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**A revision of bird skin preparation aimed at improving the
scientific value of ornithological collections**

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17 **Abstract**

18 The methods used to preserve bird skins in museums have a potentially crucial
19 impact on the feasibility and use of these specimens as a source of biological
20 knowledge, although this subject is rarely broached. Study skins of birds are
21 usually prepared with folded wings and straight legs to facilitate storage in the
22 collection; yet, this method can hamper the measurement and examination of
23 certain important features such as wing-feather moult. To make consultation
24 easier for ornithologists, alternative preparation methods such as the splitting of
25 wings and tarsi from the rest of the animal have been proposed by curators. Our
26 aim was to study whether or not preparing bird specimens with spread limbs
27 makes consultation simpler. First, we used two different methods to prepare two
28 specimens each of two common European passerine species: (1) 'traditional'
29 (folded wings and straight tarsi) and (2) 'spread' (limbs spread on one side of
30 the body). Then, we asked 22 experienced ornithologists to identify moult limits
31 and take three biometric measurements (wing chord, length of the third primary
32 feather and tarsus length) from all four specimens. Subsequently, we asked
33 which preparation method they preferred for obtaining data. The 'spread'
34 preparation was preferred for moult, third primary feather length and tarsus
35 length, whilst the 'traditional' preparation was preferred for wing chord. Data
36 obtained from the folded and spread preparations were very highly repeatable
37 within each method but only moderately to highly repeatable between methods.
38 One of the handicaps with the 'spread' preparation is the increase in storage
39 space required, a factor that should be taken into account before it is employed.
40 Nevertheless, this specimen preparation technique can greatly facilitate

consultation and therefore improve the scientific value of ornithological collections.

Keywords

Biometry, bird study skin, moult, natural history museums, taxidermy, zoological collections

Introduction

Natural history collections are repositories of an important biological heritage and have traditionally played a pivotal role in improving knowledge of the natural sciences. For instance, zoological collections are crucial in the acquisition of and increase in knowledge of bird ecology, evolution and systematics.^{1,2} The study of bird skins in zoological collections enables us to split subspecies of bird species and identify fundamental biological aspects such as differences in sex and age, moult limits and biometry,^{3,4} phylogeny^{5,6} and evolutionary processes.^{7,8} However, the methods used for preparing bird skins may affect the feasibility and use of these specimens as a source of biological information.

Bird specimens are traditionally prepared with folded wings and straight legs (Figure 1(a)) due, historically, to the need for quick and efficient preparation in the field, for specimens to be robust enough to withstand shipping, and for space-efficient packing and storage.^{9–12} This last is the final goal of the

specimen in being amenable to researchers for consultation. This method of preparation, however, does not necessarily take into account the use of specimens for scientific purposes, and may in fact hamper studies of bird biometrics, aging, moult and the identification of key plumage characteristics. For example, the examination of secondary feathers in specimens prepared using the 'traditional' method is problematic,¹³ and measuring the wing chord (frequently used for determining age, species or subspecies) is complicated if specimens have folded wings and increases the risk of specimen damage.^{14,15} Examining feather moult in specimens with folded wings can be challenging or even almost impossible, as many feathers remain hidden below the tightly packed feathers that overlay them.¹⁶ In addition, the evaluation of feather colouration and wear is difficult in specimens with folded wings.¹⁷ Therefore, the correct determination of certain commonly used wing parameters (e.g. moult, biometric measurements and colouration) is impossible for certain parts of the wing unless folded-wing specimens are mutilated.^{18–20}

To facilitate research and, in particular, biometric and moult studies using bird specimens, a variety of alternative preparation methods have been proposed by museum curators and employed, including the idea of severing one extended wing.¹⁶ In addition to facilitating the collection of moult and biometric data, these proposals aim to facilitate taxonomic studies and the evaluation of wing wear, shape and colour.^{18,19} However, to date few quantitative studies have ever attempted an objective evaluation of zoological preparation methods,²¹ despite the widespread use of collections and the calls made to use quantitative methods to improve the protocols used in zoological preparation.^{21,22} Here, we publish for the first time a quantitative approach that

objectively assesses and compares two preparation methods for bird specimens. This novel study involved the cooperation of field ornithologists and museum curators, and was designed to improve the ease with which relevant biological information can be obtained from ornithological collections. Our aim was to compare the ease of consultation of bird specimens prepared using two different methods: folded wings and straight tarsi ('traditional' *preparation*) with limbs spread out on one side of the body ('spread' preparation). We requested expert passerine ringers to take biometric and moult data from two specimens of a small-sized passerine, the Eurasian blackcap (*Sylvia atricapilla* Linnaeus, 1758), and two of a medium-sized passerine, the song thrush (*Turdus philomelos* Brehm, 1831), of which one specimen of each was prepared using the 'traditional' method and one using the 'spread' method. We also evaluated the consistency of the biometric measurements obtained from both methods by assessing the repeatability within each method (Intramethod R_i , for 'traditional' and 'spread' preparations) and between methods (Intermethod R_i , 'traditional' vs 'spread' preparations).²³ Finally, and given that the 'spread' preparation requires greater storage space, we compare the storage space required for specimens prepared using these two methods.

Material and methods

We prepared four passerine specimens, two of the Eurasian blackcap (voucher numbers: MZB 2017-0505 and MZB 2017-0508) and two of the song thrush (voucher numbers: MZB 2017-0504 and MZB 2017-0507). These species are very frequent in the migration period of the northeastern part of the Iberian Peninsula, they are frequently ringed by ornithologists based there,²⁴ and they

also are regularly donated to the Natural Science Museum of Barcelona (MCNB), added to the fact that they represent two common species of small and medium body size birds, respectively, explain why they were chosen for the present study. One specimen of each species was prepared using the ‘traditional’ method (folded wings and straight tarsi, see Figure 1(a)) (MZB 2017-0507 and MZB 2017-0508) and one using the ‘spread’ method (limbs spread on the left-hand side of the body, see Figure 1(b)) (MZB 2017-0504 and MZB 2017-0505).

Museum specimens dry out when frozen and stored for long periods (i.e. years or even decades), especially when they are not properly insulated, which can affect both the ease of preparation and their final quality (i.e. probability of breakage and their flexibility).²² To ensure similar freezing conditions, we only used specimens that had been collected in autumn 2015 in the NE Iberian Peninsula as accidental deaths and subsequently donated to the MCNB by wildlife recovery centres or ornithologists. Since we were interested in identifying wing-moult limits, we selected individuals hatched during the previous breeding season (EURING 5 or SY) that had undergone a partial moult during their first year.¹⁸ In addition, we only selected males to avoid possible differences caused by the confounding effects of plumage dichromatism.¹⁸

Measurement of observer preferences

We carried out a preference survey amongst 22 ornithologists, all experienced passerine ringers but not all with previous experience of working with zoological collections. However, given that experience with bird skins may play an

important role in assessing the feasibility of alternative preparation methods, we took into account ornithologists' experience (or lack of) with bird skins in order to obtain a better understanding of the advantages/disadvantages of each preparation method when extracting the required data. All of these expert ringers had previous experience with these two species or with similarly sized species as all fulfilled the EURING-based standards for ringers (e.g. Catalan ringers must ring at least 500 individuals of 50 species to become an 'expert ringer' and have more than 2 years of experience). Nevertheless, our aim was not to evaluate their expertise with these species but, rather, determine which preparation technique made consultation easier, regardless of the species considered and bearing in mind that they were a homogeneous group of experienced observers. For each specimen, these expert ringers were asked to fill out a moult card²⁵ and take three biometric measurements^{15,26}: maximum wing chord, length of the third primary feather (hereafter 'P3 length') and tarsus length, all information that is frequently sought by museum-based studies,^{15,19,27} and it is known by ringers of Spanish Ringing schemes (SYLVIA, PASSER).^{26,28} Subsequently, they were requested to judge for each measurement whether one preparation was easier to handle than the other by scoring each as follows: 1: slightly easier; 2: easier; and 3: markedly easier. The small sample size used in this study can be explained by the fact that we were interested in assessing the ease of consultation of each preparation method, and by the limitations of the collection. One observer only measured the song thrush but not the Eurasian blackcap so sample sizes differ between species (i.e. $N_{Turdus philomelos} = 22$; $N_{Sylvia atricapilla} = 21$).

We calculated the mean score and 95% bootstrapped intervals (size of the posterior sample = 4000).²⁹ Finally, to compare the scores, we used the Wilcoxon rank-sum (also known as the Mann-Whitney U), which is employed when a categorical scoring of preference is similar to Likert scores.³⁰ All analyses were carried out in R (version 3.6.3).³¹

Repeatability of measures within and between methods

An important assumption for validating the scientific use of the 'spread' preparation method is that the wing measurements obtained from this alternative model are as repeatable as those obtained from a folded wing. For this reason, we evaluated the repeatability of the biometric measurements used in this study for both species. Repeatability (R_i) is a statistical measurement of the reliability of repeated measurements of a single characteristic of the same specimen, and is used to quantify statistically the consistency of equivalent measurements of a particular object.³² Additionally, it can be used as an indicator of the consistency of methods.^{23,32,33} Repeatability values lie in the range 0–1, where the measurement error calculation equals $1 - R_i$. Thus, a high repeatability value (>0.70) indicates a consistent measurement and a low measurement error, although values ≥ 0.90 (very high repeatability) are generally sought in the case of biometric measurements.^{34,35}

To assess the consistency of biometric measurements using the same method (Intramethod R_i), we measured the wing chord, P3 length and tarsus length from the same body side (left-hand) in a set of specimens from the MCNB collection obtained between 2009 and 2018 (traditional method: N_{Turdus}

philomelos = 10; N_{*Sylvia atricapilla*} = 8; spread method: N_{*Turdus philomelos*} = 10; N_{*Sylvia atricapilla*} = 8). To assess the repeatability between methods (Inter-method Ri) we compared both wing and tarsus measurements from the same individual, prepared following the traditional method (right-hand body side) and the spread method (left-hand body side) (N_{*Turdus philomelos*} = 10; N_{*Sylvia atricapilla*} = 8). All of the measurements were taken by the same observer (SG).

To calculate the repeatability, we used the rptR package in R which is based on the estimation of repeatabilities within the framework of the mixed effects model. This approach uses random-effect predictors to estimate variances at different hierarchical levels.³⁶

Finally, in order to analyse the trade-off using the ‘spread’ preparation method (i.e. bearing in mind its drawbacks regarding storage space), we assessed the percentage of storage loss with the alternative preparation method compared to the traditional one by counting the number of specimens prepared according to each method that could be accommodated in a standard skin storage box (400 mm long by 300 mm wide by 120 mm deep; Euro container EU4312L Box®, Rajapack™) in the MCNB.

Results

The preliminary analyses showed that results were consistent between and within species. Thus, we pooled the scores for the two species and report here the results for the whole sample. We found that the ‘spread’ preparation was preferred for scoring moult limits and for measuring P3 and tarsus lengths. The preference for the ‘spread’ format when measuring P3 length was less than for

the two other categories, which provided clearly higher scores. Conversely, the 'traditional' preparation method was preferred for measuring chord length (Table 1, Figure 2).

When we considered whether or not observers had previous experience working with collections, similar results in method preference were obtained. There were no differences in preference between experienced observers and non-experienced observers (all p s > 0.11), except for tarsus length ($W = 133.74$; $p < 0.05$). However, all observers chose the spread method for this trait ($X_{\text{Experienced}} = 1.07$; $X_{\text{Non-experienced}} = 2.31$).

Repeatability analyses showed that all measurements had high (>0.70) or very high (>0.90) repeatabilities; the 'spread' preparation had similar or higher repeatabilities than the folded preparation for both species (Table 2). However, we obtained moderate to high repeatability when we compared the two methods. The Intermethod R_i was higher in the larger species (i.e. song thrush) than in the smaller one (i.e. Eurasian blackcap).

The results showed that for both species there was an increase in the storage space required when specimens were prepared using the 'spread' method. We could store 17 song thrushes and 19 Eurasian blackcaps prepared using the 'folded' method in the same space (Euro Box®), compared to 12 song thrushes and 14 Eurasian blackcaps prepared according to the 'spread' method. This implies a increase by 42% and 36% in storage space requirements by due to 'spread' method for the two species, respectively.

Discussion

The method used to prepare bird specimens destined for zoological collections has an important effect on how our biological heritage is used as a source of scientific knowledge. Regardless of the experience of the observer with zoological collections, the 'spread' preparation of bird skins was notably preferred when examining moult limits and measuring tarsus length, and even appeared to provide a slight advantage in the measurement of P3 length. This preparation method allows wing moult to be scored without damaging specimens since researchers do not have to unfold feathers to expose those lying beneath. This alternative technique is also likely to facilitate other biometric measurements since, for example, measurement of the wing chord is not hindered as it only requires the unfolding of one wing. Unlike the preparation of extended wings severed from the body, a method used in museums and universities such as the Burke Museum in Seattle,³⁷ the Cleveland Museum of Natural History,³⁸ the Australian Museum (LR Tsang, personal communication), the University of Puget Sound (PH Wimberger and G Shugart, personal communication) and the National Museum of Ireland (P Viscardi, personal communication), the 'spread' preparation allows researchers to obtain moult and biometric information from the whole specimen. Furthermore, the 'spread' preparation also displays the whole wing, which simplifies the inspection of feather variation, form and colour, and is ideal for artists,²⁰ illustrators of field guides³⁹ and for studies of plumage colouration.⁷

Both preparation methods provide consistent biometric measurements, as the very high repeatability values for each method demonstrate. Nevertheless, a relevant question here is how comparable or close to one another (i.e. precise) are the data obtained from the alternative 'spread' method

259 of preparation and those obtained using the 'traditional' method, in addition to
260 how accurate those measurements are with regard to the actual morphometric
261 in question. In the context of this study, precision is important given that the
262 'spread' method could negatively affect longterm studies of traits in collections
263 that have been prepared using the 'traditional' method. Our results suggest that
264 measurements resulting from the 'spread' method are partially comparable to
265 those obtained from the 'traditional' one. However, it is worth highlighting two
266 aspects of this question. First, since the idea is to unfold only one wing (left-
267 hand one), the right-hand wing can still be measured in the same way as in the
268 'traditional' preparation. Secondly, to evaluate the Intramethod Ri, we would
269 need to assess at least two independent measurements of the *same* wing after
270 preparation by both the 'spread' and 'folded' techniques, which is a drawback.
271 The only possibilities for doing so are (1) to compare the same bird before and
272 after preparing the wing/tarsus (however, previous studies have shown that
273 significant biometric differences between fresh and prepared specimens occur
274 in some traits¹⁵); and (2) to compare both wings/tarsi from the same sides. We
275 used this second approach, even though wing/tarsus asymmetry could mask
276 the repeatability of results. Thus, the measurement error obtained in our
277 analysis results from the sum of the asymmetry between wings/tarsi and the
278 actual measurement made by the observer. Nevertheless, to overcome the fact
279 that this new 'spread' design could compromise the utility of the measurements
280 in longitudinal studies, correction factors could be developed,^{15,40} which would
281 allow for each species' morphometric correction from a 'spread' specimen to a
282 'folded' specimen.

Although this alternative preparation provides new opportunities for obtaining biometric data more effectively (particularly important for moult and colouration analysis), this method may interfere with the availability of other scientific data (e.g. studies of bilateral asymmetry). This issue, however, is a recurrent trade-off in collection management and is also of concern when preparing a single wing (as in other museums) or a particular element from a specimen (e.g. skeleton).⁴¹

Spread-wing preparation entails important advantages in research, and also has advantages for collection management: it reduces the risk of damage to prepared specimens when consulted by experts, and having a single voucher for each specimen avoids double labelling and minimizes errors, which is particularly important in large collections. However, it should be acknowledged that spread-wing specimens also represent challenges for collection management. One drawback of the 'spread' method is that it increases the chances that the specimen will be damaged since unfolded wings are more fragile. Another shortcoming is that it requires more storage space (36%–42% in our study) than the 'traditional' preparation, which can be a severe limiting factor in the case of museums under increasing storage pressure.⁹ There are, however, two ways of minimising this latter impact: (1) specimens can be prepared with their unfolded limbs always on the same side, and (2) specimens can be placed in drawers with an imbricated lay-out, that is, with the body of one specimen lying on the unfolded wing of the adjacent specimen. This second strategy could significantly increase the risk that specimens will be damaged and so precautions should be taken to minimise its impact. It is also important to acknowledge that the 'spread' method could be particularly problematical when

preparing larger taxa, even if these species may also benefit from this preparation technique. Another disadvantage associated with the 'spread' preparation is that it seems to require slightly more material during the preparation work (e.g. needles and wire) and more time (20%–25%, JC-O&JM-V, pers. obs.). Despite these considerations, we still lack a thorough cost-benefit analysis of the storage issues associated with the implementation of the 'spread' preparation. Moreover, the 'spread' preparation might end up being more time-consuming as more time will be needed to repair specimens in the future (which are more likely to be damaged), to retrieve or replace specimens that overlap each other, and to pack or unpack specimens for shipping. Nevertheless, as we have seen, this method provides several functional advantages. In general terms, it is important to acknowledge the existence of a compromise between the ease of use of specimens (which is vital for their scientific usefulness), their long-term preservation so they will be available to future researchers, and storage practicalities in museums. A balanced perspective is therefore encouraged.

Natural science museums and the repositories of research collections (e.g. research centres and universities) worldwide strive to increase the value of their collections by increasing the scientific use of the biological heritage they harbour.⁴² This study highlights how research in zoological preparation can help to improve preparation techniques, and so optimise the scientific use of our accumulated ornithological heritage. We have shown that the 'spread' preparation facilitates the consultation of bird skin collections and also reduces the risk of damage to prepared specimens when handled for data acquisition. Although this format could result in more damage to specimens when they are

retrieved and returned to their storage drawers, the fact that it actually reduces the risk of damage when measurements are carried out constitutes the key advantage of this style of preparation. We recommend the implementation of this preparation method in bird skin collections if storage space is not an important limiting factor.

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Author contributions

Study conception and design: J.C.-O., J.Q.; development of methodology: J.C.-O., S.G., J.Q.; data collection: J.C.-O., J.Q., S.G., J.M.-V.; formal analysis of the study data: S.G.; drafting of the manuscript: J.M.-V., J.C.-O., S.G., J.Q.; critical revision of the manuscript: J.C.-O., J.M.-V., S.G., J.Q.; research supervision and project administration: J.Q.

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TABLES

Table 1. Wilcoxon rank-sum value analysis used to evaluate preferences by experienced ornithologists for ‘folded’ or ‘spread’ bird skin preparations.

	<i>N</i>	<i>W</i>	Difference of mean	<i>p</i> -value
Moult limits	43	1682.00	2.26	***
Wing chord	43	1370.00	−2.19	***
P3 length	43	1170.00	0.63	*
Tarsus length	42	1527.00	1.80	***

Negative values between means imply a preference for the traditional (‘folded’) preparation.

* $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

484 **Table 2.** Repeatability (Ri) values of three biometric measures within each method ('folded' and 'spread' preparation) and a
 485 comparison of both methods ('folded' vs 'spread' preparation).

	Within-method Ri						Between-method Ri		
	Folded preparation			Spread preparation			Folded vs spread prep.		
	Ri	SE	<i>p</i> -value	Ri	SE	<i>p</i> -value	Ri	SE	<i>p</i> -value
Eurasian blackcap									
Wing chord	0.97	0.03	***	0.93	0.07	***	0.54	0.25	0.07
P3 length	0.83	0.16	**	0.81	0.17	**	0.66	0.23	*
Tarsus length	0.72	0.19	*	0.96	0.05	***	0.43	0.26	0.14
Song thrush									
Wing chord	0.54	0.23	*	0.98	0.02	***	0.53	0.24	*
P3 length	0.93	0.06	***	0.94	0.06	***	0.70	0.19	**
Tarsus length	0.93	0.07	***	0.90	0.09	***	0.72	0.19	**

486

487 Ri > 0.70 is considered as a high repeatability.

488 **p* < 0.05. ***p* < 0.01. ****p* < 0.001.

489 FIGURE CAPTIONS

490 **Figure 1.** Example of two specimens of song thrush in ventral view prepared
491 with the: (a) ‘traditional’ folded method (folded
492 wings and straight tarsi) and (b) ‘spread’ method (spread left-hand wing and
493 bent left-hand tarsus).

494

495 **Figure 2.** Ornithologists’ preferences between the two specimen preparation
496 methods: ‘spread’ (one unfolded wing and tarsus on
497 one side of the body) and ‘folded’ (folded limbs). Mean and 95% Bayesian
498 bootstrapped intervals ($N = 4000$).

499

Figure 1

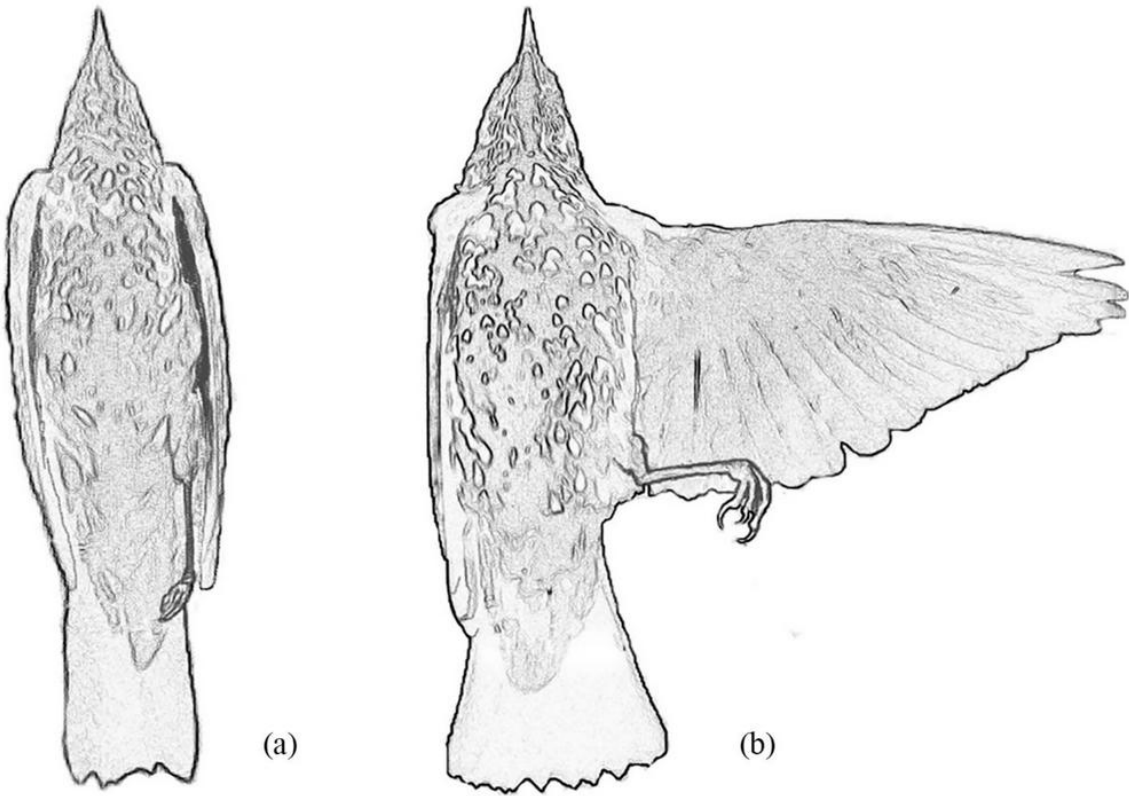


Figure 2

