

Parasitism of Native Luna Moths, *Actias luna* (L.) (Lepidoptera: Saturniidae) by the Introduced *Compsilura concinnata* (Meigen) (Diptera: Tachinidae) in Central Virginia, and Their Hyperparasitism by Trigonalid Wasps (Hymenoptera: Trigonalidae)

SHELLY K. KELLOGG,¹ LINDA S. FINK,² AND LINCOLN P. BROWER

Department of Biology, Sweet Briar College, Sweet Briar VA 24595

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ABSTRACT Parasitism rates of the nonnative tachinid fly, *Compsilura concinnata* (Meigen), on experimental populations of native luna moth caterpillars (*Actias luna* (L.)) were determined in central Virginia, where both *C. concinnata* and the gypsy moth, its biocontrol target, have become established in the past few decades. In a forest that has not yet had gypsy moth damage, we placed cohorts of second through fifth instar *Actias luna* caterpillars on understory hickory trees. At the end of each instar, surviving caterpillars were collected and reared. Four parasitoid species and two hyperparasitoid species emerged from the caterpillars and pupae. *C. concinnata* was the most common parasitoid, attacking 0% to 62% of the caterpillars recovered in each instar. Hyperparasitism of *C. concinnata* by trigonalid wasps was high: 47% and 16% in the two generations. UV light census data indicate that current saturniid populations in this area of central Virginia are robust, but the long-term effects of *C. concinnata* introductions on populations of these and other native macrolepidoptera should be monitored.

KEY WORDS *Compsilura concinnata*, hyperparasitoid, nontarget host, parasitoid, Saturniidae

FROM 1906 TO 1986, the European tachinid parasitoid *Compsilura concinnata* (Meigen) was released widely in the eastern United States as a biological control agent against the gypsy moth (*Lymantria dispar* (L.)) and other lepidopteran pests (Sanchez 1995, USDA 2002). Quite early in this period, entomologists recognized that it was also a significant enemy of native Lepidoptera (Culver 1919), and in recent discussions of nontarget effects of biological control, *C. concinnata* has been cited as posing risks to native insects (Simberloff and Stiling 1996, Hawkins and Marino 1997, Stiling and Simberloff 2000). *C. concinnata* has three life history traits that contribute to its potential negative impact on native Lepidoptera. First, the fly is multivoltine, while the gypsy moth is univoltine (Culver 1919). *C. concinnata* parasitizes gypsy moth larvae in the spring, but it then requires alternate hosts for three to four generations. Second, *C. concinnata* overwinters inside a host caterpillar or pupa, and the gypsy moth overwinters as eggs. Therefore, nontarget species must be used as overwintering hosts. Third, *C. concinnata* is an extreme generalist. In North America, it is known to parasitize more than 150 butterfly

and moth species and eight sawfly species from numerous families (Arnaud 1978, Strazanac et al. 2001).

Recently, Boettner et al. (2000) suggested that parasitism by *C. concinnata* was partly responsible for a decline in populations of saturniid moths in New England. Lepidopterists widely agree that populations of many saturniid moths in southern New England declined in the 1950s (Ferguson 1971, Schweitzer 1988), but the causes for the declines and for the lack of recovery of some species are still debated. Boettner et al. (2000) reviewed the three major hypotheses for the declines: habitat loss, outdoor lighting, and spraying of DDT and other pesticides. They found limited evidence supporting the first hypothesis and none for the second but concluded that pesticides may, in part, explain the original declines. However, large sections of New England were never sprayed with DDT, and pesticide use has been curtailed for decades, yet most northeastern saturniid populations have not recovered. In field experiments in Massachusetts, Boettner et al. (2000) tested whether parasitism by *C. concinnata* could be an additional factor in the declines and absence of recovery. Caterpillars of the saturniids *Hyalophora cecropia* (L.) and *Callosamia promethea* (Drury) were transferred from the laboratory onto natural host plants in the field for five to 8 d and then brought back into the laboratory to determine rates of parasitism. The mortality rate of *promethea* caterpil-

¹ Current address: Department of Entomology, University of Kentucky, S-225 Ag. Science North, University of Kentucky, Lexington, KY 40546.

² Corresponding author: E-mail: lfink@sbc.edu.

lars from *C. concinnata* was 67.5%. The cumulative mortality rate from *C. concinnata* for instars one through three of *H. cecropia* was 81%, sufficiently high, in combination with other mortality factors, for Boettner et al. (2000) to conclude that "no *H. cecropia* population can persist for long with these levels of larval mortality."

Saturniid declines have not been reported elsewhere in the United States. Based on Natural Heritage data, most species are considered "secure" or "apparently secure" (NatureServe 2001). However, Saturniids are little studied in the field and are poorly sampled in community level collections of caterpillars and adult Lepidoptera. In recent surveys of forest macrolepidoptera in Virginia, New Brunswick, and Missouri, for example, saturniids comprised 0%, 0.6%, and 0%, respectively, of the collected individuals (Marquis and Passoa 1989, Thomas and Thomas 1994, Thomas 1996, Le Corff et al. 2000). Saturniids are similarly poorly represented in data sets examining the effect of the spread of the gypsy moth on lepidopteran communities, comprising 0% to 1.1% of collected adults (Butler and Kondo 1991, Butler et al. 1999, Work and McCullough 2000) and 0% to 5.7% of collected caterpillars (Wagner et al. 1996, Butler and Strazanac 2000a, 2000b). Therefore, population trends for most members of the family in most of their ranges are unknown.

Alerted to the possible impact of *Compsilura concinnata* on saturniid populations, we conducted an experiment similar to Boettner et al. (2000) in central Virginia. Seasonal UV light censuses conducted from 1999 to 2002 in the Blue Ridge Mountains in Nelson County, VA, have determined that seven saturniids are common to abundant (i.e., *Actias luna*, *Antheraea polyphemus*, *Automeris io*, *Callosamia angulifera*, *Citheronia regalis*, *Dryocampa rubicunda*, and *Eacles imperialis*), and four are occasional (i.e., *Anisota stigma*, *A. virginensis*, *Callosamia promethea*, and *Hyalophora cecropia*) (L.S. Fink and L.P. Brower, unpublished data). Seven of the aforementioned saturniids are recorded hosts for *C. concinnata* (Arnaud 1978). *C. angulifera*, *C. regalis*, *E. imperialis*, and *A. stigma* lack adequate parasitoid records from areas where *C. concinnata* has become established.

A small number of *C. concinnata* were released in Washington, D.C., in 1910 against *Hemerocampa leucostigma* (Culver 1919), but large releases (totaling more than a quarter of a million flies) did not take place in Virginia until 1971 through 1986 (Sanchez 1995, USDA 2002), as the gypsy moth spread southward into the state in the 1980s (Ravlin and Stein 2001). *C. concinnata* may have arrived in Virginia on its own, since by the 1920s, its range was spreading west and south of that of the gypsy moth (Schaffner 1927, 1934).

The first known recoveries of wild *C. concinnata* in museum records for Virginia and West Virginia are National Museum of Natural History records from Charlottesville, VA, on 22 August 1979, and from Elk Garden, WV, on 25 June 1968 (Boettner, personal communication), but the parasitoid was not widely reported from the area until the mid 1980s. For ex-

ample, *C. concinnata* was not among 21 parasitoid species reared from an outbreak of *Phigalia titea* (Cramer) (Geometridae) caterpillars in eastern West Virginia in 1983 (Butler 1990), although *P. titea* is a known host (Arnaud 1978). Similarly, no *C. concinnata* were reared from 1987 to 1990 in a large study on parasites of the saturniid *Anisota senatoria* in coastal Virginia (Coffelt and Schultz 1993). Although Schaffner (1934) reported that *Anisota* spp. were seldom parasitized by *C. concinnata*, *A. senatoria* is a known host (Arnaud 1978; D. Parry, personal communication). Within 250 km of our field site, *C. concinnata* was collected from seven species of macrolepidoptera in Cooper's Rock State Forest in West Virginia from 1984 to 1985 (Butler 1993), from gypsy moths in the George Washington National Forest and Shenandoah National Park in Virginia from 1991 to 1992 (Boettner, personal communication), and from forest canopy Lepidoptera in Virginia and West Virginia from 1995 to 1996 (Strazanac et al. 2001).

Compsilura concinnata has been interacting with gypsy moths and with native insects in Massachusetts for at least 90 yr, and its effects where it is a more recent arrival may be quite different. Recent reviews of the ecological effects of biological control have called for more field studies on nontarget species, emphasizing that an absence of documentation of an effect is not equivalent to the absence of an effect (Howarth 1991, Onstad and McManus 1996, Van Driesche and Hoddle 1997, Stiling and Simberloff 2000). Therefore, the specific aim of this study was to determine the rate of parasitism by the nonnative *C. concinnata* on an experimental population of native luna moth caterpillars (Saturniidae: *Actias luna* (L.)) in central Virginia, near the current southern edge of the gypsy moth's expanding range.

Materials and Methods

Field Sites. The field site is a 10 ha section of Fern Woods on the Sweet Briar College campus, Amherst County, VA (37° 32' 43" N, 79° 05' 38" W, elevation 240 to 270 m). This 80-yr-old hardwood forest, >100 ha in extent and contiguous with other forested areas, is dominated by *Liriodendron tulipifera*, *Acer rubrum*, *Quercus alba*, *Q. prinus*, and three *Carya* species. Sweet Briar is 34 km from the Nelson County site where we census adult saturniid moths in a hardwood forest at an elevation of 450 m (37° 49' 55" N, 78° 57' 48" W). Although we have not censused moths at Sweet Briar, the habitat is appropriate for saturniids, and they are seen regularly at lights. In spring 1999, the ground below an unidentified owl roost on campus was regularly littered with numerous saturniid wings, including *A. luna*, *A. polyphemus* and *C. regalis*.

Gypsy moths have not yet been recorded at Sweet Briar, although infestations have occurred elsewhere in Amherst County. In the summer of 2001, the closest known site with moderate defoliation was on Big Piney Mountain, ≈8.5 km west of our field site (T. Tigner, personal communication).

Actias luna Field Experiment. In a protocol modeled after Boettner et al. (2000), cohorts of laboratory reared caterpillars were placed in the forest on natural hostplants for one instar, then brought back to the laboratory and reared to adult or parasite emergence or to death from other causes. The experiment was repeated with two generations of luna moth caterpillars. Early generation caterpillars were placed in the field from 5 to 29 June 2001 and late generation caterpillars from 23 July to 15 August 2001. Early generation *Actias luna* eggs were collected from a wild female attracted to the Nelson County UV light. Late generation eggs were obtained from three early generation female moths mated with wild males at the Nelson County site.

Caterpillars were fed leaves of mockernut hickory (*Carya tomentosa*), shagbark hickory (*Carya ovata*), and pignut hickory (*Carya glabra*), with new leaves provided at least every other day. The leaves came from small trees within the field site or large trees along forest edges within 2 km of the field site. Caterpillars were reared in groups in plastic shoeboxes until they were put in the field. When they were brought back in from the field, they were reared individually in 700-ml plastic boxes. They were reared on a bench top at $\approx 21^{\circ}\text{C}$ in a laboratory, with north facing windows and constant light from overhead fluorescent fixtures.

We placed four caterpillars in the same instar on separate leaves of small (up to 5-m tall) understory hickory trees (*Carya tomentosa*, *C. glabra*, and *C. ovata*). Since wild *A. luna* eggs are laid singly or in small groups (Tuskes et al. 1996) and a number of its foodplants (Covell 1984, Tuskes et al. 1996) are small forest trees (sumacs (*Rhus*), persimmon (*Diospyros virginiana*), and (further north) paper birch (*Betula papyrifera*)), this spatial arrangement is probably realistic. Each tree was marked by a flag placed near but not directly below it, and each tree was used only once. The trees used for all cohorts were interspersed through the field site. In some cases adjacent trees were in use simultaneously; in other cases, used trees were separated from other small hickories by >50 m.

Cohorts of ≈ 100 caterpillars were placed in the field during each of the second through fifth instars (only 76 fifth instar caterpillars were available in the early generation). Caterpillars were placed outside on the first day after they had molted and censused daily. The second, third, and fourth instar caterpillars were deployed for the entire feeding period of their respective instar and were collected when they showed head capsule slippage. Because the fifth instar caterpillars disappeared rapidly due to mortality and/or wandering, they were left in the field for only 4 to 5 d (i.e., early generation) or 4 d (i.e., late generation) to ensure adequate sample sizes.

Parasitoid Rearings. The caterpillars that were recovered from the field completed their development in the laboratory, and all caterpillars and their cocoons were checked daily for parasite emergence until late fall. Dead caterpillars and pupae were kept until either parasitoids emerged or evidence of disease was clear.

Healthy cocoons were placed individually in nine by 14-cm cylindrical screen cages enclosed in lengths of pantyhose, and misted with water two to three times per week to prevent desiccation. Some late generation cocoons produced adults that eclosed in September. The remaining cocoons were kept in a closed metal garbage can in a shaded open shed and misted occasionally from late fall to mid February 2002 and then brought back into the laboratory for spring emergences.

Compsilura concinnata females larviposit into their host caterpillars. Depending on temperature, the larvae then feed on a host for 10 to 16 d before emerging, forming a puparium, and eclosing as an adult 10 to 15 d later (Culver 1919). Emerged *C. concinnata* and other parasitoid larvae and pupae were kept in small plastic containers on a laboratory bench at ambient conditions until adults eclosed. A kimwipe (Kimberly-Clark, Roswell, GA) in each container was moistened every other day to prevent desiccation. In June 2002, all remaining moth pupae and unemerged *C. concinnata* parasite puparia were dissected to determine their fates. Emerged tachinid parasitoids were identified by George H. Boettner (University of Massachusetts-Amherst), and hymenopteran parasitoids and hyperparasitoids were identified by Ken Ahlstrom (North Carolina Department of Agriculture and Consumer Services). Voucher specimens of the Hymenoptera have been deposited in the North Carolina State University Insect Collection; voucher specimens of all species are held at Sweet Briar College.

***Hyalophora cecropia* and *Callosamia promethea* Experiment.** In addition to the detailed study of *A. luna*, smaller numbers of cecropia (*Hyalophora cecropia*) and promethea (*Callosamia promethea*) caterpillars were also placed in the field and similarly exposed to parasites. Cecropia caterpillars were reared and tested on red maple (*Acer rubrum*, Aceraceae); promethea caterpillars were reared and tested on spicebush (*Lindera benzoin*, Lauraceae). For cecropia, 20 caterpillars in each of the second through fifth instar were deployed on red maple trees in Fern Woods from 9 June to 2 August 2001. Caterpillars were placed four per tree either in a group (second instar) or on separate leaves (third through fifth instar). For promethea, nine second instar, and 12 each third and fourth instar caterpillars were placed in the field from 24 May to 15 June 2001. Caterpillars were placed three per plant either in a group (second and third instars) or on separate leaves (fourth instar). These caterpillars were placed in the Constitution Oaks Sanctuary, a mature oak-hickory forest on Sweet Briar campus two km from Fern Woods.

Statistical Analyses. Statistical analyses were performed using computer software (SAS Institute 1999).

Results

Luna Moth Recoveries. We recovered 325 of the 773 (42%) *A. luna* caterpillars (Table 1). Recovery rates did not differ significantly among instars in either generation (early generation, chi-square = 3.62, df =

Table 1. Fates of *Actias luna* caterpillars placed in the field for one instar in Amherst County VA, June to August 2001

Generation	Instar deployed	Sample size	Recovery % (#)	Fates (% of recovered caterpillars)			
				Adult moth	Parasitoid	Pathogen	Dead, cause unknown
Early	2	100	41 (41)	87.8	2.4	7.3	2.4
	3	100	46 (46)	95.7	0	4.3	0
	4	100	35 (35)	71.4	11.4	11.4	5.7
	5	76	36 (27) ^a	19.2	80.8	0	0
Late	2	102	44 (45) ^b	50.0	2.3	38.6	9.1
	3	98	53 (52)	78.8	0	13.5	7.7
	4	102	40 (41) ^c	42.1	21.1	23.7	13.2
	5	95	40 (38)	73.7	2.6	10.5	13.2
Total		773	42 (325)				

^a 1 fifth instar escaped in the lab; fates are based on 26 rather than 27 caterpillars

^b 1 pupa in group lost; fates are based on 44 rather than 45 caterpillars

^c 3 pupae in group lost; fates are based on 38 rather than 41 caterpillars

3, $P > 0.05$; late generation, chi-square = 5.21, df = 3, $P > 0.05$) or between the two generations (chi-square = 0.51, df = 1, $P > 0.05$).

Parasitoids. Four species of parasitoids were reared from 36 (11.1%) of the recovered caterpillars (Tables 1 and 2). *C. concinnata* accounted for 78% of the parasitism, killing 28 caterpillars. Three native parasitoids (*Lespesia aletiae* (Riley), Tachinidae; an unidentified species of Tachinidae; and *Phobocampe clisiocampae* (Weed), Ichneumonidae) killed a total of only eight caterpillars. *C. concinnata* and *P. clisiocampae* are previously described from *A. luna* (Peigler 1994, Tuskes et al. 1996). *A. luna* represents a new host record for *L. aletiae*. This result is not unexpected because *L. aletiae* is a broad generalist known from 16 families (including saturniids) and more than 40 different species of moths and butterflies (Arnaud 1978).

***Compsilura concinnata*.** *C. concinnata* was reared from early generation caterpillars deployed in the fourth and fifth instar, and from late generation caterpillars deployed in the fourth instar (Table 2). Its highest parasitism rate was in early generation caterpillars exposed in the fifth instar, with the tachinid killing 16 of 26 (62%) recovered caterpillars.

C. concinnata frequently parasitized more than one of the four luna caterpillars on an individual tree. *C. concinnata* were reared from caterpillars that had been deployed on 15 different trees; nine of these trees produced *C. concinnata* from more than one caterpillar. One to seven *C. concinnata* puparia emerged per host caterpillar (mean = 3.4, SD = 1.7).

Slightly higher numbers of parasitoids emerged per fifth instar host than per fourth instar, but the difference was not significant (fourth instar: $n = 12$, mean = 2.67, SD = 1.44; fifth instar: $n = 16$, mean = 3.88, SD = 1.75; t -test, df = 26, $t = -1.951$, $P = 0.062$).

When *A. luna* were attacked in the fourth instar, the *C. concinnata* emerged either from host larvae or from prepupae that had formed complete or partial cocoons. When hosts were attacked in the fifth instar, the *C. concinnata* emerged from prepupae. No *C. concinnata* emerged from any luna moth pupae.

Other Tachinids. *Lespesia aletiae* attacked two fifth instar caterpillars (Table 2), with one and two fly larvae per host emerging when their hosts were still in the fifth instar. An unidentified tachinid attacked four fifth instar caterpillars, with three to seven fly larvae per *A. luna* emerging after their hosts had pupated. The unidentified tachinids pupated, but none eclosed as adults.

In one instance, a fourth instar caterpillar was brought back from the field with unidentified parasitoid eggs on its head capsule but produced a healthy adult moth. Since *C. concinnata* larviposits through the host cuticle (Culver 1919), the eggs were from a different species. This caterpillar was not counted as parasitized in the data tables and analyses, since it was not killed, and no parasitoid was produced.

In two cases two different tachinids attacked the four caterpillars on an individual tree. One tree produced three caterpillars parasitized by *C. concinnata* and one by *L. aletiae*, and one tree produced one

Table 2. Number of *Actias luna* caterpillars and pupae killed by each parasitoid

Generation	Instar deployed ^a	Tachinidae			Ichneumonidae	Total
		<i>Compsilura concinnata</i>	<i>Lespesia aletiae</i>	Unknown sp.	<i>Phobocampe clisiocampae</i>	
Early	2	0	0	0	1	1
	4	4	0	0	0	4
	5	16	2	3	0	21
Late	2	0	0	0	1	1
	4	8	0	0	0	8
	5	0	0	1	0	1
Total		28	2	4	2	36

^a No caterpillars deployed in the third instar were parasitized (Table 1).

Table 3. Hyperparasitism of *Compsilura concinnata* puparia reared from *A. luna* caterpillars

Generation	Fates of <i>C.c.</i> puparia					% hyperparasitized
	<i>C.c.</i> adult	<i>O.p.</i>	<i>T.g.</i>	Trig indet.	Total	
Early	32	24	3	1	60	47
Late	16	1	2	0	19	16
Total	48	25	5	1	79	39

C.c. = *Compsilura concinnata*; *O.p.* = *Orthogonalys pulchella* (Hymenoptera: Trigonidae); *T.g.* = *Taeniogonolus gundlachii* (Hymenoptera: Trigonidae); Trig indet. = unidentifiable trigonalid dead in puparium

caterpillar parasitized by *C. concinnata*, and one by the unidentified tachinid. No successful multiparasitism occurred, in which two different parasitoid species emerge from a single host (Godfray 1994).

Hymenopteran Parasitoids. *Phobocampe clisiocampae* (Weed) (Ichneumonidae) attacked one second instar luna moth caterpillar in each generation. A single wasp larva emerged from each host while the host was in the third instar.

Hyperparasitoids. In the early generation, almost half the *C. concinnata* puparia (47%) produced two different species of hyperparasitic wasps from the family Trigonidae (Table 3). Since trigonalids lay eggs on foodplants that are ingested by the caterpillars (Clausen 1940), this value represents the minimum percentage of caterpillars that both consumed trigonalid eggs and were attacked by *C. concinnata*. Of 60 *C. concinnata* puparia from early generation *A. luna* caterpillars, 24 (40%) were hyperparasitized by *Orthogonalys pulchella* (Cresson), three (5%) by *Taeniogonolus gundlachii* (Cresson), and one, determined by dissection, by a trigonalid that was not identifiable to species. In the late generation, one of 19 *C. concinnata* puparia produced *O. pulchella*, and two produced *T. gundlachii*. This 16% attack rate was significantly lower than the early generation (chi-square = 5.88, df = 1, $P < 0.05$).

The rates of hyperparasitism of *A. luna* (calculated as the number of *A. luna* producing at least one trigonalid/total number of *A. luna* recovered) were 6%, 48%, and 7% of the early generation fourth and fifth instar, and late generation fourth instar caterpillars,

respectively. In the early generation, the *C. concinnata* were reared from 20 caterpillars that had fed on 10 different hickory trees, and hyperparasitoids were reared from 15 of these caterpillars from nine of the 10 trees. In the late generation, the two *T. gundlachii* came from two caterpillars on the same tree, while the *O. pulchella* came from a caterpillar on a different tree.

In three instances, both species of hyperparasitoid emerged from separate *C. concinnata* puparia that had emerged from a single caterpillar. Two of the three caterpillars had been on the same tree; the third caterpillar was on a separate tree.

Cecropia and Promethea Caterpillars. From 49 recovered cecropia caterpillars, two *C. concinnata* and one unidentified tachinid parasitoid were reared from three caterpillars exposed in the fourth instar (Table 4). The *C. concinnata* emerged from the caterpillars, and the unidentified tachinid emerged from a postdiapause cecropia pupa. No *C. concinnata* were reared from 18 recovered promethea caterpillars, but three puparia of an unidentified tachinid were found in one pupa of a moth exposed in the fourth instar (Table 4).

Discussion

We found that *C. concinnata* was responsible for 78% of the parasitism on *A. luna* caterpillars in a site near the leading edge of the gypsy moth's range. In combination with Boettner et al. (2000), these results indicate that the tachinid has the potential to alter the population dynamics of many saturniid moths throughout the eastern United States.

Mortality from *C. concinnata* was considerably lower for *A. luna* in this study than for *H. cecropia* and *C. promethea* in Massachusetts (Boettner et al. 2000). The fact that we tested different caterpillar species and different host plants complicates our ability to make direct comparisons, but data from our small cohort of *H. cecropia* (Table 4) can be compared. Boettner et al. (2000) tested instars one to three of *H. cecropia*, and this study tested instars two to five. The 0% *C. concinnata* parasitism in our second and third instar cecropia larvae is significantly lower than the rates of 27% and 70% in Massachusetts (combining

Table 4. Fates of *Hyalophora cecropia* and *Callosamia promethea* caterpillars placed in the field for one instar in Amherst County VA, June to August 2001

Instar deployed	Sample size	Days deployed	Recovery rate % (#)	Fates (% of recovered caterpillars)		
				Adult moth	Parasitoid ^a	Pathogen
<i>Hyalophora cecropia</i>						
2	20	7-9	55 (11)	64	0	36
3	20	8-17	80 (16)	75	0	25
4	20	6-15	55 (11)	36	27	36
5	20	5-7	55 (11)	82	0	18
<i>Callosamia promethea</i>						
2	9	11-15	78 (7)	86	0	14
3	12	7-9	50 (6)	100	0	0
4	12	6-12	42 (5)	80	20	0

^a *H. cecropia* parasitoids: *C. concinnata* (2), tachinid sp. (1); *C. promethea* parasitoid: tachinid sp.

Table 5. Number of days each cohort of *A. luna* caterpillars was deployed in the field (data for recovered caterpillars only)

Generation	Instar	N	Mean	s.d.	Range
Early	2	41	4.2 a	1.26	2 to 7
	3	46	4.8 b	0.82	3 to 7
	4	35	5.3 b	0.84	4 to 8
	5	27	4.3 a	0.47	4 to 5
Late	2	45	3.3 a	0.55	3 to 5
	3	52	6.4 b	1.00	5 to 9
	4	41	4.9 c	0.61	3 to 6
	5	38	4.0 d	0	4 to 4

Means with the same letter within a generation are not significantly different ($P > 0.05$, Games/Howell post hoc test, SAS 1999)

the data for second and third instars because of the small sample in this experiment, chi-square = 20.7, $df = 1$, $P < 0.01$). In this study, *C. concinnata* did attack two fourth instar caterpillars (18%), but the combined parasitism for second through fourth instar caterpillars is still significantly lower than the parasitism in Massachusetts for second through third instar (chi-square = 21.5, $df = 1$, $P < 0.01$). Without further experiments, we cannot determine whether the lower parasitism rate in Virginia reflects differences in parasitoid abundance, parasitoid host preferences, host-plants used (*Prunus serotina* in Massachusetts versus *Acer rubrum* in Virginia), or other habitat features. The promethea moths in our study had 0% mortality from *C. concinnata* versus 67.5% in Massachusetts, but our small sample (i.e., 18 caterpillars) and different experimental design preclude statistical comparison.

The absence of *C. concinnata* parasites in the second and third instar in all three species of caterpillars that we deployed is unexpected. In addition to parasitizing these instars of *H. cecropia* (Boettner et al. 2000), *C. concinnata* parasitizes early instars of gypsy moths (Weseloh 1982). The absence of *C. concinnata* parasitism of small hosts was not a seasonal effect since it occurred in both generations. Nor was it due to competition with other parasitoids since only two caterpillars in the early instars in any of the three species were parasitized. Finally, the early instar caterpillars did not have significantly fewer days of exposure to parasites than the late instar caterpillars (Table 5). A two-factor analysis of variance (ANOVA) testing the effect of generation and instar on number of days in the field found a significant (generation \times instar) interaction effect, so the effect of instar was analyzed for each generation separately. In both generations, there were significant differences in exposure time among instars (1-factor ANOVA, early generation: $F_{3,145} = 11.07$, $P < 0.0001$; late generation: $F_{3,172} = 201.3$, $P < 0.0001$), but in both generations, the third and fourth instars were deployed for longer periods than the second and fifth instars.

In *A. luna*, although *C. concinnata* parasitism of the caterpillars deployed in the fourth instar was higher in the late than the early generation (Table 2), no *C. concinnata* were reared from the late generation caterpillars deployed in the fifth instar. The difference is not attributable to a disappearance of adult *C. concinnata*: the parasitized fourth instar caterpillars were deployed between 5 and 11 August, and the unpara-

sitized fifth instar caterpillars were deployed between 9 and 15 August. *C. concinnata* females have been collected in the field through November in the northeastern United States (Culver 1919, Schaffner and Griswold 1934, Weseloh 1981).

Possible explanations for the absence of *C. concinnata* parasitism on the final instar *A. luna* are that the large caterpillars evaded parasites through a change in behavior or microhabitat, or that *C. concinnata* females did not accept them as hosts. We observed no differences in the resting or feeding behavior of the fifth instar caterpillars in the two generations and can identify no reason why the last instar caterpillars should have been acceptable hosts in June but unacceptable in August. *C. concinnata* could have either overwintered in them or emerged from prepupae by early September, with time to produce another generation. Overwintering hosts in Massachusetts were attacked from August through October (Schaffner 1934).

We hypothesize that the late generation fifth instar caterpillars may, in fact, have been attacked by *C. concinnata*, but that environmental cues induced diapause in the parasitoids but not in the caterpillars. Most of the *A. luna* individuals (87%) deployed in the fourth instar did diapause and emerge as adults in the spring, but 79% of the individuals deployed in the fifth instar eclosed in September. Pupal diapause in at least three saturniids is induced by short photoperiod during the fourth through fifth larval instars (Williams and Adkisson 1964, Mansingh and Smallman 1967). Because the overhead lights were left on in the laboratory continuously, the fifth instar caterpillars may not have received an appropriate cue to trigger diapause. The factors inducing diapause in *C. concinnata* are not known, although in nondiapausing *C. concinnata*, development is influenced by host hormones (Weseloh 1984). If diapause was triggered in *C. concinnata* but not in the late generation *A. luna*, the moths could have "outdeveloped" the *C. concinnata* as an artifact of our rearing conditions.

The rate of hyperparasitism of *C. concinnata* is much higher than rates found in the northeast. Culver (1919) reared unidentified hyperparasitoids (secondary parasites) from only 10% of the *C. concinnata* puparia collected in 1915 and 1916. No hyperparasitoids emerged from *C. concinnata* reared from many experimental populations of saturniid caterpillars in Massachusetts from 1995 to 2002 (Boettner, unpublished data). The suggestion that *C. concinnata*'s rapid

development may allow it to escape hyperparasitism (Peigler 2001) is clearly incorrect.

The ecology and behavior of trigonalid wasps are poorly known and merit further study. The few reports of trigonalids hyperparasitizing saturniids are individual records (Butler 1993, Peigler 1994, Tuskes et al. 1996). No study has reported a trigonalid parasitism rate similar to that found in this experiment. Trigonalids lay large numbers of eggs on leaves. The eggs can remain viable for months, but once ingested by a caterpillar, they hatch within hours (Clausen 1940). If the caterpillar has been parasitized by a primary parasite that is a suitable host, the first instar trigonalid will locate and parasitize it. The high rate of hyperparasitism in this study indicates that, in our field sites, either the hyperparasitoid eggs are exceedingly abundant, or the female hyperparasitoids locate and oviposit close to feeding caterpillars, as has been hypothesized but never well documented (Clausen 1940, Weinstein and Austin 1991).

In malaise trap sampling at eight sites in Virginia, Maryland, and West Virginia, Smith (1996) found *O. pulchella*, the major hyperparasitoid in our study, to be locally abundant in a habitat quite similar to our field site. Of close to 4,000 specimens of *O. pulchella*, almost 2,000 were collected from Blandly Experimental Farm in Clarke County, VA. At this site, almost all individuals were collected in a 90-yr-old oak-elm-hickory woodlot. *T. gundlachii* was less abundant and also less habitat-specific: as many individuals were trapped in open or semiopen habitats as in woodlands (Smith, l.c.).

Few host records are known for *Orthogonalys* spp. (Carlson 1979, Carmean and Kimsey 1998). *Orthogonalys pulchella*, the only member of the genus in North America, has been reared from *Archytas aterrimus* (Robineau-Desvoidy) (Tachinidae) from an unidentified host, and the African *O. seyrigi* has been reared from a limacodid moth. In contrast, *Taeniogonolus* spp. have been reared from many ichneumonid and tachinid parasitoids of Lepidoptera (Carlson 1979, Carmean and Kimsey 1998, Janzen and Hallwachs 2002). *T. gundlachii* (= *Poecilogonolus costalis* and *Trigonalys costalis*; Carmean and Kimsey 1998) has been reared from parasitoids from moths in at least five families, including the North American saturniids *Anisota senatoria*, *A. virginianensis*, *A. discolor*, and *Automeris io* (Butler 1993, Peigler 1994, Carmean and Kimsey 1998), and the Costa Rican saturniids *Arsenura armida*, *Copaxa moinieri*, *Hylesia lineata*, *Rothschildia lebeau*, *R. erycina*, and *Syssphinx mexicana* (Janzen and Hallwachs 2002). Although the high hyperparasitism of *C. concinnata* in this study is an unusual occurrence, it at least raises the possibility that the tachinid's impact on native Lepidoptera may be locally or temporarily ameliorated by its own enemies.

Large and colorful, saturniid moths are widely admired by lepidopterists and by others who pay attention to the natural world (Cody 1996, Tuskes et al. 1996, Kingsolver 2000). "Charismatic" insect groups, including saturniid moths, are disproportionately

more likely to be considered for conservation protection than less familiar groups, such as the Diptera or Hymenoptera, not because they are at higher risk or of higher ecological importance, but because they are better known by the public, and by amateur and professional biologists (Bossart and Carlton 2002). Therefore, if saturniid populations were to decline, whether from parasitoids, climate change, habitat destruction, or natural ecological succession, we might expect this to be noticed quickly. In fact, however, the dramatic 20th century decline of northeastern United States saturniids was poorly documented, and a reconstruction of what happened relies heavily on anecdotal memories of collectors and scientists (Schweitzer 1988).

Although the effort and cost necessary to evaluate thoroughly the impact of biological control on all nontarget organisms would be prohibitive (Ehrlich 1992, Hawkins and Marino 1997), detailed investigation of selected case studies, such as *Compsilura concinnata*, should continue. The determination of parasitism rates in cohorts of caterpillars (Boettner et al. 2000; this study) is an important tool for evaluating *C. concinnata*'s effects on the majority of native Lepidoptera that, like saturniids, tend to occur at low densities. However, short-term studies will not reveal whether the effects of *C. concinnata*, on individual species or entire communities, are major or minor. Because parasitism rates vary spatially and temporally (Le Corff et al. 2000, Stiling and Simberloff 2000), and because the effects of an introduced species may change through time (Louda et al. 1997), more extensive data from additional sites and years are needed.

Most studies of nontarget effects of biological control have the weakness that preinvasion data on the nontarget populations are lacking (Simberloff and Stiling 1996, Stiling and Simberloff 2000). Recognizing *C. concinnata*'s potential impact, entomologists should be collecting population data on forest Lepidoptera and their parasites in areas just beyond its current range in the midwest and in the southern Appalachians. In addition to its impact on Lepidoptera, its potential to displace native parasitoids should be evaluated.

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