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HOST USE AND GEOGRAPHIC VARIATION IN FALL WEBWORMS (*HYPHANTRIA CUNEA*)

A Thesis

Presented to

the Faculty of Natural Sciences and Mathematics

University of Denver

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Katrina J. Loewy

June 2013

Advisor: Shannon M. Murphy

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Title: HOST USE AND GEOGRAPHIC VARIATION IN FALL WEBWORMS (*HYPHANTRIA CUNEA*)
Advisor: Shannon M. Murphy
Degree Date: June 2013

Abstract

Host use in herbivores is determined by a variety of ecological drivers, including bottom-up and top-down selective pressures such as host abundance, host plant quality, and parasitism pressure. If the relative importance and strength of interactions among these selective conditions change over an herbivore's geographic range, local patterns of host use should change in response, evident in differing diet breadths. The fall webworm (*Hyphantria cunea*) is a widespread, polyphagous moth with two color morphs, red and black-headed. In the eastern United States, fall webworms feed on dozens of plant species and previous research demonstrated that host plant abundance was the only significant predictor of host plant use. Populations of fall webworm in Colorado are found on considerably fewer host plant species than populations farther east. We investigated the impacts of host abundance, larval performance, and parasitism on patterns of host use for fall webworm in Colorado to determine whether differences in selective pressures may explain why these populations are relatively more specialized compared to previously-studied populations. Additionally, we used DNA sequences from fall webworms collected across their geographic range to investigate genetic variation via phylogenetic tree building and AMOVAs. Using those genetic techniques, we found that red-headed and black-headed fall webworms are not reproductively isolated, but there are two genetic groups: one that is exclusively black-headed and one that is both red and black-headed. Similar to studies on eastern populations, we found that host abundance was a significant predictor of host use. We also found a trade-off between host quality, as measured by larval performance, and percent parasitism. Host plants that supported larvae with higher fitness, as measured by survival, pupal weight, feeding efficiency, and development time, also had a greater proportion of larval mortality due to parasitism. Local patterns of host plant abundance may lead fall webworms to a relatively restricted diet in Colorado compared to the east coast, while the trade-off between quality and parasitism may explain the maintenance of a generalized feeding strategy.

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CHAPTER ONE: LIFE HISTORY TRAITS AND REARING TECHNIQUES FOR FALL WEBWORMS (*HYPHANTRIA CUNEA* DRURY) IN COLORADO

Introduction

The fall webworm (*Hyphantria cunea* Drury) is a moth species native to North America and an invasive pest in Europe and Asia (Tadić 1963, Yang *et al.* 2008). The gregarious larvae spin extensive webs for protection (Ito 1977) and thermoregulation (Morris and Fulton 1970, Rehnberg 2002, 2006), usually on the outer branches of deciduous trees. *Hyphantria cunea* are noted generalists and have been recorded feeding on dozens of plant families worldwide (Warren and Tadic 1970). Their preferred hosts are deciduous, woody plants, but larvae have been observed on herbaceous plants like *Clematis* (Swain 1936) and even gymnosperms (Oliver 1964).

The taxonomic status of *H. cunea* is unclear. There are at least two genetically distinct 'races' or forms of *H. cunea* in North America (Jaenike and Selander 1980) capable of interbreeding to produce fertile offspring (Oliver 1964, Masaki and Ito 1977). Morphologically, the only distinguishing feature is larval coloration: a black-headed form and a red or orange-headed form. The two larval forms are also behaviorally distinct as fifth instar larvae; black-headed *H. cunea* leave the web during the ultimate instar and become solitary (Szalay-Marzso 1972), whereas red-headed *H. cunea* are reported to cluster within the communal web until pupation (Oliver 1964). Masaki and Ito (1977) noted a third form with a mottled head native to North America. Because red and mottled-headed larvae create a much thicker web that spreads to the crook of the host tree, similar to the behavior of tent caterpillars, they are sometimes referred to collectively as *Malacosoma*-type *H. cunea* (Masaki and Ito 1977). We do not distinguish between red and mottled-headed *H. cunea*, both of which may exist in Colorado, and refer to the *H. cunea* form that we study as red-headed. Within North America, the black-headed form is primarily in the east and northeast, whereas the red-headed form is mostly in the south and west, with large areas of sympatry (Masaki 1977). Voltinism is graded geographically; *H. cunea* populations in the northeastern United States and Canada are univoltine, and populations in the Gulf States and Mexico have been observed with four or more generations per year (Masaki 1977). Where red and black-headed forms co-occur in the mid-Atlantic, they are phenologically distinct, with a univoltine red-headed generation emerging between two black-headed generations (Masaki 1977). Host selection also differs between the two forms, however, there is some overlap (Oliver 1964).

Several researchers have published rearing techniques for *H. cunea* on both natural host plants and artificial media (Jasič and Macko 1961, Yearian et al. 1966, Morris and Fulton 1970, Lorimer and Bauer 1983) as well as studies of larval fitness on natural host plants (Jasič and Macko 1961, Morris and Fulton 1970, Greenblatt 1978, Gomi *et al.* 2005, Mason *et al.* 2011). However, all of these studies have focused on the black-headed form, which is the only form present in Europe and Asia, and there are very few studies on the red-headed form (but see Oliver 1964, Masaki and Ito 1977). For this paper we had three objectives. Our first objective was to provide the first published account of successful rearing techniques for red-headed *H. cunea*. Our second objective was to measure and report life history traits of *H. cunea* in Colorado and to compare them with other published accounts. Our third objective was to test whether female pupal mass predicts fecundity, and thus lifetime fitness, for *H. cunea* as well as other lepidopteran species.

Materials and Methods

Objective 1 – Rearing Techniques for Red-headed *H. cunea*

Larval Collection

The red-headed form of *H. cunea* is widespread in North America; in Colorado, researchers and hobbyists have recorded the moths in 15 counties (Ferguson *et al.* 2000). We collected wild larvae from multiple field sites near the cities of Boulder (Boulder County, 40.090013, -105.359962), Fort Collins (Larimer County, 40.5852602, -105.084423), and Idledale (Jefferson County, 39.746944, -105.210833) in Colorado (Fig. 1). The conspicuous webs were visible along roads and waterways, and we most commonly found webs in canyons dominated by deciduous woody plants and on the plains abutting the foothills. We collected larval *H. cunea* from July 21 through September 21 of 2010, after which webs that we searched were empty. Webs were distributed at heights greater than one meter from the ground on the outer branches of woody shrubs and trees. When webs were out of reach of hand clippers, we used an extendable tree pruner (4.9m Jameson poles, Marvin pruner head, Sherrill Tree, Greensboro, North Carolina) to remove the inhabited branch. After cutting down a web, we placed 12 larvae into a 0.5L clear plastic container (Fabri-Kal, Kalamazoo, Michigan) provisioned with a wet filter paper disc (7.5 cm diameter; VWR, West Chester, Pennsylvania) and a sprig of the natal host, and replaced the web within the branches of the tree of origin. We kept the containers cool and transported them to the laboratory on the same day.

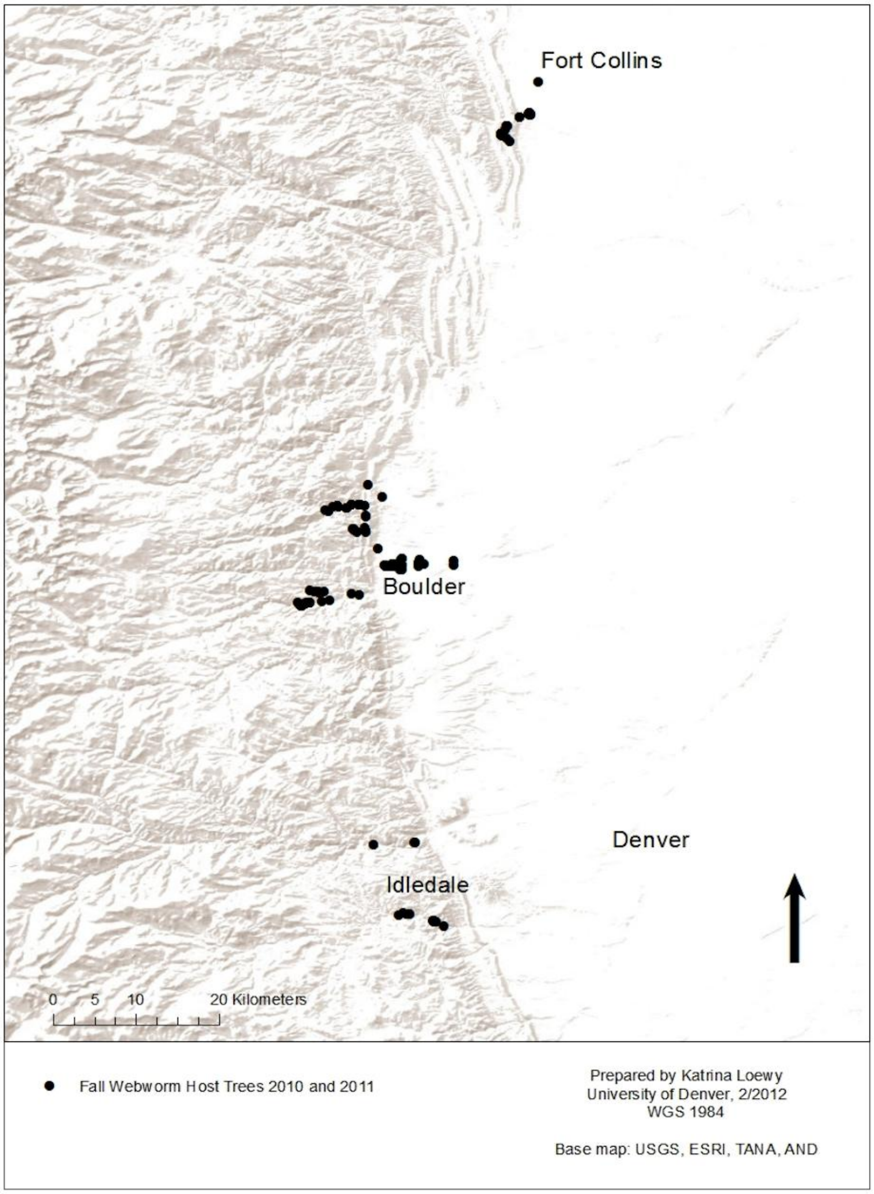

Figure 1: Map of observed *Hyphantria cunea* webs along the Colorado Front Range during the summers of 2010 and 2011. Each dot represents the location of a host tree. Dots are allowed to overlap. The directional arrow points north.

Rearing Larvae in the Laboratory

We successfully reared both wild-caught *H. cunea* larvae in 2010 and *H. cunea* larvae hatched from eggs in 2011 (see subsections below on ‘Mating adult moths’ and ‘Oviposition, Eggs and Early Instar Larvae’) in ambient conditions in our laboratory at the University of Denver in Denver, Colorado. Depending on size, we reared larvae individually or in groups of up to six individuals in the same type of clear plastic containers that we used to collect them from the field. Large larvae were housed individually and small larvae were housed in groups, with group size decreasing as they grew larger. Early instar larvae are naturally aggregative feeders and it has been shown previously that group sizes of four to eight black-headed *H. cunea* hatchlings are necessary for establishment and survival, while later instars develop faster with less crowding (Watanabe and Umeya 1968). We stored the plastic containers with *H. cunea* larvae at room temperature on shelves with exposure to ambient light from outside a nearby window.

We collected fresh host plant branches from our field sites biweekly and stored them in 49.2L plastic bags (Tall Kitchen Bags, Safeway, Pleasanton, CA) in a walk-in growth chamber (Kysor-Sherer, Marshall, MI) set at 4-10°C. For each larval container, we replaced old food plants with fresh foliage and removed frass biweekly. In 2010, we reared larvae on 5 host plant species: chokecherry (*Prunus virginiana*, n= 183), crabapple (*Malus* sp., n= 40), Lombardy poplar (*Populus nigra*, n= 20), narrowleaf cottonwood (*Populus angustifolia*, n= 167), and thinleaf alder (*Alnus tenuifolia*, n= 20); although larvae were reared on all of these host plants, any single larva was reared for the entirety of its development on the single host plant species upon which it was discovered. During feeding, we used a spray bottle filled with tap water to remoisten the filter paper, which helped to keep the host plant material fresh and to increase ambient humidity, a function performed by the web in wild populations (Morris and Fulton 1970). When larvae reached the prepupa stage, we suspended maintenance, as pupating larvae experience higher mortality when disturbed (Morris and Fulton 1970).

Rearing Larvae in Growth Chambers

In 2011, we moved a subset of 400 20-day-old, lab-reared larvae from the lab to environmental growth chambers (Percival Scientific, Pery, IA) set to a diurnal cycle calculated to mimic average field temperatures in Boulder, CO on August 15, 2011 (L14:D10 and 27ºC:19.5ºC). Individual larvae were housed in an inverted 1L plastic container (Fabri-Kal, Kalamazoo, Michigan) with a sprig of host plant from one of four plant species (choke cherry, crab apple, narrowleaf cottonwood and thinleaf alder); we divided the 400 larvae equally among the host plants such that there were 100 larvae reared on each host. The plant had a fresh water supply provided by an aquapic (7.6 cm recycled water tubes, Afloral.com, Jamestown, NY). We replaced sprigs of host plant and refilled aquapics with water biweekly. The aquapic was placed in a 1.5 cm diameter hole in the lid so that when closed, the host plant and larva were enclosed in the 1L plastic container (Fig. 2). The inverted 1L container was then placed on an upright 0.5 L container so that the entire apparatus could stand alone. Condensation built up quickly inside the containers, and it was especially important to remove standing water from containers with later instars, because too much water can prevent successful splitting and shedding of the final larval skin (Morris and Fulton 1970, Loewy, pers. obs.). We checked containers daily to record any larvae that had reached the prepupa or pupal stage.

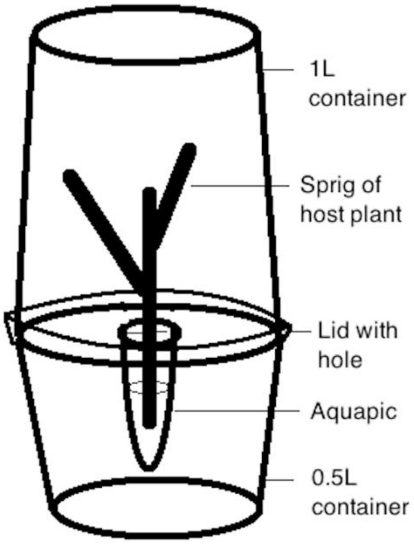

Figure 2: Diagram of rearing chamber configuration used to rear *Hyphantria cunea* larvae in environmental chambers.

Pupae

We removed hardened pupae, most of which had entered diapause, from their containers and cleaned them of debris and frass. We placed each individual pupa into a new 0.5 L plastic container that contained 2-3 cm of moist sphagnum peat (Ferti-lome peat moss, Cheek Garden Products, Austin, TX). We overwintered the containers with peat and pupae in environmental growth chambers (L0:D24 and 4 °C) for seven months, starting in early November. Morris and Fulton (1970) suggested a minimum chilling period of 6 months at 1.7 degrees C, and a maximum of 8 months, after which survival rate decreased sharply. We moistened the peat with tap water by misting the containers every two to three months to maintain ambient humidity.

Adult Emergence

In the beginning of June, we cleared pupal containers of peat, misted the pupae, and placed a moistened filter paper disc under each pupa in its container. Then we returned the containers to the environmental growth chambers set to a diurnal cycle calculated to mimic average field temperatures in Boulder, CO on June 8, 2011 (L15:D9 and 23ºC:16ºC). We recorded newly-emerged adults daily, and definitively determined their sex. Male moths have pectinate antennae and tend to have smaller abdomens, while female moths have filamentous antennae and larger abdomens, at times with greenish eggs visible within.

Mating Adult Moths

On the day of emergence, we placed a single female and one to three males into a plastic shoebox (34.6 cm x 21 cm x 12.4 cm, Sterilite Corp., Townsend, MA) lined with wax paper that served as a mating chamber, taking care that none of the females were paired with a male sibling from the same natal web to avoid potential inbreeding depression in our colony. Jaenike and Selander (1980) confirmed that black-headed larvae within a single web are full-sibs, and we assumed the same of the red-headed form. Putting the mating chambers in an environmental growth chamber with its stark transition between light and dark did not facilitate mating behavior. However, when we moved the mating chamber into the lab and exposed it to natural light, mating took place within two days. Hidaka (1977) found that mating flight is likely cued by the dim light of dawn or dusk in black-headed *H. cunea*. We did not record the precise timing of mating behavior in the lab, although we only discovered mated pairs, still in coitus, in the mornings.

Oviposition, Eggs and Early Instar Larvae

A day after copulation, we removed males from the mating chamber so that they would not disturb the ovipositing female; females were left to oviposit in the shoebox mating chamber. Oviposition began 1-3 days after mating was observed. With rare exceptions, females laid their eggs on the wax paper-lined walls of the mating chamber, rather than the floor or lid. Females slowly swung their abdomens back and forth, creating row after row of eggs, usually in a single layer. When undisturbed, most females laid all eggs in a single batch and often died with their wings covering the egg mass, a behavior also observed in wild populations by Swain (1936).

We removed egg masses from the mating chamber by cutting the wax paper around them with a X-acto knife. We kept eggs on the wax paper until hatching to avoid breakage and placed the egg masses in a new 0.5 L plastic deli container with a moist cotton ball. We kept most of the containers in the lab under ambient conditions, and moved eight into the growth chamber at 26°C. We labeled containers with information about the eggs' maternal and paternal lineage and the date that they were laid. As long as the cotton ball was moist, humidity within the container was sufficient to allow hatching. Head capsules became visible, turning the eggs dark, a day before the larvae hatched.

Once larvae began to eclose, we removed the moist cotton ball and replaced it with a moist filter paper disc. We also placed a sprig of host plant into the container. We found that if we tried to move neonate larvae to a host plant leaf with a fine paintbrush, they suffered higher mortality than neonates we allowed to locate the leaf on their own. We placed fresh leaves in the container biweekly. To minimize disturbance of delicate early instar larvae, we only removed old leaves if they started to get moldy. We misted containers lightly while introducing the fresh host plant. As the larvae grew, we divided them into smaller groups and moved them to new containers to minimize the frequency with which food needed to be replaced and make individual identification easier.

Objective 2 – Life History Traits of Red-headed *H. cunea* in Colorado

To better understand the life history of red-headed *H. cunea* in Colorado, in 2011 we recorded the timing of life events for larvae reared in the lab for the entirety of their development, including the dates of oviposition, eclosion, and pupation so that we could calculate total larval development time. For ease of comparison with the results from other studies, larval development times are only included for larvae that completed their development in the environmental growth chambers, not in the lab. After overwintering, we also recorded the number of days that passed from when the pupae were first exposed to spring conditions in the environmental chambers to adult emergence. For adult females, we recorded the number of days that passed from mating to the onset of oviposition.

Pupae deplete their fat stores over time, so we weighed all pupae exactly 30 days after pupation (to the nearest 0.01 mg; Mettler-Toledo XP6, Columbus, Ohio). We determined the sex of each individual by viewing the pupae under a dissecting microscope and noting the location of the genital slit (Fig. 3), similar to methods reported by Villiard (1975) and Carter and Feeny (1985). We confirmed our sex determinations after the moths emerged as adults the following spring.

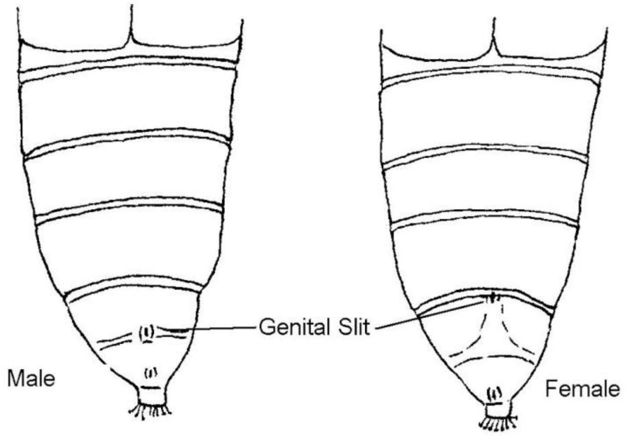

Figure 3: Diagram of pupal sex differences in *Hyphantria cunea*, with the ventral surface of a male pupa on the left and a female pupa on the right. On female pupae, the genital slit is located on the anterior edge of the fourth abdominal segment posterior to the wing covers. On male pupae, the genital slit is located on the posterior edge of the same segment. The genital slit of both sexes sometimes appears to transect two segments.

Objective 3 – Pupal Mass as a Predictor of Potential Fecundity and Lifetime Fitness in Lepidoptera

To quantify fecundity for red-headed *H. cunea*, we photographed egg masses using the macro setting on a Cannon PowerShot SD780 IS and uploaded the pictures to a computer for counting in Paint (Microsoft, Redmond, WA). For greatest accuracy, we dotted each egg with the pencil tool and kept track of the number with a tally counter. We also reviewed the literature for other studies that have investigated the relationship between female pupal mass and fecundity. We performed keyword searches on Web of Science and Google Scholar using various combinations of the following terms: fecundity, fitness, Lepidoptera, lifetime fitness, pupa* mass and realized fitness. We then limited the results to studies that ran a regression of female pupal mass by potential fecundity. Following Awmack and Leather (2002), potential fecundity is a measure of the number of eggs an insect produces, while realized fecundity refers to the number of offspring produced.

Statistical Analyses

We analyzed our results with T test and regression using JMP Pro 9.0.0 (SAS Institute Inc., Cary, NC). All means are reported ± 1 standard error.

Results

Survival

We found that our rearing technique for fall webworm was quite successful. When larvae were reared on a high-quality host plant such as chokecherry (n=100), we found that 98% of the larvae successfully pupated and 74% successfully completed their development to the adult stage. Survival was lower for larvae collected from the field or reared on lower quality host plants, but this was due to mortality related to parasitism and host plant quality, rather than our rearing technique.

Sex Determination

For our analyses, we identified morphological differences between male and female *H. cunea* pupae. Of the 129 pupae that we determined to be female, 125 emerged as female moths. Of the 141 pupae that we determined to be male, 139 emerged as male moths. Thus, we were able to successfully identify 99% of male pupae as males and 97% of female pupae as females. Our ability to distinguish males from females in the pupal stage allowed us to investigate whether male and female larvae differ in developmental life history traits without excluding individuals that did not reach adulthood.

Development Time

We found that all eggs from a single egg mass hatched on the same day and the mean development time was 13.9 ±0.2 days (*N*=44 egg masses, range=10-16 days) in the population kept in ambient lab conditions, and 11.6 ±0.6 days (*N*=7 egg masses, range=9-13 days) in the population kept in an environmental chamber set at 26°C. After the eggs hatched, we found that mean larval development time was 42.1 ±0.2 days (*N*=332, range=35-62 days) in the environmental chamber (L14:D10 and 27°C:19.5°C).

We found that female larvae took longer to develop than male larvae by 0.3 days, but the difference was not significant (t=0.9, P=0.36). Females took 42.2 ±0.3 days (*N*=167, range=36-53 days) to develop while males took 41.8 ±0.3 days (*N*=155, range=35-62 days).

After pupae were removed from the overwintering chamber, we found that it took 26.8 ±0.5 days for adults to emerge (*N*=264, range=17-52 days); of the 300 pupae that we overwintered in growth chambers from 2010-2011, 88% emerged. Females took longer to emerge than males by 1.8 days (t=1.93, P=0.055). Females emerged 27.7 ±0.7 days (*N*=125, range=17-52 days) after removal from cold storage, while adult males emerged 25.9 ±0.6 days (*N*=139, range=17-44 days) after removal from the cold.

Pupal Mass

As in most Lepidoptera, female pupae were significantly larger than males (*t*=13, *P*< 0.0001). The mean mass for male pupae was 185.66 mg (±1.9, *N*=155, range=127.78-286.00 mg), while the mean mass for female pupae was 223.25 mg (±2.19, *N*=167, range=143.58-300.52 mg), which is 120% heavier than male pupae.

Adult Female Fecundity

An average of 2.2 ±0.2 days elapsed from when female *H. cunea* were mated until they began to lay eggs (*N*=45, range=1-5 days). Females laid an average of 484.2 ±22.4 eggs (*N*=43, range=34-830 eggs). We found a significant positive relationship between a female's pupal mass and the number of eggs that she laid during her lifetime (R^2=0.34, *t*=4.59, n=43, *P*<0.0001); for every additional mg of pupal mass, a female laid an additional 2.35 eggs (Fig. 4).

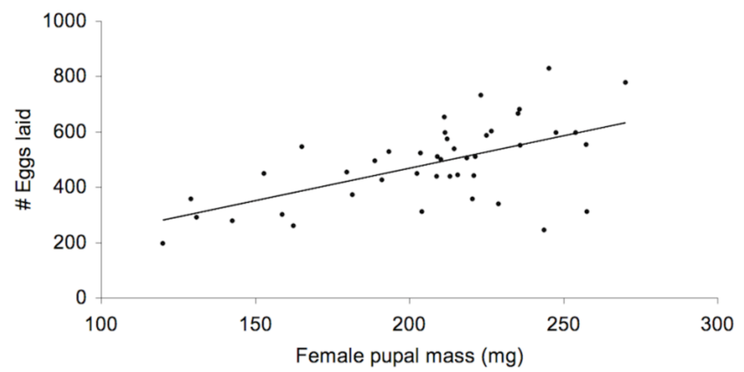

Figure 4: The relationship between female fecundity, measured by eggs laid per female, and female pupal mass (y=2.353x - 1.8343; n=43 females, R^2=0.34, T=4.59, p < 0.0001) for red-headed *Hyphantria cunea* in Colorado.

Our literature review demonstrated that there is a significant, positive relationship between pupal mass and potential female fecundity for the majority of lepidopteran species for which this relationship has been investigated (Table 1).

Table 1. Compilation of data that examines the relationship between female pupal mass and potential fecundity from studies of other Lepidoptera gathered from the literature. The slope represents the increase in the number of eggs per mg of additional pupal mass. Depending on the study, the number of eggs could refer to eggs laid, dissected out, matured, or any combination of the three. If a variable was not reported, it is noted as NR. Other values given in the table are the correlation coefficient r (all correlation coefficients are significant unless noted 'ns' for not significant) and the number of groups or individuals in the study (n).

Family	Species	Slope	r	n	Source
Erebidae	*Hyphantria cunea* (black head)	4.8	0.64	71	Jasič and Macko (1961)
		4.4	0.68	86	
		4.7	0.70	20	
		3.8	0.49	167	
		3.8	0.89	30	Morris and Fulton (1970)
	Hyphantria cunea (red head)	2.35	0.58	43	This study
	Orgyia antiqua	1.12	0.93	39	Tammaru *et al.* (2002)
	Orgyia leucostigma	1.29	0.89	187	Tammaru *et al.* (2002)
	Orgyia vetusta	NR	0.69	32	Harrison and Karban (1986)
Geometridae	*Operophtera brumata*	10.5	0.92	91	Roland and Myers (1987)
		9.14	0.96	41	
	Epirrita autumnata	2.31	NR	296	Heisswolf *et al.* (2009)

Table 1 — *Continued*

Family	Species	Slope	r	n	Source
Lasiocampidae	*Malacosoma disstria*	0.45*	0.89	12	Lorimer (1979)
		0.45*	0.82	12	
		0.45*	0.71	12	
		0.45*	0.6(ns)	9	
		0.45*	0.89	13	
		0.59*	0.81	13	
		0.59*	0.95	11	
		0.59*	0.92	14	
		0.59*	0.82	15	
	Streblote panda	0.09	0.73	55	Calvo and Molina (2005)
Limacodidae	*Acharea stimulea*	0.24	0.57(ns)	11	Murphy *et al.* (2011)
	Euclea delphinii	0.27	0.32(ns)	23	Murphy *et al.* (2011)
Noctuidae	*Mythimna convecta*	7.02	0.7	NR	Smith (1986)
	Mythimna pallens	4.36	0.61	14	Hill and Hirai (1986)
		2.29	0.57	13	
		6.05	0.83	21	
		1.97	0.53	15	
	Mythimna separata	6.24	0.9	10	Hill and Hirai (1986)
		7.99	0.92	20	
		2.46	0.39	29	
		3.43	0.51	28	
	Sesamia nonagrioides	5.73	0.49	50	Fantinou *et al.* (2008)
	Spodoptera exigua	11.2	0.73	NR	Tisdale and Sappington (2001)
		12.5	0.60	NR	
		16.8	0.81	NR	
Plutellidae	*Plutella xylostella*	28.0	0.78	15	Sarfraz *et al.* (2011)

Table 1 — *Continued*

Family	Species	Slope	r	n	Source
Saturniidae	*Antheraea polyphemus*	0.05	0.88	26	Miller *et al.* (1982)
	Callosamia promethea	0.14	0.99	25	Miller *et al.* (1983)
Tortricidae	*Choristoneura conflictana*	2.09	0.2(ns)	20	Evenden *et al.* (2006)
		4.36	0.81	13	
		-4.7	0.3(ns)	7	
		4.07	0.65	22	
		5.37	0.73	16	
		1.91	0.5(ns)	6	
	Choristoneura fumiferana	1.1	0.4(ns)	42	Lorimer and Bauer (1983)
		1.9	0.78	40	
	Cnephasia jactatana	35.7	0.62	175	Jiménez-Pérez and Wang (2004)

*Slopes were obtained by combining nine separate broods of *Malacosoma disstria* into two geographic groups.

Discussion

Here we report natural history for the red-headed form of *H. cunea* for the first time; previously, all detailed published accounts of *H. cunea* natural history have focused on the black-headed form. To our knowledge, this is also the first account of successful rearing techniques for red-headed *H. cunea*. Our results suggest that much of the phenology of red-headed *H. cunea* differs from that of the black-headed form; red-headed eggs took longer to hatch and larvae took longer to develop than those in studies of the black-headed form. Additionally, red-headed pupae were heavier than black-headed pupae.

Comparison of *H. cunea* life history traits among studies is complicated because different studies have used a variety of different host plants and temperatures for rearing caterpillars. Furthermore, voltinism differs among populations with anywhere from one to more than four generations per year; *H. cunea* in Colorado has a single generation each year (pers. obs.), but black-headed populations in Maryland are bivoltine while populations in southwestern Japan are trivoltine (Gomi and Takeda 1996, Mason *et al.* 2011). Despite these complications, we compared our results with those of other studies to better understand how life histories may differ between red-headed and black-headed *H. cunea*. At several life stages, red-headed *H. cunea* took longer to develop than the black-headed form. European, Asian, and North American measurements of embryonic development time (the time from oviposition to hatching) for black-headed *H. cunea* range from ~7 days at 27ºC to ~23 days at 16ºC (Jasič and Macko 1961, Yearian *et al.* 1966, Szalay-Marzso 1972, Gomi *et al.* 2005). We found that red-headed *H. cunea* eggs incubated at 26 ºC took 11.6 days to hatch, which is longer than in any study of black-headed *H. cunea* eggs incubated at that temperature. Red-headed *H. cunea* larvae took longer to develop than black-headed *H. cunea* larvae in similar studies; published development times for black-headed *H. cunea* larvae range from 17–47 days depending on which host plants were used as food, the temperature at which the larvae were reared, and the sex of the larva (Jasič and Macko 1961, Yearian *et al.* 1966, Morris and Fulton 1970, Gomi *et al.* 2005). The red-headed larvae we studied took 40-70% longer to develop compared with black-headed larvae in other studies. Furthermore, Jasič and Macko (1961) recorded shorter larval development times for males than for female black-headed *H. cunea*, with 1-2 days difference between the sexes. Notably, males and females in our study both took about 42 days to develop from egg hatch to pupation, with mean male development time shorter than mean female development time by only a fraction of a day.

The pupae of female red-headed *H. cunea* tend to be much larger than the female pupae of the black-headed form studied by Jasič and Macko (1961), Morris and Fulton (1970), and Gomi et al. (2005). One complicating factor when comparing pupal masses across studies is that there is a positive relationship between rearing temperature and pupal mass such that even on the same host plant, larvae develop into heavier pupae when reared at higher temperatures (Jasič and Macko 1961). Despite the use of a different host plant, white mulberry (*Morus alba* L.), Jasič and Macko (1961) reared black-headed *H. cunea* at a similar temperature to the temperatures we used to rear red-headed *H. cunea*, differing only by about 1-2 degrees. Jasič and Macko (1961) recorded a mean pupal mass for black-headed *H. cunea* that was 24% lower than the mean pupal mass we recorded for red-headed *H. cunea*, despite rearing the larvae at a mean temperature 2.1 degrees higher than that of our study.

Intraspecific variation in insect body mass is often correlated with lifetime fitness (Slansky and Scriber 1985, Honěk 1993). Two studies have previously found a positive correlation between pupal mass and potential fecundity for *H. cunea* (Jasič and Macko 1961, Morris and Fulton 1970), but both of these studies focused only on the black-headed form. Our results demonstrate that pupal mass may be used as a predictor of potential fecundity for the red-headed form of *H. cunea* as well. Furthermore, our results support and add to the limited but growing body of literature that demonstrates a positive relationship between body size and lifetime fitness for Lepidoptera. In a thorough review of the literature, we were able to find studies for 21 lepidopteran species that investigated the relationship between female pupal mass and potential fecundity (Table 1). For 19 of these 21 species, a significant, positive relationship exists between pupal mass and female fecundity (Table 1); for the two limacodid species, the relationship was still positive, but not significant. Thus, for all of the lepidopteran species studied to date, females that gain more mass as larvae are able to produce more eggs as adults. The magnitude of the effect size may depend on the feeding behaviors of adult female moths; the mass gained as larvae may be even more critical to lifetime fitness for species that do not feed as adults (Jervis et al. 2005).

Our data on the natural history of red-headed *H. cunea*, combined with genetic and molecular analyses (Jaenike and Selander 1980, McIntee and Nordin 1983) and behavioral observations (Oliver 1964), suggest that red-headed and black-headed *H. cunea* may be two distinct species or subspecies. To better understand natural history and behavioral differences between the two forms of *H. cunea*, there needs to be consistency among studies to allow for meaningful comparisons among populations of these two host forms. Previous studies have all used different rearing temperatures and diets that reflect the local climate and habitat of the focal *H. cunea* population, but this makes it difficult to compare natural history traits for the two forms across their geographic range. In Colorado, we find only the red-headed form, but in some areas of North America the black-headed and red-headed forms are sympatric, which would allow for more direct comparisons between the two forms. Investigations into ecological, phenotypic and genetic differences among black-headed and red-headed populations of *H. cunea* across the entire geographic range, both where the forms are sympatric and allopatric, would be a fruitful area of future research.

CHAPTER TWO: TRADE-OFFS IN HOST CHOICE OF AN HERBIVOROUS INSECT BASED ON PARASITISM AND LARVAL PERFORMANCE

Introduction

Of the many decisions faced by an insect herbivore, choosing a host plant is among the most important. Plants can potentially provide food, shelter, and protection from natural enemies. Herbivorous insects are often categorized as specialists, which feed on only one or a few plant families, or generalists, which feed on many different plant families; more than 90% of insect herbivores are considered specialists (Schoonhoven et al. 2005, Price et al. 2011). Generalization in insects is often considered a basal or transitional trait, and is underrepresented in host choice literature (Mercader and Scriber 2007). However, there is evidence that a species' evolutionary movement along the generalist-specialist continuum is not unidirectional, but fluctuates between wider and narrower diet breadths (Janz et al. 2001, Janz et al. 2006).

There are clear fitness benefits gained by specialization. Specialist herbivores have developed behaviors and metabolic systems to counteract and even thrive on their hosts' mechanical and chemical defenses (Schoonhoven et al. 2005). Some larval lepidopterans avoid host structures with the most concentrated allelochemicals, and some have specialized enzymatic pathways that allow them to gain nutrition from compounds that would be toxic to other herbivores (Karban and Agrawal 2002). Juvenile insect herbivores often have limited mobility, and are restricted to feeding on hosts chosen by adults, however the relationship between adult preference and larval performance has been ambiguous (Mayhew 1997). For Lepidoptera, the positive relationship between the host preference of an ovipositing female and the performance of larvae on the same plant is stronger in specialized species than in generalized ones (Gripenberg et al. 2010). However, both specialist and generalist adults and their offspring are exposed to additional environmental conditions that may favor generalization. For example, specialized females searching for rare hosts spend more time vulnerable to attack by natural enemies, and may not be able to lay a full complement of eggs. The ability to use additional hosts can reduce search time and result in more oviposition opportunities, translating into a higher percentage of eggs laid (Johansson et al. 2007). Greater resource availability may also benefit larvae that leave or fall from their original host plants (Bernays and Minkenberg 1997). Multiple host plants allow for diet mixing within the larval stage, which has been shown to improve larval performance for some generalist species (Karban et al. 2010).

Bottom-up and top-down interactions between host plants and parasitoids may also support a generalist herbivore feeding strategy. Jeffries and Lawton (1984) defined enemy-free space as a way of living that reduces a species' exposure to parasitoids and predators. Others have characterized the role that host choice plays in creating enemy-free space (Murphy 2004) and the trade-offs between enemy-free space and plant quality (Singer et al. 2004). If host plants that provide protection from natural enemies and host plants that provide high quality food are different, the trade-off insects experience when choosing among these hosts may sustain relatively polyphagous habits within insect populations (Rodrigues et al. 2010). The existence and direction of such trade-offs are expected to vary, even where the suite of host plant species remains unchanged. Both biotic and abiotic factors modify a host plant's suitability throughout its range and growing season (Michaud 1990), and variation in host plant genotype may alter its quality as a food source as well (Fox and Morrow 1981). Throughout an herbivore's range, it can experience different communities of plants, natural enemies and competitors. The resulting patchwork of selective pressures variably shape species' habits, including host use and diet breadth (Thompson 1998, Thompson 2005).

The fall webworm (*Hyphantria cunea* Drury, Erebidae) presents an ideal opportunity to test how the ecological drivers of host use by a polyphagous herbivore vary over its geographic range. Worldwide, fall webworms are known to feed on hundreds of different host plants in dozens of plant families (Warren and Tadić 1970). In North America, populations of fall webworms in the eastern United States commonly feed on dozens of different species (Berger 1906, Greenblatt 1978, Mason et al. 2011). Mason et al. (2011) measured how host abundance, larval performance, and natural enemies impacted host choice in fall webworms in Maryland and Connecticut. They found that for these populations of fall webworms in the eastern United States, neither natural enemies nor larval performance explained host choice; rather, the abundance of potential hosts emerged as the driver of patterns of host use in both states, which suggests great selective pressure to reduce search time for oviposition sites by adult females (Mason et al. 2011). Compared with the broad array of host plants used on the east coast, host use by fall webworms in Colorado is more restricted with only 19 plant species recorded as hosts, and only 3 of those species accounting for over half of the records (Swain 1936, Loewy and Murphy personal observation). The selective pressures that could have led to this restriction in diet breadth are unknown.

Here we investigate three main selective pressures that may drive host use by fall webworms in Colorado. We examined the impact of 1) host plant abundance, 2) larval performance (survival, development time, pupal mass, and feeding efficiency), and 3) mortality from parasitoids on fall webworm host choice in the Colorado foothills through an observational field experiment as well as a manipulative split-brood experiment under controlled lab conditions. Our goal was to test how these selective pressures may act individually or in concert to explain regional differences in fall webworm diet breadth and host use.

Materials and Methods

Study System

The fall webworm is a moth native to North America and invasive in Europe and Asia (Jasič and Macko 1961, Gomi and Takeda 1996, Yang et al. 2006). The moths were unintentionally introduced to Hungary and Japan in the 1940s and spread to other parts of Europe and Asia in the following decades (Tadić 1963, Yang et al. 2008). In China, fall webworms feed on 175 host tree species, including cultivated crops, and are considered a pest of economic importance (Yang et al. 2006).

In North America, fall webworms range across the United States and are found in parts of Canada and Mexico (Masaki and Ito 1977). Adults have been recovered in most Colorado counties, and relatively dense communities can be found in the foothills of the

Colorado Rocky Mountains (Ferguson et al. 2000). The gregarious larvae spin extensive webs for protection (Ito 1977) and thermoregulation (Morris and Fulton 1970, Rehnberg 2002), usually on the outer branches of deciduous trees. They are highly polyphagous, and researchers have recorded them feeding on hundreds of species from dozens of plant families throughout their range (Warren and Tadić 1970). However, their diet is limited regionally; in Colorado, we have observed them on 19 woody tree species representing 11 different genera from 8 plant families. Fall webworms in Colorado can completely defoliate trees during outbreaks (Swain 1936) but the larvae and their expansive webs are usually more unattractive than harmful.

Study Sites

Along the Colorado Front Range, fall webworm populations are concentrated in the canyon-carved foothills of the Rocky Mountains, as well as in the adjacent plains (Fig. 1). Fall webworm females preferentially lay their eggs along open edges (e.g. roads, streams), which make larvae easy to locate after they have built a web. We collected larvae from multiple field sites near the cities of Boulder (Boulder County, 40.090013, -105.359962), Fort Collins (Larimer County, 40.5852602, -105.084423), and Idledale (Jefferson County, 39.746944, -105.210833) from 1557 m to 2023 m in elevation in both 2010 and 2011. Fall webworms in these populations emerge as adults in midsummer and larval webs can be found from mid July through the end of September (see Chapter 1).

Host Plant Abundance

To quantify the abundance of host plants available to ovipositing female moths, we haphazardly chose 72 host plants with fall webworm webs in 2010 and 82 in 2011. For each host plant, we established a 30 m transect, 15 m to either side of the host, parallel with the habitat edge (e.g. road or stream). For each tree along the transect, we recorded the species' identity and stem diameter at breast height (DBH), excluding plants that rarely serve as fall webworm hosts (we never observed webs on trees <1.5 m in height or gymnosperms, but see Oliver (1964) and Warren and Tadić (1970)). We collected voucher specimens for all host plants as well as any plant species that we were unable to identify in the field for later identification (host plant voucher specimens are deposited in the Kathryn Kalmbach Herbarium at the Denver Botanic Gardens). We also recorded the number of webs in each tree, assuming that each web represented the offspring of a single mother (a brood) as suggested by Jaenike and Selander (1980).

Prior to analysis, we lumped several host plant species into larger categories because of challenges with consistent identification and low sample size. For example, "Elm" (*Ulmus*, n=31) includes Siberian and Scotch elms (*Ulmus pumila* and *Ulmus glabra*), and "Willow" (*Salix*, n= 59) includes black willows, peachleaf willows, and other willow species (*Salix nigra* and *Salix amygdaloides*). Apple (*Malus domestica*, n=24) is distinguished from the many species of crabapple (all crabapple species are lumped as *Malus* spp., n=31). We split chokecherry into two groups: chokecherry with green leaves (*Prunus virginiana*, n=130) and an ornamental variety with purple leaves, Schubert chokecherry (*Prunus virginiana* var. Schubert, n= 50). The remaining hosts were narrowleaf cottonwood (*Populus angustifolia*, n=193), plains cottonwood (*Populus deltoides*, n=75), quaking aspen (*Populus tremuloides*, n=56), Lombardy poplar (*Populus nigra*, n=11), box elder (*Acer negundo*, n=11), green ash (*Fraxinus pennsylvanica*, n=15), thinleaf alder (*Alnus tenuifolia*, n=29), plum (*Prunus americana*, n=14), white oak (*Quercus alba*, n=1), black walnut (*Juglans nigra*, n=1), and Viburnum (*Viburnum* sp., n=3). The sample size (n) associated with each genus or species is the number of times we recorded a tree of that type in a transect during the study, whether or not it served as a host (Table 2).

Table 2. Fall webworm host plants recorded in wild and cultivated sites in Colorado and in the eastern United States. Host plants in Colorado are listed alphabetically, although hosts that co-occur in wild and cultivated sites are listed first. The number of times a plant was observed in wild or cultivated sites is in parentheses. Host plants used by fall webworm populations in the eastern United States (Mason et al. 2011) are listed alphabetically. Plants in bold were observed as hosts in Colorado and the East Coast.

Wild	Cultivated	Eastern
Acer negundo (6)	***Acer negundo*** (5)	***Acer negundo***
Alnus tenuifolia (28)	*Alnus tenuifolia* (1)	*Ailanthus altissima*
Fraxinus pennsylvanica (4)	***Fraxinus pennsylvanica*** (11)	*Alnus rubra*
Malus domestica (12)	*Malus domestica* (12)	*Betula nigra*
Populus angustifolia (191)	*Populus angustifolia* (2)	*Betula pendula*
Populus deltoides (54)	***Populus deltoides*** (21)	*Betula populifolia*
Prunus americana (7)	*Prunus americana* (7)	*Carya glabra*
Prunus virginiana (124)	*Prunus virginiana* (6)	*Carya* sp.
***Salix* spp.** (37)	***Salix* spp.** (6)	*Castanea pumila*
***Ulmus* spp.** (15)	***Ulmus* spp.** (16)	*Cephalanthus occidentalis*
Populus nigra (11)	*Populus tremuloides* (56)	*Cercis canadensis*
Viburnum sp. (3)	*Juglans* sp. (1)	*Diospyros virginiana*
	Malus spp. (29)	*Fagus grandifolia*
	Prunus virginiana *var*. Schubert (50)	***Fraxinus pennsylvanica***
		Lonicera japonica
	Quercus alba (1)	*Lonicera* sp.
		Morus alba
		Morus rubra
		Nyssa sylvatica
		Platanus occidentalis
		Populus deltoides
		Prunus pensylvanica
		Prunus serotina
		Rhus trilobata
		Salix fragilis
		Salix nigra
		***Salix* spp.**
		Tilia americana
		Ulmus rubra
		***Ulmus* sp.**

Plant communities in the canyon areas (designated "wild") were generally distinct from those of the cultivated residential developments. To quantify the similarity between wild and cultivated sites in 2010 we calculated a Jaccard coefficient (Gotelli and Ellison 2004). We pooled all species recorded on transects in wild areas into a "wild" site type and all species recorded on transects in cultivated areas into a "cultivated" site type. A Jaccard coefficient (or similarity index) is calculated as C ÷ (A+B+C) in which A = number of species only in wild sites, B = number of species only in cultivated sites, and C = number of species both site types have in common. The coefficient is expressed as the percentage of species shared between the two types of sites.

We calculated relative host use as the number of webs on a single species divided by the total number of webs in a site type (cultivated versus wild). We calculated relative abundance as the number of times a host species was present on transects divided by the total number of trees, both host and non-host, recorded on transects within a site, following Mason et al. (2011). We defined host species as any species observed with one or more webs during the study. Surveys took place when webs were most visible, from August 7 through September 26, 2010 and July 21 through August 22, 2011.

Larval Performance

In 2010 and 2011, we cut branches containing fall webworm webs with a tree pruner (4.9m Jameson poles, Marvin pruner head, Sherrill Tree, Greensboro, North Carolina) and removed 5-15 fall webworm larvae from each web. We brought all caterpillars found in the field back to the lab to complete development on their natal host plant and monitored them for parasitoid emergence (for rearing methods, see Chapter 1).

The single bottom-up performance measure for field collected fall webworms among the different host plants was pupal mass, which we measured 30 days after pupation.

In order to control for maternal effects and to record performance measures that we could not obtain from field-collected individuals (e.g. development time), we conducted a split-brood experiment in 2011 with the offspring of fall webworms we collected in 2010. We selected four hosts to use in the controlled experiment that varied in quality. Host quality was determined by pupal masses of field-collected individuals in 2010; larvae on high quality plants produce heavier pupae. We chose two high quality host plants (narrowleaf cottonwood and chokecherry) and two low quality host plants (crabapple and alder). We haphazardly chose 10 egg masses from our colony (see Chapter 1 for details on colony maintenance) that were laid within a 5-day period, July 12-17 2011, to diminish the effect of foliage age on larval fitness. We then cut each egg mass into four sections, one to be reared on each host plant. After 21 days, we culled the larvae to ten per host per mother for a total of 400 individuals. At that point, the larvae were old enough to be housed in individual containers and moved to a climate controlled growth chamber at temperatures and day lengths appropriate for Boulder, CO in mid August (L14:D10 and 27ºC:19.5ºC) (see Chapter 1). We measured larval performance on host plants in four ways: survival to pupation, development time to pupation, pupal mass, and feeding efficiency. We measured development time as the number of days from hatching to pupation; shorter development time is generally correlated with higher fitness because of reduced exposure to natural enemies in the wild (Price et al. 1980). We weighed all pupae exactly 30 days after pupation (to the nearest 0.01 mg; Mettler-Toledo

XP6, Columbus, Ohio). We analyzed male and female pupal mass separately; the impact of male body size on lifetime fitness is more equivocal than that of female body size, which correlates positively with potential fecundity (Calvo and Molina 2005, Davis and Landolt 2012) (Fig. 4). We also calculated an overall fitness score for all surviving individuals that incorporated both pupal mass and development time. To calculate the fitness score for each individual we divided its pupal mass (mg) by its development time (days), which allowed us to test whether host plant abundance and relative percent use and affect overall larval performance as a single measure. We measured feeding efficiency as the slope of the line of best fit in a correlation of frass mass (fecal mass) by pupal mass. This measure of feeding efficiency controls for larvae that eat different amounts; efficient feeders convert a greater portion of the food they consume into biomass and leave less waste than less efficient feeders (Mason et al. 2011). We collected frass for each larva from 21 days of age until pupation, dried the frass in a drying oven for a week at 40°C and weighed it. Highly efficient larvae should have higher body masses with less mass contributing to waste.

Mortality from Parasitoids

Although we observed insect predators attacking and eating fall webworm larvae in the field, we limited our measurements of top-down control to parasitoids. A large proportion of larval mortality is due to parasitoids; Tadić (1963) reported fall webworm parasitism levels of 50%. We recorded parasitoid emergences for all field-collected larvae. The majority of parasitoid flies and wasps emerged prior to pupation, but some emerged from the pupal cases after overwintering. For analysis within year, we removed single-use host plants, but we reinserted them for the pooled analysis. To estimate the influence of fall webworm ontogeny on parasitoid host use, we recorded the body length (to the nearest 0.1 mm; 150mm/.1mm Super Poly Fiberglass Dial Caliper, Swiss Precision Instruments, Garden Grove, CA) of one representative fall webworm from each web upon collection from the field.

Statistical Analyses

We analyzed pupal mass and development time using a mixed-model ANOVA, with host species, sex and their interaction as main effects, and brood as a random effect, as well as the interaction between host species and origin (lab-reared or field-collected). When significant, ANOVAs were followed by post hoc Tukey's HSD tests. We used contingency tables to analyze variation in survival (yes/no) and parasitism (yes/no) by host plant; Pearson’s chi-square value is reported. We used a student's t-test to determine if the type of parasitoid (wasp or fly) that emerged from the fall webworms was predicted by the caterpillar's length when collected. To determine the selective factors with the highest impact on natural patterns of host use, we tested for correlations between relative host use and relative host abundance, survival, development time and pupal mass. We also tested for correlations between percent parasitism and performance measures, excluding feeding efficiency. We analyzed feeding efficiency with an analysis of covariance (ANCOVA), in which significant interactions between the slopes of different host plants indicate variation in feeding efficiency (Mason et al. 2011). Means are given ± standard error. All statistical analyses were performed with JMP Pro 9.0.0 (SAS Institute Inc., Cary, NC).

Results

Host Plant Abundance

The geographic area encompassed by our transects spanned ~125 km north to south, yet we found that plant communities in canyons in Jefferson and Larimer Counties were similar to those in Boulder County (69% of canyon plants in Jefferson County and 64% in Larimer County were also found in Boulder County). Furthermore, we found that canyon plant communities differed from plant communities in cultivated areas; over all transects in 2010, only 25.5% of plants, both fall webworm hosts and non-hosts, were recorded in both wild and cultivated sites. Thus, we decided that the most useful site distinction for our analyses would be wild versus cultivated. Larimer and Boulder Counties contained both wild and cultivated transects while all transects within Jefferson County were classified as wild.

Host plant use by fall webworms varied between wild and cultivated sites (Fig. 5). However, in both wild and cultivated areas, fall webworm primarily used cottonwood (*Populus)* species (narrowleaf cottonwood in wild and plains cottonwood in cultivated), followed by chokecherry (*P. virginiana* in wild and *P. virginiana* var. Schubert in cultivated). In both types of sites, host plant abundance predicted host plant use (Fig. 6a; Wild: $R^2 = 0.82$, $n = 17$, $p < 0.0001$; Fig 6b; Cultivated: $R^2 = 0.43$, $n = 17$, $p < 0.005$). Host abundance was a stronger predictor in wild sites, where potential hosts made up 85% of total trees recorded, than in cultivated sites where potential hosts comprised 70% of total woody, broadleaf trees. However, the significant trend of abundance predicting host use in wild sites was due to the effect of a single species; narrowleaf cottonwood was both far more abundant and more commonly used than other host plant species. When we removed narrowleaf cottonwood from the wild-site analysis, the relationship between host abundance and use by fall webworm disappeared ($R^2 = 0.02$, n = 16, $p > 0.5$).

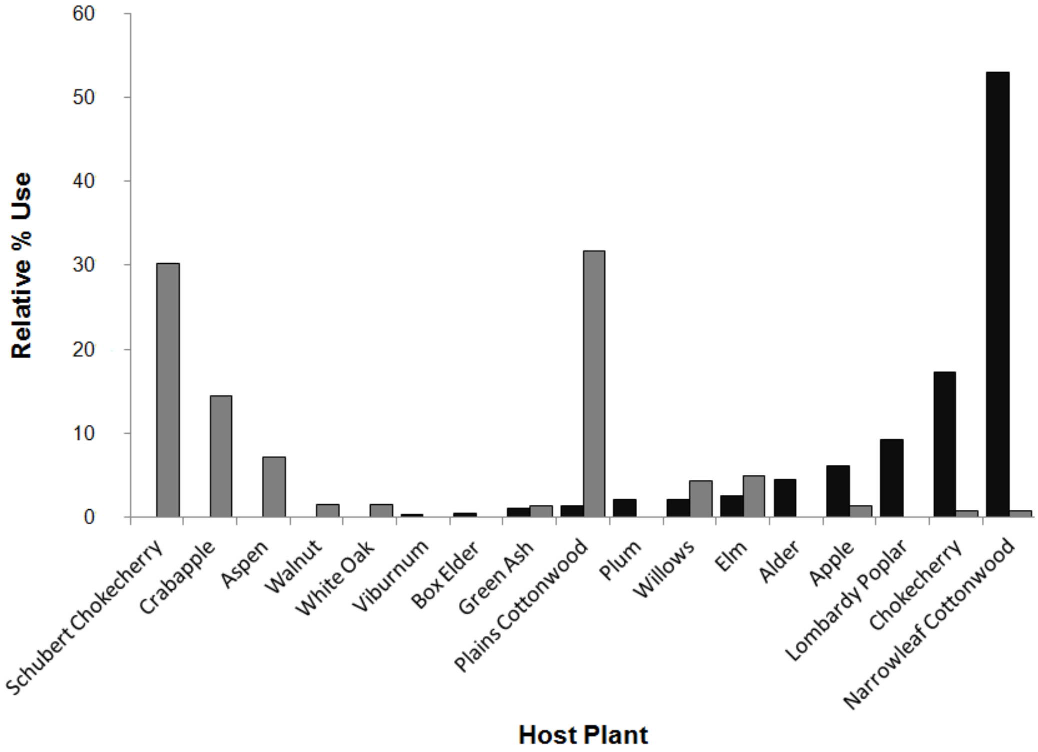

Figure 5. Patterns of natural host use by fall webworms in Colorado measured by relative percent use in wild and cultivated sites. Relative percent use is the number of webs on a host species divided by the total number of webs in the site. Data are pooled from 2010 (n = 273 webs) and 2011 (n = 244 webs). Black bars represent host species in wild sites (n = 378 webs) and gray bars represent host species in cultivated sites (n = 139 webs). Host plants without bars were not used by fall webworms within the site type.

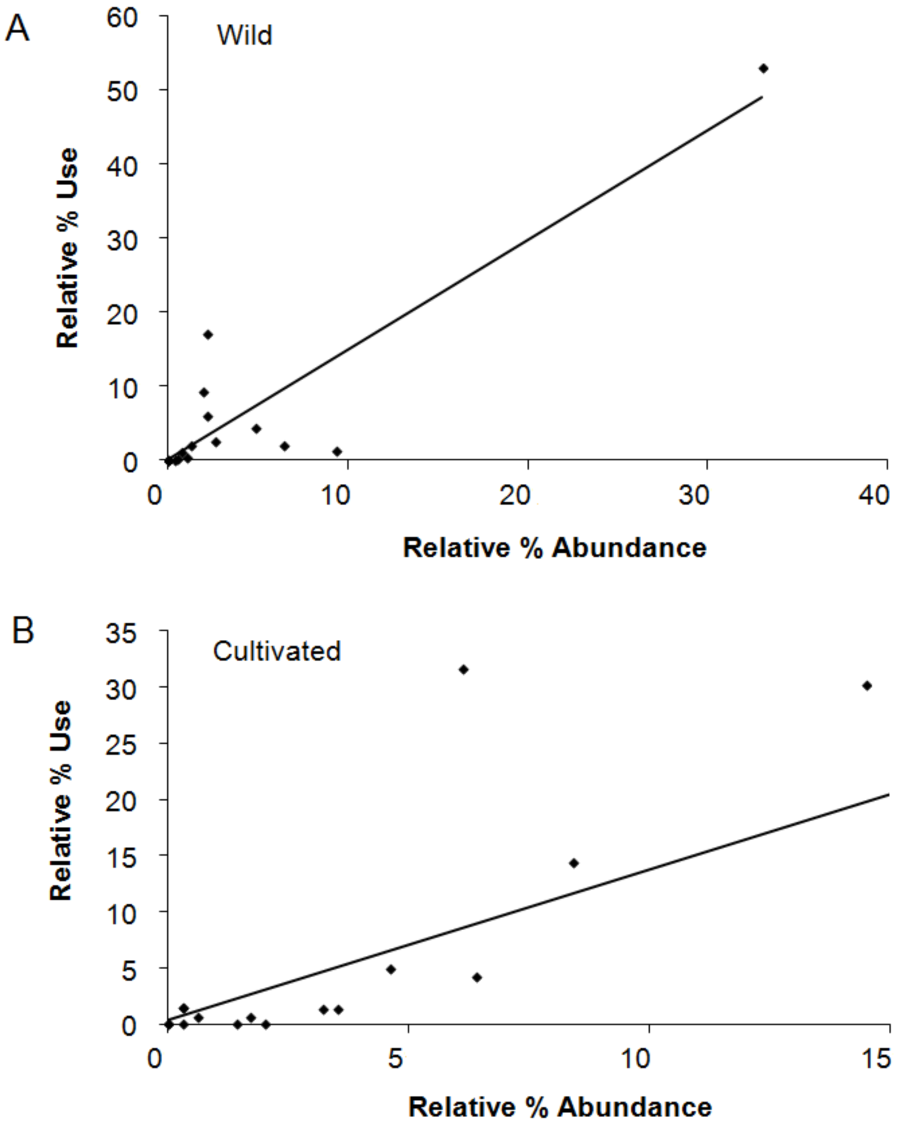

Figure 6. The relationship between fall webworm relative percent use (number of webs per host/ all webs) and relative percent abundance of potential hosts in A) wild ($N = 17$, $p < 0.0001$) and B) cultivated sites ($N = 17$, $p < 0.005$). Data are pooled for 2010 and 2011 and each point represents a host species.

Larval Performance

For fall webworm larvae that we collected from the field, pupal mass varied by sex (F = 73.87, df = 1, $p < 0.0001$), brood (F = 6.35, df = 73, $p < 0.0001$), and host species (F = 7.18, df = 12, $p < 0.0001$) when larvae from both sites and years are combined (Fig 7.). However, the interaction between host species and fall webworm sex was not significant (F = 1.29, df = 15, p = 0.2).

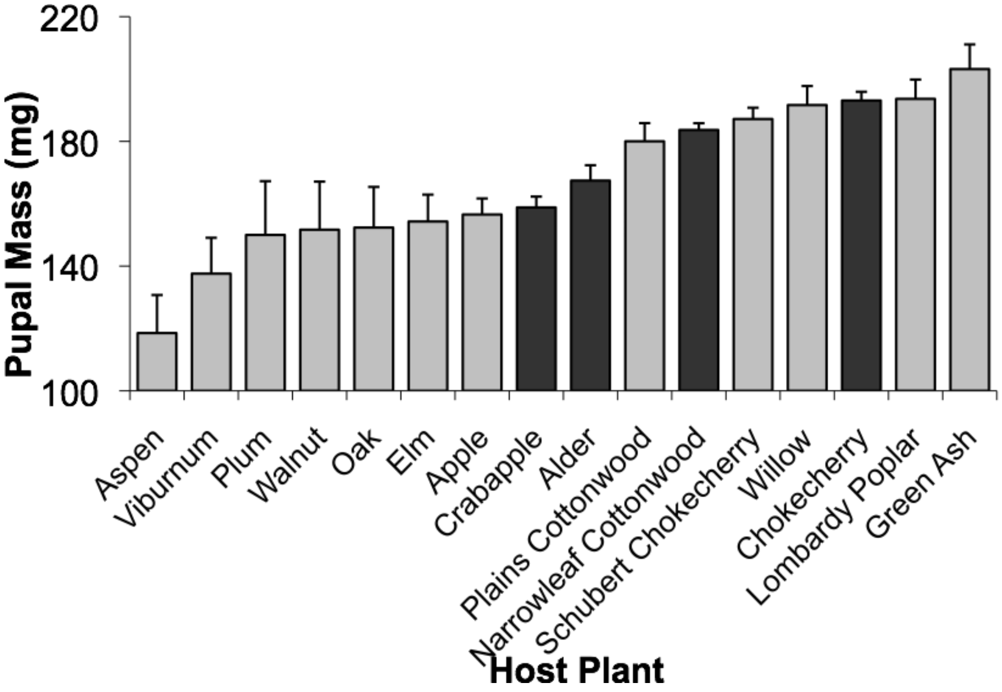

Figure 7. Mean pupal mass for fall webworms collected from the field on different host plants (N = 16). Males and females are combined. Means are given ± standard error and pooled for 2010 and 2011 and all transects. Black bars identify the host species used in the split-brood study.

For larvae reared in the lab as part of the split brood experiment, survival varied by host plant (Fig. 8a; $\chi^2 = 57.85$, df = 3, $p < 0.0001$). Fall webworm reared on chokecherry had 1.5 times more survivors than those reared on alder. Larval development time also varied by host plant (Fig. 8b; $F_{3,329} = 14.8$, $p < 0.0001$). Larvae reared on narrowleaf cottonwood had shorter development times than those on alder by ~2.7 days and larvae on chokecherry developed more quickly than those on both alder and crabapple by ~3.7 and ~2.1 days, respectively (Tukey's HSD test, $p < 0.05$).

Female pupal mass varied among host plants (Fig. 8c; $F_{3,163} = 4.91$, $p < 0.005$); larvae fed crabapple had significantly lower mean pupal mass than larvae fed narrowleaf cottonwood and chokecherry (Tukey's HSD test, $p < 0.05$). The mean female pupal mass of larvae fed on alder was not significantly different from that of any other host plant. Male pupal mass did not differ significantly among host plants ($F_{3,151} = 0.096$, $p = 0.96$; data not shown). Fall webworms reared from eggs in controlled lab conditions had higher mean pupal mass than fall webworms collected from the field when reared on the same host plant ($F_{3,504} = 6.16$, $p < 0.0004$). Fall webworms reared entirely in the lab on alder had a mean pupal mass ~23% greater than fall webworms collected from alder in the field; similarly, fall webworms reared on narrowleaf cottonwood and crabapple in the lab were ~15% larger than their field-collected counterparts (Tukey's HSD test, $p < 0.05$). The mean mass of pupae fed chokecherry did not differ between larvae that were reared entirely in the lab and field-collected individuals that completed their development in the lab (Tukey's HSD test, $p > 0.05$).

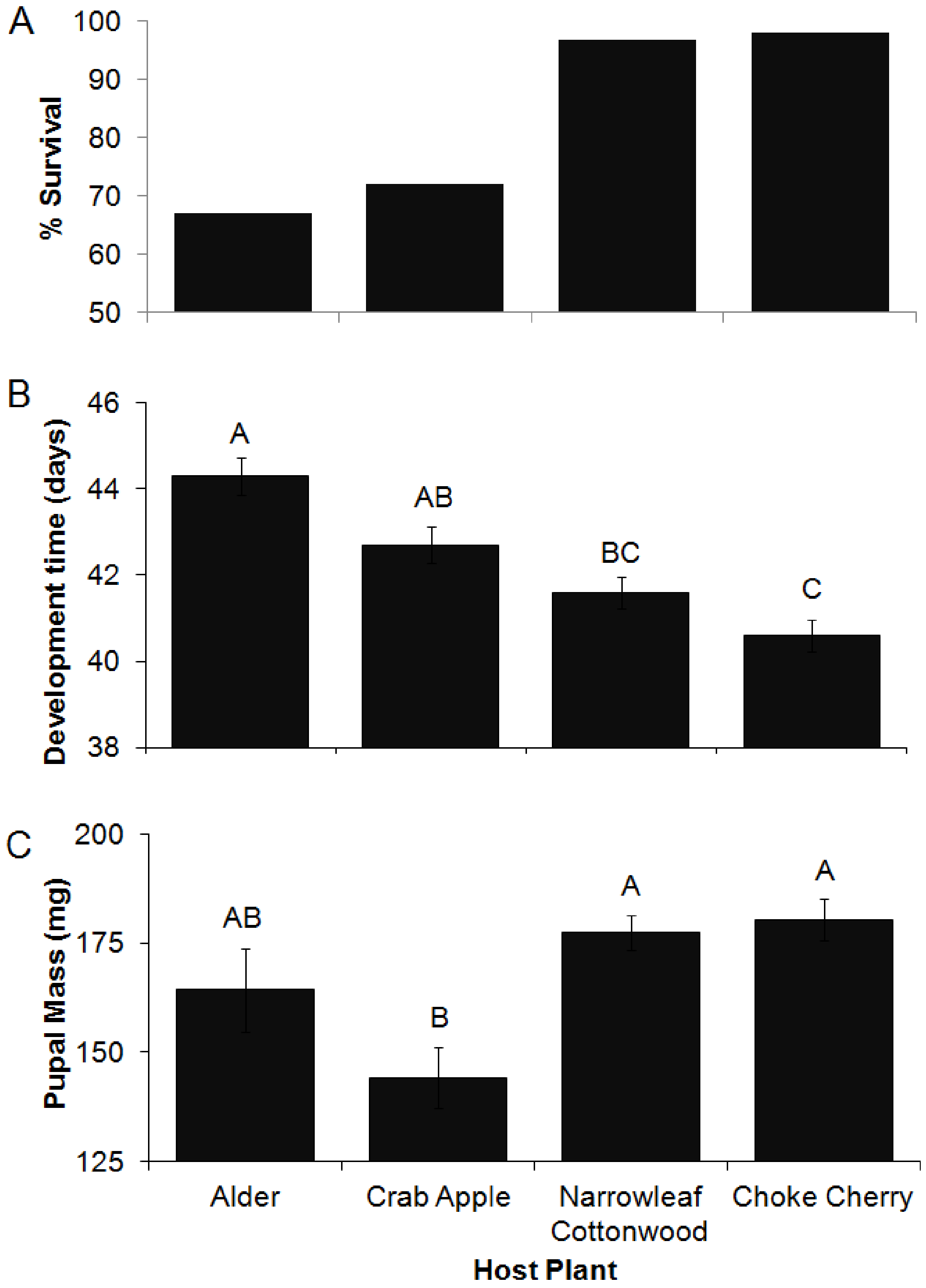

Figure 8. Larval performance, measured by A) survival, B) development time and C) pupal mass on four host plants in the split-brood experiment. Fall webworms of both sexes are included in graphs of survival and development time. Only female fall webworms are included in the graph of pupal mass. Bars represent means ± standard error. Treatments connected by the same letter are not significantly different.

Fitness score, which incorporates both development time and pupal mass, varied by host plant ($F_{3,166} = 11.00$, $p < 0.0001$). Mean larval fitness scores for larvae reared on chokecherry (5.1 ± 0.07) and narrowleaf cottonwood (5.0 ± 0.07) were significantly higher than the mean fitness scores for larvae reared on both crabapple (4.6 ± 0.08) and alder (4.7 ± 0.09)(Tukey's HSD test, $p<0.05$).

Feeding efficiency, the slope of the linear relationship between pupal mass (both sexes lumped) and frass mass, was greatest on chokecherry (slope = 0.19, $R^2 = 0.6$, n = 96, $p < 0.0001$), followed by crabapple (slope = 0.15, $R^2 = 0.33$, n = 69, $p < 0.0001$) and then alder (slope = 0.1, $R^2 = 0.13$, n = 62, $p < 0.005$)(Fig. 9). The slope of feeding efficiency for larvae fed on narrowleaf cottonwood was not significantly different from zero (slope = 0.02, $R^2 = 0.01$, n = 96, $p = 0.3$).

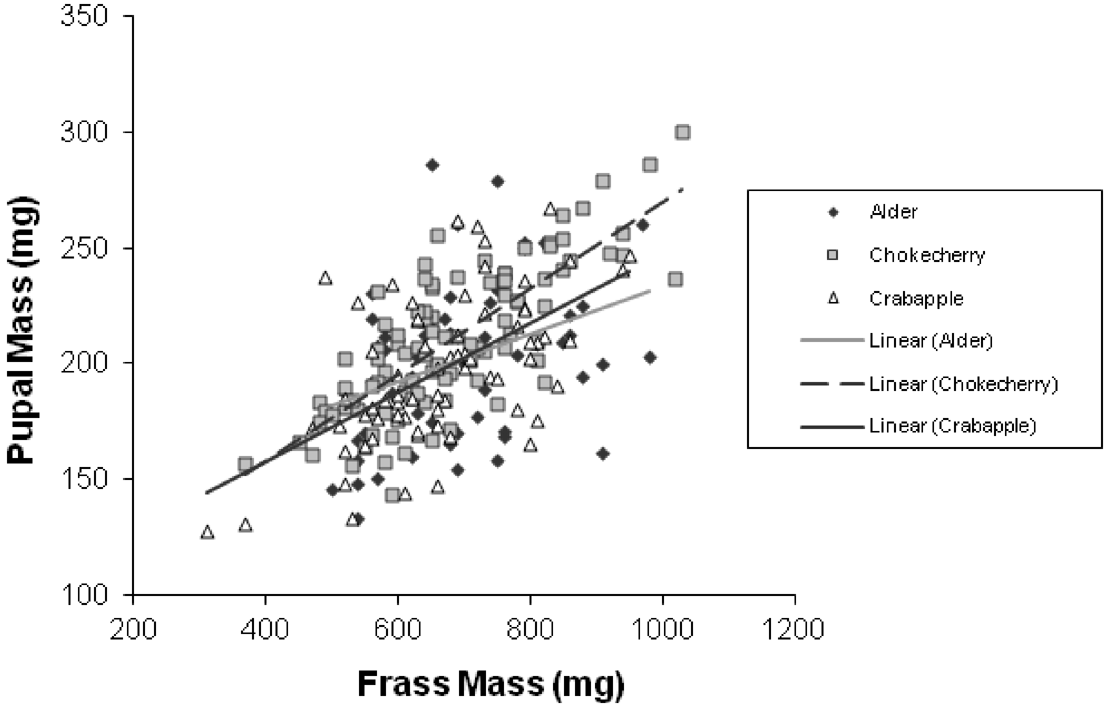

Figure 9. Variation in fall webworm feeding efficiency on three host plants. The slopes are significantly different, indicating that feeding efficiency varies between these hosts. A steeper slope indicates higher feeding efficiency (chokecherry: n = 96; crabapple: n = 69; alder: n = 62). Each point represents an individual insect. The correlation coefficient for fall webworm fed on narrowleaf cottonwood was not significant and was therefore excluded from this analysis.

None of the performance measures, either from field-collected larvae or those in the split-brood experiment, had a statistically significant relationship with relative host use. Lumping all sites and both years, the correlation of pupal mass of larvae collected from the field and relative percent host use was not significant (R^2 = 0.14, N = 16, p = 0.15). Neither survival (R^2 = 0.56, N = 4, p = 0.25), nor fitness score (R^2 = 0.42, N = 4, p = 0.35) were significantly related to fall webworm percent host use.

Mortality from Parasitoids

About one quarter (24%) of all field-collected larvae died as a result of parasitism; to be conservative, we did not include individuals that died from mold, disease, or unknown causes in our estimate of mortality from parasitism. Fall webworms that perished due to causes other than parasitism constituted 7.5% of deaths in 2010 and ~19% of deaths in 2011. The proportion parasitized differed significantly among host plants for both years in wild ($\chi^2 = 18.12$, df = 7, $p < 0.02$) and cultivated ($\chi^2 = 31.88$, df = 4, $p < 0.001$) sites and also when host plants from all sites were analyzed together (Fig. 6; $\chi^2 = 58.65$, df = 15, $p < 0.0001$). There was no relationship between percent parasitism and relative host use within wild sites ($R^2 = 0.27$, n = 7, p = 0.19) or cultivated sites ($R^2 = 0.001$, n = 5, p = 0.95).

Parasitized fall webworm larvae ranged from 6.0-31.7 mm in body length on the day we collected them from the field. The mean length-at-collection of larvae parasitized by tachinid flies was 23.4 mm (± 0.75 mm), which is ~60% longer than the mean length of larvae parasitized by wasps (14.7 mm ± 0.46 mm)($t_{262} = 9.78$, $p < 0.0001$).

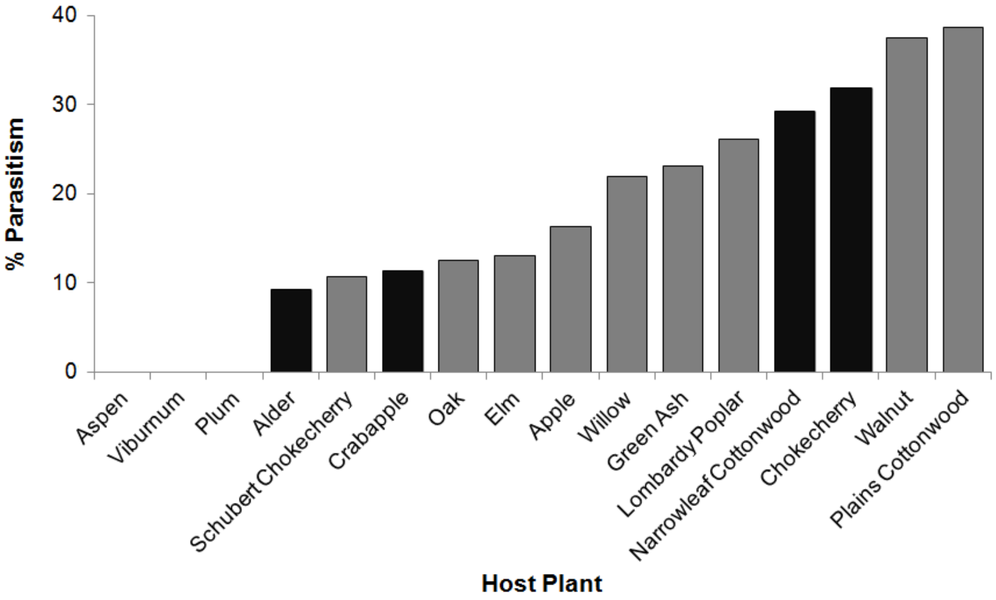

Figure 10. Percent parasitism of fall webworm larvae among all hosts. Data are pooled for all transects and both years. Each bar represents the proportion of fall webworm parasitized on a single host species (N = 16). Black bars identify the host species used in the split-brood study. No parasitoids emerged from larvae reared on host plants without bars.

Trade-offs

As the survival of fall webworms on the four host plants used in the controlled lab experiment increased, percent parasitism on that host in the field also increased (Fig. 11a; $R^2 = 0.99$, n = 4, $p < 0.005$). There was also a significant, positive relationship between fitness score and percent parasitism (Fig. 11b; $R^2 = 0.97$, n = 4, $p < 0.02$). When the analysis includes all hosts, the linear relationship between mean pupal mass of field collected larvae and percent parasitism is weaker, but it remains significant (Fig. 12; $R^2 = 0.38$, n = 16, $p < 0.02$). We identified no other tradeoffs; there was no relationship between host relative percent abundance and percent parasitism ($R^2 = 0.15$, N = 16, $p > 0.1$), pupal mass ($R^2 = 0.13$, N = 16, $p > 0.1$) or fitness score ($R^2 = 0.76$, N = 4, $p > 0.1$).

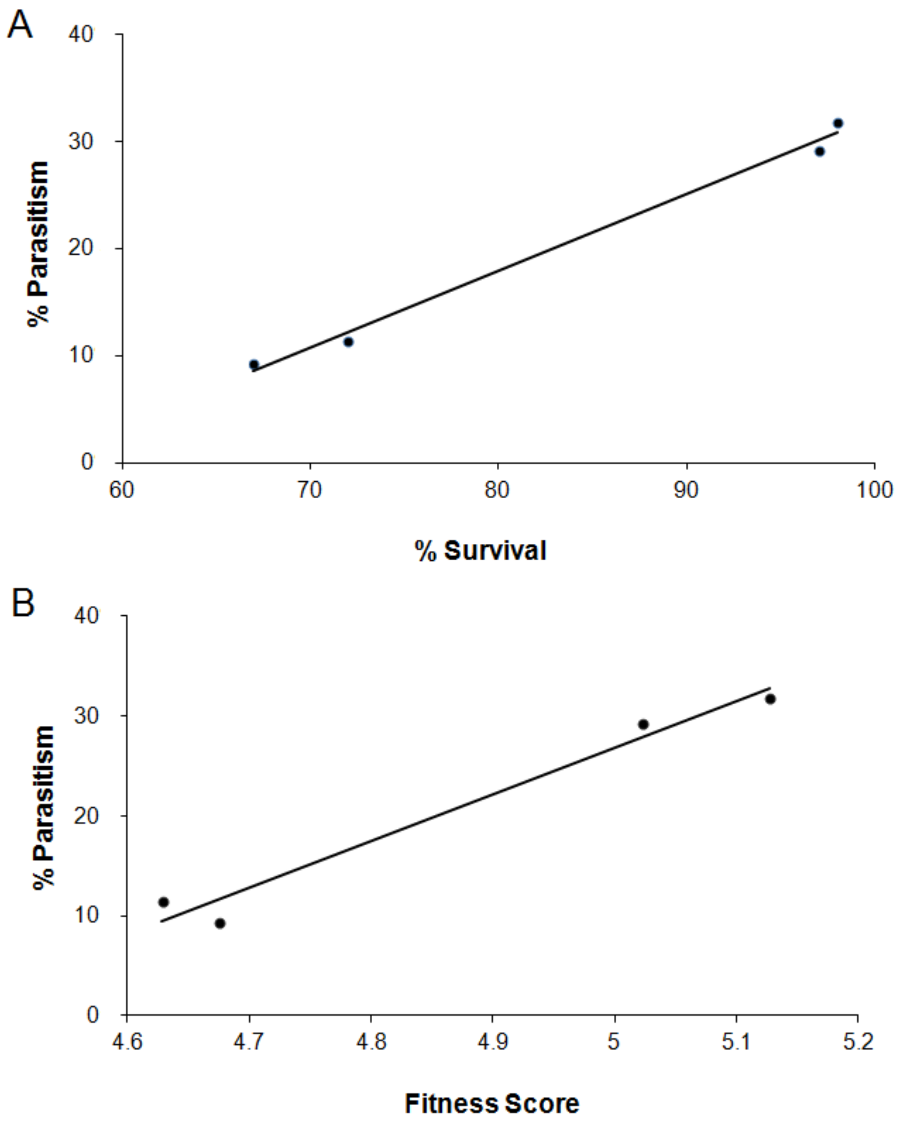

Figure 11. The linear relationship between aspects of host quality-mediated fitness and percent parasitism for four fall webworm host species (A) Survival by host use: n = 4, $p < 0.005$; B) Fitness score by host use: n = 4, $p < 0.02$). Each point represents a single host fed to fall webworm larvae under controlled conditions. Survival is the number of fall webworm of either sex that reached pupation out of 100 individuals per host. Fitness score was calculated as pupal mass (mg)/ development time (days); only female pupae are included in the fitness score.

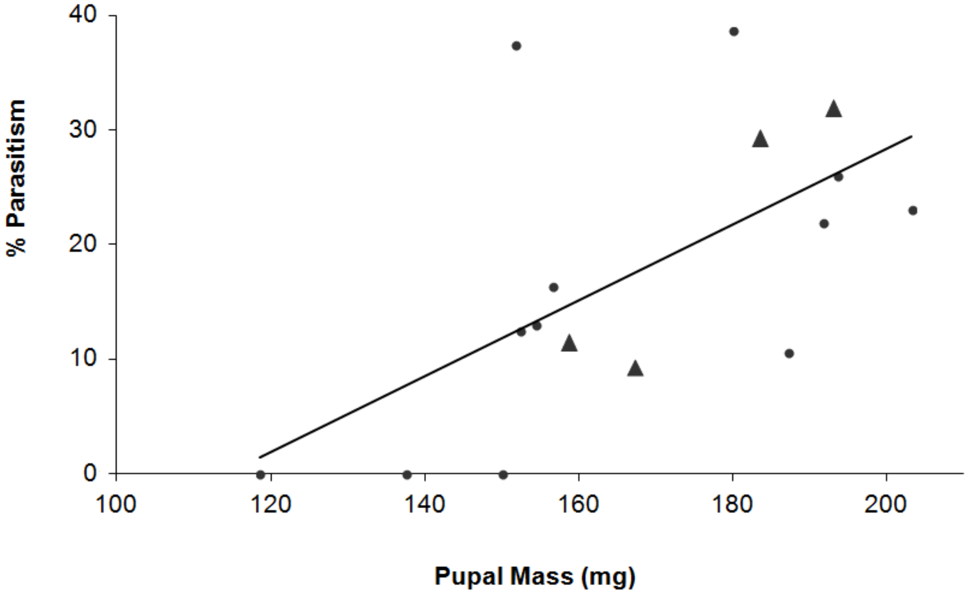

Figure 12. The relationship between pupal mass (mg) and percent parasitism among field collected fall webworm over the course of the study (N = 16). Both sexes and fall webworms of unknown sex are included. Each point represents a single host species. Triangles represent the four host species also used in the controlled experiment. Data were pooled for all sites and both field seasons.

Discussion

Of the three selective pressures that we measured, host plant abundance, larval performance and percent parasitism, only abundance predicted patterns of fall webworm host use in Colorado (Fig. 6). Our results are consistent with those of Mason et al. (2011) whose study of fall webworm populations in the eastern United States found that host availability predicted host use while performance and parasitism did not. Host abundance explained 82% (wild) and 43% (cultivated) of variation in fall webworm host use in Colorado (Fig. 6), while host abundance explained 54% of host use in Connecticut and 90% - 96% of host use in Maryland. The positive relationship between host abundance and host use in wild sites had more explanatory power than the relationship in cultivated sites, although that relationship was driven by the most abundant and most commonly used host, narrowleaf cottonwood. It is notable that host abundance explained more of the variation in host use in Colorado for wild sites than for cultivated sites, which are altered by human activity. Several private homeowners had attempted to control fall webworms on their properties through mechanical and chemical means (personal communication), which may have skewed fall webworm distribution towards taller trees (like plains cottonwood) and communally owned property in those areas.

The two high quality plants, chokecherry and narrowleaf cottonwood, were the most commonly used host plants in wild areas (Fig 5), but we did not find any evidence that plant quality, as measured by larval performance, predicted local host use. Under controlled conditions in our laboratory split-brood experiment, chokecherry and narrowleaf cottonwood emerged as significantly better hosts than crabapple and alder in terms of survival, development time, and pupal mass, which agreed with our previous host plant rankings based only on pupal mass from field-collected larvae. However, larvae reared from eggs in the lab gained significantly more mass over the course of development than did larvae collected from the field on three of the four host plants tested. There are several potential explanations for the mass difference between lab and field reared larvae. First, it is likely that differences in humidity contributed to the changes in pupal mass; fall webworms perform better with greater humidity (Morris and Fulton 1970). In Boulder, Colorado, August was the driest month of 2011, with an average low humidity of 16%. Although we did not measure the humidity within the lab rearing chambers, there was water condensation visible on the inner surfaces of the chambers at all times. Second, we observed that larvae were sometimes food limited in the field when they completely defoliated smaller host plants; in the lab, however, we ensured that larvae always had ample food. Finally, insects are known to change their behaviors in the presence of natural enemies. Studies on the tobacco hornworm have demonstrated that the larvae spend less time feeding with predators present (Thaler and Griffin 2008). In the field, we have observed pentatomid adults and nymphs feeding on fall webworm larvae within their webs several times. We have also observed *Polistes* wasps hovering near and darting towards webs full of larvae. We did not observe any instances of bird predation, but it has been documented for fall webworm at other sites (Ito 1977). These three factors are not mutually exclusive and may have acted in concert to contribute to the difference we observed in pupal masses of field-collected and lab-reared fall webworms.

Mortality from parasitoids varied among host plants, but did not predict host use in our study populations. Tachinid fly and wasp parasitoids were responsible for nearly a quarter of the mortality that we observed. Interestingly, we found evidence suggesting that parasitoid wasps attack smaller fall webworm larvae than do parasitoid flies. It is important to note that we know the size of the larval host when it was collected from the field, but not the size at which it was attacked, which would be equal or less than the size at collection. The only other studies to investigate host partitioning by parasitoid communities also found that parasitoid flies tend to attack larger larvae than parasitoid wasps (Stoepler et al. 2011, Murphy et al. in review); both of these studies focused on larvae in the family Limacodidae and our similar findings for larval hosts in the family

Erebidae lend additional support the hypothesis that parasitoid flies and wasps partition hosts by size.

Although fall webworms did not show a preference for host plants that provided better larval performance, their parasitoids did (Fig. 11). Larvae on the highest quality host plants also experienced the highest levels of mortality due to parasitism. That trade-off could reinforce polyphagy if the fitness benefits of experiencing less parasitism offset the benefits of feeding on a high quality plant. We do not know why parasitism pressure on fall webworms was higher on some plants than on others. The size of host larvae (Stoepler et al. 2011), host immune function (Schmid-Hempel 2005), host density (Lessells 1985), light environment (Stoepler and Lill In Press) and volatile cues released by herbivore-damaged plants (Turlings et al. 1990) have all emerged as important factors in determining parasitism levels.

Our findings that host plant abundance drives plant use in fall webworms suggests that fall webworms are under selective pressure to minimize the amount of time they spend searching for a suitable host, as was also found by Mason et al. (2011). The selective pressure of search time limitation was also important in a previous study in which gravid butterflies given access to an additional host species laid more eggs than butterflies with only one available host (Johansson et al. 2007). Since none of the other selective pressures we measured other than host abundance correlated with relative percent host use, our study does not explain differences in fall webworm host breadth between Colorado and East Coast populations. However, patterns of plant abundance may explain why fall webworms use fewer tree species in wild sites in Colorado than they do in Maryland and Connecticut. Apparent "local specialization" in a generalist herbivore may be due to fewer locally available host plants (Fox and Morrow 1981). Eastern forests are more species rich than the riparian forests of Colorado's foothills and adjacent plains (Waring et al. 2006); thus fall webworms may feed on the same proportion of potential hosts in both regions. Differences in diet breadth and host selection between eastern fall webworms and fall webworms in cultivated sites of Colorado need further explanation. Many more trees and shrubs, including ones common in eastern forests, are planted in Colorado residential developments (Murphy, personal observation) and are thus available to fall webworms as potential hosts. Additional plant survey data are needed for a complete comparison of the proportion of available plants used as fall webworm hosts.

Conclusions

Host availability is the primary driver of patterns of host plant use by fall webworm populations in Colorado, which agrees with the results that Mason et al. (2010) found for fall webworm populations in the eastern United States. It’s interesting to note that populations in Colorado that use a limited number of hosts appear to be driven by the same selective pressures as those in the eastern US, where they have a much broader diet breadth. Despite the importance of host availability to fall webworms in both the western and eastern US, these populations differ in important ways. Fall webworms in the eastern US do not experience trade-offs between larval performance on a host plant and percent parasitism on the same host, while the Colorado populations experienced a strong trade-off. This difference, which could potentially contribute to a generalist feeding strategy, underscores the importance of understanding the geographic mosaic of selection in wide ranging species.

CHAPTER THREE: DIFFERENTIATION IN A WIDESPREAD GENERALIST MOTH: REVISITING THE QUESTION OF HOST RACES IN THE FALL WEBWORM (*HYPHANTRIA CUNEA*)

Introduction

Herbivorous insects are small and many species spend the majority of their lives on a single plant (Funk et al. 2002). The benefits of adapting to a specific type of host plant may include emerging at a time in the growing season when the plant is available as food (Funk et al. 2002) and the physiologic ability to process the host's toxic secondary compounds (Schoonhoven et al. 2005). In sympatric populations, host plant associations can also lead to differentiation (Berlocher and Feder 2002) and as differentiation progresses, distinct host-associated lineages may form. Drès and Mallet (2002) defined host races using four criteria. First, host races use different hosts in the wild, and display fidelity to the same hosts over generations. Second, host races live, at least partially, in the same geographic location. Third, they are genetically differentiated in at least two loci. Finally, there is gene flow between host races within a species. Genetic differentiation of herbivorous insects by host plant has been supported in a number of systems including the cotton fleahopper on three host species (Barman et al. 2012) as well as a stem-galling moth, gall-making midges, a gall-making fly, and a stem boring beetle on goldenrod species (Stireman et al. 2005), and walking sticks on two host species (Nosil et al. 2002).

The fall webworm (*Hyphantria cunea*), a web-building caterpillar in the Erebidae family, is a good candidate to investigate geographic and host plant-based genetic differentiation. Fall webworms in North America have two morphologically distinct varieties. As larvae, one morph has a black head and black tubercles, and the other has an orange head and tubercles, although the "orange" can appear brown or pinkish depending on the individual and its developmental stage (Masaki and Ito 1977). The two color morphs are respectively known as black-headed and red-headed. Originally, the presence of spots on the wings of adults was also used to differentiate fall webworms, but the spotting was later found to be mutable and based largely on pupal temperature (Morris 1963).

Previous studies of fall webworms have suggested that red and black-headed morphs are genetically distinct (Oliver 1964, Masaki and Ito 1977, Jaenike and Selander 1980). Behavioral differences have also been observed between the color morphs; red-headed fall webworms spin thick, opaque webs, and only leave the web to feed at night (Oliver 1964, Masaki and Ito 1977), while black-headed larvae spin coarse webs and fed during the day (Masaki and Ito 1977). Additionally, the two color morphs are behaviorally distinct as fifth instar larvae; black-headed fall webworm leave the web during the ultimate instar and become solitary (Szalay-Marzso 1972), whereas red-headed larvae cluster within the communal web until pupation (Oliver 1964). Red and black-headed color morphs also differ in body size and the duration of larval development (see Chapter 1).

In addition to genetic differentiation, host races must use different hosts in the wild and exist in sympatry in at least part of their range (Drés and Mallet 2002). The fall webworm is highly polyphagous, and feeds on dozens of plant families throughout its worldwide range (Warren and Tadić 1970). Regionally, their diet can narrow to about a dozen locally available host plants (Mason et al. 2011, Loewy personal observation). Although as a species fall webworm is a diet generalist, individuals are functionally monophagous and feed on a single host plant. In areas where black and red-headed fall webworms co-occur, they have been noted using different host plants, albeit with some overlap. For example, black-headed fall webworms in Louisiana were found on sweetgum, persimmon and willow, while red-headed fall webworms used pecan and persimmon (Oliver 1964). In Arkansas, red-headed fall webworms were observed on pecan, walnut, and persimmon, while black-headed fall webworms were observed on mulberry, sycamore, *Populus* spp., and various species in the Rosaceae.

A study in New York tested whether red and black-headed fall webworms from a single county were genetically distinct and if genetic variation could be explained by host plant use (Jaenike and Selander 1980). Based on allele frequencies at eight genetic loci, Jaenike and Selander (1980) concluded that reproductive isolation between red and black-headed morphs was complete and that the two color morphs were separate species. Among other observations, they noted that at one locus the two morphs were fixed for different alleles. They also found no evidence of host race formation within red and black-headed morphs. However, all fall webworms in the New York study came from a single county and there have not been any additional studies on genetic differentiation of fall webworms across a greater geographic range or a greater diversity of host plants.

Here, we test the extent and patterns of differentiation between red-headed and black-headed fall webworm using individuals sampled over a large geographic area and from a variety of different host plants. The aim of this study is to 1) determine if red and black-headed morphs of fall webworm are genetically distinct across broader sampling ranges, and 2) test the relative contributions of geographic isolation and host plant associated differentiation to genetic variation in fall webworms. We use sequences of mitochondrial cytochrome oxidase 1 from fall webworms collected from 12 US states and 4 other countries to investigate genetic variation on a large geographic scale.

Materials and Methods

Geographic Origin of Specimens and Collection Technique

With help from many collaborators, we collected fall webworm larvae from 10 states within the US (Colorado, Connecticut, Maryland, Missouri, Ohio, Oklahoma, Oregon, South Carolina, Tennessee, and Texas) and Washington, DC (Table 3). We scanned for fall webworm webs at wooded edges along flyways, such as roads and rivers, where webs were easily visible on the outer branches of broadleaf trees. We removed several larvae from each web and preserved them at room temperature in glass vials with 95% ethanol until DNA extraction. For samples collected in Colorado, we identified the morph (red or black-headed) of living larvae upon collection by observing the head capsule color. For larvae from other states, morph identification took place after alcohol preservation and DNA extraction in cases where intact heads of siblings remained in the collection vials. We retrieved 12 additional sequences of fall webworm mitochondrial CO1 from Asia, Europe, and North America from NCBI GenBank (Table 3).

Table 3. Fall webworm collection locations, including CO1 sequences acquired from NCBI GenBank.

Location (number of sequences)	Accession number* (where applicable)
Ontario, Canada (1)	AF549608.1
Colorado (32)	
Connecticut (15)	
Florida (1)	DQ116194.1
Hungary (1)	DQ116183.1
Japan (1)	AB077293.1
Kentucky (1)	DQ116193.1
Maryland (10)	
Washington, D.C. (7)	
Missouri (14)	AB105317.1; AB105318.1
Ohio (12)	
Oklahoma (1)	
Oregon (1)	
South Carolina (1)	
South Korea (1)	AB105312.1
Tennessee (5)	AB105313.1, AB105314.1; AB105315.1; AB105316.1
Texas (1)	

*NCBI Genbank samples originate from three papers. Prefix "AB" = (Gomi et al. 2004), "AF" = (Hebert et al. 2003), and "DQ" = (Armstrong and Ball 2005).

DNA Data Collection

We used different procedures for DNA extraction, polymerase chain reaction (PCR) and sequencing depending on where the specimens were processed. DNA from fall webworm collected in Ohio, Connecticut, Maryland, Washington, D.C., Missouri, Texas, Tennessee, Oklahoma, South Carolina and Oregon was extracted from head capsules using Puregene DNA Purification System, Cell and Tissue Kit (Gentra Systems

Inc., Minneapolis, MN) following Stireman et al. (2008). A 684 bp segment of mitochondrial cytochrome oxidase 1 (CO1) was amplified between primers Lep-F1 and Lep-R1 (Hebert et al. 2004)(Table 4). PCR thermal cycling was done using a single preheat step at 94°C for 2 minutes followed by 35 cycles of denaturing at 94°C for 30-45 seconds, annealing at 37°C for 45–60 seconds, and extending at 72°C for 1 minute. A final 72°C extension of 4 minutes completed the reaction (modified from Stireman et al. 2005). The PCR product was shipped to Arizona Research Labs in Tucson, Arizona for purification and sequencing in both directions.

Table 4. Primers used in the amplification of fall webworm mitochondrial cytochrome oxidase 1.

Name	Sequence	Source
Lep-F1	5’-ATTCAACCAATCATAAAGATAT-3’	(Hebert et al. 2004)
Lep-R1	5’-TAAACTTCTGGATGTCCAAAAA-3’	(Hebert et al. 2004)
Lep-86F	5’-TGAGCAGGAATAGTTGGAACATC-3’	This study
Lep-734R	5’-GAGAAATTATTCCAAATCCTGGTA-3’	This study

DNA from specimens collected in Colorado was extracted from head capsules using the DNeasy Blood and Tissue Kit extraction procedure for insects (Qiagen, Germantown, MD). To increase yields, we extracted DNA of 1-5 larvae from the same mother (web) to create a single, larger sample. With reference to the fall webworm mitochondrial genome published by Liao et al. (2010), we designed primers Lep-86F and Lep-734R (synthesized by Sigma Life Science, St. Louis, MO) to flank a 647 bp region of COI that had considerable overlap with the region amplified by Lep-F1 and Lep-R1 (Table 4). PCR thermal cycling was done using a single preheat step at 94°C for 1 minute followed by 35 cycles of denaturing at 94°C for 40 seconds, annealing at 50°C for 40 seconds, and extending at 72°C for 1 minute 30 seconds. A final 72°C extension of 7 minutes completed the reaction. The amplified samples were purified via an EXO/SAP enzymatic clean-up by mixing 1 µl of exonuclease 1 (EXO) and 1 µl of shrimp alkaline phosphatase (SAP) with each 20 µl PCR product. Incubation occurred in two steps, 37°C for 45 minutes and 80°C for 45 minutes. Purified samples were sequenced in reverse from the Lep-734R primer by Eurofins MWG Operon (Huntsville, Alabama).

Analysis

We reviewed chromatograms with Sequence Scanner (Applied Biosystems, Carlsbad, CA) to trim sequence ends and replace questionable bases with “N” in cases where quality values were below 20. We aligned the trimmed sequences plus those obtained through GenBank in Mega 5 (Tamura et al. 2011) using ClustalW with the MEGA program default settings. Sequences from previous studies, and those from

Colorado, were shorter than those prepared in Ohio. The most common sequence length was 684 bp.

A previous study found that fall webworm larvae within a single web were full siblings (Jaenike and Selander 1980). In our study, there were 26 cases in which 2 or more individuals from the same web were sequenced separately. For phylogenetic reconstruction and AMOVA analyses, we haphazardly used a single individual from each web, which brought the total number of sequences to 106 (Table 3). In Colorado, multiple larvae from the same web had been sequenced as a single sample, making it impossible to throw out duplicate individuals from the same web. Accordingly, in Colorado, each sample is represented by the most dominant haplotype among supposed siblings.

A file containing sequence information from one representative of each unique haplotype was used to produce trees describing the relationships among haplotypes. Neighbor Joining trees (Saitou and Nei 1987) were calculated in MEGA5 using the "compute maximum composite likelihood of transition/transversion bias" and “pairwise deletion” options. Bootstraps were generated using 1000 replicates (Felsenstein 1985). The Kimura 2-parameter correction (Kimura 1980) was applied with a gamma shape parameter estimated through MEGA5 (Tamura et al. 2011) under the HKY+G model (Hasegawa et al. 1985). Another moth species in the Erebidae family, *Estigmene acrea* (EU119586.1), served as the outgroup to root phylogenic trees. The overall transitions/transversion bias (*R*) across all pairwise comparisons, nucleotide frequencies across all samples, and average within and between group distances (base substitutions per site) for the major clades were also calculated ± standard error (from a 1000 bootstrap test) within MEGA5 (Tamura et al. 2011).

A Bayesian phylogeny based on the same dataset was also completed using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) using a general time reversible model with six substitution types for each molecular partition allowing for a proportion of invariant sites (nst=6, rates=invgamma, shape=0.0744, Pinvar=0.8354). Four Markov-chain Monte-Carlo chains were used with interchain exchange set to a temperature of 0.2, sampling every 100 generations. Convergence was reached after 8 million generations with <0.01 standard deviation of split frequencies. The first 20,000 trees (2 million generations) were eliminated as burnin.

To prepare the trees for visual analysis, we included information on the geographic origin of the samples, the color morphs they represent, and the host plant from which they were collected. To increase overlap between distant sites, we performed all host plant analyses using genera rather than species. For example, *Prunus virginiana* was only used as a fall webworm host in Colorado, whereas *Prunus serotina* was recorded as a host in four other states (Table 5). Categorizing sequences by genus created taxonomic associations that were present in a higher number of sampling locales.

Table 5. Fall webworm host plant genera listed by collection state. Full species names are included where applicable. Numbers in parenthesis indicate the number of fall webworms sequenced from the preceding host plant. States are abbreviated and presented in alphabetical order. Maryland (MD) includes sites in Washington, District of Columbia.

Genus or Species (# individuals)	State
Acer negundo (7)	CT, MD
Ailanthus altissima (1)	CT
Alnus tenuifolia (1)	CO
Betula pendula (2)	CT
Carya sp. (5)	MD, MO
Carya tomentosa (2)	OH
Cephalanthus occidentalis (1)	CT
Cercis canadensis (6)	MD, MO, OH
Diospyros virginiana (6)	MO, TN
Fraxinus pennsylvanica (6)	CO, CT, MD
Juglans sp. (4)	CO, MD, MO, OK
Juglans regia (1)	OR
Liquidambar (2)	MD, OH
Liquidambar styraciflua (1)	SC
Malus sp. (2)	CO
Malus domestica (2)	CO
Morus sp. (1)	MD
Nyssa sylvatica (1)	MD
Oxydendron arboreum (4)	OH
Platanus sp. (1)	MD
Populus angustifolia (7)	CO
Populus deltoides (3)	CO
Populus tremuloides (1)	CO
Prunus serotina (7)	CT, MD, OH, MO
Prunus virginiana (8)	CO
Quercus sp. (1)	CO
Salix nigra (3)	CT
Ulmus sp. (2)	CO
Ulmus rubra (3)	CT
Viburnum sp. (1)	CO

We ran 6 hierarchical AMOVAs with Arlequin 3.5 (Excoffier and Lischer 2010) to incorprorate every combination of color morph, geographic origin, and host plant as major groupings and subgroupings. When the major grouping was by color morph (red or black-headed), individuals of unknown morph were excluded from the analysis. When samples were grouped by geographic origin, states and nations with a single sequence were excluded from the dataset. Finally, when samples were grouped into shared host plant genera, any genus represented by a single sequence was excluded from the dataset.

Results

Out of 26 pairs or groups of larvae collected from within the same web, 6 had more than one haplotype among presumed siblings: the remainder had sequences identical to other individuals from the same web. Of the 106 individuals that remained after haphazardly removing within-web replicates, there were 40 unique haplotypes with a total of 47 variable sites. The majority of variable sites were in the third codon position (n=43), with no change in translation. Only one position in a single individual coded for a different amino acid (an individual found on *Ulmus rubra* in Haddam State Park, Connecticut). The nucleotide frequencies across 106 sequences were 30.76% (A), 38.18% (T/U), 14.81% (C), and 16.26% (G). The overall transition/transversion bias was R = 4.74. The gamma shape parameter was calculated at 0.05.

Both Bayesian and Neighbor Joining phylogenetic trees shared similar topology. The tree is split into two clades by fall webworm head color morph (red or black) (Fig. 13). One clade contains both black and red-headed morphs, and is supported by a

Bayesian prior probability value of 94 and 86% of bootstrap test iterations. Members of the other clade, supported by a prior probability value of 88 and 99% of bootstrap iterations, are solely black-headed. Mean within group distance for the mixed clade is 0.018 ± 0.006 and 0.005 ± 0.001 for the all black-headed clade. The mean distance between mixed and black-headed clades is 0.064 ± 0.022. On two occasions, both red and black-headed individuals share identical CO1 sequences within the mixed clade. Samples from outside of the US are exclusively in the all black clade, and fall webworms from the Asian nations, Japan and South Korea, have identical sequences.

A red-headed subclade is supported by a prior probability value of 98 and a bootstrap percentage of 82. All fall webworms in that subclade originated in Colorado. In Colorado, the 32 sequenced individuals share five unique CO1 haplotypes. Four of the five haplotypes have one fixed transition and one fixed transversion present only in Colorado fall webworms. A single individual carried the fifth haplotype which lies outside the Colorado subclade in a larger clade that is supported at a prior probability value of 93 and a 80% bootstrap level, which includes sequences of both color morphs from Connecticut, Ohio, Maryland/DC and Missouri.

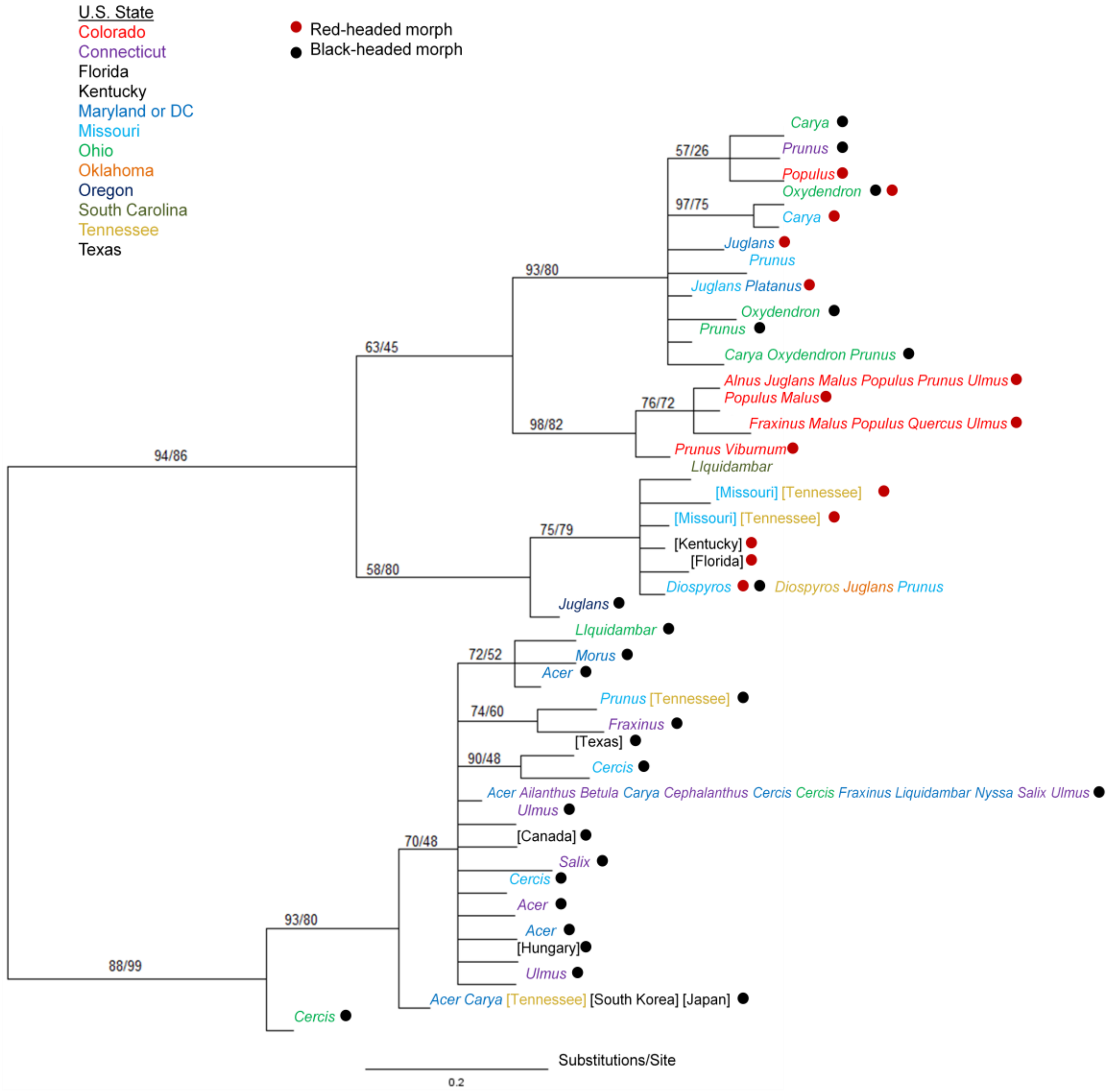

Figure. 13. A phylogenetic tree of fall webworm CO1 haplotypes showing host plant genus, state or nation of origin, and color morph (red or black-headed). Samples lacking host plant information are indicated by state or nation of origin in brackets. Color morph symbols are to the right of samples in which head capsule color is known. The tree is drawn to scale. Numbers on branches indicate the Bayesian prior probability value followed by the bootstrap value, which is the percentage of 1000 replicates in which the sequences clustered together as shown using the Neighbor Joining method. Branches with prior probability values under 50% are collapsed to the preceding node. The tree is rooted with an outgroup, *Estigmene acrea*, which has been erased for this display.

Eight host plant genera are represented by samples from more than one state (Table 5). Of those eight, *Cercis* and *Acer* are only found in the all-black clade, and

Juglans is only found in the mixed clade. *Carya*, *Fraxinus*, *Liquidambar* and *Prunus* are in both clades. The six samples of fall webworms that fed on *Diospyros* in Missouri and Tennessee all share an identical sequence.

Six AMOVAs partitioned the relative contribution of color morph (red or black), geographic location, and host plant genus to variation in fall webworm sequences (Table 6). Whether fall webworms had a red or black head capsule accounted for most of the variation (48.37% and 39.97%) followed by location and host genus. The second column, percent molecular variation among subpopulations, within groups, supports that ranking. When color morph is nested as a subpopulation, both location and host plant contributions to genetic variation become non-significant. When host plant is nested as subpopulations within the greater geographic structure, variation due to host plant subpopulations is less than a percentage point higher than the major grouping. In contrast, when geographic subpopulations are nested within host genera as a major grouping, the subpopulations are responsible for ~30 percentage points more variation in fall webworm CO1 sequences.

Table 6. AMOVA calculation results for 107 fall webworm CO1 sequences. *P*-values based on 1023 permutations are in parentheses.

Grouping criteria	Analysis of molecular variance (%)		
Major Group/ Subgroup	Among groups	Among populations within groups	Within populations
Morph/ Geography	48.37 (0.00293±0.00164)	28.23 (0.00000±0.00000)	23.40 (0.00000±0.00000)
Geography/Morph	12.30 (0.54936±0.01492)	58.86 (0.00000±0.00000)	28.84 (0.00000±0.00000)
Geography/Host	38.49 (0.00000±0.00000)	39.12 (0.00000±0.00000)	22.39 (0.00000±0.00000)
Host/Geography	23.97 (0.01564±0.00394)	54.12 (0.00000±0.00000)	21.92 (0.00000±0.00000)
Host/Morph	16.00 (0.22190±0.01244)	57.80 (0.00000±0.00000)	26.19 (0.00000±0.00000)
Morph/Host	39.97 (0.00000±0.00000)	38.67 (0.00000±0.00000)	21.35 (0.00000±0.00000)

Discussion

The color morph of a fall webworm, red or black-headed, is responsible for the majority of variation in a 684 base pair sequence in the CO1 gene. However, the local evidence for speciation found by Jaenike and Selander (1980) in New York does not fully extend to the rest of the US. In our study that covers a larger geographic range, we found that one clade is composed entirely of black-headed individuals, including the black-headed fall webworms that are now invasive pests in Europe and Asia. However, the other major clade formed by the phylogenetic tree of mitochondrial haplotypes in our more comprehensive study contains both red and black-headed individuals. Furthermore, two unique haplotypes in the mixed clade contain both red-headed and black-headed members (Fig. 13). Red and black-headed fall webworms are capable of interbreeding in laboratory conditions (Oliver 1964, Masaki and Ito 1977) and our evidence of shared haplotypes suggests that they can interbreed in natural conditions as well.

When we identified color morph from preserved and live specimens, head capsules that appeared intermediate in color were removed from the color facet of analysis. At times we observed red-headed fall webworms with some black markings, and black-headed fall webworms sometimes showed variation in head capsule coloration when stored in ethanol (Peri Mason, personal communication). Among black and red-headed fall webworms, there may be red-black hybrids, or potentially members of a third color morph, that were not identified as such for this paper. Masaki and Ito (1977) observed, measured, and made predictions about the geographic range of "mottled-headed" fall webworms in the US. To our knowledge, no additional studies have examined this possible third morph with intermediate head color. It would be interesting to make more detailed observations of head capsule coloration and determine where intermediate or "mottled-headed" morphs lie on a phylogeny constructed with nuclear markers.

Geographic location contributes to fall webworm genetic variation and color morph distribution. Only red-headed fall webworms have been observed in Colorado (Swain 1936), which is geographically isolated and ecologically distinct (mountainous) from the other collection sites. The majority of samples in both clades were collected from sites within the eastern and midwestern United States. The apparent monophyly of most haplotypes of Colorado fall webworms in the foothills of the Rocky Mountains may be an artifact of limited sampling on the Great Plains, and to the north and south of Colorado. Only four samples represent the Great Plains region; two sequences came from Warsaw, Missouri (Gomi et al. 2004), which lies near the edge of the Great Plains, and an additional two samples came from the Great Plains regions of Oklahoma and Texas. Currently, the sequence from Houston, Texas represents the only haplotype outside of the temperate forests of the Eastern US in the all black-headed clade. Additional sampling is needed to clarify the extent of the all black-headed clade in North America.

Although Jaenike and Selander's (1980) findings that red and black-headed fall webworms are separate species did not extend beyond their New York site, their conclusion that fall webworms have not developed host races cannot be refuted by the weak relationship between host plant and genetic differentiation in our study. To be considered as different host races, an insect must be more closely related to distant individuals on the same host than to nearby individuals on a different host (Drés and Mallet 2002). However, host plant was confounded with geography in our study; host species in the eastern US were different from those in the west (Table 5). We categorized host plants by genus instead of species to increase the incidence of similar hosts in different regions and of 22 host plant genera included in this study, only 8 were sampled from more than one state. Among the 8 host plant genera that we sampled from multiple states, fall webworms representing both the mixed and black-headed clades were sampled from 4 tree genera while the other 4 genera had fall webworms exclusively from a single clade. For example, fall webworms collected from *Diospyros* in two states shared the same haplotype, but these two states are also neighbors so host plant remains confounded with geography. Thus, if host race formation is occurring, it is not ubiquitous.

In our study, sampling efforts were concentrated in the eastern and midwestern US as well as the foothills of the Rocky Mountains in Colorado, leaving large swaths of the country underrepresented. It is possible that our limited sample size in some states reflects relatively low fall webworm population densities in those regions. For example, the Great Plains may be a true barrier to fall webworm gene flow. Plentiful webs are frequently visible lining the roadways in the eastern US, but they are suddenly absent from the Great Plains (Murphy and Loewy, personal observations). Adult fall webworms have been recorded in the plains of Colorado (Ferguson et al. 2000), and were collected in Oklahoma and Texas for this study, but the host plants were not identified, leaving the identity of hosts that bridge the Great Plains a mystery. Considering that host plant is confounded with geographic location in our study, greater effort to sample from the same host plants in different geographic regions throughout the United States would greatly improve analysis of fall webworm variation.

Although our results do not support host race formation, there is evidence that local adaptation has occurred. Six fall webworm samples were collected from *Acer* *negundo* in Maryland and Connecticut and all haplotypes from those samples sorted into the all black-headed clade. *Acer negundo* is also known to be a high quality host plant for fall webworms in the eastern US (Mason et al. 2011). Although *A. negundo* is plentiful in the Colorado canyons where fall webworms were sampled, only two webs (out of 517 observed across 17 different host species) were observed on *A. negundo* trees over the course of two years (Loewy, personal observation). Moreover, Colorado fall webworms reared on *A. negundo* have high mortality rates and low lifetime fitness (Murphy, unpublished data), which suggests that red-headed fall webworms in Colorado may have adapted to local host plants and have lost the ability to thrive on *A. negundo*. A cross-fostering experiment in which East Coast fall webworms from the all black-headed clade are fed *A. negundo* grown in Colorado and Colorado fall webworms are fed on the same host found in the eastern US may identify differences in feeding abilities between the clades. Although we were not able to support host-associated differentiation in this study, host use patterns should be reexamined focusing on potential hosts that exist in multiple regions.

We tested three variables that may be related to genetic variability in fall webworms: color morph (red or black-headed), geographic separation, and host plant. We found that red-headed and black-headed fall webworms are not reproductively isolated. However, we did find evidence for two genetic groups: one that is exclusively black-headed and one that is both red and black-headed. Geographic location explains more genetic variation than does host plant use, which suggests that fall webworms may be adapting locally, but are not forming host races. However, geographic location and host plant are often confounded in our study, and additional sampling from populations in the western US and on multiple, widespread hosts would clarify each factor's impact on fall webworm differentiation.

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