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INSECT DRY WEIGHT: SHORTCUT TO A DIFFICULT QUANTITY USING MUSEUM SPECIMENS

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ABSTRACT

Body mass is an important, necessary parameter in life history studies. For insects, body mass is hard to obtain for large samples of species that may be distributed worldwide, making comparative studies difficult. Dry weights of museum specimens would be convenient, but specimens are generally inseparable from their pin. Here, I provide curves that allow researchers to estimate the weight of pins based on an empirically-derived model of length, diameter, material and head type (r^2 = 0.99). Thus, pin weight can be subtracted from total specimen weight, allowing estimation of dry specimen weight. I assess inaccuracy of the method due to uncertainty in pin weight, and discard specimens where this made the dry weight estimate unreliable. This occurred primarily in insects weighing less than twice the 95% confidence interval for the weight of the pin; as a rule of thumb, I show that this method is unsuitable for insects below 11mm long. Among remaining specimens, dry weights agreed well with reported weights of oven-dried conspecifics, but were slightly heavier than predicted based solely on length—possibly indicating shrinkage in length over time. Age had no effect upon specimen weight. This is a quick and easy method for determining insect dry weight with relatively small loss of accuracy, and should greatly facilitate comparative studies of insect life history that require body mass as a covariate.

Key Words: body size, comparative method, morphology, museum collections, dry mass

RESUMEN

La masa corporal es un parámetro importante y necesario en los estudios de historia de vida. Con los insectos, la masa corporal es difícil de obtener para muestras grandes de especies que pueden ser distribuidas en todo el mundo, haciendo los estudios comparativos difíciles. El peso seco de especímenes de museos, sería conveniente, pero las muestras son generalmente inseparables de su alfiler. Aquí, presento curvas que permiten a los investigadores estimar el peso de los alfileres basado sobre un modelo empírico derivado de la longitud, el diámetro, el material y la clase de cabeza (r^2 = 0.99). Así, se puede restar el peso del alfiler del peso total del especimen, lo que permite la estimación de peso del especimen en seco. Se evalúo la inexactitud del método debido a la incertidumbre en el peso del alfiler, y se descartó los especimenes cuando este hizo que la estimación del peso seco fuera no confiable. Esto ocurrió principalmente en los insectos que pesan menos de dos veces el intervalo de confianza del 95% para el peso del alfiler, como regla general, se muestra que este método no es adecuado para los insectos que son menos de 11 mm de largo. Entre los especimenes restantes, el peso seco fue de acuerdo con el peso reportado en especimenes de la misma especie secados en un horno, pero eran un poco más pesados de lo previsto basado únicamente en la longitud - lo que posiblemente indica la contracción de la longitud de especimen en el tiempo. La edad no tuvo efecto en el peso del especimen. Este es un método rápido y sencillo para determinar el peso seco de insectos con una pérdida relativamente pequeña de precisión, y en gran medida debe facilitar los estudios comparativos de historia de vida de los insectos que requieren la medida de la masa corporal como una covariable.

A reliable measure of body size is crucial to life-history analysis (Blair Hedges 1985). Specifically, body mass is a key parameter in most life history models (see Blueweiss et al 1978; Schmidt-Nielsen 1984; Peters 1986). To test interspecific predictions arising from such models (see e.g., Savage et al 2004; Bielby et al 2007; Gilbert & Manica 2010) authors therefore frequently need to account for the body masses of a range of species whose representatives can be distributed worldwide and are therefore difficult to obtain. How, then, would we determine body mass without catching specimens in the field?

Insect life-historians have a particular problem. For students of many other taxa, comparative studies are relatively easy because there are excellent literature records for life history variables such as body mass (e.g. mammals, PanTHE-

RIA [Jones et al 2009]; birds, Bird Life International Data Zone [http://www.birdlife.org/datazone/]; fishes, FishBase [Froese & Pauly 2011]). In insects there is no equivalent resource, possibly owing to the relative sparsity of data coverage given the enormous diversity of species.

Dry weight is one measure of biomass—arguably better than fresh weight, since variation from water content is reduced (e.g. Sage 1982; Jakob et al 1996). Museum specimens, which have dried over a long period, may provide one way of obtaining species-typical dry body weight estimates. Although museum specimens are commonly used for comparative morphological studies in invertebrates (e.g. Babin-Fenske et al 2008; Shreeves & Field 2008), to my knowledge they have never been used to measure dry weight. The perceived difficulty of separating the weight of the specimen from that of the pin is one reason that could discourage researchers from weighing museum specimens (see Shreeves & Field 2008).

I describe here a technique for directly measuring insect dry weight from pinned museum specimens, which is quick and easy. I provide an accurate model of pin weight based on length, diameter, material and head type, all of which can be measured without disturbing the specimen. Estimated pin weight can then be subtracted from total specimen weight. I use this method to estimate the weights of 1104 specimens from 13 orders. I discard 222 specimens where variation in pin weight obscured meaningful variation in specimen weight, and estimate a rule of thumb that will allow researchers to choose specimens where unexplained pin weight is not a problem. For the remaining 882 specimens, I assess the accuracy of the method by comparing my weight estimates with (1) weights reported for a subset of the same species by other authors that directly measured the weights of oven-dried specimens, and (2) the weight that would be expected based upon the specimens' body length, using known regression equations from the literature.

Methods

To estimate pin weight, I first weighed 204 pins of different kinds. Museum pins were of non-standardized sizes, ages and types, and originating from many countries; and a researcher using this technique will typically be unable to determine the pin brand or model. I therefore selected pins at random. To predict the weight of a pin, I used only those pin characteristics that I would be able to measure directly without disturbing a pinned insect. I recorded pin length and diameter (using calipers precise to 0.05 mm), head type ('none'; 'small', <0.5 mm diam; or 'medium/large', >0.5 mm diam) and material (categorized as 'brass' or 'steel', determined using a magnet after weighing, to avoid effects of magnetism on the precision of the balance). Pins were assumed to be cylindrical with volume $\pi \times (\text{diameter}/2)^2 \times \text{length}$. I then constructed a linear model of pin weight with 'volume', 'material' and 'head type' as predictor variables.

I measured dry weight of pinned museum specimens at the University of Cambridge, UK (Insect Room, Museum of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ) and the Natural History Museum (Cromwell Road, London SW7 5BD). Most species measured had been previously chosen for a comparative study of life history evolution and parental care across insects (Gilbert & Manica 2010). I weighed the whole, pinned specimen to a precision of 0.001g, using a balance accurate to 0.0001g (Cambridge: Ohaus® Adventurer Pro balance; London: Sartorius® GD503 balance), then estimated the weight of the pin and subtracted it. Depending upon availability, I weighed up to 6 specimens for each species. I chose the youngest and best-condition specimens with no outward evidence of museum-beetle infestation, pin oxidization, 'verdigris' (copper corrosion that affects brass pins) or glue, to reduce effects of age, corrosion, or preservation method (e.g. Howmiller 1972; Leuven et al 1985). Insects mounted on points or cards were excluded. I recorded the specimen's age from its museum label, where recorded. The method and duration of preservation prior to mounting was not recorded on museum labels and therefore could not be assessed. I also measured body length as the anterior-most part of head, excluding projections such as pronotal spurs, to the posterior-most part of abdomen, excluding projections such as cerci, ovipositors or forceps (Sage 1982).

This method of estimating insect weight depends upon the accuracy of the estimate of pin weight that is subtracted from the total specimen weight. Thus where the insect is very small but is mounted on a large pin, the region of uncertainty in pin weight may be large relative to the unknown weight of the insect, and may cause the estimate of insect weight to be inaccurate. The degree of accuracy with which pin weight can be estimated, compared to the size of the insect, will therefore determine the accuracy with which the dry weight of the insect can be estimated. I expected that there would be a certain ratio of insect weight to pin weight at which the error in the estimate of pin weight would obscure the meaningful variation in insect weight, and the method would become useless.

I therefore established a threshold criterion for researchers to assess whether or not this technique will be useful for a given specimen. To assess the contribution of unexplained error in pin weight to the eventual estimate of insect dry weight, I used 2 principles. First, if I underestimate pin weight, I will then overestimate the insect's weight because I will subtract too small a pin from the weight of the whole pinned specimen. Conversely if I overestimate pin weight, I will then underestimate the true weight of the insect. Thus, error in estimating the pin weight should covary negatively with error in estimation of insect weight. Second, insect dry weight varies with insect length (e.g. Rogers et al. 1976). The error residuals of this regression ("dry weight error") tell us whether a given insect is heavy or light for its length. If I have overestimated an insect's weight, therefore, the corresponding residual from the weight-length regression should be positive, and vice versa. Taken together, these 2 principles suggest that if there is a large margin of error (positive or negative) in estimated pin weight relative to the weight of the insect, then the magnitude (positive or negative) of the dry weight error will be large after accounting for the insect's body size. I therefore calculated the ratio X, or the ratio of (estimated dry weight) to (95% confidence interval in estimated weight of pin). As specimens increased in size compared to their pin, "meaningful" variation in the insect's dry weight would obscure the uncertainty in the pin weight and so the ratio X would gradually become unimportant in explaining the magnitude of the dry weight error. Thus, I expected that the relationship between X and the magnitude of dry weight error would be curvilinear, beginning steeply, for those specimens whose dry weight was small compared to the range of possible pin weight. I used piecewise regression to find an appropriate threshold ratio X below which I excluded specimens from further analysis.

Once I had determined an appropriate threshold ratio X for including specimens in the study, I used two approaches to validate the method. First, I compared dry weights obtained using this method with oven-dried weights reported for the same species by other authors (n = 55). I used a standardized major axis (SMA) regression to assess whether the relationship between estimated dry weight and literature dry weight was different from a unit slope with intercept 0. Second, I compared the dry weights I obtained (n = 882) with those that would be expected based upon specimen length, using the allometric equation provided in Rogers et al (1976; Weight = -3.49 + Length$^{2.62}$), again testing whether these quantities were related by a unit slope with intercept 0.

Results

Pin weight was a mean of 0.0518 g (range 0.0009-0.2367 g), and was estimated from pin volume, head size, and metal type with r^2 = 99.0% (n = 204; Table 1). I then estimated body weight for 1,104 museum specimens from 359 species, 117 families and 13 orders (breakdown in Table 2). The full pin weight dataset is available in supplementary Table S1.

First, I investigated the effect of unexplained variation in pin weight upon the eventual dry weight estimate. I calculated the ratio X of estimated dry specimen weight to the 95% confidence interval of estimated weight of the pin. Below X ~ 2.0, this ratio X accounted for a significant proportion of the magnitude of residual dry weight after body length was taken into account (piecewise regression, Fig. 1a), but above X ~ 2.0, the ratio X was relatively unimportant in explaining the magnitude of the residual dry weight. The implication is that the technique described here for estimating dry insect weight is only useful if the insect weighs more than approximately twice the magnitude of the 95% confidence interval in the weight of the pin; below this weight, error in estimating pin weight obscures meaningful variation in specimen weight.

How should researchers determine whether a given specimen has a ratio X above 2.0? Both specimen size and pin size may be important. In order to compare the relative contributions of pin volume and insect body length in determining the ratio X, I standardized both variables by subtracting the mean and dividing by the standard deviation. Thus standardized, pin volume was relatively unimportant in explaining the ratio X compared to body length (standardized ef-

TABLE 1. PARAMETERS FOR THE REGRESSION OF MUSEUM PIN WEIGHT (MG, LN-TRANSFORMED) UPON PIN VOLUME (MM^3, LN-TRANSFORMED), WITH HEAD SIZE AND METAL TYPE AS PREDICTOR VARIABLES (R^2 = 99.0%).

	Intercept	SE Intercept	Slope	SE slope	n
Head size (brass pins)					
Large	2.793	0.043	0.775	0.023	36
Small	2.583	0.052	0.855	0.029	35
None	2.375	0.047	0.913	0.028	34
Head size (steel pins)					
Large	2.637	0.064	0.831	0.033	48
Small	2.472	0.079	0.865	0.045	20
None	2.433	0.071	0.853	0.040	31

TABLE 2. SAMPLE SIZES FOR EACH ORDER INCLUDED IN THIS STUDY.

Order	N individuals	N species
Coleoptera	319 (275)	112 (92)
Hemiptera	160 (129)	49 (41)
Lepidoptera	154 (101)	31 (28)
Hymenoptera	101 (78)	31 (24)
Diptera	89 (71)	30 (21)
Orthoptera	65 (65)	27 (20)
Dictyoptera	54 (53)	21 (17)
Trichoptera	45 (39)	18 (17)
Ephemeroptera	43 (25)	14 (14)
Neuroptera	38 (18)	14 (6)
Dermaptera	20 (17)	5 (5)
Megaloptera	9 (8)	5 (5)
Embioptera	7 (3)	2 (1)

Values in parentheses show sample sizes after discarding specimens with ratio $X < 2.0$.

fect sizes differed by a factor of 10: body length effect size = 0.611 ± 0.041, pin volume effect size = -0.063 ± 0.055). Thus, researchers should use insect body size as a rule of thumb for estimating X. Insects above approx. 11mm were almost all suitable for analysis (i.e. ratio $X > 2.0$), but below 11 mm, the proportion of unsuitable specimens increased as specimen length decreased (Fig. 1b).

After excluding specimens where $X < 2.0$, those remaining (n = 882) weighed from an estimated 0.001 g (Hemiptera: *Urentius*) to 9.546 g (Blattaria: *Macropanesthia*) after subtraction of the pin weight. All measured data for these specimens are available in supplementary Table S2. Where recorded, age was not associated with specimen dry weight for a given body length ($F_{1,283}$ = 0.40, P = 0.53), indicating that wear-and-tear over time was not a source of bias in dry weight.

To validate the method, I first compared my dry weight estimates against oven-dried weights obtained for conspecifics by other authors, where reported (n = 55). The data used for this analysis are available in supplementary Table S3. Estimated dry weights were not different from those obtained by oven-drying for the same species - the SMA regression line was not different from a unit slope with zero intercept, whether using raw data (intercept 7.76 [CI 16.75-32.26], slope 1.01 [CI 0.92-1.10], R^2 = 0.90, n = 55) or log-transformed data to reduce leverage of outliers (intercept 0.39 [CI 0.00-0.76], slope 0.93 [CI 0.84-1.02], R^2 = 0.87, n = 55, Fig. 2b). Second, I compared my weight estimates against the expected weights of the museum specimens based on their length, according to the allometric equation provided by Rogers et al (1976). The slope of the regression line was not different from 1, indicating that the allometric scaling of weight upon length was similar for both the museum specimens and the author's oven-dried specimens (Fig. 2c). The intercept was slightly but significantly higher than

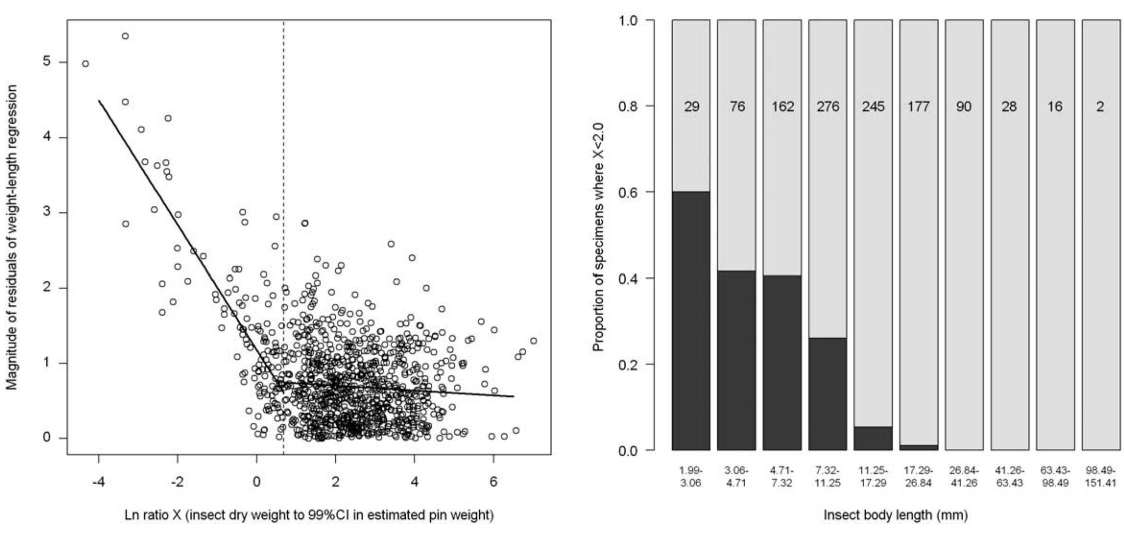

Fig. 1. (a) Effect of the ratio X upon the magnitude of dry weight error. Dashed line indicates X = 2.00. Below X = 2.00, variation in pin weight obscures meaningful variation in the insect's weight. (b) Proportion of specimens for which $X < 2.00$ within a given length range (obtained by dividing total range of ln-lengths evenly into 10 categories). Above ~11 mm, most specimens have $X > 2.00$ and are therefore suitable for analysis. Sample sizes for each bar are given.

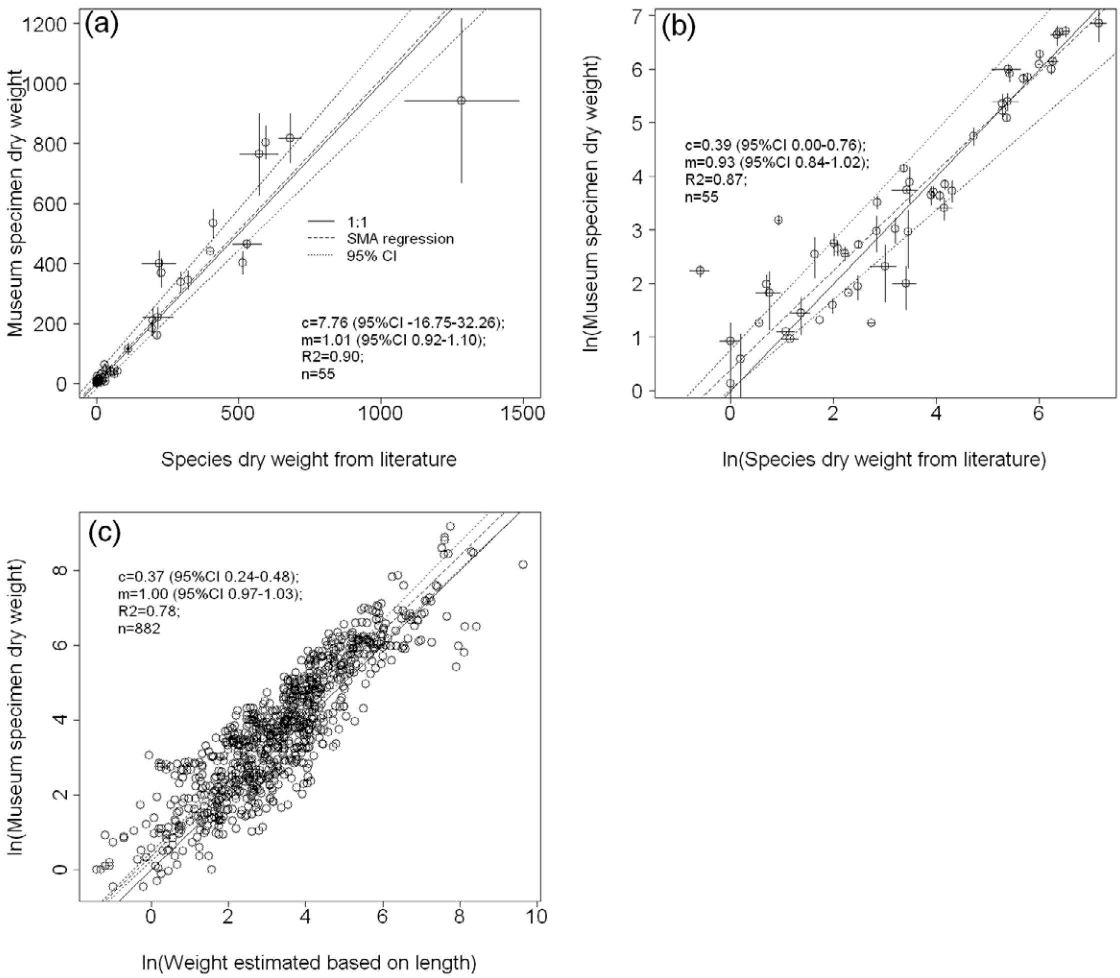

Fig. 2. (a, b) Mean ± SE dry weight of museum specimens against reported mean (±SE where reported) weight of oven-dried conspecifics, raw and log-transformed data, respectively; (c) Regression of dry weight upon predicted weight based on body length (n = 882).

0, indicating that my specimens were slightly heavier than expected for their length (intercept 0.37 [CI 0.24-0.48], slope 1.00 [CI 0.97-1.03], R^2 = 0.78, n = 882).

Discussion

Procedures for obtaining specimen dry weight are time-consuming, involving field collection and oven-drying, sometimes over several days (e.g., Sample et al. 1993). Here, I have shown that (1) dry weight obtained from museum specimens agreed well with previous findings based on oven-dried specimens, and (2) based on specimen length alone, the equation formulated by Rogers et al (1976) for oven-dried specimens was a good predictor of the dry weight of museum specimens. This suggests that museum specimens are a feasible substitute for oven-dried specimens for measuring dry weight.

Specimens were slightly heavier than expected from their length alone. This could be due to specimen shrinkage causing me to under-measure specimen length. Alternatively this may be due to a taxonomic bias towards beetles (a relatively well-sclerotized group) relative to the species set used by Rogers et al (1976), which presumably was not thus biased.

Based on my assessment of error due to uncertainty in pin weight, this method is likely to be most useful for researchers interested in estimating the weight of medium-sized to large insects, above 11 mm. Since pin size was relatively unimportant in determining the ratio *X*, though, researchers typically need not be concerned about selecting specimens according to the pin on which they are mounted. One exception may be researchers confronted with unusual types of pin not covered here, such as those with nylon heads, or made of an unusual material. In these cases I suggest that researchers construct their own models of pin weight according to the method described above.

Several important potential sources of error should be mentioned, which may introduce bias into a dataset depending on the nature of the study at hand. First, researchers wishing to use this method should be careful to ensure correct identification of species, both for dry weight data and life history data. Identification error can be reduced, although not eliminated, by critical examination of museum specimens and consultation with curators, and by careful tracing of life history data sources, respectively. Museum specimens and specimens used for life-history data collection may also have been sampled or chosen differently; unusually large and small exemplars of species are commonly chosen for display in museums, whereas ecological sampling methods used to obtain life history data may also result in size-biased subsets, for which a researcher may not be able to account. Museum specimens may be of a different ecotype, or may have been collected from different parts of the ranges of a given species, from the specimens used to obtain life history data. Although I have shown that weight was not affected by specimen age, the weight of a specimen may be affected by unrecorded methods and durations of preservation before storage in the museum (Howmiller 1972; Leuven et al 1985), unrecorded repairs to specimens such as limb reattachment, distortion or shrinkage of specimens over time, or corrosion of the pin. These caveats may be more or less important depending on the requirements of individual authors and the data or specimens available to them.

I have described a quick and relatively easy way of obtaining direct dry weight measurements from museum specimens as opposed to catching and drying specimens from the field that agrees well with existing estimates of species' dry weight. This technique is most likely to be useful in situations where species-typical dry weights for large numbers of disparate insect species are required, such as in comparative studies of insect life history, and should greatly facilitate such studies. It will also be useful when weights of experimental animals are required in retrospect once specimens are already dried and pinned.

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Disclosure

No competing financial involvements or interests are declared.

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