

Diversification of gut morphology in caterpillars is associated with defensive behavior

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Summary

The relationship between insect gut structure and foraging strategy has been studied for several hundred years; however, we know little about how, or even if, other common insect behaviors are linked to gut morphology. For example, many insects defend themselves by regurgitation, a behavior which is expected to be closely connected to gut structure. Caterpillars belong to an insect taxon, the Lepidoptera, with a particularly well-studied digestive tract and a known predilection for defensive regurgitation. I have explored whether defensive regurgitation is associated with specific gut structure by examining the relationship between defensive behavior and gut morphology in the larvae of 36 butterfly and moth species. My analysis shows that predilection to defensively regurgitate is closely associated with fore- and midgut

morphology. Species that primarily regurgitate in defense have enlarged foreguts and shortened midguts, whereas the opposite relationship is seen among those that do not readily regurgitate. These results reveal that: (i) defensive regurgitation is not the primary defense of all caterpillars, and (ii) gut morphology can be associated with other factors than the chemical and mechanical properties of ingested foods. These findings challenge long held beliefs concerning the ubiquity of defensive regurgitation in caterpillars and the causes of diversification of gut morphology, and further reinforce the gut's standing as a complex and sophisticated organ.

Key words: Lepidoptera, digestive tract, anatomy, anti-predator, larva.

Introduction

Nutritional ecology is a unifying concept that links diverse aspects of animal life history such as foraging strategy, habitat selection, defense, and reproduction (Scriber and Slansky, 1981). Foraging strategy itself is the primary driver of evolutionary diversification seen in vertebrate (Van Soest, 1996) and invertebrate (Dow, 1986) digestive tracts. Insect gut morphology is exceptionally diverse because of the multitude of food sources they have exploited over evolutionary time (Dow, 1986). For example, the simple, tubular guts of caterpillars typify specialization for rapid passage and processing of solid plant foods (Dow, 1986). This dietary specialization is thus thought to have resulted in a lack of gut structure diversity among caterpillars (Dow, 1986). However, evidence supporting this hypothesis is lacking.

Modification of insect gut morphology and function is driven by two important dietary dichotomies: (1) solid/liquid and (2) animal/plant feeding strategies (Dow, 1986). These two dichotomies result in four classes of insect feeding habits that may be used to categorize insect gut structure (Dow, 1986). In insects, the gut is essentially a cylinder that connects mouth and

anus. It consists of three physiologically distinct sections (foregut, midgut and hindgut) that become morphologically modified in response to environmental factors (Dow, 1986). The foregut is further modified into three sections: the esophagus, crop and proventriculus, and the crop's major function is generally considered to be food storage (Dow, 1986).

Larvae of butterflies and moths are the classic example of solid-plant feeders (Dow, 1986). Their guts evolved to rapidly process large quantities of abrasive plant material, which is thought to have resulted in simple, tubular guts with vestigial foreguts and expansive midguts (Dow, 1986). Given the apparent dietary homogeneity of this taxon (Dow, 1986), it is surprising that little consideration has been afforded to the potential effects of other behaviors on gut morphology. For example, defensive regurgitation is a common defensive ploy that effectively deters invertebrate attacks (Freitas and Oliveira, 1992; Gentry and Dyer, 2002; Smedley et al., 1990), and is a tactic widely used by insects, especially the vulnerable larvae of moths and butterflies (Fig. 1) (Bowers, 1993). However, the extent to which this behavior is used as a primary defensive response among lepidopteran larvae is

unknown (Peterson et al., 1987), and the degree to which it is associated with variation in gut morphology has not been explored.

The effectiveness of regurgitation as a primary defensive response relies on the volume of regurgitant that a caterpillar is able to produce, the accuracy with which it is able to direct it, and its ability to re-imbibe expelled fluid (Brower, 1984). If defense is associated with morphology then caterpillars that primarily rely on regurgitation for protection would be expected to have a gut morphology that both reflects these abilities, and differs from that of larvae that rely on other types of defenses such as spines, glands or hair. I have tested for a relationship between the behavioral strategy of defensive regurgitation and specific gut morphology. This defense hypothesis expressly predicts that primary-regurgitators will possess large crops in which to stockpile defensive regurgitant, whereas secondary- and non-regurgitators will have smaller crops as they turn to other weaponry and their reliance on regurgitation decreases.

Materials and methods

Caterpillar identification

Twenty-nine species of caterpillars from 16 families were opportunistically field-collected in Tompkins County, NY, USA throughout the months of April to August, 2004. Mature (fourth through sixth instars) caterpillars were identified to the species level with field guides (Wagner et al., 2001a; Wagner et al., 2001b) and professional taxonomic advice at Cornell University (Table A1). Four species (*Utetheisa ornatix*, *Limenitis arthemis astyanax*, *Papilio machaon aliaska*, *Callosamia promethea*) were donated from laboratory-raised populations of known species. *Utetheisa ornatix* was fed an artificial diet consisting of pinto beans mixed with its natural diet, the seeds of *Crotalaria spectabilis* (Iyengar et al., 2002). Regurgitation behavior was not expected to be affected by this laboratory diet as it was similar to the natural diet of *U. ornatix*. *Limenitis arthemis astyanax*, *Papilio machaon aliaska* and *Callosamia promethea* were fed branches or sprigs of their natural host plants.

Behavior

Regurgitation behavior was determined for three replicate mature larvae (instars IV, V or VI) of 33 species with a pinch assay that simulated predation. Only mature larvae were used because ontogenetic changes in defensive behavior often occur at the cutoff between third and fourth instars (Cornell et al., 1987). Specifically, a pinch delivered with No. 5 forceps simulated the mandibular bite of an ant (Ayre and Hitchon, 1968; Eisner et al., 1972). Caterpillars were fasted for 6 h prior to the pinch assay to control for meal size on regurgitation response. As the average meal moves through a caterpillar's gut in 2.5 h (Santos et al., 1983), a 6 h fast was sufficient to ensure that gut content was equalized among test subjects. The threshold between primary- and secondary-regurgitators was selected as 2.5 pinches because it was approximately one

standard deviation (s.d.) less than the mean number of pinches required to elicit regurgitation (mean \pm s.d.: 5.5 ± 3.3) and below the lower 99% confidence interval (CI) of the mean (lower CI=3.9 pinches). This threshold was associated with discrete defensive behaviors that allowed further distinction between the two categories, the most prominent being that primary-regurgitators re-imbibed expelled fluid and directed regurgitant with accuracy whereas secondary-regurgitators did not. Eight pinches were chosen as the cut-off for non-regurgitators because it was approximately one standard deviation above the mean and also above the upper 99% confidence interval of the mean (upper CI=7.0 pinches). If a caterpillar did not respond by ten pinches it was classified as a non-regurgitator and no more pinches were delivered. Generally, if a caterpillar did not respond by seven or eight pinches, more pinches did not elicit regurgitation. This threshold was also associated with marked defensive behaviors that allowed further distinction between the two categories, the most prominent being that non-regurgitators could seldom be induced to regurgitate whereas secondary-regurgitators would defensively ooze regurgitant from their oral cavities. These thresholds were further validated with a hierarchical clustering analysis of the number of pinches necessary to elicit regurgitation. Quantitative measurement of regurgitant volume was precluded by other defensive behaviors of caterpillars, such as thrashing and wriggling to escape.

A lack of well-resolved phylogenies at many lower taxonomic levels among the Lepidoptera prevents phylogenetically controlled comparative analyses (Costa and Pierce, 1998). However, after applying the Runs Test as implemented in Phylogenetic Independence 2.0 software (Reeve and Abouheif, 2003), regurgitation behavior was not found to be phylogenetically autocorrelated (1000 iterations, Cstat=17.48, $P=0.004$). Thus, from an evolutionary point of view, these 33 species represented 33 independent evolutionary events and could be analyzed by standard multi- and univariate analysis of variance (MANOVA and ANOVA).

Morphology

Gut morphology was measured in three replicate mature larvae (instars IV, V or VI) of 33 species. Caterpillars were fasted for 6 h prior to dissection and measurement in order to control for the effect of meal size on crop expansion and overall gut length. As the average meal moves through a caterpillar's gut in 2.5 h (Santos et al., 1983), a 6 h fast was sufficient to ensure gut vacancy. Caterpillars were killed by freezing at -4°C for 30 min then pinned into a glass-bottomed magnetic dissecting tray filled with Ringer's solution. The cuticle was cut from anal plate to head capsule along the dorsal axis and pinned to the sides to allow for measurement of the gut and its components. Caterpillars were stretched to their fullest extent to control for differences in gut elasticity. Because caterpillar guts are tubular and not coiled, stretching them in this fashion allowed for

measurement of maximum gut length. Total gut length and gut component lengths were measured with calipers to the nearest 0.1 mm under a 40 \times dissecting microscope. Caterpillars found to contain parasitic wasp or fly larvae were excluded from analysis because of potential behavioral modification resulting from infestation.

To test the association between gut structure and defensive behavior the relationship between relative gut compartment length (crop, midgut and hindgut) and defensive behavior was evaluated using the 33 species behaviorally classified as primary-, secondary- and non-regurgitators by the previously described pinch assay. To control for the effects of body size on gut component length, the proportion of each gut section length relative to total gut length was used in all dissected caterpillars for all statistical analyses.

Statistical analyses

Midgut data were not transformed because they met the assumptions of normality (Shapiro–Wilk W -test: midguts, $W=0.984$, $P=0.90$) and equality of variances (Levene's test: $F_{2,30}=2.394$, $P=0.11$). The raw data for crop and hindgut proportions did not meet the assumptions of normality (Shapiro–Wilk W -test: crops, $W=0.929$, $P=0.03$; hindguts, $W=0.927$, $P=0.03$) and were square root transformed to achieve a more normal distribution (Shapiro–Wilk W -test: square root (crops), $W=0.950$, $P=0.14$; square root (hindguts), $W=0.946$, $P=0.10$). After transformation, these data also met the assumption of equality of variances (Levene's test: crops, $F_{2,30}=0.0728$, $P=0.93$; hindguts, $F_{2,30}=2.040$, $P=0.15$). Comparisons of gut proportions among primary-, secondary- and non-regurgitators were made using MANOVA followed by univariate ANOVA. Because species to species variation was being tested, the experimental unit was reduced (i.e. a single response per species was analyzed instead of statistical replicated measures for each species) by calculating the mean response for each species (Sall et al., 2005). Statistical analyses were then performed on these mean responses. All statistical analyses were performed with JMP[®] 5.1 software.

Results

Caterpillars varied dramatically in their use of defensive regurgitation: some species regurgitated after only a single pinch, whereas others could not be induced to regurgitate regardless of how many times simulated attack occurred (Fig. 1). On average, caterpillars regurgitated after 5.5 pinches (± 3.3 s.d.), and this value ranged from 1 to 10 pinches. Of the 33 species examined, 27.3% (9) were classified as primary-regurgitators (1–2 pinches), 30.3% (10) as secondary-regurgitators (3–6 pinches) and 42.4% (14) as non-regurgitators (8–10 pinches).

Regurgitation defensive strategy clearly differed among the three types of caterpillars. Those in which the initial defense response was regurgitation (primary-regurgitators) behaved very differently than secondary-regurgitators, as measured by

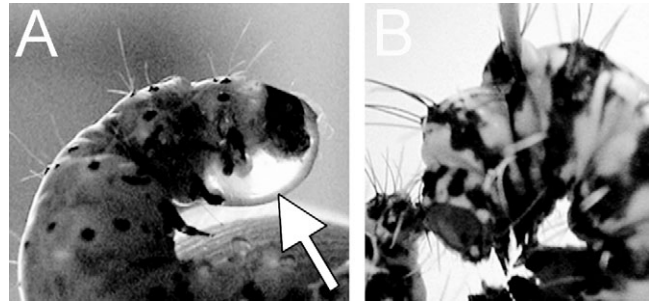


Fig. 1. Defensive regurgitation of two caterpillars in response to simulated predation. (A) *Saucrobotys futilalis*, a primary-regurgitator, responds with a copious amount of regurgitant that it directs towards the simulated predator. The arrow marks a droplet of regurgitant. (B) *Utetheisa ornatrix*, a non-regurgitator, does not produce regurgitant when attacked.

both the number of pinches required to elicit the response and the dynamics of the response itself. The initial defensive response of primary-regurgitators was regurgitation directed at the offending forceps, followed by recovery of regurgitant. Regurgitant recovery is expected from caterpillars that regurgitate frequently because of the costs associated with losing gut content nutrients expelled with regurgitant. Primary-regurgitators controlled how regurgitant was discharged and were often noted to produce regurgitant droplets of varying size in response to weaker or stronger pinches. Neither of these behaviors was noted in secondary-regurgitators, in which regurgitation was used secondarily after primary defenses such as flailing, biting or escape attempts failed to deter the simulated predator. Secondary-regurgitators did not produce a distinct droplet, but oozed regurgitant in a non-directed fashion that often resulted in as much regurgitant on the cuticle and substrate as on the forceps. Consistent with a lack of reliance on regurgitation as the primary defensive response, these caterpillars also failed to re-imbibe regurgitant after attack.

The occasional production of regurgitant by non-regurgitators seemed to be more a stress response than an antipredator response. Although not quantified, regurgitant volume appeared to be much less in these animals than in primary- or secondary-regurgitators, and non-regurgitator responses that resulted in regurgitation were often due more to exhaustion than defense. Therefore, it was clear that regurgitation was a primary or secondary defensive tactic in primary- and secondary-regurgitators, but not used as a defense in non-regurgitators. Primary-regurgitators responded by the second or third pinch, secondary-regurgitators responded after four to six pinches, and non-regurgitators required at least eight pinches to elicit regurgitation or did not regurgitate at all (Fig. 2).

A multivariate analysis revealed well-defined differences among proportions of the three major gut structures in primary-, secondary- and non-regurgitating species (Fig. 3A–C MANOVA: Wilk's Lambda $N=33$, approximate $F_{4,58}=19.5653$, $P<0.0001$). These differences are localized in

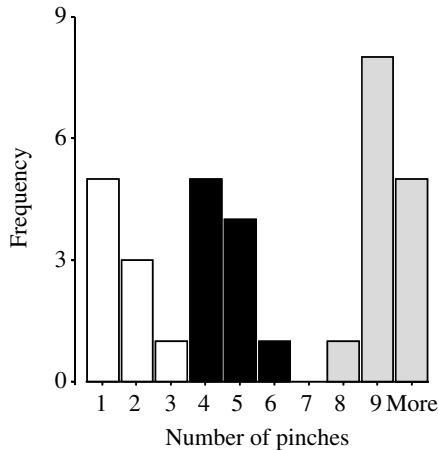


Fig. 2. Numbers of pinches required to elicit regurgitation in the caterpillars of 33 species of moths and butterflies. Those that deploy regurgitation as their primary defense (primary-regurgitators) are shown in white, as their secondary defense (secondary-regurgitators) in black, and those that do not use regurgitation as a defense (non-regurgitators) are in gray.

the crop and midgut among the different types of regurgitators (Fig. 3A–C univariate analysis of variance (ANOVA), $N=33$: crops, $F_{2,30}=62.0312$, $r^2=0.80$, $P<0.0001$; midguts, $F_{2,30}=18.7012$, $r^2=0.55$, $P<0.0001$; hindguts, $F_{2,30}=0.6922$, $r^2=0.04$, $P=0.51$). Propensity to regurgitate significantly predicted crop proportions, with the crops of primary-regurgitators [mean \pm standard error (s.e.m.)= 0.34 ± 0.02] consisting of a greater proportion of the total gut than crops of secondary-regurgitators (mean \pm s.e.m.= 0.21 ± 0.02), which in turn were greater than those of non-regurgitators (mean \pm s.e.m.= 0.09 ± 0.01 ; Fig. 3A, Tukey–Kramer HSD test: primary–secondary, $P<0.0001$; primary–non, $P<0.0001$; secondary–non, $P<0.0001$; global mean \pm s.e.m.: 0.20 ± 0.02).

Midgut proportions were smaller in primary-regurgitators (mean \pm s.e.m.= 0.50 ± 0.03) than in secondary- (mean \pm s.e.m.= 0.65 ± 0.02) or non-regurgitators (mean \pm s.e.m.= 0.73 ± 0.03 ; Fig. 3B, Tukey–Kramer HSD test: primary–secondary: $P<0.001$; primary–non: $P<0.0001$; global mean \pm s.e.m.: 0.64 ± 0.02), and showed a similar, but non-significant trend between secondary- and non-regurgitators (Fig. 3B, Tukey–Kramer HSD test, $P=0.08$), indicating that digestive capacity may be constrained in regurgitating caterpillars.

Discussion

Reviews of arthropod defenses suggest that regurgitation is a common defense in many insects, including caterpillars (Blum, 1981; Blum, 1992; Eisner, 1970; Eisner and Meinwald, 1966). However, different assemblages of defenses are important for caterpillar survival (Dyer, 1997) and, as shown here, regurgitation is not a ubiquitous primary defensive response among caterpillars. Caterpillars utilize a number of defensive strategies to ward off several types of enemies

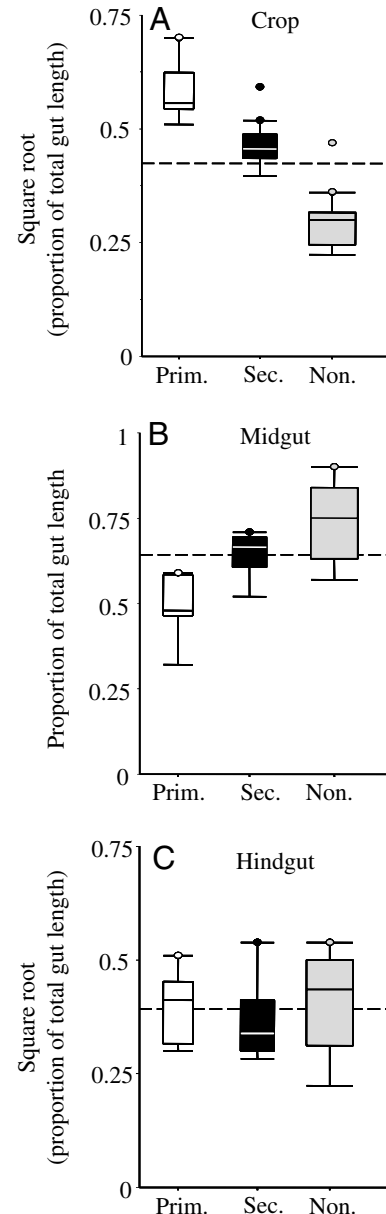


Fig. 3. Crop, midgut and hindgut proportions of total gut length in regurgitating caterpillars ($N=33$). Prim., primary-regurgitators ($N=9$); Sec., secondary-regurgitators ($N=10$); Non., non-regurgitators ($N=14$). Box plots show medians, 25th and 75th percentiles as well as outlying data points. (A) Crop proportions vary significantly with regurgitation behavior. Broken line indicates global mean of square root (crop proportions). (B) Midgut proportions vary significantly with regurgitation behavior. Broken line indicates global mean of untransformed midgut proportions. (C) Hindgut proportions were similar regardless of regurgitation behavior. Broken line indicates global mean of square root (hindgut proportions).

(Gentry and Dyer, 2002), so it is not surprising that defensive regurgitation is used to varying extents by different species. What was unexpected was the relationship between gut morphology and reliance on regurgitation as a defense. Larvae of butterflies and moths for which the primary defensive

response is regurgitation have distinct gut morphology relative to larvae for which regurgitation is a secondary defensive response or minimally used as a defense. Previous research on the relationship between lepidopteran gut structure and function logically focused on the gut's role in digestion, but overlooked defensive functions such as regurgitation (Dow, 1986). From a digestive physiology stance caterpillar guts are expected to be quite similar as a result of their similar foraging strategies (Dow, 1986). At a coarse, taxon-wide scale this proves true, and when broadly compared to other insects it is reasonable to say that caterpillars possess fairly limited foreguts, large midguts and small hindguts. However, as shown here, defensive regurgitation clearly is part of caterpillar gut function and strongly correlated with gut morphology. Future research in the area of caterpillar gut function would profit by incorporating both foraging and defense perspectives.

Primary-regurgitators possess larger crops and smaller midguts than secondary- and non-regurgitators, results that indicate that regurgitation may be costly in terms of loss of midgut function. Insect digestion and nutrient absorption take place almost exclusively in the midgut (Terra et al., 1996); therefore a decrease in midgut capacity may result in reduced digestive function relative to similarly-sized species which have larger midguts. Further research is necessary to determine if digestive efficiency is diminished in primary-regurgitators compared to secondary- and non-regurgitators. Previous research demonstrated another cost of regurgitation in terms of lost nutrition that resulted in decreased growth rates (Bowers, 2003). Primary-regurgitators appear to attempt to compensate for this loss by re-imbibing regurgitant when possible (this study). Secondary- and non-regurgitators do not exhibit this behavior, which may indicate that they are not adapted to regurgitation as a defense as well as are primary-regurgitators. Nutritional losses may also be minimized in primary-regurgitators because of their ability to control droplet volume and direct regurgitant with high accuracy (this study). These abilities were not observed in secondary- or non-regurgitators, which may make them more vulnerable to nutritional losses when they are forced to regurgitate. However, regurgitation remains an important defense in many caterpillars and protects them against parasitic wasps (Gentry and Dyer, 2002), ants (Cornelius and Bernays, 1995; Eisner et al., 1972; Freitas and Oliveira, 1992; Peterson et al., 1987; Smedley et al., 1993) and spiders (Theodoratus and Bowers, 1999). Regurgitation is an especially effective defense when it is combined with sequestered compounds from the larval host plant (Gentry and Dyer, 2002). Clearly, there is an important interplay between the costs and benefits of defensive regurgitation in caterpillars.

Taken together, these results suggest that, for caterpillars, defensive regurgitation strategy is strongly associated with gut morphology. Caterpillars that regurgitate as their primary defense have much larger crops than caterpillars that utilize other defenses. The short foregut of caterpillars is characteristic of a continuously feeding animal (Dow, 1986) and inconsistent with its sole function being one of food

storage. Caterpillars appear to have modified their vestigial crops into defensive weaponry that takes advantage of the allelochemicals inherent in their plant-based diets. Regurgitation and even defecation are used defensively by many other insect taxa (Eisner and Meinwald, 1966), as well as some birds (Clarke, 1977). Thus, gut modification to enhance defensive regurgitation ability is potentially a general phenomenon among the Lepidoptera and may apply more broadly to the Insecta as well as some vertebrate groups. Furthermore, the feeding of conspecifics and nonconspecifics by regurgitation (often termed trophallaxis in invertebrates) is a common behavior in many vertebrate and invertebrate taxa (Cammaerts, 1996; Cassill and Tschinkel, 1995; Janes, 1997; Mech et al., 1999; Pal, 2005; Rauter and Moore, 2002; Salomon et al., 2005; Schneider, 2002; Suarez and Thorne, 2000); a comparative examination of association of this type of regurgitation and gut morphology may be profitable. However, trophallaxis has not yet been reported in caterpillars of any species, perhaps because of a lack of parental care or kin selection, which appear to be key components in the evolution of trophallaxis in other invertebrate taxa such as termites, ants and honey bees (Anduaga and Huerta, 2001; Brandmayr, 1992; Sleight, 2002). Furthermore, although some species of caterpillars do live in social groups that consist of genetically related individuals (Costa and Ross, 1993; Grant, 2005; Porter et al., 1997), behavioral mechanisms of kin bias such as trophallaxis have yet to be shown to affect colony structure (Costa, 1998; Costa and Ross, 1993; Costa and Ross, 1994).

Previously, physiologists contended that gut morphology was solely a function of the influence of diet (Dow, 1986). However, this traditional approach to the organization of digestive processes in insects has some limitations, despite its general utility as a classification system (Terra et al., 1996). A full understanding of gut diversification will be enhanced by the incorporation of multiple perspectives, including behavioral ecology and phylogeny, in addition to the traditional approach of research on diet and digestive processes. The interplay between optimal defensive and foraging strategies may be an important, but generally overlooked area of predator-prey interactions.

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Appendix

Table A1. Caterpillar families, species, sample size, regurgitation prowess and mean crop and midgut proportions of total gut length

Number	Family	Species	N	Regurgitation behavior	Mean crop proportions	Mean midgut proportions
1	Tortricidae	<i>Archips cervasivorana</i> (Fitch)	3	Primary	0.31	0.59
2	Saturniidae	<i>Automeris io</i> (Fabricius)	3	Non	0.09	0.68
3	Saturniidae	<i>Callosamia promethea</i> (Drury)*	3	Secondary	0.19	0.52
4	Tortricidae	<i>Choristoneura rosaceana</i> (Harris)	3	Secondary	0.35	0.58
5	Arctiidae	<i>Cycnia tenera</i> (Hübner)	3	Non	0.06	0.75
6	Danaidae	<i>Danaus plexippus</i> (Linnaeus)	3	Primary	0.28	0.58
7	Limacodidae	<i>Euclea delphinii</i> (Boisduval)	3	Non	0.07	0.86
8	Gelechiidae	<i>Gelechia albisparsella</i> (Chambers)	3	Primary	0.28	0.50
9	Arctiidae	<i>Halysidota harrisii</i> (Walsh)	3	Non	0.09	0.62
10	Arctiidae	<i>Halysidota tessellaris</i> (Smith)	3	Non	0.09	0.67
11	Arctiidae	<i>Hyphantria cunea</i> (Drury)	3	Primary	0.49	0.32
12	Nymphalidae	<i>Limenitis arthemis astyanax</i> (Fabricius)*	3	Non	0.12	0.58
13	Arctiidae	<i>Lophocampa caryae</i> (Harris)	3	Secondary	0.20	0.69
14	Lymantriidae	<i>Lymantria dispar</i> (Linnaeus)	3	Non	0.21	0.57
15	Lasiocampidae	<i>Malacosoma americanum</i> (Fabricius)	3	Secondary	0.21	0.68
16	Lasiocampidae	<i>Malacosoma disstria</i> (Hübner)	3	Secondary	0.22	0.64
17	Geometridae	<i>Melanolophia canadaria</i> (Guenée)	3	Secondary	0.26	0.62
18	Noctuidae	<i>Morrisonia confusa</i> (Hübner)	3	Primary	0.26	0.48
19	Lymantriidae	<i>Orygia leucostigma</i> (Fitch)	3	Non	0.09	0.64
20	Papilionidae	<i>Papilio machaon aliaska</i> (Scudder)*	3	Secondary	0.16	0.68
21	Geometridae	<i>Phigalia titea</i> (Cramer)	3	Secondary	0.15	0.65
22	Pieridae	<i>Pieris rapae</i> (Linnaeus)	3	Secondary	0.19	0.72
23	Nymphalidae	<i>Polygonia interrogationis</i> (Fabricius)	3	Non	0.06	0.84
24	Noctuidae	<i>Psychomorpha epimenis</i> (Drury)	3	Non	0.07	0.74
25	Arctiidae	<i>Pyrrharctia isabella</i> (Smith)	3	Secondary	0.20	0.71
26	Pyalidae	<i>Saucrobotys futilalis</i> (Lederer)	3	Primary	0.31	0.59
27	Tortricidae	<i>Sparganothis pettitana</i> (Robinson)	3	Primary	0.35	0.45
28	Unknown	<i>Unknown 1</i>	3	Non	0.05	0.90
29	Unknown	<i>Unknown 2</i>	3	Primary	0.33	0.50
30	Unknown	<i>Unknown 3</i>	3	Primary	0.31	0.47
31	Arctiidae	<i>Utetheisa ornatrix</i> (Linnaeus)*	3	Non	0.10	0.75
32	Nymphalidae	<i>Vanessa cardui</i> (Linnaeus)	3	Non	0.07	0.84
33	Yponomeutidae	<i>Yponomeuta cagnagella</i> (Hübner)	3	Non	0.09	0.81

*Obtained from a laboratory population.

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