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ORIGINAL ARTICLE

Diapause in eastern North American populations of *Coccinella septempunctata* (Coleoptera: Coccinellidae)

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Abstract. Experiments examined the life histories and the effect of photoperiod on the expression of adult hibernal diapause in the Palearctic ladybird beetle *Coccinella septempunctata,* within 10 years of its discovery in North America. The study used beetles from two populations in eastern United States and focused on two aspects of diapause. First, the effects of daylength on the induction and depth (or duration) of diapause were examined by rearing and maintaining adult beetles from Tompkins County, New York, under six constant photoperiods until oviposition, death, or the passage of 125 days. Second, the seasonal progression of diapause maintenance and termination under natural conditions was evaluated by periodically (between October and April) testing the photoperiodic responses of overwintering adults that had been held out-of-doors; this test used beetles from Bergen County, New Jersey. Despite large variation in responses, the first test indicated that LD 16:8 had a strong, but not complete, diapause deterring effect. About 60% of females reared at LD 16:8 oviposited without delay, whereas photoperiods with shorter daylengths yielded lower incidences of oviposition without delay (18% at LD 14:10, 0% at LD 12:12 and 10:14). Only 27% of the females that experienced daylengths of LD 18:6 and 17:7 reproduced without delay. Females that survived the full length of the experiment (125 days) without ovipositing were considered likely to express an alternate (univoltine) life-history pattern that was unaffected by photoperiod. In the second experiment, results from the naturally overwintering *C. septempunctata* population indicated that females undergo significant changes in their photoperiodic responses during hibernal reproductive diapause. Based on the beetles' seasonal pattern of photoperiodic responses, we conclude that during autumn, short (and/or decreasing) daylengths play a role in diapause maintenance. By January, the photoperiodic maintenance of diapause diminishes, and by April, photoperiod no longer affects the timing of postdiapause reproduction. In our study, a small proportion of the overwintering adult *C. septempunctata* was parasitized by the koinobiont parasitoid *Dinocampus coccinellae* (Hymenoptera: Braconidae); data on the parasitoid's emergence pattern indicated that photoperiod plays a role in maintaining its diapause until the middle of January.

INTRODUCTION

The release and establishment of natural enemies into new regions for biological control of exotic pest species provides the opportunity to examine novel genotype-environment interactions, the evolutionary changes involved in range expansions, and the population dynamics of non-native species in new regions (Sethuraman et al., 2018, 2020; Zilio et al., 2023). The seven spotted ladybird beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), a Palearctic species that now occurs throughout most of North America, provides a good example. This species was first reported from North America in the 1970s, following its discovery in the Hackensack Meadowlands, Bergen County, New Jersey, USA and in Repentigny, L'Assomption Comte, Quebec, Canada (Angalet et al., 1979; Schaefer et al., 1987; Hodek & Michaud, 2008; Global Biodiversity Website, 2023). Population genetic studies using the CO1 gene indicate that the current *C. septempunctata* population in the United States resulted from the natural (or human assisted) spread of populations from the east coast of North America and also from additional importations from several Palearctic regions (Kajita et al., 2012).

This species is especially appropriate for studies of range extensions for a number of reasons. First, its recent establishment and spread throughout much of North America indicates that it possibly still is in a period of adaptation to its new homeland. Second, it expresses both intrapopulation and interpopulation variation in phenological and reproductive traits that appear to adapt it to a broad range of seasonally and regionally variable challenges. These traits include both seasonal (hibernal) dormancy and a pattern of

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† Professor Maurice J. Tauber deceased on 6 October 2014.

variable voltinism under conditions that appear favorable for growth and reproduction. Thus, the species' phenology appears complex, multifaceted, and interesting.

In addition to the above, comparative information on the seasonal phenology of *C. septempunctata* from its presumed Palearctic homeland is available. Specifically, the environmental (photoperiodic, thermal, and nutritional) influences on the timing of development, seasonal reproduction and dormancy are documented and reviewed (Hodek, 2012; Nedved & Honek, 2012). And several field-based and laboratory experiments have led to proposals of a genetic basis for at least some of the variation in voltinism among populations in central Europe (Hodek & Cerkasov, 1961; Hodek & Ruzicka, 1979; Hodek & Michaud, 2008; Hodek, 2012). These data from Eurasian populations are well summarized (Hodek, 2012) and provide focus for comparisons with populations relatively recently established in the Nearctic region.

In contrast to the *C. septempunctata* populations in Europe, during the ~50 years since its reported arrival, factors influencing the seasonal phenology of *C. septempunctata* in eastern North America have been the focus of relatively few studies. One study examined the thermal requirements for development in a single Nearctic population < 10 years after its reported discovery in North America (Obrycki & Tauber, 1981). This study documented requirements for preimaginal development similar to those of central European populations summarized by Honek (1996): K values of ~200 heat-degree-days (°C) above a threshold (*t*) of approx. 12°C. Later, selected developmental and reproductive life history traits (e.g., lengths of the preoviposition periods, interoviposition frequencies) of two Nearctic and two Palearctic populations of *C. septempunctata* were shown to have no statistically significant interpopulation differences (Phoofolo & Obrycki, 1995, 2000). Finally, an analysis of a long-term (31-year) dataset of the seasonality and habitat preferences of two nonnative predatory species *C. septempunctata* and *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) in Michigan, USA, revealed species-specific features in the temporal (based on degree day accumulations) and spatial occurrence that seem to contribute to the coexistence of these two nonnative predatory species (Arnold et al., 2023). Of the two species, *C. septempunctata* appeared to have the more stringent environmental preferences.

Here, to add to the information on the early phases of *C. septempunctata*'s postarrival establishment in North America, we report the results of phenological studies on two populations conducted soon after they were discovered in the early 1970s. Given the limited nature of these studies, our purpose here is not to provide definitive answers regarding the phenology of the North American population, rather it is to make available a relatively small set of data that we gathered from the species soon after its arrival in North America, and that hopefully can provide a basis for future comparative phenological and life history studies.

There are three parts to our study. First, we determined the influence of constant photoperiods on diapause induction and maintenance in the offspring of *C. septempunctata* adults collected in 1981 in central New York State (approx. 42.4°N, 76.5°W). Second, we determined the role of photoperiod in diapause maintenance and termination in naturally overwintering *C. septempunctata* adults collected in New Jersey, USA (approx. 40.8°N, 74.1°W) in 1976. Because diapause is a dynamic state (Tauber et al., 1986; Tougeron, 2019; Denlinger, 2022), we periodically (fall, winter, and spring) tested the photoperiodic responses of overwintering adults that had been maintained under natural conditions in the field. As part of this study (diapause maintenance and termination), we coincidentally recorded the emergence pattern of the koinobiont parasitoid, *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae) [= *Perilitus coccinellae* (Schrank)]) that had parasitized field collected *C. septempunctata* adults used in our diapause termination experiment above. This parasitoid overwinters as an egg or first instar within an adult ladybird beetle host (Kadono-Okuda et al., 1995; Ceryngier et al., 2004, 2012, 2023; Fei et al., 2023); subsequently the mature larva exits from its host. For this study, we recorded parasitoid emergence from hosts under the photoperiods used to study the field-collected *C. septempunctata*. Because this parasitoid is reported to be prevalent and attacks *C. septempunctata* in Eurasia and elsewhere, including North America (Ceryngier et al., 2012, 2023), it is unknown whether the parasitoids encountered here have native or introduced origins.

MATERIAL AND METHODS

Diapause induction in *C. septempunctata* reared at constant photoperiods

Adult *C. septempunctata* were collected in Tompkins County, New York (approx. 42.4°N, 76.5°W) in June 1981. Five male-female pairs were established in individual cages held at LD 16:8, 22°C; they were provided with water, a Wheast diet [mixture of Wheast, protein hydrolysate of yeast, sugar, and honey (volumetric ratio 1:1:1:1)] (Tauber & Tauber, 1975), and an excess daily supply of live aphids [pea aphids, *Acyrthosiphon pisum* (Harris), and green peach aphids, *Myzus persicae* (Sulzer)]. Eggs were collected daily and systematically distributed among six photoperiods (LD 18:6, 17:7, 16:8, 14:10, 12:12, 10:14), at 22°C ± 1°C (Table 1); larvae were reared individually on *A. pisum* in glass vials. Because the origins of the North American populations of *C. septempunctata* are not precisely known, we included two photoperiods (LD 18:6 and 17:7) that are representative of long summer daylengths in the northern European regions of its native range. After emergence, pairs of F1 adults were held in 0.24 L paper containers at the same preimaginal photoperiod they experienced as immatures. They were provided with water, the Wheast-protein diet described above, and an excess daily supply of live aphids (*A. pisum* and *M. persicae*). The date of first oviposition of each female was recorded. We calculated the percentage of females ovipositing at each photoperiod and the mean and median number of days for the preoviposition period under each photoperiod. Adults were maintained until oviposition, or death occurred, or for 125 days.

The oviposition pattern of *C. septempunctata* beetles does not clearly distinguish nondiapause reproduction from postdiapause reproduction. Therefore, we used a cutoff value of 22 days as the criterion for categorizing females; this value was derived as 2×

Table 1. Photoperiodic effects on the pattern of reproduction (oviposition versus reproductive diapause) in female *Coccinella septempunctata* from Tompkins County, New York, USA. F1 immatures were reared and the resulting adults were paired and maintained under one of six constant photoperiods (at 22 ± 1°C, for 125 days posteclosion).

	Daylength (L:D)						Log-rank test
	18:6	17:7	16:8	14:10	12:12	10:14	
Preoviposition period (days)[1]							
Mean ± SE	15.3±9.8	15.3±1.9	13.0±4.3	43.2±31.5	64.8±9.7	85.2±17.7	Chi^2=4.566,
Min–Max	10–30	14–18	7–19	9–78	54–80	64–118	df = 5,
Median	10.5	14.5	11	47	62	82.5	P = 0.47
No. females tested	11	15	15	11	12	12	
No. females that oviposited (% oviposited)	4 (36%)	4 (27%)	9 (60 %)	5 (45%)	6 (50%)	6 (50%)	
No. females that oviposited within cutoff time (22 days)[2]	3	4	9	2	0	0	
No. females that survived 125 days without oviposition	5	10	6	5	6	5	
No. females died within 125 days	2	1	0	1	0	1	

[1] Nonparametric log-rank analysis within row examines response to the 6 photoperiods which includes females that died or did not oviposit within 125 days (ChiSq = 4.566, df = 5, P = 0.47).

[2] The criterion for distinguishing nondiapause oviposition from postdiapause oviposition was calculated as 2× the median preoviposition period (days) observed at LD 18:6, 17:7, and 16:8. The median value for these three photoperiods was 11 days; therefore, the cutoff time for the nondiapause preoviposition period was 22 days.

the median preoviposition period (days) observed at LD 18:6, 17:7, and 16:8. In the study here the median value for these three photoperiods was 11 days; therefore, the cutoff time for the nondiapause preoviposition period was 22 days after emergence. This type of classification was used previously to separate females into diapause and nondiapause groups in studies of adult reproductive diapause in predatory hemipterans (Ruberson et al., 2000, 2001) and for three species of predatory ladybird beetles in the genus *Hippodamia* (Obrycki, 2020). For diapausing females, the length of the preoviposition period was used as an indicator of the depth of diapause the beetles experienced.

Samples of overwintering *Coccinella septempunctata* and *Dinocampus coccinellae*

Our procedures for examining the influence of daylength on the maintenance and termination of diapause followed those developed by Tauber & Tauber (1973) and which had been used on two other species of predatory ladybird beetles [*Coleomegilla maculata* (de Geer) and *Adalia bipunctata* L.] (Obrycki & Tauber, 1979; Obrycki et al., 1983). For this part of the study we focused on a well-established population of *C. septempunctata*, that was first detected in the early 1970s, in Lyndhurst Township, Bergen County, New Jersey, USA (approx. 40.8°N, 74.1°W) (Angalet et al., 1979; Schaefer et al., 1987). A cohort of adult beetles from this population was gathered in August 1976 and maintained outdoors by USDA-ARS personnel at the USDA Beneficial Insects Introduction Research Unit in Newark, Delaware, USA. Four samples of these adults were shipped overnight to our laboratory at Cornell University and experiments started on October 28, 1976, January 13, 1977, March 16, 1977 and April 13, 1977. Within 24 h of arrival on each date, we distributed 16–25 male-female pairs of beetles among five photoperiods (LD 16:8, 14:10, 12:12, 10:14, and 9:15) at 24 ±1°C (Table 2). Beetles were provided with water, the Wheast-protein mixture described above, and an excess daily supply of live aphids (*A. pisum* and *M. persicae*).

For each of the four samples, the incidence of females that oviposited as well as the mean and median preoviposition periods were recorded for all photoperiodic conditions (Table 2). These values reflected the presence or absence of diapause and the depth of diapause in females at the time of the sample. For our calculations here, we considered females that did not oviposit during the period of the test (see Table 2) as the "univoltine" part of the population. Taken together across all samples the values reveal the relative size of the univoltine part of the population as well as the seasonal progression in the photoperiodic maintenance of diapause and the estimated time of diapause termination under field conditions.

For the parasitoid *D. coccinellae* we recorded the exit dates of larvae from their *C. septempunctata* hosts. The mean ± SD number of days from sample date to larval exit was calculated for each photoperiodic condition at each sample date. Because of the low numbers of *D. coccinellae* larvae emerging from *C. septempunctata* hosts (Table 3), no statistical analysis of these data was conducted.

Statistical analysis

Because a high percentage of *C. septempunctata* females did not oviposit at each photoperiod in the diapause induction and maintenance experiment and data were not normally distributed (Table 1), we first analyzed the preoviposition periods (number of days from female eclosion to first oviposition) from all photoperiods using a nonparametric log-rank analysis (JMP 17.0.0). Because they had no preoviposition periods, females that died or did not oviposit within 125 days, the duration of the experiment, were censored. [See previous treatment of similar data from the ladybird beetle *H. axyridis* (Raak-Van Den Berg et al., 2013)]. We then examined only those *C. septempunctata* females that oviposited at each photoperiod, using 22 days as the preoviposition period that delineates nondiapause reproduction from postdiapause reproduction.

In our study of the influence of photoperiod on the maintenance and termination of diapause, the length of the preoviposition period (number of days from sample date to first oviposition) at each photoperiod was compared within the four sample dates (Table 2). Because the data were not normally distributed, we used a nonparametric Kruskal-Wallis test (JMP 17.0.0) to analyze the response to photoperiod within each sample date. To quantify the dynamic nature of hibernal diapause in this species, we also used the nonparametric Kruskal-Wallis test to compare responses to a given photoperiod among the four sample dates (Table 2). Multiple comparisons of mean preoviposition periods were analyzed using the nonparametric Wilcoxon method (JMP Pro, 2023). Females that died or did not oviposit were not included in these analyses.

RESULTS

Diapause induction in lab-reared *C. septempunctata* from Tompkins County, New York

Among the various photoperiodic treatments in the diapause induction experiment, between 40 and 67% of females did not oviposit, but they survived and appeared healthy for the 125 days of the experiment (Table 1). The highest incidence of nonreproducing females (67%) occurred in LD 17:7, and the lowest was at LD 16:8 (40%). Based on a nonparametric log-rank analysis the number of days to initiate oviposition did not vary significantly with photoperiod (ChiSq = 4.566, df = 5, P = 0.47).

Among the various photoperiodic treatments used in the diapause induction experiment, LD 12:12 and 10:14 yielded no reproduction within the 22-day cutoff period for nondiapause reproduction. Among the longer daylengths tested, LD 16:8 yielded the highest incidence of nondiapause reproduction (60% of females) and also a relatively short preoviposition period (13.0 ± 4.3 days). The intermediate daylength (LD 14:10) resulted in a relatively low incidence (18%) of nondiapause reproduction, and the two daylengths that were longer than LD 16:8 had relatively low incidences of nondiapause reproduction (27% each). Thus, it appears that the critical photoperiod for diapause induction (50% induction of diapause) in this population of *C. septempunctata* probably lies between LD 16:8 and 14:10. Although relatively few females oviposited under the longer daylengths of LD 17:7 and 18:6, the preoviposition periods of the females that did oviposit were relatively short – i.e., roughly equivalent to those at LD 16:8.

It is also worth noting the interesting pattern of variation in the preoviposition periods that were longer than the 22-day cutoff value for nondiapause oviposition. Most of the delayed oviposition occurred in the three treatments with short daylengths: LD 14:10 to 10:14 (Table 1). Within these

Table 2. Seasonal effects on the progression of dormancy (diapause and postdiapause development) in *Coccinella septempunctata* held under natural conditions out-of-doors in Bergen County, New Jersey, USA. On four dates during the overwintering period, samples were taken from the overwintering site and placed under five constant photoperiods (at 24°C ± 1°C). For each sample date, the length of time (days) from the transfer to oviposition was the measure of the photoperiodic influence on the progression of diapause under natural conditions. Nonparametric Kruskal-Wallis Test: (1) Within rows, the test examined the response to photoperiod within each sample date (results are in the right column). (2) Within columns, the test examined the response to each photoperiod among the four sample dates (results are in the row at the bottom).

	Daylength (L:D)					Kruskal-Wallis test
	16:8	14:10	12:12	10:14	9:15	
Oct 28, 1976[1]						
No. females[2]	15	15	14	15	14	
No. females ovipositing (% ovipositing)	5 (33%)	14 (93%)	7 (50%)	4 (27%)	7 (50%)	
Time (days) from sample to oviposition:						
Mean ± SE[3]	18.2 ± 15.7 A ab	13.5 ± 3.5 A a	23.7 ± 2.9 A b	24.8 ± 1.0 A b	43.1 ± 21.6 A b	$\text{Chi}^2 = 20.74$,
Min–Max	9–46	8–22	19–27	24–25	13–70	df = 4,
Median	13	13	24	24.5	43	P = 0.0004
Jan 13, 1977						
No. females[2]	21	24	22	24	21	
No. females ovipositing (% ovipositing)	11 (52%)	17 (71%)	11 (50%)	9 (38%)	8 (38%)	
Time (days) from sample to oviposition:						
Mean ± SE[3]	10.7 ± 1.7 A	9.4 ± 2.6 B	12.0 ± 3.9 B	11.0 ± 4.7 B	13.9 ± 5.5 B	$\text{Chi}^2 = 7.10$,
Min–Max	9–13	6–15	8–19	5–20	9–24	df = 4,
Median	11	9	11	9	12.5	P = 0.1305
March 16, 1977						
No. females[2]	22	18	21	23	25	
No. females ovipositing (% ovipositing)	13 (59%)	13 (72%)	13 (62%)	15 (65%)	14 (56%)	
Time (days) from sample to oviposition:						
Mean ± SE[3]	6.9 ± 1.3 B	8.6 ± 3.5 B	9.8 ± 5.3 BC	7.1 ± 4.2 C	9.6 ± 5.3 C	$\text{Chi}^2 = 8.59$,
Min–Max	5–10	5–18	6–24	4–21	6–26	df = 4,
Median	7	7	8	6	7.5	P = 0.0721
April 13, 1977						
No. females[2]	16	17	17	17	16	
No. females ovipositing (% ovipositing)	14 (88%)	10 (59%)	11 (65%)	11 (65%)	13 (81%)	
Time (days) from sample to oviposition:						
Mean ± SE[3]	7.2 ± 2.4 B b	6.2 ± 1.5 C b	6.6 ± 1.7 C b	9.4 ± 1.7 B a	7.2 ± 2.5 C b	$\text{Chi}^2 = 14.56$,
Min–Max	4–12	3–8	5–11	7–13	5–14	df = 4,
Median	6.5	6	6	9	6	P = 0.0057
Kruskal-Wallis test	$\text{Chi}^2 = 21.42$, df = 3, P = 0.0001	$\text{Chi}^2 = 25.94$, df = 3, P = 0.0001	$\text{Chi}^2 = 25.48$, df = 3, P = 0.0001	$\text{Chi}^2 = 19.67$, df = 3, P = 0.0002	$\text{Chi}^2 = 24.27$, df = 3, P = 0.0001	

[1] For the October sample, beetles were maintained for at least 80 days; maintenance was slightly shorter for the Jan, March, and April samples. Exact termination dates were not recorded.

[2] Number of females at each photoperiodic condition that were not parasitized by the parasitoid *Dinocampus coccinellae*. The number of adults (females/males) initially placed in each photoperiod for each sample date: October 28 (16/16), January 13 (25/25), March 16 (25/25), April 13 (20/20).

[3] Means within rows followed by the same lowercase letter are not significantly different (Wilcoxon Method); Means within columns followed by the same uppercase letter are not significantly different (Wilcoxon method).

three photoperiods, the mean, as well as the median, minimum, and maximum values of the preoviposition periods appeared to be inversely related to daylength. Daylengths longer than LD 16:8 were not associated with further shortened preoviposition periods. Finally, a substantial number of females survived the 125 days of the experiment, but did not oviposit.

Diapause maintenance and termination in field-collected *C. septempunctata*

The preoviposition periods of *C. septempunctata* females transferred from the field to five LD conditions in October varied significantly (P = 0.0004) in response to photoperiod (Table 2). The median values increased from 13 days at LD 16:8 and 14:10, to approximately 24 days at LD 12:12 and 10:14, and 43 days at LD 9:15 (Table 2). Whether this pattern of response resulted from the actual sustained daylength that the beetles experienced after the transfer or from the size of the change in daylength at the time of their transfer is not distinguishable from our data. By the middle of January, the median preoviposition periods tended to be shorter at each photoperiod, and the response of females in the various photoperiodic conditions was similar. In the March and April samples, the median preoviposition periods ranged from 6 to 9 days under all photoperiods (Table 2), and the actual range of variation (in days) was small. There was significant variation in the preoviposition periods in response to the five photoperiods over the four sample dates (Table 2 – analyses within columns). Within each of the five photoperiodic conditions there was a consistent pattern of a gradual decline in the preoviposition periods over the four samples from late October to the middle of April (Table 2).

Diapause maintenance and termination in the parasitoid *Dinocampus coccinellae*

Because of the relatively low numbers of *D. coccinellae* larvae that exited from *C. septempunctata* hosts (Table 3), we did not conduct statistical tests on parasitoid emergence. However, we observed that for each sample date, 4%–10% percent of the beetles yielded parasitoids and except for LD 9:15 in the March sample, larval parasitoid exit occurred under all treatments. In general, the time for parasitoid emergence after transfer from the field tended to decrease with sample date.

Table 3. Mean number of days ± SD for *Dinocampus coccinellae* larvae to exit from adult *Coccinella septempunctata* hosts after transfer from outdoors (New Jersey, USA) to five constant photoperiods (all at 24 ± 1°C) on four sample dates. Number in parenthesis = number of parasitoid larvae that exited from hosts.

Sample date	Daylength treatment (L:D)				
	16:8	14:10	12:12	10:14	9:15
Oct 28, 1976	16	22	22.5±0.7	1	31.5±2.1
(No.)	(1)	(1)	(2)	(1)	(2)
Range			22,23		30,33
Jan 13, 1977	12.0±1.4	9	14.0±5.2	12	13.8±2.2
(No.)	(4)	(1)	(3)	(1)	(4)
Range	10–13		11–20		11–16
March 16, 1977	10.0±3.6	11.1±2.3	9.0±1.4	8.5±2.1	–
(No.)	(3)	(7)	(4)	(2)	(0)
Range	7–14	9–14	8–11	7,10	
April 13, 1977	8.8±1.7	11.0±2.6	6.7±0.6	8.7±2.3	8.3±2.1
(No.)	(4)	(3)	(3)	(3)	(4)
Range	7–11	8–13	6–7	6–10	6–11

DISCUSSION

Seasonal phenology of European and North American *C. septempunctata*

The results from our study indicate that within 10 years of its introduction the North American population of *C. septempunctata* expressed numerous similarities in its life history with populations in its presumed European homeland. Most noticeable is the expression of two forms of life-history trajectories – one with a photoperiodically based univoltine, bivoltine or multivoltine life cycle, another with an apparently obligatory univoltine life cycle.

These types of life histories were reported for European populations (Hodek & Okuda, 1997; Hodek, 2012). And, in our study, the pattern of dual life-histories was observed in the diapause induction experiment in which 47 to 67% of females, including many under long daylengths and all with sufficient aphid prey, did not oviposit, but survived and appeared healthy for the full 125 days of the experiment (Table 1). The possibility of dual responses also is supported in the overwintering study, in which relatively large numbers of females survived for long periods without ovipositing (Table 2). The percentages of non-reproductives apparently were not affected by either sample date or by post-sample photoperiod. Although this type of life cycle has not been fully described and its physiological and perhaps genetic bases have not been elucidated, the frequent, but variable, reports of non-reproductives among *C. septempunctata* geographical populations (Hodek, 2012; Wang et al., 2013) make it an enticing focus for future ecophysiological and genetic exploration.

The life-history trajectory with a photoperiodically based diapause, as expressed by *C. septempunctata,* is a seasonal response commonly found among coccinellids; it can result in univoltine, bivoltine or multivoltine life cycles, and it includes reproductive dormancy induced and maintained by photoperiod (Table 2). In general, long daylengths, optimal temperatures, and abundant prey tend to promote reproduction, and the inverse conditions (short daylengths, lower temperatures, and prey restrictions) induce reproductive diapause, dormancy, and/or migration (Hodek & Ruzicka, 1979; Hodek, 2012). However, the potentially high reproductive capacity of a multivoltine population may come with the cost of high mortality if seasonal conditions become unpredictable. In contrast, the alternate trajectory (an obligatory univoltine life-history) presumably is associated with reduced requirements for food and perhaps increased resilience against extreme (unpredictable) conditions (Hodek & Michaud, 2008). However, the benefits to survival associated with this trajectory come with the cost of reduced reproductive capacity during favorable conditions.

It has been proposed that evolutionary selection for females with dual pathways of reproductive variability could underlie adaptation to an unpredictable food supply and/or periods of range expansion or environmental disruption, including global warming (Bradshaw & Holzapfel, 2006; Hodek & Michaud, 2008; Zilio et al., 2023). In this respect, the univoltine part of the *C. septempunctata* population remains largely unexplored. For example, it is not known when, or under what circumstances, univoltine females become reproductive. Nor are the physiological or genetic bases for the dual life cycle known for *C. septempunctata* or any other ladybird beetle. Theoretically, if the life-history trajectory is genetically determined and heritable, it could serve as a form of bet-hedging response to unpredictable environmental change, which could reduce the temporal variance in fitness associated with uninterrupted reproduction in a fluctuating/variable environment (Joschinski & Bonte, 2020).

We propose that the variation in *C. septempunctata*'s seasonal phenology may be described as a continuum ranging from (i) nonreproductive (univoltine) females undergoing some form of prolonged period of suppressed reproduction, possibly combined with migration (see discussion in Zilio et al., 2023) to (ii) individuals that express a seasonal cycle of reproduction and dormancy based on diapause in response to annually recurring local environmental conditions, and (iii) nondiapause females that reproduce rapidly [within approx. 14 days of eclosion (Phoofolo & Obrycki, 1995)]. We suggest that the molecular and physiological tools are now available for use in combination with experimental and observational studies to elucidate the basis of the variation in the complex seasonal phenology of *C. septempunctata* populations (Qi et al., 2015; Ren et al., 2016; Kostal et al., 2017; Xiang et al., 2021; Nadeau et al., 2022; Denlinger, 2023).

Future studies

The ability to use the molecular and physiological tools to investigate the complex variation in coccinellid life-history adaptations can be used in conjunction with the substantial natural history and experimental data currently available from a broad range of coccinellid species. In the section below, we highlight some of the available studies that show a broad range of features shared with *C. septempunctata* and some significant areas of diversity. From these comparisons, we also make some recommendations for future explorations.

Comparisons – diapause induction

The incidences of photoperiodic induction of adult hibernal reproductive diapause, as well as the occurrence of a nonreproductive (univoltine) life style, have been shown to be highly variable within and among European populations of *C. septempunctata* (reviewed by Hodek, 2012). In contrast, Wang et al. (2013) observed relatively uniform phenological responses in a population of *C. septempunctata* from Beijing, China (approx. 40° N). Given this broad range of geographic variation, future studies might consider examining the responses of interpopulation hybrids to explore the genetic basis for the observed response patterns [See experiments conducted with lacewing predators (Tauber & Tauber, 1982)].

Compared with other North American species of ladybird beetles (with the rare exception of *Coccinella novemnotata*; see Table S1), *C. septempunctata* shows uniformly high incidences (80–100%) of diapause induction in response to short daylengths (LD 12:12 and 10:14). The response to LD 14:10 in our population of *C. septempunctata* (82% diapause) is also similar to the responses of three other tested species: *Hippodamia parenthesis* (Say), *Hippodamia variegata* (Goeze) and *Propylea quatuordecimpunctata* (L.) (Table S1). However, among all the species and populations, our *C. septempunctata* population showed the lowest incidence of nondiapause oviposition (60%) at LD 16:8 (which was the longest daylength tested in all populations except for *C. novemnotata*) (Table S1). The physiological and genetic bases for this variable responsiveness to daylengths are unknown and of considerable interest.

Similar to our results for *C. septempunctata,* photoperiod (LD 16:8 to 10:14) also had a significant effect on the lengths of the preoviposition periods of females in three North American populations of *Hippodamia* (from approx. 42°N latitude)*: Hippodamia convergens Guérin-Méneville, H. parenthesis* (Say), and *H. variegata* (Goeze) (Table S2). At LD 14:10, these three species exhibited a wide range of median preoviposition periods (e.g., from 11 days for *H. convergens* to 90 days for *H. parenthesis*) (Table S2). Moreover, the trend of an inverse relationship between daylength (LD 16:8, 14:10, 12:12, and 10:14) and the length of the preoviposition period that we documented in *C. septempunctata* was not observed in any of the *Hippodamia* species. These results indicate that the four species from similar latitudes may respond very differently to photoperiod under laboratory conditions; future studies are needed to determine if these differences correspond to differences in seasonal phenologies.

Comparisons – diapause maintenance and termination

The results from our North American population of *C. septempunctata* are similar to those reported for Palearctic populations in which responses to long and short photoperiods during diapause were highly variable, but short daylengths tended to prolong diapause in autumn (Hodek & Ruzicka, 1979; Hodek, 2012). Studies also showed that the photoperiodic sensitivity (responsiveness) of *C. septempunctata* females generally declined over the autumn and showed little change during winter (Table 2). This type of pattern is similar to that observed in two other ladybird beetle species that overwinter as adults, *Coleomegilla maculata* and *Adalia bipunctata* (Obrycki & Tauber, 1979; Obrycki et al., 1983), but the findings indicate a broad range of variability. For example, photoperiodic sensitivity in diapausing *A. bipunctata* females ends by the end of January, while a portion of *C. maculata* females continue to respond to photoperiod until early March. Within the *C. septempunctata* population we studied, a portion of females appeared to end photoperiodic responsiveness by the end of January, but even in April, 25% to 40% of females appeared to be photosensitive as indicated by their delayed reproduction under short daylengths.

Other recent studies have quantified similarly complex and variable seasonal responses in native and nonnative populations of another species of ladybird beetles, *Harmonia axyridis* in Eurasia (Reznik & Vaghina, 2011, 2013; Raak-Van Den Berg et al., 2013; Reznik et al., 2015; Belyakova et al., 2021). One study of *H. axyridis* maintained outdoors in the Netherlands from October to December indicated that females have a relatively short and weak diapause response to photoperiod (Raak Vanden Berg et al., 2013). After introduction into European Russia, nonnative populations of this species exhibited an even further reduced role for photoperiod in regulating diapause, accompanied by an increased influence of nutrition/prey availability in diapause induction (Ovchinnikova et al., 2016; Reznik et al., 2015). These findings from *H. axyridis* indicate that rapid and substantial changes in diapause and other life-history characteristics, at least in some coccinellid species, may occur over relatively short time periods. These response patterns should be examined when the species is introduced to new habitats.

Finally, the role of decreasing daylength in maintaining autumnal diapause demonstrated here in at least a portion of *C. septempunctata* females appears to be similar to its role in the diapause of the lacewing predator *Chrysoperla* (= *Chrysopa*) *carnea* Stephens (Neuroptera: Chrysopidae) (Tauber & Tauber, 1973). This type of response pattern warrants further investigation.

Diapause in the parasitoid *Dinocampus coccinellae*

Our results showing that *D. coccinellae* larvae exited from 4 to 10% of field-collected beetle hosts from New Jersey are similar to levels (1% to 6%) previously reported by Angalet et al. (1979) for *C. septempunctata* also collected in New Jersey between 1975 and 1978. Studies of Palearctic populations of *C. septempunctata* have documented higher levels of parasitism (15% to 25%) by *D. coccinellae* (Ceryngier et al., 2012).

Among the four samples (Oct–April), the one taken in Oct showed notably longer times for *D. coccinellae* larvae to exit from hosts than those from the other sample dates. Exit times in the January, March, and April samples appeared to be similar at all photoperiods. Thus, we conclude that *D. coccinellae* overwintering in North American *C. septempunctata* hosts probably terminate diapause by the middle of January. Thus, the duration of diapause in *D. coccinellae* overwintering within *C. septempunctata* adults appears similar to that reported when the host was the Nearctic ladybird beetle *Coleomegilla maculata* (Obrycki & Tauber, 1979; Tauber et al., 1983). This period is within that observed previously in a study of *D. coccinellae* overwintering in a French population of *C. septempunctata*; in that population the response to photoperiod ended between early December and late March (Hodek et al., 1977).

CONCLUSION

Hodek (2012) speculated that the following factors may interact in the diapause syndrome of European populations of *C. septempunctata*; photoperiod, temperature, nutrition (prey availability), and possibly a density dependent effect of adult population. Although limited, our current study provides data on *C. septempunctata*'s life history and photoperiodic responses during an early period in its establishment and adaptation to seasonal conditions in northeastern USA. As show above, these data could be used, perhaps as a basis for comparison, in future studies aimed at documenting current or future changes in phenological and photoperiodic responses that accompany the species' adaptation to North American seasonal and geographic conditions. We hope that such studies will be conducted.

An understanding of the historical and current phenological responses of North American populations of *C. septempunctata* is valuable as a basis for comparison with its ancestral population(s) and thus for understanding the recent and unfolding evolutionary history of *C. septempunctata* on its new continent, involving interactions of photoperiod, temperature, prey availability, and intraguild relationships (Hodek & Michaud, 2008; Hodek, 2012; Arnold et al., 2023). Such an understanding could also influence our ability to use this predator in biological control of aphid pests and to understand its influence on the biodiversity of native and nonnative species of ladybird predators (Rondoni et al., 2020; Li et al., 2021; Soares et al., 2023).

Finally, *C. septempunctata* is just one of many ladybird species now established in North America that is of potential value in biological control. Many of these species have been studied and exhibit significant forms of seasonal and photoperiodic variation in their life histories (Table S1). As an aid in documenting and comparing results across species, we compiled relevant information and references in Tables S1 and S2.

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Supplementary Tables S1 and S2 follow on next pages.

Table S1. Summary of published studies on the photoperiodic induction of reproductive diapause in North American populations of native and nonnative species of predatory ladybird beetles (Family Coccinellidae; Tribe Coccinellini). Unless noted, immatures were reared and adults were maintained under constant photoperiods and temperatures. The criteria used to determine reproductive diapause are defined for each species. NT = response to this L:D was not tested.

		Percentage (%) of Females in Diapause							
		Photoperiod (L:D)							
Species	Population / °N Lat	18:6	17:7	16:8	15:9	14:10	12:12	10:14	Reference
Native									
Adalia bipunctata [1]	NY-USA / 42°N	NT	NT	0	0	46	100	100	Obrycki et al., 1983
Coccinella novemnotata [2]	CA-USA / 37°N								McMullen, 1967
	15.6°C	82	NT	0	NT	50	89	100	
	21.1°C	20		0		5	70	96	
	26.7°C	7		0		0	10	40	
	32.2°C	10		0		11	10	20	
Coccinella transversoguttata [3]	ME-USA / 47°N	NT	NT	20		67	100	NT	Storch, 1973
Cycloneda munda [4]	KY-USA / 38°N	NT	NT	0	NT	5	83	100	Obrycki, 2022
Hippodamia convergens [5]	IA-USA / 42°N	NT	NT	9	NT	12	100	88	Obrycki et al., 2018
	KS-USA / 39°N			17		0	82	93	
	TX-USA / 31°N			5		29	94	91	
Hippodamia parenthesis [6]	NY(North)-USA / 44°N	NT	NT	15	NT	100	100	100	Obrycki, 2020
	NY(Central)-USA / 43°N			19		50	100	100	
	IA-USA / 42°N			19		100	100	100	
	IL-USA / 40°N			17		65	100	100	
Hippodamia tredecimpunctata [7]	ME-USA / 47°N	NT	NT	0	NT	23	73	NT	Storch & Vaundell, 1972
Non native									
Coccinella septempunctata [8]	NY-USA / 42°N	73	73	40	NT	82	100	100	Current study
Hippodamia variegata [9]	NY-USA / 44°N	NT	NT	15	NT	100	100	100	Obrycki, 2018
	IL(North)-USA / 42°N			21		27	100	100	
	IL(Central)-USA / 40°N			13		81	100	100	
Propylea quatuordecimpunctata [10]	QUE-CAN / 45.5°N			22		80	90	90	Jones et al. (2023)
	NY-USA / 44°N			8		100	100	100	

[1] Data from Table 1 (Obrycki et al., 1983), Temp = 23° ± 1°C; Criterion for diapause = pre-oviposition period > 14 days.

[2] Data from Table III (McMullen, 1967), Study examined response to photoperiod at 4 temperatures and 3 prey levels (5, 10, 20 pea aphids/day/adult). Data presented here are for the highest prey level at each L:D and temperature tested in the study. Under reduced prey levels there were slightly higher incidences of diapause in 75 % of the treatments; reduced prey did not appear to affect the incidence of diapause in 20% of treatments; at L:D14:10 (32.2°C) lower incidences of diapause occurred with lower prey levels. Criteria for diapause (all treatments) = no oviposition within 21 days after eclosion at 15.6°C, within 14 days of eclosion at 21.1°C , within 10 days at 26.7°C, and within 7 days at 32.2°C; followed by dissection to confirm lack of ovogenesis and accumulation of fat body.

[3] Data from Table 1 (Storch, 1973), Study examined larval and adult responses to 4 photoperiods (LD 24:0, 16:8, 14:10, and 12:12) at 21°C ± 2°C. Preimaginal development was at one of the four photoperiods; newly eclosed females were placed in one of the four LD conditions; males were placed into LD 16:8. Ten days after eclosion, each female was paired with a male; oviposition was recorded for 20 days. Criterion for diapause = no oviposition during the 20 day period. Data presented in this table are from immatures and adults reared at the same LD condition (LD 16:8, 14:10, and 12:12).

[4] Data from Table 2 (Obrycki, 2022), Temp = 22°C ± 1°C. Criterion for diapause = median preoviposition period (days) > 2× median preoviposition period (days) at LD 16:8 or did not oviposit during the 110 day experiment.

[5] Data from Table 2 (Obrycki et al., 2018), Temp = 22°C ± 1°C. Criterion for diapause = preoviposition period > 2× median preoviposition period at LD 16:8 or did not oviposit during the 95 day experiment.

[6] Data from Table 6 (Obrycki, 2020), Temp = 22°C ± 1°C. Criterion for diapause = median preoviposition period > 2× median preoviposition period at LD 16:8 or did not oviposit during the 120 day experiment.

[7] Data from Table 2 (Storch & Vaundell, 1972), Study examined larval and adult responses to 4 photoperiods (LD 24:0, 16:8, 14:10, and 12:12) at 21°C ± 2°C. Preimaginal development occurred at one of four photoperiods, newly eclosed females were placed in one of the four LD conditions, males were placed in LD 16:8. Ten days after eclosion, each female was paired with a male; oviposition was recorded for 20 days. Criterion for diapause = no oviposition during the 20 day period. Data presented in this table from immatures and adults reared at the same LD condition (LD 16:8, 14:10, and 12:12).

[8] Data from Table 1 of current study. Criterion for diapause = median preoviposition period > 2× median preoviposition period at LD 16:8, 17:7, and 18:6 or did not oviposit during the 125 day experiment.

[9] Data from (Obrycki, 2018), Temp = 22 ± 1°C. Criterion for diapause = median preoviposition period > 2× median preoviposition period at LD 16:8 or did not oviposit during the 100 day experiment.

[10] Data for populations from Jefferson County, New York and Montreal, Quebec, Canada from Table S9 (Jones et al., 2023), Temp = 22 ± 1°C. Criterion for diapause = median preoviposition period > 2× median preoviposition period at LD 16:8 or did not oviposit during the 120 day experiment.

Table S2. Preoviposition period (days) as a measure of diapause depth in *Coccinella septempunctata* (Tompkins County, NY), *Hippodamia convergens* (Boone and Story Counties, IA), *Hippodamia parenthesis* (Johnston County, IA), *Hippodamia variegata* (McLean County, IL). First laboratory generation individuals were reared under constant photoperiods: L:D 16:8; 14:10; 12:12; and 10:14 at 22°C ± 1°C. Preoviposition period: Mean (days ± SE); Min–Max days; Median (days); [N] = number of ovipositing females. Nonparametric log-rank analysis bottom row of each column compares responses to each photoperiod by the four species.

Species	Daylength treatment (L:D)				Reference
Latitude 42°N	16:8	14:10	12:12	10:14	
Coccinella septempunctata (NY)					
Mean ± SE	13.0 ± 4.3	43.2 ± 31.5	64.8 ± 9.7	85.2 ± 17.7	Current study
Min–Max [N]	7–19 [9]	9–78 [5]	54–80 [6]	64–118 [6]	Data from Table 1
Median	11	47	62	82.5	
Hippodamia variegata (IL-North)					
Mean ± SE	13.1 ± 2.2	26.5 ± 1.5	–	–	Obrycki, 2018
Min–Max [N]	6–31 [14]	18–32 [11]	67 [1]	– [0]	
Median	9	23	–	–	
Hippodamia convergens (IA)					
Mean ± SE	8.8 ± 1.0	10.8 ± 0.6	47.7 ± 5.9	42.1 ± 4.4	Obrycki et al., 2018
Min–Max [N]	6–23 [23]	7–15 [17]	17–80 [12]	5–58 [13]	
Median	7	11	49	51	
Hippodamia parenthesis (IA)					
Mean ± SE	12.4 ± 2.4	87.4 ± 8.0	–	–	Obrycki, 2020
Min–Max [N]	4–43 [16]	49–120 [8]	– [0]	– [0]	
Median	8.5	90.5	–	–	
Log-Rank Chi^2,	24.2717,	65.1572,	43.8946,	46.5705,	
df,	3,	3,	3,	3,	
P	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	