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1. Introduction:

Animals, especially birds, furthermore of the order of the Passeriformes, which are usually relatively small, have great energy needs. It is commonly thought that songbirds are more energy-limited, than other members of the Vertebrates (CIERLIK *ET AL.*, 2004). It is thus important that passerines, particularly small passerines, have to function as energy efficiently as possible, especially so during winter months at northern latitudes (> N 45°). Suboptimal weather conditions, food bottlenecks, disease or parasites may cost the birds unaffordable energy.

All birds are thought to harbour some kind of parasite at some stage of their lives, either endo- or ectoparasites. Ectoparasites in European birds range in size from mites (Acarina), which are almost undetectable with the naked eye, through feather-lice (Mallophaga), fleas (Siphonaptera) up to louse-flies (Hippoboscidae, also known as flat-flies), which have the size of a common housefly. All of these parasites feed on blood, feathers or skin of the passerine host (ROTHSCHILD and CLAY, 1957). Some mite genera e.g. *Pterolichidae* also feed on oil droplets from the bird's uropygial gland (DUBININ, 1955) and others on fungi that grow within the bird's feathers, e.g. *Pterodectes rutilus* (Proctophyllodidae) and *Pteronyssoides nuntiaeveris* (Avenzoariidae) (BLANCO and TELLA, 2001). Today 2000 species in 33 families of feather mites are known (GAUD and ATEYEO, 1996), and about 3000 species of feather lice.

Interestingly, many bird species that have been found to be free of haemoparasites are highly infested with ectoparasites (MARTINEZ-ABRAIN *ET AL.*, 2004). Good examples of this can be seen in the long-lived Procellariiformes and alpine swifts, which are highly infested with ectoparasites but apparently free of haemoparasites (GONZALEZSOLIS AND ABELLA, 1997); (MERINO AND MINGUEZ, 1998); (TELLA *ET AL.*, 1998). Although ectoparasites can incur some cost to their host's fitness (MERINO *ET AL.*, 1999), they do not cause death as some endoparasites do, especially haemoparasites, e.g. plasmodium. Under certain circumstances, ectoparasites may even have an overall positive effect on their hosts (COX, 2001).

Although from an evolutionary perspective, research suggests that many bird species, especially birds of prey (e.g. Falconiformes), but also several species of songbirds for example the European Starling (*Sturnus vulgaris*) that re-use their nests from year to year, adopted 'medical skills'. They use green plants to fumigate their nests with insect repelling qualities or even insecticidal compounds to tackle ectoparasite burden (WIMBERGER, 1984) or to ward off endoparasite vectors, e.g. *Culex quinquefasciatus* as vector for avian malaria (LEVIN *et al.*, 2009).

Furthermore, Eastern Screech-Owls (*Megascops asio*) bring live blind snakes (*Leptotyphlops dulcis*) to their nestlings, whereas all other prey is delivered dead. Some of the snakes are eaten, but most remain unharmed in the nest debris, where they feed on insect larvae. Consumption of the larvae may reduce larval parasitism. Nestlings in nests containing a snake grow faster and their mortality rate is reduced. GEHLBACH AND BALDRIDGE (1987) suggest a commensalistic relationship in which the Screech-Owl benefits reproductively and the blind snake remains unharmed.

So far, observations of successful adaptations to fight off parasites in European songbirds have not been widely documented. MENNERAT *et al.* (2008) hypothesised, that

aromatic plants brought to the nests by blue tits (*Cyanistes caeruleus*) on Corsica would have anti-blow fly effects during the chick-rearing stage, but no significant relation was found between amount of aromatic plants in nests and blow fly infestation intensity.

These findings raise the question as to whether ectoparasites in European passerines affect their host at all. In this report, the effects of ectoparasites on the physiology of passerine birds will be assessed, with special regards to weight and fat reserves, as weight and body fat are easily measureable physiological factors to obtain a broad image of the overall condition of an individual. The main subjects in this work are Yellowhammers (*Emberiza citrinella*) and Blackbirds (*Turdus merula*) as a representative species for relatively small and relatively large respectively, passerine winter residents in Europe [**Fig.1**].

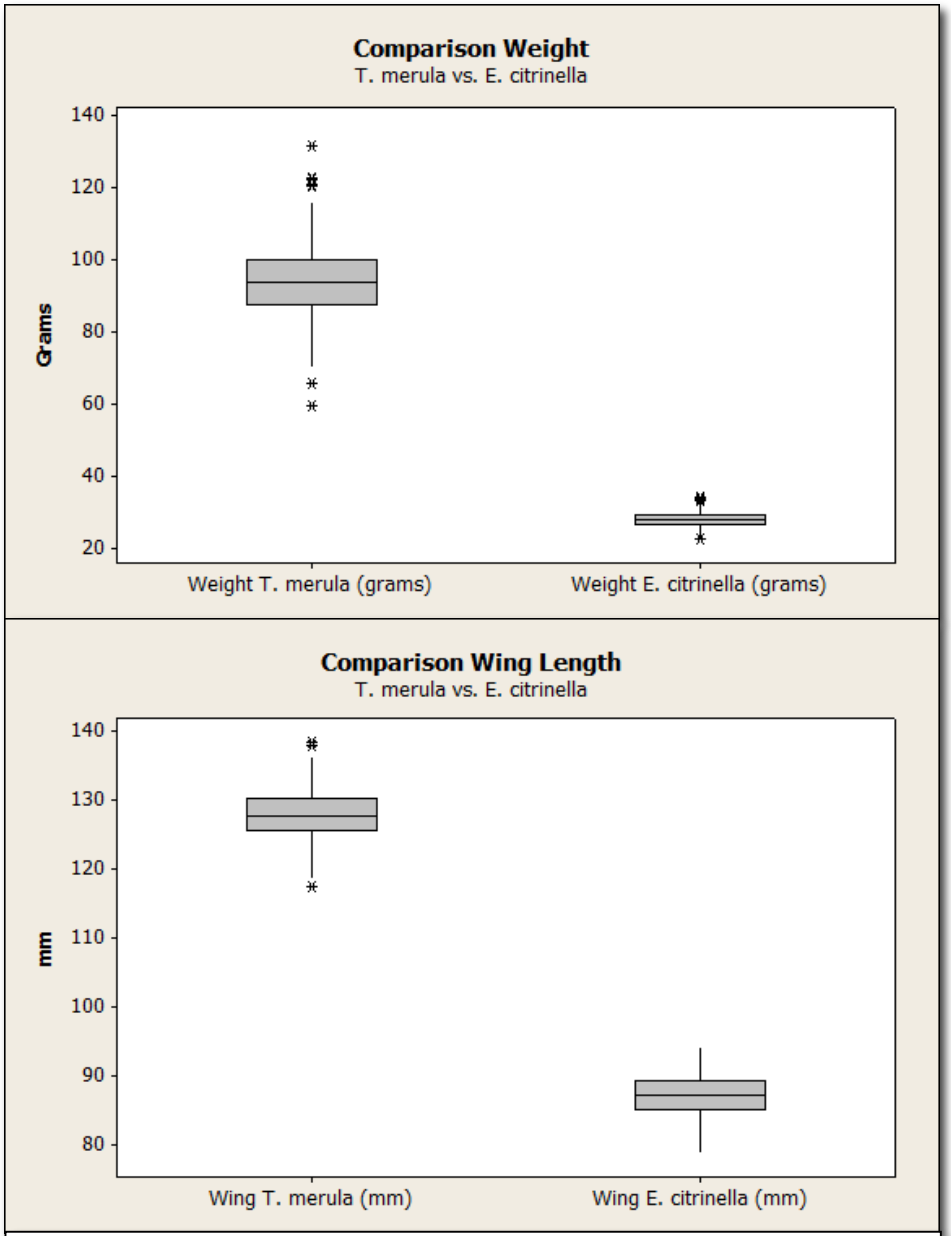

Fig.1: Boxplots, comparing weight and wing length between Blackbirds and Yellowhammers.

1.1 Main Hypotheses:

1) A bird with high ectoparasite burden possesses lower subcutaneous fat reserves, than a bird with few or no ectoparasites.

2) A bird with high ectoparasite burden has lower body weight, than a bird with few or no ectoparasites.

3) *Turdus merula* have, relative to body size, significantly higher ectoparasite burdens than *Emberiza citrinella*, due to different feeding and nesting behaviour, i.e. a blackbird feeds on the ground and some reuse their nests from year to year (McBride, 1978), whereas Yellowhammers feed mainly in shrubs and trees and use fresh nests every year.

4) Ectoparasites are readily transmitted horizontally during capture and handling, due to the reuse of infested bird bags.

Fig.2: 'Schlammwiss', 150 ha ringing station in Uebersyren, Luxembourg. Most of the ringing for this work was carried out here.

Fig.3: 'Sunflower Pad', ~2.7 ha ringing area in Ystrad Mynach, Wales. Ringing in November was carried out here.

2. Methods:

Bird ringing was carried out in two locations, 'Schlammwiss' in Uebersyren, Luxembourg (49°38'18.48"N; 6°16'35.79"E) [**Fig.2**], a wet meadow on 150 ha along the river Syre and at Ystrad Mynach in and around a sunflower pad north of Cardiff, Wales (51°38'9.60"N; 3°14'28.85"W) [**Fig.3**], during November, December 2009 and January 2010. During the ringing sessions bird species, age and sex were identified and biometric measures, such as subcutaneous fat deposits, weight and wing length were recorded. Fat deposits were measured using the system with degrees from 0 to 8 as proposed by (1993). Age and sex determination was recorded using a method outlined by SVENSSON (2006). Birds, known to belong to stress-insensitive species, were than fumigated using a device as proposed by (BEAR, 1995), but subject to a few slight changes in design.

2.1 The Ectoparasite Collection Device (ECD v6.2):

Fig.4: Ectoparasite collection device (ECD v6.2); 1) Flexible Thera-Band®; 2) Hole, to allow the bird free breathing, without inhaling chloroform gases; 3) Lit, with a perch fitted inside, to allow the bird to rest comfortably; 4) Jessops® Rocketblower; 5) 22 mm hole for gas circulation; 6) Wine kork; 7) Overpressure absorber; 8) Cylindrical Tupperware® container; 9) Whatman® 110 mm filter paper.

The device consists of a cylindrically shaped Tupperware ™ container (inside height: 175 mm, inside diameter: 110 mm) [8]. The centre of the lid was core-drilled to form a circular 60 mm opening. The opening was resealed with latex Thera-Band® (strength: 0.25 mm) used in physiotherapy with 2-component glue [1]. The Thera-Band®, in the centre of the opening, was hole-punched to form a flexible 10 mm circular gap [2], allowing the bird's head to emerge, without being exposed to the gas inside the container, but leaving sufficient room to allow it to breath normally. Attached, inside the lid [3], was a perch for the bird to rest comfortably during the procedure. Five more holes were drilled through the walls of the cylinder. Four of these were 22 mm holes at a 90° angle apart from each other at approximately one third of the height from the top [5], allowing the gasses to be circulated between the bird's feathers, using a Jessops® Rocket Blower [4] (usually used in photography for the cleaning and maintenance of cameras). In addition, there was a 6 mm hole with a party balloon emerging from it [7] to capture the overpressure resulting from the Jessops® Rocket Blower. The four 22 mm-holes were sealed, when not in use, with wine corks [6]. The bottom of the container was covered with 110 mm Whatman® filter paper [9] to collect the parasites [**Fig.4**].

2.2 Fumigation:

Ringed birds were fumigated with chloroform, using the Ectoparasite Collecting Device (ECD) described above. At the start of the procedure, the filter paper was drained with 5 ml of analytical reagent grade chloroform. The bird's head was then pushed through the hole in the Thera-Band® and secured with two fingers on both sides of their neck. The lid was placed back on the ECD and a stopwatch was started. After three minutes the Jessops® Rocket Blower was used in each of the 22 mm holes to create a circulation of chloroform between the bird's feathers and under its wings. Each time during gas circulation only the hole in use was opened. Circulation took approximately one minute and was repeated after another 3 minutes, before the bird was released again after a total of 7 minutes.

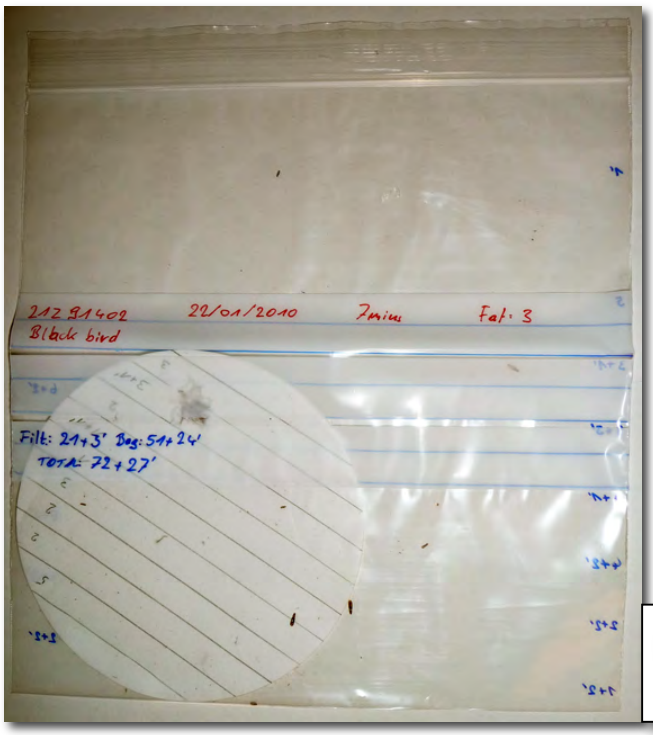

Fig.5: Sealable plastic bag, after microscopic analysis; containing the sample of a Blackbird who had 72 mites and 27 feather lice.

2.3 The samples:

After fumigation, the filter paper was carefully removed from the ECD and placed in a sealable plastic bag [**Fig.5**]. Any parasites that ended up off the filter paper were carefully put back, using a soft paintbrush. The ring number, date of fumigation and fumigating time were noted on each bag, which was than placed into an air tight Tupperware™ container with the others, leaving enough space to avoid damage to the samples.

After each procedure, the ECD was cleaned and the filter paper replaced.

The samples were then analysed using a stereomicroscope (1x – 6.3x magnification). The Whatman® filter paper was removed from the bag. To avoid double counting of parasites, lines were drawn onto the paper in intervals of 1 cm using a HB pencil (ø 0.3 mm) [**Fig.5**]. Line by line, the parasites were counted and classified as mites or lice. The value was written at the end of each line (e.g. if 12 mites and 2 lice were identified: 12 + 2' was noted). Detached body parts were ignored. Then the plastic bag was analysed, since its electrostatic charge attracted most of the arthropods from the filter paper. The bag was fixed to a piece of graph paper of the same size, using Bostik™ BluTack®, at the very top and very bottom edges of the sample bag. Where the bag was not transparent (e.g. the area to write on), a permanent marker pen was used to draw lines on in 1 cm intervals. It then was analysed in the same way as the filter paper. After the filter and the bag were analysed the numbers of mites and lice were added and recorded in the same Excel spreadsheet as the birds biometrics.

In total, 15 *E. citrinella*, 15 *T. merula*, 3 *Carduelis chloris*, 1 *Parus major* and 1 *Passer domesticus* were analysed and recorded [see Appendix I].

2.4 Data analysis:

Collected data from ringing and sampling was put onto a general worksheet in the following form:

- Bird species (e.g. Blackbird *Turdus merula*),
- Ring number (e.g. 21Z91088),
- Ringing and fumigation date (e.g. 15/12/2009),
- Time i.e. hour of day (e.g. 15),
- Fumigation time in minutes (e.g. 7),
- Age in two categories (e.g. 1 for juvenile bird and 2 for adult bird),
- Sex in two categories (e.g. 1 for male bird and 2 for female bird),
- Weight in grams, to the nearest 0.1 g (e.g. 110.8),
- Subcutaneous fat deposit in quantitative levels (SVENSSON, 2006) (e.g from 0-8),
- Wing length in millimetres, to the nearest 0.5 mm (e.g. 134.5),
- Number of mites found in sample (e.g. 52),
- Number of feather-lice found in sample (e.g. 3)

Data analysis software, Minitab 15® and Microsoft® Excel:mac2008's StatPlus:mac add-in, were used to carry out data analysis. All linear and/or continuous variables (weight, subcutaneous fat deposit, wing length and the number of mites and lice) were checked for normal distribution, in blackbirds and yellowhammers separately, using Anderson-Darling's test for normality (AD). Mites on blackbirds were not normally distributed [**Fig.6**], for they were showing leptokurtic (Kurtosis: 5.003) and positively skewed (Skewness: 1.86) distribution, giving an Anderson-Darling p-value of 0.038. Taking the square root of the mite-numbers transformed the data eventually to normal distribution [**Fig.7**].

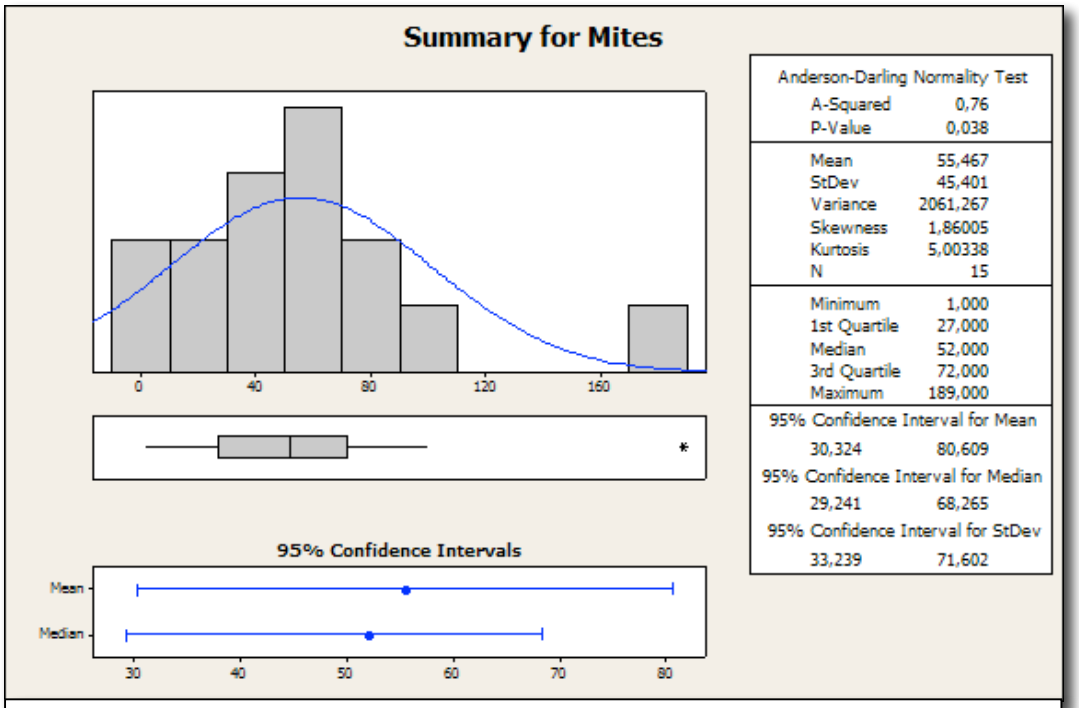

Fig.6: Graphical summary of mite numbers in Blackbirds. The Anderson-Darling test (P-value = 0.038 < 0.05) shows that the data is not normally distributed.

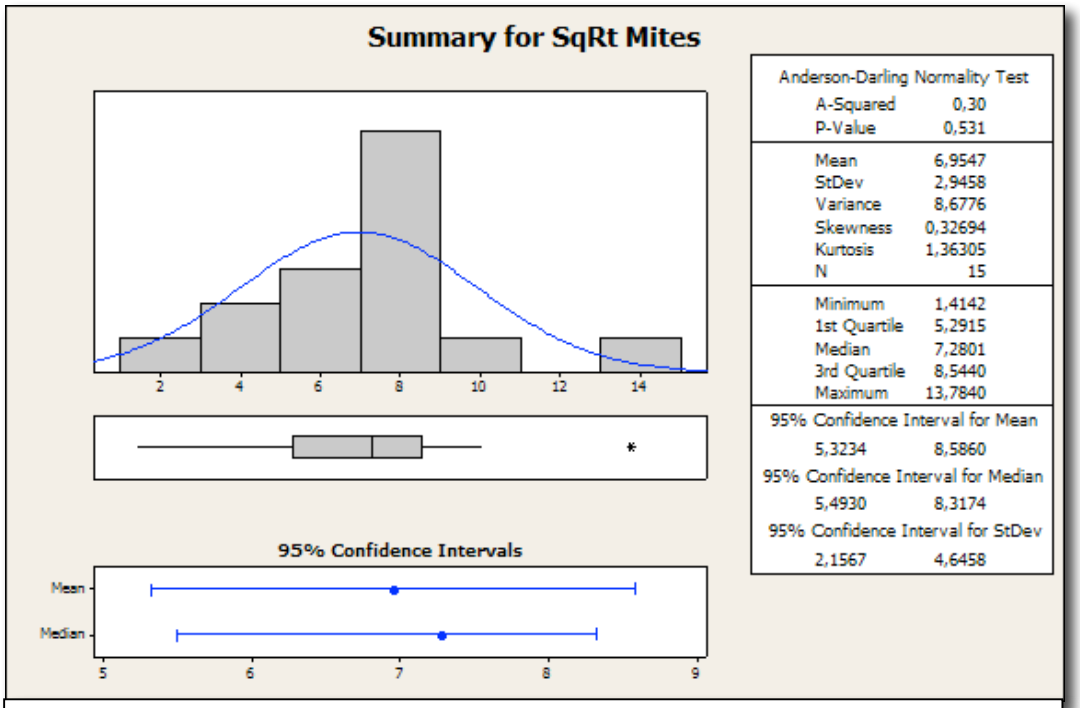

Fig.7: Graphical summary of the square root of mite numbers in Blackbirds. After transformation – square root of mite numbers - the Anderson-Darling test (P-value = 0.531 > 0.05) shows that the data is now normally distributed.

After normal distribution was achieved for all variables, Pearson's correlation coefficients were calculated between the birds' linear biometrics and mite numbers or louse numbers respectively. Spearman's rank correlation coefficient was used to compare categorical
values (i.e. age and sex) against ectoparasite numbers. Lice only occurred – in reasonable numbers – on blackbirds, and were therefore ignored on yellowhammers.

3. Results:

Table 1 summarises the results from Pearson's and Spearman's rank correlation coefficients. Cells shaded in red and pink contain values that show no correlation. Green and deep green contain values that show correlation. For example there is highly significant correlation between square root of mite numbers and louse numbers in blackbirds: r = 0.674 and P = 0.006. 0.674 > 0.514 (critical r in Pearson's correlation coefficient) and P < 0.01, which means that there is highly significant correlation between the variables at > 99% level. **Fig.8** provides a graphical representation of the correlation between transformed mite numbers and louse numbers in black-birds. No correlations were found between any of the other variables.

n = 15		Pearson	Spearman
	critical r=	0.514	0.518

		Turdus merula		*Emberiza citrinella*	
		SqRt Mites	Lice	Mites	Rank Lice
Weight (g)	r=	-0.017	-0.249	-0.31	0.163
	p=	0.499	0.37	0.216	n/a
Fat	r=	-0.081	-0.189	0.037	0.083
	p=	0.775	0.499	0.895	n/a
Wing (mm)	r=	-0.162	-0.005	n/a	n/a
	p=	0.597	0.986	n/a	n/a
Rank Wing	r=	n/a	n/a	0.408	0.106
Rank Age	r=	0.196	0.323	-0.079	0.11
Rank Sex	r=	0.101	0.237	-0.422	-0.413
Mites	r=	n/a	n/a	1	0.374
	p=	n/a	n/a	n/a	n/a
SqRt Mites	r=	1	0.674	n/a	n/a
	p=	n/a	0.006	n/a	n/a
Lice	r=	0.674	1	n/a	n/a
	P=	0.006	n/a	n/a	n/a

Table 1: Summary of Pearson's and Spearman's rank correlation coefficients in *T. merula* and *E. citrinella*.

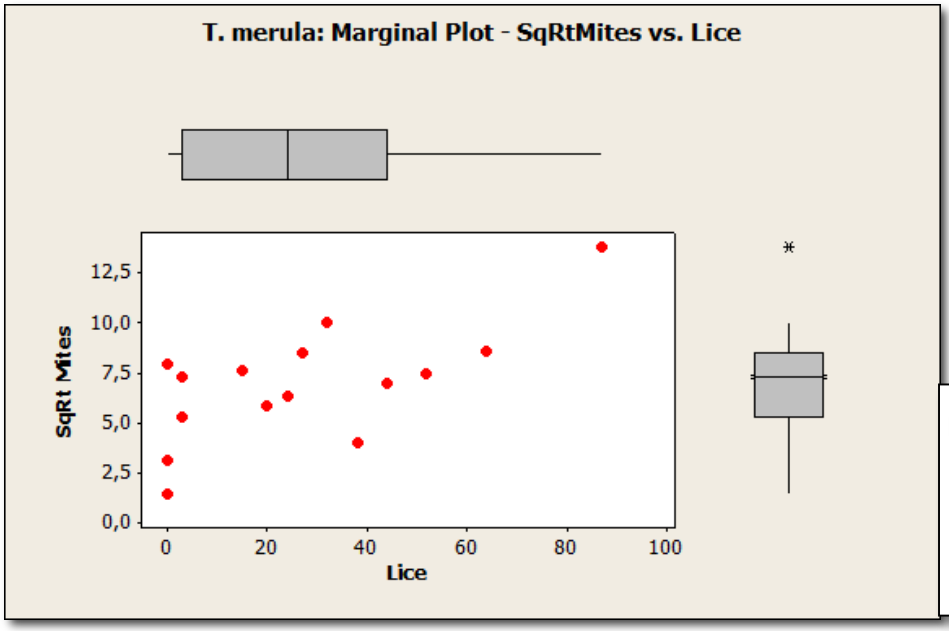

Fig.8: Marginal plot of correlation between the square root of mite numbers and louse numbers in *T. merula*.

4. Conclusion & Discussion:

There was only one significant correlation found: $\sqrt{\text{Mites in Blackbirds}}$ correlate positively with lice in Blackbirds [**Table 1** and **Fig.8**]. A single explanation for this is virtually impossible to find, because during the microscopic analysis, no effort was taken do determine the exact species of neither mites nor lice. Retrospectively I recognise that the interactions between birds, lice and mites are too diverse, to form a conclusion if the information about species identification is unknown. PROCTOR (2003) describes relationships, where feather lice feed on mites, where mites parasitize lice and where lice act as vectors for mites, i.e. phoresis. A possible explanation may also be that habitat preferences in *T. merula* tend to involve parasite-infested environments, e.g. feeding on the ground or feeding on decaying fruit. That would suggest that lice and mites are likely to spend time in these environments between two hosts. Furthermore, some Blackbirds have been reported to reuse their nests from year to year (MCBRIDE, 1978), equivalent observations on Yellowhammers are not known. These nests may build up on arthropod infestation from reuse to reuse, therefore this correlation may be observed in Blackbirds, but not in Yellowhammers.

Other correlations could not be found, i.e. between subcutaneous fat deposits and parasite numbers or weight and parasite numbers. Therefore the results appear to support the null hypothesis, that there is no correlation between fat deposits or weight and parasite numbers. If this is true then birds must be able to support the parasite burden without too much difficulty, i.e. energy expended, due to lice and mites, is not significant compared with what the birds need to support themselves. If we don't believe this, then possible reasons for the lack of correlation may include:

- Sample size to small. In total, 136 birds were fumigated, but the microscopic analysis process took much longer then anticipated, e.g. one sample took between 1 hour and 1.5 hours, rather then 10 minutes. Problems are explained below.

- Non-random samples. Maybe parasite-infested birds are more likely to get caught in the mist nets, then birds that are free of parasites? Further tests maybe needed to confirm this.
- Winter-feeding. Perhaps food was so abundant on the ringing sites that, whether the birds were highly infested with parasites or not at all, didn't reduce their ability to put on energy reserves [**Fig.9**].

4.1 Problems and Potential Error Sources:

Other problems, which may have affected the outcome of the experiment, are described here.

Fig.9: 20 of these bird feeders are installed and replenished daily in 'Schlammwiss'.

During early fumigation in Ystrad Mynach (i.e. November 2009) I realised, that executing the fumigation procedure in open air, in moist to wet weather conditions, brought with it many potential errors. One example of this would be that many small arthropods would stick to the walls of the Tupperware® container, due to humidity, and therefore wouldn't end up in the plastic sample bags, making these samples useless for further analysis. At the LNVL ringing site in Uebersyren, Luxembourg [**Fig.2**], ringing and fumigation was carried out in a heated hut, such as those that may be found on building sites [**Fig.10**], so the humidity problem was eliminated.

The first few weeks of ringing, in Ystrad Mynach [**Fig.3**], were still the developmental stage of the fumigation methods, e.g. the final design of the ECD, used for most of the fumigation, was version 6. Although BEAR (1995) suggested a different design, I felt some modifications were necessary. In ECD v1.0, cotton wool balls were drained with chloroform, on which a large fraction of the parasites would end up, therefore making it hard or even impossible, to carry out the microscopic analysis. In addition, the perch used in the first version, was an overly complicated cage-style construction, before I realised that a simpler way, using a bent copper wire (ø 3 mm), was much easier to handle and far more efficient. After many more similar properties of the ECD were changed, it finally seemed fit for its task.

Sampling was carried out during the winter months only; I think if it had occurred during the breeding season or the summer months, the results would have turned out very differently. Many species migrate out of Europe for the winter, including Barn Swallows, *Hirundo rustica*. From July to September 2009, I helped ringing about 30'000 *H. rustica* in 'Schlammwiss' and about a third of them were infested with flat-flies. I think much more conclusive results might have been possible through working with them. Flat-flies also act as flying vectors for lice and mites (PROCTOR and OWENS, 2000), bringing about very different circumstances.

Another factor that might have altered the outcome is that intensive winter-feeding occurred in 'Schlammwiss' [**Fig.9**]. Feeders were installed in every area on the LNVL ringing site and replenished on a daily basis. Apples from last autumn were laid out at some of the mist nets too. In mist nets with apples, the bulk of the Blackbirds were captured. This winter-feeding might result in higher body mass and larger subcutaneous fat deposits, indepen-dently from ectoparasite burden, be-cause food was so abundant.

Fig.10: Site hut, in 'Schlammwiss' where ringing and fumigation were carried out.

Better results too, are likely to have come from longer fumigation time. Eight birds were fumigated for more then 12 minutes. These individuals don't figure in my data set so as to avoid skewing the results. From observing these samples in situ, more feather lice were found on these birds during fumigation, than from birds treated for only seven minutes. However since no statistical tests have been carried out, this cannot be confirmed. Seven minutes fumigation time was used, nevertheless, for the study, to expose the subjects to the least stress possible, whilst still getting useful results.

Birds caught on early mornings, which ended up in the lower pockets of the mist nets [**Fig.11**], may have become wet, while hanging around in the tall grass. Obviously lice and mites may stick better to moist feathers, even if they are dead, then to dry plumage. It is therefore possible that – even with the rocket blower – fewer parasites fell onto the filter paper, because they stuck firmer to the bird.

Another potential error source was the microscopic analysis in its own right. As mentioned earlier, the sealable plastic bags [**Fig.5**], used for storing the Whatman® filter papers with the parasites on them, had an electrostatic charge, so that most of the parasites were adducted by the plastic on insertion of the filter. Therefore the bag had to be analysed with the stereomicroscope too. By moving the bag under the lens it may have happened (although, it doesn't have to have happened), that the arthropods were moved around between the graph paper lines, so that double counting or missing of individual parasites may have occurred. Probably though, both possibilities cancelled each other out.

Fig.11: Mist net for capturing birds in 'Schlammwiss'.

4.2 Suggestions:

To improve the experiment, I'd suggest a sample size of at least 120 birds of each species, collected over a period of a year, of equal size for every season. That would provide 30 samples for Yellowhammers and Blackbirds respectively, for each of the four seasons, i.e. winter, spring, summer and autumn. Extra feeding should not occur at the ringing sites. Furthermore, try to use individual holders for the samples, which are about the same size than the filter paper and preferably not electrostatically charged, e.g. flat petry dishes of the adequate size.

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