

# Semi-quantitative assessment of wing feather mite (Acarina) infestations on passerine birds from Portugal

## Evaluation of the criteria for accurate quantification of mite burdens

J. Behnke<sup>1</sup>, P. McGregor<sup>1,\*</sup>, J. Cameron<sup>1</sup>, I. Hartley<sup>2</sup>, M. Shepherd<sup>3</sup>, F. Gilbert<sup>1</sup>, C. Barnard<sup>1</sup>, J. Hurst<sup>1,†</sup>, S. Gray<sup>1</sup> and R. Wiles<sup>4</sup>

<sup>1</sup>School of Biological Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, U.K.

<sup>2</sup>Biological Sciences Division, Lancaster University, Lancaster LA1 4YQ, U.K.

<sup>3</sup>Designated Areas and Site Branch, Research and Advisory Services Directorate, Scottish Natural Heritage, 2 Anderson Place, Edinburgh EH6 5NP, U.K.

<sup>4</sup>Department of Biology, University of Buckingham, Buckingham MK18 1EG, U.K.

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### Abstract

Wing feather mite burdens on seven species of passerine birds (*Carduelis carduelis* – goldfinch; *C. chloris* – greenfinch; *Serinus serinus* – serin; *Sylvia atricapilla* – blackcap; *Sylvia melanocephala* – Sardinian warbler; *Turdus merula* – blackbird; *Passer domesticus* – house sparrow) from Portugal were assessed by the subjective semi-quantitative scoring system of Behnke *et al.* (1995) in order to evaluate more fully the accuracy and reliability of the technique. Our analysis indicated that in all species, scores allocated to flight feathers showed a significant positive relationship with mite counts as assessed through microscopical examination of the same feathers. However, there were differences between species of birds. Of the species examined, goldfinches and greenfinches showed the weakest relationships between assigned mite scores and actual mite numbers indicating that the technique was less accurate when applied to these species compared with the remaining five. No evidence was found that anything more was to be gained from scoring both wings, rather than just one. Feather mites (*Proctophylloides* spp., *Trouessartia incisa*) were also detected on tail feathers, but the assessment of these feathers presented additional problems and it was concluded that in the interests of minimizing handling time of birds, tail scores had little more to offer. We conclude that scoring all the flight feathers (including all primary, secondary, and tertiary feathers) on one entire wing, but alternating between left and right wings of birds within a species, represents an acceptable compromise between sufficiently detailed examination and minimization of bird handling time in the field.

**Key words:** passerine birds, feather mites, Acarina, infestation levels

### INTRODUCTION

The study of bird parasites and in particular their effects on host fitness is a topical issue because current hypotheses postulate that parasites have had a significant influence on the evolution of coloration and song repertoire in birds (Hamilton & Zuk, 1982; Folstad & Karter, 1992; Clayton & Moore, 1997; John, 1997). However, gathering data on bird parasite infestations in the field to test these hypotheses is problematic for a number of reasons, but especially if the birds are to be released unharmed after assessment. In an earlier paper (Behnke *et al.*, 1995) we reported that wing feather mite

infestations were sufficiently frequently encountered on passerine birds examined in Portugal to enable semi-quantitative analysis of infestation levels in relation to other measures of bird fitness during routine ringing. The technique we described allowed mite infestations to be quantified in a few minutes of handling, additional to that routinely allowed for ringing and recording of standard morphometric measurements, prior to release. We demonstrated that the primary feathers could be scored on a range of 0–3 depending on visual assessment of the proportion of the feather surface covered by mites. Calibration of these scores by correlation with actual mite counts on sampled feathers across all the bird species examined yielded a highly significant positive relationship, indicating that the scores accurately reflected mite infestations on feathers.

Our previous paper was based on examination of only the 10 primary flight feathers of the left wing, and whilst

\* Present address: Zoological Institute, University of Copenhagen, Tagensvej 16, DK-2200, Copenhagen N, Denmark

† Faculty of Veterinary Science, University of Liverpool, Liverpool L69 3BX, U.K.

this was sufficient to yield semi-quantitative data enabling statistical analysis and allocation of birds to groups differing in the intensity of infestation, the technique begged several questions. Was it sufficient to score just the primary feathers on one wing to obtain an accurate indication of the overall infestation on individual birds? Could additional pertinent information be obtained by inclusion of other feathers (e.g. secondary and tertiary flight feathers, tail feathers, wing coverts, body feathers)? Do different species of birds present specific problems with this type of assessment, i.e. is the scoring technique equally valid for all species?

In this paper we re-appraise the technique of Behnke *et al.* (1995), including some of the original data-sets as well as new data collected in the 3-year period following the original study. We address the specific issues of whether there is anything more to be gained by scoring secondary and tertiary feathers on the left wing, by scoring the flight feathers of the right wing and by including tail scores in the overall assessment of mite infestations. An all important consideration throughout our study was that handling time of birds should be minimized to reduce stress and enable subsequent release without harm

## MATERIAL AND METHODS

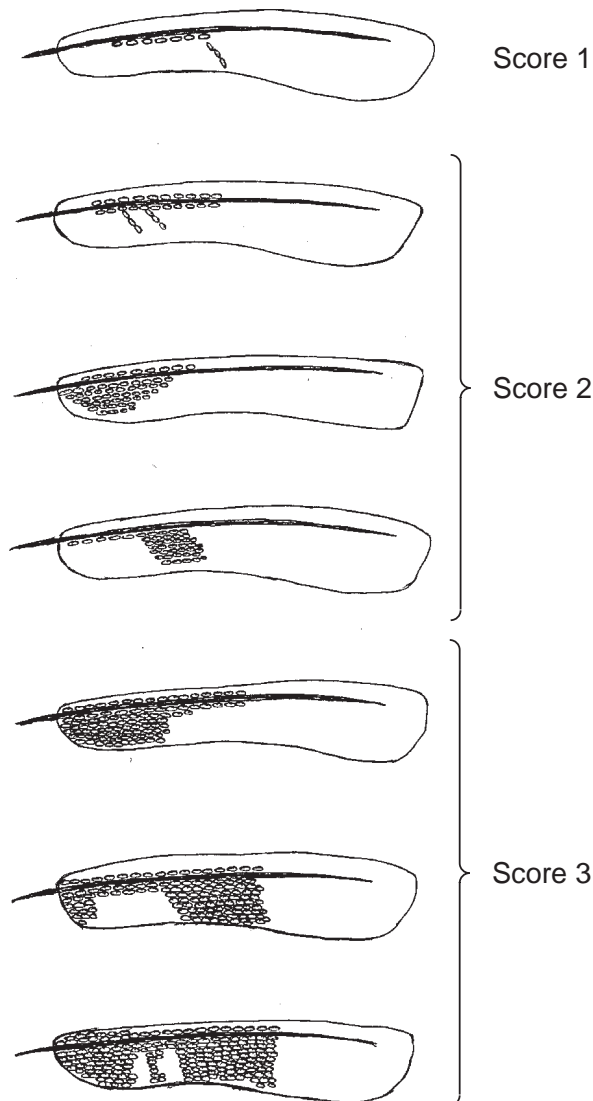
### Study site

The work presented in this paper was carried out during a 2-week period each year in mid-April from 1990 to 1997, as part of the Department's Behavioural Ecology Field Course by second year undergraduate students under close supervision of academic staff. Most birds were caught at the Quinta de São Pedro, located in Sobreda on the Setubal Peninsula near Lisbon, but 2 other sites were also used with the landowners' permission: the Quinta Niza, about 3 km further south-east; and Pancas, a protected site in the north-east of the Tejo estuary.

In 1990, only limited data were collected, some of which have already been published (Behnke *et al.*, 1995). The present study is based on data collected from 1991 to 1997, during which 1130 birds (representing 33 species) were examined. This paper concentrates on the 7 species that were netted annually in sample sizes suitable for quantitative analysis: *Carduelis carduelis* (goldfinch); *C. chloris* (greenfinch); *Serinus serinus* (serin); *Sylvia atricapilla* (blackcap); *S. melanocephala* (Sardinian warbler); *Turdus merula* (blackbird); and *Passer domesticus* (house sparrow), collectively representing 71.7% of the birds examined.

### Capture and treatment of birds

All the birds were caught in standard mist-nets. Birds were handled in accordance with British Trust for



**Fig. 1.** Illustration of mite clusters as seen by eye, corresponding to scores of 1 (1–10 mites), 2 (11–30 mites) and 3 (> 30 mites).

Ornithology (BTO) established procedures (Spencer, 1984), by trained, licensed bird ringers. Throughout the study close contact was maintained with the Centro de Estudos de Migrações e Protecção de Aves (CEMPA) who approved the techniques used in the study.

### Examination of birds for mites

On completion of morphometric measurements, the left wing of each bird was extended and held up to ambient light. The complete length of the primary feathers was exposed, primary coverts and underwing coverts being moved gently aside if necessary and both sides of each feather were examined by eye. Feather mites were visible along the shaft of each infested feather and, in more intense infestations, ran along the barbs and barbules. It was thus possible to examine each of the 10

primaries in a consistent and rapid manner without undue distress to the birds. We employed the technique of Behnke *et al.* (1995), based on a modification of the method reported by McClure (1989) and followed Svensson's (1992) ascendent numbering scheme for feathers, i.e. from the outside of the wing in towards the body. All the flight feathers from primary 1 (the outermost primary) to primary 10, across the secondary feathers (s1–s6) and the 3 tertiary feathers (on some species primary 1 is extremely small and was not assessed) were assessed visually for evidence of mite infestation and a score in the range 0–3 was allotted to each feather depending on whether there were no mites visible through to intense accumulations covering over 50% of the wing surface (Fig. 1). Full details are given elsewhere (Behnke *et al.*, 1995). In the period 1995–1997 we also scored all flight feathers on the right wing and the 12 tail feathers. Additional body feathers examined included samples from the neck, head, belly, and back (maximum of 5 feathers/bird, often released during handling but occasionally plucked).

### Sampling feathers for microscopical examination

In order to calibrate the scoring system it was necessary to remove feathers from birds for microscopical examination. Only one flight feather from the left wing was taken from each bird, although birds showing signs of distress (overt disease, weakness, damage) or juveniles with incomplete adult plumage were not sampled. Where possible, feathers were plucked rather than cut because plucking is considered to lead to faster feather regrowth (C. M. Perrins, pers. comm.). In the period 1991–1993 we removed only the feather showing the apparently heaviest signs of mite infestation from among primary 3 to primary 10. Primary feathers 1 and 2 were not sampled because of their presumed importance in flight. In 1994 and 1995 we systematically sampled primary 3 through to primary 10 in a numerical sequence specific for each species to ensure that sampled feathers included the widest range possible, and in 1996 and 1997 we also included the secondary and tertiary feathers.

Each removed feather was placed between 2 microscope slides in a Petri dish on which the identity, reference number of the bird and feather were recorded. Binocular dissecting microscopes were used in the field in sunlight, with only the microscope stage shaded. In general, light transmitted from the substage mirror was used to highlight the mites, although occasionally surface light also aided examination. The mites were counted from one end of the feather to the other on one side and then on the reverse and each microscopist recorded his or her own count for each feather. Examination of each feather was completed within 5 min of removal from the birds. During the 7 years of the study a different microscopist was responsible in each year, although in 2 years we had 2 microscopists working independently, and unaware of each other's values until data collation.

All feathers, confirmed by microscopy as being infested with mites, were placed into labelled vials containing 70% ethanol and 10% glycerol for subsequent identification of the mites.

### Identification of mites

Feather mites of the genus *Proctophyllodes* Robin, 1868 were identified using the keys of Atyeo & Braasch (1966) and other genera using Zumpt (1961) and Dubinin (1951, 1953, 1956).

### Abbreviations

In the present work we use the following abbreviations: PMIS: the sum of mite infestation scores of the 10 primary flight feathers, equivalent to PTMIS in Behnke *et al.* (1995). We use PMIS to avoid confusion with TOTMIS.

2/3MIS: the sum of the mite infestation scores of the 6 secondary and 3 tertiary flight feathers.

TOTMIS: the sum of mite infestation scores of the primary, secondary and tertiary flight feathers.

TAILMIS: sum of the mite infestation scores of the 12 tail feathers.

p1–p10: primary flight feather 1 through to primary flight feather 10.

s1–s6: secondary flight feather 1 through to secondary flight feather 6.

t1–t3: tertiary flight feather 1 through to tertiary flight feather 3.

### Data presentation and statistical procedures

As the study progressed from one year to the next, we modified data collection to meet new objectives and hence all data are not available across the whole period. Thus specific analyses are restricted to birds caught in years when relevant data were collected.

Where appropriate the data are presented as mean  $\pm$  the standard error of the mean (SEM). Non-parametric statistical procedures were employed because ordinal data, showing overdispersed distribution, were involved. Correlations between variables were examined with the Spearman rank order correlation test and  $r_s$  values are given as appropriate. Probabilities are 2-tailed unless indicated otherwise.

## RESULTS

### Numbers of birds by species and year

Table 1 summarizes the number of birds of the seven species selected for this analysis, examined by species per year and overall totals.

**Table 1.** Numbers of birds sampled by species and year

Species	1991	1992	1993	1994	1995	1996	1997	Total
Blackbirds	7	3	8	9	15	5	15	62
Blackcaps	44	24	24	22	31	30	34	209
Goldfinches	19	11	10	17	25	17	34	133
Greenfinches	3	1	10	13	17	10	10	64
House sparrows	34	20	25	22	25	34	27	187
Sardinian warblers	11	5	9	13	14	15	17	84
Serins	1	4	4	7	21	10	24	71

### Species of mites recovered

All the birds in the study were affected by mites from the genus *Proctophyllodes* as described by Behnke *et al.* (1995). Greenfinches and goldfinches both carried *P. pinnatus* (Nitzsch, 1818), serins carried *P. serinus*, blackcaps and Sardinian warblers both had *P. sylviae* Gaud, 1957, although *P. clavatus* Fritsch, 1961 was also recorded on Sardinian warblers. House sparrows had *P. truncatus* Robin, 1877. Blackbirds carried *P. musicus* Vitzthum, 1922, and *Trouessartia incisa* Gaud, 1957.

### How accurately do assigned scores represent actual mite numbers present?

The relationships between the assigned subjective scores on the sampled feathers of the seven species of birds and the actual mite counts, as revealed by microscopical examination, are shown in Fig. 2. The overall relationship across the seven species (Fig. 2h) was computed from the means for each species. All relationships gave significant positive correlations. However, examination of the correlation coefficients indicated that the strongest relationships between scores and actual mite numbers were for blackcaps and Sardinian warblers. Those for goldfinches and greenfinches, whilst significant, were much weaker.

Analysis of all the species combined showed relatively small standard errors and a highly significant correlation (Fig. 2h). However, the scales differed between the species, a score of 3 for house sparrows equating to an average mite count of about 120, whilst those for all the other species were considerably lower (range 23 [goldfinches] to 57 [blackbirds]). Moreover, the score of 0 did not reflect total absence of mites, although there were clear differences between the species in this context. For blackbirds, blackcaps, Sardinian warblers and serins, 0 scores corresponded to an average mite count of less than 3. For house sparrows, greenfinches and goldfinches the 0 scores gave mean mite counts of 6.7, 8.7, and 10.7, respectively. Quite clearly the poorest relationship between scores and mite counts was among the goldfinches, the calibration curve giving the shallowest gradient of all, but the sample size for the higher scores were low.

Figure 3 shows the percentage of scores that were accurately allocated to the respective categories on the

basis that a score 0 should actually represent 0 mites present, a score of 1 should cover a range of 1–10 mites, a score of 2 a range of 11–30 mites and a score of 3 a range of greater than 30 mites. It can be seen that for scores of 0, 1, and 3, >60% were accurately assessed. The greatest discrepancy was for a score of 2, 35.5% of which should have been given scores of 3 and 21.5% a score of 1. Many of these errors occurred among greenfinches and goldfinches, as already emphasized above. House sparrows also posed a problem with 16 of the 27 house sparrow feathers given a score of 2, having mite numbers corresponding to a score of 3.

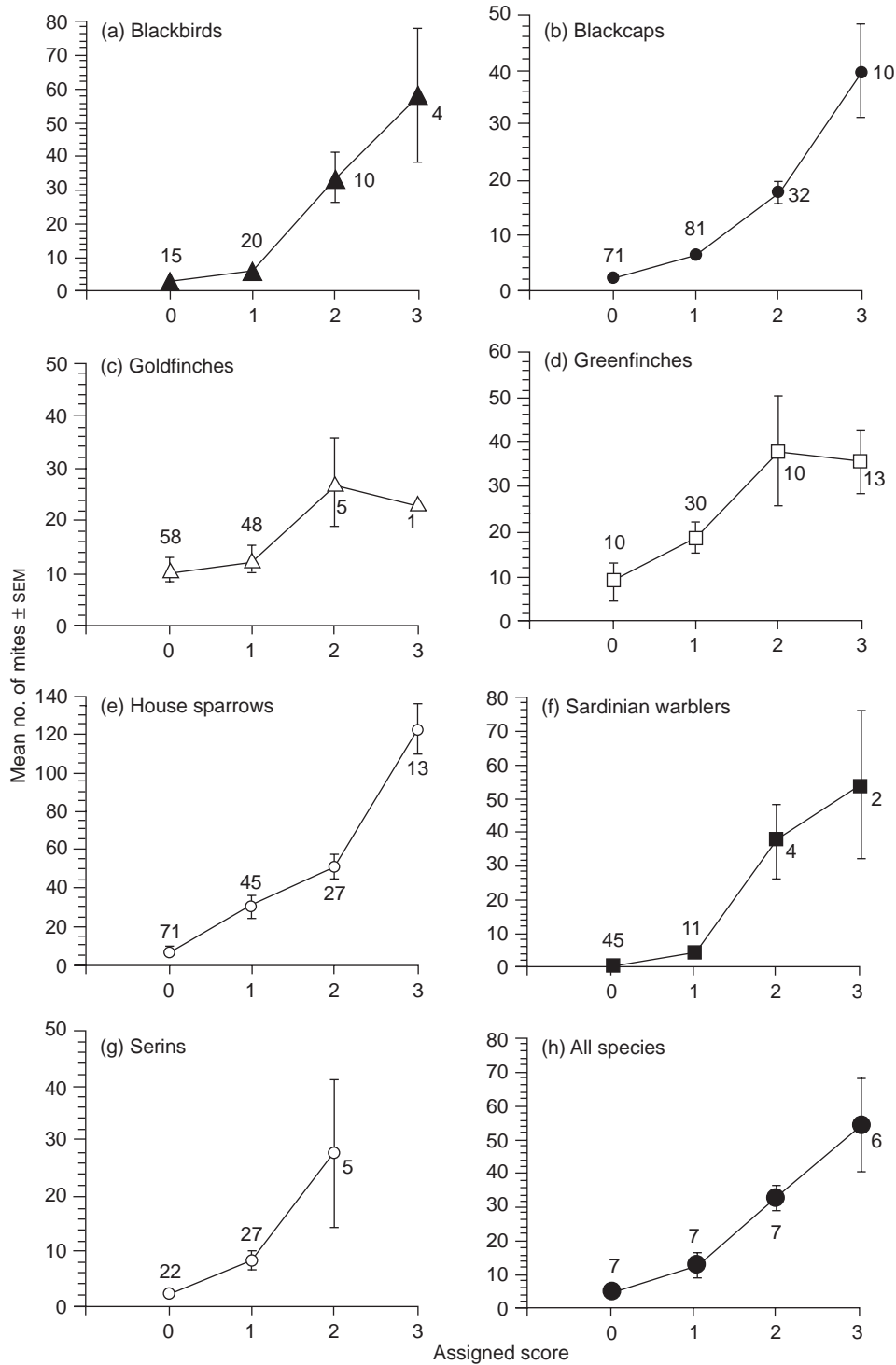
### How accurate and consistent is the microscopical examination?

In order to assess the accuracy of the microscopical examination, in each of 2 years of the study, feathers were examined independently by two microscopists. Additionally a third microscopist (JMB) spot-checked some of the feathers. This data set is based on 28 of the 30 species of birds examined in those years and is illustrated in Fig. 4. The relationship between mite counts obtained by microscopist 1 compared with microscopist 2 showed little discrepancy. A second analysis, based only on the seven species on which this paper concentrates, gave very similar results ( $r_s = 0.984$ ,  $n = 212$ ,  $P < 0.001$ ).

### Is it sufficient just to score the left wing or is there any advantage to be gained from scoring both wings?

In 1995–1997, we scored all primary, tertiary, and secondary feathers on both right and left wings for the seven species in this study. The values were very similar within species although the means differ quite markedly between species. Statistical analysis by one-way ANOVA (Kruskal-Wallis) with left TOTMIS, right TOTMIS, left PMIS and right PMIS as the dependent variables and species as the factor gave  $H = 71.1$ , 69.8, 53.3, 54.1, respectively ( $P < 0.001$  for all).

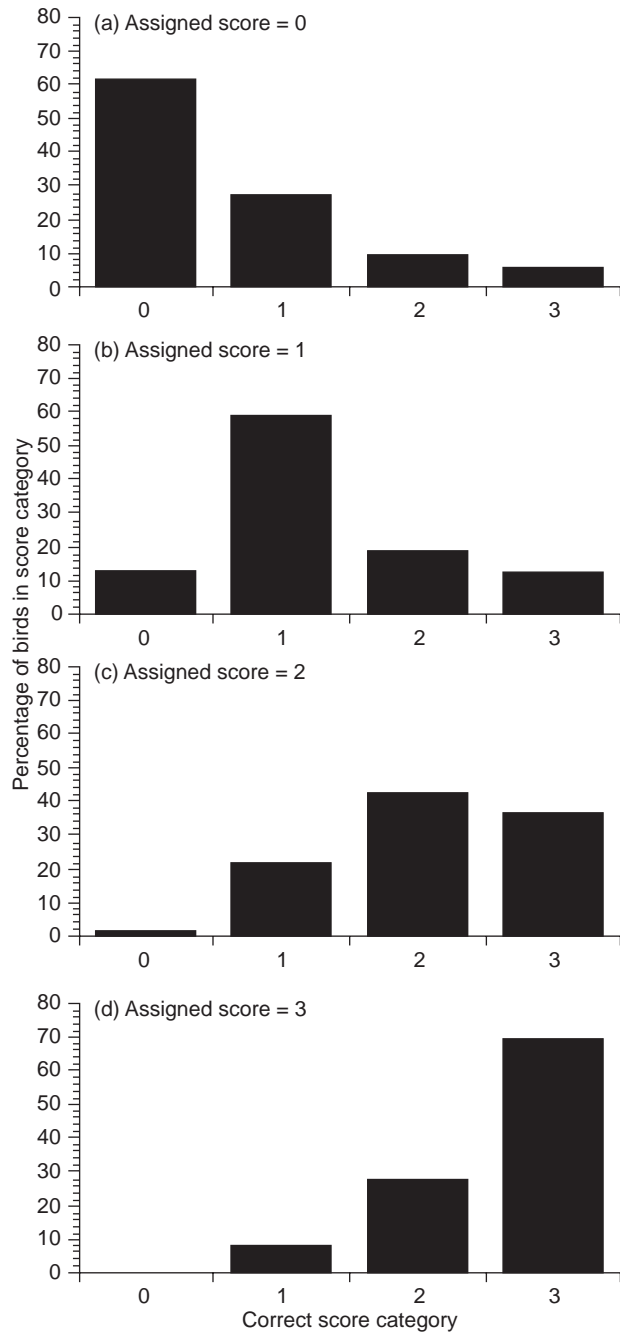
Close examination of the within species differences revealed very little discrepancy, as illustrated in Fig. 5, with some species showing a slight right bias (house sparrows) whilst others showed a slight left bias (serins and blackcaps). The maximum mean difference was for



**Fig. 2.** Relationship between the assigned scores and the actual mite counts for the sampled feathers on all seven species of birds, and for all species combined (h, calculated from means from each species). The figures beside the symbols give (a–g) number of birds at each score level and (h) number of species contributing to the mean. (At the assigned score of 3,  $n = 6$  because no feathers with this score level were sampled from serins.) Statistical analysis of results: (a)  $r_s = 0.699$ ,  $n = 49$ ,  $P < 0.001$ ; (b)  $r_s = 0.761$ ,  $n = 194$ ,  $P < 0.001$ ; (c)  $r_s = 0.211$ ,  $n = 112$ ,  $P = 0.026$ ; (d)  $r_s = 0.488$ ,  $n = 63$ ,  $P < 0.001$ ; (e)  $r_s = 0.684$ ,  $n = 156$ ,  $P < 0.001$ ; (f)  $r_s = 0.704$ ,  $n = 62$ ,  $P < 0.001$ ; (g)  $r_s = 0.602$ ,  $n = 54$ ,  $P < 0.001$ ; (h)  $r_s = 0.853$ ,  $n = 27$ ,  $P < 0.001$ .

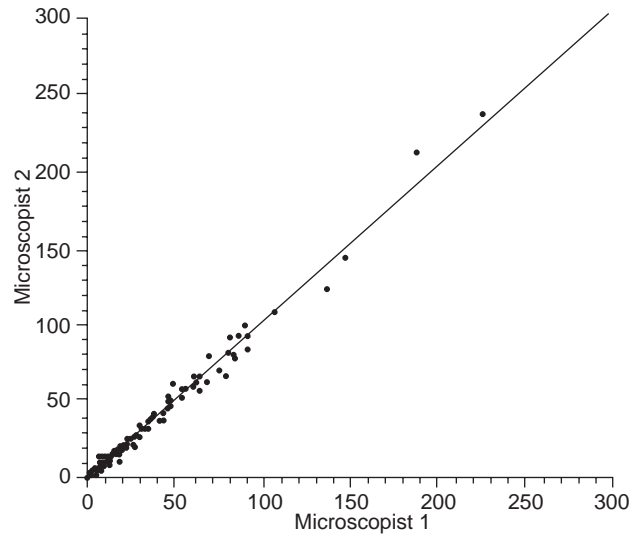
serins, with a left–right TOTMIS of 1.6, but all others were considerably smaller and represented only a fraction of the mean value in each case. However, analysis of the differences between left and right wing scores by

one-way ANOVA (Kruskal-Wallis) with left–right TOTMIS and left–right PMIS as the dependent variables and species as the factor gave  $H = 2.7$  and 1.6 respectively ( $P = 0.032$  and 0.044, respectively). Thus

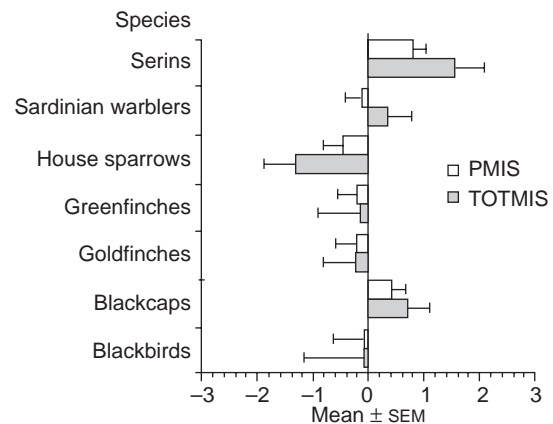


**Fig. 3.** Frequency distributions of category of mite number within each class of score assigned by observers. (Categories defined as zero in category 0; 1–10 in category 1; 11–30 in category 2; >30 in category 3.) Sample sizes are: (a) 0 = 178, 1 = 77, 2 = 24, 3 = 13; (b) 0 = 34, 1 = 154, 2 = 45, 3 = 29; (c) 0 = 1, 1 = 20, 2 = 39, 3 = 33; (d) 0 = 0, 1 = 3, 2 = 11, 3 = 29.

there were significant differences between the species in the extent of the differences between left and right wing scores. We calculated *t* for departure from zero for each species and the only significant values were for blackcaps (TOTMIS,  $t = 1.746$ ,  $n = 56$ ,  $P < 0.05$ ), house sparrows (TOTMIS,  $t = 2.33$ ,  $n = 42$ ,  $P < 0.025$ ), and serins (TOTMIS,  $t = 2.968$ ,  $n = 30$ ,  $P < 0.005$ ; PMIS,  $t = 3.786$ ,  $P < 0.0005$ ).



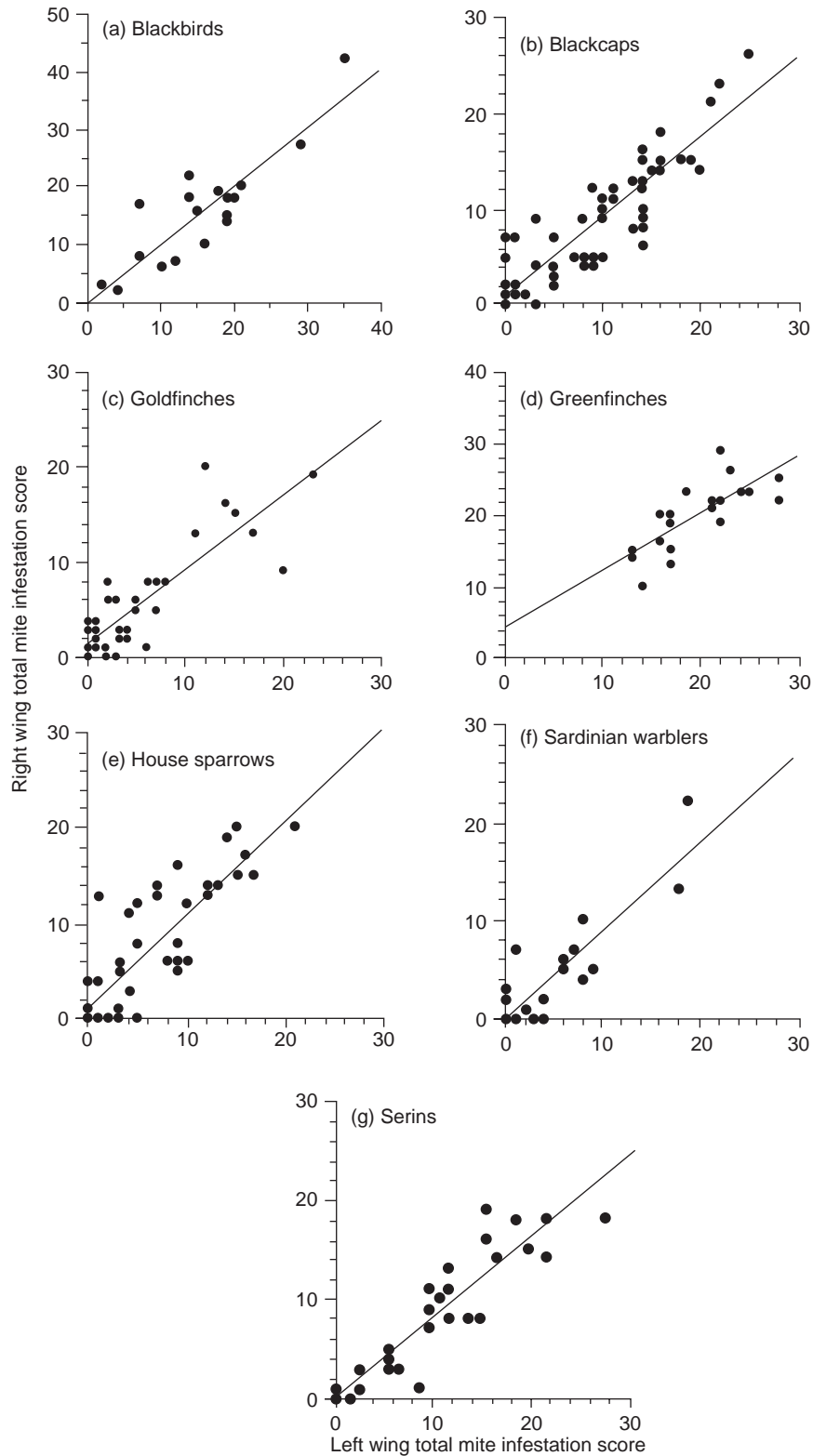
**Fig. 4.** Comparison of mite counts by two microscopists working independently on sampled feathers during 2 years of the study ( $r_s = 0.989$ ,  $n = 303$ ,  $P < 0.001$ ).



**Fig. 5.** Comparison of mean difference between left and right wing total mite infestation scores across species (left TOTMIS minus right TOTMIS).

Figure 6 shows the correlations between the left and right wing TOTMIS, for all seven species. The data for PMIS are not illustrated. All relationships (including TOTMIS and PMIS) were highly significant, although again some species showed better correlation coefficients than others. Surprisingly, the highest correlation coefficient was calculated for serins, which had shown a significant difference between left and right wing scores in the earlier analysis. (For TOTMIS see legend to Fig. 6, for PMIS  $r_s = 0.913$ ,  $n = 30$ ,  $P < 0.001$ .) The lowest correlation coefficients were for blackbirds. (For TOTMIS see legend to Fig. 6, for PMIS  $r_s = 0.886$ ,  $n = 18$ ,  $P < 0.001$ ).

We also examined the distribution of mean mite scores by feather across the left and right wings of three of the species in the study. All left and right wing scores for comparable feathers in the sequence from p1–t1 were very similar. Thus a peak on the outermost feathers of blackbirds (p2–p3), blackcaps and green-



**Fig. 6.** Relationship between the total mite infestations on the left and right wing flight feathers (primary, secondary and tertiary feathers): (a) blackbirds  $r_s = 0.747$ ,  $n = 18$ ,  $P < 0.001$ ; (b) blackcaps  $r_s = 0.883$ ,  $n = 56$ ,  $P < 0.001$ ; (c) goldfinches,  $r_s = 0.762$ ,  $n = 34$ ,  $P < 0.001$ ; (d) greenfinches,  $r_s = 0.801$ ,  $n = 20$ ,  $P < 0.001$ ; (e) house sparrows,  $r_s = 0.842$ ,  $n = 42$ ,  $P < 0.001$ ; (f) Sardinian warblers,  $r_s = 0.789$ ,  $n = 28$ ,  $P < 0.001$ ; (g) serins,  $r_s = 0.921$ ,  $n = 30$ ,  $P < 0.001$ .

**Table 2.** Relationship between PMIS and secondary + tertiary MIS on the left wing

Species	<i>n</i>	<i>r<sub>s</sub></i>	<i>P</i>
Blackbirds	18	0.480	0.044
Blackcaps	56	0.647	< 0.001
Goldfinches	34	0.719	< 0.001
Greenfinches	20	0.227	NS
House sparrows	42	0.485	0.001
Sardinian warblers	28	0.696	< 0.001
Serins	30	0.760	< 0.001

All probabilities are two-tailed; PMIS: sum of mite infestation scores on primary flight feathers; MIS: mite infestation scores.

finches (p3–p4) was evident in the scores of both left and right wings for each species. Likewise the dip across the inner primaries (p5–p9) was reflected in lower scores and the rise in the secondaries by higher scores on both wings.

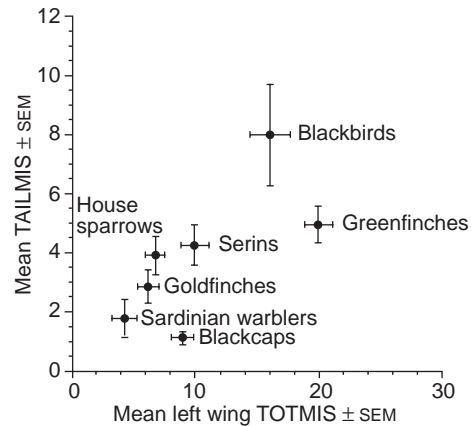
#### Is there anything to be gained by including secondary and tertiary scores?

Table 2 gives the results of Spearman's rank order correlation tests for relationships between PMIS and secondary + tertiary MIS (2/3MIS) scores on the left wing of the birds, by species. With the exception of greenfinches all relationships were significant, although those for house sparrows and blackbirds were low.

#### Is there anything to be gained by including tail feather scores?

Figure 7 presents the relationship between left wing TOTMIS and tail scores (TAILMIS) across species. In general, species with high left wing TOTMIS also had high TAILMIS and vice versa, although the relationship across species was not perfect. Highest TAILMIS were recorded on blackbirds even though greenfinches had higher left wing TOTMIS. Blackcaps had higher left wing TOTMIS scores than Sardinian warblers, goldfinches, and house sparrows but lower TAILMIS.

We also analysed the relationships between left wing TOTMIS, PMIS, and TAILMIS within species. Blackbirds, blackcaps, house sparrows, Sardinian warblers and serins all showed a significant correlation of left wing TOTMIS with TAILMIS as illustrated in Fig. 8, whereas goldfinches and greenfinches did not. House sparrows, Sardinian warblers, and serins additionally gave significant correlations between left wing PMIS and tail MIS (data not illustrated;  $r_s = 0.462$ ,  $P < 0.001$ ;  $r_s = 0.555$ ,  $P = 0.001$ ;  $r_s = 0.473$ ,  $P = 0.002$ , respectively). As can be seen from Fig. 8, however, even the significant relationships were generally weak and there were many exceptions to the central trend for each species, suggesting that despite the significant correlations, the relationship between TOTMIS and TAILMIS was not



**Fig. 7.** Comparison of mean left wing total mite infestation scores and tail mite infestation scores across species: blackbirds,  $n = 23$ ; blackcaps,  $n = 60$ ; goldfinches,  $n = 40$ ; greenfinches,  $n = 36$ ; house sparrows,  $n = 55$ ; Sardinian warblers,  $n = 30$ ; serins,  $n = 40$ .

clearcut. For example, many blackcaps, house sparrows, Sardinian warblers, and serins had detectable and even high TOTMIS, but no evident mites on their tail feathers. The reverse was rare, although exceptions are evident in Fig. 8b and 8d.

#### Is there anything to be gained by including wing covets, breast, back, neck, and head feathers?

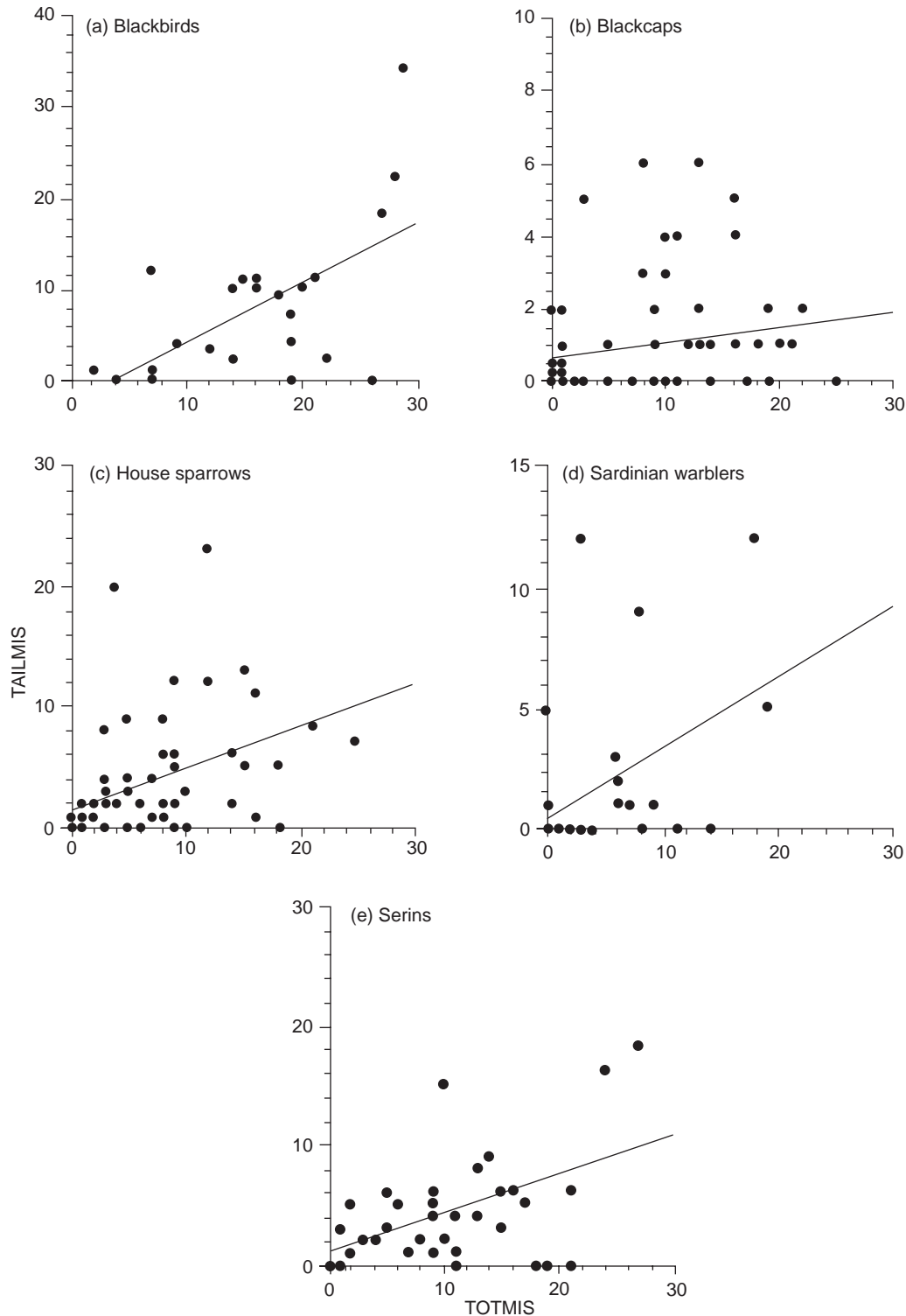
None of these feathers can be examined easily in the field *in situ*. Examination is dependent on their removal, and only a limited number of such feathers can be plucked without causing undue distress and harm to the host. We were therefore unwilling to adopt more than a cautious approach and limited ourselves to five feathers per bird. Only occasionally were mites detected, the vast majority of such feathers examined by us being without mites.

## DISCUSSION

The data and analyses presented in this paper provide clear support for our earlier claim that feather mite infestations on the flight feathers of passerine birds can be assessed quantitatively by a subjective scoring technique based on a range of 0–3 for each feather examined (Behnke *et al.*, 1995).

Calibration of scores allocated to feathers with actual mite counts showed that for each of the seven bird species there was a highly significant positive correlation indicating that scores of 0 through to 3 reflected a general increase in mite infestations. The exceptions to this were the goldfinches and greenfinches. In goldfinches the score of 3 was lower than that for 2, but there was only one such feather assessed. This arose because we adopted a systematic sequentially ordered approach for sampling feathers in order to ensure that the full range





**Fig. 8.** Within species correlations between left wing total mite infestation scores and tail mite infestation scores: blackbirds  $r_s=0.455$ ,  $n=23$ ,  $P=0.029$ ; blackcaps,  $r_s=0.258$ ,  $n=60$ ,  $P=0.046$ ; house sparrows,  $r_s=0.441$ ,  $n=55$ ,  $P=0.001$ ; Sardinian warblers,  $r_s=0.465$ ,  $n=30$ ,  $P=0.01$ ; serins,  $r_s=0.36$ ,  $n=40$ ,  $P=0.023$ .

of flight feathers was sampled for each species. Unfortunately, only one of the sampled feathers corresponded to a score of 3. Nevertheless this is not the only problem, because the low correlation coefficients suggest that many other errors were made in the assess-

ment of scores 0, 1 and 2 for these species of birds. In our experience, an important hindrance to accurate assessment of feathers on the goldfinches is the tendency for mites to cluster on the pigmented (yellow), narrower outer web of the rachis, rather than, as in other species,

on the broader inner web. In this location they are significantly less apparent to visual inspection, but awareness of this problem results in marked improvement of scores.

Another possible source of error was considered to be inaccurate microscopical assessment of mites on sampled feathers. However, comparison of counts from two independent microscopists showed that they arrived at virtually identical counts and spot-checks by one of us (JMB) concurred in all cases with their results. Therefore the difficulties which give rise to inaccuracies reside at the visual inspection stage. A further problem is generated by possible confusion between mites, their larvae (nymphs) and eggs. Whilst these are easy to separate under the microscope they present problems for visual inspection because several small larvae can appear to occupy the space of fewer adults. Thus for some low visual scores, mite counts were higher than they should have been because such feathers had large numbers of nymphs. However, we do not consider this to be an important source of error because even after microscopical analysis few eggs and nymphs were recorded on the flight feathers. Rather, the eggs of *Proctophylloides* spp. appear to be laid preferentially on the wing coverts and nymphs probably reside here for the most part before subsequent migration onto the flight feathers. Occasionally specks of dirt were also confused for mites but with experience such problems were avoided.

We compared the assessment of left and right wing scores for birds for which complete datasets were available, and it is evident from the three types of analysis undertaken that no important differences were detected. Thus average PMIS and TOTMIS scores were very similar numerically even though some significant differences were detected. In all species correlations between left and right wing scores were very high and when the distribution of scores across the individual flight feathers was compared within species, no significant differences were found. We conclude, therefore, that there is little to be gained from examining both wings of each bird. In order to minimize handling time, examination of one wing is sufficient and is reflective of the situation on the other wing but in order to compensate for the slight discrepancy between TOTMIS on the left and right wings of some species, we propose that the wings chosen for assessment are alternated among birds of each species under study.

The question now arises as to whether restricting analysis to just the 10 primary feathers is sufficient. Given that in some species large numbers of mites also accumulated on the secondary feathers, by failing to score these, important additional information would have been missed. The relationships between 2/3MIS and PMIS scores on the left wing were mostly highly significant, but this did not hold true for greenfinches and blackbirds. Thus, although it is possible to conclude that birds with high PMIS scores are also likely to have many mites on their 2/3 feathers and therefore, PMIS is reflective of overall infestation intensity, this is not

entirely accurate for all species. Given that there is additional information to be gained from the high mite infestations on the 2/3 feathers in some species, we conclude that, for consistency, it is preferable to score all flight feathers rather than concentrating on just the primaries. Perhaps in a study concentrating on just one species of bird with high PMIS and low 2/3MIS, it might be justifiable to record only PMIS to save on handling time. However, in our experience, none of the seven species in this study fully conformed to these requirements. Therefore, our view is that on balance the entire wing should be scored.

It is interesting to note that the order of intensity of infection, from the species harbouring the lowest mite infestations to that harbouring the most intense gave Sardinian warblers < goldfinches < house sparrows < blackcaps = serins < blackbirds < greenfinches. This is much the same as that reported in our previous paper for PTMIS scores (equivalent to PMIS in the present work) based on data collected from 1991–1993, with only one exception in that previously we had found house sparrows to be higher placed in this league ahead of blackcaps but lower than blackbirds.

Finally we looked to tail feathers for additional sources of data on mite infestation. Our analysis of relationships between TAILMIS and TOTMIS indicated that there were significant differences between bird species. Thus, whilst blackbirds in general had high infestations on both tail and wing feathers, greenfinches, whose wing feathers showed higher scores, had lower tail scores. Blackcaps seldom showed evidence of tail infestations. Of the species examined only rarely did a bird have mites on its tail whilst none were evident on its left wing. (See points on vertical axes in Fig. 8, for blackcaps, house sparrows, and Sardinian warblers.) Examination of tail feathers also presented additional problems. It is difficult to see the entire length of these feathers because their bases are covered by body feathers. The ground, shrub inhabiting species (particularly blackbirds) often had highly abraded and occasionally missing tail feathers. For these reasons we conclude that there is little more to be gained from including a systematic examination of tail feathers in future.

In this paper we have drawn attention to the benefits of estimating the intensity of infestation with bird feather mites through a subjective scoring system, which we have shown reflects actual mite counts. Our study was carried out with a new inexperienced group of students in each of the 7 years in which it was conducted and we believe that each group rapidly became skilled in carrying out the inspections in a reliable and accurate manner, despite some errors, as with the greenfinches and goldfinches. The problems raised by the assessment of these particular species will be addressed in the next phase of our project but it is worth pointing out that in longer term projects in which a single assessor takes responsibility for scores, the levels of accuracy are likely to be much higher than those we could hope to achieve in a 2 week learning period with each batch of new

students. Following Behnke *et al.* (1995) we now conclude that all the flight feathers should be scored on one of the two wings from each bird examined but little more is to be gained from including both wings and the tail feathers. The data gained in this way reflect the overall infestation level of each bird and represent an acceptable compromise between sufficiently detailed examination and minimization of bird handling time in the field.

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### REFERENCES

- Atyeo, W. T. & Braasch, N. L. (1966). The feather mite genus *Proctophyllodes* (Sarcoptiformes: Proctophyllodae). *Bull. Univ. Nebr. State Mus.* **5**: 1–354.
- Behnke, J. M., McGregor, P. K., Shepherd, M., Wiles, R., Barnard, C., Gilbert, F. S. & Hurst, J. (1995). Identity, prevalence and intensity of infestation with wing feather mites on birds (Passeriformes) from the Setubal Peninsula of Portugal. *Exp. Appl. Acarol.* **19**: 443–458.
- Clayton D. H. & Moore J. M. (Eds) (1997). *Host–parasite evolution. General principles and avian models*. Oxford: Oxford University Press.
- Dubinin, V. B. (1951). Feather mites (Analgesoidea). Part I. Introduction to the study. *Fauna U.S.S.R. Arachnida* **6**: 1–363. (In Russian.)
- Dubinin, V. B. (1953). Feather mites (Analgesoidea). Part II. Families Epidermoptidae and Freyanidae. *Fauna U.S.S.R. Arachnida* **6**: 1–411. (In Russian.)
- Dubinin, V. B. (1956). Feather mites (Analgesoidea). Part III. Family Pterolichidae. *Fauna U.S.S.R. Arachnida* **6**: 1–813. (In Russian.)
- Gaud, J. (1957). Acariens plumicoles (Analgesoidea) parasites des oiseaux du Maroc. I. Proctophyllodidae. *Bull. Soc. Sci. Nat. Phys. Maroc* **37**: 105–136.
- Folstad, I. & Karter, A. J. (1992). Parasites, bright males, and the immunocompetence handicap. *Am. Nat.* **139**: 603–633.
- Hamilton, W. D. & Zuk, M. (1982). Heritable true fitness and bright birds: a role for parasites? *Science* **218**: 384–387.
- John J. L. (1997). The Hamilton-Zuk theory and initial test: an examination of some parasitological criticisms. *Int. J. Parasitol.* **27**: 1269–1288.
- McClure, H. E. (1989). Occurrence of feather mites (Proctophyllodidae) among birds of Ventura County Lowlands, California. *J. Field Ornithol.* **60**: 431–50.
- Spencer, R. (1984). *The ringer's manual*. 3rd edn. Beech Grove: British Trust for Ornithology.
- Svensson, L. (1992). *Identification guide to the European passerines*. 4th edition. Stockholm: Uggå.
- Zumpt, F. (1961). The arthropod parasites of vertebrates in Africa south of the Sahara. I. *S. Afr. Inst. Med. Res.* **9**: 1–457.