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Plant-Insect Interactions

Investigating the effect of host plant identity on instar number in fall webworm, a common generalist herbivore

Mykaela M. Tanino-Springsteen[1,*], Dhaval K. Vyas[1], Audrey Mitchell[1], Catherine Durso[2], Shannon M. Murphy[1]

[1]Department of Biological Sciences, University of Denver, Denver, CO, USA, [2]Department of Computer Science, University of Denver, Denver, CO, USA *Corresponding author, mail: mykaela.tanino-springste@du.edu

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For herbivorous insects with a broad diet breadth, host plant identity can influence larval development by either accelerating or delaying growth. For some species of Lepidoptera, the number of larval instars varies depending on the host plant's identity. Fall webworm (*Hyphantria cunea*, Drury) is a polyphagous herbivore that feeds on over 450 host plants worldwide. Of the 2 morphotypes (red- and black-head) of fall webworm, the number of instars for the red-head fall webworms has not been characterized. Given its broad diet breadth, fall webworm developmental stages may vary with plant identity. We investigated whether host plant identity affected the number of instars observed during red-head fall webworm development. We measured the head capsules of over 6,000 fall webworm larvae reared on 6 different plants commonly eaten by fall webworms in Colorado. We modeled head capsule widths as Gaussian mixture models, with a Gaussian distribution that corresponded to each instar. We show that our red-head fall webworms varied in number of instars depending on the identity of their host plant upon which they fed. We found that red-head fall webworm exhibited 7 instars on 5 of the host plants and 8 instars on 1 host plant that we studied. Our results for the number of instars for red-head fall webworm are consistent with reports of the number of instars for black-head fall webworm. Our research provides insight into the influence of host plant identity on fall webworm development, which can be used to advance lab and field research of this species.

Key words: bottom-up effect, diet-mediated growth, stadia, larval development, supernumerary instar

Introduction

Plants have a significant influence on the immature stages of herbivorous insects. Larval growth is contingent on the plant's nutritional quality and the anti-herbivore physical and chemical defenses (Coley et al. 2006, Gallon and Smilanich 2023). As nutritional quality and plant defenses can change with plant ontogeny, the age of a plant can also impact larval growth (Hamilton and Zalucki 1993, Quintero et al. 2014, Quintero and Bowers 2018). For herbivorous insects with a broad diet breadth, the plant species changes the outcome of larval development by either accelerating or delaying growth (Tikkanen et al. 2000, Engelkes and Mills 2013, Friedrichs et al. 2022). For a variety of insects, adult fitness is directly associated with the larval diet, which influences adult traits such as female fecundity (Awmack and Leather 2002). However, for many insect species, we either lack data on how diet affects larval growth or we have a limited understanding of this relationship.

Among Lepidoptera, components of diet quality such as host plant identity, nutritional content, and defense chemicals have significant effects on larval development (Bentacourt et al. 2004, Lee et al. 2012). To assess dietary impacts on lepidopteran development, instars (developmental stages) are a valuable metric because they provide measurements of discrete, stage-specific growth. Notably, for some species of Lepidoptera the number of larval instars can vary depending on host plant identity and quality (e.g., Calvo and Molina 2008, Mo et al. 2013). For example, when feeding on a low-quality diet, some lepidopteran larvae will undergo an extra instar, or supernumerary instar, to compensate for slower growth (Kidd and Orr 2001, Esperk et al. 2007a, Lee et al. 2012, Tuan et al. 2015, Abarca et al. 2020).

A lepidopteran's instar affects its interactions with its natural enemies (e.g., parasitoids), and these ecological interactions have significant impacts on applied research, such as integrated pest management (IPM). For example, parasitoids are commonly used as

biological control agents for IPM and many parasitoids target specific host instars (Keller and Tenhumberg 2000, Stoepler et al. 2011, Murphy et al. 2014) which are indicators of host quality and suitability. In turn, successful parasitoid larval development and survival are affected by the changes in the host's physiology and nutritional status as it develops (Vinson and Iwantsch 1980, Godfray 1994). In addition to biological control measures, IPM includes age-specific pesticide applications, the efficacy of which is highly dependent on the developmental stage of the pest insects (Stuijzand et al. 2000, Huseth et al. 2017). Indeed, studies of instars are commonly conducted on pest lepidopterans with the goal of improving the timing of age-specific pesticide application (McClellan and Logan 1994, Godin et al. 2002, Irigaray et al. 2006, Calvo and Molina 2008, Delbac et al. 2010, Chen and Seybold 2013, Mo et al. 2013). Pest management decisions are improved by studies that assess the bottom-up influence of diet on larval growth (Irigaray et al. 2006, Calvo and Molina 2008). Thus, from both conceptual and applied perspectives, we must understand how the bottom-up effects from plants alter instar number and duration since important ecological interactions are dependent on the larval developmental stage.

Fall webworm (*Hyphantria cunea*, Drury) (Lepidoptera: Erebidae) (hereafter FW) is a geographically widespread, polyphagous herbivore and is an ideal system for studying how diet affects top-down and bottom-up ecological interactions (e.g., Murphy and Loewy 2015, Vidal and Murphy 2018, Vyas and Murphy 2022). FW are extreme dietary generalists feeding on more than 450 host plant species worldwide (Schowalter and Ring 2017), but in some populations, diet breadth is often much narrower (Vidal et al. 2019). Diet breadth also appears to depend on morphotype. FW has 2 morphotypes distinguished by their head capsule color, black-head and red-head, and these 2 morphotypes are most likely genetically distinct species (Vidal et al. 2019). These 2 morphotypes are allopatric or sympatric and where they are sympatric, they often have population peaks that are separated temporally. The black-head FW usually have a wider diet breadth than red-head FW (Murphy and Loewy 2015) and black-head FW are the only morphotype to have been introduced to Asia and Europe (Gomi et al. 2004). In Japan, black-head FW larvae undergo 6–7 instars depending on sex, diet, and temperature (Gomi et al. 2005, Gomi 2006). Previous studies on red-head FW larvae have shown that overall development time, survival to pupation, and pupal mass are all associated with the species of plant consumed (Lowey et al. 2013, Murphy and Lowey 2015, Vidal and Murphy 2018, Vidal et al. 2020). However, little is known about the number of instars experienced by red-head FW, or how diet may influence the number of instars of this morphotype.

Here we conduct the first thorough investigation into how the host plant identity may affect the developmental pattern and instar number of red-head FW. Specifically, we investigate whether FW displays differences in instar number based on which host plant larvae are fed. A better understanding of how many instars red-head FW have will enable a comparison of life histories between red-head FW and the better-studied black-head FW. Furthermore, as we examine the effect of herbivore diets on insect development, we are better able to inform the applications that rely on comprehending the ecology of plant–insect interactions.

Methods

Study System

We studied the red-head FW morphotype from our colony housed in our laboratory at the University of Denver (Denver, Colorado). We established this colony in 2019 with FW we collected from Larimer, Boulder, and Jefferson counties in Colorado, and we add new genetic lines every year to maintain genetic diversity. FW emerge from diapause in mid to late June and we then mate females with unrelated males from different maternal lines in clear plastic shoe boxes following methods described in Loewy et al. (2013) and Robinson-Castillo et al. (2021). Females lay their egg masses, which can contain 34–830 eggs (Loewy et al. 2013), however, not all of these eggs are viable. The number of viable eggs a female lays depends heavily on female pupal mass (Loewy et al. 2013), which is highly correlated with the mother's diet and whether she fed upon a high- or low-quality diet (Murphy and Lowey 2015, Vidal and Murphy 2018). After larvae hatch, they take an average of 42 days (±2 days) to reach pupation, with most larvae in Colorado reaching pupation by late October (Loewy et al. 2013). However, the duration of larval development depends heavily on their host plant (Murphy and Loewy 2015) and temperature (Morris and Fulton (1970).

Experimental Design

Head capsules are ideal for quantifying larval growth in Lepidoptera and assessing larval body size because they are sclerotized and can only change size during molts between instars, thus head capsule size provides a reliable measure of discrete growth (Delbac et al. 2010). Furthermore, head capsules exhibit geometric growth patterns, allowing use of successive head capsule widths when estimating the number of instars (Dyar 1890)). As a result, head capsule width and instar number are often examined in conjunction.

In the summers (June–September) of 2020, 2021, and 2022, we reared FW egg masses from a total of 173 matrilines sourced from our FW lab colony in separate 0.95 L clear, plastic deli containers according to the host plant and hatch date. To control for environmental influences, we reared all FW larvae in ambient conditions in our laboratory (~22°C, constant room temperature); larvae were exposed to ambient daylight through the windows (~14 h daylight at this time of year). For all rearing, we followed the methods described in Loewy et al. (2013) and Robinson-Castillo et al. (2021), which provide more information on rearing protocols. We chose 6 host plants in 3 different plant families that are commonly eaten by FW in Colorado for our study and that vary considerably in quality; plant quality has been determined by measures of survival, pupal mass, and development time from our past studies (Loewy et al 2013, Murphy and Lowey 2015). We studied 1 host plant in the Betulaceae, thinleaf alder (*Alnus tenuifolia,* Nuttall), which is a low-quality host plant for FW. We studied 2 host plants in the Rosaceae, apple (*Malus domestica,* Borkhausen) and chokecherry (*Prunus virginiana,* Linnaeus); apple is a low-quality host plant and chokecherry is a high-quality host plant for FW. Lastly, we studied 3 host plants in the Salicaceae, broadleaf cottonwood (*Populus deltoides,* Marshall), narrowleaf cottonwood (*Populus angustifolia,* Torrey), and black willow (*Salix nigra,* Marshall); narrowleaf cottonwood is currently a low-quality host plant and broadleaf cottonwood and black willow are both high-quality host plants for FW. Because of logistical constraints, not all the host plants were used in all years, and not all possible matriline and host plant combinations were represented in any single year (Supplementary Table S1). We cleaned each larval container by removing old leaves, frass, and deceased larvae at least twice per week. We provided larvae with freshly clipped leaves as needed and collected this foliage from a variety of mature trees/shrubs at our field sites and in Denver; we purposefully collected leaves from a variety of plants to avoid any biases due to plant individuals with stronger or weaker plant defenses.

To measure larval head capsule widths throughout development, we destructively sampled larvae from each host plant frequently. All larvae were placed in individual 1.5 mL centrifuge tubes, killed by freezing and kept frozen (−20°C) until they could be measured at a later date. We attempted to sample larvae every day, but this was sometimes not possible (especially in 2020 during lockdowns); we made sure to sample larvae at least 5 days per week. For each sample, when possible, we aimed to haphazardly select 5 larvae (range: 1–10) from each host plant treatment within each matriline. Sometimes we were not able to sample 5 larvae if they were at the end of development and fewer than 5 individuals were still alive in the host plant treatment after having destructively sampled sibling larvae every previous day. Sometimes we sampled more than 5 larvae, especially in the earlier instars when getting an accurate count was challenging due to small size and thus, we preferred to over-sample than under-sample. The youngest larvae were collected hours after hatching (defined here as 0.5 days old) and the oldest larvae collected were 66 days old. In 2020 and 2021, we split egg masses onto multiple host plants so that the matriline and host plant were not confounded, but this meant that some matrilines with small egg masses were not represented later in development because the sample size per host was too low. In 2022, to increase sample sizes per host plant, we reared entire egg masses on a single host plant. Due to limitations in the numbers of eggs laid by individual females, not all matrilines could be collected every day from hatch through pupation (e.g., 5 larvae harvested each day × 48 days = 240 eggs, which is not consistently available as females can lay far fewer viable eggs). Thus, for some matrilines, most larvae were collected early in development (especially in 2020 and 2021) while for other matrilines we let larvae develop and selected them later so that all ages would be relatively well represented.

We measured the head capsule width of each larva with a digital micrometer (Electronic Digital Vernier Caliper, LOUISWARE, China), and recorded widths to the nearest 0.01 mm. Widths were measured as the distance between the most distant lateral sides of the head capsule as outlined by Delbac et al. (2010). When measuring head capsule widths, we positioned each larva dorsal side up under a dissecting microscope (M80 Stereomicroscope, Leica). We measured a total of 6,319 larval head capsules across 173 maternal lines (Supplementary Tables S1–S4).

Statistical Analyses

We modeled head capsule widths as Gaussian mixture models (GMMs), with a Gaussian distribution corresponding to each instar (Wu et al. 2013). For each diet, we estimated the number of Gaussian distributions, their parameters, and the mixing proportions using GMMs. For each diet, we fit GMMs for a range of possible numbers of instars. We used the R function emV (Scrucca et al. 2016) to estimate the parameters for each GMM. The inputs for the emV algorithm are the observed values, the number of Gaussian distributions (number of possible instars), the starting values for means and variances for each Gaussian distribution, and the proportions in which each instar is represented in the data. The fitted model is the GMM estimated by an estimation–maximization algorithm that finds the parameters maximizing likelihood based on the inputs. The fitted model depends strongly on the number of Gaussian distributions. After some exploratory analysis, we chose to fit models with 5–8 Gaussian distributions. Given the number of Gaussian distributions, the fitted GMM depends to some extent on the starting values for the means, variances, and proportions. To generate starting values under the assumption of n instars, we calculated n head capsule widths consistent with Brooks–Dyar spacing with the smallest value positioned at a small quantile q_1 of the observed head capsule widths and the largest value positioned at a large quantile q_n of the observed head capsule widths. Setting the ratio between successive values to $c = \sqrt[n-1]{\frac{q_n}{q_1}}$ gives the sequence of values q_1, cq_1, c^2q_1, … $c^{n-1}q_1 = q_n$ for the first estimate of the head capsule widths for each instar. We then performed 1 step of k-means clustering with these values as seeds for the cluster means. We used the means of the n resulting clusters as starting values for the means of the Gaussian distributions, the variances of the head capsule widths in each cluster as starting values for the variances of the Gaussian distributions, and the proportions in each cluster as starting values for the mixing proportions of the Gaussian distributions. We tuned the quantiles to the characteristics of the sample. For the apple host plant treatment, we used the 0.05 quantile for the smallest value and the 0.99 quantile for the largest value. For the remaining host plant treatments, we used the 0.05 and 0.95 quantiles.

We based our initial model selection on the biological premises that the Gaussian distributions corresponding to successive instars should have increasing head capsule widths and that the variance of the distributions should generally be increasing because of differential growth rates per larva. After this initial model selection, we then selected models with better consistency with Crosby's growth rule (Craig 1975) (see Supplementary Figure S1 for models). If multiple models had acceptable consistency with Crosby's growth rule, we selected models with low Akaike information criterion and Bayesian information criterion values. Next, we conducted several tests to address the plausibility of the selected models for each host plant as well as the sensitivity of the model parameters to a specific sample. First, to assess the plausibility of the selected GMM for each host plant, we drew 100 parametric bootstrap samples from the fitted GMM and compared the log likelihood of the observed data under the selected model to the log likelihood of each simulated data set. As a separate test, for each host plant, we drew 5 random samples of half the head capsule widths, fitted the selected model to each sample, and then applied the Kolmogorov–Smirnov test to the other half of the data that was not included in that random sample. To examine the sensitivity of the model parameters to the specific sample, we used non-parametric bootstrapping for each diet at each sampling time to generate 500 simulated data sets. When multiple matrilines were represented in a sampling time, we resampled the matrilines, and then measurements from those matrilines were sampled. We compared the GMM parameters from a range of models on the observed data to GMM parameters from that range of models fitted to the bootstrap samples.

To compare the fitted mean head capsule widths at each instar between samples from different host plants with 7 Gaussian models, we used pairwise comparisons. For each pair of host plants, we fit a GMM to the merged data of the observations from those 2 host plants and drew parametric bootstrap samples from that model for each of the 2 populations according to the proportions of each instar in the fitted model for that population. Then we fit GMMs to each of the 2 bootstrapped populations using the same methods applied to the observed populations. We compared the distance between the vectors of fitted mean head capsule widths for the observations to the set of distances between the vectors fitted to the bootstrapped population pairs. This gives a measure of whether the difference between the pair of models for the observed data is consistent with the difference between a pair of models based on samples from the pooled data.

Results

Our data were consistent with the GMM approach. The selected models satisfied the plausibility and sensitivity checks. In comparisons

of the log likelihood of the observations under the selected model to the log likelihood of parametric bootstrap samples under their fitted models, the observed log likelihood under the selected model was larger than at least 30% of the log likelihoods of samples from the Gaussian mixture under their fitted GMMs. In the Kolmogorov–Smirnov analysis, all the tests had *p*-values above 0.2. These checks alone do not produce definitive model selection because many of the rejected GMMs were similarly consistent with the data. The sensitivity of the fitted means for the Gaussian distributions was moderate. For example, for all hosts, the interquartile range of the bootstrapped fitted means of each instar was strictly above the bootstrapped interquartile range of the previous instar (Supplementary Figure S2). The 0.025 quantile of the bootstrapped fitted means of each instar was strictly above the 0.975 quantile of the bootstrapped fitted means of the previous instar except for the seventh instar for chokecherry and the sixth and seventh instars for narrowleaf cottonwood. The means estimated from the observed data are between the 0.15 and the 0.85 quantiles of the bootstrapped fitted means.

We found different numbers of instars for red-head FW larvae reared on different host plants (Figure 1, Table 1). For 5 of our host plant species (chokecherry, black willow, narrowleaf cottonwood, apple, and thinleaf alder), we selected models with 7 Gaussian distributions (instars) and for the remaining host plant (broadleaf cottonwood), we selected a model with 8 Gaussian distributions (instars). In the case of 1 host plant (alder), the model with 7 Gaussian distributions (instars) and the model with 8 Gaussians (instars) performed comparably, but the 7 Gaussian (instar) model was less sensitive to resampling and thus preferred (Figure 1, Supplementary Figure S2).

When we compared fitted mean head capsule widths at each instar between samples from different host plants with 7 Gaussian models, we found that most pairs did not differ significantly. For 2 pairs, black willow compared to alder and black willow compared to chokecherry, the observed mean head capsule widths at each instar differed more than would be typical if the observations were from the pooled data, which indicates that head capsule width differs significantly at the same instar between these host plant pairs. Specifically, larvae feeding on black willow had larger head capsule widths than larvae feeding on alder and chokecherry starting at the fourth instar with the largest differences found during the sixth instar (Table 1). For the black willow comparison with alder, the observed difference in mean head capsule widths by instar was larger than all 500 simulated comparisons. For the black willow comparison with chokecherry, the observed difference in mean head capsule widths by instar was greater than all but 24 of the 500 simulated comparisons, or a proportion of 5%. For the remaining pairs, the proportion of simulated comparisons that were less than the observed difference was at least 10%, indicating that the other pairwise comparisons across host plants did not show strong evidence of a difference in mean head capsule widths by instar.

Discussion

We found that the number of developmental stages (instars) depended on the larval host plant. For FW reared on 5 of the 6 host plants that we used in our study, we found that larvae undergo 7 instars before pupating. Notably, for 1 of our host plants, broadleaf cottonwood, larvae develop through 8 instars. Seven instars

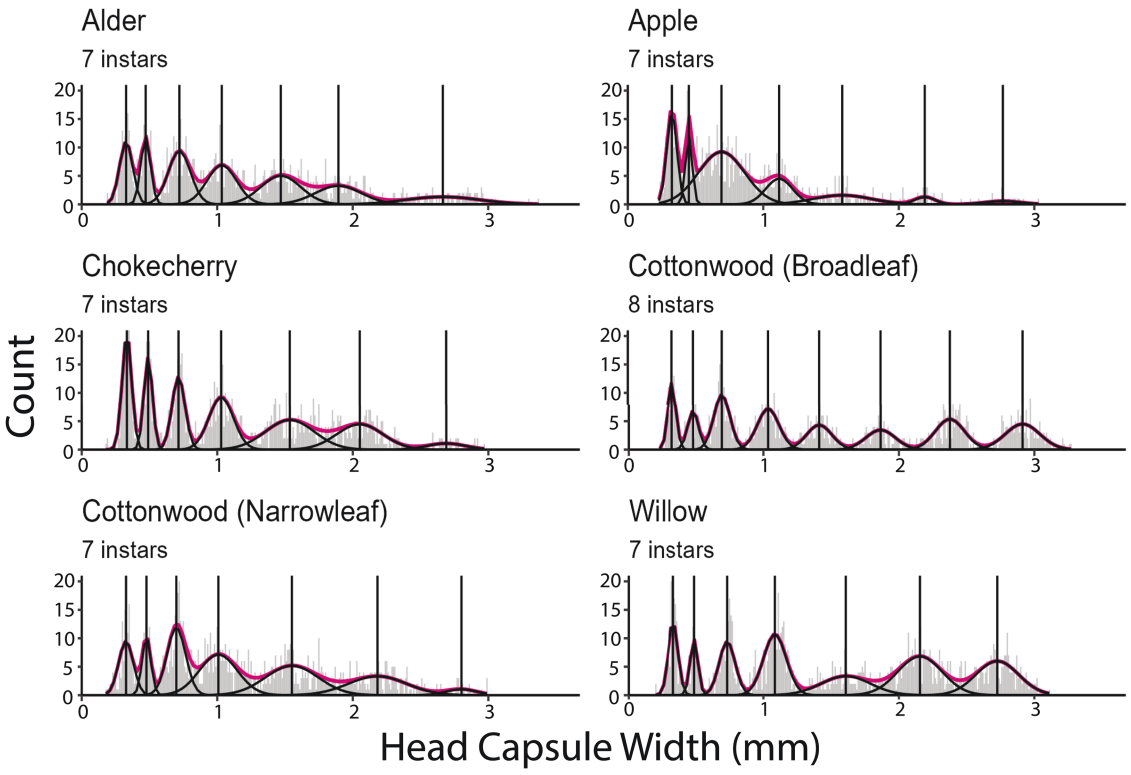

Fig. 1. Estimates for the number of red-head fall webworm (*Hyphantria cunea*) instars on 6 different host plants. For each host plant, we plot the frequency (count) of each head capsule width (in mm) with light gray bars representing the observed frequency distributions of head capsule widths for larvae on that host plant. Vertical lines represent the fitted mean head capsule width for each larval instar. The black curves show frequency predictions for each instar based on the GMM. Magenta (online color version, dark grey in print version) curves show the GMM frequency predictions for the instars collectively within a host plant species.

Table 1. Fitted values of red-head fall webworm (*Hyphantria cunea*) head capsule widths (measured in mm; mean ± SD) for each instar on 6 different host plants across 3 different plant families: Betulaceae: thinleaf alder (*Alnus tenuifolia*); Rosaceae: apple (*Malus domestica*) and chokecherry (*Prunus virginiana*); and Salicaceae: broadleaf cottonwood (*Populus deltoides*), narrowleaf cottonwood (*Populus angustifolia*), and black willow (*Salix nigra*). Importantly, the SD is the fitted standard deviation for the Gaussian representing the instar, not a measure of the precision with which the model estimates the mean

	Host Plant					
	Betulaceae	Rosaceae		Salicaceae		
Instar	Alder	Apple	Chokecherry	Cottonwood (Broadleaf)	Cottonwood (Narrowleaf)	Willow
1	0.33 ± 0.05	0.33 ± 0.04	0.33 ± 0.04	0.32 ± 0.03	0.33 ± 0.05	0.33 ± 0.04
2	0.47 ± 0.04	0.45 ± 0.02	0.49 ± 0.03	0.48 ± 0.04	0.48 ± 0.03	0.49 ± 0.03
3	0.72 ± 0.08	0.69 ± 0.17	0.71 ± 0.05	0.69 ± 0.06	0.70 ± 0.07	0.73 ± 0.06
4	1.03 ± 0.11	1.12 ± 0.10	1.03 ± 0.09	1.03 ± 0.08	1.01 ± 0.14	1.08 ± 0.08
5	1.48 ± 0.17	1.58 ± 0.21	1.54 ± 0.19	1.41 ± 0.09	1.55 ± 0.19	1.60 ± 0.17
6	1.92 ± 0.16	2.19 ± 0.08	2.06 ± 0.16	1.86 ± 0.11	2.18 ± 0.22	2.15 ± 0.17
7	2.66 ± 0.27	2.77 ± 0.13	2.69 ± 0.015	2.38 ± 0.11	2.80 ± 0.11	2.73 ± 0.16
8				2.91 ± 0.13		

is consistent with studies of black-head FW, which experience 6–7 instars (Itô and Miyashita 1968, Warren and Tádic 1970 , Morris and Fulton 1970 , Gomi et al. 2005), but it is interesting that diet can cause red-head FW to undergo one more instar than black-head FW do (black-head: 6–7 instars; red-head: 7–8 instars). There is only 1 study on the number of red-head FW instars to which we can compare our results; Swain (1938) suggested that red-head FW have 8–11 instars, but the sample size of this study was very small (n = 20 larvae from only 2 matrilines and 2 larvae died before pupation) and Swain did not examine instars on different diets. To our knowledge, ours is the first study to show the host-plant-dependent effects on instar number for red-head FW larvae.

Supernumerary instars result from several environmental and biological variables, one of which can be an insect's diet. For herbivores, plant host quality can affect the number of larval instars in Lepidoptera, with some species undergoing additional instars if they are faced with a poor-quality diet (Esperk et al. 2007a). For example, sometimes a plant with low nutrients or certain secondary defense chemicals can elicit a supernumerary instar in lepidopteran larvae (Lee et al. 2012, Abarca et al. 2020). However, our results contradict this pattern because our host plant on which FW larvae experienced an extra instar (broadleaf cottonwood, 8 instars) is generally a favorable host plant for FW larvae in Colorado. FW larvae that feed on broadleaf cottonwood have high rates of survival and pupal mass (Murphy, unpublished data) and broadleaf cottonwood has been commonly used by some FW populations (Murphy and Loewy 2015). It is unclear if any nutritional or chemical components of this host plant influenced the additional instar; we plan to study and compare the metabolomics of broadleaf cottonwood and the other host plants used in our research in the future.

Both sex and temperature have also been shown to affect the number of larval instars in Lepidoptera (Esperk et al. 2007a, 2007b), but these variables cannot explain our results. In Japanese populations of black-head FW, Gomi et al. (2005) found sexual dimorphism in the number of instars as females were more likely to have 7 instars compared to the 6 instars of males, regardless of their diet. An extra instar for females may provide a fitness advantage to females as larger size is correlated to higher fecundity (Esperk et al. 2007a). We could not test for differences between sexes because sex is only observable in the pupal stage and larvae used for our study were destructively sampled before pupation; however, given that this species typically has 1:1 sex ratios (Oliver 1963), it is unlikely that we sampled only female FW on broadleaf cottonwood. Moreover, we would expect that a difference in instar according to sex would be exhibited across diets, as found by Gomi et al. (2005), so this would not explain why larvae feeding on broadleaf cottonwood underwent an additional instar. Temperature can also affect supernumerary instars, and Gomi (2006) found that higher temperatures increase the likelihood of female FW having 7 instars. However, temperature cannot explain our broadleaf cottonwood results either because all larvae were reared under the same temperature across hosts for our experiments. Thus, for the variables that are most often thought to cause supernumerary instars in insects, host plant identity seems the most likely to be causing FW to add an additional instar, but further investigation is needed to understand this mechanism.

Head capsule widths for red-head FW differed across host plant families, but interestingly these effects only manifested during the fourth instar. During the first 3 instars, larvae on all host plants were about the same size in terms of mean head capsule width when they molted to the next instar (Table 1). Once larvae reached the fourth instar, however, differences in head capsule width across host plants were observable; larvae reared on black willow (Salicaceae) had greater mean head capsule widths compared to larvae reared on choke cherry (Rosacea) and alder (Betulaceae). The fourth instar may be the stage when maternally provisioned resources from the egg, which supported growth during the first 3 instars, begin to decline and subsequent larval growth rate becomes more dependent on the host plant. However, because we destructively sampled FW larvae for our experiment, we were unable to measure growth rate for each instar and thus it remains unknown how many days FW larvae spend within each instar and this should be investigated in the future.

We demonstrate that bottom-up effects of host plant can influence the development of an insect that feeds on wild plants. We conducted the first thorough investigation into how host plant identity affects developmental pattern and instar number of red-head FW. We found that the instar number depended on which host plant larvae were fed, with larvae reared on most plants having 7 instars and 1 host plant eliciting a supernumerary eighth instar. Research on insect instars is largely done to inform the management of pest insects of major crop systems (Coombs et al. 1997, Castañeda-Vildózola et al. 2016, McClellan and Logan 1994, Godin et al. 2002, Irigaray et al. 2006, Calvo and Molina 2008, Delbac et al. 2010, Chen and Seybold 2013, Mo et al. 2013, Kekeunou et al. 2018). FW are a pest

species in Europe and Asia where this species has been introduced, but in North America where we study them, they are not often a pest species. Our research is important because FW is becoming an ideal system for ecological and evolutionary research on herbivore diet breadth and we need a better understanding of how developmental patterns vary between their morphotypes in order to ask key research questions. For example, knowing how many instars red-head FW have allows us to study life history differences between red-head FW, which has a narrower diet breadth, and the better-studied black-head FW, which has a broader diet breadth. Furthermore, as we examine the effect of herbivore diets on insect development, we are better able to inform the applications that rely on comprehending the ecology of plant–insect interactions.

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Author contributions

Mykaela Tanino-Springsteen (Data curation [lead], Funding acquisition [equal], Investigation [lead], Methodology [equal], Supervision [equal], Visualization [equal], Writing—original draft [lead], Writing—review & editing [equal]), Dhaval Vyas (Conceptualization [equal], Funding acquisition [equal], Investigation [supporting], Methodology [lead], Visualization [equal], Writing—original draft [supporting], Writing—review & editing [equal]), Audrey Mitchell (Conceptualization [supporting], Funding acquisition [equal], Investigation [supporting], Methodology [supporting], Writing—review & editing [supporting]), Catherine Durso (Data curation [equal], Formal analysis [lead], Methodology [equal], Validation [equal], Writing—original draft [supporting]), and Shannon Murphy (Conceptualization [equal], Funding acquisition [lead], Methodology [equal], Project administration [equal], Resources [equal], Supervision [equal], Visualization [equal], Writing—original draft [supporting], Writing—review & editing [equal])

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Data availability

Data are archived from the Zenodo repository: 10.5281/zenodo.10278727

Supplementary material

Supplementary material is available at *Environmental Entomology* online.

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