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Immature Stages of the Masked Birch Caterpillar, *Drepana arcuata* (Lepidoptera: Drepanidae) With Comments on Feeding and Shelter Building

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Abstract

The masked birch caterpillar, *Drepana arcuata* (Lepidoptera: Drepanidae) is an excellent model for studying vibratory communication and sociality in larval insects. Vibratory communication occurs throughout development, but the functions of signals are reported to change as larvae change from gregarious to solitary lifestyles. To better understand the sensory ecology of these caterpillars, it is important to study their life history. Here, we describe the morphological and behavioral characteristics of larvae by confirming the number of instars, identifying their distinguishing morphological features, and noting changes in feeding and shelter construction. Five instars were confirmed based on the number of head capsules collected for individuals throughout development, and by using Dyar's rule, which predicts the number of instars based on geometric growth patterns of head capsules. Frequency distributions of head capsule widths showed five separate peaks, indicating that this is a useful parameter for distinguishing between instars. Other morphological features including body length, shape, and banding patterns of head capsules, and morphology of thoracic verrucae are helpful in distinguishing among instars. Feeding behavior changes from leaf skeletonization in first and second instars to leaf cutting in fourth and fifth instars, with third instars transitioning between these feeding styles as they grow. Early instars typically construct communal silken shelters whereas late instars live solitarily in leaf shelters. These results provide essential life history information on the masked birch caterpillar that will enable future investigations on the proximate and ultimate mechanisms associated with social behavior and communication in larval insects.

Key words: caterpillar, behavior, leaf shelter, ontogeny, morphology

Larvae of the arched hooktip moth, *Drepana arcuata* Walker (Lepidoptera: Drepanidae) have been studied as a model for larval vibratory communication, a poorly understood mode of communication in juvenile insects (Yack 2016). Late instars are territorial and generate ritualized acoustic signals to defend leaf shelters from conspecifics (Yack et al. 2001, 2014; Scott et al. 2010; Guedes et al. 2012). Early instars, unlike late instars, are reported to live in small groups and a recent study demonstrates that vibrational signaling is associated with recruitment of conspecifics (Yadav et al. 2017). In addition, vibration signals are proposed to function in other social interactions among early instars living in groups (Matheson 2011). The masked birch caterpillar (adults are referred to as arched hooktip moths) offers great potential for studying the roles of vibratory communication and mechanisms mediating social interactions in larval insects. To proceed with such investigations, it is important to be able to identify the larval stages, and to understand how life history traits change with each stage.

D. arcuata is broadly distributed throughout northern and east-southeastern North America (Rose and Lindquist 1997). Host plants of *D. arcuata* include *Betula papyrifera* Marshall (Fagales: Betulaceae), *Betula populifolia* Marshall (Fagales: Betulaceae), *Betula glandulosa* Michx. (Fagales: Betulaceae), *Betula alleghaniensis* Britton (Fagales: Betulaceae), *Alnus rubra* Bong. (Fagales: Betulaceae), and *Alnus rugosa (incana)* (L.) Moench (Fagales: Betulaceae) (Handfield 1999). Previous studies have reported on various life history, morphological, behavioral, and physiological traits (Packard 1890, Dyar 1895, Beutenmuller 1898, Stehr 1987). Adults are medium-sized, broad-winged with hooked tips on the forewings (Fig. 1). They possess abdominal ears that are ultrasound-sensitive and proposed to function in bat detection (Surlykke et al. 2003). Previous reports on immature stages provide mostly anecdotal details on the morphology or behavior of late instars (sometimes referred to as 'mature' larvae) (e.g., Dyar 1895, Beutenmuller 1898, Stehr 1987). A more detailed study by Packard (1890) provides

Fig. 1. Adult moth *D. arcuata* in resting position on a birch leaf. Scale bar: 5 mm.

morphological descriptions of larval stages, but this study was limited in that it followed only a few, unspecified numbers of individuals and did not provide objective or quantifiable measures for distinguishing between larval stages. Furthermore, Packard (1890) provided limited information on instar-specific behaviors, and for only certain instars. Previous studies focusing on vibratory communication (Yack et al. 2001, 2014; Scott et al. 2010; Matheson 2011; Guedes et al. 2012; Yadav et al. 2017) described some characteristics of leaf shelters, conspecific communication, predator detection, and morphological features associated with signal production in unidentified 'early' or 'late' instars. Currently, there are no formal studies documenting instar-specific morphological and behavioral traits. The goals of this study are to document the number of instars, identify morphological criteria for distinguishing between instars and to note stage-specific behaviors associated with sociality including feeding, grouping, and shelter building.

Methods

Insect Collection and Rearing

D. arcuata (Lepidoptera: Drepanidae) were collected as moths from ultraviolet lights at the Queen's University Biology Station (Chaffey's lock, ON, Canada, 44.5788° N, 76.3195° W) and a few other locations close to Ottawa, Ontario, Canada (45.4215° N, 75.6972° W) between May and September, 2010–2015. Gravid females were held in glass jars where they oviposited on paper birch (*B. papyrifera*) cuttings or brown paper bag clippings. Using a fine paint brush, neonates were transferred to fresh birch cuttings held in plastic vials and reared indoors at room temperature (21–23°C and 16 h: 8 h light:dark).

To determine the number of instars, neonate larvae were followed throughout their development. On the day of hatching, four to six neonates (first instars, 59 in total and obtained from >10 females) were transferred to leaves contained in a polystyrene petri dish (Falcon, 100 × 15 mm) (number of petri dishes = 14) lined with moistened paper towels. Larvae of the same age (i.e., hatched within 12 h of each other) were placed in petri dishes in small groups. First instars were kept in petri dishes instead of jars to facilitate collection of their very small head capsules. After molting to second instar, larvae were transferred to twigs of paper birch with 5–10 leaves. Birch twigs were inserted into the lids of water-filled plastic vials and care was taken to seal the bases of the twigs using reusable adhesive putty (Staples) to prevent wandering larvae from drowning. Each twig, in turn, was enclosed in large glass jars (23.5 × 14 cm) lined with moistened paper towels (number of jars = 10). Early instars were reared in small groups

of 4–6 because of their lower survivorship when reared individually (personal observation, J. E. Yack). Jars and petri dishes were examined daily to collect head capsules for subsequent measurements, to take photographs, to monitor feeding and shelter building activities, and to refresh food supplies. This enabled us to keep track of molting and keep track of head capsules for each individual caterpillar. A few individuals of each instar were preserved in 75% ethanol.

Morphology

Morphological features were assessed from live individuals, ethanol preserved specimens and from shed head capsules. Each live individual was examined at least once within 24 h of molting, and the final (fifth) instar was examined for an additional time period to document the prepupal stage. A number of morphological features, including color, setae, verrucae, and body length were recorded from live larvae in their natural resting positions on leaves. Shed head capsules were measured across the widest part for each larval stage (Dyar 1890) for instars I–IV. Because head capsules were deformed following ecdysis from fifth instar, these measurements were taken directly from live larvae on days 3–4 of the fifth instar.

Photographs were taken using a stereomicroscope (Leica M205 C, Leica, Wetzlar, Germany) equipped with a camera (Leica DMC4500, Leica, Wetzlar, Germany). Measurements, *z*-stacked images, and videos were obtained using Leica application suite V 4.2. A Nikon Coolpix camera (4500, Nikon, Japan) was used to obtain images of eggs, pupae, and adults. For scanning electron micrographs, head capsules were air-dried and mounted on aluminum stubs, sputter-coated with gold-palladium, and examined using a Tesca Vega-II XMU scanning electron microscope (XMU VPSEM; Brno, Czech Republic). Identification and naming of various morphological traits followed the nomenclature of Stehr (1987) and Scoble (1992).

Behavioral Observations

Behaviors were monitored and documented with photographs and videotapes at various times following 24 h after the molt for each instar. Feeding style was noted as being either by skeletonization (feeding only on the green tissue between the leaf veins) or cutting (whereby the mandibles cut through the full leaf). Shelter construction behaviors, including the patterns of silk deposition and location on the leaf, were recorded. Records were also made on each instar's tendency to live solitarily or in groups. However, because the nature of the rearing process (designed to follow individuals to collect head capsules) may have impeded the caterpillars' natural tendencies to group, a separate study on instar-specific grouping behaviors would be required.

Measurements to Distinguish Larval Stages

We measured head capsule widths and body lengths of larvae in each instar in order to confirm the total number of instars and to distinguish between larval stages. To confirm the number of instars, in addition to counting the number of shed head capsules by following individuals, we also used Dyar's rule (Dyar 1890, Gaines and Campbell 1935, Cazado et al. 2014). Dyar's growth ratio was calculated by dividing mean head capsule width of one instar by the mean head capsule width of the preceding instar and then calculating the average growth ratio for all instars. We plotted the natural log of the mean head capsule width for each instar against the number of instars and conducted linear regression analysis to determine if larvae follow a regular geometric growth progression (Dyar 1890, Gaines and Campbell 1935). To identify morphological traits for distinguishing between larval stages, we plotted both normal

distribution values of head capsule widths and body lengths (within 24 h of molting from preceding instar) of larvae in each instar and conducted analysis of variance (ANOVA) by using one-way ANOVA tests followed by Tukey's HSD in R Studio 1.0.136 (R Core Team 2016) package Agricolae (de Mendiburu 2016). All the statistical analyses in this study were performed at $P < 0.05$.

Results

General Comments on Immature Stages and Rearing

Our results confirm that *D. arcuata* has five larval instars. Twenty-two individuals were followed from neonatal to pupal stages. More data were collected for early than late instars for two reasons (1) higher mortality was observed in early instars (25%) and; (2) 1–3 representatives for each instar were preserved in 75% ethanol for reference (Instar I, II, III = 3 larvae of each, IV, V = 2 larvae of each). Development time from hatching to pupation took 16–22 d (mean = 19.32 ± 1.73, $n = 22$).

Eggs

Morphology

Eggs were smooth, polished, flattened and oval (Fig. 2). Diameter: 0.64–0.84 mm (mean = 0.77 ± 0.03 mm, $n = 46$). Adults laid eggs in rows of 2–14 on both the upper and lower surfaces of leaves, as well as on the plant stems, paper bag clippings, and the sides of the glass jars. The color of fertilized eggs changed from yellow when laid, to orange-brown, reddish-brown, and then to black as they neared hatching. It took approximately 9–11 d for eggs to hatch. Neonates hatched at different times, with the exit holes about one-third diameter of the eggs shells, and oriented away from adjacent eggs.

First Instar

Morphology

Head capsule width: 0.26–0.31 mm (mean = 0.29 ± 0.01 mm, $n = 42$), body length: 1.75–2.93 mm (mean = 2.36 ± 0.33 mm, $n = 42$) (Figs. 3 and 4; Tables 1 and 2). Head capsule black, shiny, granulated, triangular, rounded on top with a slight notch at the dorsal end of epicranial suture; head is approximately the same width or slightly wider than the body. Body mostly dark brown-black with bright pale colored prothoracic segment (T1) and abdominal segments A1, A7; two dark brownish black parallel lines on the

subdorsal surface running along the segments from T1 to A10. Pairs of brown-black dorsal and subdorsal structures called tubercles by Packard (1890) and verrucae by Stehr (1987) are present on thoracic (T1, T2, T3) and abdominal segments. We follow Stehr's term "verrucae" to provide descriptions of these setae. Verrucae on thoracic (T1, T2, T3) and abdominal segments (A9, A10) are slightly more prominent than those on other segments. The subdorsal verrucae on T1 are the most prominent. Dorsal verrucae on thoracic (T2, T3) segments bear one seta whereas the subdorsal verrucae bear two setae. Greyish-black, elongated thoracic legs are present on T1–T3; 4 pairs of light brown, thick, rounded abdominal prolegs are present on A3–A6, and the anal proleg on A10 is absent. Clear, forked setae arise from the dorsal and lateral surfaces on all thoracic and abdominal segments. A uranal plate is formed by the last abdominal segment (A10) and has a small, brownish, conical, bifurcated projection (called a "knob" or "process" by Stehr, 1987), is covered with very short bristles, and two long setae emerging from the bifurcated tip.

Developmental time (mean ± SD, $n = 42$) = 4.36 ± 0.90 d.

Feeding and Shelter Construction

Post hatching, neonate larvae wander individually until they find a location to feed and build a shelter, eventually forming small groups. Larvae construct a tent-like silk shelter by first laying a silk mat on either the upper or lower leaf surface, followed by spinning silk threads, slightly folding the leaf edge. Larvae begin constructing the shelter by first spinning two silk strands on either side of the shelter which they then extend into multiple cell-like units by attaching smaller silk strands with slight webbing. The location of the shelter is variable, with most shelters (~85%) formed at the edges of leaf. The size of the shelter also varies depending on the size of group contributing to shelter construction (e.g., 0.7 cm for a group of two individuals to 2 cm with seven individuals). First instars typically make only one shelter during this stage, and molt within the same shelter. Shelter building activity alternates with resting, walking, and feeding behaviors within the shelter. Larvae attach frass to the silk canopy of the shelter (Fig. 4B). Larvae skeletonize the leaf surface within the shelter, with feeding spots of variable size and number depending on the number of individuals residing within the shelter. When several larvae reside together in shelters they often, but not always, work on edges of the same feeding spot. They extend both the feeding spots and shelters as they skeletonize the leaf tissue. Larvae tended to form small groups within 24 h of placing them in petri dishes.
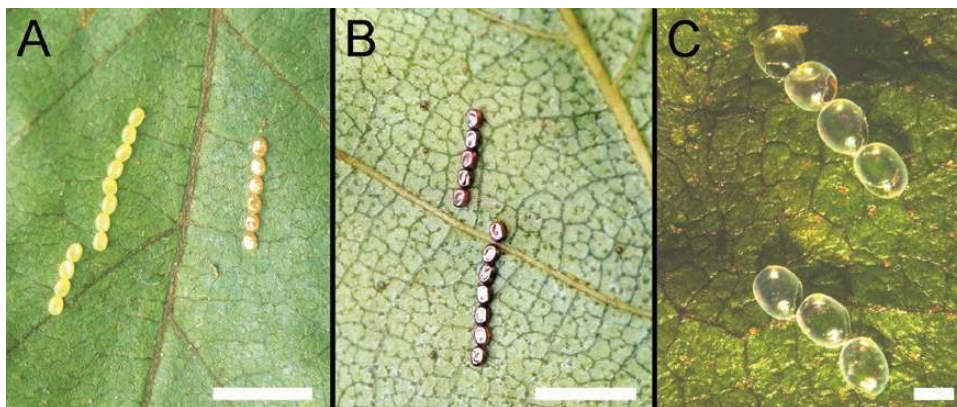

Fig. 2. Eggs of *D. arcuata* laid in rows on birch leaves. (A) One day old yellow eggs (left) and 3 days old light orange colored eggs (right) laid on the upper surface of a birch leaf. Scale bar: 4 mm; (B) Nine days old dark brown-black colored eggs laid on the underside of a birch leaf. Scale bar: 4mm. (C) Hatched eggs showing exit holes. Scale bar: 500 μm.
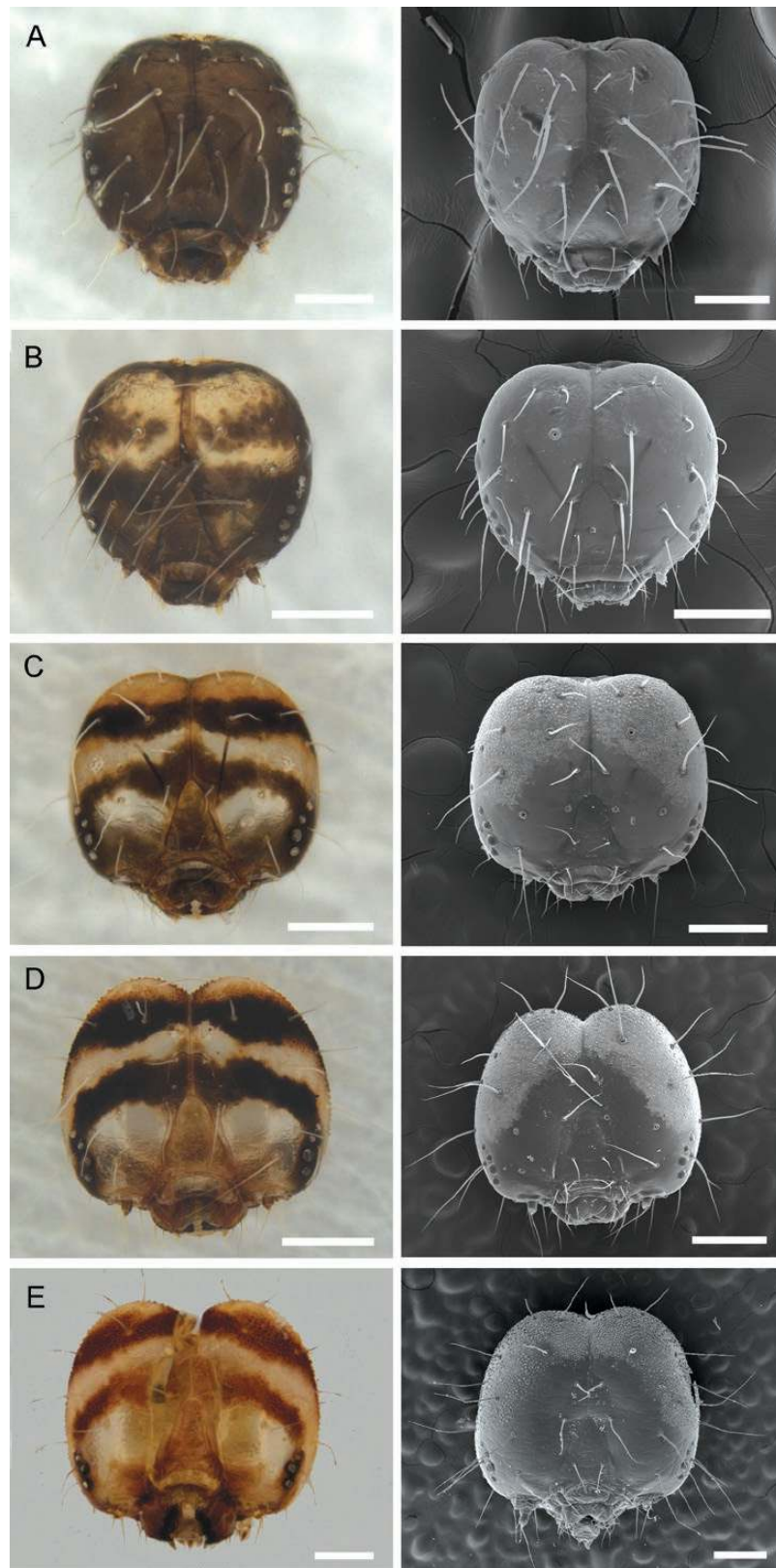
Fig. 3. Light micrographs (left) and scanning electron micrographs (right) of *D. arcuata* head capsules: (A) first instar. Scale bar: 100 µm; (B) second instar. Scale bar: 200 µm; (C) third instar. Scale bar: 300 µm; (D) fourth instar. Scale bar: 500 µm (E) fifth instar. Scale bar: 500 µm.
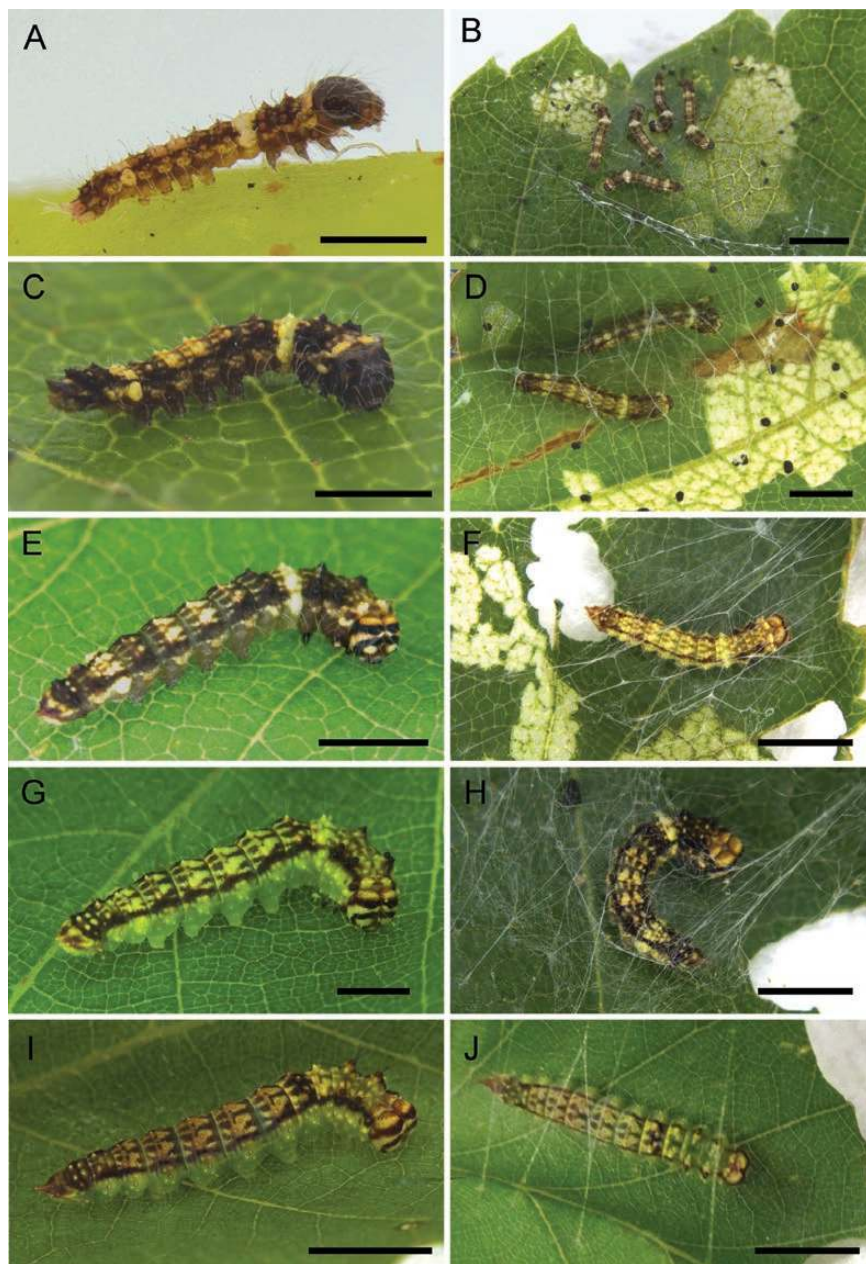
Fig. 4. Lateral (left) views of each instar and dorsal views of larvae within their characteristic shelter (right). (A) First instar lateral view. Scale bar: 500 μm; (B) first instars in shelter. Scale bar: 2,000 μm; (C) second instar lateral view. Scale bar: 1,000 μm; (D) second instars in shelter. Scale bar: 2,000 μm; (E) third instar lateral view. Scale bar: 2,000 μm; (F) third instar in shelter. Scale bar: 3,000 μm; (G) fourth instar lateral view. Scale bar: 2,000 μm; (H) fourth instar in shelter. Scale bar: 3,000 μm; (I) fifth instar lateral view. Scale bar 5,000 μm; (J) fifth instar in shelter. Scale bar: 5,000 μm.

Second Instar

Morphology

Head capsule width: 0.46–0.56 mm (mean = 0.52 ± 0.03 mm, $n = 32$), body length: 3.09–5.58 mm (mean = 3.85 ± 0.67 mm, $n = 32$) (Figs. 3 and 4; Tables 1 and 2). Head capsule differs from the first instar primarily in color and banding pattern, with the color becoming lighter brown and the appearance of two, not so well pronounced, dark brown transverse bands across the head. Overall, the body color is lighter brown but with the same color pattern as first instars. Lateral verrucae on thoracic segment T1 similar in size to the dorsal and subdorsal verrucae on thoracic segments T2 and T3; dorsal and subdorsal verrucae are present on T2, T3, and lateral verrucae on abdominal segment A9 are slightly more prominent and conspicuous than in the first instar. Bifurcation of the

conical projection at the end of the suranal plate is less well pronounced than in first instars, covered with more conspicuous black setae. A pair of setae, emerging from the bifurcation, is shorter than the projection itself.

Developmental time (mean ± SD, $n = 32$) = 4.21 ± 0.64 d.

Feeding and Shelter Construction

Following the molt from first instar, the exoskeleton is consumed in most cases (in >90% of the cases) while the head capsule is often attached to the overhanging strands of the silk shelter (in >80% of the cases), or present on the floor of silk shelter (<20% of the cases). Second instars either continue extending the same first instar shelter or make a new shelter on the same leaf. Feeding and shelter construction behaviors are similar to those observed in the first instar.

Third instar
Morphology
Head capsule width: 0.77–1.02 mm (mean = 0.92 ± 0.06 mm, n = 28), body length: 4.20–7.50 mm (mean = 5.72 ± 0.91 mm, n = 28) (Figs. 3 and 4; Tables 1 and 2). Head capsule differs from the second instar in shape, color, and banding pattern; the shape becomes slightly round, the color is yellowish-green, and there are two clear, brown transverse across the head. Overall, the body color changes to yellowish green with a similar pattern as previous instar. Lateral verrucae on T1 are reduced in size compared to dorsal and subdorsal verrucae on T2 and T3; dorsal and subdorsal verrucae on thoracic segments T2, T3, as well as other verrucae on abdominal segments, are much more prominent than in the second instar; verrucae on abdominal segments are lighter yellow to red. Thoracic legs are blackish-brown, and abdominal prolegs are yellowish-brown to green. The conical projection at the end of suranal plate is rust colored with a black tip and more conspicuous black setae.

Developmental time (mean ± SD, n = 28) = 3.75 ± 0.81 d.

Feeding and Shelter Construction
Following the second instar molt, third instars continue to feed within the same shelter by skeletonizing the leaf tissue, but then transition to cutting the leaf edges, moving inwards and consuming silk strands as they feed. Within 24 h of molting, third instars move out of the early instar shelter and make a new solitary shelter on the edge or tip of a leaf. Leaf skeletonization is no longer observed after this point in their development. Shelter construction involves more folding of the leaf compared to early instars with occasional attachment of frass to the overhanging silk strands. Shelters consist of thicker silk strands joining one end of leaf to another, thus folding the leaf more so than in earlier instars. Prior to molting into fourth instar, third instars construct a "premolting" shelter that can either be a new shelter or a modification of the existing shelter. The premolting shelter comprises additional layers of silk, making it denser.

Fourth Instar
Morphology
Head capsule width: 1.20–1.75 mm (mean = 1.46 ± 0.12, n = 25), body length: 6.62–10.60 mm (mean = 8.50 ± 1.08, n = 25) (Figs. 3 and 4; Tables 1 and 2). Head capsule differs from previous instar mainly with respect to shape, becoming completely round, and bilobed with a prominent, well-defined notch at the epicranial suture. Overall body color changes to lighter green, with a green thorax and abdominal segments green mottled with brown spots; the pattern is the same as the preceding instar. Lateral verrucae on T1 reduced in size, becoming flat; dorsal and subdorsal verrucae on T2, T3 are more conspicuous and prominent than others on abdominal segments and compared to the verrucae on T2, T3 of the previous instar; dorsal verrucae on T2, T3 are bigger than subdorsal ones with a yellowish-green base and black tip; distinctly visible oval, green spiracles with brown outline present on T1, A1–A8. Thoracic legs and abdominal prolegs are green. The conical projection at the end of A10 is bright rust-red colored with a small pair of setae emerging from the significantly reduced bifurcation.

Developmental time (mean ± SD, n = 25) = 3.55 ± 0.80 d.

Feeding and Shelter Construction
Larvae lay a silk mat on the leaf surface as do previous instars but while constructing the shelter fold the leaf significantly more so than third instars. Fourth instars also construct feeding and molting shelters as described for third instars. Larvae may make multiple shelters for resting and feeding that consist of only a few (2–4) thick silk strands with no cells and webbing. Some larvae were observed to remove frass from the shelter by either backing up and flicking over the edge of the leaf, or by picking it up in their mandibles, walking to the leaf edge and dropping it. Like late third instars, fourth instars feed by cutting the leaf and consuming the shelter as they feed. Once the larva consumes the entire leaf, it wanders in search of a new leaf to construct a shelter.

Fifth Instar
Morphology
Head capsule width: 1.56–2.21 mm (mean = 2.01 ± 0.18 mm, n = 22), body length: 10.00–20.50 mm (mean = 13.95 ± 3.01 mm, n = 22) (Figs. 3–5; Tables 1 and 2). Morphology and color patterns of head capsules and body do not change significantly from fourth instar; dorsal abdominal body color turns rust-brown. Dorsal and subdorsal verrucae on thoracic segments T2, T3 more prominent than other verrucae on the body and compared to T2, T3 thoracic verrucae on the previous instar; dorsal verrucae almost double the size of subdorsal ones with a yellow base and bright red tip; lateral verrucae on A9 green and inconspicuous. Prepupal larvae become enlarged, with the head capsule noticeably more narrow than the body; dorsal surface of abdominal segments turns brownish-red with green thoracic segments; prominent verrucae are only present on thoracic segments T2, T3.

Developmental time (mean ± SD, n = 22) = 4.41 ± 0.80 d.

Feeding and Shelter Construction
Same as observed in fourth instar. Prior to pupation, larvae may wander and eat multiple leaves before settling into a silk shelter that they construct specifically for pupation. A fifth instar feeds for 2–3 d before entering prepupal stage and then continues feeding for 1–2 d before folding the leaf. Once the leaf is entirely folded, it takes 2–3 d for the pupa to form. This shelter consists of several (~5–10) thick silk strands that fold the leaf edge tightly, encasing the pupa.

Pupa
Morphology
Length: 11.19–13.84 mm (mean = 12.49 ± 0.88, n = 10); width: 3.67–4.40 mm (mean = 3.98 ± 0.30, n = 10) (Fig. 5). The pupa is medium to dark brown colored with fine hair-like setae present on abdominal segments. Darker brown to black colored spiracles are also present on the abdominal segments.

Duration: the duration of the pupal stage is temperature dependent. We did not formally measure this, but in general, the duration is about 2 wks at room temperature (~21–23°C). Pupae can also successfully overwinter for over a year at 4–6°C (J. E. Yack, unpublished observations).

Comparisons Between Sizes of Instars
Five head capsules were collected for each of the individuals followed throughout the larval development from hatching to pupation. When we plotted the distribution of head capsule widths using normalized values (Fig. 6A), five distinct peaks were observed. Furthermore, head capsule widths of each instar were significantly different from each other at $P < 0.05$ (one-way ANOVA; $F_{4,144}$ = 1,797, $P < 0.0001$ and Tukey's HSD test). This indicated that there are five distinct instars and head capsule width is a good indicator of instars. Furthermore, the natural log of head capsule widths plotted against instars showed geometric larval growth through development (Fig. 7; Dyar's average growth ratio: 1.63) hence further confirming five instars for
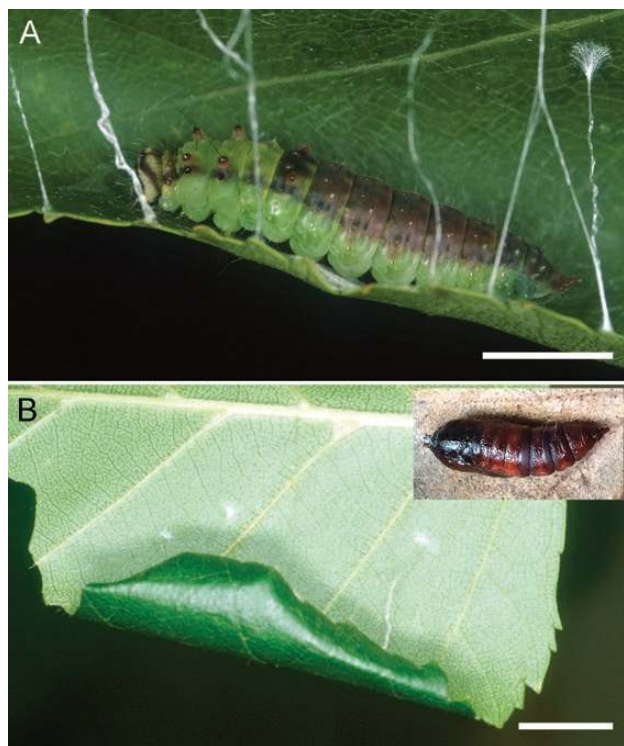
Fig. 5. Light micrographs of prepupa and pupa of *D. arcuata*. (A) Prepupal phase showing thick strands characteristic of pupal shelters. Scale bar: 500 µm. (B) As silk dries, it contracts forming a tight leaf enclosure for the pupa. Pupa is shown in the inset. Scale bar: 500 µm.
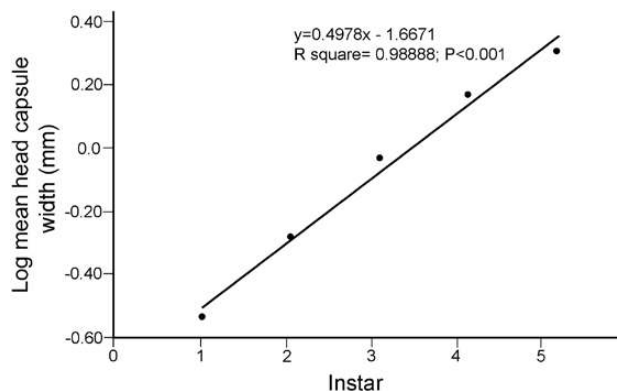

Fig. 7. Natural log of mean head capsule widths plotted against the number of instars. The figure shows a linear geometric progression (R^2 = 0.98, P = 0.0005), confirming five instars for *D. arcuata*.

Table 1. Head capsule measurements for instars (I–V) of *D. arcuata*

Instar	N	Range (mm)	Mean ± SD (mm)	Coefficient of variation	Dyar's ratio[a]
I	42	0.26–0.31	0.29 ± 0.01	0.04	—
II	32	0.46–0.56	0.52 ± 0.03	0.05	1.79
II	28	0.77–1.02	0.92 ± 0.06	0.06	1.79
IV	25	1.20–1.75	1.46 ± 0.12	0.08	1.57
V	22	1.56–2.21	2.01 ± 0.18	0.09	1.40

[a]Dyar's ratio (e.g., for instar II): mean head capsule width of Instar II/Mean head capsule width of instar I

Table 2. Body length measurements for instars of *D. arcuata*

Instar	N	Range (mm)	Coefficient of variation	Mean ± SD (mm)
I	42	1.75–2.93	0.14	2.36 ± 0.33
II	32	3.09–5.58	0.17	3.85 ± 0.67
III	28	4.20–7.50	0.16	5.72 ± 0.91
IV	25	6.62–10.60	0.13	8.50 ± 1.08
V	22	10.00–20.50	0.22	13.95 ± 3.01

Fig. 6. Distribution of head capsule widths and body lengths for *D. arcuata* instars. (A) Normalized distribution of head capsule widths; (B) normalized distribution of body lengths recorded at 24 h following molt for each instar.

D. arcuata. Body lengths for larvae in each instar were also measured within 24 h of molting. Although we observed five peaks corresponding to five instars with each significantly different from each other (one-way ANOVA; $F_{4,144}$ = 328.8, P < 0.0001 and Tukey's HSD

test) but as expected there was more overlap in lengths across instars with higher coefficients of variation for each instar than for head capsule widths (Tables 1 and 2; Fig. 6B).

Discussion

Two main goals of this study were to confirm the number of instars and to establish morphological criteria to distinguish between these instars. By following molts and collecting head capsules for multiple individuals, we confirmed that *D. arcuata* larvae undergo five instars. We further confirmed five instars using Dyar's rule (1890). Dyar showed a more or less constant, geometric progression in larval head capsule widths for 28 larval lepidopteran species. Dyar's rule (1890) has been used to identify the number of larval stages in several insects (e.g., McClellan and Logan 1994, Delbac et al. 2010, Velásquez and Viloria 2010, Barrionuevo and San Blas 2016). Head capsule width is the most reliable way to identify a larval stage, as it does not change within an instar.

In addition to head capsule width and body length, a number of other morphological characteristics differed between instars. First
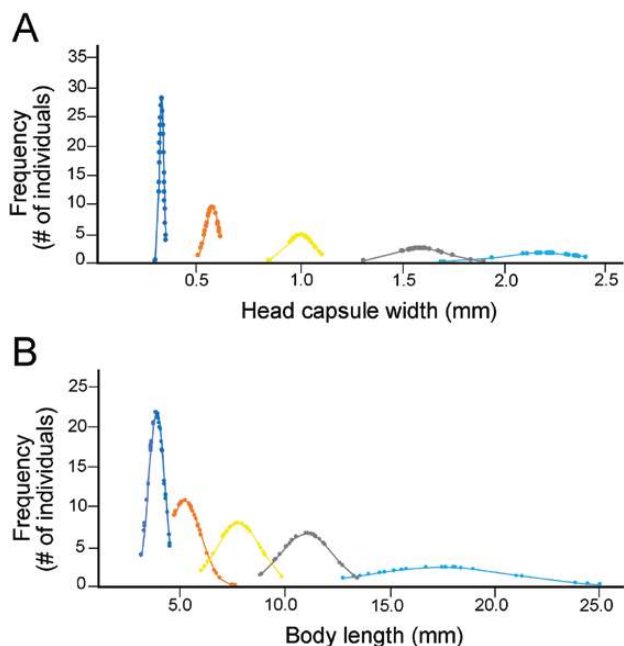
instars differ from all others based on their characteristic black, triangular head, and dark brown body. Second instars develop faint brown bands on their heads with comparatively more prominent verrucae on thoracic and abdominal segments. Third instars are the first to develop two clear, distinct brown transverse bands on the head, with the head becoming more rounded than oval. Between third and fourth instar, the head becomes more rounded, thoracic legs and abdominal prolegs become green in color, and the overall body color becomes lighter green mottled with brown on abdominal segments. Aside from head capsule width and body size, the most noteworthy change from fourth to early fifth instar is the very prominent bright red-tipped dorsal verrucae on thoracic segments T2 and T3. However, as fifth instars reach the prepupal stage their dorsal abdominal surface becomes rust-brown, and the overall body becomes enlarged with the head size almost half as wide as the body. While we did find consistency in body color within instars, this morphological feature should be further studied in larvae reared on different colored leaves, it has been noted that body color of late instars may vary depending on the leaf color (personal observation, J. E. Yack), and therefore this feature should be used cautiously in distinguishing between instars, and warrants further attention.

Our study is the first to document instar-specific behavioral changes in this species, focusing on feeding style and shelter construction. Feeding style changes from exclusively skeletonizing the leaf surface in first and second instars, to exclusively cutting the leaf in fourth and fifth instars. Third instars exhibit both behaviors, and transition from one to the other as they mature. Changes in feeding style from skeletonizing in early instars to cutting in late instars have been previously noted for a number of Lepidoptera (Hochuli 2001). These changes could be attributed to the size of the head and mandibles, and changing nutritional requirements (Hochuli 2001). We did not assess how late instars cut the leaf or how they processed the leaf material after cutting the edge (i.e., whether they snipped, crushed, or chewed the leaf material), as this would require further analysis of the mandible structure and gut contents (e.g., Bernays and Janzen 1988).

All instars were observed to lay a silk mat on the leaf surface in addition to building silk shelters. Silk mats in many Lepidoptera are suggested to help larvae feed efficiently on leaves with high-trichome density (Fordyce and Agrawal 2001) and also to provide protection from predators as the larvae grip on the silk mat when attacked (McClure and Despland 2011). All *D. arcuata* instars constructed intricately woven silk shelters for resting, molting, and feeding. Many lepidopteran larvae construct silk shelters (Stehr 1987, Scoble 1992), with potential benefits such as defense and improved microclimate (Hunter and Willmer 1989, Costa and Pierce 1997). Leaf folding, which was more pronounced in the solitary shelters of late instars could have been aided by contraction of drying silk strands (see Fitzgerald et al. 1991, 1994). First, second, and occasionally third instars attached frass to silk shelters. Fourth and fifth instars were observed to remove frass from their shelters, possibly to eliminate olfactory cues to avoid being detected by predators and parasitoids (Weiss 2003). The number and types of shelters change as larvae mature and change from a gregarious to solitary lifestyle as seen in several other lepidopterans (e.g., Abarca et al. 2014). These changes could be attributed to differences required for shelter and protection from predators and parasitoids, or different feeding habits with increasing body size (Lind et al. 2001).

Our study shows that early instars (I, II) form small groups within silk shelters whereas late instars do not (IV, V), supporting previous observations in both the lab (Yack et al. 2014, Yadav et al. 2017) and field (J. E. Yack, unpublished observations). Post

hatching, early instars (I, II) use vibrational signaling in the formation of small groups (Yadav et al. 2017), whereas in late instars (IV, V) vibrational signaling is used in territorial encounters (Yack et al. 2001, 2014; Guedes et al. 2012). It is still unknown how early instar groups are maintained, but vibratory signals likely play a role. Third instars exhibited both behaviors in that they resided in the communal shelter for a period following their molt, but within 24–48 h of molting became solitary. Gregariousness is an important life history trait observed in a number of lepidopteran larvae with a large number of species living gregariously either throughout or through a part of their development (Costa and Pierce 1997, Costa 2006). However, the mechanisms mediating sociality in larval Lepidoptera requires further study. The masked birch caterpillar is an excellent model to investigate the multimodal mechanisms involved, as they clearly transition from being gregarious to solitary during development, produce vibratory signals throughout their development, while at the same time possibly using chemical cues associated with silk in their shelters. In particular, the interesting transitional features of third instars provide opportunities to study physiological and genetic mechanisms underlying social behavior in larval insects.

Acknowledgments

References Cited

Abarca, M., K. Boege, and A. Zaldívar-Riverón. 2014. Shelter-building behavior and natural history of two pyralid caterpillars feeding on *Piper stipulaceum*. J. Insect Sci. 14: 39.

Barrionuevo, M., and G. San Blas. 2016. Redescription of immature stages of the soybean looper (Lepidoptera: Noctuidae: Plusiinae). Can. Entomol. 148: 247–259.

Bernays, E. A., and D.H. Janzen. 1988. Saturniid and sphingid caterpillars: two ways to eat leaves. Ecology. 69: 1153–1160.

Beutenmuller, W. 1898. Bombycine moths of vicinity of N.Y. Bull. Am. Mus. Nat. Hist. 17: 388–389.

Cazado, L.E., G.A. Van Nieuwenhove, C.W. O'Brien, G.A. Gastaminza, and M.G. Murúa. 2014. Determination of number of instars of *Rhyssomatus subtilis* (Coleoptera: Curculionidae) based on head capsule widths. Fla. Entomol. 97: 639–643.

Costa, J.T. 2006. The other insect societies. The Belknap Press of Harvard University Press, Cambridge.

Costa, J.T., and N.E. Pierce. 1997. Social evolution in the Lepidoptera: ecological context and communication in larval societies, pp. 407–422. In: J. C. Choe and B. J. Crespi (eds.), The evolution of social behavior in insects and arachnids. Cambridge University Press, Cambridge.

Delbac, L., P. Lecharpentier, and D. Thiery. 2010. Larval instars determination for the European Grapevine Moth (Lepidoptera: Tortricidae) based on the frequency distribution of head-capsule widths. Crop Protection. 29: 623–630.

Dyar, H. G. 1890. The number of molts of lepidopterous larvae. Psyche 5: 420–422.

Dyar, H.G. 1895. Notes on drepanid larvae. J.N.Y. Entomol. Soc. 3: 66–69.

Fitzgerald, T. D., and K. Clark. 1994. Analysis of leaf-rolling behavior of *Caloptilia serotinella* (Lepidoptera: Gracillariidae). J. Insect Behav. 7: 859–872.

Fitzgerald, T. D., K. Clark, R. Vanderpool, and C. Phillips. 1991. Leaf shelter-building caterpillars harness forces generated by axial retraction of stretched and wetted silk. J. Insect Behav. 4: 21–32.

Fordyce, J.A., and A. Agrawal. 2001. The role of plant trichomes and caterpillar group size on growth and defence of the pipevine swallowtail *Battus philenor*. J. Anim. Ecol. 70: 997–1005.

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.

Guedes, R. N. C., S. M. Matheson, B. Frei, M. L. Smith, and J. E. Yack. 2012. Vibration detection and discrimination in the masked birch caterpillar (*Drepana arcuata*). J. Comp. Physiol. A. 198: 325–335.

Handfield, L. 1999. Le guide des papillons du Quebec, vol. 1. Broquet inc., Quebec, Canada

Hochuli, D.F. 2001. Insect herbivory and ontogeny: how do growth and development influence feeding behavior, morphology and host use? Aust. Ecol. 26: 563–570.

Hunter, M. D., and P. G. Willmer. 1989. The potential for interspecific competition between two abundant defoliators on oak: leaf damage and habitat quality. Ecol. Entomol. 14: 267–277.

Lind, E. M., M. T. Jones, J. D. Long, and M. R. Weiss. 2001. Ontogenetic changes in leaf shelter construction by larvae of *Epargyreus clarus* (Hesperiidae), the Silver-spotted Skipper. J. Lepidop. Soc. 54: 77–82.

Matheson, S.M. 2011. Vibratory mediated spacing in groups of insect larvae (*Drepana arcuata*, Lepidoptera; *Scolytus multistriatus*, Coleoptera). M.Sc. thesis, Carleton University, Canada.

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.

McClure, M., and E. Despland. 2011. Defensive responses by a social caterpillar are tailored to different predators and change with larval instar and group size. Naturwissenschaften 98: 425–434.

de Mendiburu, F. 2016. Agricolae: statistical procedures for agricultural research. R package version 1.2–4. https://CRAN.R-project.org/package=agricolae

Packard, A. S. 1890. The life-history of *Drepana arcuata*, with remarks on certain structural features of the larvae and on the supposed dimorphism of *Drepana arcuata* and *Dryopteris rosea*. Proc. Boston Soc. Nat. Hist. 24: 482–493.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rose, A. H., and O. H. Lindquist. 1997. Insects of eastern hardwood trees. Canadian Forestry Service, Ottawa, Forestry technical report 29.

Scoble, M. J. 1992. The Lepidoptera: form, function and diversity. The Natural History Museum & Oxford University Press, London.

Scott, J. L., A. Y. Kawahara, J. H. Skevington, S. H. Yen, A. Sami, M. L. Smith, and J. E. Yack. 2010. The evolutionary origins of ritualized acoustic signals in caterpillars. Nat. Commun. 1: 1–9.

Stehr, F.W. 1987. Order Lepidoptera, pp 288–596. In: F. W. Stehr (ed.), Immature insects, vol. 1. Kendall/Hunt, Dubuque.

Surlykke, A., J. E. Yack, A. J. Spence, and I. Hasenfuss. 2003. Hearing in hook-tip moths (Drepanidae, Lepidoptera). J. Exp. Biol. 206: 2653–2663.

Velásquez, Y., and A. Viloria. 2010. Instar determination of the neotropical beetle *Oxelytrum discicolle* (Coleoptera: Silphidae). J. Med. Entomol. 47: 723–726.

Weiss, M. R. 2003. Good housekeeping: why do shelter-dwelling caterpillars fling their frass? Ecol. Lett. 6: 361–370.

Yack, J. E. 2016. Vibrational signaling, pp 99–123. In: G. S. Pollack, A. C. Mason, R. R. Fay, and A. N. Popper (eds.), Springer handbook of auditory research: insect hearing. Springer, New York.

Yack, J. E., M. L. Smith, and P. J. Weatherhead. 2001. Caterpillar talk: acoustically mediated territoriality in larval Lepidoptera. P. Natl. Acad. Sci. USA 98: 11371–11375.

Yack, J. E., S. Gill, D. Drummond-Main, and T. N. Sherratt. 2014. Residency duration and shelter quality influence vibratory signaling displays in a territorial caterpillar. Ethology. 120: 354–364.

Yadav, C., R. N. C. Guedes, S. M. Matheson, T. A. Timbers, and J. E. Yack. 2017. Invitation by vibration: recruitment to feeding shelters by vibrating caterpillars. Behav. Ecol. Sociobiol. 71: 5.