The failure to distinguish these categories can lead to the rejection of useful information, or the interpretation of ecologically meaningless information (Jackson 1991, Jackson *et al.* 1992).

Several statistical models with simulation matrices were tested to obtain useful approaches to component evaluation. The most promising models (broken-stick or boot-strapped eigenvalue-eigenvector) suggest from one quarter to half of the components should be interpreted (Jackson 1993). In this study, principal component scores were computed from the covariance matrix of log-transformed data (alpine accentors) and correlation matrix of original data (dunnocks). We verified that both types of matrices yield approximately the same results (see also Somers 1986). PCA of correlation matrix tends to incorporate less shape into the first component and separate the new vectors more efficiently than PCA of the covariance matrix (Somers 1986, Leonart *et al.* 2000). PCA of the covariance matrix of logarithmically transformed data effectively describes relative changes in the measured characters within the size component (PC1) and reflects the

changes in shape which are highly dependent on size (Mosimann and James 1979, Strauss 1987, Yazdi 2014). In the comparison of *Ph. modularis,* the individuals of *Ph. emiliae* from alpine accentors were measured in all seasons of the year, so the technique with covariance matrix was more useful to explore how shape varies with size among seasonal generations of lice. Five components were retained in both analyses (*Ph emiliae* and *Ph. modularis*), then PC scores were compared between louse sexes and between seasons to test for differences between groups. The differences among mean scores were tested by One-way and Two-way (factorial) ANOVA with testing of interactions. The significance of differences between groups of lice were tested in the Tukey multiple range test at $P = 0.05$ (software STATISTICA 12).

RESULTS

The PCA technique was useful to explore how morphology of lice varies among different seasons. In both parasite species *Ph. emiliae* and *Ph. modularis,* abdominal shape varied with size, indicating that the abdomen increases proportionally with size (PC1, Tables 1 and 2), where adult lice are larger than nymphs (Fig. 1). Another source of variation, independent of body size was head size (PC5

Table 1. Eigenvectors of the first five principal axes for 163 specimens of *Philopterus emiliae* from alpine accentors, and the percent variance accounted for by each principal component. The component scores of PC1 and PC5 varied among seasons. PC5 is shape which does not vary with size). BL – total body length, AL – abdomen and thorax length, AW – abdomen width at level of segment three, HL – head length at midline, HW – head width taken at widest point, AFW – width of arcus frontalis between two frontal setae, PW – prothorax width.

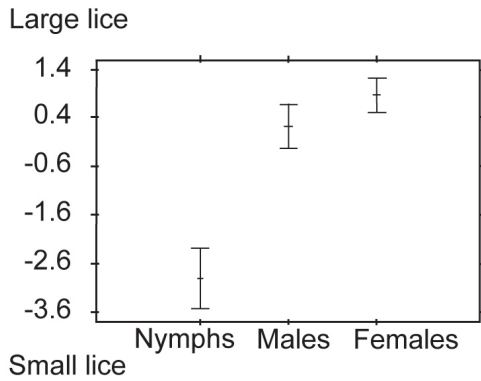| Variable	PC1	PC2	PC3	PC4	PC5
	Size	Shape	Shape	Shape	Shape
BL	0.40	- 0.03	- 0.30	- 0.21	0.09
AL	0.49	- 0.04	- 0.48	- 0.43	- 0.26
AW	0.41	- 0.02	- 0.19	0.85	- 0.27
HL	0.24	- 0.03	0.04	0.17	0.76
HW	0.33	- 0.10	0.06	- 0.05	0.44
AFW	0.40	- 0.49	0.71	- 0.12	- 0.25
PW	0.32	0.86	0.37	- 0.07	- 0.08
Variation (%)	84.4	4.9	4.1	4.0	1.8

Large lice

Small lice

Fig. 1. Size of lice *Philopterus emiliae* from alpine accentors (expressed by PC1, Y axis – means of principal component scores with intervals of standard errors); the females (n = 82) and males (n = 53) did not differ, nymphs (n = 28) were significantly smaller than adults (One way ANOVA: n = 163, F = 38.5, *P* = 0.0000 and Tukey multiple range test at *P* = 0.05)

in Table 1 and PC4 in Table 2). The components refer to the contrast between size of the head and thoracic/abdominal part of lice.

Variation in size

As expected, nymphs were significantly smaller than adult lice, and females were significantly larger than males in *Ph. modularis* (One way ANOVA: n = 103, F = 17.5, *P* =

0.0001) and slightly larger than males in *Ph. emiliae* (Fig. 1). Evaluation of louse adult body size in relation to different seasons produced the same results for both species of parasites. On dunnocks, adult lice were largest in April (n = 17), intermediate in size in May (60), and smallest in June (29) and July (16). The lice were significantly different in body size among the seasons (One way ANOVA: n = 122, F = 3, *P* = 0.03 and Tukey multiple range test at *P* = 0.05). In alpine accentors, the largest lice occurred in winter and the smallest in September (Fig. 2).

In alpine accentors, the relationship between louse size and shape and overall host size (mass) was also evaluated. No significant relationship existed between louse size and the body mass of the host (r = 0.1, n = 139, F = 2.37, *P* = 0.12). But a marginally significant positive relationship existed between wing length of an individual host and the size of parasites (r = 0.26, n = 135, F = 7.1, *P* = 0.05). Feathers are the substrate in which Ischnoceran lice interact with their hosts most directly.

Long-term variation in size. In the Great Fatra National Park, the wintering group of adult alpine accentors was continuously monitored and colour-ringed from 1988 to 1999. There were several generations of adult birds; in each year some ringed birds disappeared and new birds were found. The male lice from birds caught in 1988 tended to be

Table 2. Eigenvectors of the first five principal axes for 122 specimens of *Philopterus modularis* from dunnocks, and the percent variance accounted for by each principal component. The component scores of PC1 and PC4 varied among seasons. BL – total body length, AL – abdomen and thorax length, AW – abdomen width at level of segment three, HL – head length at midline, HW – head width taken at widest point, AFW – width of arcus frontalis between two frontal setae, PW – prothorax width.

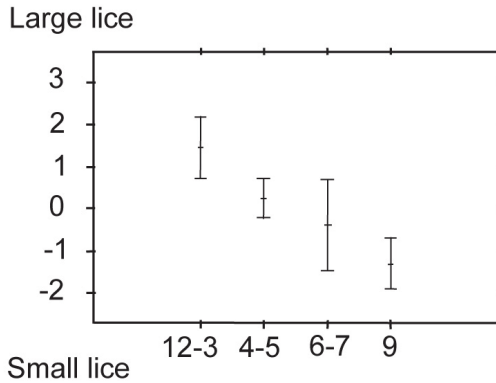| Variable	PC1	PC2	PC3	PC4	PC5
	Size	Shape	Shape	Shape	Shape
BL	-0.87	0.37	0.13	- 0.14	0.13
AL	-0.88	0.32	0.21	0.09	- 0.04
AW	-0.85	0.15	-0.46	0.20	0.05
HL	-0.91	- 0.15	-0.13	- 0.30	0.01
HW	-0.93	- 0.21	-0.05	- 0.14	-0.04
AFW	-0.86	- 0.37	0.18	0.19	0.22
PW	-0.92	-0.09	0.10	0.12	- 0.31
Variation (%)	76.0	6.9	4.7	3.3	2.4

Fig. 2. Size of the adult lice *Philopterus emiliae* from alpine accentors in different seasons (expressed by PC1, Y axis – means of principal component scores with intervals of standard errors). 12–3: December to early March, wintering of Accentors (n = 27), 4–5: April – May – arrival of host females, premating and mating, first attempts of breeding (n = 64), 6–7: June – July, the main breeding period of accentors in the West Carpathians (n = 13), 9: September – molting (n = 35). The largest lice were found in winter and spring, they significantly differed from lice collected in autumn, the lice from summer were at the intermediate level (One way ANOVA: n = 139, F = 10, P = 0.0000 and Tukey multiple range test at P = 0.05).
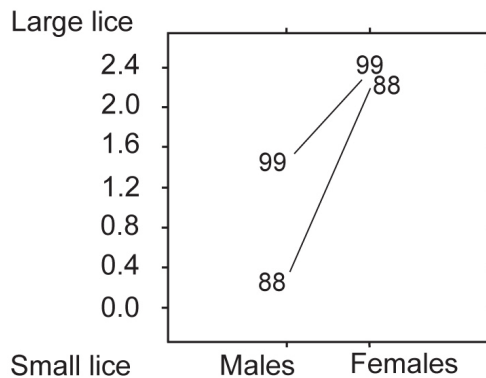

Fig. 3. Comparison of size (Y axis – PC1 scores, n = 35) of adult lice *Philopterus emiliae* in eleven year period (88 – 1988, 99 – 1999) from birds at the same wintering site. The adult lice did not differ significantly in size after eleven years (Two factorial ANOVA with interactions: n = 35, F = 2.6, P = 0.110). The sexes of lice differed in size (F = 11.2, P = 0.002) significantly equally in both periods ($F_{interaction}$=1.4, P = 0.24).

smaller than male lice from 1999, but the results were not statistically significant. Females also did not differ in size. (Fig. 3)

Variation in shape

To examine the relationship of parasite shape (independent of size) to the age/sex of lice, and among seasons, the component scores between the different groups of parasites were compared. The greatest variation was in head size (PC5 in Table 1 and PC4 in Table 2). The relative size of the head (in terms of head width and length) in relation to the other measured body variables (abdomen and prothotorax) was the largest in louse females. Males and nymphs had proportionally smaller heads than females (*Ph. emiliae*: One-way ANOVA: n = 162, F = 19.3, P = 0.000, PC 5 in Table 1, *Ph. modularis*: One-way ANOVA: n = 104, F = 4.8, P = 0.01, PC4 in Table 2).

The relative size of the head in adult lice of alpine accentors also differed between seasons. In winter, the head was large, while in the autumn, head size was smallest in proportion to other body variables (Fig. 4). Adult lice from dunnocks also tended to have relatively larger heads in April than in July but the differences were not statistically significant.

Long-term variation in head shape. Different sexes of adult lice from birds of the same wintering area did not differ in shape, although the males tended to have proportionally smaller heads, relative to the thorax and abdomen. However, the shape of both sexes changed statistically significantly from 1988 to 1999. Both sexes of lice had relatively larger heads in 1999 than in 1988 (Fig. 5).

DISCUSSION AND CONCLUSION

Many studies on lice provide support for the hypothesis that parasites on large-bodied hosts are larger than those on small-bodied hosts (Rózsa 1997, Tompkins and Clayton 1999, Clayton *et al.* 2003, Johnson *et al.* 2005). This positive relationship of parasite and host body size is known as Harrison´s rule. But feather lice eat mainly feathers. At the intraspecific level, the length of feathers and body size (*i.e.* body weight in many studies) reflect such factors as level of postnatal development, nutrition in birds, local geography, and seasons. Some birds may be heavier and short winged, while other individuals may be the reverse (Janiga 1986). These slight differ-

ences may also be found among individuals in a population. This is what we call shape
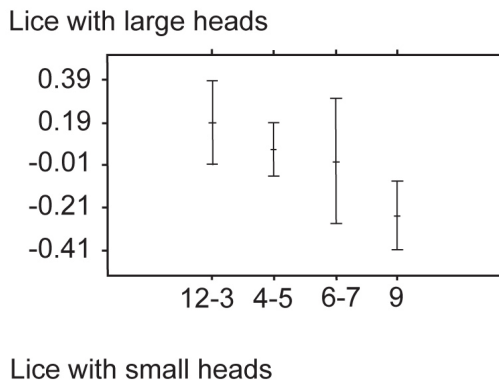
Lice with large heads

Lice with small heads

Fig. 4. Shape of the adult lice *Philopterus emiliae* in different seasons (expressed by PC5, Y axis means of principal component scores with intervals of standard errors). 12–3: December to early March, wintering of Accentors (n = 27), 4–5: April – May – arrival of host females, premating and mating, first attempts of breeding (n = 64), 6–7: June – July, the main breeding period of accentors in the West Carpathians (n = 13), 9: September – moulting (n = 35). Relative to thoracic and abdominal size, lice had larger heads in winter than in autumn (One way ANOVA: n = 139, F = 3.9, P = 0.01, lice from winter and spring significantly differed from lice collected in autumn, shape of lice from summer was at the intermediate level, Tukey multiple range test at P = 0.05).
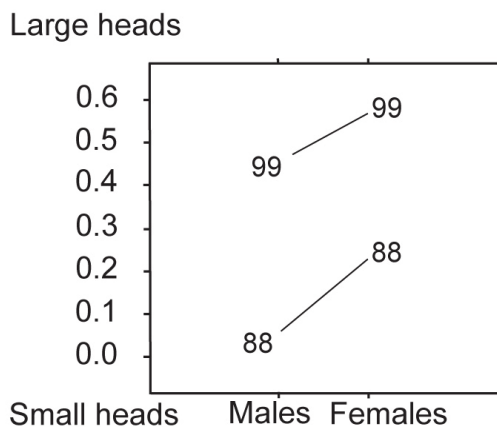
Large heads

Small heads   Males Females

Fig. 5. Comparison of shape (Y axis – PC5 scores, n = 35) of adult lice Philopterus emiliae over an eleven year period (88 – 1988, 99 – 1999) from Alpine Accentors from the same wintering area. The adult lice significantly differed in shape after eleven years, relative to thoracic and abdominal size lice had larger heads in 1999 than in 1988 (Two factorial ANOVA with interactions: n = 35, F = 7.3, P = 0.01). The sexes of lice did not differ in the shape (F = 1.15, P = 0.28), nor in 1988, nor in 1999 (Finteraction = 0.04, P = 0.83).

in birds and the components of shape variation in birds can be quantified with the help of multifactorial statistics. Morphology of lice may be consistent with variation in the body size and shape of different host populations (Hughes 1984). Johnson *et al.* (2005) examined the basic morphology of 78 species of diverse bird lice and their hosts. In feather lice they found that body size of the parasite was highly correlated with wing feather interbarb space of hosts, while there was no clear relationship between louse body size and host size. Evaluation of the alpine accentor's feather louse size in relation to the host body size and wing length produced similar results; parasite size did not significantly relate to host body size, but was positively correlated with wing length of accentors. In terms of allometry, long-winged and lighter individuals in accentors may host more feather lice than short-winged and heavier birds. At an intraspecific level, host body shape may have an important influence on parasite size and a pattern known as Harrison's rule should be tested in relation to shape of the host bird. Moreover, feather morphology may differ across years. At the end of the breeding period and before molting, when the feathers show wear and tear in many bird species – including accentors – the size of feathers need not correspond to the body mass of the bird. The feather damage that lice cause reduces host mating success (Clayton 1990), thermoregulatory ability (Booth *et al.* 1993) and survival (Clayton *et al.* 1999).

Head shape variability. The genus *Philopterus* exhibits great divergence in body shape among populations (Hughes 1984). The head morphology of Ischnoceran lice is particularly diverse, so the significant variation between seasonal generations of lice was not unexpected. The variation in size of the head probably reflected better feeding conditions in winter, as during this period the parasites were big with proportionally larger heads. The head shape may go some way to explaining niche segregation, in cases where multiple species of lice occur on the same species of host (Smith 2001). Results from the morphometric analysis of the shape of *Ph. emiliae* correlates well with the data described for size in seasons. Although the comparisons between eleven-year periods confirm that causes of

variation in body size and shape of lice may be independent, size was more influenced by sex while shape was more influenced by differences in the environment in 1988 and 1999. Seasonal data, as well as data from different years indicates that proportional size of the head (when related to other body parts) may be a variable which is very sensitive to change in environmental conditions.

Seasonal population dynamics of lice and variation in their size. Sychra (2005) examined 120 chukars (*Alectoris chukar*) for chewing lice. He found four species of Ischnoceran lice with the most abundant species being *Goniocotes microthorax* (the other three species occurred less frequently, with intensities ranging from 10 to 100 times lower than that of *G. microthorax*). Similar to the high occurrence of *Ph. emiliae* in winter (Janiga and Kubašková 2000, Janiga and Mičková 2004), the highest intensity of *G. microthorax* occurred in winter (December – February), while from March to October intensity of lice decreased approximately five-fold. Additionally, in many other common passerine species a marked peak of seasonal infestation by feather lice occurs in winter, or just prior to the birds´ breeding season. A peak for blackbirds (*Turdus merula*) usually occurs in February, and for chaffinch (*Fringilla coelebs*) in April. Robins (*Erithacus rubecula*) usually reach a peak of infestation earlier than the chaffinches; in March. Generally speaking, many species of birds show an increase in infestation during winter months (Zekhnov 1950, Ash 1960, Kettle 1983, Hamstra and Badayev 2009). The most favourable period for feather eating lice is winter. At this time, the number of lice increases (Janiga and Kubašková 2000), and the size of adults is largest, possibly due to a decrease in preening-mediated selection. The second peak of increased number of Ischnoceran lice occurs in alpine accentors in September (Janiga and Mičková 2004) during molting, but the bodies of adult lice are smaller as an adaptation to avoid preening. In this period, the amount of time spent preening increases in the daily cycle of accentors (Janiga and Romanová 1997). Janiga and Mičková (2004) collected *Ph. emiliae* from 13 alpine accentors in September, from 13 birds in October through November, and from 6 birds in December through February.

85 percent of the birds were infested in September, no birds were infested in the October – November period, and 50 percent of birds were infested in the December – February period. The data suggests that intensive preening during a moult period rapidly reduces louse population sizes. Similar reductions in louse populations were reported for robins (Ash 1960) and trumpeter finches (Carrillo *et al.* 2007). The gradual decrease of infestation in robins occurred between March and late-summer, with zero infestation occurring in September and October. This trend was unsurprisingly slightly earlier than in accentors, as robins breed at lower elevations. In October and November, alpine accentors compose feeding aggregations that can include hundreds of birds. Their body mass can increase from 40 to more than 50 grams (Janiga, pers. obs.), and they have new plumage. The few individuals of lice that survive moult may trigger an increase that leads to larger intensities of lice in winter. Wintering birds at high latitude, such as those in this study, usually spend most of the short daylight period feeding and resting without preening. Less frequent preening, reduced daylight in winter, and relatively new plumage provides the lice with a very favourable environment. The result may be large lice. Our comparison with dunnocks from the Tatras confirmed that the winter is an excellent period for the growth and development of Ischnoceran lice. In April and May, approximately 60 percent of dunnocks were infested by *Ph. modularis* while in June – July, the lice were found on only 40 percent of birds (Popelářová 2012). Lice found on adult dunnocks were largest in April, and smallest in July.

At wintering feeding sites, males alpine accentors often warbled sub-song. The activity increased toward spring, and was accompanied by preening, which serves to control ectoparasites (Reiczigel and Rózsa 1998). Experimental manipulation of preening provides direct evidence that it is an important host defence that controls lice in birds (Clayton 1990, 1991). When we consider that the ratio of male to female lice did not differ from unity except during spring, and in the other seasons female lice were more common than male lice (Janiga and Mičková 2004), I hypothesize that preening played the most im-

portant role in the louse reduction in early spring. Moreover, from February through March, the fat stores of birds are quickly reduced (Heer 1998), reflecting changes in their behaviour and physiology. The lice found on the bodies of birds were smaller in the spring than in winter but nymphs were found, indicating an occurrence of a new parasite generation.

The alpine accentor is a double-brooded species (Dyrcz 1976, Talposh 1977, Gómez-Manzaneque 1989, Davies *et al.* 1995, Heer 1998, del Hoyo *et al.* 2005). Fledglings from the first broods may occasionally be seen at the end of May and at the beginning of June, and fledglings from the second broods begin to appear in mid-July. In altricial birds, the nesting period plays the most important role in the development of lice with occurrence of a high proportion of louse eggs and nymphs on nestlings. Therefore, females of accentors seem to be extremely important for the transmission of summer generations of lice because they perform the main portion of parental care such as territory defence against other females, choice of nest-site, nest-building, incubation, brooding and feeding of nestlings. The males never incubate (Heer 1998) but sometimes they sing after entering the nest site. After egg hatching, males begin to bring food, usually starting from the third day of the nestling-rearing period. Females perform the majority of feedings, and male feedings are short and less frequent than those of females, generally lasting from just one to a few seconds. Because one male can control several nest-sites and thus several females (Nakamura 1995), it may be hypothesized that while host females cause direct vertical transmission of lice from adult to young birds, the males of accentors are responsible for 'horizontal' transmission between contemporary as well as successive nests. The short fertile period enables males to guard one female after the other in succession, because the females in polygynandrous families breed often asynchronously (Davies *et al.* 1995, Nakamura 1998,). Alpine lice *Ph. emiliae* must be adapted for transmission during this short period. The transmission of lice from parents to offspring likely occurs mainly by nymphs as relatively more nymphs than adult lice were found on young nestlings

and the nymphs are smaller than adults (Janiga and Mičková 2004). The incubation period of the louse eggs may last 4–5 days and each of three nymphal stages may require approximately three days (Stockdale and Raun 1960). The generation time of most lice is about a month (Marshall 1981). Therefore one would expect small nymphs transmitted to nestlings to mature and begin laying eggs one month later. After the summer peak of infestation, the number of lice decreased to zero in August indicating that summer is unfavourable period for the development of lice. The results of this study call into question previous visual studies documenting a decrease in louse abundance associated with moult (*e.g.* Marshall 1981), because in September many small adult lice on fully or partially moulted birds were found. Host physiological constraints may allow ectoparasites plenty of time to circumvent moult in most cases. The observations of lice using pinfeathers as refuges suggest that lice are at least capable of identifying new feathers that will not be lost as a result of moult (Moyer *et al.* 2002). The habitat features of the alpine accentor are predictably unfavourable. In this environment, the decreasing of adult lice to a minimum in late autumn suggests that the alpine accentors are able to effectively preen away some of the lice (cf. Clayton *et al.* 2005). On the other hand, studies on both Prunellidae species and their parasites confirm that feather lice are highly adaptable to the cold environment, as the small adult lice found in autumn can survive on both adult and young birds after moulting and are able to establish a viable wintering population of many big individuals. This is probably the first study to quantify lice ecomorphology in alpine habitats during all seasons. Given the available data, it has been shown that the probability of a bird being infested with lice is different between seasons and could be driven by species behaviour.

REFERENCES

Ash J.S. 1960 – A study of the Mallophaga of birds with particular reference to their ecology – Ibis, 102: 93–110.

Balát F. 1955 – Mallophaga of the Tatra National Park – Zool. a entomol. listy, 4: 389–398.

Balát F. 1959 – Řád Všenky - *Mallophaga* (In: Klíč zvířeny ČSR III, Ed: J. Kratochvíl) – ČAV, Prague, pp. 243–269

Bartholomew D.J. 2007 – Three faces of factor analysis (In: Factor analysis at 100: Historical developments and Future Directions, Eds: R.R. Cudec, R. MacCallum) – Erlbaum Associates, Lawrence, pp. 9–22.

Bear A. 1995 – An improved method for collecting bird ectoparasites – J. Field Ornithol. 66: 212–214.

Booth D.T., Clayton D.H., Block B.A. 1993 – Experimental demonstration of the energetic cost of parasitism in free-ranging hosts – Proc. R. Soc. B, 253: 125–129.

Carrillo C.M., Valera F., Barbosa A., Moreno E. 2007 – Thriving in an arid environment: High prevalence of avian lice in low humidity conditions – Ecoscience, 14: 241–249.

Clayton D.H. 1990 – Mate choice in experimentally parasitized rock doves: lousy males lose – Amer. Zool. 30: 251–262.

Clayton D.H. 1991 – Coevolution of avian grooming and ectoparasite avoidance (In: Bird - parasite interactions, Eds: J.E. Loye, M. Zuk) – Oxford University Press, Oxford, pp.258 – 289.

Clayton D.H., Bush S.E., Goates B.M., Johnson K.P. 2003 – Host defense reinforces host-parasite cospeciation – Proc. Natl. Acad. Sci. USA, 100: 15694–15699.

Clayton D.H., Drown D.M. 2001 – Critical evaluation of five methods for quantifying chewing lice (Insecta: Phthiraptera) – J. Parasitol. 87: 1291–1300.

Clayton D.H., Lee P.L.M., Tompkins D.M., Brodie E.D. 1999 – Reciprocal natural selection on host-parasite phenotypes – Am. Nat. 154: 261–270.

Clayton D.H., Moyer B.R., Bush S.E., Jones T.G., Gardiner D.W., Rhodes B.B., Goller F. 2005 – Adaptive significance of avian beak morphology for ectoparasite control – Proc. Biol. Sci. 272: 811–817.

Cramp S. 1988 – Handbook of the Birds of Europe, the Middle East and North Africa - The Birds of the Western Palearctic, Vol. V. – Tyrant Flycatchers to Thrushes, Oxford University Press, Oxford, New York, London, 1084 pp.

Davies N.B., Hartley I.R., Hatchwell B.J., Desrochers A., Skeer J., Nebel D. 1995 – The polygynandrous mating system of the Alpine Accentor, *Prunella collaris*. I. Ecological causes and reproductive conflicts – Anim. Behav. 49: 769–788.

Del Hoyo J., Elliot A., Christie, D. 2005 – Handbook of the birds of the World. 10. Cuckoo shrikes to Thrushes – Lynx Editions, Barcelona.

Denny H. 1842 – Monographia Anoplurorum Britanniae – Henry G. Bohn, London.

Dyrcz A. 1976 – [The materials on biology of Alpine Accentor *(Prunella collaris)*] – Notatki Ornithol. 17: 79–92 (in Polish, English summary).

Dyrcz A., Janiga M. 1997 – Alpine Accentor *Prunella collaris* (In: The EBCC atlas of European breeding birds: their distribution and abundance, Eds: E.J.M. Hagemeijer, M.J. Blair) – T and AD Poyser, London, pp. 510–511.

Gómez-Manzaneque A. 1989 – Quelques données sur la reproduction de l'Accenteur alpin (*Prunella collaris*) dans la Sierra de Gredos, Espagne Centrale – Alauda, 57: 223–225.

Hafner M.S., Sudman P.D., Villablanca F.X., Spradling T.A., Demastes J.W., Nadler S.A. 1994 – Disparate rates of molecular evolution in cospeciating hosts and parasites – Science B, 265: 1087–1090.

Hamstra T.L., Badyaev A.V. 2009 – Comprehensive investigation of ectoparasite community and abundance across life history stages of avian host – J. Zool. 278: 91–99.

Heer L. 1996 – Cooperative breeding by alpine accentors *Prunella collaris*: polygynandry, territoriality and multiple paternity – J. Ornithol. 137: 35–51.

Heer L. 1998 – The polygandrous mating system of the Alpine Accentor *Prunella collaris*, individual reproductive tactics, breeding adaptations on high mountain conditions and winter ecology – Ph. D. thesis, University of Bern, Bern, 152 pp.

Heer L. 1999 – Age and sex determination in the Alpine Accentor *Prunella collaris* – Ornithol. Beob. 96: 241–248.

Hughes J.M. 1984 – Morphometric variation in the Mallophaga of the Australian Magpie (*Gymnorhina tibicen* Latham) – Aust. J. Zool. 21: 467–477.

Jackson J.E. 1991 – A user´s guide to principal components analysis – John Wiley and Son, New York, 569 pp.

Jackson D.A. 1993 – Stopping rules in principal component analysis: A comparison of heuristical and statistical approaches – Ecology, 74: 2204–2214.

Jackson D.A., Somers K.M., Harvey H.H. 1992 – Null models and fish communities evidence of nonrandom patterns – Am. Nat. 139: 930–951.

Janiga M. 1986 – The feather growth of young *Columba livia f. domestica* as an ecological indicator – Biologia (Bratisl.), 41: 1031–1038.

Janiga M. 2018 – Different coevolutionary breeding strategies of Ischnoceran lice on *Prunella collaris* and *P. modularis* in high mountains – Pol. J. Ecol. 66: 182–193.

Janiga M., Kubašková Ľ. 2000 – The biology of the Alpine Accentor *Prunella collaris.* III. The coevolution of Alpine Accentors and lice (*Phthiraptera*) – Oecol. Montana, 9: 24–28.

Janiga M., Mičková A. 2004 – The biology of the Alpine Accentor *Prunella collaris* V. The sex ratio and transmission of lice *Philopterus emiliae* – Oecol. Montana, 13: 17–22.

Janiga M., Romanová E. 1997 – The biology of the Alpine Accentor *Prunella collaris* II. Behaviour: Rhytmic aspects of maintenance activities – Oecol. Montana, 6: 45–48.

Johnson K.P., Bush S.E., Clayton D.H. 2005 – Correlated evolution of host and parasite body size: Tests of Harrison´s rule using birds and lice – Evolution, 59: 1744–1753.

Johnson K.P., Drown D.M., Clayton D.H. 2001 – A data based parsimony method of cophylogenetic analysis – Zool. Scripta, 30: 79–87.

Jolicoeur P. 1984 – Principal components, factor analysis, and multivariate allometry. A small sample direction test – Biometrics, 40: 685–690.

Jolicoeur P., Mosimann J.E. 1960 – Size and shape variation in the painted turtle. A principal component analysis – Growth, 24: 339–354.

Kettle J. 1983 – The seasonal incidence of parasitism by Phiraptera on starlings (*Sturnus vulgaris*) in England – New Zeal. Entomol. 7: 403–408.

Klembara J., Janiga M. 1993 – Variation in *Discosauriscus austriacus* (Makowsky, 1876) from the Lower Permian of the Boskovice Furrow (Czech Republic) – Zool. J. Linnean Soc. 108: 247–270.

Leonart J., Salat J., Torres J. 2000 – Removing allometric effects of body size in morphological analysis – J. Theor. Biol. 205: 85–93.

Marshall A.G. 1981 – The ecology of ectoparasitic insects – Academic Press, London, 459 pp.

Møller A.P., Rózsa L. 2005 – Parasite biodiversity and host defenses: Chewing lice and immune response of their avian hosts – Oecologia, 142: 169–176.

Mosimann J.E., James F.C. 1979 – New statistical methods for allometry with applications to Florida red-winged blackbirds – Evolution, 33: 444–459.

Moyer B.R., Drown D.M., Clayton D.H. 2002 – Low humidity reduces ectoparasite pressure: implications for host life history evolution – Oikos, 97: 223–228.

Nakamura M. 1990 – Age determination in the Alpine Accentor *Prunella collaris* by discriminant analysis of morphological measurements – Jpn. J. Ornithol. 39: 19–24.

Nakamura M. 1995 – Territory and group living polygynandrous Alpine Accentor *Prunella collaris* – Ibis, 137: 477–483.

Nakamura M. 1998 – Multiple mating and cooperatives breeding in polygynandrous alpine accentors. II. Male mating tactics – Anim. Behav. 55: 277–289.

Popelářová S. 2012 – [Host and parasite relation - Dunnock (*Prunella modularis*) and *Philopterus modularis*] – M.Sc. thesis, University of Zvolen, Zvolen, 71 pp. (in Slovak, English summary).

Price R.D., Hellenthal R.A., Palma R.L., Johnson K.P., Clayton D.H. 2003 – The Chewing Lice: World Checklist and Biological Overview (Special Publication 24) – Illinois Natural History Survey, Illinois, 501 pp.

Ramli R.L., Cusack M., Curry G.B., Furness R.W. 2000 – Morphological variation of chewing lice (Insecta: Phthiraptera) from different skua taxa – Biol. J. Linn. Soc. 71: 91–101.

Reiczigel J., Rózsa L. 1998 – Host-mediated site segregation of ectoparasites: An individual based simulation study – J. Parasitol. 84: 491–498.

Rohlf F.J., Bookstein F.L. 1987 – A comment on shearing as a method for ´size correction´ – Syst. Zool. 36: 356–367.

Rózsa L. 1997 – Patterns in the abundance of avian lice (Phthiraptera: Amblycera, Ischnocera) – J. Avian Biol. 28: 249–254.

Smith V.S. 2001 – Avian Louse Phylogeny (Phthiraptera: Ischnocera). A cladistic study based on morphology – Zool. J. Linn. Soc. 132: 81–144.

Sokal R.R., Rohlf F.J. 1981 – Biometry – Freeman and Co., San Francisco, 776 pp.

Somers K.M. 1986 – Multivariate allometry and removal of size with principal component analysis – Syst. Zool. 35: 359–368.

Stillwell R.C., Blanckenhorn W.U., Teder T., Davidowith G. 2010 – Sex differences in phenotypic plasticity affect variation in sexual size dimorphism in insects: from physiology to evolution – Annu. Rev. Entomol. 55: 227–245.

Stockdale H.J., Raun, E.S. 1960 – Economic importance of the chicken body louse – J. Econ. Entomol. 53: 421–423.

Strauss R.E. 1987 – On allometry and relative growth in evolutionary studies – Syst. Zool. 36: 72–75.

Sychra O. 2005 – Chewing lice (Phthiraptera: Amblycera, Ischnocera) from chukars (*Alectoris chukar*) from a pheasant farm in Jinacovice (Czech Republic) – Vet. Med. 50: 213–218.

Talposh V.S. 1977 – [Breeding biology of Alpine Accentor in USSR] – Nauchnye doklady vysshei shkoly, Biologicheskie nauki, 9: 54–59 (in Russian).

Tompkins D.M., Clayton D.H. 1999 – Host resources govern the specificity of swiftlet lice: size matters – J. Anim. Ecol. 68: 489–500.

Tryjanowski P., Szczykutowicz A., Adamski Z. 2007 – Size variation in chewing lice *Docophorulus coarctatus*: how host size and louse population density vary together – Evol. Ecol. 21: 739–749.

Walther B.A., Clayton D.H. 1997 – Dustruffling: a simple method for quantifying ectoparasite loads of live birds – J. Field Ornithol. 68: 509–518.

Yazdi A.B. 2014 – Application of geometric morphometric to analyse allometry in two species of the genus *Myrmica* (Hymenoptera: Formicidae) – Soil Org. 86: 77–84.

Zekhnov M.I. 1950 – [Adaptive features in the breeding of lice of *Coloeus monedula*] – Russian J. Zool. 29: 478–480 (in Russian, English summary).

Zlotorzycka J. 1964 – Mallophaga parasiting Passeriformes and Pici III. Philopterinae – Acta Parasitol. Polonica, 12: 401–430.