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Estimating Maggot Age from Weight Using Inverse Prediction

REFERENCE: Wells, J. D. and LaMotte, L. R., **"Estimating Maggot Age from Weight Using Inverse Prediction,"** *Journal of Forensic Sciences,* JFSCA, Vol. 40, No. 4, July 1995, pp. 585–590.

ABSTRACT: Forensic entomological evidence is most often used to estimate the postmortem interval (PMI). Satisfactory techniques have not been available to quantify the precision of such a PMI estimate. For *Cochliomyia macellaria* (F.) (Diptera: Calliphoridae), we describe construction of a confidence interval on age of a larva, given its weight. The method requires a controlled experiment by which weights of larvae are observed at ages spread over sufficient range to cover the time from egg hatch up to postfeeding stage. A statistical model relating distributions of weights to age is formulated and fit to these data. We assumed a simple model in which both means and variances of weight distributions are linearly interpolated between sampled ages. The weight of a larva of unknown age is then compared to the fitted model via inverse prediction to compute the confidence interval on age of the larva.

KEYWORDS: forensic science, forensic entomology, *Cochliomyia macellaria,* Calliphoridae, prediction statistics, postmortem interval, maggot growth rate

The use of insects to investigate cases of wrongful death has increased dramatically in recent years [*1*]. The chief contribution of the forensic entomologist is in the estimation of the postmortem interval (PMI), although other inferences may be made [*2*].

Two general lines of entomological evidence are used in PMI determination [*3*]. In the first, the age of specimens collected from a victim may be estimated to provide a minimum time period since death. Most developmental data have been obtained for the Diptera (true flies), particularly blow flies or Calliphoridae [*4*]. This approach requires detailed knowledge of the fly species used and the conditions at the crime scene, but is relatively conservative if one assumes no knowledge of the interval between human death and the deposition of insect eggs or larvae.

The second approach takes advantage of the succession of arthropod species commonly observed on a wide variety of carrion [*5*]. Unlike the previous technique, successional analysis may be used to estimate both a minimum and maximum PMI [*6*]. The succession of arthropods within a body, however, is a more complicated phenomenon than larval development [*7,8*]. Investigators who use succession (as opposed to development) data must deal with larger number of complicating factors and, presumably, a larger number of sources of uncertainty.

Substantial scientific progress has been made for both approaches in recent years. Carrion succession is a classical subject in ecology [*9–11*], and recent studies have stressed observations applicable to criminal investigations [*12,13*].

Development data have also been gathered for a large number of carrion species [*4,14–17*].

As forensic entomology has matured, investigators have also considered the effects of complicating factors such as freezing and thawing of the body [*18*], drugs in a victim's tissues [*19*], and the elevated temperature produced by a maggot mass [*4,17,20*]. Mathematical and computer models have been developed in an effort to bring greater accuracy to PMI estimates [*21*], and to aid in the management of large data sets [*6*].

Little has been done, however, to deal objectively with the variation in entomological data applied to forensic cases. Specifically, no statistical techniques have been employed to express the uncertainty associated with any PMI estimate. Such methods are essential for establishing the precision of the estimate, and for evaluating any conflict of opinion among experts (that is, different PMI estimates may not be, in fact, significantly different). In this study, we develop and illustrate construction of a confidence interval on the age of a fly of known weight but unknown age.

Materials and Methods

Cochliomyia macellaria (F.) larvae were collected from carrion near Baton Rouge, LA. The resulting adults were allowed to emerge in a cage and were supplied with H_2O, granulated sucrose and ground beef. Eggs from these adults were obtained over a nine-day period.

Eleven 250 mL plastic containers were assigned to ages 0.5 d, 0.75 d, 1.0 d, 1.25 d, 1.5 d, 1.75 d, 2.0 d, 2.5 d, 3.0 d, 3.5 d, and 4.0 d, respectively. The order in which they were to receive their egg samples was randomized. Each container was prepared with 180 g of refrigerated (4°C) ground beef an hour before it received eggs. The container was then kept at room temperature until the eggs were deposited.

Eggs were collected during a one-hour period so that they would be the same age. When an adult in the cage was observed to deposit a clump of eggs, the clump of eggs and a small amount of the surrounding ground beef were transferred to one of the 250 mL plastic containers. The parallel arrangement and size of each clump indicated, albeit not with complete certainty, that all its eggs came from one mother. The container was then sealed with a paper towel and placed in an incubator (Quincy Lab Inc., Model 10-100, Chicago) at 28°C. After an elapsed time corresponding to the age designated for the container, all the larvae in the container were preserved in Kahle's solution [*22*]. The instar (stage of development) of each preserved larva was determined according to the number of spiracular slits [*2*]. All larvae from the container were dried together for 48 h at 50°C in an oven (Dispatch Oven Co., Model 288-A, Minneapolis). The dried larvae were weighed on a

Received for publication 27 July 1994; revised manuscript received 26 Oct. 1994; accepted for publication 28 Oct. 1994.

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balance (Metler, Type B5, Zurich) that provided weights as small as 0.0001 g.

Preliminary observations indicated that hatching and larval molts occur within two days after eggs are laid, so we designated sample ages every six hours from 0.5 d to 2.0 d. Beyond 2.0 d, we chose sample ages 12 h apart. Postfeeding larvae wander from the food and decrease in size [4]. Preliminary observations indicated that larvae at 4.0 d are near but not at this stage, so we chose 4.0 d as the maximum sampled age.

The 0.5 d sample contained no useable larvae; egg hatch occurred between 0.5 d and 0.75 d. The larvae obtained at ages up to 1.25 d were so small that they had to be weighed in sets of several larvae each. At 0.75 d, these sets had 25 larvae each, while at 1.0 d and 1.25 d they had 10 larvae each.

After considerable scrutiny of the weights of the larvae obtained at these ten sampled weights, and about a year after these larvae had been obtained, we sampled 84 larvae at an age of 2.3 d using the same procedure. The eggs from which these larvae came were observed to come from one mother. We chose 2.3 d because it appeared that that age would test most severely the assumptions and approximations that we had devised to analyze the correspondence between age and weights.

Results and Discussion

The data consist of 907 dry weights of larvae at specific ages. Twenty of these are weights of sets of twenty-five or ten larvae each. Although combining weights of different numbers of larvae requires some technical details, it is straightforward. We shall describe these details shortly. Our purpose at this point is to describe our objective and how we have tried to attain it.

Table 1 shows the composition of the larvae by instar at each age. With the exceptions of 1.50 d and 2.00 d, larvae at each age are all the same instar. Those at 1.50 d include 1% of instar I, and those at 2.00 d include 8% of instar II. Although mixtures of instars at these ages may affect the average and dispersion of weights, the percentages of the minority instars are so small that it appears that their effects are negligible.

Table 2 summarizes the weight data. As an example to explain the entries in each row, consider the row for larvae 1.00 d old. There were $n_i = 80$ larvae weighed in eight sets of ten larvae per set. The average of these eight weights is 10×0.055 mg, so the average dry weight per larva is 0.055 mg. The sample variance of the weights of the eight sets of larvae is $10 \times 0.000{,}857$ mg^2.

TABLE 1—*Proportion of larvae in each developmental stage by age.*

Age (d)	egg	Larval Instar I	II	III	*n*
0.50	1.00				nc
0.75		1.00			50
1.00		1.00			85[a]
1.25		1.00			102[a]
1.50		0.01	0.99		90
1.75			1.00		48
2.00			0.08	0.92	91
2.50				1.00	147
3.00				1.00	202
3.50				1.00	151
4.00				1.00	158

[a]Some larvae at these ages (5 and 2, respectively) were not weighed because *n* was not divisible by 10.

TABLE 2—*Summary of the data.*

Age (days)	Avg. weight per larva (mg)	Larvae/ set	Sets	df$_i$	n_i	s_i^2 per larva	Pooled	df
0.75	0.020	25	2	1	50	0.000,800	0.001,435	17
1.00	0.055	10	8	7	80	0.000,857	0.001,435	17
1.25	0.062	10	10	9	100	0.001,956	0.001,435	17
1.50	0.224	1	90	89	90	0.010,407	0.010,407	89
1.75	0.521	1	48	47	48	0.017,429	0.017,429	47
2.00	2.558	1	91	90	91	1.269,12	1.269,12	90
2.50	3.397	1	147	146	147	0.721,635	0.721,635	146
3.00	19.420	1	202	201	202	10.977,2	10.977,2	201
3.50	19.524	1	151	150	151	7.292,548	7.292,48	150
4.00	20.782	1	158	157	158	9.838,99	9.838,99	157

Since each set weight is a total of ten individual weights, the sample variance of the eight set weights estimates ten times the variance of an individual weight. Thus the corresponding estimate of the variance of an individual weight is 0.000,857 mg^2. However, because it is proportional to a sample variance of eight weights, it has $8 - 1 = 7$ degrees of freedom. The additional details required for weights of sets of larvae are not required for ages greater than 1.25 d, where individual weights are recorded.

Sample variances differ considerably with age. For the least three ages, though, we chose to pool the three sample variances in order to attain greater combined degrees of freedom and because the three sample variances were not greatly different.

Figure 1 depicts the data. Average weight increases steadily with age, but not in a smooth, sigmoid shape usually associated with growth curves. Variation among weights differs greatly with age, but it is fairly stable within four ranges of age: .75–1.25 d, 1.50–1.75 d, 2.00–2.50 d, and 3.00–4.00 d. For ages 3.00–4.00 d, variation is increased greatly by a few very small larvae and a few atypically large larvae.

Constructing a Confidence Interval on Age

Our objective is to describe how the weight of a single *Cochliomyia macellaria* larva can be used to establish its age, and to

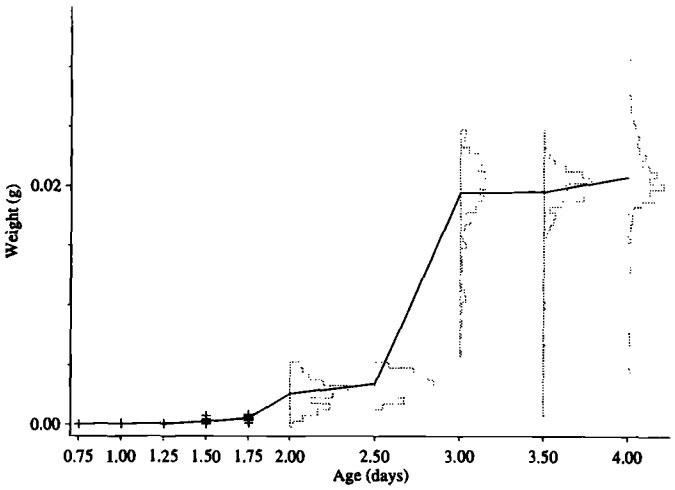

FIG. 1—*Distributions of dry weights by age. Weights of sets are indicated by + for ages up to 1.75 days. Histograms of weights are shown for ages 2.00 days and greater. The solid line connects average weights at each sampled age.*

describe the precision of the result. The procedure we shall use is straightforward in concept, although it is a little busy in its details. Using the data in Table 2, for each value of age between 0.75 d and 4.0 d we shall construct a range of values within which it is reasonable to think the weight of a randomly sampled larva of that age might fall. Such a range is called a prediction interval. Thus for each age we will construct a prediction interval on the weight of a larva. Then, given the weight of a larva, we shall find all values of age for which that weight falls in the corresponding prediction interval. For each such age, the given weight would not be particularly rare among larvae of that age. In formal terms, the list of all such ages comprises a confidence set on age for the given weight (as shown in [23], p. 79). This set is typically, but not always, an interval of values of age.

Figure 2 illustrates how this confidence interval (or confidence set, if it is not an interval) is constructed. Imagine drawing a vertical line through a given age, say 2.5 d. That line intersects the two light lines at the extremes of a 95% prediction interval on the dry weight of a larva 2.5 d old. The light lines connect these prediction bounds computed for all ages between .75 d and 4.00 d. Given the weight of a larva (0.009 g is illustrated in Fig. 2), find all ages for which the weight lies within the prediction bounds. That set of ages forms an approximate 95% confidence set on the age of the larva. In Fig. 2, that set is the range of ages from 2.580 d to 2.847 d.

The basic procedure is to construct a prediction interval on weight at each age. Take age = 2.00 d as an example. A 95% prediction interval on weight is

$$\bar{y} \pm t_{.025,\mathrm{df}} \sqrt{\hat{\sigma}^2_{2.00}\left(1 + \frac{1}{n_i}\right)}$$

where $\bar{y}$ is the observed average weight and n_i is the number of larvae whose weights are averaged in $\bar{y}$. The population variance of weights of 2.00 d-old larvae is denoted by $\sigma^2_{2.00}$. We need an estimate of this variance, which is indicated by the hat. Since we have a sample from this particular population (namely, larvae 2.00 d old), we can estimate the population variance with the sample variance, which is $s^2_{2.00} = 1.269{,}12$ mg^2. It has 90 degrees of freedom. The critical value $t_{.025,\mathrm{df}} = t_{.025,90} = 1.987$ is the upper 2.5 percentile of Student's t distribution with 90 degrees of freedom. Putting these together, the interval is

$$\begin{aligned} 2.558 \pm 1.987 &\sqrt{(1.269{,}12)\left(1 + \frac{1}{91}\right)} \\ &= 2.558 \pm 2.251 \\ &= 0.307 \text{ mg to } 4.809 \text{ mg}. \end{aligned}$$

Prediction intervals on weight at other ages for which there are observations can be constructed similarly.

In order to construct prediction intervals on weight at ages that were not sampled, we must assume some relation between the variance of weight and age. Although other more complicated models are possible, we shall assume that both mean weight and the variance of weight are linear with age between the sampled ages. That is, we shall linearly interpolate both means and variances of weight with respect to age. For the means, this corresponds to connecting the means with straight lines, as illustrated in Fig. 1.

The next four paragraphs describe the construction of prediction intervals on weight at each age in the range from 0.75 d to 4.00 d. The procedure will be illustrated in terms of constructing a prediction interval on weight of a 2.75 d-old larva. Three fundamental ingredients are required to construct a prediction interval: an estimator of the population mean weight of 2.75 d-old larvae; an estimate of the variance of this estimator; and an estimate of the population variance of weights of 2.75 d-old larvae.

With the assumed model relating weights to age, we can estimate the population mean weight at ages that were not directly observed. For example, at age 2.75 d, the estimate of the population mean weight obtained by linear interpolation is $\hat{\mu}_{2.75} = .5 \times 3.397 + .5 \times 19.420 = 11.41$ mg. This estimator is a linear combination of two sample means, the average weights for the 2.5 d-old larvae and the 3.00 d-old larvae. Therefore its variance can be expressed in terms of the two population variances, and hence its variance can be estimated in terms of the two sample variances as

$$\begin{aligned} \hat{\mathrm{V}}\mathrm{ar}(\hat{\mu}_{2.75}) &= (.5)^2 \times \frac{\hat{\sigma}^2_{2.50}}{147} + (.5)^2 \times \frac{\hat{\sigma}^2_{3.00}}{202} \\ &= (.5)^2 \times \frac{0.721{,}635}{147} + (.5)^2 \times \frac{10.9772}{202} \\ &= 0.01481. \end{aligned}$$

To construct a prediction interval on weight of 2.75 d-old larvae requires an estimate of the variance of weights of 2.75 d-old larvae. We have no data on 2.75 d-old larvae, and we are not willing to assume that the variance of weights of 2.75 d-old larvae is the same as the variance of weights of 2.50 d-old larvae or of 3.00 d-old larvae. A reasonable approximation is to linearly interpolate variances between data points. Assuming a linear interpolation model for the variances of weights, for 2.75 d-old larvae we would estimate the population variance of weight to be $.5 \times 0.721{,}635 + .5 \times 10.9772 = 5.84942$ mg^2.

By linearly interpolating population mean weights between observed ages and by linearly interpolating variances of weights

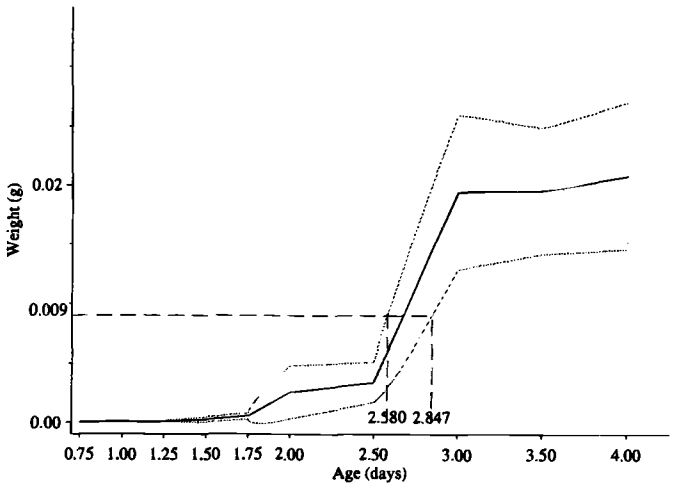

FIG. 2—*Construction of a confidence interval on age. Light lines connect approximate 95% prediction bounds for each age. Construction of a confidence interval on age for a larva weighing 0.009 g is illustrated.*

between ages we have the ingredients required for a prediction interval. The prediction interval has the form

$$\hat{\mu}_{2.75} \pm t_{.025,df}\sqrt{\widehat{\mathrm{Var}}(\hat{\mu}_{2.75}) + \hat{\sigma}^2_{2.75}} = 11.41 \pm t_{.025,df}\sqrt{0.014{,}81 + 5.849{,}42}.$$

The one remaining question is how to determine degrees of freedom for the critical value $t_{.025,df}$. It may be seen that the quantity inside the square root is a positive linear combination of sample mean squares, namely $\left(.5 + \frac{.5^2}{147}\right)s^2_{2.50} + \left(.5 + \frac{.5^2}{202}\right)s^2_{3.00}$. Satterthwaite [24] described an approximation to degrees of freedom for this situation, and we have used that approximation here. For a linear combination $c_1s_1^2 + c_2s_2^2$ of mean squares, Satterthwaite's approximation to df is

$$df = \frac{(c_1s_1^2 + c_2s_2^2)^2}{\frac{(c_1s_1^2)^2}{df_1} + \frac{(c_2s_2^2)^2}{df_2}}.$$

This calculation yields $df = 227.0$ for age = 2.75 d, which gives a critical value of $t_{.025,227} = 1.97$. Putting all this together, the 95% prediction interval on weight of a larva 2.75 d old is the range from 6.64 mg to 16.18 mg.

Although these calculations appear tedious, they are easy to program and compute for each specified value of age. Figure 2 shows upper and lower bounds of 95% prediction intervals plotted against age. These bounds form an envelope around the lines that connect the observed average weights. With appropriate caveats for the assumptions and approximations that have gone into computing these bounds, we can derive an approximate confidence interval on the age of any larva for which we are given the weight. For example, for a larva that weighs 9 mg, the approximate 95% confidence interval on its age is the range from 2.580 d to 2.847 d, a span of about 6.4 hours.

Several approximations and assumptions are involved in the construction of the prediction bounds. In order to get some idea of the validity of this procedure, we sampled larvae at 2.3 d. We chose 2.3 d because it appeared to be an age at which the greatest departure from the linear interpolation model might occur. Following the same experimental procedure as described above, 84 larvae were obtained and weighed at this age; their average weight was 3.12 mg, and the sample variances of their weights was 0.889 mg^2. Figure 3 shows an enlargement of Figure 2 with a histogram of the additional weights for the 2.30 d-old larvae superimposed. The average weight of the 2.30 d-old larvae is very close to the linear interpolant. Of the 84 weights, all but one fell within the 95% prediction interval on weight at 2.30 d.

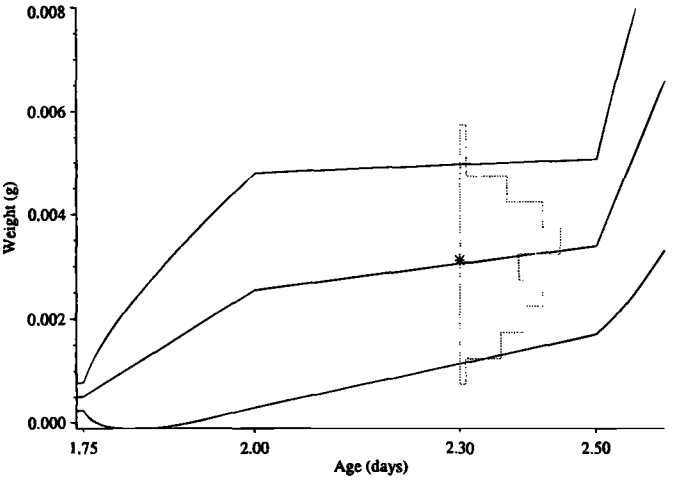

FIG. 3—*The distribution of weights of 84 2.3-day-old* C. macellaria *larvae. The histogram of weights is outlined by the light line. The average weight of these 84 larvae is indicated by *. Solid lines connect average weights at each sampled age (not including 2.3 days) and 95% prediction bounds.*

Discussion, Caveats and Limitations

The limitations of any procedure to distinguish age of a larva from its weight are evident in Fig. 1. Just looking at the distributions of weights at different ages, it is clear that there is much overlap. It will be very difficult to distinguish among ages 0.75 to 1.25 d, between 1.50 d and 1.75 d, between 2.00 d and 2.50 d, and among 3.00 to 4.00 d. Within these ranges of ages, the confidence intervals we have described reflect this difficulty. They do not improve the situation, but they do quantify the lack of resolution. For example, for a larva weighing 0.0003 g, the 95% confidence interval based only on its weight (its instar would provide greater resolution here than its weight) is 1.38 d to 2.00 d, a span of about fifteen hours. Resolution is greatest for weights in the range from 0.005 g to 0.013 g, corresponding to ages from 2.50 d to 3.00 d, where lengths of 95% confidence intervals on age are from four to eight hours.

Interpretation of a confidence interval is straightforward. For example, the 95% confidence interval on the age of a larva that weighs 0.009 g is the range from 2.58 d to 2.85 d, as shown in Fig. 2. For a larva less than 2.58 d old, a weight as great as 0.009 g would be very unlikely; for a larva greater than 2.85 d old, a weight as small as 0.009 g would be very unlikely. For ages within the interval, such a weight would not be particularly unlikely. Corresponding to the 95% level of confidence, what constitutes "very unlikely" is an event that occurs with probability less than 5%.

In constructing the confidence intervals on age, it is assumed that the weight in question is the weight of one larva that can be reasonably regarded as having been sampled at random from the population of larvae of the same age and under the same conditions. Further, in order to be certain that the probabilistic properties of the confidence intervals hold it is necessary that the distribution of weights of larvae in the sampled population be normal. This condition is not verifiable in practice because many flies are likely to have deposited eggs on the victim, and the siblings of the sampled larvae cannot be identified (perhaps this could be done with DNA fingerprinting). However, the probabilities involved in the confidence intervals are good approximations if the distribution of weights of larvae of a given age is reasonably mound-shaped. The data used here are reasonably mound-shaped, except for stragglers like those noted in Fig. 1. The stragglers are numerous enough that they have two effects. First, they increase the sample variance, thus making the prediction intervals on weight wider than they would be if the stragglers were not present. Second, they indicate that in populations of larvae it is reasonable to expect such stragglers with some probability, albeit rather small. These two effects compensate for one another to some extent in their effects on the validity of the confidence interval.

We believe that the growth model presented here could be

directly applied to *C. macellaria* from a forensic scene in which the air temperature had fluctuated only a few degrees about 28°C, the victim was shaded, and in which the density of larvae was relatively low (no shortage of food or elevated maggot mass temperature). Often, however, the conditions under which the subject larva grew are different from those under which the experimental larvae were grown. A large number of factors (for example, the fly species, whether the fly is in a state of diapause (developmental arrest), abiotic environmental conditions) might influence maggot growth rate. In the absence of accurate observation and measurement of these conditions, one must use some qualitative judgment and what limited knowledge of conditions exists in order to assess whether the subject larva might have grown faster or slower than the experimental larvae. Investigators should develop their own baseline data for conditions relevant to any particular location, insect species and environment likely to be encountered in the field, and we hope that the type of analysis performed in this study will be applied to such experiments.

Most studies of maggot development report only one measure of size (for example, length after larvae are heated, wet weight or dry weight). Obviously one must measure a larva of unknown age in the same manner as in the reference study to be used. Wells and Kurahashi [*25*] discuss the relative merits of developing growth curves based on length compared to dry weight.

Bias in selecting the subject larva can occur if, for example, the larva is selected because of its size. If an investigator deliberately chooses the largest available larva, then the bounds on age computed for that larva will be too high. That is not to say that investigators should deliberately ignore larvae that appear to be large or small. It is to point out a limitation of the confidence intervals that we have described here because they are based on the assumption that the larva is randomly selected from a population of larvae of a given age.

Many scientists may be uncomfortable with a statistical procedure not found in textbooks or software packages. Statistical advice, however, is provided by most academic institutions, and we encourage forensic scientists to form such collaborations.

The procedure we have described, in which a model relating a response (weight) to a factor (age) is fit to experimental data, then used to infer the factor value of a subject based on its measured response, is called calibration or inverse prediction in the statistical literature. It has been applied in a great variety of settings. See [*26*] for a nice description of its application to determine fetal age from ultrasound measurements. Details of the model fit to the experimental data may differ greatly with the application, but the concepts and technical basis are the same.

Although we have used fly development data in this paper, the forensic uses of these methods extend far beyond entomology. Inferences based on relations such as we have examined here are common: estimation of a victim's age based on skeletal measurements [*27*] and estimation of the post-mortem interval based on body temperature [*28*] or on concentrations of chemicals leached from the victim into the soil [*29*], for example. In each such setting, use of confidence intervals on the target quantity, obtained as we have described here, provides both an estimate of the quantity and, by the length of the interval, an assessment of the precision of the estimate.

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