SHORT COMMUNICATION

Reduced *Compsilura concinnata* parasitism of New England saturniid larvae

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Abstract
1 In the northeastern U.S.A., 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 that *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, this field experiment was repeated 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.

Keywords Community ecology, invasive species, parasitoid, saturniid.

Introduction

Populations of silk moths (Lepidoptera: Saturniidae) in the northeastern U.S.A. have declined precipitously over the past half-century (Boettner *et al.*, 2000; Wagner, 2012; Goldstein *et al.*, 2015). All saturniids have been affected: 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, because 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 1940s and 1950s, 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 because deer browse plants containing moth eggs and larvae and birds prey heavily upon larvae and pupae (Wagner, 2012). Although each factor is likely important, they cannot explain why saturniids outside of New England appear largely unaffected.

Research has 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 the control of *L. dispar* and 12 other pests (Elkinton *et al.*, 2006). *Compsilura* established quickly in North America, where it can attack and kill over 180 native insect species (Arnaud Jr, 1978). *Compsilura* was even used against one native saturniid moth, the range caterpillar *Hemileuca oliviae*, although without any apparent success (Boettner *et al.*, 2000). Stamp and Bowers (1990) documented high *Compsilura*-induced mortality on the saturniid *H. lucina*, although a study of the closely related *H. maia* found lower parasitism rates (Selfridge *et al.*, 2007). Between 1995 and 1998, Boettner *et al.* (2000) assessed the impact of *Compsilura* on the saturniids *Hyalophora cecropia* and *Callosamia promethea*. They found that 60–80% of larvae were parasitized over a three-instar period, a rate high enough (if extrapolated over

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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, representing 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). Although the effect of the fly on saturniids is now accepted (Wagner & Van Driesche, 2010), it is still not known how *Compsilura* populations are affected by fluctuations in the densities of *L. dispar* or other hosts (Hajek *et al.*, 2015).

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, and then assayed parasitism rates. We found that parasitism was substantially lower on *H. cecropia* larvae than in the previous study and virtually non-existent on *C promethea* larvae. Although the results reported in the present study only comprise a 2-year snapshot, they suggest that the ecological importance of this parasitoid has changed.

Materials and methods

Eggs of both *H. cecropia* and *C. promethea* were obtained by pairing unrelated captive individuals from New England (Rhode Island and Connecticut) populations collected the previous year. Eggs were incubated in polypropylene cups (240 mL) (Pactiv, Lake Forest, Illinois). As described by 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 Agribon bags (length 1 m) (Berry Plastics, Evansville, Indiana) to exclude predators and parasitoids. Pilot experiments found these bags effective at predator/parasitoid exclusion and the larvae reared outdoors within them experienced minimal mortality (A. Baranowski, unpublished data).

Once larvae had reached the third-fifth 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). In accordance with their prior studies, three larvae (third-fifth 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 matched that described by Boettner *et al.* (2000), who found no evidence for density-dependent parasitism. We also conducted our surveys at the same time of the year as their prior studies: Boettner *et al.* (2000) deployed *C. promethea* larvae on 22–24 July 1995, whereas *H. cecropia* larvae were deployed on 19–24 June and 2 July 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 5 min. 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 laboratory in polystyrene cups (473 mL) (Pactiv) 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, Ohio). During 2018 rearing, cut host branches were briefly immersed in a 1% beach solution and shaken dry before placement into rearing cups; this was carried out aiming to reduce the spread of larval pathogens. Larvae were inspected and waste removed from cups daily and their status (live/dead/pupated) was recorded. Dead larvae were held individually for 10 days to check for parasitoid emergence, then frozen for dissection to determine whether they contained larval parasitoids. Cocoons were inspected for parasitoid emergence, then held outdoors in a mesh cage until moth emergence the next 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 the present study versus the data reported in Boettner *et al.* (2000), using likelihood ratio chi-squared tests. All data were analyzed in JMP, version 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 10 *Compsilura* pupae in total. 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 ($\chi^2 = 4.59$, d.f. = 1, $P = 0.032$), although both years of our study differed greatly from the results reported in Boettner *et al.* (2000) ($\chi^2 = 98.5$, d.f. = 2, $P < 0.001$).

In 2017, we deployed 295 *C. promethea* larvae and recovered 190 (64.4%) (Table 1); approximately 40% ($n = 77$) of the

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

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

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 with respect to how *Compsilura* overwinters that 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. Because 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 ($\chi^2 = 0.45$, d.f. $= 1$, $P = 0.5$); again, however, there was a highly significant difference between our results and those of the previous study ($\chi^2 = 223$, d.f. $= 2$, $P < 0.001$). We dissected all larvae of both moth species that died prior to pupation to determine cause of death and count any immature larval parasitoids.

Despite lower rates of *Compsilura* parasitism, the *H. cecropia* larvae that 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 colour, 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). Although 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 laboratory-associated pathogen, the stress of multiple moves (from field cages to survey trees to the laboratory over a 7–9 day period), or some combination of the two factors.

We found no larval parasitoids in the dissected larvae. Although 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. *Compsilura* inserts its hatchlings into and lurks within the immune system-neglected peritrophic membrane of its host (Caron *et al.*, 2008), which makes it unlikely that immune activity of sick larvae could reduce survival of this parasitoid.

Although care must be taken when extrapolating from a 2-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 (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 the average (mean $\pm$ SE) temperature was 22.4 ± 3.1 °C and weekly rainfall was 2.4 ± 1.13 cm (NOAA, 2018). During our work in 2017 and 2018, the temperature was 23.0 ± 1.5 °C and weekly rainfall was 4.0 ± 1.1 cm (NOAA, 2018). Although rainfall values are higher in the present study, our means include one extremely wet week (28 June to 4 July 2018) in which 8.1 cm of rain fell; by excluding, we reduce our average weekly rainfall 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 approximately 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) reported 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 & Van Driesche, 2010). Starting in the late-1980s, the establishment of *E. maimaiga* in the northeastern U.S.A. 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 such as *Compsilura* are unable to complete their development (Hajek & van Nouhuys, 2016). *Compsilura* also tends to attack *L. dispar* during early instars, whereas *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, although 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 aim to explore interactions between *Compsilura* and other lepidopteran hosts. Specifically, it is 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, both biotic and abiotic, are also relatively unexplored. Although much work remains to be carried out, the results of the present study highlight the importance of longitudinal survey work that is capable of detecting cryptic but important changes in community structure.

Acknowledgements

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