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Running head: Silkmoth parasitism by *Compsilura*

Reduced *Compsilura concinnata* parasitism of New England saturniid larvae

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Abstract

1. In the northeastern United States, the non-native generalist parasitoid *Compsilura concinnata*, introduced in the early 20th century to control forest pests, has been linked to the decline of giant silk moths (Lepidoptera: Saturniidae).

2. Field research conducted in New England in the late 1990s on two saturniid species, *Hyalophora cecropia* and *Callosamia promethea*, found *C. concinnata* parasitized 81% and 68%, respectively, when larvae were reared outdoors and replaced weekly. These parasitism rates, extrapolated over the larval period, would prevent any larvae from reaching pupation.

3. In 2017 and 2018, we repeated this field experiment using these same two saturniid species for the same duration, and at the same site, location, and time of year. In 2017, *C. concinnata* parasitized only 19% of *H. cecropia* larvae and 1% of *C. promethea* larvae; in 2018, parasitism rates were 3% and 0%, respectively.

Key Words

saturniid, parasitoid, invasive species, community ecology

Introduction

Populations of silk moths (Lepidoptera: Saturniidae) in the northeastern United States have declined precipitously over the past half-century (Boettner et al. 2000, Wagner 2012, Goldstein et al. 2015). All saturniids have been affected, and the regal moth (*Citheronia regalis*) has been extirpated from the New England mainland, the imperial moth (*Eacles imperialis*) is a Massachusetts Division of Fish and Wildlife Threatened Species, and the barrens buck moth (*Hemileuca lucina)* is a Species of Special Concern in Massachusetts (Wagner 2012, Goldstein et al. 2015). Several anthropogenic causes for this decline have been hypothesized: these include habitat loss, human development (including electric lighting), and control efforts for gypsy moth

(*Lymantria dispar*) and other forest pests (Fitzgerald 1995, Wagner 2012). The latter threat reflects the fact that since saturniids and gypsy moth larvae overlap in host usage, pesticide use on deciduous trees should affect both groups. Because most pesticide use was localized and heavier in the 1940's and 1950's, however, silk moths should have recovered in the past 70 years. The increasing abundance of deer and birds in the wake of increased human development is another potential cause of decline, since deer browse plants containing moth eggs and larvae and birds prey heavily upon larvae and pupae (Wagner 2012). While each factor is likely important, they cannot explain why saturniids outside of New England appear largely unaffected.

Researchers have suggested that the parasitoid *Compsilura concinnata* (Meigen 1824) (Diptera: Tachinidae; hereafter *Compsilura*) might help explain saturniid declines (Boettner et al. 2000, Kellogg et al. 2003, Wagner 2012, Goldstein et al. 2015). This multivoltine tachinid was first introduced to New England in 1906 (and repeatedly over the next 80 years) for control of *L. dispar* and 12 other pests (reviewed in Elkinton et al. 2006). *Compsilura* established quickly in North America, where it can attack and kill over 180 native insect species (Arnaud 1978). *Compsilura* was even used against one native saturniid moth, the range caterpillar *Hemileuca oliviae*, but without any apparent success (Boettner et al. 2000). Stamp and Bowers (1990) documented high *Compsilura*-induced mortality on the saturniid *Hemileuca lucina*, although a study of the closely related *H. maia* found lower parasitism rates (Selfridge et al. 2007). Between 1995-1998, Boettner et al. (2000) assessed the impact of *Compsilura* on the saturniids *Hyalophora cecropia* and *Callosamia promethea*. They found 60-80% of larvae were parasitized over a three-instar period, a rate high enough (if extrapolated over the larval period) to kill the entire experimental larval cohort (n=965). Their results suggested that *Compsilura* may have played a major role in saturniid decline and disappearance in the Northeast. In the Appalachian region of Virginia, saturniids remain common (Kellogg et al. 2003) despite the presence of *Compsilura*. *Actias luna* larvae deployed in the region were frequently (16-60% of cases) hyperparasitised by trigonalid wasps after *Compsilura* parasitism, a degree of top-down control that may dampen the suppression of moth populations (Kellogg et al., 2003). More recently, surveys failed to detect *Compsilura* on New England islands with high saturniid densities (Goldstein et al. 2015). While the fly's effect on saturniids is now accepted (Wagner and Van Driesche 2010), how *Compsilura* populations are affected by fluctuations in the densities of *L. dispar* or other hosts (Hajek et al. 2015) is unknown.

We report the results of surveys conducted in 2017 and 2018 that repeated work described in Boettner et al (2000). To investigate whether parasitism rate of saturniids by *Compsilura* have changed over the past 20 years, we deployed *C. promethea* and *H. cecropia* larvae in conditions replicating those of the previous study, then assayed parasitism rates. We found that parasitism was substantially lower on *H. cecropia* larvae than in the previous study and virtually nonexistent on *C promethea* larvae. Our results, although only a two-year snapshot, suggest that the ecological importance of this parasitoid has changed.

Methods

Eggs of both *H. cecropia* and *C. promethea* were obtained by pairing unrelated captive individuals from New England (RI and CT) populations collected the prior year. Eggs were incubated in 240mL polypropylene cups (Pactiv, Lake Forest, Illinois). As per Boettner et al. (2000), the emerging *H. cecropia* and *C. promethea* larvae were reared on *Prunus serotina* and *Sassafras albidum,* respectively. Larvae were reared outdoors on live hosts within one-meter-long agribon (Berry Plastics, Evansville, Indiana) bags to exclude predators and parasitoids. Pilot experiments found these bags effective at predator/parasitoid exclusion, and larvae reared outdoors within them experienced minimal mortality (A. Baranowski, *unpublished data*).

Once larvae had reached the 3rd-5th instar, they were deployed on naturally growing understory *P. serotina* and *S. albidum* saplings, respectively, along transects in Cadwell Memorial Forest (Pelham, Massachusetts). Both this site, and the location of the transects within it, were the same as reported in Boettner et al. (2000). As per the prior studies, three larvae (3rd-5th instar) were placed on each plant, which were spaced 1-3 m apart and marked with flagging tape; different transects were used for the two saturniid species. The 'stocking density' of three larvae/plant follows Boettner et al. (2000), who found no evidence for density-dependent parasitism. We also conducted our surveys at the same time of the year that the previous study did; Boettner et al. deployed *C. promethea* larvae on July 22-24 1995, while *H. cecropia* larvae were deployed on June 19-24 and July 2 1997.

The survey began on 2 July when we deployed 89 *C. promethea* larvae. We retrieved these larvae on 8 July; if all three larvae were not found, we searched the surrounding vegetation for five minutes. Following retrieval, we deployed an additional 104 *C. promethea* larvae. We retrieved these larvae on 15 July. That same day, we deployed 72 *H. cecropia* larvae; these were retrieved on 22 July. The final survey took place when we deployed 102 *C. promethea* larvae on 12 August (retrieved 19 August).

Retrieved larvae were reared individually in the lab in 473mL polystyrene cups (Pactiv, Lake Forest, Illinois) on fresh leaves from the appropriate host; wilted leaves were replaced with fresh foliage. Rearing cups were covered with cheesecloth held in place with a rubber band, and cups were placed on a bench over a moist section of absorbent liner to maintain humidity. Indoor rearing temperatures were 21-28° C during both years. Host sprigs were kept fresh by means of moist floral foam (Smithers-Oasis brand, Kent, OH). During 2018 rearing, cut host branches were briefly immersed in a 1% beach solution and shaken dry before placement into rearing cups; this was done to reduce the spread of larval pathogens. Larvae were inspected and waste removed from cups daily, and their status (live/dead/pupated) recorded. Dead larvae were held individually for ten days to check for parasitoid emergence, then frozen for dissection to determine if they contained larval parasitoids. Cocoons were inspected for parasitoid emergence, then held outdoors in a mesh cage until moth emergence the following spring. Parasitoids were identified to species whenever possible.

In 2018, we repeated the above procedures for both moth species. We deployed *H. cecropia* and *C. promethea* larvae on 28 June, 5 July, 12 July, and 20 July; they were collected on 5 July, 12 July, 20 July, and 27 July, respectively. Numbers of *H. cecropia* larvae deployed per day were 63, 87, 71, and 16, respectively. Numbers of *C. promethea* larvae deployed per day were 89, 104, and 102 (we did not deploy this species on 20 July), respectively.

We analyzed data on the number of *Compsilura*-parasitized larvae in 2017 versus 2018, and in both years of our study versus the data reported in Boettner et al. (2000), using likelihood ratio χ^2 tests. All data were analyzed in JMP 9.0.0 (SAS 2010).

Results and Discussion

In 2017, we deployed 72 *H. cecropia* larvae and recovered 31 (Table 1); seven of the 31 (22%) survived to pupation. Six dead larvae had been parasitized by *Compsilura* (19.3%): these produced a total of ten *Compsilura* pupae. One prepupal larva was killed by the *H. cecropia* tachinid specialist *Lespesia samiae*; this single cecropia larva produced 22 adult parasitoids. None of the pupated *H. cecropia* had *Compsilura* emerge from them. In 2018, we deployed 198 larvae and recovered 32; only one survived to pupation. *Compsilura* killed one prepupal larva

(3.1%) and produced a single parasitoid pupa; no other parasitoid species emerged. Parasitism rates were slightly higher in 2017 than in 2018 ($\chi^2_{1\,df} = 4.59$, p=0.032), but both years of our study differed greatly from the results reported in Boettner et al. (2000) ($\chi^2_{2\,df} = 98.5$, p<0.001).

In 2017, we deployed 295 *C. promethea* larvae and recovered 190 (64.4%) (Table 1); approximately 40% (n=77) of the retrieved larvae pupated. No *Compsilura* emerged from the dead larvae; a single *C. promethea* larvae was parasitized and killed by an unknown ichneumonid. Two *Compsilura* emerged in spring 2018 from *C. promethea* cocoons, having evidently overwintered inside them. This is the first record of how *Compsilura* overwinters we can find in the literature. No other cocoons contained either *Compsilura* pupae or adult flies. A subsequent examination revealed two cocoons that each had one hole in its side, indicative of parasitoid escape. Since no other parasitoids emerged from these cocoons, we suspect the two *Compsilura* each emerged from a unique host larva. In 2018, we deployed 155 larvae and recovered 48. Thirty (55%) of the recovered larvae pupated; no parasitoids emerged from dead larvae. There was no difference in *Compsilura* parasitism rates between 2017 and 2018 ($\chi^2_{1\,df} = 0.45$, p=0.5); again, however, there was a highly significant difference between our results and those of the previous study ($\chi^2_{2\,df} = 223$, p<0.001). We dissected all larvae of both moth species that died prior to pupation to ascertain cause of death and count any immature larval parasitoids.

Despite lower rates of *Compsilura* parasitism, the *H. cecropia* larvae we recovered from the field experienced high rates of pathogen-related mortality in both 2017 (55%) and 2018 (94%). This also affected *C. promethea* larvae (58% and 45% in 2017 and 2018, respectively). Pathogen-killed larvae went from apparently healthy to dead, with their integument turning a distinctive black color, in a short (1-2 day) period. Pathogen presence was confirmed via dissection and microscopy (G.H. Boettner, *unpublished data*); the dark fluid that we found oozing from the dead larvae is typical of viral epizootics in captive stocks of other saturniid moths (Skowron et al. 2015). While larvae occasionally died in the field sleeves, none exhibited the same symptoms as those dying post-retrieval. The >10-day lag between retrieval and the death of pathogen-killed larvae, in combination with the rapid decline and death of 'healthy' individuals, further suggests that the mortality resulted from a lab-associated pathogen, the stress of multiple moves (from field cages to survey trees to the lab over a 7-9 day period), or some combination of the two factors.

We found no larval parasitoids in the dissected larvae. While this might have resulted from the high rates of pathogen-induced mortality, the time from retrieval to death of pathogen-killed *H. cecropia* larvae was 10.8±1.5 days, versus 6.2±0.9 days for *Compsilura*-killed *H. cecropia* larvae. Because *Compsilura* develops quickly within its host, this difference in time suggests that any *Compsilura* larvae present should have grown to sizes detectable by dissection. The fact that *Compsilura* inserts its hatchlings into, and lurks within, the immune system-neglected peritrophic membrane of its host (Caron et al. 2008), also makes it unlikely that immune activity of sick larvae could reduce survival of this parasitoid.

While care must be taken when extrapolating from a two-year survey, the decline in *Compsilura* parasitism suggests that important changes in the ecological impact of this tachinid may be occurring. There are many possible explanations for our results; we discuss below several of the more compelling hypotheses for the apparent decline.

One possible explanation for our results involves climatic differences: extreme temperatures can alter or disrupt host-parasitoid interactions (Hance et al. 2007), and changes in precipitation can impact caterpillar abundance (e.g., Karban et al. 2017). An analysis of daily weather data from the periods when caterpillars were deployed in 1995 (22-31 July) and 1997

(19-24 June and 2-9 July) revealed that temperatures averaged 22.4° ± 3.1° (SE) C and weekly rainfall averaged 2.4 ± 1.13 cm (NOAA 2018). During our work in 2017 and 2018, temperatures averaged 23.0° ± 1.5° (SE) C and weekly rainfall averaged 4.0 ± 1.1 cm (NOAA 2018). Although rainfall values are higher in our study, our means include one extremely wet week (28 June - 4 July 2018) in which 8.1 cm of rain fell; excluding this week reduces our weekly rainfall means to 3.25 ± 0.86 cm.

The reduction in parasitism may also have resulted from increased predation on *Compsilura* itself. Kellogg et al. (2003) found that ~50% of *Compsilura* pupae collected in Virginia were hyperparasitized by trigonalid wasps and hypothesized that this might explain the continued local abundance of silk moths. We assessed hyperparasitism by rearing the 14 *Compsilura* pupae we collected: 13 emerged as adults and one died without producing any hyperparasitoids. In addition, no hyperparasitoids emerged from dead saturniid larvae.

A third possibility is that *Compsilura*-saturniid interactions are influenced by the interplay between *L. dispar* and its fungal pathogen *Entomophaga maimaiga*. *Compsilura* was introduced to control *L. dispar*, and Culver (1919) found lower *C. promethea* densities at tachinid release sites. *Compsilura* parasitizes early season *L. dispar* caterpillars (Gould et al. 1990), and the ensuing generations of parasitoids attack both early and late season saturniids (Wagner and Van Driesche 2010). Starting in the late-1980s, the establishment of *E. maimaiga* in the northeastern U.S. reduced both the frequency and magnitude of *L. dispar* outbreaks (Hajek 2007, Hajek et al. 2015). This pathogen also kills *L. dispar* larvae so quickly that co-infecting parasitoids like *Compsilura* are unable to complete their development (Hajek and van Nouhuys 2016). *Compsilura* also tends to attack *L. dispar* during early instars, while *Entomophaga* kills later instar larvae. If early-season hosts determine mid and late season *Compsilura* densities, the impact of *E. maimaiga* on *L. dispar* could affect the ability of *Compsilura* to suppress saturniids, however, there is little evidence to confirm this.

In summary, we consistently found lower rates of saturniid parasitism by *Compsilura* than previously reported (Boettner et al. 2000) from the same location. If *Compsilura* is not presently the primary driver and sustainer of saturniid decline, then the reasons for it, particularly in the Northeast region, remain indeterminate. In addition to surveying for parasitoid presence or absence, future studies should explore interactions between *Compsilura* and other lepidopteran hosts. Specifically, it seems important to investigate the phenology of both *L. dispar* and its natural enemies for possible overlap in pathogen and parasitoid activity. The factors responsible for wild mortality in other life stages of these declining moths, biotic and abiotic, are also relatively unexplored. While much remains to be done, our results highlight the importance of longitudinal survey work capable of detecting cryptic but important changes in community structure.

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Table 1. Larval retrieval and parasitoid-related mortality for *Hyalophora cecropia* and *Callosamia promethea* in 2017 and 2018 and as reported in Boettner et al (2000) for the same location and site in the late 1990s.

		Larvae	Days in	Larvae	Parasitoid-related rearing mortality	
Saturniid species	Data set	deployed	field	retrieved (%)	*C. concinnata*	Other parasitoids
Hyalophora cecropia	Boettner et al. 2000	300	5-7	134 (45%)	81%	2%
	This survey (2017)	72	7	31 (43%)	19%	3%
	This survey (2018)	198	6-7	32 (16%)	3%	0%
Callosamia promethea	Boettner et al. 2000	665	6-8	117 (18%)	68%	21%
	This survey (2017)	295	6-7	190 (65%)	1%	1%
	This survey (2018)	155	6-7	48 (31%)	0%	0%