Application of a Frequency Distribution Method for Determining Instars of the Beet Armyworm (Lepidoptera: Noctuidae) From Widths of Cast Head Capsules

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ABSTRACT Instar determination of field-collected insect larvae has generally been based on the analysis of head capsule width frequency distributions or bivariate plotting, but few studies have tested the validity of such methods. We used head capsules from exuviae of known instars of the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), to determine the larval instars with the frequency distribution method and Dyar's rule. Head capsule widths of *S. exigua* ranged from 0.313 to 1.446 mm. The number of instars from the analytical method matched that from the observed data. Based on misclassification rules derived from nonlinear least square fitting of the head capsule width data, the theoretical misclassification rates ranged from 0.62 to 1.92%. Comparing the theoretical distribution to the observed data, the observational misclassification probabilities ranged from 1.18 to 3.03%. There were also 10 head capsules, eight third instars and two fourth instars, not classified into any of the known instars based on the theoretical distributions. Dyar's growth ratios of successive instars ranged from 1.41 to 1.65, and those based on the observed data and theoretical distribution were similar. Both approaches yielded a linear relationship between the natural logarithm of the mean head capsule width and the instar number, which indicates full representation of the larval instars. The results demonstrated that the frequency distribution-based method was robust, although we recommend caution when using such methods to classify head capsules into specific instar classes. Application of computer algorithms should also be accompanied by visual inspection to determine instars from the frequency distribution.

KEY WORDS *Spodoptera exigua* (Hübner), nitrogen, instar determination method, kernel density estimation

Knowing the developmental stages of an insect population is critical in selecting and applying effective pest management strategies. Beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), is an economically important pest of many crops (Pearson 1982, Ruberson et al. 1994). The single most important parasitoid causing *S. exigua* mortality in the southeastern United States is *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) (Ruberson et al. 1994). *C. marginiventris* generally attacks early instar *S. exigua* larvae (Beckage et al. 2003, Chen 2007). Therefore, for successful biological control of *S. exigua* populations, *C. marginiventris* needs to be released before *S. exigua* reaches the third instar. Gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), is most susceptible to *Bacillus thuringiensis* (*Bt*) when it is in the first or second instar and timing of *Bt* application needs to be planned accordingly (U.S. Department of Agriculture 1989).

Because of these practical needs, instar determination from field-collected larvae is necessary and methods based on frequency distribution have been developed and used widely (Dyar 1890, Caltagirone et al. 1983, Fink 1984, Daly 1985, Got 1988, Beaver and Sanderson 1989, McClellan and Logan 1994, Logan et al. 1998). These methods have been applied to insects from many taxa (Table 1). Compared with the wealth of studies that determined instars with these methods, research has rarely been directed to test the accuracy of the methods with exuvial head capsules from known instars (but see Gaines and Campbell 1935, Schmidt et al. 1977). The objectives of this study were to test the accuracy of 1) determining the total number of instars; 2) classifying head capsules of known width to the correct instar with head capsule data from known instars of *S. exigua;* and 3) verifying Dyar's rule that the sizes of successive instars follow a geometric progression.

Materials and Methods

Head capsule width data were from a study examining effects of nitrogen fertilization on *S. exigua* de-

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Table 1. Selected examples of insects whose instars have been determined through analysis of frequency distribution methods

Order	Family	Species	Common name	Literature
Coleoptera	Cerambycidae	*Plectrodera scalator* (Fab.)	Cottonwood borer	Forschler and Nordin 1991
	Chrysomelidae	*Diabrotica barberi* Smith & Lawrence	Northern corn rootworm	Hammack et al. 2003
	Chrysomelidae	*D. virgifera virgifera* LeConte	Western corn rootworm	Hammack et al. 2003
	Chrysomelidae	*Oulema melanopus* (L.)	Cereal leaf beetle	Hoxie and Wellso 1974
	Curculionidae	*Pissodes nitidus* Roel.; *Pissodes castaneus* (De Geer)	A pine weevil; a pine weevil	Kishi 1971; Panzavolta 2007
		Sitona hispidulus (F.)	Clover root curculio	Leibee et al. 1980
	Scolytidae	*Dendroctonus ponderosae* Hopkins	Mountain pine beetle	Logan et al. 1998
	Scolytidae	*Pityophthorus juglandis* Blackman	Walnut twig beetle	Dallara et al. 2012
Ephemeroptera	Baetiscidae	*Baetisca roger* Berner		Fink 1984
	Leptohyphidae	*Tricorythodes minutus* (Trico)		Fink 1984
Hymenoptera	Tenthredinidae	*Pikonema alaskensis* (Rohwer)	Yellowheaded spruce sawfly	Vanderwerker and Kulman 1974
Lepidoptera	Gelechiidae	*Aroga argutiola* Hodges		Wilson 1974
	Lasiocampidae	*Malacosoma disstria* Hübner	Forest tent caterpillar	Smith et al. 1986
	Lymantriidae	*Lymantria dispar* (L.)	Gypsy moth	Higashiura 1987; Jobin et al. 1992; McClellan and Logan 1994
	Noctuidae	*Helicoverpa armigera* Hübner	Cotton bollworm	Mohammadi et al. 2010
		Heliothis obsoleta (Fab.)	Corn earworm	Gaines and Campbell 1935
	Pyralidae	*Amyelois transitella* (Walker)	Navel orangeworm	Caltagirone et al. 1983; Beaver and Sanderson 1989
		Ostrinia nubilalis (Hübner)	European corn borer	Got 1988
	Sesiidae	*Synanthedon rhododendri* (Beutenmüller)		Neal 1984
	Tortricidae	*Archips negundanus* (Dyar)		Parker and Moyer 1972
	Tortricidae	*Choristoneura viridis* Freeman		Schmidt et al. 1977
	Tortricidae	*Laspeyresia molesta* Busck.	Oriental peach moth	Peterson and Haeussler 1928
	Tortricidae	*Rhyacionia frustrana* (Comstock)	Nantucket pine tip moth	Fox et al. 1972

velopment (Chen et al. 2008). Newly hatched *S. exigua* larvae were reared individually in petri dishes and fed on cotton, *Gossypium hirsutum* L., leaves grown under 42 and 196 ppm nitrogen. Larval molting was checked daily and cast head capsule widths were determined with an ocular stereomicroscope (40×) until pupation. Each nitrogen treatment was repeated five times (replications) and there were 10 larvae in each replicate. As a result there were 50 larvae per treatment at the onset of the experiment. A few larvae died during the process, and at the end of the experiment we had measured 100, 100, 99, and 85 head capsules from first, second, third, and fourth instar larvae, respectively. Because the cast head capsules of later instars (i.e., fifth and sixth instars) were severely malformed they were not measured and included in the study.

Instar Determination. We modified an estimation-maximization algorithm for instar determination (Beaver and Sanderson1989, McClellan and Logan 1994, Logan et al. 1998). Briefly, a frequency distribution of the whole set of head capsule widths was constructed separately by nitrogen treatment (Proc UNIVARIATE) (SAS Institute 2010). A histogram width class of 0.02 mm was selected based on 10 frequency classes per peak (Logan et al. 1998) and based on the satisfactory resolution of the resultant histogram in this study. The number of peaks in the frequency distribution was determined by using Kernel density estimation, instead of by visual determination as in other studies. Kernel density estimation is a nonparametric technique to estimate the probability density function of a random variable with a Gaussian density. The number of peaks corresponds to the number of instars (k). The lowest points separating the peaks are the separation points for instars. Head capsule width data were then separated into subsets (i.e., instars) based on the separating points; the means ($\bar{x}_i$) and variances (s_i^2) of each subset were calculated. Counts of the most frequent width class in each subset were also computed. Each data subset was fitted to equation 1 with the nonlinear least squares procedure (Proc NLIN) (SAS Institute 2010).

$$y_i = a_i e^{-b_i(x - c_i)^2},\ i = \text{instar } 1, \ldots, k \qquad [1]$$

where y_i is the frequency of each width class, x is the head capsule width, a_i is a scaling parameter, b_i = one-halfs_i^2, and c_i is the mean of each subset. The initial estimates for α_i, b_i, and c_i were derived from the counts of the most frequent width class, one-halfs_i^2, and $\bar{x}_i$. The estimates of the initial nonlinear least squares parameters, α_i, b_i, and c_i, from equation 1 were further simultaneously fitted to equation 2 to obtain final nonlinear least squares estimates (Proc NLIN).

$$h_i = \sum_i^k a_i e^{-b_i(x - c_i)^2} \qquad [2]$$

where h_i is the counts of the head capsule width classes in the i-th instar. The final nonlinear least squares parameters b_i and c_i were then substituted into equation 3 and misclassification probabilities (P_i) were computed from the intersections between the frequency distributions of the instars.

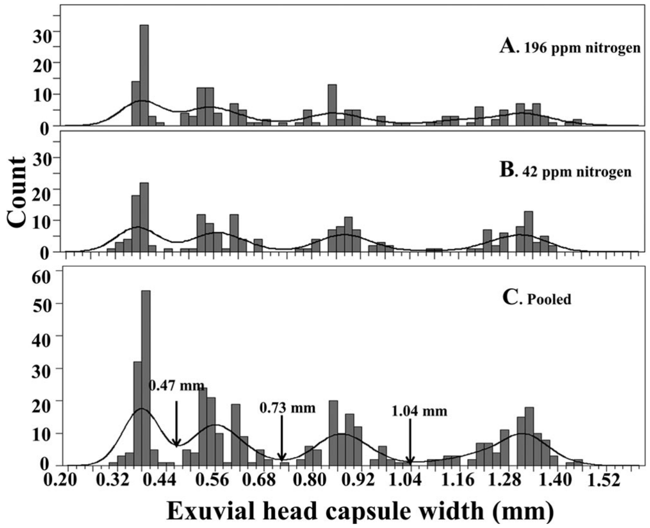

Fig. 1. Frequency distributions of *Spodoptera exigua* exuvial head capsules and kernel density estimation. (A) Head capsules of *S. exigua* larvae reared on cotton plants grown under 196 ppm nitrogen; (B) head capsules of *S. exigua* larvae reared on cotton plants grown under 42 ppm nitrogen; (C) head capsules pooled over both nitrogen treatments.

$$f_i = \frac{1}{\sigma_i\sqrt{2\pi}}e^{-\frac{(x-u_i)^2}{2\sigma_i^2}} \quad [3]$$

where σ_i^2 = one-halfb_i. The misclassification probabilities of classifying instar i to $i + 1$ and instar i to $i-1$ were calculated by using two methods. Theoretical misclassification probabilities (method 1) were obtained by solving equations 4 and 5. The intersection points (l_i) were visually determined from the distributions.

$$P_{i\ to\ i+1} = \int_{l_i}^{\infty} f_i dx \quad [4]$$

$$P_{i\ to\ i-1} = \int_{-\infty}^{l_i} f_i dx \quad [5]$$

Observational misclassification probabilities (method 2) were computed by equations 6 and 7.

$$P_{i\ to\ i+1} = \quad [6]$$

the observed number of head capsules belonging to i-th instar that was classified into $i + 1$-th instar based on equation 2/the total number of i-th head capsule (6)

$$P_{i\ to\ i-1} = \quad [7]$$

the observed number of head capsules belonging to i-th instar that was classified into i - 1-th instar based on equation 2/the total number of i-th head capsule (7).

The two main assumptions for the procedure are 1) data subsets from each instar follow a normal distribution and 2) the total distribution (i.e., pooled across instars) of h_i is the sum of individual normal distributions from each data subset. Normality of individual and total distributions was tested with Kolmogorov–Smirnov's D statistic (Proc UNIVARIATE) (SAS Institute 2010).

Dyar's growth ratios (i.e., ratio of consecutive instar head capsule widths) based on the frequency distribution and observation were calculated. Constant succeeding growth ratios indicate geometric growth of the head capsules (Dyar 1890). A regression of the natural log of the mean head capsule width for each instar against the corresponding instar number was conducted (Proc REG) (SAS Institute 2010), and a linear relation indicates full representation of the larval instars (Dyar 1890, Daly 1985).

Table 2. Parameter (i.e., a_i, b_i, and c_i) estimates of head capsule frequency distributions of the first four *Spodoptera exigua* instars

Instar	Initial estimates			Initial NLLS			Final NLLS		
	a_i	b_i (mm^2)	c_i (mm)	a_i	b_i (mm^2)	c_i (mm)	a_i	b_i (mm^2)	c_i (mm)
1	54	1,301.54	0.38	53.61	1,293.1	0.39	53.46	1,302.7	0.39
2	25	192.23	0.57	21.44	156.9	0.55	21.72	174.9	0.55
3	20	165.29	0.88	17.01	166.6	0.87	16.99	165.0	0.87
4	18	80.12	1.29	15.76	168.3	1.31	15.76	168.3	1.31

The initial estimates for a_i, b_i, and c_i were derived from the counts of the most frequent width class, $1/2s_i^2$, and $\bar{x}_i$; the initial and final nonlinear least square (NLLS) estimates were derived from equations 1 and 2, respectively.

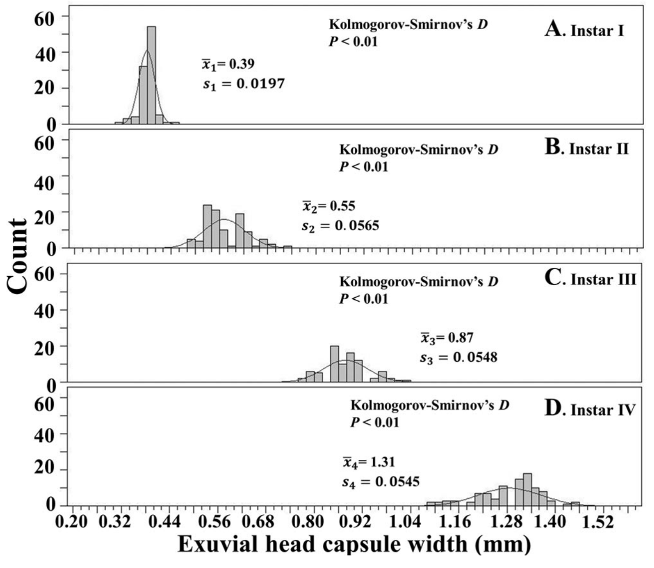

Fig. 2. Frequency distributions of *Spodoptera exigua* exuvial head capsules of the first four instars and their fitted normal curves. $\bar{x}_i$ and s_i were computed from b_i and c_i that were obtained from the initial nonlinear least squares estimation (i.e., equation 1), respectively.

Results

The head capsule widths of the *S. exigua* ranged from 0.313 to 1.446 mm. The kernel density estimation produced four peaks (i.e., instars) regardless of the nitrogen treatment (Fig. 1A,B). Because the peak patterns in response to different nitrogen treatments were similar, the data were pooled across the nitrogen treatments (Fig. 1C). The separation points for the four peaks for the initial nonlinear least squares estimation were ≈0.47, 0.73, and 1.04 mm (Fig. 1C).

The initial nonlinear least squares estimates of a_i, b_i, and c_i of each instar were very close to the corresponding final nonlinear least squares estimates (Table 2), indicating the validity of the assumption that the total distribution was the sum of the individual distributions. None of the individual and total distributions (Figs. 2 and 3) was normally distributed ($P < 0.01$ for all the Kolmogorov–Smirnov *D*s). The intersecting point between the distributions of the first and second instars was 0.439 (Fig. 4A); the theoretical probability of misclassifying a first as a second instar head capsule was 0.62%, and the probability of misclassifying a second into a first instar head capsule was 1.92% (Table 3). No intersection was observed between the second and third instars (Fig. 4B) or between the third and fourth instars (Fig. 4C). The head capsule width ranges for each instar from the total distribution were ca. < 0.439, 0.439≈0.710, 0.711≈1.040, and >1.160 mm (Figs. 3 and 4). Based on these ranges, there were 3 out of 99 (i.e., an observational misclassification probability of 3.03%) head capsules of third instars classified as fourth instars, 2 out of 99 (i.e., 2.02%) head capsules of third instars classified as second instars, and 1 out of 85 (i.e., 1.18%) head capsules of fourth instars classified as third instars (Table 3). There were also 10 head capsules, 8 third instars, and 2 fourth instars, not classified into any of the 4 instars (Fig. 4C, circled).

Dyar's growth ratios ranged from 1.41 to 1.65, and the growth ratios of the theoretical distribution and the observed data were similar (Table 4). The regressions between the natural logarithms of the mean head capsule widths and their corresponding instar numbers followed straight lines (theoretical distribution: Fig. 5A; adj. $R^2 = 0.9957$, $P < 0.01$; observed data: Fig. 5B; adj. $R^2 = 0.9964$, $P < 0.01$), indicating that no larval instars were missing in either case.

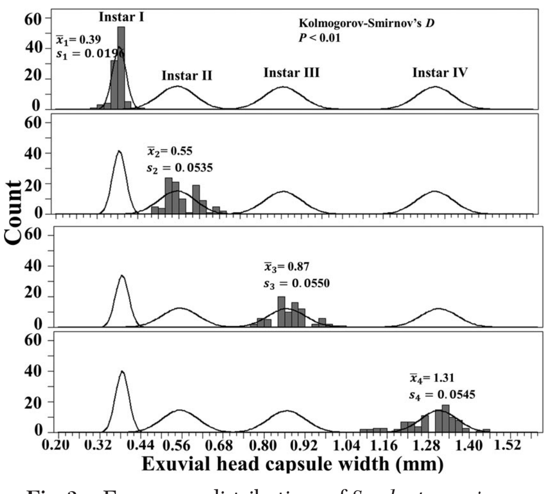

Fig. 3. Frequency distributions of *Spodoptera exigua* exuvial head capsules of the first four instars and their fitted normal curves. $\bar{x}_i$ and s_i were computed from b_i and c_i that were obtained from the final nonlinear least squares estimation (i.e., equation 2), respectively.

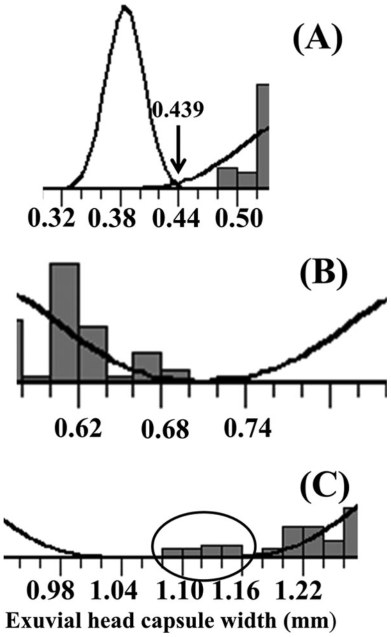

Fig. 4. Close-up of the regions of potential overlap between individual normal distributions of *Spodoptera exigua* exuvial head capsules from Fig. 3. (A) Intersection between the first and second instars; (B) no intersection between the second and third instars; (C) no intersection between the third and fourth instars.

Discussion

S. exigua normally passes through five instars before pupation (Campbell and Duran 1929, Wilson 1934, Eveleens et al. 1973, Chen and Ruberson 2008), although six, or even seven instars have been reported (Fye and McAda 1973, Pearson 1982, Chen et al. 2008). Most *S. exigua* larvae reared on cotton plants in Chen et al. (2008) underwent six larval instars before pupation, irrespective of nitrogen treatments (i.e., 42 or 196 ppm nitrogen). However, the proportion of larvae that underwent six larval instars was greater when feeding occurred on the low rather than on the high nitrogen treatment. Because the cast head capsules of later instars (i.e., the fifth and sixth instars) were severely malformed, they were not measured, that is, the head capsule data included in this study were from the first four larval instars.

Instar determination consists of establishing 1) the number of larval instars; and 2) the instar classification of head capsules with known widths. The number of instars determined from our data matched the number of instars that were suggested by kernel density estimation and by the visual inspection of the frequency distribution. However, visual inspection alone should be avoided if possible when determining the number

Table 3. Probabilities (%) of misclassifying *Spodoptera exigua* instar *i* to *i* + 1 and instar *i* to *i* − 1 based on frequency distribution method and observed data

Instar, *i*	Theoretical misclassification probability[a]		Observational misclassification probability[b]	
	Instar *i* to *i* + 1	Instar *i* to *i* − 1	Instar *i* to *i* + 1	Instar *i* to *i* − 1
1	0.62	—	0	—
2	0.00	1.92	0	0
3	0.00	0.00	3.03 (3)	2.02 (2)
4	—	0.00	—	1.18 (1)

[a] Probabilities calculated from equations 4 and 5.
[b] Probabilities calculated from equations 6 and 7.
The no. of head capsules belonging to each instar was $n_{first} = 100$, $n_{second} = 100$, $n_{third} = 99$, $n_{fourth} = 85$. Numbers in parentheses are the numbers of misclassified head capsules.

Table 4. Means and SDs of head capsules of the first four *Spodoptera exigua* instars and Dyar's rule growth ratios based on the theoretical frequency distribution and observation

Instar	Based on theoretical frequency distribution			Based on observation		
	Mean	SD	Growth ratio	Mean	SD	Growth ratio
1	0.39	0.0196	1.41	0.38	0.0181	1.50
2	0.55	0.0535	1.65	0.57	0.0493	1.60
3	0.87	0.0550	1.51	0.91	0.1214	1.43
4	1.31	0.0545		1.30	0.0681	

Dyar's rule growth ratios: ratio of consecutive instar head capsule widths.

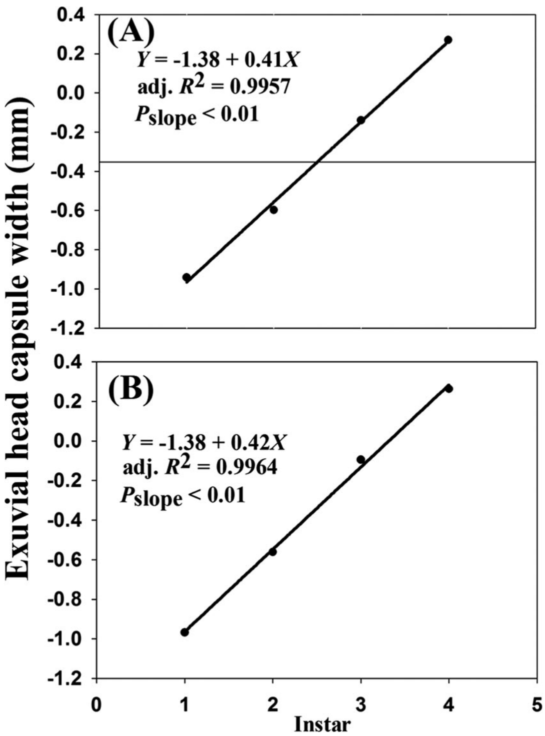

Fig. 5. Regressions of the natural log of *Spodoptera exigua* mean head capsule width for each instar against the corresponding instar number. A linear relation indicates full representation of the larval instars. (A) Based on theoretical distribution; (B) based on observed data.

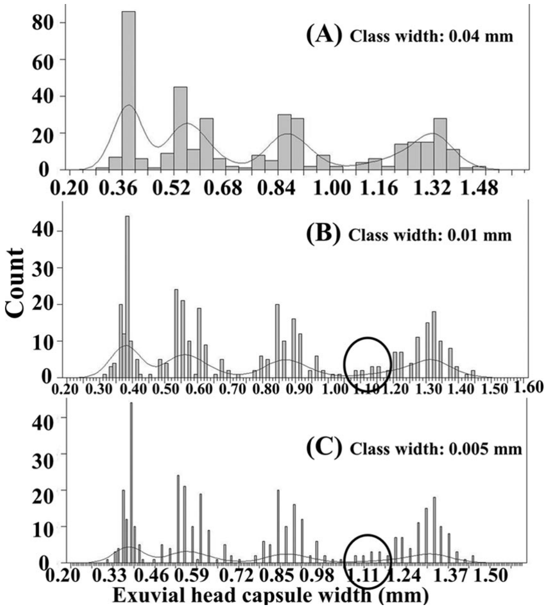

Fig. 6. Frequency distributions and kernel density estimation of *Spodoptera exigua* exuvial head capsules with various class widths. Head capsule class width affects visual determination of distribution peaks, but it does not affect kernel density estimation. (A) 0.04 mm; (B) 0.01 mm; and (C) 0.005 mm.

of instars (i.e., peaks) from frequency distributions because the number of peaks might be subjective to class width of the distribution and other potential factors (Schmidt et al. 1977, Fink 1984). For example, when the class widths were 0.02 (Fig. 1C) and 0.04 (Fig. 5A) mm, four peaks were observed. A fifth peak appeared when the class widths were narrowed to 0.01 (Fig. 6B, circle) and 0.005 mm (Fig. 6C, circle). Regardless of the class widths, the kernel density estimation suggested four peaks.

Assigning head capsules to a certain instar based on the nonlinear least squares fitting could be risky when the accuracy of instar information is critical. There was a small proportion of the head capsules of certain instars that were misclassified based on the misclassification probabilities and instar width ranges derived from the nonlinear least squares fitting. Using the head capsule width ranges from the nonlinear least squares fitting, misclassified head capsules occured for both the third and fourth instars. For instance, 3.03% of the observed third instar head capsules were misclassified into the fourth instar, whereas 1.18% of the observed fourth instar head capsules were classified into the third instar. Furthermore, there were 10 head capsules excluded from any of the instars. Of the 10 head capsules classified into none of the four instars, 8 were from the third instars that underwent five instars before pupation, and 2 were from the fourth instars that underwent six instars before pupation. Head capsules from insects grown under normal conditions were wider than those of the same instar that developed supernumerary instars (Chen and Ruberson 2008). As a result, head capsules from larvae grown under normal conditions might readily be misclassified into the same group with the next older instar that had undergone a supernumerary instar. It is well known that many ecological conditions (e.g., food quality, temperature, and starvation) might induce supernumerary development of insects (Pipa 1976, Jones et al. 1981, Chen and Ruberson 2008). Therefore, a supernumerary number of instars might interfere with the validity of frequency distribution-based instar determination, and researchers should approach these systems with caution.

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References Cited

Beaver, R. J., and J. P. Sanderson. 1989. Classifying instars of the navel orangeworm (Lepidoptera: Pyralidae) based on observed head capsule widths. J. Econ. Entomol. 82: 716–720.

Beckage, N. E., M. Hongskula, D. Vickerman, and F. F. Tan. 2003. Development of the solitary braconid endoparasitoid *Cotesia marginiventris* in the sphingid host, *Manduca sexta.* J. Invertebrate Pathol. 83: 253–256.

Caltagirone, L. E., W. Getz, and D. W. Meals. 1983. Head capsule width as an index of age in larvae of naval orangeworm, *Amyelois transitella* (Lepidoptera: Pyralidae). Environ. Entomol. 12: 219–221.

Campbell, R. E., and V. Duran. 1929. Notes on the sugarbeet armyworm in California. Calif. Dep. Agric. Monthly Bull. 18: 267–275.

Chen, Y. 2007. Tritrophic effects of nitrogen on cotton ecosystem. Ph.D. dissertation, University of Georgia, Athens, GA.

Chen, Y., and J. R. Ruberson. 2008. Starvation effects on larval development of beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae). J. Entomol. Sci. 43: 247–253.

Chen, Y., J. R. Ruberson, and D. M. Olson. 2008. Nitrogen fertilization rate affects feeding, larval performance, and oviposition preference of the beet armyworm, *Spodoptera exigua,* on cotton. Entomol. Exp. Appl. 126: 244–255.

Dallara, P. L., M. L. Flint, and S. J. Seybold. 2012. An analysis of the larval instars of the walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Scolytidae), in northern California black walnut, *Juglans hindsii,* and a new host record for *Hylocurus hirtellus.* Pan-Pac. Entomol. 88: 348–366.

Daly, H. V. 1985. Insect morphometrics. Annu. Rev. Entomol. 30: 415–438.

Dyar, H. G. 1890. The number of molts of Lepidopterous larvae. Psyche (Camb.) 5: 420–422.

Eveleens, K. G., R. van den Bosch, and L. E. Ehler. 1973. Secondary outbreak induction of beet armyworm by experimental insecticide applications in cotton in California. Environ. Entomol. 2: 497–503.

Fink, T. J. 1984. Errors in instar determination of mayflies (Ephemeroptera) and stoneflies (Plecoptera) using the simple frequency, Janetschek, Cassie and Dyar's law methods. Freshwater Biol. 14: 347–365.

Forschler, B. T., and G. L. Nordin. 1991. Instar determination in the cottonwood borer, *Plectrodera scalator* (Fab.) (Coleoptera: Cerambycidae). Coleopt. Bull. 45: 165–168.

Fox, R. C., N. H. Anderson, S. C. Garner, and A. I. Walker. 1972. Larval head-capsules of the Nantucket pine tip moth. Ann. Entomol. Soc. Am. 65: 513–514.

Fye, R. E., and W. C. McAda. 1972. Laboratory studies on the development, longevity, and fecundity of six lepidopterous pests of cotton in Arizona. Tech. Bull. No. 1454. U.S. Dep. Agric. 1–72.

Gaines, J. C., and F. L. Campbell. 1935. Dyar's rule as related to the number of instars of the corn ear worm, *Heliothis obsoleta* (Fab.), collected in the field. Ann. Entomol. Soc. Am. 28: 445–461.

Got, B. 1988. Determination of instar of the European corn borer (Lepidoptera: Pyralidae) based on a distribution model of head capsule widths. Ann. Entomol. Soc. Am. 81: 91–98.

Hammack, L., M. M. Ellsbury, R. L. Roehrdanz, and J. L. Pikul, Jr. 2003. Larval sampling and instar determination in field populations of northern and western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 96: 1153–1159.

Higashiura, Y. 1987. Larval densities and a life-table for the gypsy moth, *Lymantria dispar*, estimated using the head-capsule collection method. Ecol. Entomol. 12: 25–30.

Hoxie, R. P., and S. G. Wellso. 1974. Cereal leaf beetle instars and sex, defined by larval head capsule widths. Ann. Entomol. Soc. Am. 67: 183–186.

Jobin, L., C. Hébert, and J.-P. Bourassa. 1992. Head capsule width of larval populations of the gypsy moth (Lepidoptera: Lymantriidae) in Quebec, with reference to Dyar's hypothesis. Environ. Entomol. 21: 89–93.

Jones, D., G. Jones, and G. Bhaskaran. 1981. Dietary sugars, hemolymph trehalose levels, and supernumerary molting of *Manduca sexta* larvae. Physiol. Zool. 54: 260–266.

Kishi, Y. 1971. Reconsideration of the method to measure the larval instars by use of the frequency distribution of head-capsule widths or length. Can. Entomol. 103: 1011–1015.

Leibee, G. L., B. C. Pass, and K. V. Yeargan. 1980. Instar determination of clover root curculio, *Sitona hispidulus* (Coleoptera: Curculionidae). J. Kans. Entomol. Soc. 53: 473–475.

Logan, J. A., B. J. Bentz, J. C. Vandygriff, and D. L. Turner. 1998. General program for determining instar distributions from headcapsule widths: example analysis of mountain pine beetle (Coleoptera: Scolytidae) data. Environ. Entomol. 27: 555–563.

McClellan, Q. C., and J. A. Logan. 1994. Instar determination for the gypsy moth (Lepidoptera: Lymantriidae) based on the frequency distribution of head capsule widths. Environ. Entomol. 23: 248–253.

Mohammadi, D., R.F.P. Abad, M. R. Rashidi, and S. A. Mohammadi. 2010. Study of cotton bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) using Dyar's rule. Mun. Entomol. Zool. 5: 216–224.

Neal, J. W., Jr. 1984. Bionomics and instar determination of *Synanthedon rhododendri* (Lepidoptera: Sesiidae) on rhododendron. Ann. Entomol. Soc. Am. 77: 552–560.

Panzavolta, T. 2007. Instar determination for *Pissodes castaneus* (Coleoptera: Curculionidae) using head capsule widths and lengths. Environ. Entomol. 36: 1054–1058.

Parker, D. L., and M. W. Moyer. 1972. Biology of a leafroller, *Archips negundanus*, in Utah (Lepidoptera: Tortricidae). Ann. Entomol. Soc. Am. 65: 1415–1418.

Pearson, A. C. 1982. Biology, population dynamics, and pest status of the beet armyworm, *Spodoptera exigua*, in the Imperial Valley of California. Ph.D. dissertation., University of California, Riverside, CA.

Peterson, A., and G. J. Haeussler. 1928. Some observations on the number of larval instars of the oriental peach moth, *Laspeyresia molesta* Busck. J. Econ. Entomol. 21: 843–852.

Pipa, R. L. 1976. Supernumerary instars produced by chilled wax moth larvae: endocrine mechanisms. J. Insect Physiol. 22: 1641–1647.

Ruberson, J. R., G. A. Herzog, W. R. Lambert, and W. J. Lewis. 1994. Management of the beet armyworm (Lepidoptera: Noctuidae) in cotton: role of natural enemies. Fla. Entomol. 77: 440–453.

SAS Institute. 2010. SAS 9.2 help and documentation. SAS Institute, Cary, NC.

Schmidt, F. H., R. K. Campbell, and S. J. Trotter, Jr. 1977. Errors in determining instar numbers through head capsule measurements of a Lepidopteran - a laboratory study and critique. Ann. Entomol. Soc. Am. 70: 750–756.

Smith, J. D., R. A. Goyer, and J. P. Woodring. 1986. Instar determination and growth and feeding indices of forest tent caterpillar, *Malacosoma disstria* (Lepidoptera: Lasiocampidae), reared on tupelo gum, *Nyssa aquatica* L. Ann. Entomol. Soc. Am. 79: 304–307.

U.S. Department of Agriculture. 1989. Final environmental impact statement: Appalachian Integrated Pest Management (AIPM) gypsy moth demonstration project. USDA–Forest Service, Southern Region. Mgmt. Bull. R8-MB 33.

Vanderwerker, G. K., and H. M. Kulman. 1974. Stadium and sex determination of yellowheaded spruce sawfly larvae, *Pikonema alaskensis*. Ann. Entomol. Soc. Am. 67: 29–31.

Wilson, J. W. 1934. The asparagus caterpillar: its life history and control. Fla. Agric. Exp. Stn. Tech. Bull. 271: 1–26.

Wilson, L. F. 1974. Life history and habits of a leaf tier, *Aroga argutiola* (Lepidoptera: Gelechiidae), on sweet fern in Michigan. Can. Entomol. 106: 991–994.

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