SCIENTIFIC NOTE

Identifying larval stages of *Orgyia antiqua* (Lepidoptera: Erebidae) from British Columbia, Canada

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The rusty tussock moth, *Orgyia antiqua* (Linnaeus, 1758) (Lepidoptera: Erebidae) is being used as an ecological surrogate to measure the impact of native natural enemies on the establishment of European gypsy moth, *Lymantria dispar* (Linnaeus, 1758), in British Columbia, Canada. To measure stage-specific mortality rates, one must be able to identify accurately different life stages of the species under study, ideally with characteristics that can be used in the field. The existing literature describing the number, size, and colouration of larval instars for *O. antiqua* is highly inconsistent (Table 1). The number of reported larval instars varies from 5–6 in males and 5–7 in females. Only two papers report the width of larval head capsules, with substantial disagreement between them (Dyer 1893; Payne 1917). Later instars of *O. antiqua* are characterised by dense tufts of setae on the dorsal surface of segments 4–7. These have been variously described as white, yellow, rusty brown, dark grey or black, and have been proposed by some authors to be aposematic warnings (Sandre *et al.* 2007a) that vary among instars. Through careful rearing of individual larvae and consistent measurements of head capsule width, we sought to clarify the number of larval instars and identify unique morphological characters that would facilitate the determination of instar in the field.

Table 1

Published descriptions of larval *O. antiqua* with respect to the colour patterns of the four dorsal tufts and the corresponding head capsule widths. Listed colours should be read as tuft colour from anterior to posterior starting on the first abdominal segment. B=Black, Y=Yellow, W=White, G=Grey, Br=Brown, ?=not reported.

	Dyer 1893	Gentner 1915	Hardy 1945	Payne 1917	Sandre 2007a	Sandre 2007b
Instar			Colour Pattern of Dorsal Tufts			
3	B-B-Y-Y	G-G-W-W	B-B-W-W	G-G-W-W	?-?-?-?	?-?-?-?
4	B-B-Y-Y	?-?-Y-Y	Y-Y-Y-Y	G-G-Y-Y	B-B-Y-Y (Pied)	B-B-Y-Y *
5	B-B-Y-Y	W-W-W-W	?-?-?-?	W-W-W-W	Y-Y-Y-Y (Bright)	Y-Y-Y-Y *
6	W-W-W-W	-	-	W-W-W-W	Br-Br-Br-Br (Dull)	Br-Br-Br-Br *
7	W-W-W-W	-	-	-	-	
			Head Capsule Width (mm)			
1	0.55			0.518 - 0.537		
2	0.75			0.812 - 0.875		
3	1.1			1.16 - 1.35		
4	1.55			1.80 - 2.02		
5	2.1			2.24 - 2.64		
6	-			3.0 - 3.5		
7	-			-		

*Sandre 2007b reports that this is the typical pattern but that "nearly all other combinations were also present."

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In May 2017, 33 *O. antiqua* egg masses were collected from a small population in Burnaby, BC, Canada (49.258821 N, 123.009661 W), that was feeding on an isolated Colorado spruce (*Picea pungens* Engelm) planted as a landscaping tree. Larvae were reared for one generation on *Alnus rubra*. In May 2018, 40 newly eclosed larvae from the second generation were reared individually in 50-mm plastic Petri dishes (20°C, 18L:6D) and fed fresh foliage of locally collected Himalayan blackberry (*Rubus armeniacus*) every 1–3 days. Head capsule widths for each larval instar were measured, using a Leica M5S dissecting microscope with an ocular micrometer with a precision of 0.012 mm, on live larvae that had been chilled for approximately 10 minutes at 5°C. Shed head capsules were retained for each individual larva in order to confirm the number of moults.

In a separate trial, a small sample of 10 newly eclosed *O. antiqua* larvae were reared on coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) foliage to determine if they could complete development on this host. The head capsule widths for these larvae were measured for 4th and successive instars only. Representative photographs were taken of individual larvae from each instar using a Nikon D7000 digital camera equipped with a Nikon Speedlight SB-700 flash unit. After pupation and subsequent emergence, the gender of adults was recorded.

Of the 40 larvae reared on blackberry, eight died of unknown causes before the 3rd instar and were excluded from the analysis. Males (n = 17) invariably had five instars, whereas females (n = 15) typically had six instars, with the exception of one female that pupated after the 5th instar. For the first four instars, the head capsule widths of the larvae grew exponentially, closely following Dyar's rule (Fig. 1). For male and female larvae, 5th instar head capsules were smaller than expected, based on the progression of the first four instars. Similarly, head capsules of 6th instar females were also smaller than expected. There was no overlap in the head capsule widths of successive instars for either sex through 1st to 6th instar (Fig. 2), and our measurements closely matched those of Payne (1917). Larvae reared on Douglas-fir foliage had very similar head capsule widths to those reared on blackberry (Fig. 2). Consistent with the blackberry-reared larvae, the male larvae reared on Douglas-fir (n=5) had five instars, and the female larvae (n=5) predominantly had six instars with the exception of one female, which pupated following the 5th instar.

The morphological appearance of the first three instars closely matched the descriptions previously published in the literature (Table 1, Fig 3). First instar larvae are characterised by the absence of orange tubercles on the 6th and 7th abdominal segments. These tubercles are present in the 2nd instar larvae, but this stage lacks lateral pencils on the 1st thoracic segment. The 3rd instar is characterised by distinct lateral pencils on the 1st thoracic segment, as well as by the appearance of dorsal tufts on the 1st to 4th abdominal segments. We found that the dorsal tufts of 4th instar larvae always had a "pied" (Table 1) or two-toned colouration that varied considerably between individuals (e.g., larvae 6 and 7 in Fig. 3). Fifth instar males had four monochromatic tufts that ranged in colour from white to yellow to a rusty brown. In females that had six instars, the 5th instar was pied (e.g., Fig. 3, larvae 6 and 28). Sixth instar females looked the same as 5th instar males, with four monochromatic tufts ranging from white to yellow and rusty brown.

In conclusion, it is difficult to unambiguously discriminate between 4th, 5th, and 6th instars in the field based solely on the colouration of the dorsal tufts. An individual with tufts of different colours could be a 4th instar of either sex or a 5th instar female that will eventually moult into a 6th instar. An individual with tufts of a single colour could be a 5th instar of either sex or a 6th instar female. Head capsule width, however, could be used to discriminate unambiguously between each of the instars. In our sample, there was no overlap in the size distributions for each instar, even when we reared the larvae on Douglas-fir, which we considered a sub-optimal host based on previously observed slower growth rates.

It is interesting to note that when females had an 'extra' instar, it was not a typical supernumerary instar as has been reported in other lepidopteran larvae (Leonard 1970; Retnakaran, 1973; Hatakoshi *et al.* 1988) but rather a repetition of the 4th instar; the additional instar was morphologically similar to the 4th instar, only larger. Only one of the 15 females reared on blackberry did not have a 6th instar and that individual had the largest head capsule of all the female larvae from the 3rd to 5th instars. This suggests to us that the physiological trigger for an extra instar is related to size and that this is triggered at some point during or before the 4th instar.

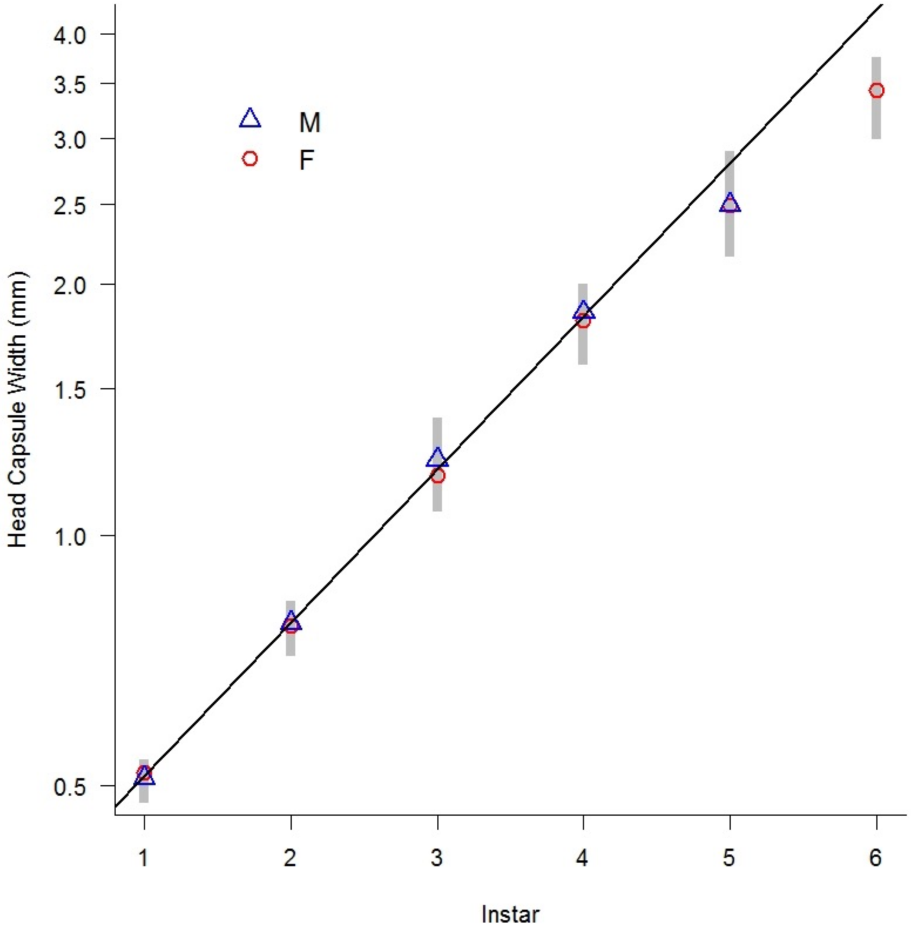

Figure 1. Average head capsule widths for female (circles) and male (triangles) *Orgyia antiqua* larvae according to instar number. The linear regression line was fitted to the first four instars only as the head capsule widths for the final two instars deviated significantly from a linear relationship $\log_{10}(HCW) = 0.185 \times Instar - 0.475$, ($R^2 = 0.999$, $F_{1,6}=7620$, $P<0.001$). Vertical grey bars represent the range of measurements for each instar.

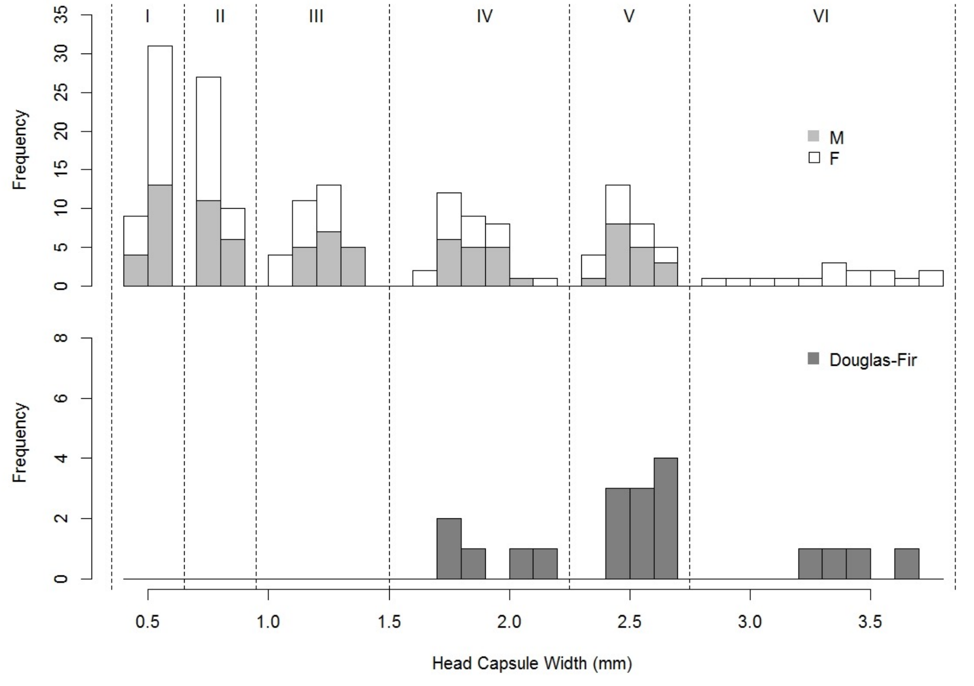

Figure 2. Distribution of head capsule sizes according to instar and sex when reared on Himalayan blackberry (top panel) and Douglas-fir (bottom panel). Instars were assigned based on the number of observed moults. Vertical dashed lines indicate proposed cut-off points to discriminate field collected larval instars.

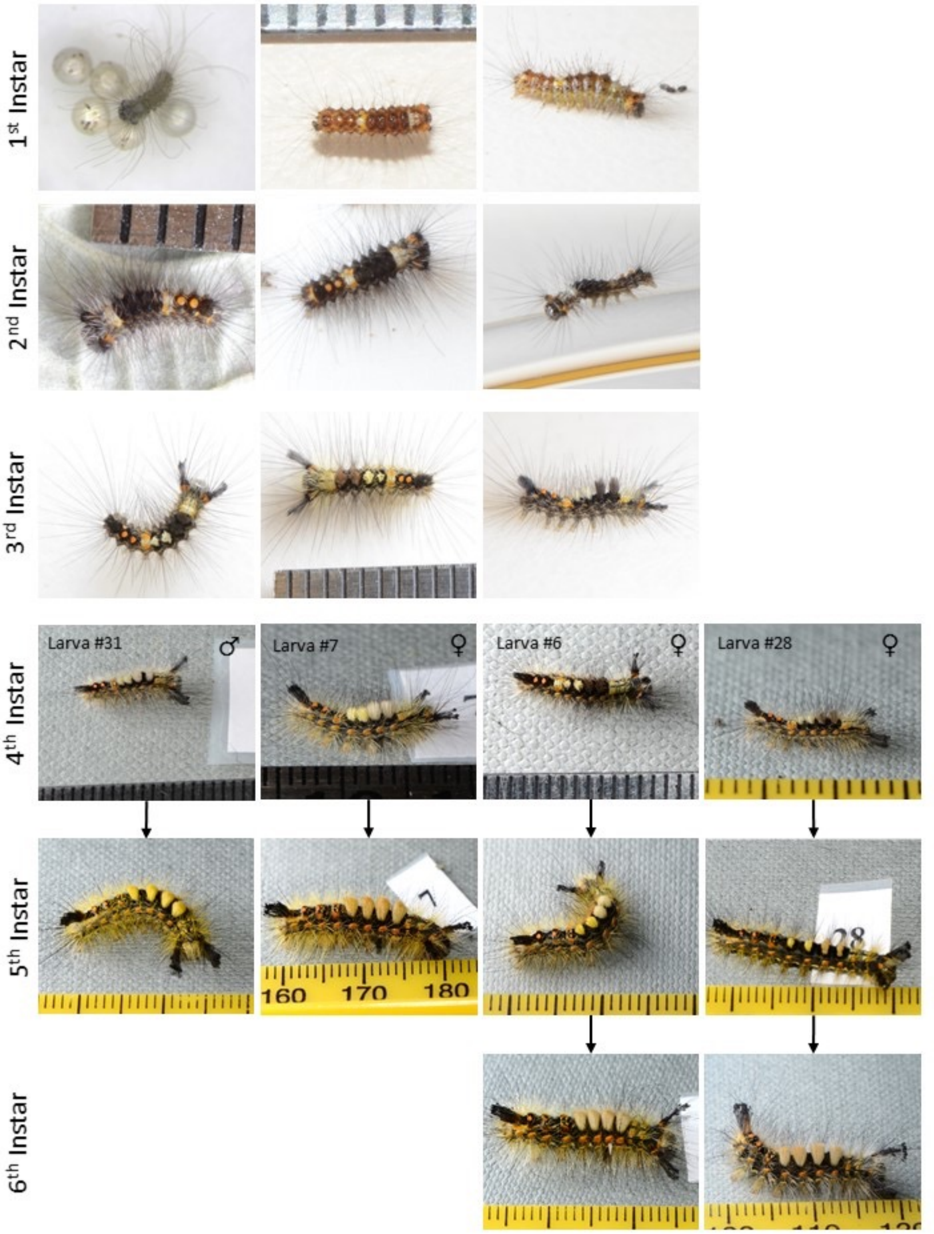

Figure 3. Representative images of *Orgyia antiqua* larvae reared on Himalayan blackberry leaves. Larvae in instars 1–3 (first three rows) exhibited little variation in colouration. The dorsal tufts were always uniformly coloured in the final instar, which was 5 in males and either 5 or 6 in females. Arrows between images indicate successive images of the same individual.

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