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Feather holes of rock ptarmigan are associated with amblyceran chewing lice

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Feather holes have traditionally been suggested to be feeding traces of chewing lice (mallophagans). There is controversy whether mallophagans are the real source of feather holes. We studied mallophagan infestations and holes in tail feathers of 528 rock ptarmigan *Lagopus muta* collected 2007–2012 in northeast Iceland. Three mallophagans were found, *Amyrsidea lagopi* (prevalence 13%), *Goniodes lagopi* (72%) and *Lagopoecus affinis* (51%). The prevalence of feather holes was 15% and based on pattern the holes could be separated into two groups termed feather hole swarms (FHS), prevalence 9%, and single holes (SH), prevalence 6%. Holes for FHS were concentrated in the central tail feathers and decreased outwards, but holes for SH did not show any such pattern. There was a significant positive relationship between the number of holes for FHS birds and *A. lagopi* number, and the prevalence was similar. No other combinations of FHS or SH and the mallophagans indicated any relationship. The observed differences between FHS and SH suggest that feather holes have different origin. Our thesis based on known feeding habits of amblycerans like *A. lagopi* is that the holes in FHS are created during the pin feather stage when the lice bite the pin feather to draw blood. The holes in FHS were often in lines parallel to the feather shaft and the distance between adjacent holes was similar to the daily growth band, and where apparent the holes were sitting in the light portion of the band suggesting diurnal rhythm in lice feeding activity. Concluding, feather holes in ptarmigan may have various origins, but there is a clear correlation between the presence and numbers of *A. lagopi* and FHS. This is a novel finding for the grouse family and the genus *Amyrsidea* and should be a valuable contribution to the studies of feather hole formation.

Feather holes are found in many different bird species such as the domestic chicken, *Gallus gallus* forma *domestica* (Wilson 1933, Crutchfield and Hixson 1943, Trivedi et al. 1991), the barn swallow *Hirundo rustica*, and some other passerine species (Møller 1991, Vas et al. 2008). Feather holes are thought to have been created by the feeding activities of chewing lice (Mallophaga, Order: Phthiptera). Mallophagans are often site-specific and their morphology correlates with the sites preferred (Bush et al. 2001). There are two main groups of mallophagans, the suborders Ischnocera and Amblycera. Ischnocerans are highly specialized, live in the plumage, and feed primarily on keratin of feather barbules of down parts (Johnson and Clayton 2003, Møller and Rózsa 2005, Clayton et al. 2008). In contrast, amblycerans tend to occur in contact with host skin and feed on the blood of their hosts by biting the skin or pin feathers, but also by shearing or scraping feathers and skin with their mandibles (Bishopp and Wood 1917, Crutchfield and Hixson 1943, Ash 1960, Johnson and Clayton 2003, Møller and Rózsa 2005).

Recently, there has been controversy whether mallophagans are the causative agent of feather holes (Vágási et al. 2011, Vágási 2014), and furthermore what species or group of mallophagans are responsible for the holes (Møller 1991, Vas et al. 2008). Feather holes have been used in several influential studies as a proxy for mallophagan load (Kose et al. 1999, Moreno-Rueda 2010), in order to examine the impact of lice infestations on such traits as flight performance (Barbosa et al. 2002), mate choice (Moreno-Rueda and Hoi 2012), reproductive success (Pap et al. 2005), moult (Moreno-Rueda 2014), or survival (Pap et al. 2005). Vágási (2014) proposed three non-mutually exclusive hypotheses for the creation of feather holes: 1) they are created by chewing lice, 2) they are created by feather-degrading bacteria, and 3) they are one form of fault bars. Fault bars are growth defects and seen as straight, translucent lines perpendicular to feather barbs (Wood 1950, Solomon and Linder 1978).

Feather holes have been found on the rock ptarmigan *Lagopus muta* (hearafter ptarmigan; Nielsen unpubl.). Three

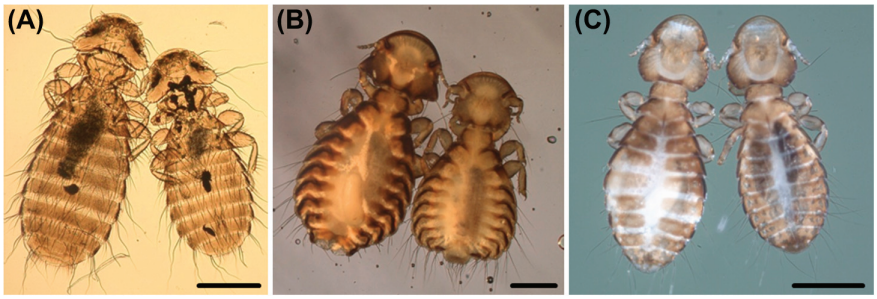

Figure 1. Chewing lice (mallophagans) infesting the Icelandic rock ptarmigan (A) *Amyrsidea lagopi*; (B) *Goniodes lagopi*; (C) *Lagopoecus affinis*. Females (shown on the left) are larger than males. Bar lengths: 0.5 mm.

species of mallophagans parasitize the ptarmigan, the amblyceran *Amyrsidea lagopi* and the ischnocerans *Goniodes lagopi* and *Lagopoecus affinis*. Most common is *G. lagopi* (prevalence, adult hosts 47%, juvenile hosts 86%), followed by *L. affinis* (adults 25%, juveniles 65%), and *A. lagopi* (adults 3%, juveniles 18%). All three species show significant host age related differences, with higher prevalences in juvenile hosts (Stenkewitz et al. unpubl.). *Amyrsidea lagopi* has an elongated body and its head is bell-bottomed, but not bulky (Fig. 1A). This species is agile and runs quickly across skin and feathers (Johnson and Clayton 2003). *Goniodes lagopi* (Fig. 1B) and *L. affinis* (Fig. 1C) have a rather short but elongated body form with a rounded head which characterizes sluggish body lice that occupy lush feathers of the body and escape preening by burrowing in the downy basal regions of feathers (Johnson and Clayton 2003, Clayton et al. 2008). Infestation of the three mallopagan species and ptarmigan body condition are not significantly associated (Stenkewitz et al. unpubl.). There is though a negative relationship between preen gland mass and prevalence of all three mallophagan species (González 2014), and also between the mass of the bursa of Fabricius – an organ of immune function in young birds – and the prevalence of *G. lagopi* and *L. affinis* (Stenkewitz et al. 2015). Both of these observations imply that there are physiological costs associated with mallophagan infestations in ptarmigan.

In 2006, we began studying the relationship between ptarmigan health and population change (Nielsen and Skírnisson 2009). Soon we noted that some of the birds sampled had a peculiar pattern of holes in tail feathers and we called those 'feather hole swarms' (Fig. 2). We wanted to examine this further and propose that this pathological

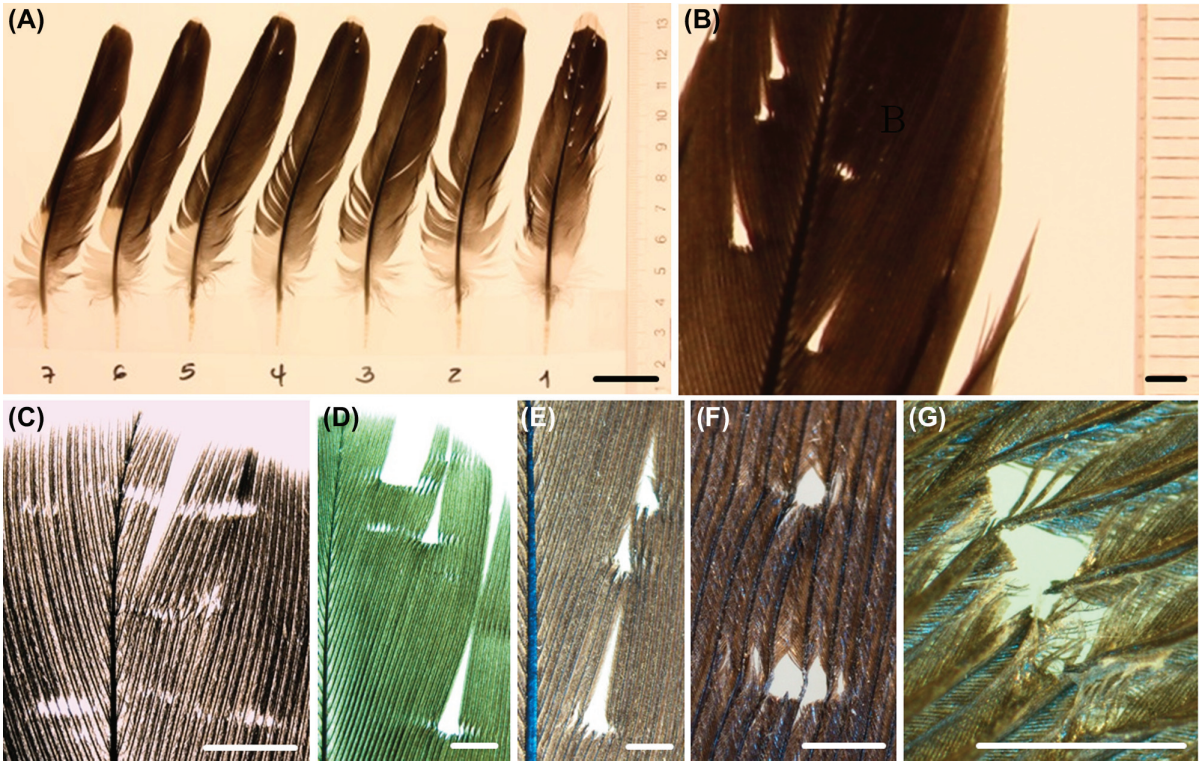

Figure 2. Feather holes and fault bars in tail feathers of Icelandic rock ptarmigan. (A) right tail side with feather hole swarms (FHS). Feather holes on five of the seven feathers are showed. (B) five feather holes. (C) four fault bars at the tip of a tail feather seen as transluscent lines. (D) feather breakage at fault bar. (E–G) closeups of feather holes. Bar lengths: (A) 2 cm, (B) 2 mm, (C) 2 mm, (D) 2 mm, (E) 1 mm, (F) 1 mm, (G) 1 mm.

character is created by mallophagan feeding activities. A priori we do not know if any one particular mallophagan species is responsible, but we expect a relationship between prevalence and abundance of the mallophagan accountable and feather holes in the combined sample and also individual years 2006–2012.

Methods

Birds used for this analysis were collected specifically for a long-term study on the relation between ptarmigan population change and ptarmigan's health related parameters (Skírnisson et al. 2012, Stenkewitz et al. 2015). To do all the sampling and analysis required for the study at large it was necessary to sacrifice birds. But it should be noted that the ptarmigan is very common in Iceland and a popular game bird and since 1995 between 40 and 160 thousand birds have been shot every year (<www.ust.is>).

The ptarmigan were collected (shot) from moorlands, lava fields and alpine areas west, east and north of Lake Mývatn in northeast Iceland (65°37′N, 17°00′W). The birds were collected in the first week of October 2007–2012 outside the hunting season authorised by the Icelandic Inst. of Natural History. Each bird was tagged immediately after collection. To avoid cross-contamination, each bird was wrapped in absorbent paper and placed in a paper bag, then sealed by interfolding and stapling. Birds were cooled to 4°C and processed within three days of collection.The first week of October was chosen as reference point to control for seasonal changes in parasite and feather hole prevalence and abundance. The annual number of juvenile birds analysed for the health study is 60 (equal sex ratio). The number of adults has varied between 18 and 41 birds. The average proportion of juveniles in autumn on the study area is 80% (Nielsen et al. 2004) so each year juveniles were collected in excess and individuals for analysis were selected at random from this catch. All adults caught were analysed, but as adult females are partly migratory (Garðarsson 1988), males dominated the adult catch (Table 1).

During dissection, the tail was removed, kept frozen, and later checked for feather holes. Mallophagans were collected according to Skírnisson et al. (2012) using a handheld vacuum cleaner (Princess, Turbo tiger, type 2755). The plumage of the intact bird was vacuum-cleaned for about two min; within this time the whole bird can be vacuumed systematically and thoroughly. The vacuum cleaner was modified for this purpose. The nozzle (4 × 1.5 cm) was connected to an external collection chamber fitted with a circular sack-like filter (92 cm², diameter of pores 2–30 μm). The filter was kept frozen until analysis when its contents were transferred to a 400 ml glass jar using the beam of a water-filled washbottle. Seven drops of the surfactant TritonH X-100 were added to the jar to reduce adhesive forces and to promote particle settling. The jar was fitted with a lid and shaken vigorously by hand and allowed to settle overnight. Mallophagans were collected under a stereoscope and embedded on a slide in Hoyer's medium (Anderson 1954) for later identification (Timmermann 1950, Scharf and Price 1983) and quantification.

Table 1. Annual sample of rock ptarmigan for feather hole studies in northeast Iceland, early October 2007–2012.

	Adult		Juvenile		
	Males	Females	Males	Females	Total
2007	13	5	29	29	76
2008	12	13	28	27	80
2009	11	7	30	28	76
2010	27	12	30	30	99
2011	24	16	30	30	100
2012	31	9	29	28	97
Total	118	63	176	172	528

An intact ptarmigan tail has 14 rectrices. The plucked tail feathers were mounted on a transparent plastic film in a right order, numbered, and photographed with illumination from below (Fig. 2A). A feather hole generally looks triangular, cone-shaped or drop-like and is 0.5–1.5 mm wide at the base where barbs are missing and sharp towards the top where the surrounding barbs close the created gap (Fig. 2B, 2E–F). Occasionally, particularly when the barbs are damaged or deformed but not broken, the gap consists of missing barbules only (Fig. 2F–G). We documented feather hole swarms (FHS) and single holes (SH) in tail feathers. Feather hole swarms always consisted of holes that were exhibited in a single line parallel to the feather shaft; sometimes there was more than one line. The distance between adjacent holes in FHS was frequently 2–4 mm, though there were exceptions, and on feathers where the growth band could be seen the holes were located in the light portion of the band. For analysis, feather holes from every feather from each tail were counted using the images. Only tails containing seven or more feathers were used to study the relationship between the number of feather holes and mallophagans. In case of missing feathers (255 out of 7392 or 3.5%), we used the same number of holes as on the equal feather from the other side of the tail to calculate total number of holes for that individual. This is justified by the low number of missing feathers involved and similar mean number of holes on equivalent feathers (Fig. 3). We only had access to dissected tails to study feather holes. We did not have access to other feathers except for wings of 26 birds that had *Amyrsidea lagopi*, but no holes in the tail feathers.

We performed statistical analyses using the software package R ver. 3.1.0 (<www.r-project.org>). Prevalence was defined as the proportion of birds with feather holes or mallophagans (Bush et al. 1997). To test if the prevalence of FHS, SH and mallophagans differed, we applied a Fisher's exact test. To test if the number of holes for birds with FHS and SH differed, we applied a Mann–Whitney U test. To test if the number of feather holes and mallophagans were related over the years, we applied generalized linear models, fitting quasipoisson family. To account for the individual years, we included factor year as interaction term and considered a fixed factor in each model. The model with the combined sample was corrected for type III error using drop1 function in R. For the model with the interaction term, the summary function was used to be able to present each year as well as the kind of relationship (positive or negative expressed in the t-value). We did not include age in the model as we are mainly interested in the relationship between the number of holes and mallophagans, and also because of sample size.

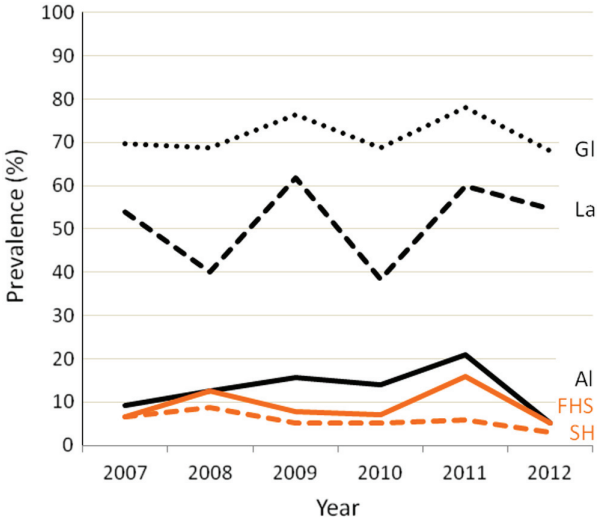

Figure 3. Prevalence of feather hole swarms (FHS), single holes (SH), and mallophagans in Icelandic rock ptarmigan 2007–2012 (n = 528). Al = *Amyrsidea lagopi*; Gl = *Goniodes lagopi*; La = *Lagopoecus affinis*.

To test for birds with feather holes, whether the frequency of feather holes or the mean number of feather holes was associated with the position of a feather within the tail, we used χ^2-tests with Yates' correction as well as non-parametric Friedman ANOVA tests, respectively. All tests were two-tailed and statistical significance deemed when $p \leq 0.05$.

Results

Five-hundred and twenty eight ptarmigan were examined for feather holes and louse abundance (raw data in Supplementary material Appendix 1). Sixty-nine (13%) had *Amyrsidea lagopi*, 378 (72%) *Goniodes lagopi*, 271 (51%) *Lagopoecus affinis*, and 79 (15%) had holes in tail feathers. From the latter, 49 birds (9%) had FHS and 30 (6%) SH in their tail feathers, this difference in prevalence was significant (Fisher's exact test $p = 0.035$). The mean number of holes for intact tails with FHS was 43.5 (n = 40, range 7–79, SE = 3.26) and with SH was 3.3 (n = 28, range 1–9, SE = 0.43). The difference in hole number between FHS and SH was highly significant (Mann–Whitney U = 2.00, $n_{FHS} = 40$, $n_{SH} = 28$, $p < 0.001$).

Location of holes within tail

For FHS, there was a significant difference with respect to the position of the tail feather and both the proportion of feathers affected (Yates' $\chi^2 = 54.2$, df = 6, $p < 0.001$) and the number of holes in affected feathers (Friedman ANOVA $\chi^2 = 136.0$, df = 6, $p < 0.001$). Both the frequency and the number of holes were highest in feathers in the mid part of the tail and decreased outwards (Fig. 4). Most holes were located on the distal half of each tail feather, and holes located on the proximal half were right below the center line and only found on the two innermost tail feathers on either side (Fig. 2A). For SH, there was no difference with either the proportion of tail feathers affected (Yates' $\chi^2 = 9.582$, df = 5, $p = 0.088$) or the number of holes in affected feathers (Friedman ANOVA $\chi^2 = 11.4$, df = 6, $p = 0.077$) and the position of the feather within the tail.

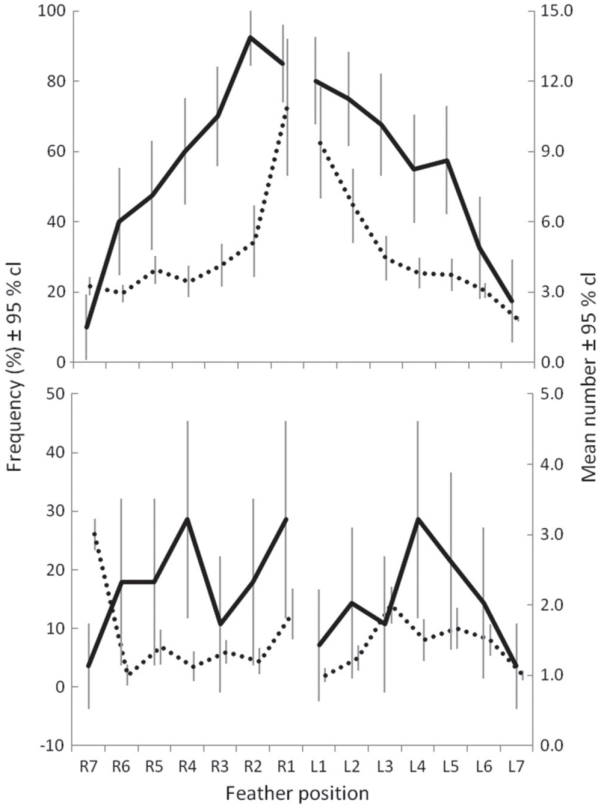

Figure 4. Frequency (continuous line; ± 95% confidence intervals) and mean number (dotted line; ± 95% confidence intervals) of feather holes for feather hole swarms (FHS; top) and single holes (SH; bottom) in tail feathers of Icelandic rock ptarmigan 2007–2012. The calculations are based on birds with intact tails only (FHS: n = 40, SH: n = 28).

FHS and mallophagans

Prevalence of FHS (9%) and the amblyceran *A. lagopi* (13%) in the total sample did not significantly differ (Fisher's exact test $p = 0.063$). In two years the prevalence of FHS was significantly lower than the prevalence of *A. lagopi* (Fig. 3, Table 2). Out of 75 birds with FHS and *A. lagopi*, 43 (57%) had both FHS and *A. lagopi*, 6 (8%) had only FHS, and 26 (35%) had only *A. lagopi*. Of the 26 birds that had no FHS in their tails, 8 (32%) had holes in secondary feathers. Also, two of the 26 birds had what we termed as SH in the tail feathers.

FHS and *A. lagopi* showed a significant positive relationship ($t = 6.5$, df = 74, $p < 0.001$). With respect to the individual years, four years showed a significantly positive relationship between the numbers of FHS and *A. lagopi*, and for two years significance was just above the rejection limit (Table 3).

Prevalence of FHS (9 %) and the ischnocerans *G. lagopi* (72 %, Fisher's exact test $p < 0.001$) and *L. affinis* (51%, Fisher's exact test $p < 0.001$) differed significantly, the ischnocerans were much more prevalent in all years (Fig. 3, Table 2). Further, numbers of FHS and *G. lagopi* (t = –0.7,

Table 2. Results from Fisher's exact tests for the prevalence of feather hole swarms (FHS) and mallophagans for Icelandic rock ptarmigan 2007–2012. Significant values ($p \leq 0.05$) indicate differences in prevalence.

Feather holes	Year	n	p
Amyrsidea lagopi	2007	7	0.569
	2008	10	1.000
	2009	12	0.014
	2010	12	0.073
	2011	22	0.021
	2012	6	0.596
Goniodes lagopi	2007	48	<0.001
	2008	52	<0.001
	2009	57	<0.001
	2010	66	<0.001
	2011	73	<0.001
	2012	65	<0.001
Lagopoecus affinis	2007	38	<0.001
	2008	31	<0.001
	2009	45	<0.001
	2010	37	<0.001
	2011	55	<0.001
	2012	51	<0.001

df = 382, p = 0.467) or *L. affinis* (t = –1.1, df = 283, p = 0.277) did not show any relationship, neither in the combined sample nor the individual years (Fig. 5, Table 3). Out of 383 birds with FHS and *G. lagopi*, holes and *G. lagopi* co-occurred in 44 (11%), 5 (1%) had only holes, and 334 (87%) had *G. lagopi* only. Out of 284 birds with FHS and *L. affinis*, holes and *L. affinis* co-occurred in 36 (13%), 14 (5%) had only holes, and 235 (83%) birds had only *L. affinis*.

SH and mallophagans

The prevalence of SH (6%) was significantly lower than the prevalence of any of the three mallophagan species (Fisher's exact test $p < 0.001$ for each). Also there was no relationship between the number of any of the three mallophagans and the number of holes (*A. lagopi*: F = 6.0, p = 0.063 after Holm–Bonferroni correction, *G. lagopi*: F = 0.1, p = 0.816, *L. affinis*: F = 0.4, p = 0.527; Fig. 5). For *A. lagopi* and SH there was very little overlap in occurrence, and out of 97 birds with either or/and, 2 birds (2%) had both SH and *A. lagopi*, 28 (29%) only SH, and 67 (45%) only *A. lagopi*.

Table 3. Results from generalized linear models between the number of feather holes for rock ptarmigan with feather hole swarms (FHS), and mallophagan numbers, northeast Iceland, early October 2007–2012. Significant values ($p \leq 0.05$) indicate a relationship between mallophagan numbers and the number of feather holes.

Feather holes	Year	n	t	p
Amyrsidea lagopi	2007	8	1.7	0.092
	2008	12	3.3	0.002
	2009	12	3.8	<0.001
	2010	14	1.8	0.073
	2011	22	5.6	<0.001
	2012	7	2.0	0.050
Goniodes lagopi	2007	54	–1.6	0.108
	2008	57	–0.9	0.372
	2009	58	–0.8	0.419
	2010	69	–1.7	0.084
	2011	78	–0.4	0.676
	2012	67	–1.8	0.069
Lagopoecus affinis	2007	43	–0.9	0.379
	2008	36	–0.9	0.346
	2009	49	–1.3	0.209
	2010	43	–1.3	0.194
	2011	60	–0.3	0.783
	2012	53	–1.3	0.206

Discussion

Different origins of feather holes

Our studies showed that 15% of ptarmigan had holes in their tail feathers. We classified the holes into two groups based on their patterns: FHS and SH. FHS were more prevalent then SH (9% versus 6%) and affected tails with FHS had more holes than SH (mean number 43.5 versus 3.3 holes). How the holes were distributed within the tail for the two groups also differed clearly. Hole frequency of occurrence and mean number of holes per feather for birds with FHS showed the highest values for the innermost tail feathers and decreased outwards. Holes for birds with SH did not form any such pattern and looked randomly distributed. This difference justified treating the two groups separately in the analysis of a relationship with the mallophagans.

The only significant relationship between feather holes and mallophagans was between abundance of FHS and the amblyceran *Amyrsidea lagopi*. Numbers of feather holes in tails with FHS were related with numbers of *A. lagopi* in the combined sample and all the individual years showed a positive relationship between the two variables. Also the prevalence of FHS and *A. lagopi* was similar. No relationship was observed between the two ischnocerans, *Goniodes lagopi* and *Lagopoecus affinis*, and FHS nor between SH and any of the three mallophagans. These observations further strengthen our view that the two types of feather holes have different origin and that only holes in FHS are created by mallophagans and in our case the amblyceran *A. lagopi*. The only other attempt to correlate the number of feather holes and mallophagans, to our knowledge, is by Møller (1991) who found a significant relationship between the number of feather holes and mallophagans on barn swallows. This study was however based on a small sample (n = 20 birds) and the mallophagans found were not identified to species level.

We do not have any explanation for the SH pattern. Also, some of the FHS holes could be breakage at a fault bar. At least 4% of 452 feathers with holes had some holes that were clearly associated with fault bars. That is, a fault bar touched or ran through the feather hole, most often at the base of the hole, but also at any level. Also, ptarmigan in our study population are known to harbour feather degrading bacteria (Sveinsdóttir et al. 2015), but no attempt has been made to associate them with feather holes.

How does *Amyrsidea lagopi* create holes?

We find it unlikely that *A. lagopi* bites the holes while eating keratin in full grown feathers. The amblycerans to which

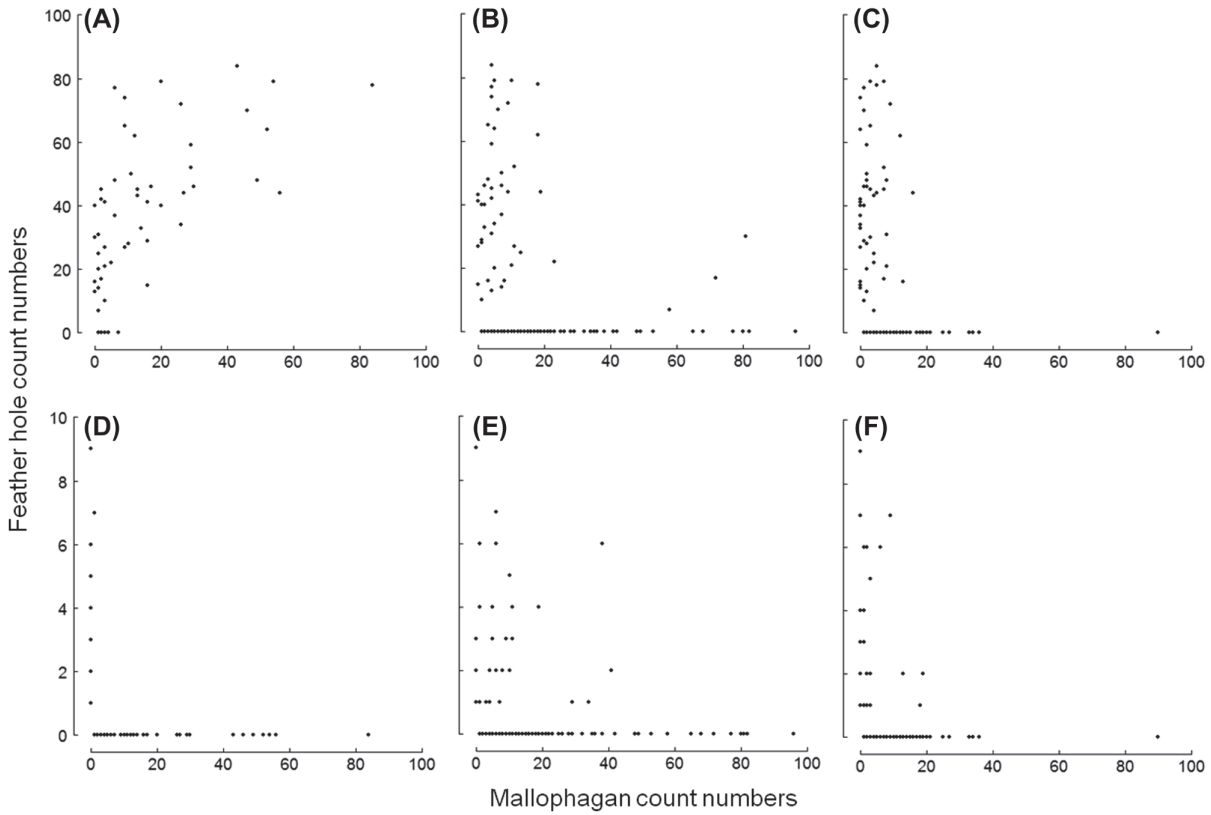

Figure 5. Associations between the number of feather holes and mallophagans for Icelandic rock ptarmigan 2007–2012. Birds with feather hole swarms (FHS) are depicted in (A–C) and single holes (SH) in (D–F). (A) and (D) *Amyrsidea lagopi*; (B) and (E) *Goniodes lagopi*; (C) and (F) *Lagopoecus affinis.*

the *A. lagopi* belongs are known to feed on living tissue rather than keratin (Crutchfield and Hixson 1943, Johnson and Clayton 2003), and also the diameter of a ptarmigan tail feather barb is at least 2–4 times greater than the mouth aperture of a fully grown *A. lagopi*. This suggests that biting damage by *A. lagopi* is done during the pin feather stage. Pin feathers are developing feathers that have a blood supply flowing through them (Lucas and Stettenheim 1972). Amblycerans are known to feed on pin feathers (Bishopp and Wood 1917, Crutchfield and Hixson 1943). Wilson (1933) observed the amblyceran poultry body louse *Menacanthus stramineus* (= *Menopon stramineum*) sucking blood from pin feathers, and also recorded old bite marks on pin feathers. Stockdale (1964) observed that most *M. stramineus* were feeding on the liquid portion (lymph and blood) of wounds and freshly plucked pin feathers. Also, Agarwal et al. (1983) demonstrated that up to 88% of *Menacanthus eurysternus* infesting the common myna *Acridotheres tristis* fed exclusively on host blood obtained from pin feathers. All mentioned amblycerans as well as *A. lagopi* from our study belong to the family Menoponidae. Our thesis is that the damage rendering feather holes in FHS is done when the amblyceran gnaws through the corneal sheath of the pin feather to draw blood and thereby damaging the developing barb.

The match between birds with FHS and *A. lagopi* was not perfect, six birds had only FHS and no *A. lagopi*, and 26 birds had only *A. lagopi*. Regarding the first group then we assume that feather holes are created during growth of the tail feathers and that takes place in July and early August, so there is a two months gap between creation of holes and the collection of mallophagans. Accordingly, extinction of *A. lagopi* could be part of the explanation for this mismatch or that we simply missed them during collecting of mallophagans. Additionally, amblycerans like *A. lagopi* are more mobile than ischnocerans and more likely to leave the host when handled or dying (Ash 1960, Bush et al. 2001, Clayton et al. 2008). The fact that we find *A. lagopi* but no FHS in tail feathers could be due to the mallophagan utilizing habitat other than the tail. Supporting this claim are feather holes found in the secondary wing feathers in 8 of these 26 birds in this category. Also, the distinction of FHS and SH is based on the pattern formed by the holes and there could be ambiguity involved and 2 of the 26 birds with *A. lagopi* but no FSH were defined to have SH. Another possible explanation would be for *A. lagopi* feeding on other live tissues than pin feathers.

Location of feather holes for FHS

The innermost tail feathers were clearly preferred, seen in both frequency of occurrence and number of feather holes. Possible explanations for this symmetrical pattern could have to do with security for *A. lagopi* by avoiding host preening and getting lost during takeoff and landing, or it reflects structural differences of feathers (outer tail feathers are more stiff) affecting access to blood, but in general the causes for this pattern remain unclear.

Feather holes were almost exclusively found on the distal part of the feathers. This suggests that *A. lagopi* feed on the pin feathers during the first phase in their growth. Growth bars (Wood 1950) in tail feathers of juvenile ptarmigan are 2 to 4 mm wide and between 3 and 6 mm in adults (Stenkewitz unpubl.). Similar feather growth rates have been reported for blue grouse *Dendragapus obscurus* (Bendell 1955). The maximum length of an affected distal part of a tail feather is 70 mm. Assuming a feather growth of ca 3 mm day^{-1} for juveniles and 4 mm day^{-1} for adults suggests that the time window available for the mallophagans to inflict their damage is approximately three weeks long. As the pin feather grows longer, the feather tip unfolds while blood supply is maintained in the lower part. At this stage in the development of the feather, according to our thesis, conditions are such that the mallophagans cannot draw blood from it anymore.

Why do the holes align themselves up in lines? This could be the result of repeated bites from the same louse either clinging to the pin feather or approaching it from the same angle for each feeding event. The distance between adjacent holes was frequently similar to the width of the daily growth band, 2–4 mm. Further, on feathers where growth bands were apparent the holes were associated with the light portion of the band, the part produced during the night (Wood 1950). This suggests a diurnal feeding rhythm of the lice.

Conclusions

Based on the observed relationship, we conclude that feather holes in ptarmigan have multiple origins with the feeding activity of the amblyceran *A. lagopi* during the pin feather stage is very likely one of those factors. The latter finding is supported by the diameter of fully grown barbs, the morphology of *A. lagopi* mouth parts, the known feeding habits of amblycerans, the relationship between the number of *A. lagopi* and feather holes, and similar prevalence of the two. To our knowledge this is the first time anyone has shown a quantitative relationship between a specific mallophagan species and feather holes. These findings are novel for the grouse family and the genus *Amyrsidea* and should be an important contribution to future studies of feather hole formations. Because feather holes in tails can be easily detected on live and dead birds, their presence can serve as a proxy for *A. lagopi* presence and hole numbers as one indicator of the health status of the bird.

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Supplementary material (available online as Appendix wlb-00255 at <www.wildlifebiology.org/appendix/wlb-00255>). Appendix 1.